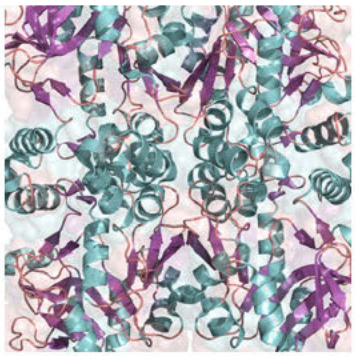
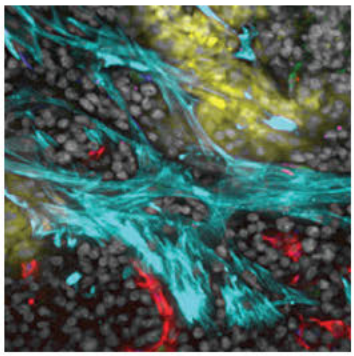
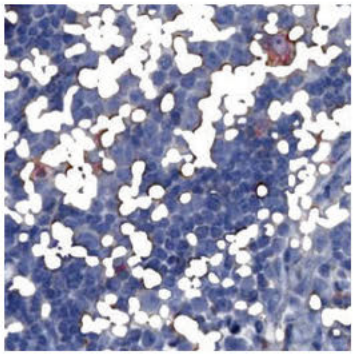
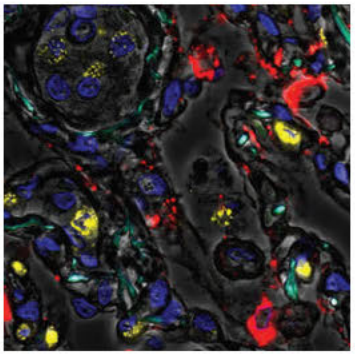
2019 2020 ANNUAL REPORT









Frederick National Laboratory
for Cancer Research

sponsored by the National Cancer Institute

Leidos Biomedical Research, Inc.

Operations and Technical Support Contractor for the Frederick National Laboratory for Cancer Research

2019–2020 Annual Report

Leidos Biomedical Research, Inc., operates for the National Cancer Institute under Contract No. 75N91019D00024.
P. O. Box B, Frederick, Maryland 21702-1201

The cover image depiction: **Top left:** Ribbon representation of the crystal structure of the Yersinia pestis UDP-glucose pyrophosphorylase at 2.17 angstroms resolution, obtained by the Basic Science Program. **Top right:** Multiplex immunofluorescence used to study hypoxia and inflammation in mouse tumor tissue, conducted by the Cancer Research Technology Program. **Bottom left:** CD4+ T cells (shaded in white) detected in a stained tissue section via AI models, conducted by the Biomedical Informatics and Data Science Directorate. **Bottom right:** Fluorescence micrograph image using the RNAscope technique to visualize SARS-CoV-2 viral RNA (red) and the host ACE2 receptor (green) in lung tissue from a COVID-19 patient. Neutrophils (yellow) were identified with anti-myeloperoxidase antibody, and nuclei (blue) were stained. Image captured by the AIDS and Cancer Virus Program.

Leidos Biomedical Research, Inc.

2019–2020 Annual Report

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Executive Summary

Frederick National Laboratory
for Cancer Research

sponsored by the National Cancer Institute

Leidos Biomedical Research, Inc.
Annual Report
September 1, 2019–August 31, 2020
Executive Summary

Leidos Biomedical Research, Inc., (Leidos Biomed) is pleased to submit this annual report for the Frederick National Laboratory for Cancer Research (FNL) Federally Funded Research and Development Center (FFRDC) sponsored by the National Cancer Institute (NCI), contract number: 75N91019D00024, for the period of September 1, 2019, to August 31, 2020.

This is the only FFRDC exclusively dedicated to biomedical research. FNL provides NCI and other institutes, such as the National Institute of Allergy and Infectious Diseases (NIAID), with this unique national resource to accelerate the development and delivery of effective preventive, diagnostic, and therapeutic products to people living with cancer, HIV/AIDS, and emerging health challenges like Zika virus, Ebola virus, and COVID-19.

This annual report documents the breadth of activities performed by Leidos Biomed in support of NCI's mission. These activities span the research and development continuum, including investigator-initiated, hypothesis-driven research into cancer and AIDS; advanced technology programs focused on genetics and genomics, proteins and proteomics, imaging, nanotechnology, bioinformatics, and laboratory animal sciences; clinical operations in support of NCI- and NIAID-sponsored clinical trials; NCI drug discovery and development efforts; and management and operations of biopharmaceutical development and manufacturing programs under current Good Manufacturing Practice conditions for NCI and NIAID. Essential administrative, financial, safety, security, and facilities services are provided to these research and development activities through state-of-the-art business processes.

National Resource

This annual report portrays the wide scope of activities conducted at FNL in support of its role in advancing the vital mission to reduce suffering from cancer, AIDS/HIV, and infectious diseases. The scope of activities conducted in support of its customers reflects FNL's service as a national resource.

FNL Customers

FNL provides direct program support to diverse divisions, offices, and centers within NCI. These include:

- **Center for Strategic Scientific Initiatives** – Develops and implements exploratory programs that invest in research and creates opportunities for scientists through initiatives, tools, and public resources. The objective of these discoveries and innovative technologies is to accelerate the pace of cancer research and the translation of research results into novel therapies, diagnostics, and preventive agents.
- **Center for Cancer Genomics** – Aligns NCI's activities in cancer genomics by synthesizing research in different fields of cancer genomics—structural, functional, and computational—in order to improve patient outcomes.
- **Center for Global Health** – Provides assistance and guidance to nations as they develop and implement cancer control plans; trains international investigators; and strengthens regional, national, bilateral, and multilateral collaborations in health research, cancer research, and cancer control to advance global cancer research, build expertise, and reduce cancer deaths worldwide.
- **Center for Biomedical Informatics and Information Technology** – Collaborates across the NCI to plan, provide, and coordinate technology, standards, and scientific computing in support of NCI's mission to speed discovery, facilitate open science, and make progress toward precision treatment in cancer care and a learning healthcare system.
- **Coordinating Center for Clinical Trials** – Facilitates efforts across NCI to enhance the effectiveness of NCI's clinical trials enterprise through collaboration and harmonization among NCI programs and extramural stakeholder communities.
- **Center for Cancer Research** – A distinguished and productive community of NCI intramural basic researchers, clinicians, and translational scientists who integrate basic and clinical research discovery to develop novel therapeutic interventions that better treat adults and children living with cancer or HIV/AIDS.
- **Division of Cancer Epidemiology and Genetics** – Conducts population and multidisciplinary research to discover the genetic and environmental causes of cancer and ways to prevent it.

- **Division of Cancer Biology** – Encourages and facilitates continued support of basic research in all areas of cancer biology to provide a research foundation that advances our understanding of cancer biology leading to new approaches for prevention, diagnosis, and treatment.
- **Division of Cancer Prevention** – Conducts and supports research to elucidate ways to prevent and detect cancer, and to prevent or relieve symptoms from cancer and its treatments.
- **Division of Cancer Treatment and Diagnosis** – Supports the translation of promising research into clinical applications to improve the diagnosis and treatment of cancer in areas of unmet need that are often too risky or difficult for industry or academia to advance alone.
- **Division of Cancer Control and Population Sciences** – Supports an integrated program of genetic, epidemiological, behavioral, social, applied, and surveillance cancer research to reduce risk, incidence, and death from cancer and enhance the quality of life for cancer survivors.

FNL also provides vital support to these NIAID divisions and centers:

- **Division of Intramural Research** – Conducts basic and clinical research across the breadth of disciplines related to immunology, allergies, and infectious diseases.
- **Division of Clinical Research** – Provides multi-disciplinary trans-NIAID services for facilitating clinical research and special projects as directed by NIAID leadership. One current example of this is how FNL rapidly pivoted its staff, expertise, and resources to combat the COVID-19 pandemic. This led to the facilitation of an international, multi-center, placebo-controlled remdesivir trial for acutely ill COVID-19 patients, who were hospitalized and oxygen-dependent or on a ventilator, which reduced time in the hospital, as published in the *New England Journal of Medicine*. Ongoing trials in concert with NIAID examine randomized combination regimens with remdesivir and other agents to combat COVID-19, antibody-based therapeutics (as part of Operation Warp Speed), and other active trials. This pivot is enhanced by efforts supported by the NCI in these areas of COVID-19 response: (i) serology, (ii) genetic susceptibility, (iii) discovery of novel therapeutics, and (iv) other initiatives.
- **Division of Acquired Immunodeficiency Syndrome** – Supports a global research portfolio on HIV/AIDS and its related coinfections and comorbidities.
- **Vaccine Research Center** – Conducts research that facilitates the development of effective vaccines for human disease.

Support is also provided to approximately 15 other institutes within the National Institutes of Health (NIH) and other federal agencies.

FNL National Programs and Initiatives

- **ATOM** – FNL is a founding member of Accelerating Therapeutics for Opportunities in Medicine (ATOM). The consortium is a public–private partnership working to transform drug discovery by dramatically accelerating the timeline for developing effective new cancer therapies.
- **RAS Initiative** – FNL is an integral part of a diligent national initiative to advance scientific insights of cancers driven by RAS mutations. Established by NCI, the initiative connects cancer scientists around the country and worldwide in a focused effort to make progress against *RAS*-driven cancers by using new approaches and technologies. Reagents and discoveries are shared with the scientific community.
- **Cancer Moonshot℠** – This initiative aims to make more therapies available to more patients faster, while also improving the ability to prevent cancer and detect it at an early stage. FNL-supported projects include: The Moonshot Pediatric Core, the high-performance computing/U.S. Department of Energy partnership, the precision medicine clinical trial, the Genomic Data Commons, and an effort to make cancer research more accessible.
- **National Cryo-Electron Microscopy Facility** – This shared user facility addresses the timely national need for structural biologists as well as cancer scientists to have access to advanced microscopes that support high-resolution cryo-electron microscopy studies. The facility opened in 2017 and already has collaborations with 39 institutions, more than 370 cancer-related data collections, and 25 high-impact publications in *Cell*, *Science*, *Nature*, and elsewhere.
- **Operation Warp Speed** – In concert with NIAID and as part of Operation Warp Speed, FNL is facilitating randomized placebo-controlled international clinical trials to combat COVID-19. These include small-molecule inhibitors as well as antibody-mediated therapeutics. A recent successful outcome with NIAID was published in the *New England Journal of Medicine* using remdesivir in a placebo-controlled international trial in hospitalized COVID-19 patients who were oxygen-dependent or on a ventilator. Other trials are underway.
- **HPV Serology Laboratory** – Launched in 2017, the laboratory promotes harmonization and proficiency of human papillomavirus (HPV) serology testing in vaccine trials. The work should expedite the implementation of HPV vaccines worldwide, especially in resource-limited countries where cervical cancer is a major public health burden. This HPV Serology Laboratory has pivoted its work to include COVID-19 serology and qualification of testing for the FDA.

COVID-19 Pivot

In addition to its COVID-19 serology efforts, FNL is also working with NCI to elucidate genetic susceptibility to COVID-19 and using tethered library screens in the RAS Initiative to identify novel candidate agents to combat COVID-19.

FNL Organization

President's Office: Ethan Dmitrovsky, M.D., President, Leidos Biomedical Research, Inc., and Laboratory Director, FNL

Three Leidos Biomed key staff report to Dr. Ethan Dmitrovsky, and each of them leads one of the three operating groups within this contract. Within the groups, there are 18 major directorates, offices, and programs, each of which has either a primary technology focus or is aligned with a primary customer. Support to the federal customers and performance of the RAS national mission are accomplished through collaboration across FNL.

Science and Technology Group: Leonard Freedman, Ph.D., Chief Science Officer

The Science and Technology Group (STG) provides scientific expertise and cutting-edge support for basic and applied research along with data management to the NCI, NIH, and the extramural scientific community. This laboratory-based research focuses on cancer, AIDS, and emerging infectious diseases, encompassing these areas:

- Discovery science
- Advanced core facility support for the NCI
- Collaborative science in concert with the NIH
- Team science led by FNL (i.e., RAS Initiative)
- Advanced technology to support the extramural community (i.e., National Cryo-EM Facility and the Nanotechnology Characterization Laboratory)

As an FFRDC, the FNL can pivot rapidly to apply substantial resources and innovative expertise to different and urgent public health challenges, as we are now doing to combat the COVID-19 pandemic.

The STG comprises five directorates and one office:

- **AIDS and Cancer Virus Program (ACVP)** – Studies viruses involved in cancer and pursues investigations that have potential or direct relevance to preventing, effectively vaccinating against, and treating HIV infection or AIDS.
- **Basic Science Program (BSP)** – Covers a wide spectrum of research activities with a focus on immunology and genetics in support of CCR.
- **Cancer Research Technology Program (CRTP)** – Serves as the program hub for the RAS Initiative and provides expertise in genomics, proteomics, imaging, informatics, and nanotechnology to NCI and external partners.
- **Laboratory Animal Sciences Program (LASP)** – Provides an integrated portfolio of research animal programs, including the development of genetically engineered mouse models, cryopreservation and assisted reproduction, pathology and histotechnology, small-animal imaging, molecular diagnostics, and animal husbandry.
- **Biomedical Informatics and Data Science (BIDS)** – Develops state-of-the-art technologies in large-scale data modeling, analysis, and integration. Working together with the NCI and Department of Energy, BIDS plays a substantial role in expanding the use of high-performance computing in cancer research. The partnership supports the government's Cancer Moonshot℠ and Precision Medicine initiatives by developing high-performance computing capabilities to meet the challenges of modeling problems in cancer on large-scale computing systems.
- **Partnership Development Office (PDO) –** Manages multiple partnering programs to facilitate innovative, collaborative research with external investigators. The PDO's mandate includes facilitating contractor Cooperative Research and Development Agreements (cCRADAs) and other partnership agreements; managing the Technical Services Agreements; and interacting with the regional, national, and international academic and scientific communities to identify strategic partners. There are over 159 unique partners and 53 executed cCRADAs in place.

Clinical Group: Barry Gause, M.D., Chief Medical Officer

The Clinical Group is composed of five directorates and three programs that provide broad support to the NCI and NIH for clinical research including direct involvement in patient care. The Clinical Group supports the intramural and extramural communities, including international studies related to emerging infectious diseases like Ebola virus, Zika virus, and SARS-CoV-2. The support includes:

- Patient care
- Clinical and laboratory monitoring of clinical trials
- Regulatory support
- Next-generation sequencing and proteomic analysis
- Biospecimen storage
- Biopharmaceutical and vaccine development
- Quality assurance and quality control

The Clinical Group includes:

- **Clinical Research Program Directorate (CRD)** – Provides quality assurance for the Biopharmaceutical Development Program (BDP) and the Vaccine Clinical Materials Program (VCMP), supports tumor sample acquisition, manages the collection and analysis of tumor and normal tissue on a molecular level, and provides technical support to research and development initiatives. CRD supports and performs assay development and research analyzing nucleic acid (genomic, transcriptomic, and metabolomic) and proteomics for epidemiologic evaluation and treatment assignment for patients with cancer, HIV, and other infectious diseases. CRD also provides technical project management expertise in support of contracts for drug development. Programs supported by CRD include:
  - Biological Resources Program
  - Molecular Characterization Center
  - Drug Discovery and Development Program
- **Clinical Monitoring Research Program Directorate (CMRPD)** – Provides comprehensive clinical research management, regulatory support, pharmacovigilance services, and protocol navigation/ protocol development support to NCI, NIAID, and other NIH Institutes. The directorate supports 11 clinical trials involving SARS-CoV-2, including trials for remdesivir, baricitinib, interferon beta, and hyperimmune immunoglobulin.
- **Applied and Developmental Research Directorate (ADRD)** – Provides clinical and biological monitoring, regulatory support, biospecimen processing and storage, assay development, and project management support to NCI and NIAID clinical programs.
- **Vaccine Clinical Materials Program (VCMP)** – Manufactures and provides biological agents used in NIAID-sponsored clinical trials both nationally and internationally. Areas of focus include HIV vaccines and monoclonal antibodies, Ebola vaccines, Malaria monoclonal antibodies, mosaic influenza vaccines, and SARS-CoV-2 cell line development.
- **Biopharmaceutical Development Program (BDP)** – Manufactures and provides biological agents used in NCI clinical trials. Recent high priority projects include CD33 and GD2 CAR T cells for pediatric acute myeloid leukemia and sarcoma/ neuroblastoma, respectively.

Operations Group: Kathy Terlesky, Ph.D., Chief Operating Officer

The Operations Group provides facility and business infrastructure to the NCI-Frederick government employee base and all FNL employees. Facility infrastructure includes the facilities, logistics, and information technology infrastructure and services necessary to operate the FFRDC. Improvements in facility infrastructure continued this year with renovation in progress for 30,000 square feet in various buildings on the 62-acre campus at Ft. Detrick. The Operations Group manages facilities in the NCI-Frederick government property and provides logistics specialists to deliver 180,000 packages across the campus annually.

Business infrastructure required to support the scientific programs at NCI-Frederick includes procurement, travel, conference planning, and subcontracting to support the associated scientific programs. Business infrastructure necessary for executing the contract includes the Financial Operations Directorate, the Project Management Operations Office, and the Contracts and Acquisitions Directorate. In addition, the Human Resources Directorate; Environment, Health, and Safety Directorate; and Communications Office deliver necessary services to support the workforce and create a safe workplace.

The Operations Group ensures personnel work efficiently and effectively to support vital scientific and clinical program areas. Performance is optimized by designing and implementing systems to facilitate portfolio management.

The Operations Group comprises six directorates and two offices:

- **Contracts and Acquisitions (C&A) Directorate** – Provides contract administration, purchasing, research and construction subcontracts oversight, intellectual property, and logistics support to FNL.
- **Human Resources (HR) Directorate** – Provides core services and supports competencies related to recruitment and staffing, employee relations and counseling, organizational and employee development, compensation and benefits, HR information management, and regulatory compliance.
- **Facilities Maintenance and Engineering (FME) Directorate** – Plans, designs, and executes facility improvements and advances for the technologically complex NCI-Frederick facilities.
- **Environment, Health, and Safety (EHS) Directorate** – Provides comprehensive security and protective services, environmental management, occupational and facility safety services, occupational health services, and emergency management to FNL and NCI-Frederick.
- **Business Services Directorate (BSD)** – Provides mission infrastructure services to the scientists at the NCI-Frederick campus. This includes procurement support; the Scientific Library; Glassware; Travel and Conference Planning; and the Scientific Publications, Graphics, and Media group.
- **Financial Operations Directorate (FOD)** – Oversees all finance-related activities for FNL, including the following functions: general accounting, payroll, accounts payable, billing, accounts receivable, financial planning and analysis, core business information systems, and audit and compliance.

- **Enterprise Information Technology (EIT) Directorate** – Provides services and infrastructure to support the communication backbone, data storage and computing, information security, help desk, and design and implementation services for software needs across the campus.
- **Project Management Operations Office (PMOO**) – Provides standardization, guidance, oversight, and leadership for the development, implementation, improvement, and extension of project management standards and processes across FNL.
- **Communications Office** – Supports FNL's National Laboratory mission through a program of external communications and community relations, internal business communications, and creative and effective multimedia services.

Global Impact

FNL activities are not limited to domestic programs. Leidos Biomed helps manage clinical research for the NIH Clinical Center, NCI, NIAID, and several other institutes within NIH, and is engaged in domestic and international studies related to cancer, AIDS, infectious diseases, emerging health threats, and various other diseases. FNL medical and clinical research professionals support numerous NIH clinics. FNL has global influence by facilitating clinical research programs and initiatives, such as the NCI Center for Global Health, NIAID's Ebola clinical research programs in Africa, and the International Network for Strategic Initiatives in global HIV trials. FNL is also part of an international network of collaborating institutions to conduct definitive clinical trials to combat COVID-19.

Community Involvement

FNL supports an extensive array of nonprofit organizations and charity fundraisers within the communities where our employees live and work. This past year, FNL remained steadfast in its approach to community outreach despite navigating through the ongoing pandemic.

Before the onset of the COVID-19 pandemic, employees devoted their time to volunteer at many local organizations, such as Habitat for Humanity, the Frederick Rescue Mission, Blessings in a Backpack, and the NIH Children's Inn. Reflecting our core value of compassion, we strive to serve organizations whose causes serve the community and match our employees' values.

Employee-driven fundraising continues to be an important component of our charitable giving. In-person fundraising focused on supporting disabled veterans, cancer research, and genetic diseases, and employees quickly pivoted to virtual events to support other charities amidst the pandemic.

Another facet of our community outreach involves helping community members weather the effects of the economic downturn and the pandemic. An ongoing series of fundraising campaigns focus on supporting underserved families and children, healthcare workers, and businesses and local food banks impacted by recent events. Our leadership and employees support business outreach initiatives; create research and training opportunities for undergraduate and graduate students through academic, biotechnology, and pharmaceutical collaborations; and remain involved at the city, county, state, national, and international levels.

FNL will continue to look for new ways to contribute to the well-being of the communities where we work and live. With help from our dedicated employees, we will strive to address some of our community's most pressing problems and produce positive change.

Thank you for reviewing our annual report. We respect and appreciate the opportunity for Leidos Biomed to collaborate with NCI, NIAID, and other NIH Institutes and colleagues along with the extramural community. Our partnership advances the public's health by improving the lives of those suffering from cancer, AIDS, or emerging health challenges.

Sincerely,

Ethan Dmitrovsky, M.D.
President, Leidos Biomedical Research, Inc.
Laboratory Director, Frederick National Laboratory for Cancer Research

Support to the **National Cancer Institute**

Frederick National Laboratory
for Cancer Research

sponsored by the National Cancer Institute

NATIONAL CANCER INSTITUTE

Center for Biomedical Informatics and Information Technology

Support Provided by the Applied and Developmental Research Directorate

DOE Collaboration Support

KEY ACCOMPLISHMENTS

- All 10 of the DOE-directed studies (10 patient-derived xenograft [PDX] models × 7 single agents) selected based on early machine learning modeling have been completed by the NCI Patient-Derived Models Repository (PDMR). Data from seven of these studies have been deposited with the DOE, and the final three are undergoing quality control review before analysis.
- Qualitative evaluation of over 20 new studies from the Rare Tumors PDX preclinical effort directed by the Division of Cancer Treatment and Diagnosis have been deposited with the DOE. Data were presented at a team meeting in Chicago (January 2020).
- The PDMR has implemented several additional response metrics for the preclinical studies and shared these with the DOE to determine whether the metrics (either alone or in combination) will help the computer modeling for prediction of preclinical response. The first set of drug responses in PDMR organoid models, performed by the In Vitro Screening & Molecular Pharmacology Group, has been shared with the DOE. These organoid studies were matched to existing PDX *in vivo* data, which will allow for building better prediction computer models for the ensemble transfer learning pipeline between *in vitro* response data and prediction of *in vivo* response, a key component of the Joint Design of Advanced Computing Solutions for Cancer (JDACS4C) Pilot 1.
- The PDMR works closely with the PDXNet Consortium and provides preclinical scientific oversight as part of the project. Data from more than 400 unique PDX models, including patient treatment history, whole-exome sequencing data, RNA-Seq data, proteomics, and preclinical response data, are being shared with the DOE to help increase the total data set used for building machine learning/deep learning computational models for the preclinical Pilot 1 effort. The team met with principal investigators from all participating PDXNet members to begin coordinating data transfer.
- Image featurization is also being investigated as a collaborative effort between JDACS4C Pilot 1 and Pilot 3. To this end, two whole-slide hematoxylin and eosin PDX images (*.sis) files were sent to the DOE to determine if the format collected by the PDMR would be of use for a future machine learning project in image recognition.

Characterization of KRAS:Membrane Interactions

The objective of the RAS structure and dynamics in cellular membranes project, a component of JDACS4C, is to create a multiscale computational framework that uses experimental input parameters, including diffusional data from single-particle tracking of RAS in the membranes of live cells (Goswami et al., *eLife*, 2020). The framework can be used to explore the conformation and dynamics of the RAS protein alone or in complex with effectors at length and time scales than cannot be interrogated by experimental approaches alone. The first phase of this project focused on the behavior of RAS on lipid membranes of varying complexity. Three discrete conformations of RAS on a simple bilayer consisting of anionic and neutral lipids were characterized using a variety of biophysical measurements. There was correlation between these experimental results and coarse-grained molecular dynamics (CG-MD) simulations performed by Los Alamos National Laboratory (Van et al., *Proc Natl Acad Sci U S A*, 2020).

Macroscale simulations of RAS on a membrane containing eight lipids from the Multiscale Machine-Learned Modeling Infrastructure (MuMMI) were performed, selecting 120,000 unique membrane patches that resulted in 200 milliseconds of aggregated CG-MD simulations. Distinctive patterns of local lipid composition correlate with interfacially promiscuous RAS multimerization and lateral diffusion (Ingolfsson et al., *Nat Struct Mol Biol*, 2020). Simulations predicted that the presence of higher concentrations of phosphatidylinositol bisphosphate (PIP2) was associated with higher RAS colocalization and confined diffusional behavior, which was confirmed by surface plasmon resonance and single-particle tracking experiments on supported lipid bilayers. The bilayers, composed of eight lipids, were systematically varied to correspond to the compositions that favored (high PIP2) or disfavored (low PIP2) RAS multimerization. Under these varying conditions, we found that the diffusion behavior of RAS and lipids correlated with the simulations. This highlights the value of the project: the simulation provides a wealth of hypotheses that can now be tested experimentally. New parameters derived experimentally can then be used to parameterize subsequent molecular dynamics simulations to refine the model.

Phase 2 of this project focuses on MuMMI simulations between membrane-bound RAS and RAF kinase. The X-ray crystal structure of the RAS-binding domain/cysteine rich domain (RBD-CRD) bound to KRAS has been solved (Tran et al., *Nat Commun*, 2020) and will be used to initiate CG-MD that results from the MuMMI simulations. This structure was solved in the absence of a membrane, so nuclear magnetic resonance experiments were conducted to solve the structure of RBD-CRD

bound to a membrane mimetic. The nuclear magnetic resonance data indicate that leucine 149, phenylalanine 158, and leucine 160 from the CRD penetrate the acyl chains of the lipid bilayer with the RBD, making transient membrane interactions. In cell-based experiments, we are measuring the membrane residence time of RDB-CRD. When we mutate these key residues, we see a decrease in the residence time and decreased interactions with RAS (as measured in protein–protein interaction assays). We are also measuring the diffusion behavior of fluorescently labeled RBD-CRD and KRAS on supported lipid bilayers using simultaneous two-color, single-particle tracking experiments. Using these innovative experimental approaches, we can test the hypotheses generated from the next round of MuMMI simulations.

Support Provided by the Biomedical Informatics and Data Science Directorate

Biomedical Operations Support

Clinical and Translational Imaging Informatics Program

Intramural Biomedical Informatics and Software Support

KEY ACCOMPLISHMENTS

- Successfully deployed 12 new software versions, one each month
- Added thirteen laboratories from National Cancer Institute (NCI) divisions, offices, and centers (DOCs) to the user group, from which they adopted the application for data set archiving and retrieving
- Developed and deployed a separate web application for Department of Energy (DOE) laboratories to deposit large machine learning data sets into the Data Management Environment archive
- Developed and deployed datascience.cancer.gov, the new web presence for the Center for Biomedical Informatics and Information Technology (CBIIT)

The services provided by the Frederick National Laboratory for Cancer Research (FNL) staff include computational genomics capabilities to assist intramural investigators in the Center for Cancer Research (CCR) and the Division of Cancer Epidemiology and Genetics (DCEG). With this assistance, these investigators receive help with biomedical informatics research in the form of scientific applications and database subject-matter expertise; consulting; and support services, including portfolio, program, project, task, and activity management.

Semantic Operations and Software Development

KEY ACCOMPLISHMENTS

- Deployed three major releases to production on the Protégé project, greatly helping NCI Thesaurus curators do their work more efficiently
- Developed the first version of the Enterprise Vocabulary Browser (EVS) Representational State Transfer (REST) application programming interface (API) to support external systems, including the Cancer Data Standards Registry and Repository (caDSR) and Clinical Trials Reporting Prograom (CTRP), with faster data throughput. This was deployed to the cloud.
- Implemented JSON and XML export functionality for samples within the cancer Nanotechnology Laboratory (caNanoLab) portal. When users view or edit an individual sample or use the sample search functionality, the capability is now in place to export sample information associated with General Information, Composition, Characterization, and Publication.

The Biomedical Informatics and Data Science (BIDS) Directorate provides essential tooling development, maintenance, and technical support for the creation, curation, and publication of controlled terminology resources. Semantic Operations and Software also includes helping the biomedical community store, process, and make accessible terminologies through terminologies servicers. BIDS is responsible for:

- Development and maintenance of the tools necessary to describe, share, and reuse human-readable and computer-readable descriptions of data
- Creation of new applications to validate and store data
- Secondary uses of data using standard framework conforming to International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 11179 and any new standard mandated by NCI

Semantic Operations and Software also includes the management and maintenance of the Enterprise Vocabulary applications, including but not limited to NCI Thesaurus, NCI MetaThesaurus, and the Enterprise Vocabulary Services. The team executed the following projects in FY2020:

- Continued operation and maintenance of the EVS
- Enhancements to the EVS Terminology Editing Software
- Continued development and support of the Center for Expanded Data Annotation and Retrieval (CEDAR) and Ptolemy
- Deployment of and updates to the EVS Metadata Validation Service. It is now available directly from the Genomic Data Commons.

Imaging Informatics and Infrastructure

KEY ACCOMPLISHMENTS

- Completed 10 releases of the National Biomedical Imaging Archive (NBIA) software and its components (search interface, data retriever, server, APIs)
- Handled and visualized several hundred pathology whole-slide images generated by the Surveillance, Epidemiology, and End Results (SEER) Virtual Tissue Repository project
- Conducted tumor segmentation and tumor-infiltrating lymphocyte analyses using machine learning algorithms
- Successfully executed the imaging challenges contest through the Medical Imaging Challenge Infrastructure (MediCI)

BIDS's support for imaging informatics includes the improvement and harmonization of data standards, tools, and workflows. This research area encompasses imaging informatics for precision medicine support, imaging archive and retrieval for NBIA and The Cancer Imaging Archive (TCIA), data harmonization, development, and maintenance of imaging analysis workflows. This task aims to enable semantic interoperability among various NCI initiatives by aligning on common clinical metadata elements. Following standards such as the Biomedical Research Integrated Domain Group (BRIDG), Clinical Data Interchange Standards Consortium (CDISC), and Digital Imaging and Communications in Medicine (DICOM), BIDS supports the development of prototypes to enable query across disparate data sets linked to relevant images. Initiatives covered under this research area include:

- Imaging Informatics for Precision Medicine
- SEER
- Digital pathology
- NBIA and TCIA support

The team supported the Imaging Informatics for Precision Medicine project in 2020 through the following subprojects:

- NBIA/TCIA: Provided development and project management support for the NBIA, a free and open-source service and software application that enables users to securely store, search, and download diagnostic medical images. NBIA is a core part of the TCIA software stack.
- DI-Cubed: Enabled semantic interoperability among various NCI initiatives by aligning on common clinical metadata elements and supporting use cases that connect clinical, imaging, and genomics data. This effort is conducted in collaboration with the University of Chicago's Center for Research Informatics. The objectives are to demonstrate that standards such as BRIDG, CDISC, and DICOM will support the interoperability goal and to develop a prototype that supports the ability to query across disparate data sets by leveraging the clinical data and linking that to images.
- MediCI Help Desk: Supported use of the MediCI Challenge Management System for image analysis algorithm development and validation
- Pediatric Oncology: Focused on improving and harmonizing imaging data standards, tools, and workflows
- Digital pathology in support of SEER Virtual Tissue Repository: The NCI Surveillance Research Program has initiated the SEER linked Virtual Tissue Repository Pilot. CBIIT and the Surveillance Research Program will design a workflow for combining custom-annotated biospecimens with whole-slide image files for a set of pancreatic and breast cancer cases with rare survival outcomes.

Websites and Scientific Web Applications

KEY ACCOMPLISHMENTS

- Performed all aspects of site design, requirements analysis, user interface design, testing, verification, and maintenance
- Ensured the reliability and accuracy of all custom web applications
- Developed more than 50 event registration websites for many NCI groups in FY2020 by using the https://events.cancer.gov Drupal system. Those sites have collected more than 1,500 total registrations.
- Redeveloped a website devoted to the BRIDG data standard by using Drupal running on NCI's infrastructure

BIDS provides CBIIT with a full-stack web development team, primarily using Drupal to deliver timely content for NCI. System development involved requirement analysis and owner sign-off, while system deployments involved close coordination with CBIIT for hosting on their CloudOne environment.

The Scientific Management Support Team

NCI is responsible for issuing and managing more than 10,000 grant applications for more than $3 billion per year in grants-based research. NCI uses a complex set of processes and procedures to determine which grants should be funded, the requirements for the grant, and the necessary associated information to be tracked with the grant. As one of the largest institutes of the National Institutes of Health (NIH), NCI builds on the NIH electronic Research Administration's Information for Management, Planning, Analysis, and Coordination (IMPAC) II system to track these grants. By extending from NIH's system, NCI has built a workflow that supports cancer-specific research and still integrates directly with the NIH grant process. The NCI system, called IMPAC II Extensions (I2E), follows the complete life cycle of a grant and provides all the tools needed for management.

During FY2020, the Scientific Management Support Team accomplished several major activities, including:

- Released a redesign of the Awaiting Receipt of Application (ARA) system. ARA allows NCI program staff to electronically initiate and submit ARA forms with information pertaining to specific (as yet not received or processed) grant applications; route them to the respective division, office, or center approvers if required; and then route them to the NCI referral officer for quality control and approval.
- Integrated several partner systems into the NCI Extramural Access Request system. This system provides a workflow that supports the request, federal approval(s), and provisioning of account access and privileges for NIH IMPAC II, NCI I2E, and other NCI systems.
- Developed web services to provide Greensheets information to the National Institute of Environmental Health Sciences
- Made updates and performance improvements to the Grant Portfolio Management and Tracking System

Cancer Research Data Commons

KEY ACCOMPLISHMENTS

- As part of outreach, the Cloud Resources (CRs) have completed more than 10 workshops, including "Train Your Colleague" and the NCI IT Engagement Seminar. More than 110 NCI personnel participated in the engagement seminar.
- For FY2020, the CRs have compiled a publication list, which now contains more than 30 unique publications from the last few years.
- In June, the Data Commons Framework (DCF) initiated development for the RAS Integration, which will be used by the CRs in the next few months.

NCI launched its Cancer Research Data Commons (CRDC) initiative in 2017. This is a cloud-based open infrastructure to enable community-wide access to NCI's funded research data. Groups store research data in nodes in the cloud, where users can then access and run analyses so the need for local computing environments is negated, thereby decreasing barriers to access. The CRDC involves the creation of nodes where data is stored and provisioning of resources to give researchers tools to work with and analyze the data. CBIIT charged FNL with implementing and managing the following main projects:

- DCF
- Expand Data Commons
- Integrated Canine Data Commons

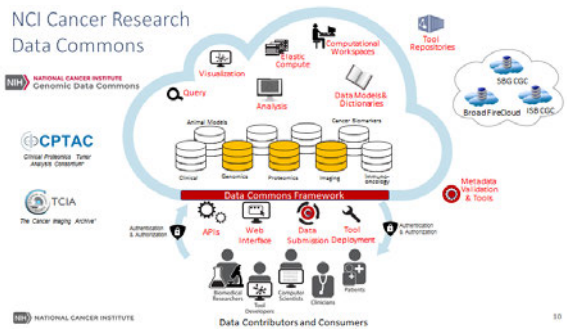


Figure 1. NCI Cancer Research Data Commons.

Data Commons Framework

The DCF project has three main deliverables:

- Develop a website to provide the community with information about the CRDC
- Build open-source infrastructure components that can be used by groups, both intramural and extramural, to more quickly and economically create data commons nodes that reside in the CRDC
- Maintain and extend the three cloud resources:
- Seven Bridges Genomics
- Institute for Systems Biology
- The Broad Institute

A new website, https://datacommons.cancer.gov/, was created for the first deliverable and went live in early summer 2020. It provides an overview of the CRDC and focuses on the component parts.

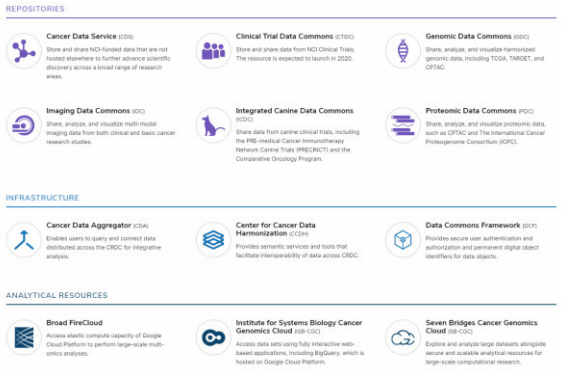


Figure 2. The Explore portion of the CRDC web page, datacommons.cancer.gov.

For the second deliverable, the team has focused on the authorization and authentication components, building and deploying solutions that are being used by other nodes. In FY2020, the authorization and authentication

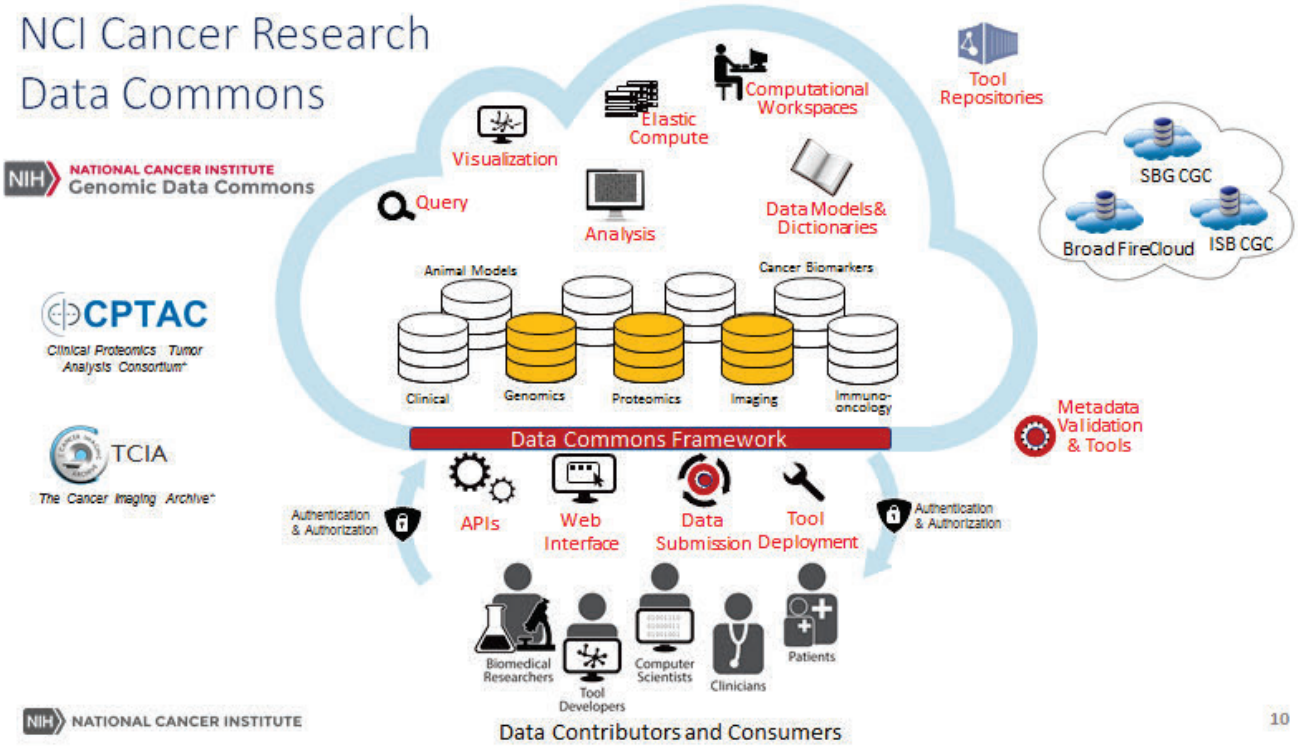


Figure 1. NCI Cancer Research Data Commons.

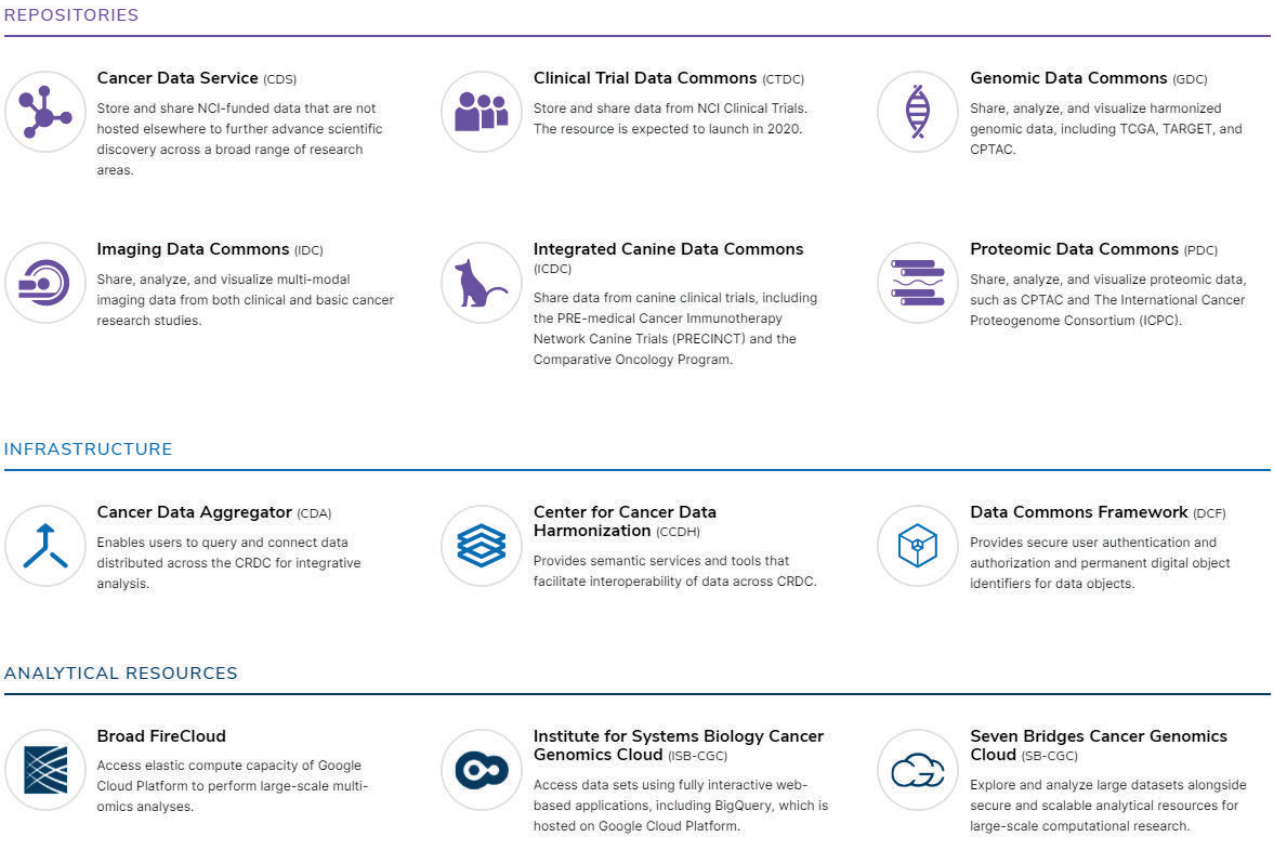


Figure 2. The Explore portion of the CRDC web page, datacommons.cancer.gov.

component (FENCE) was extended and is being used by multiple CRDC nodes. For the third deliverable, Seven Bridges Genomics, the Institute for Systems Biology, and the Broad Institute all worked on adding features to enable connectivity with CRDC repository nodes, providing state-of-the-art analysis environments, and engaging in community outreach with the intention of extending the utility the community derives from these CRDC cloud resources.

BIDS is also working on other CRDC components under the Expand Data Commons task order. Details are provided elsewhere in this report.

Biomedical Informatics Software Development

The Clinical Trials Reporting Program

KEY ACCOMPLISHMENTS

- Set up a new help desk team that includes staff and software to better support cancer centers across the United States
- Developed a trial coding system to support COVID-19-related studies
- Redesigned trial accrual counters so that a single trial may now support accrual counting methodologies that differ between cancer centers
- Substantially enhanced searching so that study participants and accrual counts may be found based upon the properties of the study in which they have enrolled. For example, a person may now determine how many study participants with malignant melanoma were enrolled in Phase II trials that opened in 2019.

The CTRP was established in response to a recommendation from the NCI Clinical Trials Working Group to the National Cancer Advisory Board and was reiterated by the Institute of Medicine's report titled, "A National Cancer Clinical Trials System for the 21st Century: Reinvigorating the NCI Cooperative Group Program." CTRP is a comprehensive database of regularly updated information, including accrual, on all NCI-supported clinical trials. This database of the entire NCI portfolio helps identify gaps in clinical research and duplicative studies to facilitate effective clinical trial prioritization, and it enhances patient accrual by making physicians aware of relevant opportunities for participation in clinical trials.

CTRP is built entirely in the Amazon Web Services (AWS) US-East cloud using a continuous integration and continuous deployment model. It is secured at the Federal Information Security Management Act (FISMA) Moderate level and has an Authority to Operate through September 2020.

This year, the CTRP team continued updating the Scientific Trial Analytics Platform (STRAP) application to improve security, reporting, and performance. The latest release presents another major evolution in the user interface, infrastructure, architecture, and software used for this important application. There are now new searching capabilities allowing authorized users to find participants by trial properties. For example, a person may determine how many patients with breast cancer enrolled in a solid tumor study or how many patients enrolled in a National Clinical Trials Network trial. STRAP has a FISMA Authority to Operate through June 2021.

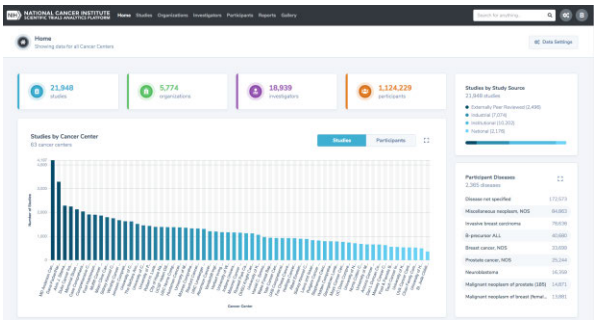


Figure 3. The STRAP application, a member of the CTRP suite of products.

National Cancer Informatics Program

KEY ACCOMPLISHMENTS

- Registered 185 patients over the last 12 months
- Evaluated 128 novel mutations for eligibility
- Designed, developed, and deployed the new Designated Laboratory Automation Program (DLAP) to production in the AWS cloud
- Enhanced NCI Molecular Analysis for Therapy Choice (NCI-MATCH) and NCI–Children's Oncology Group (NCI-COG) Pediatric MATCH to provide Human Genome Variation Society annotation, comparison, and assignment)

NCI Molecular Analysis for Therapy Choice

MATCH is an NCI-sponsored project to develop an informatics system for assigning precision medicine to patients with solid tumors or lymphomas based on the genetic profile of their tumors. The goals of the project include the following:

- Identify mutations/amplifications/translocations in patient tumor samples to use as eligibility criteria for trial enrollment
- Assign patients to relevant agents/regimens
- Perform tumor biopsies and sequencing at progression to illuminate resistance mechanisms
- De-identify samples submitted to central laboratories
- Provide umbrella protocols for multiple single-arm Phase II trials
- Match each molecular subgroup to a targeted agent

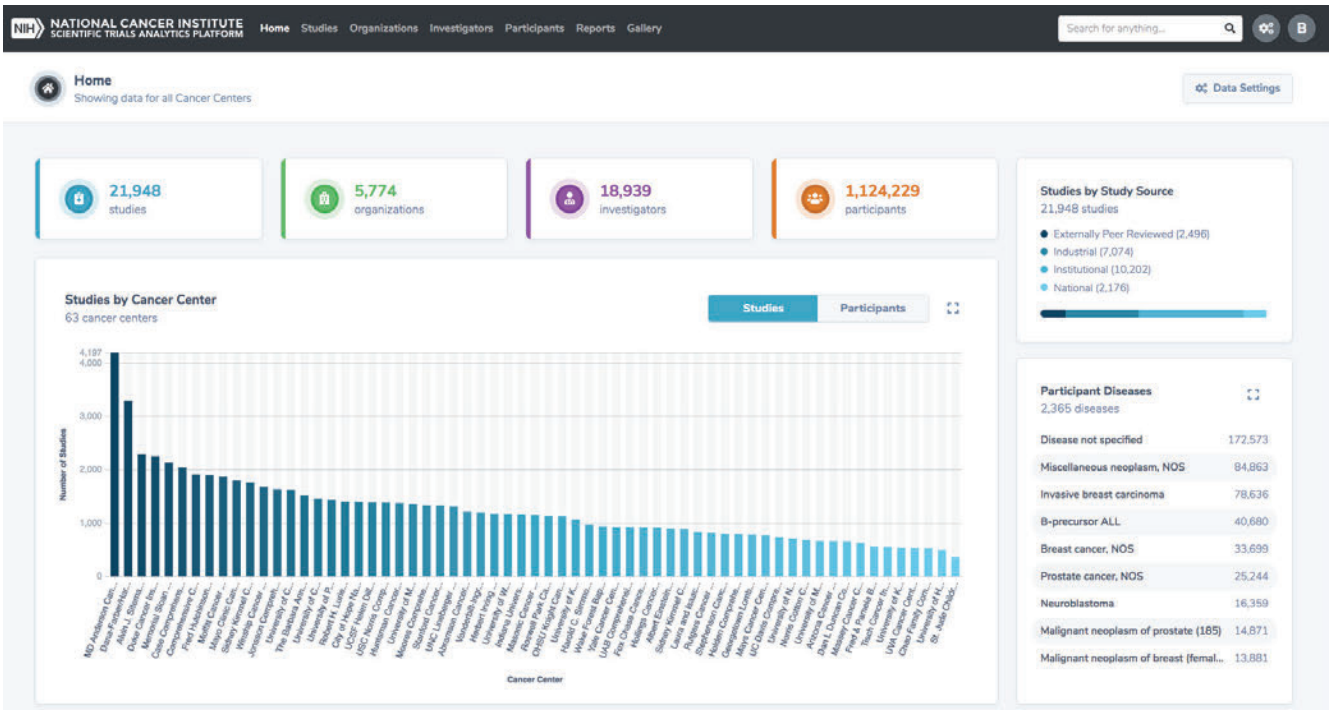


Figure 3. The STRAP application, a member of the CTRP suite of products.

- Use a protocol template developed by the Cancer Therapy Evaluation Program for each Investigational New Drug
- Allow arms to be added or deleted without affecting other arms
- Hold device discussions with the Center for Devices and Radiological Health
- Focus initially on single agents (commercial or experimental)
- Consider combinations for targets that have validated combination targeted therapy
- Establish minimum dose/safety in Phase I trials
- Submit study to the NCI Central Institutional Review Board for review

Eligibility criteria for the study include the following:

- The patient has either solid tumors or lymphomas that have progressed following at least one line of standard therapy.
- Histologies are excluded from a given arm of a clinical trial if they are already approved by the U.S. Food and Drug Administration for that indication or if a lack of efficacy has been documented.
- The tumor is accessible for biopsy, and the patient is willing to undergo biopsy.
- The patient is at least 18 years of age.
- The patient's Eastern Cooperative Oncology Group (ECOG) performance status is 0–2.
- Organ function is adequate.

Participation in the study includes the following:

- The Eastern Cooperative Oncology Group/American College of Radiology Imaging Network (ECOG-ACRIN) leads with the full cooperation of the NCI Clinical Trials and Translational Research Advisory Committee's National Clinical Trials Network.
- National access is handled through the Cancer Trials Support Unit.

The informatics portion of the NCI-MATCH trial is designated as NCI-MATCHBox. NCI-MATCHBox integrates data from several external systems at partner organizations with an internal expert system to process genomic, histological, and demographic data according to scientific rules to find targeted treatment for the patient.

The MATCH trial has closed biopsy screening two years earlier than anticipated due to massive public interest, with more than 6,000 participants registered to the trial. However, a second phase of MATCH has begun, continuing to enroll patients in rare variant arms using designated biopsies and sequencers. This phase required updates to NCI-MATCHBox to support the workflow and called for substantial expansion of the manual bioinformatics work. This year, to support the continued use of external data, the NCI-MATCHBox team added a next-generation sequencing variant standardization and annotation pipeline (VarSAP) that allows NCI-MATCHBox to compare data submitted in a harmonious, streamlined manner. This pipeline normalizes all data to standards published by the Human Genome Variation Society and reports any data submission errors it may find. This greatly improves the quality of data in the system and eases the burden on data science activities associated with heterogenous laboratory comparisons.

MATCHBox is built entirely in the AWS US-East cloud using a continuous integration and continuous deployment model. It is secured at the FISMA Moderate level and has an Authority to Operate through January 2021.

Pediatric MATCH

Pediatric MATCH is a Phase II trial with substudies (arms) for each targeted drug being investigated. Whereas NCI-MATCH focuses on adults, Pediatric MATCH is a separate trial for children and adolescents aged 1–21 who have solid tumors—including non-Hodgkin lymphomas, brain tumors, and histiocytoses—that no longer respond to standard treatment or have recurred after treatment. The trial has two enrollment steps. Each patient initially enrolls in a screening study, in which a sample of his or her relapsed tumor will undergo DNA and RNA sequencing to detect genetic abnormalities. If a genetic abnormality is identified in the tumor that matches a drug target under study in Pediatric MATCH, the patient can then enroll in the corresponding treatment arm, assuming he or she meets all eligibility criteria.

The informatics portion of the Pediatric MATCH trial is designated as Pediatric NCI-MATCHBox. The pediatric version uses the same expert system built for NCI-MATCH; however, it also has a germline component that allows for comparison of blood-normal variants to tumoral variants. The Pediatric MATCH trial went live on July 24, 2017, and continues to expand with additional arms, new rules, and new functionality requests.

Over the past year, the Pediatric MATCH team added workflow functionality, supported the addition of new arms, and increased reporting functionality.

Pediatric NCI-MATCHBox is built entirely in the AWS US-East cloud using a continuous integration and continuous deployment model. It is secured at the FISMA Moderate level and has an Authority to Operate through September 2020.

Designated Laboratory Automation Program

With the success of using designated outside laboratories for the enrollment of patients in clinical trials, and with the expansion of precision medicine trials, has come the need to streamline and automate much of the variant submission process associated with working with variant data coming from these laboratories. To this end, FNL staff in BIDS created and deployed DLAP to production as a stand-alone application that allows outside laboratories to automate the submission of data from their internal systems; allows for the central editing and collection of data; and allows for data to be sent to VarSAP, with results being automatically sent to the

designated laboratories' systems. This new application greatly reduces the manual labor associated with data collection, correction, and annotation and thus speeds the patient enrollment process for precision medicine trials. The application is FISMA-compliant; deployed in the AWS cloud; secured behind Okta and iTrust; and compatible with the precision medicine trials NCI-MATCH, NCI-COG Pediatric MATCH, MyleoMATCH, ComboMATCH, and iMATCH.

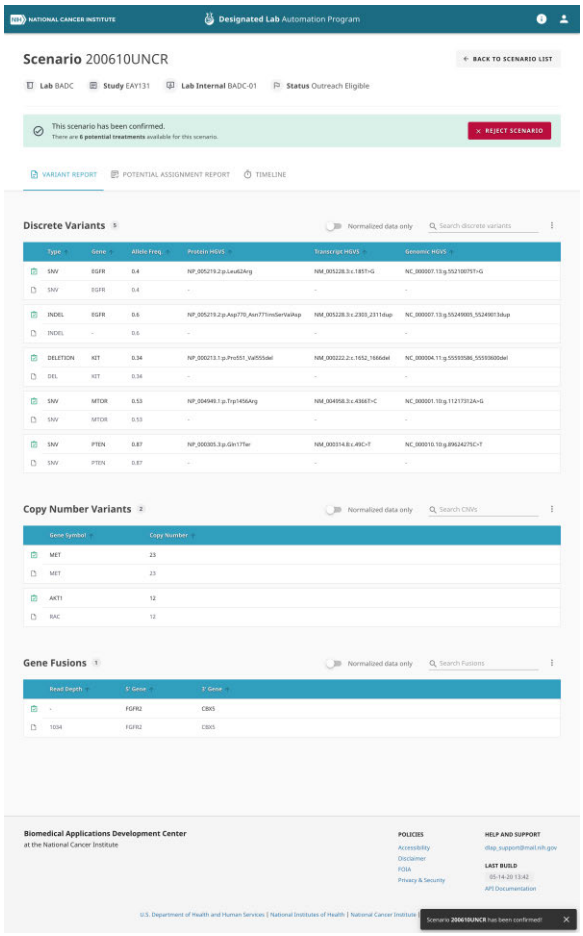


Figure 4. A hypothetical study participant scenario in DLAP.

Scientific Computing Program Development

Protected Data Cloud

KEY ACCOMPLISHMENTS

- Completed Authorization to Operate renewal by meeting all security authorization requirements
- Completed agreement for the transfer of de-identified human data to the University of Chicago controlled computing cloud
- Supported GenoMEL using Bionimbus Protected Data Clouds (PDC) and GenoMEL Data Analysis environment
- Supported operation of The Cancer Genome Atlast (TCGA) data distribution employing the Bionimbus environment
- Updated the NIH Trusted Partner Status application to Childhood Disease Commons data, including Kids First data

The FNL staff has continued working across NCI as part of ongoing pilot efforts to use and apply the Bionimbus PDC to provide cloud-based data management support for advancing cancer research. The Bionimbus PDC is a secure biomedical cloud operated at the FISMA Moderate level as infrastructure as a service with an NIH Trusted Partner status for analyzing and sharing protected data sets. It is a collaboration between the University of Chicago Center for Data Intensive Science and the Open Commons Consortium. The Bionimbus PDC allows users authorized as an NIH Trusted Partner to compute human genomic data in a secure, compliant fashion. The Bionimbus environment is uniquely positioned as an externally operated, scalable general informatics platform capable of supporting the functions of collaborative data deposition, analysis, and distribution. In collaboration with CBIIT and DCEG, the FNL staff in BIDS has continued a partnership with the University of Chicago to bring together germline genomic data on melanoma from multiple international sources.

BIDS delivered a cloud-based data management environment meeting the operational requirements specified in the task order and continued to support the specific data analysis environment used by the GenoMEL consortium.

BIDS's support of TCGA through the Bionimbus PDC gave scientists and investigators broader access to the large genomic data sets for analysis.

BIDS successfully updated Bionimbus to use Database of Genotypes and Phenotypes (dbGaP) and the current NIH Authentication Service to authenticate data placed in the Childhood Data Commons and authorize its distribution to the intramural and extramural research communities.

BIDS's update of the NIH Trusted Partner Status application to the Childhood Disease Common data was completed within the required timeframe. The total monthly storage used on Bionimbus for the Childhood Disease Commons was 2.89 PB.

Cancer Data Science Outreach and Engagement

KEY ACCOMPLISHMENTS

- Drove, organized, and supported data science training workshops targeted for various levels of experience
- Increased engagement in the NCI Data Science Training Huddle

- Established a new virtual community: the NCI Data Science Learning Exchange

Driven by NCI and CBIIT strategic goals, cancer data science outreach and engagement focused on building the capacity to engage more broadly with the NCI intramural research community in several areas. These include development of data science capabilities education and training to advance NCI's mission, support for the development of NCI Data Science Communities of Interest and Communities of Practice, attendance and presentations at meetings and conferences, and support for strategy development and execution of trans-NCI data science training collaboration and communication. A new, remote data science training initiative was created to serve NCI during the required telework period due to COVID-19.

Data Science Training Workshops Targeting Various Levels of Experience

In FY2020, the FNL staff continued to collaborate with the scientists at the CBIIT Cancer Informatics Branch, the CBIIT Infrastructure and Information Technology Operations Branch, the NCI at Frederick Office of Scientific Operations, and FNL to deliver data science training workshops. This was a continuation of the initiative launched in FY2019 to address workforce development needs in data science.

Workshops were led by experts from NCI's DCEG, DOE's Argonne National Laboratory, the FNL's Advanced Biomedical Computational Science group in BIDS, and the NIH National Institute of Diabetes, and Digestive and Kidney Disease (NIDDK). Notably, two of these workshops resulted from recommendations BIDS received from scientists in DCEG and were based on the scientists' knowledge of the NCI data science learning community's priority needs and a belief that CBIIT's workshops reach a broader audience than their training efforts could achieve.

The FNL staff in BIDS continued to build the NIH.AI Forum, which BIDS co-developed. NIH.AI is a trans-NIH group focused on artificial intelligence (AI), machine learning, and deep learning for intermediate to advanced practitioners of data science and AI. All workshop presentations and recordings were posted to the NCI Hub site for future reference.

The FNL staff organized and supported the following eight workshops:

1) *Interacting with NCI Bioinformatics and Data Science Resources and Framing Your Technical Questions to Receive the Support You Need* – September 16, 2019
2) *NIH.AI Forum Workshop: Applications of Machine Learning for Next Generation Sequencing and Drug Data* – October 23, 2019
3) *How to Create a Machine Learning Model Using Keras* – November 6, 2019
4) *Organizing Data Science Projects* – December 12, 2019
5) *Evaluation of Deep Learning-Based Segmentation of Nuclei from Fluorescence Microscopy* – March 3, 2020
6) *Fluorescence Image Restoration and Denoising, A Biologist's View* – January 30, 2020
7) *Machine Learning Image Analysis: A Practical Hands-on Tutorial Using Aivia* – January 31, 2020
8) *Evaluation of Deep Learning-Based Segmentation of Nuclei from Fluorescence Microscopy Images* – March 3, 2020

CBIIT Scientific Computing Program Team

In support of another CBIIT strategic goal, the FNL staff coordinated and supported the meeting to launch a cohesive effort for the CBIIT Scientific Computing Program Team, led by Dr. Carl McCabe. The group aims to help connect NCI staff to the scientific computing community and includes BIDS experts in data management and storage, high-performance computing, and outreach and engagement, along with the NCI federal staff.

NCI Data Science Training Huddle

The FNL staff guided, coordinated, facilitated, and performed outreach for the monthly NCI Data Science Training Huddle of NCI training directors and stakeholders. Interest in participating in the Huddle continued to increase in FY2020. New members include Dr. David Goldstein, associate director of the CCR Office of Science and Technology Resources, and Dr. Oliver Bogler, director of the NCI Center for Cancer Training (CCT).

The Huddle has become a synergistic information-sharing and planning resource. For example, a long-term goal identified by CBIIT leadership, NCI training directors, and stakeholders is to develop a cohesive communication strategy for data science training across NCI. Discussions among the Huddle on this important topic are ongoing. Highlights of the Huddle's other collaborative endeavors include:

- Collaborative input in the CCT, DCEG, and Division of Cancer Control and Population Sciences (DCCPS) survey of fellows' preferences for data science and training topics. Along with feedback from the CBIIT/FNL workshop attendees, four main areas of interest (data science, machine learning, statistics, and software engineering) were identified and helped guide the CBIIT/FNL staff in planning.
- Being invited to present an overview of CBIIT resources to two sessions of CCT's orientation for new fellows (at NCI at Frederick and NCI at Bethesda)
- Discussion on common data science training needs and challenges, such as attracting fellows who are skilled in data science and promoting the need and opportunities for cancer scientists to learn basic coding

- Sharing information about data science training programs across NCI, including:
  - Bioinformatics Training and Education Program (BTEP) resources and workshops
  - CBIIT's Computational Genomics and Biomedical Informatics training
  - FNL's "Statistics for Lunch" series and Bioinformatics Users Forum
  - DCEG's Cloud4Bio – Weekly Hackathon on Cloud Computing and Data Science (focusing on the use of cloud and web computing for cancer research)
- Reporting (by the FNL staff) on data science training initiatives across NIH that were presented at the NIH Data Science Town Hall meetings, including the National Library of Medicine's Data Science Training Program and the Data Science Online Learning Community. In addition, the FNL staff engaged with the directors of NIH programs and with the NCI at Frederick librarian to discuss common interests and share resources and information.

NCI Data Science Learning Exchange

At the onset of required telework resulting from the COVID-19 pandemic, the FNL staff in BIDS collaborated with the CBIIT Infrastructure and Information Technology Operations Branch to create and launch a remote virtual learning network within three weeks. The NCI Data Science Learning Exchange is a virtual community that engages NCI staff to learn data science skills with peer-to-peer support. The community includes fellows, staff scientists, program staff, and others who want to build data science skills.

As part of the CBIIT strategic initiative on cancer data science training, the Learning Exchange was originally envisioned to provide remote learning opportunities during telework and to connect NCI scientists interested in cancer data science.

Developed in consultation with NCI training directors through the NCI Data Science Training Huddle and with feedback from their teams, the Learning Exchange offers numerous resources, including: aggregated learning resources for all levels of learners, from novice to advanced; a user-friendly website on GitHub; and a Microsoft Teams site, with 16 channels on the data science topics and tools most requested by the community, including Biowulf, data visualization, Git/GitHub, Python, R, and others.

Based on ongoing feedback from users, the Learning Exchange also offers access to structured instructional content, including licenses for the Biostar Handbook, a bioinformatics virtual textbook. The FNL staff facilitated the procurement of licenses for Dataquest.io, a collection of interactive data science guides and tutorials.

The Learning Exchange is growing and is expected to become a long-term, self-sustaining learning resource for data science at NCI. The community includes enthusiastic data science learners, from beginner to advanced; frequent feedback and updates; and support from NCI Data Science Training directors.

Since the launch in mid-April, more than 138 federal staff and contractors have joined the community. Scientists from nearly every NCI DOC have participated, including various laboratories in CCR, the Center for Research Strategy, DCCPS, DCEG, the Division of Cancer Prevention, and several programs (e.g., CCR Radiation Oncology Branch, Laboratory of Cancer Biology and Genetics Microscopy Core, Macromolecular Crystallography Laboratory, Laboratory of Cellular Oncology, Laboratory of Pathology, and Molecular Imaging Clinic). The National Institute of Allergy and Infectious Diseases, NIDDK, and scientists from FNL also participated, along with others from CBIIT, NCI's Office of Communications and Public Liaison, and NCI's Office of the Director.

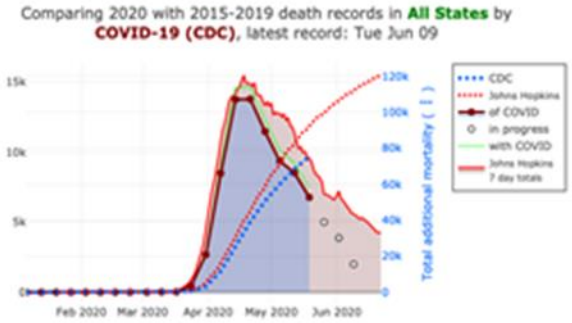


Figure 5. Comparing 2020 with 2015–2019 death records in all states by COVID-19, latest record Tuesday, June 9.

The Learning Exchange also includes remote, hands-on tutorials, workshops, and presentations. The CBIIT/FNL data science training workshops are now offered as part of the NCI Data Science Learning Exchange. In June, DCEG's chief data scientist, Dr. Jonas De Almeida, presented the COVID-19 Mortality Tracker to an audience of 220 remote attendees from across NCI, including NCI Division of Extramural Authorities scientific review officers.

In July and August, FNL scientists presented tutorials on *Machine Learning for Drug Classification* and *Exploratory Data Analysis (EDA) for Clinical Datasets*.

Milestones:

- Announced via an all-NCI email on April 7, 2020, and on MyNCI
- Promoted by NCI training directors and others in the NCI Data Science Training Huddle
- Received more than 138 registrations
- Held a kickoff webinar on April 16, 2020, presented by the FNL staff
- Provided scientific software licenses (Dataquest.io and the Biostars Handbook)

- Hosted the DCEG webinar on the COVID-19 Mortality Tracker with over 220 attendees
- Offered hands-on tutorials on Machine Learning for Drug Classification

Cancer Data Science: Data Services

Data Services Program Support and User Support

KEY ACCOMPLISHMENTS

- Archived over 600 TB of scientific data and analyses from nine user groups across NCI
- Onboarded four new groups onto the Data Management Environment (DME)
- Implemented and deployed the DME Archival Workflow, enabling end-to-end automation of the archival process
- Facilitated data-sharing with external collaborators
- Deployed several operational and performance improvements to DME
- Setup Cloudian as the default S3 provider, with the ability to easily switch between multiple object stores
- Introduced multipart upload to improve throughput and enable easier recovery from network issues
- Expanded existing technical documentation to include videos detailing the archival and data retrieval processes

Data Services Program Support and User Support includes providing technical support for scientific groups and third-party applications using data services, helping with onboarding to the DME, providing data curation and data archiving support, and ensuring continuity of operation of the DME. The following were the focus areas of this group during FY2020:

- User Group Onboarding: The below groups onboarded onto DME in FY2020:
  - National Cryo-Electron Microscopy (Cryo-EM) Facility: More than 175 TB of annotated cryo-EM images from 69 projects have been archived since October 2019.
  - Surgery Branch: More than 65 TB of annotated genomics data from 375 patients have been archived since March 2020.
  - Cancer Imaging Program: More than 16 TB of annotated data from five genomic data sets have been archived since October 2019.
  - NIH Intramural Cryo-EM Facility: More than 22 TB of annotated cryo-EM images have been archived since June 2020.
- Archival Workflow: A generic, configurable workflow was designed and deployed to enable large-scale automated archiving. This is an extension of the prototype developed in FY2019 for archiving High-Throughput Imaging Facility data sets. Salient features include:
  - Fault tolerance and multi-threading for uninterrupted operation and high throughput
  - Ability to scan a directory path at configured intervals
  - Ability to ingest custom metadata from a spreadsheet and to decipher it from source folder structures, file names, and user-defined rules
  - Option to tar and/or compress files at any depth, as well as include or exclude files or directories
  - Automated reporting of archival status.
- User Support: User support activities performed in FY2020 included technical support, user training, data archiving support, assistance with data transfer to and from external collaborators, and assistance with Globus account setup. As in FY2019, detailed technical documentation was provided for all new features and enhancements deployed during the monthly software releases.
- Operational and Performance Improvements: As part of the continuous improvements to provide a best-in-class, round-the-clock archival system, the FNL staff deployed several enhancements to the DME targeted toward improving system reliability, system use, and transaction isolation. In addition, automated retries were built into registration and data retrieval commands to improve error handling, and multipart upload was introduced to improve throughput and enable easier recovery from network issues.

Data Services Environment Development

KEY ACCOMPLISHMENTS

- Enhanced and expanded the Data Services API core and added new command line utilities
- Enhanced the DME web application to provide new graphical user interface features
- Enabled enrollment of non-NCI users from other institutes/centers across NIH
- Integrated Google Drive as a data source option (in addition to AWS S3 and Globus end point)
- Integrated AWS S3 as an archival store option, enabling extension of archiving to the cloud
- Added the ability to configure S3 provider and object stores separately for each DOC
- Added support for integration with third-party applications, including a single-sign-on authentication scheme
- Added support for selectively permissioning personally identifiable information metadata

This effort aims to address the ongoing need for storing scientific data and analyses generated at NCI. The primary functions include, but are not limited to,

expanding and enhancing the capabilities of data services environments, including the DME, while extending integrations with the cloud and other computing resources. The following were the areas of focus in FY2020:

- Data Services API Core: New REST APIs were added to the Data Services API suite, and several existing APIs were enhanced to provide expanded capabilities in the area of data retrieval, data archiving, data and metadata management, and user account management.
- Web Application Enhancements: The DME web application was enhanced to improve user experience; simplify onboarding; and provide enhanced data discovery, permissions management, and data archiving capabilities. The FNL team also introduced soft links to enable users to set up custom views without data duplication.
- Google Drive Integration: In accordance with the strategy to give users flexibility in their choice of data sources, the FNL staff added support for performing transfers to and from Google Drive. Data can now be archived from and downloaded to Google Drive through the DME web application. This is in addition to the existing integrations with AWS S3 and Globus end points.
- AWS S3 Archiving: The FNL staff added support for archiving data to AWS S3 buckets, in addition to on-premises object stores. This capability, along with the ability to configure multiple object stores for a DOC through separate base paths, will enable a DOC to distribute data sets across on-premises and cloud storage.
- Flexible S3 Provider Configuration: In order to provide flexibility in the choice of the S3 object store for a DOC, the DME was enhanced to support multiple S3 providers, with the ability to independently configure the object store for each group.
- Third-party Integration: The FNL staff implemented single sign-on to enable integration with third-party applications and web clients. This was successfully piloted on the Palantir platform, enabling users to access archived data sets through DME from Palantir. In addition, new APIs were added to enable third-party applications to access data, metadata, and configurations information for an authenticated user.

Scientific Computing Program Development and Intramural Research Program Support

KEY ACCOMPLISHMENTS

- Defined, developed, and ran three data science pipelines/analysis related to nuclei instance segmentation, tumor microenvironment quantification, and accelerated computing for radiation therapy
- Engaged the NCI and the NIH community by preparing, organizing, and deliverating four data science workshops

Description of work:

- Education and outreach (Lead: Dr. Carl McCabe, CBIIT):
  - Planned and delivered two workshops on next-generation sequencing, drugs, and image denoising in collaboration with the NIH.AI forum
  - Prepared and delivered two data science workshops for NCI staff on image segmentation and exploratory data analysis
  - Built a website for the NCI Data Science Learning Exchange
- Tumor-immune microenvironment cell interaction (Principal Investigator: Dr. Houssein Abdul Sater, CCR):
  - Developed features of densities and probability density functions for describing spatial heterogeneity in the tumor-immune microenvironment
  - Working on finding patterns in these spatial heterogeneity features using machine/deep learning methods in an effort to assess effects of an experimental drug
- Test and evaluation of an accelerated computing server for radiation therapy simulation (Lead: Dr. Jeff Buchsbaum, Radiation Research Program):
  - Ran accelerated computing test program on 20 field-programmable gate array cards
  - Identified limitation of the server and communicated recommendations for features in an updated server
- Evaluation of neural network models for instance segmentation (Lead: Dr. Gianluca Pegoraro, CCR):
  - Designed, implemented, tested, and ran in production a snakemake pipeline for evaluation of deep learning instance segmentation models: Feature Pyramid Network2 - WaterShed (FPN2-WS) and Mask-RCNN (MRCNN).

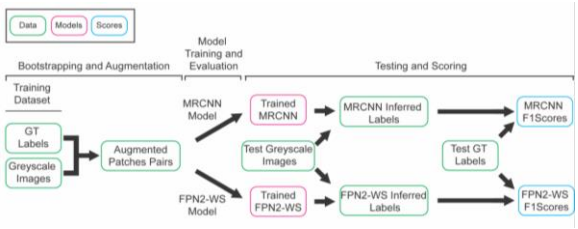


Figure 6. Deep learning nuclear segmentation pipelines.

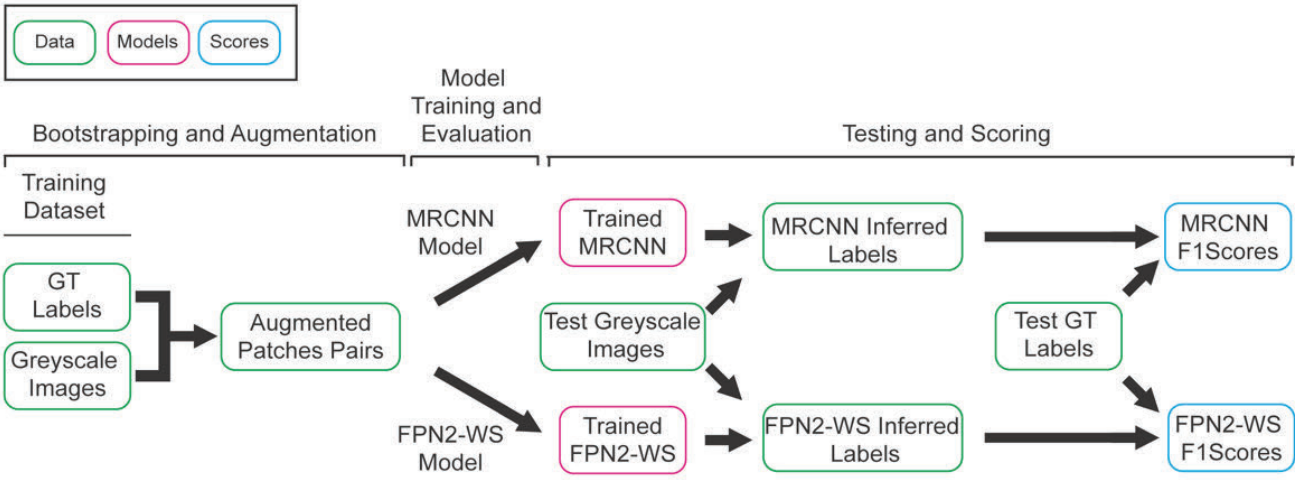


Figure 6. Deep learning nuclear segmentation pipelines.

- Openly shared the work on GitHub; submitted a paper describing the work for review; and compared the pipeline with Jacobkie, a state-of-the-art solution.

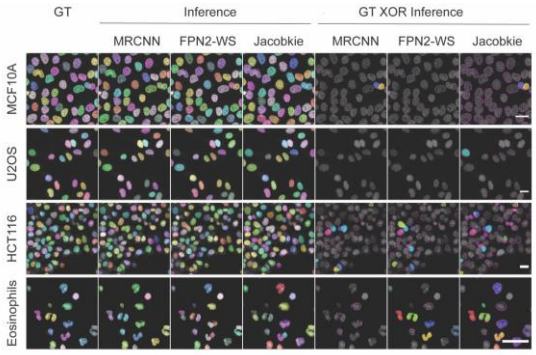


Figure 7. Visualization of nuclear segmentation inference performance of the optimized training strategy for the MRCNN and FPN2-WS model architectures as compared to the pre-trained Jacobkie model architecture.

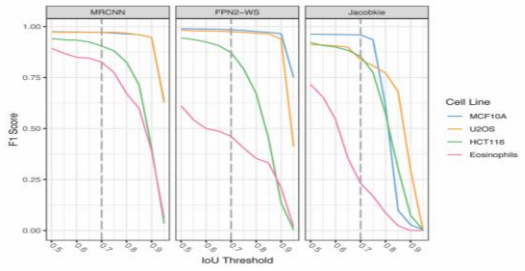


Figure 8. Line plot of the test F1 score (higher is better) at increasing Intersection over Union thresholds for the MRCNN and FPN2-WS models and for the pretrained Jacobkie model.

Prior work with the High-Throughput Imaging Facility group that has been published:

- "Transcriptional Bursting and Co-bursting Regulation by Steroid Hormone Release Pattern and Transcription Factor Mobility" (Stavreva et al., *Mol Cell*, 2019)
- **Best Poster Award:** "Live Imaging of Gene Expression in High-Throughput and at the Single Transcription Site Level" (Wan et al., Society of Biological Imaging and Informatics, 2019)

The FNL staff conducted the following outreach activities in FY2020:

- Continued to support the CBIIT group (Dr. Keyvan Farahami) as they prepared to host the CodaLab challenge platform
- Advised and defined, shared, and engaged in a collaboration between Bellvitge Biomedical Research Institute (Barcelona, Spain) and DCEG. The collaboration explores applying machine learning methods for early diagnosis and classification of endometrial cancer.

Cancer Data Ecosystem

KEY ACCOMPLISHMENTS

- FNL continues to hold workshops supporting the Cancer Data Ecosystem (CDE).
- FNL procured a consultancy agreement with Dr. Bradley Malin and hired Synectics for Management Decisions. Together, they identified top candidates for software to support CDE.
- FNL is procuring test licenses for the top four candidates for further evaluation.
- FNL has established a partnership to conduct the performance evaluation.

The CDE is a part of the National Cancer Data Ecosystem (NCDE). CDE is an NCI research initiative that aims to create an ecosystem where researchers, clinicians, and patients will be able to participate, contribute, and analyze the cancer data. While the NCDE's scope is envisioned to be broader than NCI, FNL is laying the groundwork for the infrastructure.

FNL is managing the following tasks:

- Task 1 – Provide meeting support, convening the stakeholders to gather the expert community's advice. FNL will use this counsel to guide the CDE's architectural design and guide identification of key data elements and contributors. These meetings are envisioned as a platform to educate, engage, and learn from the cancer research community.
- Task 2 – Conduct a systematic research and evaluation of the available privacy-preserving patient record linkage software (P3RLS) and recommend a short list of the best P3RLS for NCI's consideration. FNL will recommend record linkage tools that have the ability to connect disparate data sources at the patient level, making it available to researchers but without compromising patient privacy or confidentiality. This connectivity should include data generated from a variety of sources and representing different aspects of each cancer patient's experience over time. This task has two phases:
  - Phase one is a landscape analysis of P3RLS, where a meta-analysis of the available P3RLS is performed based on market research and publications review.
  - Phase two is the performance evaluation of the candidate P3RLS identified in phase one.

For Task 1, three of the five planned workshops have been held. One occurred in 2018; the other two were held in FY2020:

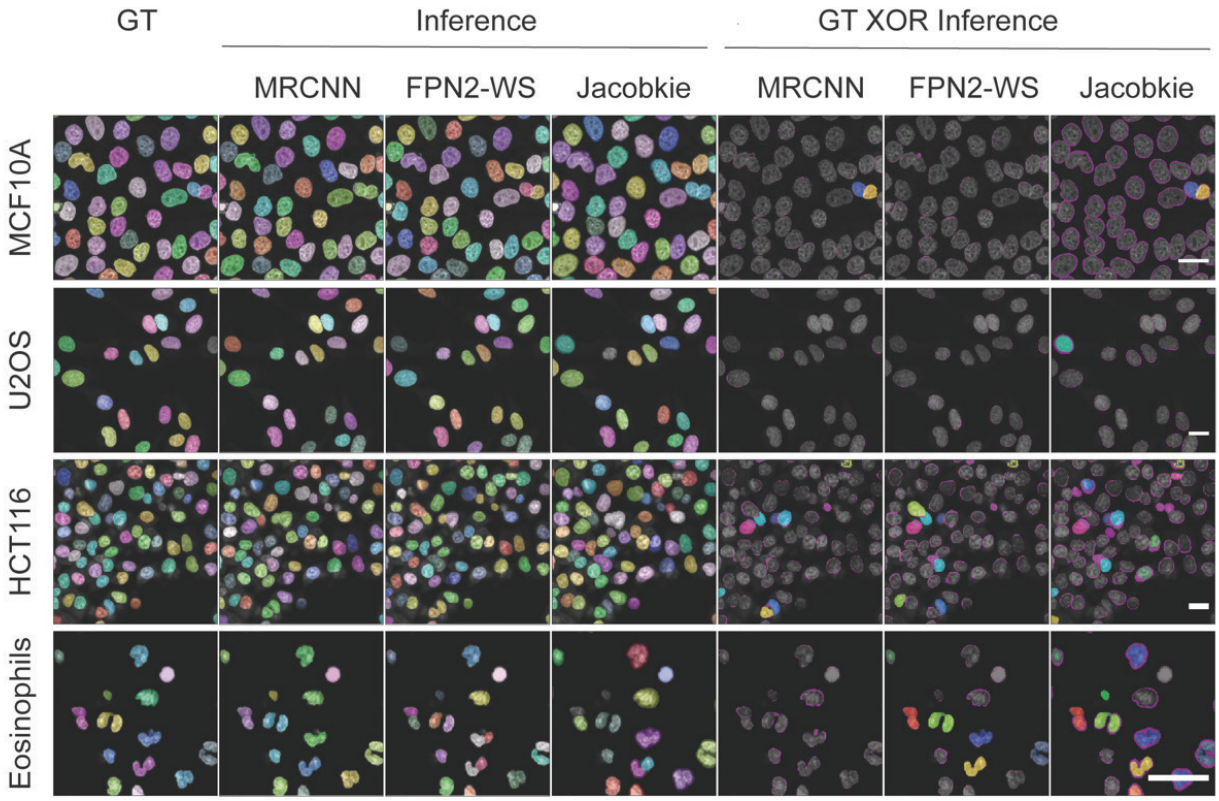


Figure 7. Visualization of nuclear segmentation inference performance of the optimized training strategy for the MRCNN and FPN2-WS model architectures as compared to the pre-trained Jacobkie model architecture.

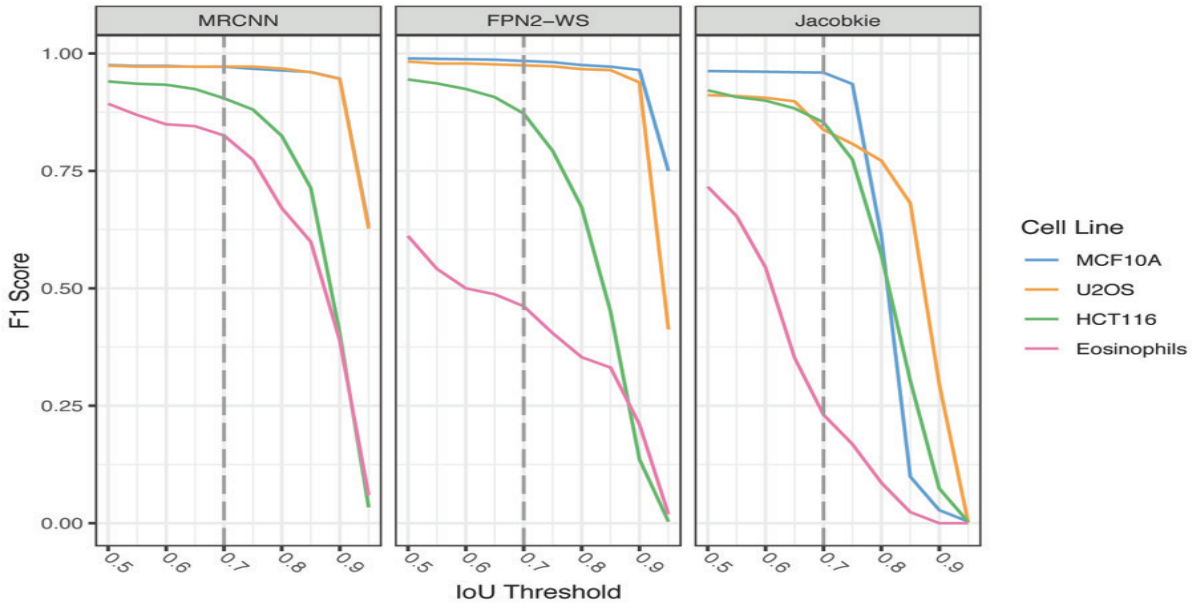


Figure 8. Line plot of the test F1 score (higher is better) at increasing Intersection over Union thresholds for the MRCNN and FPN2-WS models and for the pretrained Jacobkie model.

- Participation in the "Architectural think tanks - AMIA 2019," held November 19–20, 2019.
- "Workshop on De-Identification of Narrative Clinical Text Documents," held February 25–26, 2020.

For phase one of Task 2, FNL procured a consultancy agreement with Dr. Bradley Malin, a leading expert in the field of de-identification and of health data. The FNL staff also hired Synectics for Management Decisions to conduct the landscape analysis of existing P3RLS, working with FNL, the NCI federal leads, and Dr. Malin. After eight months of meticulous requirements gathering, researching, interviewing the P3RLS vendors, Synectics gave FNL a detailed report of its systematic research identifying top P3RLS candidates for further performance evaluation.

Based on Synectics' report, FNL has initiated phase two of Task 2, working closely with NCI to procure test licenses for the top four candidate P3RLS for further evaluation. FNL has established a partnership to conduct the performance evaluation. The partner is the honest broker for the cancer registries, and the data from some of the cancer registries will be used as test data for the evaluation. FNL anticipates completing the evaluation of the chosen candidates by July 2021.

Expand Data Commons

KEY ACCOMPLISHMENTS

- FNL initiated the Center for Cancer Data Harmonization (CCDH) on September 26, 2019.
- FNL started the Cancer Data Aggregator (CDA) project on May 6, 2020.
- The Cancer Data Services (CDS) is fully operational on NCI Cloud One and is providing data from multiple projects to the CRDC Cloud Resources.
- The first draft of the Clinical Trials Data Commons (CTDC) was completed on time on April 14, 2020.

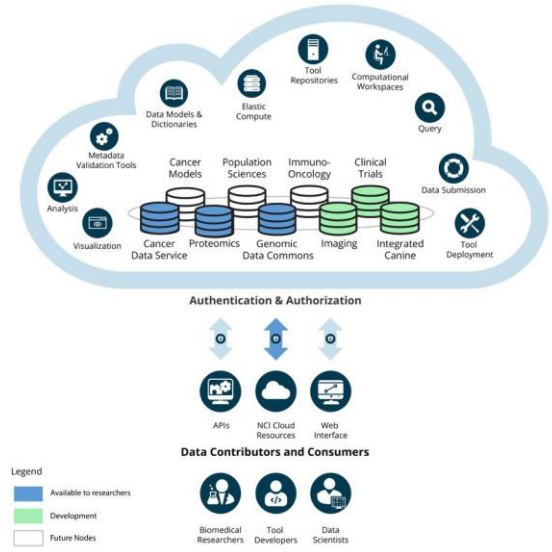


Figure 9. NCI Cancer Research Data Commons.

The Expand Data Commons project supports a variety of data nodes, search capabilities, and semantics that enhance the capabilities and utility of the NCI CRDC.

During FY2020, the IDC partnership kicked off the development of the IDC with a minimum viable product release scheduled for late summer 2020. This FISMA Low repository will initially host images from TCIA and make those images available to all CRDC users. The partner will develop the IDC on Google Cloud. This has resulted in substantial interest from Google as IDC will make use of the Google Healthcare API and Google Data Loss Prevention API, which focuses on image de-identification.

The CCDH project is running well and has completed its first two major deliverables. The first deliverable, a preliminary version of the harmonized CRDC-H data model, was presented in February 2020. This model will provide a core reference point for all of CRDC, especially the CDA. The CCDH project then applied the lessons learned from the first phase and delivered a significant update in May 2020. This update includes mapping to the BRIDG data model standard from the Food and Drug Administration and NCI as well as mapping to the Health Level Seven International Fast Healthcare Interoperability Resources standard. These mappings will help the CRDC stay in sync with international data standards. The CCDH's rapid progress will be a significant benefit to all of CRDC as new data come in.

The launch of the CDA in May 2020 represents the start the last major project originally envisioned in Expand Data Commons project. This project, led by the Broad Institute, involves all of the current CRDC Cloud Resources, which is a major benefit as the Cloud Resources will also be the first significant customers of the CDA project. While this project is only just getting underway,

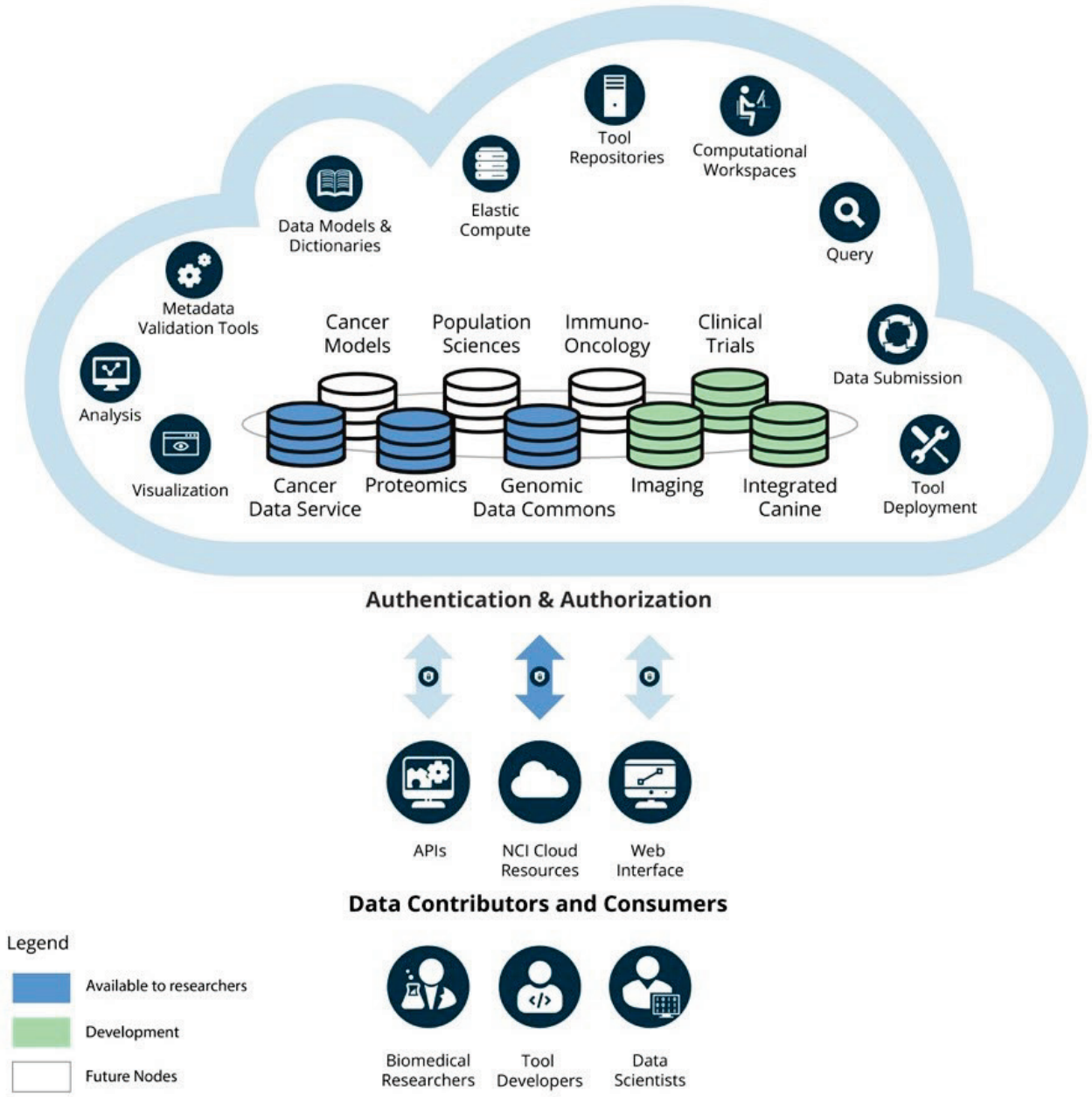


Figure 9. NCI Cancer Research Data Commons.

rapid progress is expected, as the CDA software will use software from a similar project that Broad has been working on for the NIH data commons efforts.

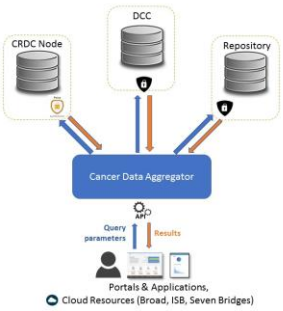


Figure 10. Cancer Data Aggregator.

The CDS project is fully operational and is currently housing data from several projects, including Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO), Advanced Prostate, and the LCCC 1108 study at the Lineberger Comprehensive Cancer Center. CDS is designed to house data that cannot be accepted by the Genomic Data Commons and is expected to have significant future growth. In addition, CDS has been actively contributing to investigations with the SEER; the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; and the Applied Proteogenomics Organizational Learning and Outcomes Network to determine whether CDS can serve as an in-project data repository and provide cloud storage to the project teams as they analyze their data.

The CTDC project attained its goal of being ready for deployment by April 14, 2020. CTDC re-uses components originally developed for the Integrated Canine Data Commons, which resulted in a rapid development and deployment cycle. CTDC will initially support NCI-MATCH as it publishes data. CTDC is currently in a holding pattern as NCI-MATCH finalizes the clinical data for release. During this hold, CTDC will continue to pursue an Authorization to Operate at FISMA Moderate.

CBIIT–DOE Collaboration Support

In 2016, NCI and the DOE established a collaboration supported through a memorandum of understanding to jointly accelerate cancer research and develop exascale-ready tools, algorithms, and capabilities through a new program—JDACS4C.

Three JDACS4C integrated precision oncology pilot projects were created:

- Pilot 1: Predictive Modeling for Pre-Clinical Screening, led by Argonne National Laboratory (ANL), NCI Division of Cancer Treatment and Diagnosis, and FNL
- Pilot 2: Improving Outcomes for RAS-Related Cancers, led by Lawrence Livermore National Laboratory (LLNL) and FNL
- Pilot 3: Population Information Integration, Analysis, and Modeling for Precision Cancer Surveillance (population level), led by Oak Ridge National Laboratory and the NCI Surveillance Research Program and supported by FNL

Two cross-cutting efforts were also created:

- Cancer Distributed Learning Environment (CANDLE), led by ANL
- Uncertainty Quantification, led by Los Alamos National Laboratory

The Accelerating Therapeutics for Opportunities in Medicine (ATOM) Consortium is also part of the NCI–DOE Collaboration project, distinct from the JDACS4C program. ATOM founding members are GlaxoSmithKline; LLNL; FNL; and the University of California, San Francisco.

The BIDS Directorate is responsible for the overall coordination of the NCI–DOE collaboration. These activities include capabilities transfer, community engagement and development, and support for the identification and development of new collaborative projects. They are described below.

Capabilities Transfer

Data, Model, and Software Capabilities Sharing

The Model and Data Sharing Clearinghouse was developed in support of NCI's efforts to transition software and computational capabilities developed in the NCI–DOE collaborations. As part of the deliverables identified for Phase 1 of the project, a public-facing web application was deployed in the NIH security domain to enable sharing of JDACS4C data sets with the broader cancer research community. It provides a graphical user interface for the NCI–DOE researchers to enter large annotated data sets into a data-sharing repository. This application leverages the Data Services API suite in the back end to provide access to the S3 object store and data- and metadata-management capabilities. Salient features include:

- Generic, expandable data hierarchy and metadata structure
- A three-level data management policy:
  - Controlled (restricted) access to upload data sets to the repository and add/edit metadata
  - Open, registered (through user accounts) access to perform data downloads
  - Open, un-registered access to execute metadata-based searches and view metadata associated with any program, study, or data set

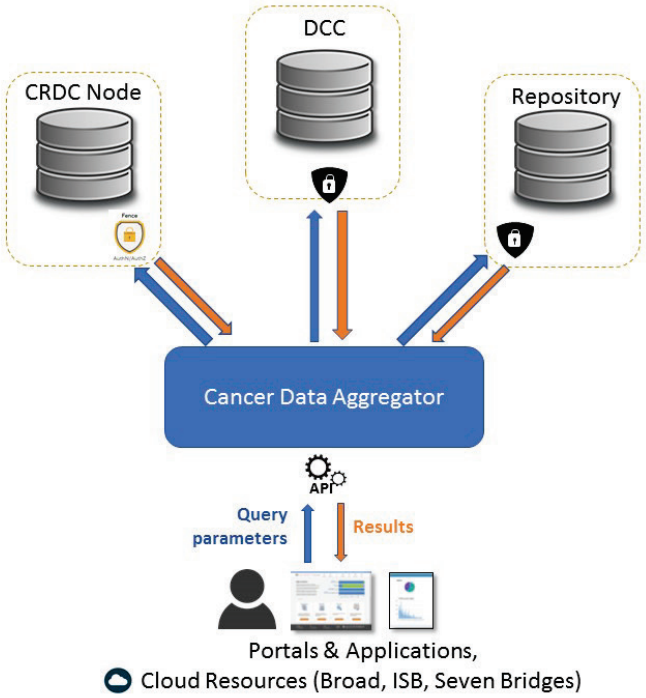


Figure 10. Cancer Data Aggregator.

- Ability to keep data sets private or restricted (group-level access) until ready for sharing (e.g., useful for pre-publication data)
- Support for data transfers to/from Globus and AWS S3 endpoints
- Ability for users to track the status of their uploads and downloads

Phase 2 of the project will enable public access to the ATOM Consortium's generated data, software, and models in accordance with the ATOM Cooperative Research and Development Agreement data- and model-release policies. This includes expanding the current metadata structure, enabling connectivity through REST APIs from the ATOM Modeling Pipeline (AMPL), and supporting conversions to/from standard model formats. Efforts are also underway to enable NCI intramural investigators to leverage the application to share data and models with external collaborators.

NCI-DOE Collaboration Capabilities Transfer and Support

KEY ACCOMPLISHMENTS

- Coordinated with ATOM to share AMPL with the NCI community via cloud resources and FNL's high-performance computing cluster, FoRCE
- Continued to develop usability enhancements, education, and training to enable CANDLE's use within the NCI and NIH community
- Developed and presented a workshop demonstrating how to generate small-molecule features and use them to build models for predicting drug function

This year, the FNL staff has accelerated its efforts to transfer the capabilities generated as part the NCI–DOE collaboration.

- ATOM:
  - The team has contributed to training and supervising interns from Butler University who are supporting ATOM this summer.
  - The ATOM team is planning a project that will address several important questions (efficacy, uncertainty, and transfer learning) in COVID-19 drug discovery. The long-term goal is to extend the findings beyond COVID-19.
- CANDLE:
  - The team has continued its work to enable CANDLE use for the NCI and NIH community.
  - The CANDLE interface was simplified by combining all the configuration parameters for CANDLE into a single file.
  - A new feature has been added to the CANDLE interface to simplify hyperparameter optimization of programs called from bash scripts, the typical mechanism of executing works on Biowulf. This feature reduces the effort to port existing applications to use CANDLE.
  - A known task inefficiency of CANDLE, impacting runs requiring only central processing units, was addressed to improve performance of these jobs.
  - Formulated and presented a workshop to the NIH's graduate school Foundation for Advanced Education in the Sciences class on using CANDLE to run hyperparameter optimization using variational autoencoder, a common machine learning algorithm. An additional CANDLE training workshop will be offered in late summer 2020.
  - Continued participation in CANDLE developer hackathons with DOE colleagues.
- JDACS4C Pilot 1:
  - Working with the project management team to create a Knowledge/Capability-Transfer Workflow standard. This workflow can be applied to other projects (e.g., ATOM).
  - Using machine learning models for classification of next-generation sequencing data: Pilot 1 has been creating models and tools to predict drug response on cancer cell lines and PDX models. Part of the capabilities transfer is to document, reproduce, and share these data science workflows with the cancer research community. Initial focus has been on the Pilot 1 benchmark, TC1, that classifies cancer type based on RNA-Seq data. The following improvements have been carried out, and these steps will form the basis for future knowledge-transfer efforts:
    - Developed a protocol for creating genomic expression data sets from public sources (e.g., TCGA), which forms the basis for many Pilot 1 projects
    - Created documentation and supporting Jupyter Notebooks for reproducibility
    - Wrote a stand-alone code for carrying out machine learning. This code with the new data has similar performance to the Pilot 1 TC1 project model.
    - Created a procedure for using models for inference
    - Worked with the Uncertainty Quantification Team (DOE) to improve the model performance
  - Educational Workshops:
    - The first workshop will introduce concepts and tools in machine learning to generate molecular descriptors for drug function classification. Details of the workshop are available at https://github.com/ravichas/ML-predict-drugclass.

    - The second workshop will show how the TC1 model has been created and trained by taking genomic expression profiles of cancer samples as input and creating a machine learning model for classifying cancer types (e.g., breast, lung, etc.). The model in our hands performs well (approximately 95 percent accuracy) and will be a great tool for cancer research. Codes and supporting documents are available from the following GitHub repository: https://github.com/ravichas/ML-TC1.
- Uncertainty Quantification: Actively working with the Los Alamos National Laboratory team to apply uncertainty quantification to one of the classification models related to next-generation sequencing data. This model will be shared with the NCI community to run in inference mode.
- Outreach to the intramural community:
    - Therapeutically Applicable Research to Generate Effective Treatments (TARGET) classification (Dr. Daoud Meerzaman): Performed classification of pediatric cancer types by developing machine/deep learning models using gene expression data from NCI's TARGET program
    - Exploratory data analysis, machine learning/modeling, and feature selection of acute myeloid leukemia cancer omics data:
        - Using the Meerzaman laboratory's acute myeloid leukemia data, the FNL staff addressed common issues with omics data modeling and provided solutions for the following problems: high feature space dimensionality, feature selection, outcome class imbalance, and hyper-parameterization.
        - To accomplish this task, CANDLE scripts were developed to demonstrate the ease of hyperparameter tuning in a high-performance computing (HPC) environment. The FNL staff used R and Python technologies for Exploratory data analysis and machine learning. Importantly, using extensive modeling/analysis, improvements were proposed to Dr. Meerzaman's laboratory's machine learning pipeline, especially in the areas of cross-validation for imbalanced data.
        - The FNL staff presented the results in a seminar and shared the results, markdown files, and HPC Biowulf batch script files with the Meerzaman laboratory.

For one of FNL's prominent publications on these efforts, please see Bhattachary et al., *Front Oncol*, 2019, in Appendix B.

Community Engagement and Development

Envisioning Computational Innovations for Cancer Challenges Community Engagement and Development

KEY ACCOMPLISHMENTS

- Envisioning Computational Innovations for Cancer Challenges Community (ECICC) held Cancer Challenges and Advanced Computing MicroLabs:
    - Second MicroLab: Developed use cases and identified critical next steps to shape future research in computational oncology
    - Third MicroLab: Focused on the Digital Twin cancer challenge area and generated interest in the ideas laboratory held in July 2020
- ECICC five-day ideas laboratory: Toward Building a Cancer Patient "Digital Twin"

ECICC Cancer Challenges and Advanced Computing MicroLabs

ECICC community engagement efforts focused on expanding participation among thought leaders in cancer research, artificial intelligence, and advanced computing technologies to develop concepts and refine the aspirational computational innovation challenge ideas generated at the 2019 ECICC Scoping Meeting (ECICC Hub Site: https://ncihub.org/groups/cicc).

Second MicroLab: Building on the breakout discussions from the first MicroLab *(*held in June 2019), a multidisciplinary group of more than 100 clinicians, researchers, and academics in cancer and computational sciences participated in our second virtual, ECICC Community MicroLab. Participants developed use cases for real-life situations and then identified the research challenges that need to be overcome to achieve them. The use cases were based on various personae derived from the four cancer challenge areas developed at the ECICC Scoping Meeting held in March 2019. The breakout session notes are posted on the ECICC Hub Site.

Third MicroLab: This virtual, interactive event brought together more than 120 scientists from 41 organizations, 61 of whom were new to ECICC events. Participants included cancer researchers, clinicians, biomedical engineers, bioinformaticians, AI researchers, data scientists, computational scientists, and mathematical modelers. The focus of this MicroLab was to identify the necessary elements of a road map for cutting-edge research in support of digital twin technology; provide a preview of the forthcoming ideas laboratory; and offer opportunities to connect with other applicants and join interactive breakout sessions. Forty-seven people participated in breakout sessions to discuss approaches for exploring the next steps required to build a digital twin of an individual cancer patient. The breakout session notes are posted on the ECICC Hub Site.

ECICC Ideas Laboratory: Toward Building a Cancer Patient "Digital Twin"

The five-day virtual ideas laboratory, *Toward Building a Cancer Patient "Digital Twin,"* was a focal point for community-engagement activities in 2020 and is an outgrowth of the ECICC Scoping Meeting. The goal of the ideas laboratory was to develop innovative cross-disciplinary collaborations and shape the future of predictive modeling across scales from biology to clinical care. The FNL team worked with Knowinnovation to frame, plan, coordinate, and facilitate the event. A steering committee from across NCI and DOE provided guidance. The ideas laboratory was originally planned as an in-person workshop at FNL, but due to the COVID-19 pandemic, the planning team decided to hold an all-virtual event. Knowinnovation's unique, proprietary technologies and vast experience in holding virtual events was essential to the success.

The FNL team did extensive promotion, including diversity outreach, across NCI, NIH, academia, national laboratories, and professional associations in cancer and scientific computing. As a result, more than 130 people with expertise in all the targeted areas—cancer research, computational and data science, mathematical modeling, AI, bioinformatics, biomedical engineering, healthcare delivery, and clinical practice—applied for 30 spots to attend the workshop.

Of note, a long-standing objective of ECICC community engagement has been to recruit more oncologists and clinical care providers, with the goal of identifying key issues that impact patients. This year, the FNL team recruited two prominent oncologists to serve as mentors for the ideas laboratory. Six mentors representing medical oncology, radiation research, mathematical modeling, HPC and data analytics, computational science, and deep learning/HPC and AI for healthcare guided project teams on research project proposal development. FNL and the DOE Office of Science provided opportunities and mechanisms for the successful project teams to apply for seed funding after the ideas laboratory, with awards expected in FY2021.

ECICC Outreach and Engagement

KEY ACCOMPLISHMENTS

- Led, developed, and moderated workshops and presentations at the Supercomputing 19 (SC19) meeting (held in November 2019), including:
  - Fifth *Computational Approaches for Cancer Workshop* (CAFCW-2019). The work presented by the JDACS4C Pilot 2 team at the workshop was selected as best paper at SC19.
  - Panel: "Edge to Exascale: Computational Innovations in Cancer and the Future for Learning Health Systems"
  - Birds of a Feather session: "Impacting Cancer with HPC: Challenges and Opportunities"
- Moderated a panel at the DOE X-Lab Artificial Intelligence Summit
- Developed and promoted workshops and presentations for the upcoming Supercomputing 20 (SC20) meeting, including the Sixth *Computational Approaches for Cancer Workshop* (CAFCW20)

Fifth Computational Approaches for Cancer Workshop

FNL staff in BIDS coordinated and facilitated CAFCW-2019 in conjunction with SC19: The 31st International Conference for High Performance Computing, Networking, Storage, and Analysis. The day-long workshop focused on cross-disciplinary collaborations and future innovations to accelerate the progress in computationally and data-driven cancer research and clinical applications, specifically the role of computing in drug discovery for cancer. The workshop was organized by the BIDS Directorate head and colleagues from NCI, the DOE, and the extramural community. Participants included academicians; industry representatives; NCI–DOE collaboration JDACS4C pilot leads; and principals from the DOE Office of Science, DOE national laboratories, NCI, and FNL.

The JDACS4C Pilot 2 team presented a paper entitled "Massively Parallel Infrastructure for Adaptive Multiscale Simulations: Modeling RAS Initiation Pathway for Cancer," which was named best paper at SC19. The paper describes the workflow driving a first-of-its-kind multiscale simulation on predictively modeling the dynamics of RAS proteins. The Pilot 2 team delivered a presentation on their paper at the CAFCW-2019 workshop.

Edge to Exascale: Computational Innovations in Cancer and the Future for Learning Health Systems Panel at SC19

The FNL staff coordinated and facilitated the "Edge to Exascale: Computational Innovations in Cancer and the Future for Learning Health Systems" panel at SC19. This event focused on (i) how innovations (e.g., medical Internet of things devices, gathering and integrating rapidly growing volumes of information, and use of scalable computing from the edge and at exascale) affect the practice of medicine and (ii) how to frame new challenges and explore new opportunities to envision a dynamic learning environment for improving cancer research, prevention, diagnosis, and—ultimately—care.

Birds of a Feather Session: Impacting Cancer with HPC: Challenges and Opportunities

The FNL staff helped to coordinate the SC19 Birds of a Feather session, "Impacting Cancer with HPC: Challenges and Opportunities." The session focused on opportunities in cancer research and clinical applications for HPC, emphasizing the data challenges and opportunities with artificial intelligence in key applications, such as drug discovery and disease diagnosis. Specific topics included data availability, model validation, sharing and adoption of developed models, and opportunities for broader collaborative efforts.

DOE X-Lab Artificial Intelligence Summit

The FNL staff moderated a panel session on the use of AI in precision medicine at the DOE X-Lab Artificial Intelligence Summit. Panelists discussed AI in drug discovery, including an emphasis on ATOM. Sponsored by the DOE's new Artificial Intelligence & Technology Office, the session engaged with scientists from several additional DOE laboratories, including existing collaborators at ANL, Brookhaven National Laboratory, Oak Ridge National Laboratory, and LLNL. It also identified new partners at Ames National Laboratory interested in integrated biological modeling (https://blogs.anl.gov/aixlab/ai-at-doe/).

Sixth Computational Approaches for Cancer Workshop

The core planning team for last year's workshop led the development of the CAFCW20 workshop (https://ncihub.org/groups/cafcw20). This day-long event is planned for November 13, 2020, in conjunction with SC20: The 32nd International Conference for High Performance Computing, Networking, Storage, and Analysis (https://sc20.supercomputing.org/). A special emphasis for CAFCW20 is the role of HPC and artificial intelligence to address research challenges when data are limited by availability, variability, and size, as is frequently found in clinical applications and cutting-edge research activities.

Broader Outreach and Engagement

The FNL staff continued to foster community engagement and build new relationships by attending meetings with key stakeholders and groups, including the NIH Data Science Town Halls; NIH National Institute of Biomedical Imaging and Bioengineering Interagency Modeling and Analysis Group meetings; the NIH artificial intelligence healthcare workshop, *Artificial Intelligence Healthcare: From Prevention & Diagnostics to Treatments* (https://datascience.nih.gov/news/artificial-intelligence-healthcare-oct-1-2019); the AI for Science Town Hall (the culmination of four DOE events held in various U.S. cities); the NIH Common Fund Stimulating Peripheral Activity to Relieve Conditions (SPARC) MicroLab and ideas laboratory (https://commonfund.nih.gov/SPARC); and others.

JDACS4C Cross-Agency Communication

The FNL staff facilitated a refreshed JDACS4C Communications Committee meeting with NCI, DOE, and FNL communications liaisons and led collaborative development of a strategic and comprehensive communication plan to identify goals, target audiences, dissemination vehicles and individuals/teams for outreach, and communications and capabilities transfer for the work of the NCI–DOE Collaboration.

ATOM: Accelerating Therapeutics for Opportunities in Medicine

KEY ACCOMPLISHMENTS

- Released AMPL software
- Developed baseline molecular optimization models
- Developed models to predict pharmacokinetic (PK) properties
- Developed models to predict drug-induced liver injury (DILI)

Supported by NCI, FNL is a founding member of the ATOM Consortium (https://atomscience.org), a public–private partnership that aims to transform drug discovery by accelerating the development of more effective therapies for patients. ATOM is developing a preclinical drug design and optimization platform that leads with computation to help shorten the drug discovery timeline. ATOM's approach employs data-driven modeling and generative molecular design to determine design criteria that consider pharmacology, safety, efficacy, and developability in the context of lead optimization. ATOM's active learning design platform aims to selectively incorporate results from mechanistic simulation and human-relevant experimentation to generate and optimize new drug candidates significantly faster and with greater success than conventional processes.

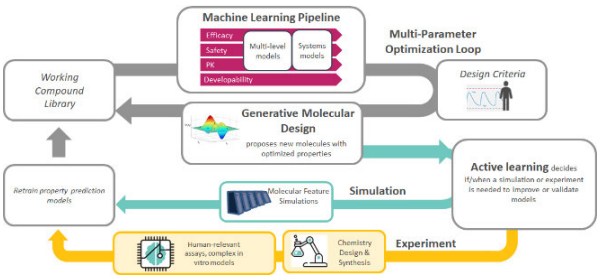


Figure 11. The ATOM platform, an active learning drug discovery framework.

Released ATOM Modeling Pipeline Software

The ATOM Consortium has developed and released AMPL, an open-source, comprehensive machine learning modeling pipeline for predicting molecular activities and properties for drug discovery (https://github.com/ATOMconsortium/AMPL). AMPL enables reproducible and reusable models specifically tailored for quantitative structure–activity relationship and drug discovery applications. AMPL supports a range of machine learning models, including chemical deep learning models, through DeepChem.

AMPL has been evaluated on a diverse set of pharmaceutical PK and safety panel data sets. Different model features and model parameters were tested, resulting in more than 11,000 models fit for these tasks.

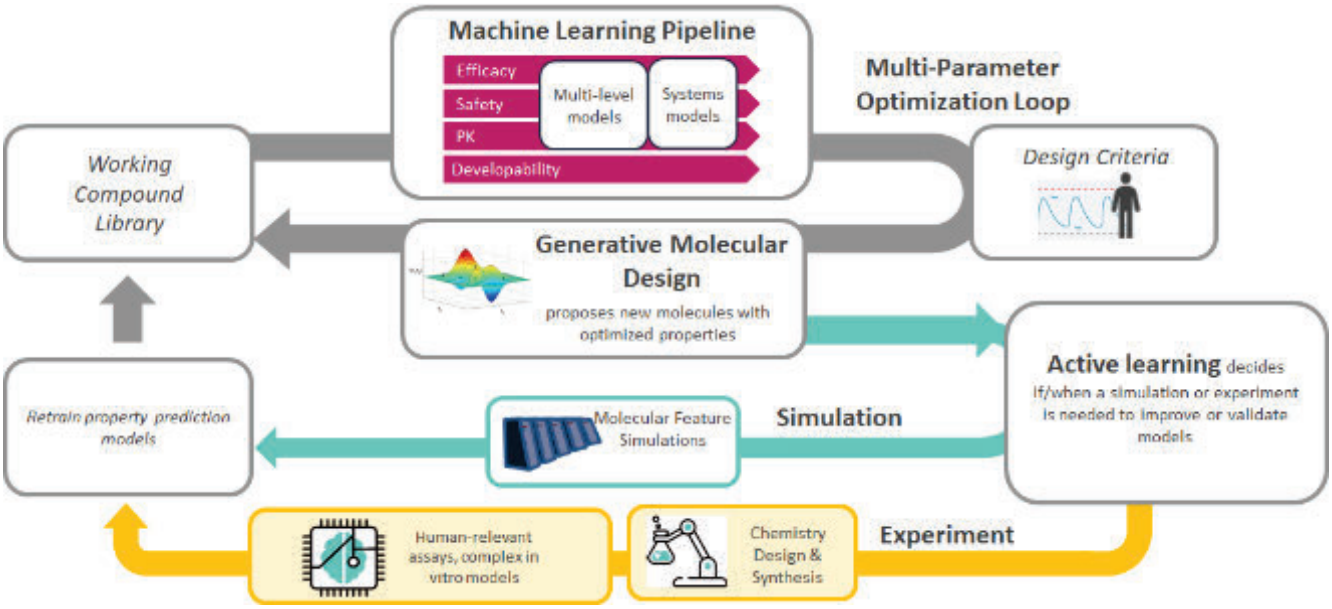


Figure 11. The ATOM platform, an active learning drug discovery framework.

Accurate models with high utility were developed for a wide range of PK and safety properties.

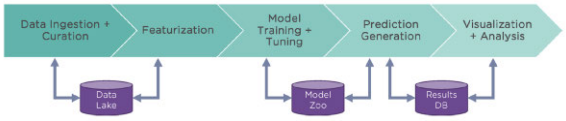


Figure 12. AMPL, software for reproducible, end-to-end, data-driven modeling.

Baseline Molecular Optimization Models

The models fit with AMPL form a baseline set to predict bioassay activities, including efficacy, physicochemical, PK, and toxicity properties. This panel is useful for a variety of modeling purposes, including *de novo* molecule property prediction, virtual screening, and optimization, and forms a critical part of generative molecular design machine learning models. In this way, the models may be used to inform and optimize key medicinal chemistry and PK parameters of lead compounds in molecular design methods.

Pharmacokinetic Property Prediction

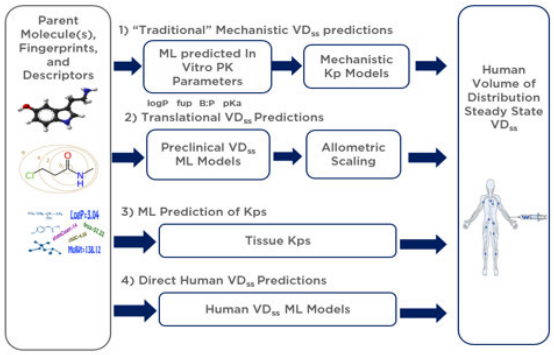


Figure 13. Pharmacokinetic property prediction methods for human steady-state volume of distribution.

ATOM has developed and tested a collection of models to predict animal and human PK properties. One key PK parameter is the steady-state volume of distribution (VDss) of a compound, defined as a theoretical volume of blood (plasma) necessary to contain the amount of drug present in the body at the same concentration as in the plasma. Lead compounds must have volumes of distribution high enough to enable exposure to their target.

ATOM has conducted a comprehensive study to compare *in vitro* and computational models to predict *in vivo* VDss for more than 1,000 compounds. Three major approaches were compared:

- Direct machine learning models to predict *in vivo* volume of distribution
- Allometric scaling of predictions of animal volume of distribution
- Mechanistic models using experimental molecular physicochemical properties or computationally predicted properties to predict how compounds partition into tissues

In addition, empirical studies were completed for more than 250 compounds to collect new physicochemical property data and cellular partitioning data. This will be a significant and valuable public data set for PK volume of distribution studies.

Drug-Induced Liver Injury Prediction

One important yet difficult-to-predict source of toxicity is DILI. ATOM colleagues have developed multiple *in vitro* and biochemical assays toward predicting DILI in animals and humans. These include high-throughput cell health assays, cellular imaging assays, and assays for inhibition of liver transporters linked to DILI. These assays will be an important source of empirical data for the models developed to predict DILI and other liver toxicity.

An important DILI is cholestatic liver injury, caused by the buildup of bile salts within hepatocytes. This condition is associated with inhibition of bile salt export pump (BSEP) proteins, which may be caused by compounds that bind and inhibit bile salt transport. AMPL was applied to fit classification models using BSEP inhibition data. These models are useful to accurately identify BSEP inhibitors and will be used as input for further mechanistic DILI models. The models to predict BSEP inhibition have been released as part of the AMPL distribution. A manuscript for this work is under review.

For two of FNL's prominent publications on ATOM in FY2020, please see Minnich et al., *J Chem Info Model*, 2020, and Hinkson et al., *Front Pharmacol*, 2020, in Appendix B. We have also submitted two additional manuscripts for publication.

Center for Cancer Research

Support Provided by the Applied and Developmental Research Directorate

ADRD: Clinical Laboratory Support

Clinical Laboratory Support

KEY ACCOMPLISHMENTS

- In support of the Pediatric Oncology Branch, the Clinical Trials Processing section of the Clinical Services Program Clinical Support Laboratory (CSL) received more than 643 samples of whole blood, serum, plasma, leukapheresis products, elutriation

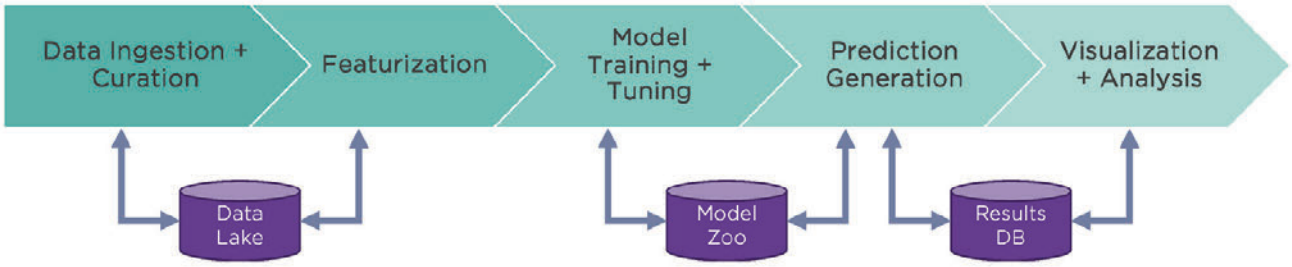


Figure 12. AMPL, software for reproducible, end-to-end, data-driven modeling.

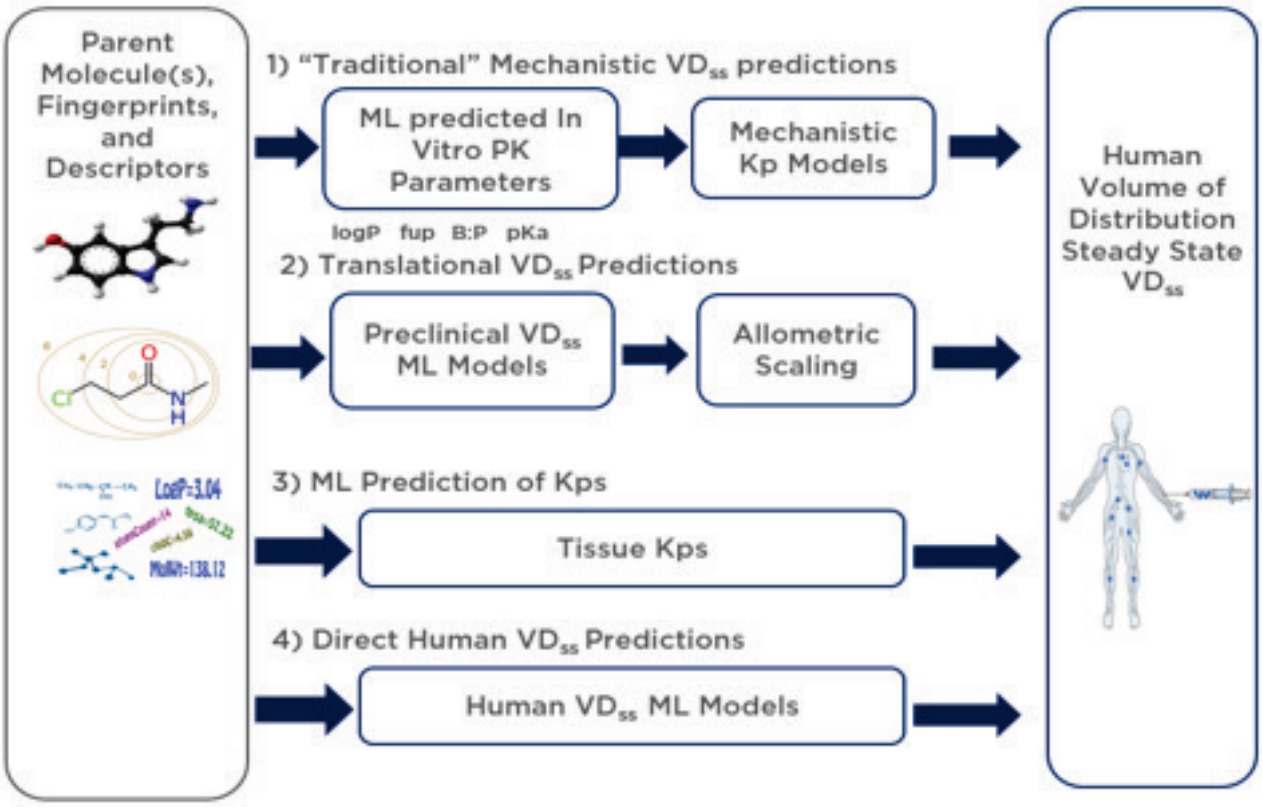


Figure 13. Pharmacokinetic property prediction methods for human steady-state volume of distribution.

cell fractions, bone marrow, cerebrospinal fluid, and DNA from six clinical research protocols, with approximately 1,926 vials produced. In addition, another 87 patient specimens were processed for DNA nucleotide extraction. Additionally, the CSL Lymphokine Testing section performed short turnaround time cytokine testing (for four inflammatory cytokines) under a stringent lab-based assay validated for the Clinical Laboratory Improvement Amendments (CLIA) to support the chimeric antigen receptor immunotherapy clinical trial. For research purposes only, an additional 441 human plasma samples were submitted as 11 requests for tests using a panel of 13 cytokines on the Meso Scale Discovery (MSD) platform. A total of 5,733 sample data points was provided to the requester. Finally, the CSL Flow Cytometry section finished developing its immunophenotyping panels to perform on patient samples coming from the Rare Tumor Initiative, directed by Dr. Karlyne Reilly. A technical advance was to combine six individual panels into two 14-plex panels. This was accomplished through adoption of a more advanced flow cytometer, the LSR Fortessa. After staff training and assay validation, a total of 30 patient samples and normal control samples have been run to date.

- In support of the Surgery Branch, the CSL Clinical Trials Processing section processed 196 blood samples from Dr. Steven Rosenberg's patients that were received in Cell Preparation Tube vacutainer tubes for isolation and cryopreservation of mononuclear cells, with 860 vials produced. The laboratory responded to multiple investigator requests to pull samples from the repository for return to the Surgery Branch for their testing. The Clinical Trials Processing section also processed 123 blood samples from Dr. James Kochenderfer's patients for isolation of their immune cells. Like other investigators' vials that CSL handles, these 504 vials generated were sent to the NCI at Frederick Central Repository for storage.
- In support of the Neuro-Oncology Branch, the CSL Clinical Trials Processing section processed 90 blood samples, generating 428 cryopreserved vials, from Dr. Mark Gilbert's clinical trial to treat glioblastoma by adding pembrolizumab to the standard treatment of radiation and temozolomide.
- In support of the Lymphoid Malignancies Branch, the CSL Clinical Trials Processing section coordinated sample processing in support of seven clinical research protocols, including three off-site trials of the Cytokine Immunology and Immunotherapy section. A total of 376 clinical specimens were processed, resulting in the generation of 1,538 vials of serum or cells cryopreserved for Dr. John Janik. In support of Dr. Wyndham Wilson of the Lymphoma Therapeutics section of the Lymphoid Malignancies Branch, the Clinical Trials Processing section processed an additional 796 samples in support of nine clinical research protocols, with approximately 1,843 vials of cells or serum produced. The CSL Cell-Mediated Immunity section performed its six-day cell proliferation testing on two samples, resulting in a total of 45 data points for Dr. Thomas Waldmann, co-chief of the Lymphoid Malignancies Branch (Metabolism Branch). Dr. Waldmann also requested CLIA-regulated assays to support assessments of patient status with testing of anti-interleukin-15. Each assay requires testing each patient sample at multiple dilutions. Clinical testing included 31 tests on a total of 40 patient samples. His clinical specimens were also tested for other analytes by the Lymphokine Testing (biomarker) section, though these were performed as "research use only" assays, and included the testing of 36 samples in a cytokine 6-plex panel as well as sIL2-Ra.
- Support of the Experimental Transplantation and Immunology Branch, specifically Dr. Christian Hinrichs' work on T-cell receptor discovery, continued. His approach is for CSL to expand and select only those low-abundance T cells expressing receptors to novel antigens that are unique to tumor cells. The CSL Cell-Mediated Immunity section continued to perform and refine the standard operating procedures from Dr. Hinrichs' laboratory. CSL work product is to operate these procedures that include three sequential stimulations of donor cells with unique peptides, a consecutive antigen-presenting cell preparation, followed by co-culture of the antigen-presenting cells and peptide-stimulated T cells. The tests performed include separate enzyme-linked immunosorbent assays (ELISAs) (Interferon-gamma) of co-cultures and flow cytometry to measure for positivity to tetramer-selected cells. This year, eight rounds of expanding and selecting T cells to tumor cell antigens has occurred on a total of 46 human samples. In conjunction with this project, the CSL Lymphokine Testing (biomarker) section measured the IFN-γ responses coming from all 3,072 unique conditions. In a separate project for Dr. Hinrichs, 76 clinical samples were received for evaluation using MSD's 7-plex cytokine panel for a total of 532 data points.
- In support of the Vaccine Branch, the CSL Clinical Trials Processing section received 190 human blood samples for processing from four clinical research protocols being overseen by Dr. Hoyoung Maeng, with 529 vials produced. She also requested the CSL Cell-Mediated Immunity section evaluate seven clinical patients using enzyme-linked immunospot (ELISpot) to identify the presence of anti-HER2 T-cell immunity following vaccination, for a total of 802 data points. Additionally, Dr. Barbara Felber requested ELISpot scanning on 13 samples from the Cell-Mediated Immunity section. Finally, in support of Dr. George Pavlakis, the CSL Lymphokine Testing (biomarker) section performed MSD

multiplex electrochemiluminescence cytokine testing on 757 samples. These were multiplex texts with investigations to assess 10-plex or 61-plex nonhuman primate analytes in a single sample. The total number of resulting data points generated was 46,177. One of those projects was to advance a mRNA/liposomal nanoparticle formulation used in their HIV RNA vaccine in order to inform their industry collaborator, who wished to test it in their COVID-19 vaccine under development.

- The CSL Clinical Trials Processing section provided support to 28 clinical research protocols under the direction of Dr. Jeffrey Schlom and Dr. James Gulley, Laboratory of Tumor Immunology and Biology, including several trials conducted at offsite locations that required additional coordination of couriers and close interaction with clinical staff. A total of 1,015 samples were received, including peripheral blood mononuclear cells from blood and leukapheresis products, serum, plasma, urine, and swabs, with over 8,109 vials of clinical materials produced. CSL also performed MSD multiplex electrochemiluminescence ELISA testing on 226 mouse samples in 10-plex. The total number of measured cytokine data points was 2,260.
- In response to six requests from the Cancer and Inflammation Program for multiplex testing of human and mouse samples, a total of 23 sets of cytokine assays were performed on 1,514 total samples, resulting in the evaluation of 6,683 biomarker data points.
- For Dr. Adam Tai Chi Cheuk and Dr. Javid Khan of the Genetics Branch, the CSL Lymphokine Testing (biomarker) section performed MSD multiplex (3-analyte) testing on 628 samples.
- In response to a request from Dr. Christina Annunziata, head of the Translational Genomic Section of the Women's Malignancies Branch, the CSL Cell-Mediated Immunity section performed antibody-dependent cell-mediated cytotoxicity testing. A total of 66 monoclonal antibody samples were assayed, resulting in the generation of 1,634 data points. In addition, the CSL Lymphokine Testing section evaluated 113 human samples for Dr. Annunziata using the MSD 10-plex assay for a total of 1,130 data points.
- In support of the Thoracic and Gastrointestinal Malignancies Branch, the CSL determined the quantity of LMB-100 (RO6927005) immunotoxin in 305 blood samples from patients using 17 free-drug pharmacokinetic assays. Additionally, the CSL Lymphokine Testing section evaluated samples from patients treated with recombinant immunotoxin using ELISA to detect the presence of specific antibodies to the LMB-100 immunotoxin by performing 10 immunotoxin neutralization assays on 115 samples and also provided a newly developed assay to a university collaborator of Dr. Ira Pastan that measures antibodies directed to D2C7, a related immunotoxin, on a total of 157 samples. Finally, using a newly adopted Luminex platform, the Lymphokine Testing section tested 41 samples derived from co-cultures of liver immune cells with supernatants derived from tumor-cell cultures for 33 cytokines and chemokines to identify potential candidates that drive plasticity in an immune-cell population for Dr. Tim Greten.
- In support of Dr. Brid Ryan, head of the Integrative Molecular Epidemiology Unit of the Laboratory of Human Carcinogenesis, the CSL Lymphokine Testing section quantified immune and inflammation proteins using the 26-plex MSD assay on 836 human samples for a total of 21,736 data points and evaluated 143 human serum samples using the MSD 10-plex assay for a total of 1,430 data points.
- In response to a request from Dr. Laura Schmidt of the Urologic Oncology Brach, CSL offered transformed B-cell lines from five patients' blood with Epstein-Barr virus immortalization.
- In support of Dr. Anish Thomas of the Developmental Therapeutics Branch, the CSL Cell-Mediated Immunity section performed IFN-gamma ELISpot assays on peripheral blood mononuclear cells from nine patients, in which three peptides were narrowed down after Dr. Thomas previously screened tumor-specific neoantigens. Fifty data points were generated.
- The CSL also performed the following NCI at Frederick Accessioning System requests for other non-Center for Cancer Research (CCR) NCI support laboratories and various NIH institutes:
    - Division of Cancer Prevention (DCP) – The Flow Cytometry section supported Dr. Yurong Song and other Frederick National Laboratory for Cancer Research (FNL) staff in Applied Developmental Research Directorate (ADRD) with a flow cytometry panel involving multiple fluorescence channels to immunophenotype seven cancer cell lines. The work product was a total of 50 conditions.
    - Division of Cancer Epidemiology and Genetics (DCEG) – The table following (Table 1) lists totals of blood samples (left) and skin samples (right) that were received and completed by the Cell Culture section from DCEG investigators into EBV-transformed B-cell lines and fibroblast cell cultures:

Investigator	EBV*	Primary Fibroblast**
Dr. Sharon Savage/ Dr. Kenneth Kraemer	22	21
Dr. Blanche Alter	3	7
Dr. Doug Stewart	3	3

* - Completed
** - Collaborator

- Biopharmaceutical Development Program (BDP) – The Lymphokine Testing section supported Dr. Trevor Broadt, FNL, by performing two bioassays to assess the biological activity of a manufacturing lot (current Good Manufacturing Practice [cGMP]) of lyophilized interleukin-12 drug product. This stability test has been performed annually using this method for the material being maintained in a stability program. Assays were performed to evaluate product stability and confirm assay performance. A total of two assays were set up to evaluate four test samples.
- National Institute of Environmental Health Sciences (NIEHS) – CSL provided ongoing support to five studies. After another FNL lab, BioProcessing and Trial Logistics, prepared and shipped specimen-collection kits to trial participants, CSL received and processed clinical specimens and extracted DNA. In addition, RNA extraction was accompanied by the measurement of the RNA Integrity Number using agarose gel electrophoresis. Multiple specimens from patients or family members were shipped to CSL from outside sources. The laboratory processed 271 clinical specimens, with 1,247 vials of cells, DNA, RNA, or serum produced. The number of these samples on which nucleotide isolations were performed included 58 and 51 specimens for DNA and RNA, respectively.
- National Center for Advancing Translational Sciences (NCATS) – CSL provided newly established support to Dr. Marc Ferrer, head of the Early Translation Branch. Using Luminex human magnetic bead assays, the lab characterized the cytokine/chemokine expression profile (with a 37-Plex and 3-Plex panels) of culture supernatants from a virus-infected human 3D-lung model. If a body's immune response has overactive cytokines, it begins to attack its own cells rather than just the virus, resulting in severe lung damage. Prior to using laboratory strains of SARS-CoV-2, they used influenza virus for this preliminary work in the 3D lung model. The Lymphokine Testing section of the laboratory generated 1,760 data points.
- National Institute of Neurological Disorders and Stroke (NINDS) – In support of clinical trial 12N0137, CSL continued the processing and cryopreservation of peripheral blood mononuclear cell samples from patients. The laboratory processed four blood samples, with eight vials produced. Additionally, the Cell-Mediated Immunity section performed seven sets of ELISpot assays on a total of 99 stored patient samples and generated 1,244 datapoints to monitor the patients for T-cell recognition to their earlier viral vector treatment in response to a request from Dr. John Heiss.
- National Institute of Dental and Craniofacial Research (NIDCR) – In support of clinical trial 15D0129, CSL continued the processing and cryopreservation of peripheral blood mononuclear cell samples from patients receiving gene therapy for Dr. John Chiorini. The laboratory processed 12 blood samples, with 40 vials produced. The generation of primary fibroblast cell lines was also performed by the Cell Culture section, with three completed.
- National Human Genome Research Institute (NHGRI) – CSL received seven whole-blood samples from patients enrolled in clinical research protocols 00-HG-0209 (Dr. Elizabeth Garabedian) for density gradient separation into mononuclear cell fractions. Each day that samples were received, a healthy donor research sample was also received for processing. The Cell-Mediated Immunity section utilized these cells to compare to samples from the seven patients with a variety of immunodeficiency disorders using flow cytometry proliferation assays, totaling 268 endpoints.

- In support of Dr. Robert Yarchoan**,** the AIDS Monitoring Laboratory (AML) provided immunological monitoring for eight active clinical research protocols involving patients with HIV/AIDS, AIDS-related malignancies, and viral-induced tumors. The laboratory is responsible for receiving, processing, and cryopreserving a variety of clinical specimens. The laboratory also performs immunophenotypic analysis of whole-blood specimens and performs ELISAs for a wide range of serum/plasma biomarkers. This work resulted in the processing of 333 whole-blood specimens, 240 serum specimens, 456 plasma specimens, 26 pleural effusions, 41 cerebral spinal fluids, 17 bronchoalveolar specimens, 93 urine specimens, and 3 ascites fluids. AML performed 57 cell immunophenotype determinations by flow cytometry and 1,905 cytokine measurements. AML cryopreserved and stored 1,049 vials of patient peripheral blood mononuclear cells (PBMCs), 2,183 vials of serum, 574 vials of pleural effusion fluid, 45 vials of cerebral spinal fluid, 873 vials of urine, 2,918 vials of plasma, 77 vials of

bronchoalveolar fluid, 45 vials of ascites fluid, and 830 vials of PBMC pellets. AML coordinated 10 shipments of clinical specimens to investigators and institutions located at various domestic sites.

- In support of clinical protocols 16-C-0047 and 16-C-0171, AML examined programmed death-ligand 1 (PDL-1) expression on monocytes and neutrophils in whole blood using a custom, three-color immuno-phenotyping panel. PD-L1, also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1), is a protein that, in humans, is encoded by the CD274 gene. PDL-1 has been thought to play a significant role in suppressing the immune system during events such as autoimmunity. In mouse models, activated monocytes have been shown to greatly upregulate PD-L1. PD-L1 has been shown to act as a positive co-stimulatory molecule in intracellular infection. Additionally, AML analyzed PD-L1 expression in 42 patient specimens.
- In support of Drs. Ramya Ramaswami and Robert Yarchoan, AML measured 10 biomarkers in 127 patient sera specimens.

Support Provided by the Basic Science Program

CCR Basic Science Program

Molecular Immunology Section, Cytotoxic Cell Studies Group

The Cytotoxic Cell Studies Group supports the Laboratory of Cancer Immunometabolism (LCIM) by uncovering the innate immune response's function and its potential application to cancer treatment. This group also provides expertise in molecular biology in support of LCIM. The characterization of receptors that regulate the activation of natural killer (NK) cells is a major focus. This group cloned and characterized many of the murine receptors (*Ly49* gene family) for major histocompatibility antigens that control NK-cell activation. The study of the *Ly49* gene family led the group to a major discovery in the field of gene regulation: a probabilistic transcriptional switch that controls *Ly49* gene activation. This discovery has important implications for the control of stem cell differentiation and may lead to techniques for modulating cell fate in differentiating systems such as bone marrow cultures.

FNL staff's current research is focused on the human killer-cell immunoglobulin-like receptor (KIR) proteins, a family of receptors for major histocompatibility complex class I molecules, and the regulation of the *HLA-C* gene, the principal ligand for the KIR receptors. A novel NK-specific promoter has been identified in the *HLA-C gene* and has been shown to drive differential expression of *HLA-C* in NK cells during development. Probabilistic switches have been identified in the *KIR* genes, even though this gene family is not related to the murine *Ly49* genes. In addition, each *KIR* gene has been found to contain multiple promoters that are active at different stages of NK development. The current research and future plans for this project will test the proposed role of each KIR promoter in the control of gene expression. Direct targeting of the four promoter regions described has been performed using the CRISPR-Cas9 system, and the phenotypic effects of deleting or altering promoter elements are currently being tested in NK cells and stem cells differentiated into NK cells in vitro. In addition, a novel project that expands the scope of the investigation of bidirectional promoters in development has been initiated. A series of lineage-defining transcription factors have been identified that produce stable antisense transcripts, and the ratio of sense to antisense transcripts varies with cell type, suggesting an important role of bidirectional transcription in their regulation. The novel RNAscope® Technology has been used to demonstrate that a bidirectional promoter in the *RORC* gene acts as a binary switch. Future work will investigate the switching between transcripts at the single-cell level by RNA sequencing and in situ hybridization, and how this switching affects differentiation.

KEY ACCOMPLISHMENTS

Further investigation of an NK-specific promoter that was discovered in the *HLA-C* gene (Li, et al., *PLoS Genet*, 2018), has revealed that NK-specific transcripts are differentially spliced in NK cells derived from blood as compared to bone marrow and spleen. The splice isoforms display a wide range of translatability that may serve to modulate NK activity in specific tissues (Goodson-Gregg, et al., *Front Immunol*, 2020). The NK-intrinsic regulation of *HLA-C* represents a novel mechanism controlling the lytic activity of NK cells during development. Overall, these findings provide insight into the mechanisms of NK cell development and the tuning of lytic activity, as well as a method to identify individuals with high NK activity that may provide superior outcomes in hematopoietic stem cell transfer (Goodson-Gregg, et al., *Immunogenetics*, 2020). Current and future work on this project will examine the developmental and tissue-specific control of NK cell *HLA-C* expression as well as the specificity and function of upstream transcripts from the *HLA-A* and *HLA-B* genes.

Computational Structural Biology Section

The Computational Structural Biology Section (CSBS) provides support to the Laboratory of Integrative Cancer Immunology (LICI) by advancing experimental techniques, accumulating unprecedented genome-scale experimental data, and addressing fundamental questions on cellular behavior under physiological conditions and disease. These questions relate to molecular interactions, principles of biomolecular recognition, and mechanisms of signal propagation involving functionally impaired mutant molecules. To function, biomolecules must interact in specific ways involving distinct atomistic

interactions and are regulated by certain signals and cellular events involving dynamic behavior, which is modulated by the extracellular and intracellular environment. Understanding these dynamics as a function of conditions, such as other binding, covalent or noncovalent, or mutational events, is essential for grasping the mechanistic underpinnings of molecular—and thus cellular and organismal—function. Perturbations in intra- and intermolecular communications often lead to cellular malfunction and disease. CSBS seeks to obtain an in-depth grasp of the biophysical principles underlying individual interactions as well as their organization in cellular networks, processes, and mechanisms. We target protein function and dysfunction in disease and attempt to unravel key factors that could aid drug discovery. CSBS focuses on cancer and inflammation, aiming to figure out the mechanism of key oncogenic proteins, such as KRAS4B, their signaling pathways, and pathways that may emerge in drug resistance and thus may conceivably be targeted prophylactically. In line with the Center for Cancer Research (CCR), National Cancer Institute (NCI), and the National Institutes of Health (NIH) at large, CSBS has also begun exploring pathogenic microbiota and their mode of hijacking cell signaling. CSBS has also taken steps in personalized cancer medicine to help inform clinical decisions at the point of care.

KEY ACCOMPLISHMENTS

- CSBS resolved the mystery of Rap1 suppression of oncogenic RAS. Decades ago, Rap1, a small GTPase very similar to RAS, was observed to suppress oncogenic RAS, reverting its transformation. The proposed reason was that there is competition between RAS and Rap1 for a common target. Yet, none was found. Another thing that puzzled researchers was Rap1's suppression of *RAF1* versus activation of *BRAF*. CSBS recently envisaged Rap1 as a model for RAS suppression by inhibitors. CSBS explained these observations and showed that a potent Rap1-like inhibitor appears unlikely.
- GRB2 is an adaptor protein that recruits SOS1, a RAS-specific guanine nucleotide exchange factor, to the plasma membrane. SOS1 exchanges guanosine diphosphate by guanosine triphosphate, activating RAS. GRB2 consists of an SH2 domain flanked by N- and C-terminal SH3 domains (nSH3/cSH3). CSBS showed that nSH3/cSH3 binding peptides, which effectively interrupt GRB2–SOS1 association, can serve as tumor suppressors. The GRB2–SOS1 mechanism that CSBS outlined offers new venues for future therapeutic strategies for upstream mutations in cancer, such as in *EGFR*.
- PI3K lipid kinases phosphorylate signaling lipid PIP_2 to PIP_3 in the PI3K/Akt/mTOR pathway to regulate cellular processes. PIP_2 and PIP_3 are the second- and third-most frequently mutated lipids in cancer, respectively, and, to date, there are still no drugs to target them. CSBS determined the PI3Kα activation mechanism at the atomic level and proposed two new paradigms for their inhibition.
- CSBS showed that oncoviruses can drive cancer by rewiring signaling pathways through interface mimicry. Oncoviruses rewire host pathways to subvert host immunity and promote their survival and proliferation. However, exactly how is challenging to understand. By employing the first interface-based host-microbe interaction prediction method, CSBS explored a pivotal strategy oncoviruses use to drive cancer: mimicking binding surfaces, or interfaces, of human proteins. CSBS showed that oncoviruses can target key human network proteins and transform cells by acquiring cancer hallmarks.

Molecular Genetic Epidemiology Section, Basic Research Laboratory

The Molecular Genetic Epidemiology Section of the Basic Research Laboratory is focused on understanding the genetic basis for global health disparities in the U.S. and in Africa, particularly for complex conditions such as chronic kidney disease (CKD) and cardiovascular disease and the interaction between host genetic factors and infectious diseases. The APOL1 protein, a component of high-density lipid particles, lyses most strains of trypanosomes, causing trypanosomiasis, but not the two strains responsible for human African trypanosomiasis. Two coding variants in the *APOL1* gene restore protection against human African trypanosomiasis at the cost of increased risk for CKD in homozygotes. These variants are prevalent throughout Africa and the African diaspora. Approximately 13 percent of African Americans carry high-risk genotypes, which cause a spectrum of CKDs, including progressive HIV-associated nephropathy and COVID-19-associated nephropathy.

KEY ACCOMPLISHMENTS

We have led or contributed to multiple studies investigating extrarenal manifestations of *APOL1*, including a meta-analysis demonstrating that *APOL1* high-risk genotypes, in conflict with some other studies, were not associated with incident cardiovascular events or death (Grams et al., *J Am Soc Nephrol*, 2019). Although *APOL1* is strongly associated with kidney failure, and accounts for nearly all excess risk of progressive CKD in African Americans, we found that *APOL1* is associated with decreased all-cause and specific-cause mortality (Gutiérrez et al., *Am J Kidney Dis*, 2019). The apparent decrease in mortality among carriers of *APOL1*-variant genotypes may be because variant *APOL1* is associated with decreased intimal arteriosclerosis calcification, a risk factor for cardiovascular disease. We contributed to a study showing that *APOL1* is associated with a 25 percent increased risk of incident sepsis, which disproportionality affects African Americans (Chaudhary et al., *Clin J Amn Soc Nephrol*, 2020).

Previously, we published the first report that *APOL1* high-risk status of the fetus is associated with a two-fold higher risk of preeclampsia in the mother, accounting for approximately one eighth of pregnancies complicated by preeclampsia, a leading cause of fetal prematurity, low birth weight, and maternal and fetal morbidity and mortality. Preeclampsia is two-fold more common in Blacks compared to others. We have now shown that fetal *APOL1* high-risk genotype as well as fetal-maternal *APOL1* allele mismatch are independently associated with increased preeclampsia in African Americans (Hong et al., under revision).

We are investigating serum uric acid levels in health and disease, using genetics to identify uric acid transporter gene targets for uric acid level lowering drug development. Dr. Sung Kweon Cho, using genome-wide association study data from 6,881 Koreans, identified two low-frequency and six common, independent variants associated with serum uric acid levels and developed a polygenic risk score, which was validated in an independent cohort (Cho et al., *Sci Rep*, 2020).

In collaborative studies with investigators at Vanderbilt University, Duke University, Georgetown University, Icahn School of Medicine at Mt. Sinai Hospital, and King's College London, we are investigating the long-term consequences of *APOL1* genotypes in patients with COVID-19 in the setting of treated HIV-infection and in the general population of COVID-19 survivors. We are investigating the role of *APOL1* and sickle-cell trait in Africans and African Caribbeans with HIV infection living in the United Kingdom on kidney function pre- and post-COVID-19 exposure. We are also contributing to a clinical trial in Nigeria assessing the efficacy of RAS blockade on prevention of kidney function decline in persons with HIV-associated proteinuria.

- Invited to write an editorial for *Frontiers in Genetics* (An et al., "Editorial: Host Genetics in Viral Pathogenesis and Control," *Fron Genet*, 2019)
- Invited to write a commentary for the *Clinical Journal of the American Society of Nephrology* (Kopp and Winkler, "Genetic Testing for APOL1 Genetic Variants in Clinical Practice: Finally Starting to Arrive," *Clin J Am Soc Nephrol*, 2020)
- Awarded two NIH Bench-to-Bedside and Back Program awards for single-cell transcriptome studies in preeclampsia and nephrotic syndrome

Hematopoiesis and Stem Cell Biology Section

The Mouse Cancer Genetics Program, Hematopoiesis and Stem Cell Biology Section (HSCBS) is working to define the molecular events that regulate hematopoietic stem cell (HSC) quiescence, survival, self-renewal, and cell-fate decisions, and translate these findings into therapies to treat hematopoietic malignancies. HSCBS is currently focused on defining the physiological function of uncharacterized transcriptional regulators in these processes. In addition, we are focused on how these transcription factors are integrated into wider transcriptional regulatory networks and how combinatorial transcription factor interactions within these networks maintain HSC quiescence, promote self-renewal, and drive lineage-specific gene expression programs. HSCBS is pursuing these studies to improve our understanding of stem cell quiescence and self-renewal; methods to expand and transplant HSCs for regenerative medicine and gene therapy; and treatment of hematopoietic malignancies, myeloproliferative disorders, and/or anemia.

KEY ACCOMPLISHMENTS

HSCBS has continued its efforts to define the physiological function of inhibitors of DNA-binding proteins in normal and malignant hematopoiesis. Specifically, HSCBS found that *Inhibitor of differentiation 1 (Id1)-/-* HSCs show enhanced self-renewal in serial bone marrow transplantation (BMT) assays. HSCBS showed that HSCs were preserved during serial BMT and that transplanted HSCs show increased quiescence under chronic proliferative stress. HSCBS found that $Id1^{-/-}$ HSCs are protected from exhaustion in other models of chronic stress including chronic genotoxic and inflammatory stress and aging. Thus, targeting *Id1* may be therapeutically useful for improving HSC survival and function during BMT and other models of chronic proliferative stress and aging. Current studies are focused on evaluating if small-molecule inhibitors of *Id1* can protect mouse and human bone marrow cells from exhaustion during BMT. Since *Id* genes are induced in HSPCs downstream of oncogene activation in myeloproliferative neoplasia's with *Flt3*, *Jak2*, and *Tet2* mutations, HSCBS hypothesized that *Id* genes could contribute to leukemia progression by promoting HSPC proliferation and increasing mutational load. HSCBS found that reducing *Id1* levels in a mouse model of myeloid leukemia, $Tet2^{-/-}$ mice, rescued HSPC expansion and myeloid cell skewing and increased the mice's survival. HSCBS found that ablation of *Id1* in $Tet2^{-/-}$ mice reduces genomic instability and is evaluating if mutation load is decreased in HSPCs from $Tet2^{-/-}Id1^{-/-}$ progenitors compared to $Tet2^{-/-}Id1^{+/+}$ mice. We have planned future studies to determine the molecular mechanism(s) of *Id1* action in $Tet2^{-/-}$ HSPCs. In contrast to *Id1*, HSCBS found that *Id2* is highly expressed in HSCs and that the intrinsic loss of *Id2 in vivo* results in HSC exhaustion and BMT failure indicating that *Id2* is required to maintain HSCs during steady-state hematopoiesis, a function quite distinct from *Id1*. HSCBS provided preliminary evidence that ablation of *Id2* in HSCs results in HSC activation that is linked to the loss of HIF-1α expression. Current studies are investigating the proliferation/activation status of $Id2^{-/-}$ HSCs, if reduced HIF-1α levels in $Id2^{-/-}$ HSCs mediate loss of HSC numbers and function, how *Id2* regulates HIF-1α levels in HSCs, and if *Id2* regulates quiescence in human HSCs and leukemic stem cells. Finally, HSCBS completed studies demonstrating that targeted loss of *Id* genes in adult endothelial cells results in a progressive

disruption in sinusoidal integrity that results in HSC activation and exhaustion that becomes more severe over time. Mechanistically, sinusoidal endothelial cells (SECs) and type-H vessels that connect SECs to arteries showed reduced proliferation mediated by an increase in cyclin-dependent kinase inhibitor expression and increased apoptosis that resembled aging. Collectively, these studies showed that *Id1* and *Id3* are required for the survival and steady state regeneration of bone marrow (BM) SECs, which maintain HSC quiescence and survival (Gadomski, S., *Cell Rep*, 2020).

Epigenetics Section

The Mouse Cancer Genetics Program, Epigenetics Section's research efforts include dynamic regulation of chromatin accessibility as a key feature of cellular differentiation during embryogenesis, but the precise factors that control access to chromatin remain largely unknown. The Epigenetics Section has discovered Lymphoid Specific Helicase (Lsh), an ATPase-dependent SNF2 family member that regulates chromatin accessibility *in vitro* and *in vivo*. Lsh modulates nucleosome structure, the smallest unit of chromatin, and controls chromatin organization and DNA-methylation pattern establishment during cellular differentiation. The Epigenetics Section has previously shown that targeted deletion of Lsh in mice is perinatal lethal, and Lsh mutant embryos display multiple organ and stem cell defects.

Mutation of human Lsh causes an Immunodeficiency, Centromeric Instability and Facial Anomalies (ICF) 4 syndrome, which is characterized by recurrent fatal respiratory and gastrointestinal infections associated with hypogammaglobulinemia and immunodeficiency. Despite the discovery of *HELLS* mutations associated with the ICF syndrome, the pathophysiological pathways underlying the disease remain unresolved including the causes of reduced immunoglobulin levels. Because constitutive deletion of Lsh in mice is lethal, the Epigenetics Section generated a conditional knockout mouse to study Lsh's role in the hematopoietic system and to identify the causes of severe immunodeficiency. Their mouse model provides novel insights into the molecular pathways of Lsh's function and into the pathophysiology of ICF4.

Key Accomplishments

To uncover the role of Lsh in ICF4 and to study the effect of Lsh in the hematopoietic system, the Epigenetics Section created a conditional Lsh knockout mouse that allows tissue-specific deletion of exon 9 and exon 10 of the *Lsh* gene. Exon 9 and 10 comprise an ATP-binding domain and a DEAD-box motif that are critical for the chromatin remodeling function of Lsh. To delineate the role of Lsh in adult hematopoietic stem cells, the mice were crossed with Mx1-Cre and Vav-Cre recombinase transgenic mice that allow cre-recombinase specific expression in blood cells and lead to specific deletion of Lsh in hematopoietic stem cells.

Using a combination of competitive and non-competitive BMTs, the Epigenetics Section found that Lsh-depleted hematopoietic stem cells exhibited significantly reduced reconstitution potential and impaired ability for B-lymphocyte development. The reconstitution assays revealed that the defect in B-cell differentiation was caused by hematopoietic cell-autonomous effects of Lsh deletion rather than indirect effects via the bone marrow microenvironment and that Lsh is required for a specific step in B-lymphocyte differentiation.

Mice that had not received BMTs suffered from severe immunodeficiency due to low immunoglobulin serum levels and Lsh-depleted purified B cells failed to generate *in vitro* certain immunoglobulin isotypes in response to stimulation. Resting naive B cells express IgM but undergo class-switch recombination upon stimulation, a process which reshuffles genes in order to express immunoglobulin isotypes. A series of cell biologic and molecular analysis revealed that Lsh-deficient B cells failed to undergo class-switch recombination, which resulted in lower levels of immunoglobulin isotype surface expression and isotype secretion. Notably, cellular expansion and apoptosis of Lsh-deleted B cells were not affected, indicating that the failure to generate immunoglobulins could not be simply explained by compromised cell growth or survival. To delineate the nature of the immunoglobulin deficiency, the Epigenetics Section assessed initiation of class-switch recombination, a process in which genomic DNA is modified through the generation of double-stranded DNA breaks, deletion of intervening DNA, and an end-joining process that generates a novel genomic segment encoding a specific immunoglobulin isotype. A combination of protein, RNA expression, and a novel technique, known as EnD-seq analysis, in which double-stranded DNA breaks are captured with streptavidin-coated beads and detected by high-throughput sequencing, revealed that the initiation phase of class-switch recombination was not affected. This indicated that Lsh-deficient cells were capable of DNA cleavage in switch regions and that the defect in switch recombination was downstream of chromatin accessibility and the formation of double-stranded breaks. To determine directly whether the defect in immunoglobulin production is at the level of recombination, they assessed the capacity to generate switch junctions using digestion-circularization polymerase-chain-reaction analysis and high-throughput, genome-wide translocation sequencing. These assays allow researchers to track the formation of DNA breaks in donor- and acceptor-switch regions, which are then joined by a nonhomologous end-joining repair pathway. The analysis of frequency and genomic distribution of junctions suggested a reduced efficiency of the classical end-joining pathway, while microhomology analysis suggested that there was no increase in alternative joining process in the absence of Lsh.

Employing a chromosomal double-stranded break reporter assay that induces genomic breaks at specific genomic locations through Cas9 expression, the Epigenetics Section identified the molecular defect in Lsh-deficient B cells. Lsh depletion diminishes the ability to perform DNA end joining efficiently which impairs class switch recombination proficiency, reduces immunoglobulin isotype generation, and leads to severe immunodeficiency.

In summary, the Epigenetics Section uncovered the molecular defect leading to ICF4. Using conditional Lsh knockout mouse models, the Epigenetics Section discovered a hematopoietic cell-intrinsic role of Lsh in B-cell development and a failure of Lsh-deficient B cells to conduct the step of DNA end joining that is required to yield a variety of immunoglobulin isotypes during class-switch recombination. Their results indicate an essential role of Lsh in immunoglobulin production, which could lead to improved therapeutic options to treat ICF4 patients.

HLA Immunogenetics Section

The Laboratory of Integrative Cancer Immunology, HLA Immunogenetics Section aims to understand the genetic basis for resistance or susceptibility to human disease conferred by polymorphic-immune-response loci through direct testing for such effects on specific disease outcomes followed by molecular and cellular biological approaches to determine the biological basis for the genetic association. We study a large variety of disorders including cancer, transplantation outcome, autoimmunity, and infectious diseases.

KEY ACCOMPLISHMENTS

Complete and precise human leukocyte antigen (HLA)-matching of unrelated donors is used to lower the risks of complications after haemopoietic stem cell transplantation (HSCT); however, many patients do not have compatible donors, particularly patients of non-white backgrounds. Although transplantation from donors with one HLA mismatch can offer life-saving therapy, severe acute graft-versus-host disease (GVHD) limits the broad utility of HLA-mismatched transplantation. The features that define risky HLA mismatches remains an important research question. HLA-B is the most polymorphic protein-encoding locus in the human genome. Clinically relevant variation can include epitopes of the expressed HLA-B molecule as well as leader peptides. Not currently considered in clinical practice are the HLA class I leader peptides, which are preferentially bound by HLA-E, the ligand for CD94/NKG2 NK receptors. The rs1050458C/T dimorphism at position –21 of exon 1 of HLA-B gives rise to leader peptides with either methionine (M) or threonine (T) at the second residue of the processed leader peptide, whereas virtually all HLA-A and HLA-C leaders have M. M and T leaders differentially influence HLA-E expression and the strength of inhibitory and activating NK and T-cell responses. Given the extreme polymorphism of HLA-B, and the high risks of GVHD associated with HLA-B mismatching, we sought to evaluate the role of the HLA-B leader dimorphism in GVHD in collaboration with Dr. Effie Petersdorff. Using multivariate regression models, we showed that among HLA-B-mismatched transplantations, acute GVHD risk was higher with leader mismatching than with leader matching (OR = 1·73; p = 0·042 for grade 2–4) and with an M-leader-shared allotype compared with a T-leader-shared allotype (OR = 1·98; p = 0·0001 for grade 3–4). In a further study, we showed that the impact of the patient-leader genotype on acute GVHD and mortality varied across different mismatched HLA loci. Non-relapse mortality was higher among HLA-DQB1-mismatched MM patients compared with HLA-DQB1–mismatched TT patients (hazard ratio=1.35; p = 0 .01). Grades-III-to-IV GVHD risk was higher among HLA-DRB1-mismatched MM or MT patients compared with HLA-DRB1-mismatched TT patients (OR = 2.52 and 1.51, respectively). The data suggest that success of HLA-mismatched unrelated transplantation might be enhanced through the judicious selection of mismatched donors for a patient's leader genotype.

The group previously reported that the risk of GVHD associated with HLA-DPB1 mismatching was influenced by the HLA-DPB1 rs9277534 expression marker. Among recipients of HLA-DPB1-mismatched transplants from donors with the low-expression allele, recipients with the high-expression allele had a high risk of GVHD. We subsequently confirmed the significance of HLA-DPB1 expression for the risk of acute GVHD in a large independent cohort. In transplant recipients matched to HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1, donor mismatching against one high-expression patient HLA-DPB1 increased moderate (OR = 1.36; p = 0.001) and severe acute GVHD (OR = 1.32; p = 0.0016) relative to low-expression patient mismatches, regardless of the expression level of the donor's mismatched HLA-DPB1. Among transplant recipients with one HLA-A, HLA-B, HLA-C, HLA-DRB1, or HLA-DQB1 mismatch, the odds of acute GVHD increased with increasing numbers of HLA-DPB1 mismatches but not with the level of expression of the patient's mismatched HLA-DPB1 allotype. Thus, the level of expression of patient HLA-DPB1 mismatches informs the risk of GVHD after unrelated HSCT matched to HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1, and the total number of HLA-DPB1 mismatches informs the risk of GVHD after HLA-mismatched unrelated HSCT. Prospective consideration of HLA-DPB1 may help to lower GVHD risks after transplantation.

Additionally, FNL staff found that HLA-DPB1 expression is also a risk factor for severe aplastic anemia (SAA). The etiology of acquired SAA is not understood but is likely related to abnormal immune responses and environmental exposures. In collaboration with investigators in the DCEG, a genome-wide association study of individuals with SAA genetically matched to healthy controls was performed. The top SNP

(rs1042151A > G; Met76Val) that associated with SAA (OR = 1.75, $p = 1.94x10^{-13}$) is located in the P4 peptide binding pocket of HLA-DPB1. We determined that the risk variant G associates with significantly higher HLA-DP cell-surface expression in healthy individuals. Imputation of HLA-DPB1 alleles revealed increased risk of SAA associated with Val76-encoding alleles (rs1042151G). The data suggest that alteration of HLA-DP peptide-binding specificity, as well as changes in HLA-DP cell-surface expression and/or other factors affecting HLA-DP function, contribute to SAA etiology.

It is unknown whether humoral and/or cellular immune responses protect against SARS-CoV-2. The highly polymorphic HLA genes are major determinants of the level of efficacy of these immune responses across subjects; class I for CD8+ cytotoxicity responses and class II for CD4 T-cell helper responses. HLA genotyping is central for identification of viral epitopes that should be targeted in vaccine design. The Immunogenetics Section concentrates heavily on genotyping the HLA class I and II genes, the most polymorphic genes in the human genome. This is the only CCR core laboratory that performs high-resolution HLA genotyping using next-generation sequencing technology. Cohorts of individuals infected with or exposed to SARS-CoV-2 as part of the Greater Boston Consortium on Pathogen Readiness are being studied in detail across many disciplines of science. These cohorts will include hospitalized patients and home-care patients with active COVID-19, patients cured of COVID-19, healthcare staff with likely exposure to SARS-CoV-2, and individuals with likely community exposure to the virus. The laboratory is currently performing genetic analyses of these cohorts, which includes HLA, NK cell receptors, cytokines involved in COVID-19 lung disease, and receptors/molecules essential for viral entry into cells. These studies are important for designing vaccines and as potential biomarkers for therapeutic interventions. The FNL team is also contributing data to the COVID-19 Host Genetics Initiative, a collaboration among the human genetics community worldwide to share, generate, and analyze data. More than 190 studies are currently underway. This data will be combined with that from other laboratories for meta-analysis, adding statistical power to results.

Molecular Targets Group

The Molecular Targets Group is organized into three subgroups: (i) assay development and screening, (ii) natural products chemistry, and (iii) protein chemistry and molecular biology. All three groups work extensively with each other and collaborate with CCR investigators to develop and apply assays focused on specific cancer-related targets and/or pathways. Their goals are to identify bioactive molecules through high-throughput screening (HTS) and to subsequently characterize the activities of active compounds. The group aims to identify novel compounds (and novel activities of known compounds) from natural product extracts obtained from the NCI Natural Products Repository and academic collaborators. A typical workflow starts with the development of highly reproducible, cell-based, biochemical or biophysical assays compatible with HTS and for use with natural product extracts. This is followed by a screen of pure compound libraries (currently up to roughly 70,000 compounds) and of natural product extracts (more than 200,000 partially purified samples and approximately 40,000 crude extracts from various sources) for samples able to affect the molecular target or pathway of interest. Active components from active extracts are identified, purified, and characterized. The group works directly with the CCR Molecular Targets Program (MTP) and has more than a dozen currently active collaborations of interesting molecules in further development with other CCR laboratories (or sections), CCR clinical branches, and non-NCI (including international) laboratories. The group coauthored two reviews on applications of HTS to natural products drug discovery (Grkovic et al., *ACS Chem Biol*, 2020; and Wilson et al., *Nat Prod Rep*, 2020).

KEY ACCOMPLISHMENTS

For the purposes of this report and because the work of the three Molecular Targets Group subgroups is closely coordinated and highly interactive, their accomplishments are combined. Many targets are being investigated in the laboratory. The Molecular Targets Group had 15 to 20 active projects at any given time in the past year. Each subgroup contributed to focus on the following targets (references noted were coauthored by FNL personnel in the Molecular Targets Group):

Covid-19 related projects. The group is working on three projects targeting the SARS-CoV-2 virus and focusing on targets and/or platforms that are also applicable to cancer targets and cellular phenotypes.

- Pseudovirus (PsV) model for identification of inhibitors of infectivity. The group has developed PsV preps containing the SARS-CoV-2 spike protein (required for binding to target cells) and a luciferase reporter-based infectivity assay (measurement of luciferase incorporation into target cells).
- Human topoisomerase 3β has been identified as a host factor required for efficient replication of different types of viruses including coronaviruses and SARS-CoV-2. The group has established a cell-based assay to identify selective inhibitors targeting cells with wild-type human topoisomerase 3β to inhibit viral replication in cells. HTS is underway.
- An assay to identify compounds that can bind to the SARS-CoV-2 spike-protein receptor binding domain using differential scanning fluorimetry (DSF) is being developed using purified spike proteins. Preliminary feasibility studies have been completed and a possible HTS configuration is being tested.

Antiviral proteins.

- The group has initiated structural analysis of a novel anti-HIV protein, isolated from a coral, genus *Alertigorgia*.
- The group has demonstrated the ability of Griffithsin, as well as an engineered trimeric variant of Griffithsin, a previously identified antiviral protein, to block entry of Nipah viral infectivity in cellular and animal models (Lo et al., *J Infect Dis*, 2020).

Cbl-b. Screening of natural product extracts by the group is near completion. Thirty-nine pure compounds have been isolated from 11 active marine and fungal extracts. Biochemical evaluation of their activity and publication of the assay are in progress.

Dendrocyte activation. A novel approach to cancer immunotherapy based on activation of dendritic cells in tumors has been proposed. The group is characterizing a reporter cell line to detect activation of dendritic-like cells for feasibility for HTS assay development.

DUOX2 (inhibition of reactive oxygen species generation). The group has developed and validated a low-to-medium-throughput screening assay to gauge the effect of test compounds and extracts on reactive oxygen species generation after ionomycin stimulation. Over 20,000 prefractionated natural product extracts have been screened using this assay.

Glypican3 (protein binding). To discover and develop imaging agents that can potentially also treat hepatocellular carcinoma, the group has developed an HTS DSF-based screen with human Glypican3. It is in the final stages of optimization.

IRG1 (protein binding). The group has optimized a DSF screening platform with the current batch of IRG1 proteins in order to identify compounds that bind and either stabilize or destabilize the protein's conformation. Binders are being further assessed using a novel liquid chromatography–mass spectrometry method for profiling enzymatic product formation (itaconic acid) for functional evaluation of hits.

MALT1 (inhibition of a protease implicated in B-cell lymphoma). The group has completed structural and biochemical characterization of active natural products, including identification of a series of active fungal metabolites. (Tran et al., *Phytochemistry*, 2019).

Merkel cell carcinoma (MCC). The group has completed HTS with a cell-based assay to identify substances able to preferentially kill MCC cells and/or to distinguish between virus(+) and virus(-) forms of MCC. Characterization of active compounds identified by the screen is underway, particularly focusing on cell death mechanisms.

Multidrug resistance (*ABCG2*). In a follow-up study, the activity of botryllamide G (natural product discovered after HTS) as an *ABCG2* inhibitor was analyzed in a mouse model of drug uptake in collaboration with an NCI molecular pharmacology laboratory (Strope et al., *Cancer Biol Ther*, 2020).

Osteosarcoma metastatic potential. An assay for differential inhibition of proliferation of high metastatic potential osteosarcoma cells along with characterization of a series of fungal metabolites from an active extract was published by the group and collaborators (Long et al., *Chem Biol Drug Des*, 2020).

PAX3-FOXO1 (fusion transcription factor involved in rhabdomyosarcoma). The group has initiated development of spheroid mono- and mixed-cell cultures for further characterization of active inhibitors. The binding of the *PFI-90* hit that emerged in the screen has been evaluated for binding interaction with the *KDM3B*, a histone lysine demethylase involved in the *PAX3-FOXO1*'s chromatin dysregulation pathway.

Programmed cell death receptor 1/programmed death ligand 1. Griffithsin, originally discovered in the MTP as an antiviral lectin-like protein, is being investigated by the group for its ability to bind peptides from physiologically/therapeutically relevant cellular proteins. In particular, anti-programmed-death-ligand-1 antibodies and peptides known to perturb the protein-protein interaction are being assessed for potential modulation of the cellular immune response against cancer cells.

RAS-RAF (modulation of dimerization). The group completed screening on two flavors of this assay, inhibition and enhancement of RAS-RAF binding. Purification of compounds from active natural product extracts has identified multiple structural families that are being analyzed for effects on RAS-RAF-MEK-ERK signaling. A novel series of inhibitory cyclic peptides from an active marine extract have been characterized and published in collaboration with natural products chemists (Kim et al., *J Nat Prod*, 2020).

Tbata (reversal of Tbata-induced growth arrest). The group has developed and validated an HTS assay using mifepristone-induced Tbata-expressing thymus cells. Screening has started with pure compounds and prefractionated extracts.

***TDP1* assay** (inhibition of phosphodiesterase activity). The group has completed structural characterization of the allosteric peptide modulator recifin (discovered via HTS and follow-up of active extracts). Based on its unique structure and function, an Employee Invention Report has been filed and a manuscript is in preparation.

***TDP2* assay** (inhibition of phosphodiesterase activity). The group continues to isolate new compounds from active extracts for submission for secondary assay evaluation with our collaborators. Analogs of several hits have been obtained from an academic chemistry collaborator for structure–activity relationship analysis.

Yeast chemical genomics. The group's application of this platform has provided novel insight into the activity of a series of natural products and synthetic derivatives of madecassic acid as disrupters of mitochondrial function (Valdeira et al., *J Nat Prod*, 2019).

Other support activities. The group has been very active in supporting the continued expansion and application of new MTP-screening libraries, both new

pure compound samples and novel natural product extracts. The group has also continued evaluating potential new MTP projects (six formal project proposals and multiple informal discussions in the last year) and managing new projects (including projects under the specific guidance of group members along with others to which the group contributes).

Biophysics Resource Group

The Biophysics Resource Group (BRG) maintains scientific instruments and computational infrastructure operations supporting research in the Structural Biophysics Laboratory (SBL), and by operating open-access biophysics resource and nuclear magnetic resonance (NMR) facilities, they provide support to a large group of CCR researchers.

Each BRG member is embedded in separate sections of the SBL:

- The SBL NMR Facility, which operates six NMR spectrometers dedicated to structural biology research and provides one open-access walk-up spectrometer to all CCR researchers. The NMR facility currently has more than 40 active users across all systems. Spectrometers were optimized for remote access to minimize in-person presence in the facility. NMR facility staff provided user training and technical support, which extends beyond the SBL NMR Facility to include all NMR spectrometers currently in operation in Fort Detrick and at the Advanced Technology Research Facility (ATRF), a total of 13 spectrometers.
- The Biophysics Resource is an open-access facility operated by SBL, which provides multidisciplinary support to CCR and NIH laboratories by maintaining and operating a broad range of scientific instruments and offering user training, protocol development, and multilevel cooperation.
- The Information Technology (IT) Infrastructure Support group maintains and expands high-end computational infrastructure that supports structural biology research. This includes maintaining over 20 scientific workstations, computational clusters, and high-capacity storage for data archiving and providing instruction to staff on high-end computing needs.

KEY ACCOMPLISHMENTS

- NMR Facility: set-up and configuration for remote operation of Bruker Neo 700 MHz spectrometer at the ATRF NMR Facility. It is now in full operation, serving RAS research efforts.
- BioPhysics Resource: FNL staff trained 45 new users, supervised their work, and performed studies on a collaborative basis with nine research teams, resulting in coauthorship on one publication and eight acknowledgements in peer-reviewed publications.
- IT Infrastructure Support: designed and implemented high-speed data transfer protocols and set up high-capacity data storage for the Arctica cryo-electron microscopy instrument for the CryoEM facility.

Bioinformatics and Structural Biology Section

The Bioinformatics and Structural Biology section provides computational and experimental support for various fields. Computational approaches are utilized for RNA design, RNA bioinformatics, computer-aided drug design, and immunogenetics, as well as for the refinement and analysis of protein structures. Experimental work in the area of structural biology focuses on X-ray crystallography and cryo-electron microscopy.

KEY ACCOMPLISHMENTS

- Previously, RNA nanostructures resembling cubes as well as hexagonal rings were reported and characterized. It was shown that they form stable complexes that can be delivered into cells using delivery agents such as bolaamphiphiles. Such RNA nanostructures can be connected via linking RNA helices into even larger supramolecular structures. Recently this has been accomplished in the form of an RNA nano-architecture resembling a truncated tetrahedron that consists of six hexameric ring units interconnected by RNA helices. FNL staff developed a novel algorithm for the in-silico design and characterization of such RNA-nanostructures and performed molecular dynamics simulations.
- FNL staff developed a convolutional neural network that is designed for recognizing RNA structural motifs of various sizes. In a recent publication, we showed that the predicted scores of selective 2′-hydroxyl acylation analyzed by primer extension correlate well with the experimental data. We also showed that the approach outperforms computational approaches solely based on thermodynamics, especially in regions with known sequence motifs such as translation start sites. This is an important step toward utilizing modern machine-learning approaches for predicting RNA properties.
- **Computational chemistry utilized for identifying inhibitors targeting C-terminal binding protein.** The C-terminal binding protein (CtBP) is associated with poor outcome in a variety of cancers. Therefore, CtBP represents an attractive target for small molecule inhibitors.

FNL staff used a quantitative structure-activity relationship approach as well as computational docking to identify a set of candidate small-molecule inhibitors targeting CtBP. From these initial candidates, four lead compounds were identified using functional assays. Further characterization of the impact of CtBP inhibition with the identified compounds demonstrated a disruption of CtBP dimerization as well as decreased cellular invasion and migration in the triple-negative breast-cancer

cell line MDA-MB-231. This suggests that CtBP inhibition is a promising avenue for cancer therapy. The utilized methodology also demonstrates the synergy between computational chemistry approaches and experimental functional assays for expediting the identification of small molecule lead compounds that are potent inhibitors of a cancer drug target.

- **Crystal structure of the *Yersinia pestis* UDP-glucose pyrophosphorylase (UGP) enzyme.** The bacterium *Y. pestis* causes the bubonic plague. While *Y. pestis* is treatable with a small number of antibiotics, resistant strains have been reported. One potential novel drug target is the bacterial UGP. UGPs exhibit a high amount of amino acid sequence similarity between bacterial species but not with their eukaryotic counterparts. This makes UGP an attractive target for the development of antibacterial therapeutics.

  Recently, the crystal structure of UGP has been solved to 2.17 Å resolution. FNL staff crystallized the protein, and collected, processed, and analyzed the data. A structural comparison with other bacterial UGP homologs revealed a highly conserved active site. This suggests that the design of broad-spectrum UGP inhibitors may be feasible and a promising approach for developing anti-plague therapeutics.

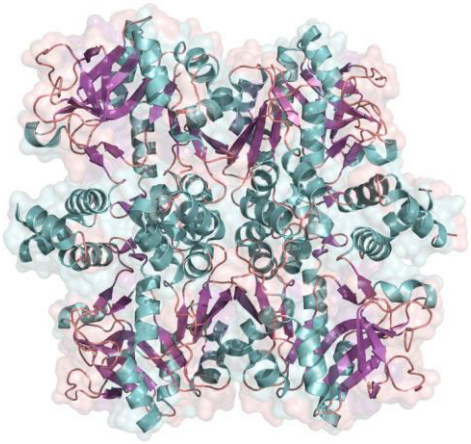


Figure 1. Ribbon representation of the crystal structure of the Yersinia pestis *UDP-glucose pyrophosphorylase at 2.17 angstroms resolution.*

- **Structural Characterization of Tyrosyl-DNA phosphodiesterase 1 (Tdp1) Inhibitors.** Tdp1 has been implicated with counteracting topoisomerase I inhibitors that are used in cancer therapy. Therefore, Tdp1 inhibitors could be beneficial for augmenting topoisomerase I inhibitor-based therapy. Using a crystallographic screening campaign, two molecular fragments were identified that bind the Tdp1 active site and have inhibitory activity. FNL staff designed the experiment, crystallized the complex with fragments, and collected, processed, and analyzed the data. The crystal structures of the protein-ligand complexes revealed that the bound fragments had a similar orientation to a previously published Tdp1 inhibitor. Next, molecular derivatives of the identified two fragments were characterized biochemically. These results are an important step toward developing therapeutic Tdp1 inhibitors.
- **Crystallographic and calorimetric analysis on *Pleurotus ostreatus* lectin and its sugar complexes.** Carbohydrate recognition is established as a property of lectins and implicated in many functions including immunity and defense against pathogens. Many lectins are characterized and proposed for various applications owing to the above-said recognition. The crystal structure of a lectin from *P. ostreatus* has been determined and shown to be calcium-dependent. The overall structure is a tandem repeat of two β-jelly roll domains, a new fold for lectins. The calcium dependence of sugar binding is analyzed in detail through isothermal titration calorimetry. The serendipitous observation of malonate and glycerol, the intentional N-Acetyl-D-galactosamine, D-Galactose and L-Rhamnose binding to *P. ostreatus* lectin by Ca2+ coordination revealed that the binding site is promiscuous. Among these sugars, Rhamnose-binding was found to be thermodynamically most favorable. In all these structures, a vicinal diol motif, one at axial and the other at equatorial positions, could be established as a specific requirement for binding. Interestingly, when compared with other calcium-mediated lectin structures; this geometric requirement is found conserved. This observation could lead to the conclusion that lectins are not "molecule-specific" but "geometry-specific," such that any molecule not necessarily a sugar may be recognized by this lectin if the geometry exists. FNL staff conducted the crystallographic study and analyzed the data.
- **Structural study of fluorescent protein FusionRed.** The crystal structure of monomeric red fluorescent protein FusionRed (λex/λem 580/608 mn) has been determined at 1.09 Å resolution and revealed two alternative routes of post-translational chemistry, resulting in distinctly different products. FNL staff conducted the crystallographic study and collected, processed, and analyzed the data. The refinement occupancies suggest the 60:40 ratio of the mature Met63-Tyr64-Gly65 chromophore and uncyclized chromophore-forming tripeptide with the protein backbone cleaved between Met63 and the preceding Phe62 and oxidized Cα-Cβ bond of Tyr64. We analyzed the structures of FusionRed and several related red fluorescent proteins, identified structural elements causing hydrolysis of the peptide bond, and verified their impact by single-point mutagenesis. These findings advance the understanding of the post-translational chemistry of GFP-like fluorescent

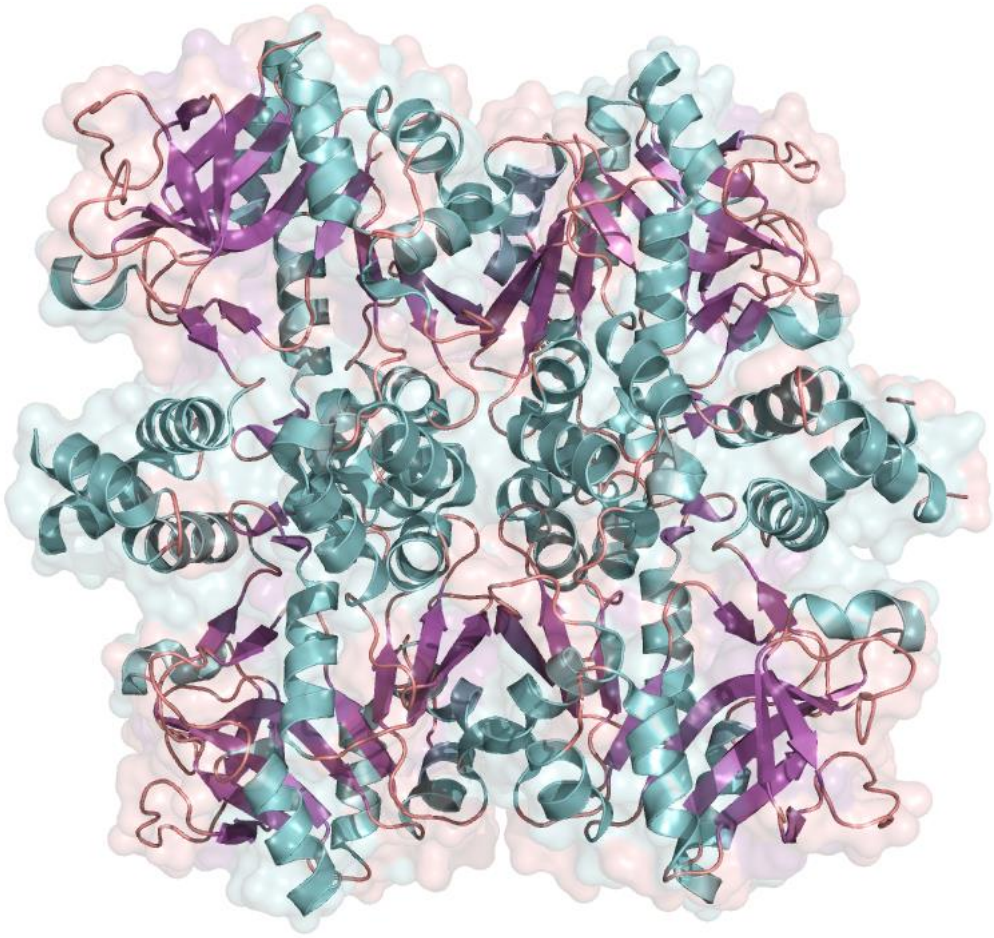


Figure 1. Ribbon representation of the crystal structure of the Yersinia pestis *UDP-glucose pyrophosphorylase at 2.17 angstroms resolution.*

proteins beyond the canonical cyclization-dehydration-oxidation mechanism. They also show that impaired cyclization does not prevent chromophore-forming tripeptide from further transformations enabled by the same set of catalytic residues. Our mutagenesis efforts resulted in inhibition of the peptide backbone cleavage, and a FusionRed variant with approximately 30 percent improved effective brightness.

- **Engineering of receptor tyrosine kinases controlled with near-infrared light.** Optically controlled receptor tyrosine kinases (opto-RTKs) allow regulation of RTK signaling using light. Until recently, the majority of opto-RTKs were activated with blue-green light. Fusing a photosensory core module of *Deinococcus radiodurans* bacterial phytochrome (DrBphP-PCM) to the kinase domains of neurotrophin receptors resulted in opto-RTKs controlled with light above 650 nm. To expand this engineering approach to RTKs of other families, we combined the DrBpP-PCM with the cytoplasmic domains of EGFR and FGFR1. The resultant Dr-EGFR and Dr-FGFR1 opto-RTKs are rapidly activated with near-infrared and inactivated with far-red light. The opto-RTKs efficiently trigger ERK1/2, PI3K/Akt, and PLCγ signaling. Absence of spectral crosstalk between the opto-RTKs and green fluorescent protein-based biosensors enables simultaneous Dr-FGFR1 activation and detection of calcium transients. DrBphP-PCM represents a versatile scaffold for engineering of opto-RTKs that are reversibly regulated with far-red and near-infrared light. FNL staff designed the experiments, analyzed the data, and performed structural modeling.
- **Structure-based rational design of two enhanced bacterial lipocalin blc tags for super-resolution microscopy.** Super-resolution fluorescent imaging in living cells remains technically challenging due in large part to the photodecomposition of fluorescent tags. Protein point accumulation for imaging in nanoscale topography (protein-PAINT) is the only super-resolution technique available for prolonged imaging of proteins in living cells. It is realized with complexes of fluorogen-activating proteins, expressed as fusions, and solvatochromic synthetic dyes. Once photobleached, the dye in the complex is replaced with a fresh fluorogen available in the sample. With suitable kinetics, this replacement creates fluorescence blinking required for attaining super resolution and overcomes photobleaching associated with loss of irreplaceable fluorophores. We have developed two protein-PAINT tags based on the 1.58 Å crystal structure of DiB1-M739 complex: an improved green-emitting DiB3/F74V-M739 and a new orange-emitting DiB3/F53L-M739 that outperform previously reported DiB-based tags to become best-in-class biomarkers for protein-PAINT. These tags advance protein-PAINT from the proof-of-concept to a reliable tool suitable for prolonged super-resolution imaging of intracellular proteins in fixed and living cells and two-color PAINT-like nanoscopy with a single fluorogen. FNL staff designed the experiment, contributed to the crystallographic study, and analyzed the results.

Bacterial Genetics Group

The Bacterial Genetics Group (BGG) uses the bacterial virus λ and its host, *Escherichia coli*, as paradigms for ongoing developmental and gene-regulation studies. Coevolution of λ with *E. coli* has produced genetic systems that are exquisitely connected to the most basic functions of the bacterial host. By examining the interface between λ and host systems, BGG follows the trail of the virus to understand what is most important and vital to cellular life and how all viruses might exploit cellular systems. The virus provides clues as to how those cellular functions work and how to study them. Recent characterizations of the λ genetic network have provided a framework for systems biology approaches using λ as a prototype for theoretical modeling methodologies, which have become important for addressing signal transduction, cancer development, and other complex genetic networks of eukaryotes.

Key Accomplishments

- BGG has developed recombineering, a highly efficient technology for precise *in vivo* DNA manipulation in bacteria using homologous recombination and requiring only short homologies. Recombineering is used to modify genome segments on bacterial artificial chromosomes (BACs); altered genomic sequences can then be introduced back into the organism of interest.

  Recombineering with double-strand DNA can precisely replace a defined region with a drug marker, enabling the creation of null gene mutants. Recombineering with single-strand DNA oligonucleotides can be extremely efficient, with greater than 50 percent of viable cells becoming recombinant. Single-strand recombination can be used to introduce point mutations on large DNAs, such as BACS; recombinants are identified with a polymerase chain reaction screen. This technology enables high-efficiency targeted mutagenesis. Recombineering can also be used to assemble multiple linear DNA fragments with short terminal homologies int o intact replicating plasmids *in vivo*. Mutation of a 3′ single-strand exonuclease, ExoI, stimulates recovery of linear DNA assembly plasmid products. *In vivo* assembly has a low error rate and provides a viable alternative to Gibson assembly.
- BGG is collaborating on a project exploring the possibility of making vaccines by displaying potential antigenic epitopes on the surface of bacteriophage λ virions. Using recombineering, BGG

has constructed many such λ phages displaying different heterologous proteins. The recombinant λ display phages are promising candidates for vaccine production targeting three diseases and/or their causative agents: (i) chronic lymphatic leukemia; (ii) *Plasmodium falciparum*, the malaria parasite; and (iii) SARS-CoV-2, the coronavirus causing COVID 19. BGG makes the λ phage display constructs and passes them on to the Developmental Genetics Section, Laboratory of Molecular Biology within CCR. The Developmental Genetics Section confirms the presence of displayed proteins in the purified phages by Western blot and makes large amounts of high-titer phage purified free from bacterial endotoxins. The reactivity of these purified recombinant λ phages will be tested for immunogenicity in animals in collaboration with other laboratories. For λ SARS-CoV-2 display phages, reactivity will be tested against serum from humans who have had COVID-19 but survived the disease, since it is assumed these individuals have antibodies against the SARS-CoV-2 coronavirus in their serum. [redacted].

The bacterial virus λ has two developmental lifestyles: active viral reproduction and lysogeny, or viral latency, where the virus exists as a prophage. Bacterial viruses are excellent model systems for mammalian viruses, which are responsible for a number of human diseases, including cancer. Understanding how this quiescent *E. coli* virus reactivates will enhance our understanding of viral reactivation in general. BGG is characterizing the region of the viral chromosome responsible for maintenance of lysogeny to better understand repression and reactivation. For a λ prophage, the repressor protein CI prevents expression of most viral genes by binding to operator sites flanking lytic promoters. A second repressor, Cro, binds to the same operator sites with a different pattern of affinities, shutting off CI repressor expression and allowing lytic growth to proceed. The interaction of CI and Cro with the operator sites forms a bistable genetic switch. BGG has found that two bacteriophage functions, RexA and RexB, modulate this switch, and an imbalance in the two proteins makes viral reactivation defective (Lynn Thomason, *Mol Microbiol*, 2019). BGG has recently demonstrated that the RexA protein stabilizes the lytic configuration of the switch, and that the RexB protein antagonizes the effect of RexA, elucidating another layer of regulation controlling the phage λ bistable switch.

- As RNA polymerase transcribes the ribosomal RNA (rRNA) operons in bacteria, it complexes with a set of proteins collectively called Nus that confer enhanced rates of transcription elongation, correct folding of rRNA, and proper rRNA assembly with ribosomal proteins to generate a functional ribosome. BGG studies the role of transcription pausing mediated by the Nus transcription factors and the transcription termination factor Rho and their effect on cellular function. Experimentally, *E. coli* and *Bacillus* strains are depleted for these activities, then the number of transcription pauses and their distribution across the bacterial genomes is compared to a wild-type reference strain, using RNA-Seq and NET-Seq in combination with bioinformatic analysis. Since most genes encoding the Nus factors and Rho are essential, depletion is accomplished using a gRNA/dCas9 system made by recombineering. All the transcription factors are efficiently repressed under optimized growth conditions and the results show that several of these factors, particularly Nus, play roles in transcription pausing.

Urologic Oncology Group

The Urologic Oncology Group (UOG) within the Urologic Oncology Branch of CCR discovers and characterizes kidney cancer susceptibility genes through studies of families with rare, inherited renal cancer syndromes and deep sequencing of sporadic histologically defined renal tumors, providing insight into the molecular mechanisms that lead to the development of renal cancer. In-depth study of the metabolic pathways dysregulated in these inherited renal cancer syndromes and their sporadic counterpart tumors has informed the design of potential molecularly targeted therapies for renal cancer. UOG is developing genetically engineered mouse models of renal cancer as an important research tool for *in vivo* functional studies and efficacy studies of novel therapeutic agents. In collaboration with the Dana Farber Cancer Institute, UOG developed an orthotopic syngeneic mouse model of clear cell renal cancer using CRISPR/Cas9 gene editing of kidney cancer susceptibility genes.

Characterization of mouse tumors from this model has demonstrated clear cell histology and successful gene editing. Targeted therapies are currently being evaluated in this model, which, if successful, will provide the basis for a clinical trial in kidney cancer patients. UOG has also been successful in the generation of a genetically engineered mouse model of TFE3-translocation kidney cancer (tTfe3-RCC) which is a highly aggressive form of kidney cancer that mainly affects children and young adults for which there are no effective therapies. Renal tumors from these mice were successfully implanted orthotopically in immunocompetent recipient mice, thereby enabling the evaluation of immunotherapies to treat this aggressive disease.

Synthetic Biologics and Drug Discovery Facility, Laboratory of Cancer Immunometabolism

The Synthetic Biologics and Drug Discovery Facility develops chemical biology tools and designs and generates drug candidates for various molecular targets, mainly focusing on “undruggable” proteins. This year, we

identified candidates by virtual screens and rational design that act as potent small-molecule inhibitors for important targets such as SARS-CoV-2 NSP9, NSP10, NSP15, NSP16, Nucleocapsid, and Spike proteins, which play a vital role in infectivity and replication of the coronavirus, and the KRAS G12V mutant protein, a key player in many cancers. For SARS-CoV-2 proteins, novel technologies such as nano-differential scanning fluorimetry and microscale thermophoresis have permitted the performance of binding experiments with inhibitors to identify the best leads, which are now being tested in fluorescence and functional assays. The most potent KRAS compounds, identified through toxicity assays in different human-lung-carcinoma cell lines and Nano-Differential Scanning Fluorimetry binding experiments, are now being used in the Microscale Thermophoresis and Fluorescence Polarization assays for binding affinity determination. Importantly, one of the peptide KRAS inhibitors developed in the laboratory is undergoing preclinical studies in tumor mouse models.

Vaccine Branch

The Vaccine Branch focuses on developing new immunotherapy procedures. The hetIL-15 trial developed by the Vaccine Branch and supported by Novartis, generated large numbers of clinical samples for further study of the pharmacokinetics and pharmacodynamics revealed by this first-in-human trial, which were evaluated by advanced flow cytometry, RNAseq, and other techniques to identify optimal treatment protocols and biomarkers for further clinical development. We demonstrated that hetIL-15 has activity against tumors and optimized administration in mouse orthotopic breast cancer models. Additionally, the Vaccine Branch has studied the tumor microenvironment using transcriptomics, proteomics, and metabolic measurements leading to the discovery of a new type of intratumoral dendritic cell found only after hetIL-15 treatment, which was presented at the recent American Association for Cancer Research 2020 meeting. A substantial finding was that these dendritic cells correlate with tumor regression and the development of long-term memory against the tumor, which does not permit tumor formation after re-challenge. Further metabolic and immunological analysis of tumor samples during hetIL-15 treatment has revealed important intratumoral changes that facilitate tumor treatment.

Chemoattractant Receptor and Signal Section, Laboratory of Cancer and Immunometabolism

The Chemoattractant Receptor and Signal Section is focused on defining the critical role of a group of G-protein-coupled chemoattractant receptors named formylpeptide receptors (FPRs) in host defense against cancer and inflammatory responses. This year, we identified FPR1, which plays a role in exacerbating diabetes-promoted cancer progression. The group has also demonstrated a key role of two mouse FPR analogues, FPR1 and FPR2, in host defense against *E. coli* infection. Additionally, we have performed studies with germ-free mice to address the contribution of the host microbiome to the development of colon crypts and colitis.

Microbiome and Genetics Core, Laboratory of Integrative Cancer Immunology

The Microbiome and Genetics Core (MGC) of the Laboratory of Integrative Cancer Immunology runs its microbiome facility with a team of two technicians, two bioinformaticians, one scientist, and one postbaccalaureate. The group is focused on characterizing the role of the microbiota in cancer and inflammatory processes. Having established reliable and reproducible methods to isolate and characterize nucleic acids of microbiota isolated from feces and other sources, the core has worked with a range of source materials and about 50 principal investigators in 10 National Institutes, to help effectively determine changes in microbial representation between experimental samples.

Robotic sample preparation platforms (Eppendorf 5073 and 5075) are used to maximize throughput and reproducibility, both for nucleic-acid isolation and for barcoded-library preparation. Quantification is accomplished using quantitative polymerase chain reaction or spectroscopy. Following purification, barcoding, and quantification, an Illumina MiSeq® is used to sequence amplicons of 16S rRNA genes. For genomic approaches, the same DNA isolation process is used, and as little as one ng of DNA is subjected to breakage and library preparation by transposon-driven "tagmentation." Whole-genome sequencing from isolates is done on the MiSeq platform, and shotgun metagenomes of the microbiota are run on the higher-capacity NextSeq or NovaSeq®. Shotgun approaches are being used in LICI and NCI projects as well as for collaborators from other NIH institutes, and terabytes of sequenced base pairs of data are being generated and analyzed from these platforms. Across the projects, different challenges have been met successfully, including finding a way to isolate DNA from high-bacterial or lower-bacterial biomass sources, developing a method to partition analyses from different sources, and determining which treatments maximize the signal-to-noise ratio of experiments. We are handling samples associated with both clinical and basic scientific research.

Illumina's cloud server as well as a backup system at FNL's computer center address the bioinformatic challenges of storing, delivering, and backing up large amounts of information. This FNL team favors the QIIME analytical approach to determining microbial abundances from 16S data. The analyses are also limited by the quality of databases of rRNA. We continue to develop a database of fully vetted, high-quality rRNA sequences for use in identifying components of the microbiome in samples.

The core continues to expand its process repertoire from 16S ribosomal amplicon-based metagenomics through whole-genome sequencing of microbial isolates to shotgun metagenomics. MGC has isolated microbiomal

DNA from a variety of sources, including swabs, fecal pellets, tissue samples, and a variety of source organisms while metatranscriptomic approaches to microbiome characterization are underway. We have incorporated fungal characterizations by 18S ITS amplicon analysis into our repertoire. The broad experimental repertoire will enable examination of potential metabolic pathway changes induced by changes in composition, gene content, and transcriptional activity of the microbiota. This has enabled MGC to undertake microbiome characterizations across a range of health fields from cancer to vaccinations and autoimmune and infectious disease studies.

For shotgun metagenomics, we utilize JAMS, a versatile pipeline developed at CCR (McCulloch et al., in preparation), which offers insights into the genomic data generated by interogating taxonomy, metabolic pathways, and gene family abundances. Continuing work includes characterization of shotgun metagenomes in checkpoint inhibition therapy of cancer; therapeutic responses to fecal transplantation; microbiota in human esophageal biopsy; microbiota and bile acid metabolism in liver cancer; microbiome characterization in hematopoiesis reconstitution; microbiota from human oral samples related to outcomes from transplantation; characterization of salivary microbiota in hepatitis infection; and shotgun metagenomic analysis of human tooth microbiota in two distinct monogenic neutrophil deficiency syndromes. New undertakings include characterization of an 880-individual mother-infant cohort and a case-control study in chronic fatigue syndrome. We have worked with microbiota in mouse models for other diseases or health interventions. Over the past year, MGC members have coauthored eight papers in addition to multiple acknowledgments elsewhere.

The core continues to support analysis in genetics, particularly regarding the role of HLA expression in autoimmune disease, infections, and transplantation medicine. The MGC has recently been involved in publications on topics including uncovering the role of HLA-B leader peptide polymorphisms in determining transplantation outcomes (Petersdorf et al., *Lancet Haematol*, 2020) and determination of the role of HLA-DPB in severe aplastic anaemia (Savage et al., *Am J Hum Genet*, 2020).

Small-Angle X-ray Scattering Core

The Small-Angle X-ray Scattering Core group supports a wide range of investigators and laboratories, including CCR laboratories, as well as NIH intramural and extramural groups, in structural investigations of biomacromolecules and their complexes in solution. We have supported 23 users/groups in the past year, excluding the time period from March 1, 2020 through June 30, 2020 due to service interruption caused by COVID-19. We have contributed to one doctoral thesis and 13 peer-reviewed publications, including seven coauthorships.

Flow Cytometry Core

The CCR-Frederick Flow Cytometry Core Laboratory is an essential resource for NCI-Frederick investigators. Administratively embedded in the LCIM, the core primarily serves CCR investigators; requests from investigators outside CCR but within FNL are accepted on a limited basis.

The core's primary services are: (i) *Analysis* using flow cytometry including data collection, statistical reduction, and presentation; (ii) *Cell Sorting*, or physical separation of defined cells from mixed samples; (iii) *Training* investigators for cytometer use; (iv) *Technology Oversight* including instrument maintenance, quality assurance, life-cycle planning, and evaluation of emerging instruments, software, reagents, and applications; and (v) *Consulting* for assay development and troubleshooting.

The core currently operates 10 cytometers: seven analytical and three sorting instruments, housed across two adjacent laboratories, including a nested biosafety level (BSL)-2+ laboratory for human cell sorting. The instruments are configured with optical layouts such that experiments designed and analyzed using a particular instrument may be replicated on other instruments within the laboratory, providing flexibility in scheduling.

To date, the core has performed 1,170 billable services including 163 cell sorts. The core recorded 62 users from the following programs:

- Cancer and Developmental Biology Laboratory
- Division of Cancer Treatment and Diagnosis
- HIV Dynamics and Replication Program
- Laboratory Animal Sciences Program
- Laboratory of Cancer Immunometabolism
- Laboratory of Cell and Developmental Signaling
- Laboratory of Integrative Cancer Immunology
- Laboratory of Molecular Cell Biology
- Laboratory of Protein Dynamics and Signaling
- Mouse Cancer Genetics Program
- RNA Biology Laboratory

KEY ACCOMPLISHMENTS

- **Response to COVID-19:** The core leadership joined with other NCI cytometry cores to draft and publish guidelines for handling human samples within NCI flow cytometry laboratories; guidance was reviewed by laboratory chiefs and distributed to investigators. Recognizing the need for clear communication describing precautions and evolving guidance to the core's users, we developed and distributed a modified reservation policy to investigators in a one-sheet visual summary.
- **Enhancement of Training Program:** The core joined with other NCI cytometry cores to develop and present flow cytometry educational lectures at the NIH Bethesda campus in May and November

2019. We coordinated vendor training days with lectures and supervised hands-on training for two new cytometers procured and installed in 2019 by Miltenyi Biotec and BDBiosciences. We continue to improve our ad-hoc user trainings with facility-specific software tutorials recently converted to self-paced YouTube-style videos.

- **Keeping Pace with Evolving Cytometry:** As of August 2020, the core is installing two high-dimensional cytometers, each with five lasers, capable of measuring more than 30 simultaneous parameters, thus keeping pace with emerging trends in cytometry and proteomics. The Cytek Aurora represents a new class of cytometer that uses the entire spectrum, from ultraviolet to infrared and deconvolute-fluorescent signals; this added analytical power can derive more usable data from limited samples like tumor-infiltrating leukocytes. The BD Biosciences Symphony S6 is capable of high-speed separation of finely defined immune cell subsets into up to six distinct types simultaneously or depositing single defined cells into 96- or 384-well plates for cloning or deep sequencing. Two instruments manufactured in 2007 and 2009 with pending vendor obsolescence will be retired.
- **Special Recognition:** A member of the core team earned the Specialist in Cytometry credential by the American Society of Clinical Pathology, indicating "broad proficiency of technical and operational aspects of cytometry and shared resource (core) laboratories."

Media Laboratory

The Media Laboratory has been in operation since 1984, and employees are skilled at making microbiological media for both bacterial and yeast work. They routinely make buffers and other reagents for biochemistry, molecular biology, and genetics research. All media are custom-made (liquids and plates); thus, additives such as antibiotics, isopropyl-B-D-1-thiogalactopyranoside, counter-selective agents, anti-cancer drugs, and reverse-transcriptase inhibitors can be added at the request of the researcher. (Ingredients not commonly used by most laboratories need to be provided by the ordering laboratory).

The Media Laboratory is usually able to accommodate requests for new reagents when provided with a recipe. Because it is located on the NCI Frederick Campus, staff members are available to answer questions. They can also accommodate requests for dispensing products in a variety of sizes (e.g., 10 bottles of LB at 100 ml/bottle). All products use the highest-quality reagents: only Difco Agar, Tryptone, and Yeast Extract are used. Most products are delivered within three business days after the Media Laboratory receives the order. The laboratory provides service to about 57 laboratories. On-site media and reagent preparation is a highly cost-effective and valuable resource for many scientific laboratories that require microbiological media or other molecular biology reagents.

A reasonable cost-sharing pricing system is utilized for media pricing. Under the cost-sharing system, buffers, plates, and reagents fall into different pricing tiers, depending on the cost of the reagents and labor involved. The Media Laboratory bills monthly to facilitate everyone's bookkeeping.

This year, during the COVID-19 pandemic mandatory laboratory closures, Media Laboratory employees came into the laboratory on a few occasions, by special request, to make media for COVID-19-related research.

Media Laboratory Products

- Bacterial growth media: LB, TB, and Minimal Broth and plates using Difco products, with or without antibiotics or other additives
- Diagnostic growth media: MacConkey agar with carbohydrate of choice, EMB agar with carbohydrate of choice
- Yeast growth media: YEPD, YEA, Amino acid drop-out plates
- Buffers and solutions: Tris buffers, Q-buffers
- Gel electrophoresis and transfer buffers: Tris-glycine buffer, SDS-Page buffer, Tris-acetate EDTA buffer
- Bacteriophage growth media for phage lambda and M13: TB, NZY, YT.

The Media Laboratory provides services to NCI CCR Laboratories at two Frederick locations, the NCI Campus at Frederick and the ATRF, as well as to laboratories at NIH Bethesda.

Support Provided by the Biomedical Informatics and Data Science Directorate

Bioinformatics and Computational Science

KEY ACCOMPLISHMENTS

- National Cancer Institute (NCI) Comprehensive Oncologic Molecular Pathology and Sequencing Service (NCI-COMPASS): Analytical and clinical validation of TruSight Oncology 500 was completed in September 2019. The analytical validation of whole-exome sequencing assay and RNA-Seq was also completed.
- The Center for Cancer Research (CCR) Sequencing Facility received 302 requests and processed more than 14,000 samples, including Illumina short-read, Pacific Biosciences long-read, and single-cell projects, delivering more than 133 trillion base pairs of data to more than 190 investigator laboratories across NCI, the National Institute of Allergy and Infectious Diseases (NIAID), and the National Institutes of Health (NIH).

- The CCR Collaborative Bioinformatics Resource (CCBR) performed data analysis for Dr. Christian Hinrichs' laboratory to study regression of epithelial cancer in patients following engineered T-cell therapy. This was a clinical trial of engineered T-cell therapy targeting human papillomavirus (HPV)-16 E7 for the treatment of metastatic HPV-associated epithelial cancers. It was discovered that one of the only patients to have almost no response to the treatment also had evidence of a nonsense mutation at human leukocyte antigen (HLA)-A*02:01, a necessary component of the E7 T-cell receptor target peptide HLA complex.
- The Bioinformatics Training and Education Program (BTEP) provided expanded bioinformatics training and workshops for NCI staff who were teleworking due to the COVID-19 pandemic. More than 300 participants have accessed the resources during this time.

COVID-19 and Teleworkforce Support Activities

- HIV Dynamics and Replication Program: FNL staff screened the human genome to make sure there are no cross-reactions of the single-copy assay primers to the human genome. They also worked to screen the primer/probe sets to make sure there are no cross-reactions to the human genome and to other severe acute respiratory syndrome coronaviruses.
- CCBR developed an automated way to download the latest SARS-CoV-2 complete genomic sequences from GeneBank/RefSeq and run multiple sequence alignment in an automated manner to find a consensus sequence for Dr. Thomas Schneider's sequence logo generator (makelogo: http://users.fred.net/tds/lab/delila/makelogo.html). CCBR also created a Docker container that includes multiple alignment using fast Fourier transform (MAFFT) and a wrapper script called `covid` that allows automated download of the latest sequences and runs multiple sequence alignment. This Docker image is freely available on Docker Hub here: https://hub.docker.com/repository/docker/nciccbr/ccbr_mafft.
- BTEP online classes and webinars during the COVID-19 extended telework period: The enforced teleworking introduced in response to the COVID-19 pandemic has resulted in a great interest in bioinformatics training. Fortunately, this timing coincided with the expansion of BTEP's online training initiatives that had been piloted (DataCamp) in the previous year within the Staff Scientists and Staff Clinicians Technical Enrichment Program. Thus, BTEP has been able to offer "homebound" researchers two new tracks for learning bioinformatics skills:
    - BTEP allocated 76 of 100 purchased licenses for the Biostar Handbook. The group has been holding a class "Bioinformatics for Beginners using the Biostar Handbook" since April 2020 and is currently on week 10 of class.
    - The group also made licenses available to dataquest.io, an online platform for learning programming in Unix, Python, R, SQL, and more. To date, the group is using 80 licenses for NCI/CCR scientists.
- Additionally, in response to the enforced telework, BTEP has moved all its seminars and workshops to webinars and is setting up virtual workshops in which CCR scientists can pose questions to a panel of subject-matter experts.
    - Workshops, Seminars, and Software
        - ChIP-Seq Data Analysis: Probing DNA-Protein Interactions (303 attendees)
        - Biostars/Bioinformatics Recipes (led by Dr. István Albert [Pennsylvania State University], 280 attendees)
        - Data Science Using Apache Spark for Biomedical Applications (35 attendees)
        - Single-Cell RNA-Seq Analysis (91 attendees)
        - Overview of Common Statistical Tests (47 attendees)
        - Variant Analysis in NGS data (50 attendees)
        - Qlucore Omics Explorer (several sessions, 10–20 attendees per session)
        - Partek Flow bulk and single-cell RNA-Seq (several sessions, attendees limited to 20 per session)
        - Palantir/NIH Integrated Data Analysis Platform training courses (26 in-person classes conducted in FY2020)
- CCR Sequencing Facility (priority efforts for COVID-19)
    - VirScan to Determine the History of Viral Exposure Including COVID-19 Patients (Dr. Xin Wei Wang, NCI): The FNL staff delivered primary/secondary/tertiary analysis for three batches, including 576 samples for 159 patients.
    - Identifying Combinations of Small Molecules for Targeting COVID-19 Replication (Dr. Eytan Ruppin, NCI): The FNL staff provided data analysis support, including building a custom reference, which was generated with *Chlorocebus sabaeus* (African green monkey) and Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1.

Sequencing Facility

Bioinformatics Support for Production Next-Generation Sequencing Data Analysis

Since September 2019, the FNL staff in the Sequencing Facility received 302 requests. The team has processed more than 14,000 samples, including Illumina

short-read, Pacific Biosciences long-read, and single-cell projects, and delivered more than 133 trillion base pairs of quality-controlled data to more than 190 investigator laboratories across CCR, NIAID, NIH, and academic institutes. The samples increased more than 20 percent from the same period in FY2019.

Highlights for Scientific Collaboration Studies

- FNL staff provided bioinformatics analysis support for serological profiling of the viral infection history in 899 individuals from an NCI–University of Maryland case-control study using a synthetic human virome, VirScan. The viral exposure signature defines early onset of hepatocellular carcinoma. The resulting manuscript has been published (Liu et al., *Cell*, 2020).
- FNL staff provided sequencing analysis for bulk RNA-Seq and single-cell RNA-Seq, as well as T-cell immune profiling analysis, to explore the role of mannose receptor (CD206) activation in tumor-associated macrophages. The results of this study have been published (Jaynes et al., *Sci Transl Med*, 2020).
- FNL staff provided sequencing quality control and variant analysis to the NCI Data Science Laboratory for a study of whether targeting purine synthesis in ASS1-expressing tumors enhances the response to immune checkpoint inhibitors.
- FNL staff provided single-cell sequencing data analysis to the NCI Laboratory of Immune Cell Biology (Dr. Remy Bosselut) to analyze the response of tumor-specific CD4+ tumor-infiltrating lymphocytes and draining lymph node (dLN) T cells. (Magen et al., *Cell Rep*, 2019).
- FNL staff provided collaborative bioinformatics support for analyzing whole-genome sequencing (WGS), whole-exome sequencing (WES), and RNA-Seq for "Evaluation of Next-Generation Sequencing of DNA and RNA from Archival FFPE Pancreatic Cancer Tissue: A Pilot Study of the SEER-Linked Virtual Tissue Repository."
- FNL analysts analyzed Pacific Biosciences Iso-Seq data to identify alternative splicing events for the RNA Biology Laboratory and found that novel, abundant *Drosha* isoforms are deficient in miRNA processing in cancer cells.

Contributions to Community-Wide Research and Best Practice Development

FNL staff and the Food and Drug Administration single-cell team, as well as other members from Sequencing Quality Control Phase II consortium, have conducted a multicenter cross-platform benchmarking study of single-cell RNA-Seq using two well-characterized cellular reference samples.

CCR Collaborative Bioinformatics Resource

Representative scientific collaborations:

- *Regulatory relationships between* CLIC4 *and miR-142-3p in head and neck squamous cell cancers* (Carofino et al., *Oncotarget*, 2019)
- *Bioinformatic support for the Retroviral Replication Laboratory* resulted in publication of the first evidence that CRISPR/Cas9 technology can be applied to knock out the *ORF57* gene from all approximately 100 copies of the Kaposi sarcoma–associated herpesvirus genome (BeltCappellino et al., *J Virol*, 2019).
- *Epigenetic modifications in breast cancer metastasis demonstrating the effect of DNMT3B in epigenetic modifications, allowing cancer cells to colonize different tissues*. FNL staff provided analytical support for Methyl-Seq analysis showing differences in methylation between metastases and primary tumors. (So et al., *Cancer Res*, 2020).
- *Induction of phenotypic changes in HER2-postive breast cancer cells* in vivo *and* in vitro is a collaboration with Dr. Gilbert Smith's group (Basic Research Laboratory) and Dr. Brian Booth's laboratory (Clemson University). The FNL staff analyzed RNA-Seq data sets derived from mice that received transplants of different ratios of co-cultured normal epithelial cells and cancer-derived cells. The analysis showed that the cancer-derived cells appeared to have nearly normal expression patterns when co-cultured with sufficient normal cells.
- ZBP1, *not* RIPK1, *mediates tumor necroptosis during tumor development*. In this study, the FNL staff provided a critical link to *in vivo* conditions by identifying 15 cancer types for which appropriate data were available through The Cancer Genome Atlas and analyzing them for the expression of relevant genes. Additionally, *ZBP1* was found to be significantly upregulated in all stages of breast cancer, with a slight decrease in stage IV cancers, which supports their model of *ZBP1*'s involvement with metastasis.
- *Regression of epithelial cancer in patients following engineered T-cell therapy*. In collaboration with Dr. Hinrichs' laboratory, this was a clinical trial of engineered T cells targeting HPV-16 E7 for the treatment of metastatic HPV-associated epithelial cancers. The FNL staff focused on RNA-Seq and exome sequencing data sets derived from tumor and normal samples taken from patients. An interesting finding of this analysis was the discovery that one of the only patients to have almost no response to the treatment also had evidence of a nonsense mutation at HLA-A*02:01, a necessary component of the E7 T-cell receptor target peptide HLA complex.

Bioinformatics Support for the Vaccine Branch

Representative projects include:

- Characterization of type 2 natural killer T-cell immunosuppressive subsets by RNA-Seq analysis
- Vaccine-induced microRNA expression in rhesus monkeys and their correlation with protection from simian/human immunodeficiency virus challenge
- Effects of peritumoral-administered interleukin-15 treatment on tumors and draining lymph nodes in macaque breast cancer models

Bioinformatics Support for the Radiation Oncology Branch

- *Release of SL-BioDP: a comprehensive web tool for predicting synthetic lethality* (Deng et al., *Cancers (Basel)*, 2019)
- *Investigation of radiation-driven evolution of glioblastoma orthotopic xenografts* (McAbee et al., *Cancer Res*, 2019)

Laboratory and Bioinformatics Support for the Laboratory of Pathology and NCI-COMPASS

- The FNL staff completed analytical and clinical validation of TruSight Oncology 500 (TSO500) in September 2019. The TSO500 assay is in production and has provided clinical results since October 2019, with a weekly sample volume of 24. FNL also completed analytical validation of a WES assay and RNA-Seq. The project is currently at the final phase of the clinical validation of both the WES and RNA-Seq assays. These clinical next-generation sequencing assays are expected to be in production in the third and fourth quarters of 2020, respectively. Installation of the NovaSeq6000 (Illumina, Inc.) was completed.
- During FY2020, the FNL analysts embedded in the NCI-COMPASS computational team performed the following tasks:
    - Developed a bioinformatics pipeline to analyze the DNA and RNA data sequenced through Illumina TSO500 and TSO170 panels, respectively. The Variant Call Format (VCF) files and RNA fusion detection output from the TSO500 pipeline are used as input to custom Python scripts that create a data package. This data package is sent programmatically to QIAGEN Clinical Interpretation services for data interpretation and clinical sign-out.
    - The Methylation Classifier pipeline was developed to analyze the DNA methylation data sequenced through the Illumina EPIC array. In brief, raw data generated by the Illumina iScan reader are used as input for the classifier. Quality control plots are generated for staining, hybridization, specificity, and restoration. A customized classifier report is created with plots for copy-number variants and promoter methylation predictions for the MGMT gene locus. Finally, t-distributed stochastic neighbor embedding (t-SNE) plots are generated for each sample using the IDAT files as input.

Support for Dr. Javed Khan's Laboratory, Oncogenomics Section, Genetics Branch

Multi-omic analyses of patients with disease that is sensitive vs. resistant to CD19 CAR-T treatment. The project involves several data types and analyses, such as single-cell RNA-Seq data analysis and analysis for copy-number variations, high-parameter cytometry (CyTOF), bulk RNA-Seq, methylation, and cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq).

CCR Office of Information Technology Labmatrix

Labmatrix is currently used by more than 80 principal investigators from more than 30 NCI laboratories/branches to support their clinical and translational research. The FNL staff's efforts in FY2020 were focused on users and laboratory support, training, and data migration.

Support for Molecular Targets Program

FNL staff bioinformatics and chemoinformatics support in FY2020 was expanded to streamline data management in the laboratory and develop web interfaces for automating specimen requests, tracking, and analysis workflows.

Support for the RNA Biology Laboratory

FNL staff developed custom analysis pipelines to interrogate high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (CLIP-seq), RNA-Seq, RNA-Seq for noncoding RNAs, and SHAPE data. This has allowed identification of the presence of RNA editing sites, RNA-binding protein targets, and noncanonical *Drosha* targets, as well as the detection and measurement of poly(A) tails in preRNA and small-molecule binding structures in RNA riboswitches.

T-Cell Receptor Epitope Discovery

KEY ACCOMPLISHMENTS

- Processed more than seven cell lines
- Identified several epitopes that are promising candidates
- Began to cross-correlate candidate epitopes between antibody experiments by Dr. Hinrich's laboratory and mass spectrometry experiments on the same cell lines

This is an investigator-initiated project to identify human T-cell receptor epitopes that putatively could be immune system targets. FNL was tasked with helping

coordinate the necessary activities that involve multiple principal investigators: Dr. Hinrichs (CCR), Dr. Clint Allen (National Institute on Deafness and Other Communication Disorders), Dr. John Sunwoo (Stanford University), Dr. Joshua Elias (Stanford University), and Dr. Ellis Reinherz (Dana-Farber Cancer Institute). Target human tumor cells are immunoprecipitated and then analyzed via mass spectrometry to identify putative T-cell receptor epitopes. The aim is to more fully document/characterize the naturally processed and presented (via HLA-associated complex) epitopes of high-therapeutic-potential and public (not unique to one tumor in a patient) antigens from tumor cells. Most epitopes of interest for targeting are unknown and cannot be deduced *in silico* with bioinformatic algorithms. Discovery of the most relevant epitopes will enable more focused efforts on developing effective T-cell receptor therapy.

This project aims to identify tumor antigen epitopes that tumor cells naturally process and high-allelic-frequency HLA molecules present to T cells. The method of discovery requires immunoprecipitation of HLA molecules (complexed with peptides) from the surface of tumor-based cell lines (prioritized and provided by NCI). Following immunization, samples are exposed to mass spectrometry, and the output spectra are analyzed to identify the peptides bound to the HLA molecules. Experiments are being performed in an iterative manner, with the target antigens and HLA allele frequency determining the priority. The number of antigens and alleles under study are dependent on the success rate for each discovery experiment. Also notable is that two different types of mass spectrometry are being used: data-dependent acquisition and data-independent acquisition. Input cell-line-derived samples are being provided by NCI, but Dr. Sunwoo will also generate mouse xenografts from tissue sourced and appropriately consented as part of his clinical practice. This is a multi-year project.

Support Provided by the Business Services Directorate

Center for Cancer Research

The Business Services Directorate (BSD) provides purchasing, logistics, operational, and administrative support across many division and centers across the Federally Funded Research and Development Center Task Order. At this time, the BSD Purchasing & Logistical Support Team is primarily focused on supporting the research needs of the Center for Cancer Research. These orders were for laboratory supplies, biologicals/chemicals/reagents, sequencing and oligos, radioisotopes, and capital equipment. The Purchasing & Logistical Support Team also facilitates several seminar series, as well as scientific travel for Frederick National Laboratory employees who make up the Basic Science Program (BSP).

KEY ACCOMPLISHMENTS

- 6,633 blanket orders were placed (19,304 lines), totaling $3,670,333.
- 5,123 credit card orders were placed (10,946 lines), totaling $3,570,579.
- 1,160 purchase requests (3,534 lines) were submitted to Purchasing or Research Subcontracts for action, totaling $27,158,695.
- 26 travel packages for Frederick National Laboratory employees were processed.
- 24 seminar speakers' travel was facilitated, and expenses and honoraria were reimbursed.

Support Provided by the Cancer Research Technology Program

Center for Cancer Research Dedicated Cores

Protein Expression Laboratory (PEL)

The six full-time equivalent (FTE) employees in the Center for Cancer Research dedicated Protein Expression Laboratory (CCR-PEL) carry out cloning, protein expression, and protein purification in support of CCR activities. In FY2020, the group continued to focus on protein production projects supporting structural biology and drug discovery projects. They also provided urgent support for COVID-19–related projects.

- The Cloning and Nucleic Acids Group, led by Carissa Grose, constructed 107 entry and expression clones for 29 investigators in FY2020. Most of these clones were developed for use in protein expression within PEL, but a small number were follow-on clones for projects previously carried out in the laboratory. All cloning and subcloning was done using Gateway recombinational cloning on our in-house-developed combinatorial cloning platform.
- The Protein Production Group (PPG), led by Jane Jones, provides protein purification and production of cells and cell-derived products via expression in bacterial, insect, and mammalian systems. Primary expression activities include production of cytoplasmic and secreted recombinant proteins from *E. coli*, baculovirus-infected insect cells, and transfected mammalian cells. Following expression, PPG carries out high-throughput, micro-scale purification methodologies to screen samples for positive lead constructs and/or expression conditions and then proceeds to scale up purification using affinity, ion exchange, and size-exclusion chromatography to purify native and recombinant proteins. During the time period covered in this report, PPG worked on 47 projects for 33 investigators. PPG carried out more than 50 protein purification scouting projects and performed scale-up purification for a total of 34 projects.

- CCR-PEL supported COVID-19 projects by rapidly generating a series of essential proteins during the period in which work was restricted to mission-critical operations. In this time, we produced eight SARS-CoV-2 proteins for Dr. Nadya Tarasova to support *in silico* drug discovery validation efforts, over 20 mg of SARS-CoV-2 receptor binding domain protein for Dr. Liang Cao for serology assay development, and SARS-CoV-2 nucleocapsid and spike proteins for Dr. Barbara Felber for vaccine research. In addition, staff consulted with various CCR investigators on matters related to SARS-CoV-2 spike protein production for serology assays.

Protein Characterization Laboratory (PCL)

The laboratory continued to support CCR on diverse projects involving global and targeted proteomics and metabolite analysis, macromolecular interactions (protein–protein, peptide–protein, and protein–DNA), and analysis of protein posttranslational modifications. Many of the proteomics projects involving both global protein and posttranslational modification analysis incorporate tandem mass tag labeling for higher-precision quantification. We worked on multiple projects developing targeted assay for a variety of metabolites, followed by quantitative measurement of the metabolite of interest. The Protein Characterization Laboratory (PCL) established a process to conduct a global untargeted metabolite analysis, and we also started developing procedures to carry out both targeted and untargeted metabolite isotopic tracing analysis. In addition, we provided binding affinity and kinetic-constant estimation for interactions between two molecules using surface plasmon resonance.

- One substantial effort focused on MHC I peptide discovery in collaboration with Dr. James Yang and Dr. Christian Hinrichs. Our work with Dr. Yang has resulted in the identification of multiple potential neoantigens that have now been validated. We continued to develop HLA peptide identification methods using our continuing Laboratory Directed Exploratory Research program funding and in collaboration with CCR investigators.
- PCL worked on several large global and phosphorylation proteomics projects using a tandem mass tag multiplexing experimental configuration for several CCR investigator (Dr. Johnson, Dr. Shern, Dr. Doroshow, and Dr. Lal). We worked in collaboration with Dr. Lisa Jenkins on global phosphorylation analysis for Dr. Merlino.
- PCL started focusing on developing methods and procedures for in-depth proteomics analysis of very low abundant samples and establishing methods for affinity purification of common posttranslational modification other than phosphorylation, which is very well established in the laboratory.
- We worked on several highly challenging posttranslational modification analyses, such as GalNAc modification on Tyr with Dr. Gildersleeve and cystine modification with Dr. McVicar.
- We carried out two global untargeted metabolite analyses for Dr. Winkler and Dr. Roberts.
- Our novel affinity capturing/liquid chromatography–mass spectrometry analytical assay for quantifying highly charged Host Defense Peptides in complex biological matrices was published in collaboration with Dr. Udo Rudloff (O'Neill et al., *J Pharm Biomed Anal*, 2020).

Optical Microscopy and Analysis Laboratory (OMAL)

OMAL collaborates with CCR labs in the area of quantitative microscopy for spatial–temporal understanding of carcinogenesis at the molecular and tissue level. Technical developments achieved by OMAL make it an integrated resource for for analysis of biological samples across multiple scales, from atoms to animals.

OMAL provides substantial optical microscopy, atomic force microscopy (Figure 1), sample preparation, and image analysis support to over 40 CCR labs (Zakrevsky et al., Nanoscale, 2020; Gadomski et al., Cell Rep, 2020; Fernandez et al., J Virol, 2019; Leng et al., Nucleic Acids Res, 2020). Some research highlights from the reporting period follow.

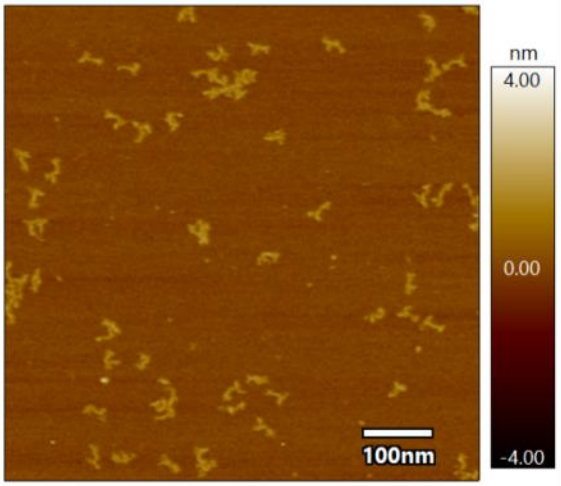


Figure 1. 356 nt fragment of the 5'UTR region of HIV-1 RNA imaged in buffer by atomic force microscopy. In support of Dr. Connie Rink and Dr. Alan Rein, CCR.

With Dr. David Wink (CCR), we are investigating the immune-suppressive role of inflammation and hypoxia in cancer using the 4T1 mouse model of human triple negative breast cancer (Somasundaram et al., Redox Biol, 2020; Philippou et al., Br J Cancer, 2020). The approach is two-fold: 1) a live-cell hypoxia-gradient chamber to model the *in vitro* tumor micro environment (Figure 2),

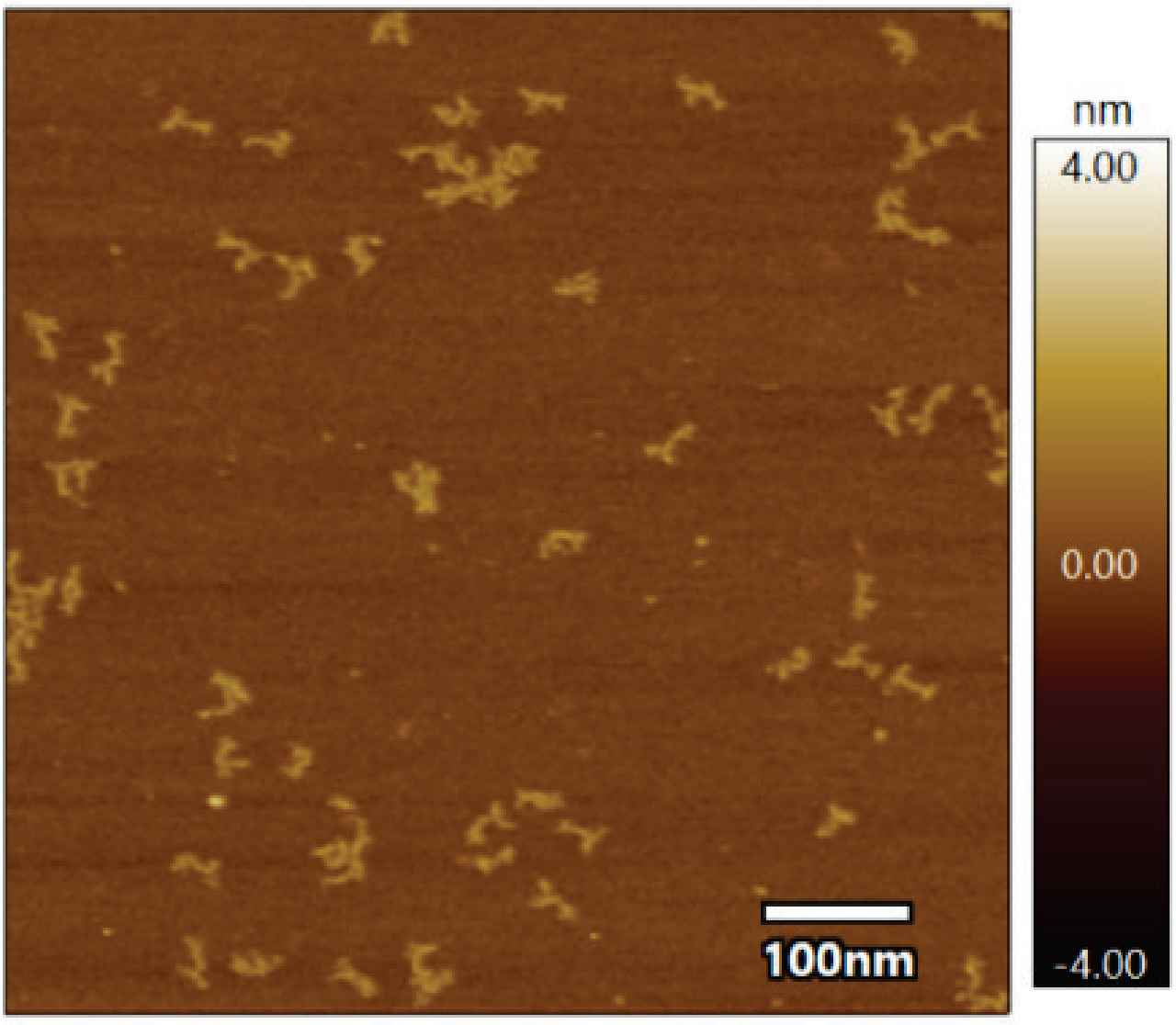
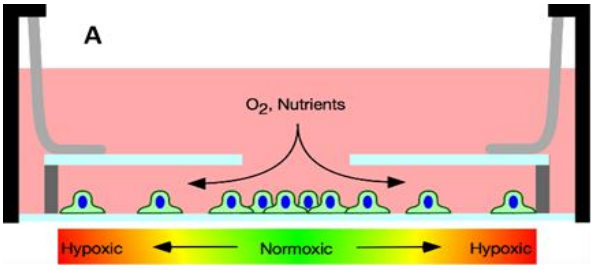


Figure 1. 356 nt fragment of the 5'UTR region of HIV-1 RNA imaged in buffer by atomic force microscopy. In support of Dr. Connie Rink and Dr. Alan Rein, CCR.

and 2) multiplex immunofluorescence (MIF) done in house and in collaboration with Dr. Noemi Kedei and Dr. Jinqiu Chen (Collaborative Protein Technology Resource) to spatially phenotype the cells comprising actual tumors (Figure 3). The chamber was shown to recapitulate key features of tumorigenesis (epithelial to mesenchymal transition, metabolism, expression of inflammation-associated enzymes Nos2 and Cox2), including alterations in these features when 4T1 cells were co-cultured with macrophages (manuscript submitted). A key result using MIF was conversion of immunologically cold 4T1 tumors in mice to hot tumors using the anti-inflammatory indomethacin (unpublished). While MIF analyzes scores of molecular markers in each tissue sample, quantitative accuracy is limited because only an arbitrary fraction of each cell is present in the thin (5 μm) section. Therefore, in collaboration with the Division of Cancer Treatment and Diagnosis and through collaboration and a contract with Oxford University, UK, we are developing methods to analyze thick sections to preserve whole cells in their 3D context. Physical expansion of thick tissue leads to an effective four-fold increase in spatial resolution (Figure 4) (Sahabandu et al., *J Microsc*, 2019). Super-resolution fluorescence microscopy is a rapidly evolving field, and OMAL utilizes this technique to support CCR (Figure 5).



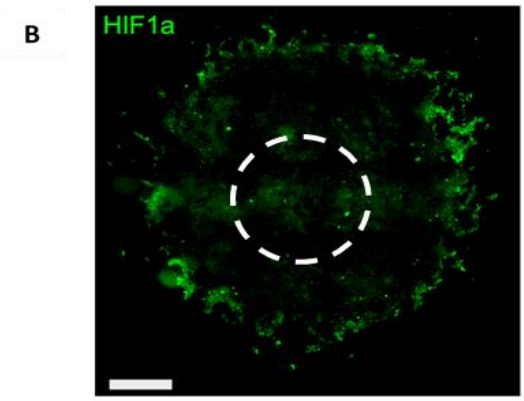


Figure 2. A, Schematic of the hypoxia-gradient chamber. Cells cultured in the chamber generate hypoxic gradients by consuming oxygen diffusing through the opening in the chamber top. B, 4T1 cells cultured in the chamber for several days form disks. The image shows the disk immunofluorescence labeled against hypoxia inducible factor 1-alpha (HIF1α), a marker of intercellular hypoxia. Cells are present inside the HIF1α- positive ring but are unlabeled. The dashed circle represents the hole in the chamber top. Scale bars = 600 μm.

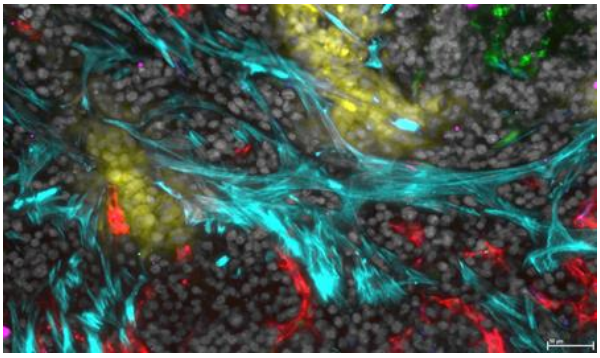


Figure 3. MIF used to study hypoxia and inflammation in mouse tumor tissue. Scale bar 50 μm.

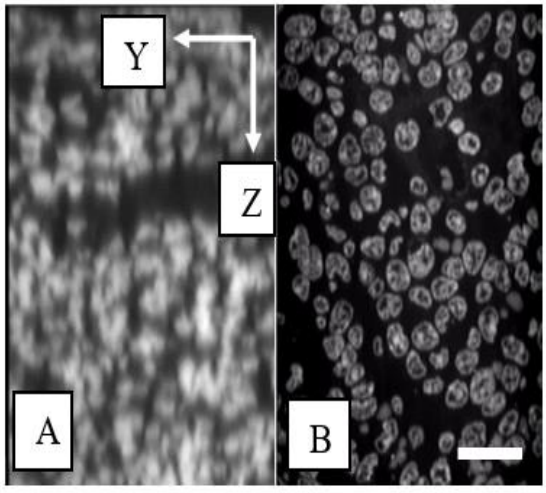
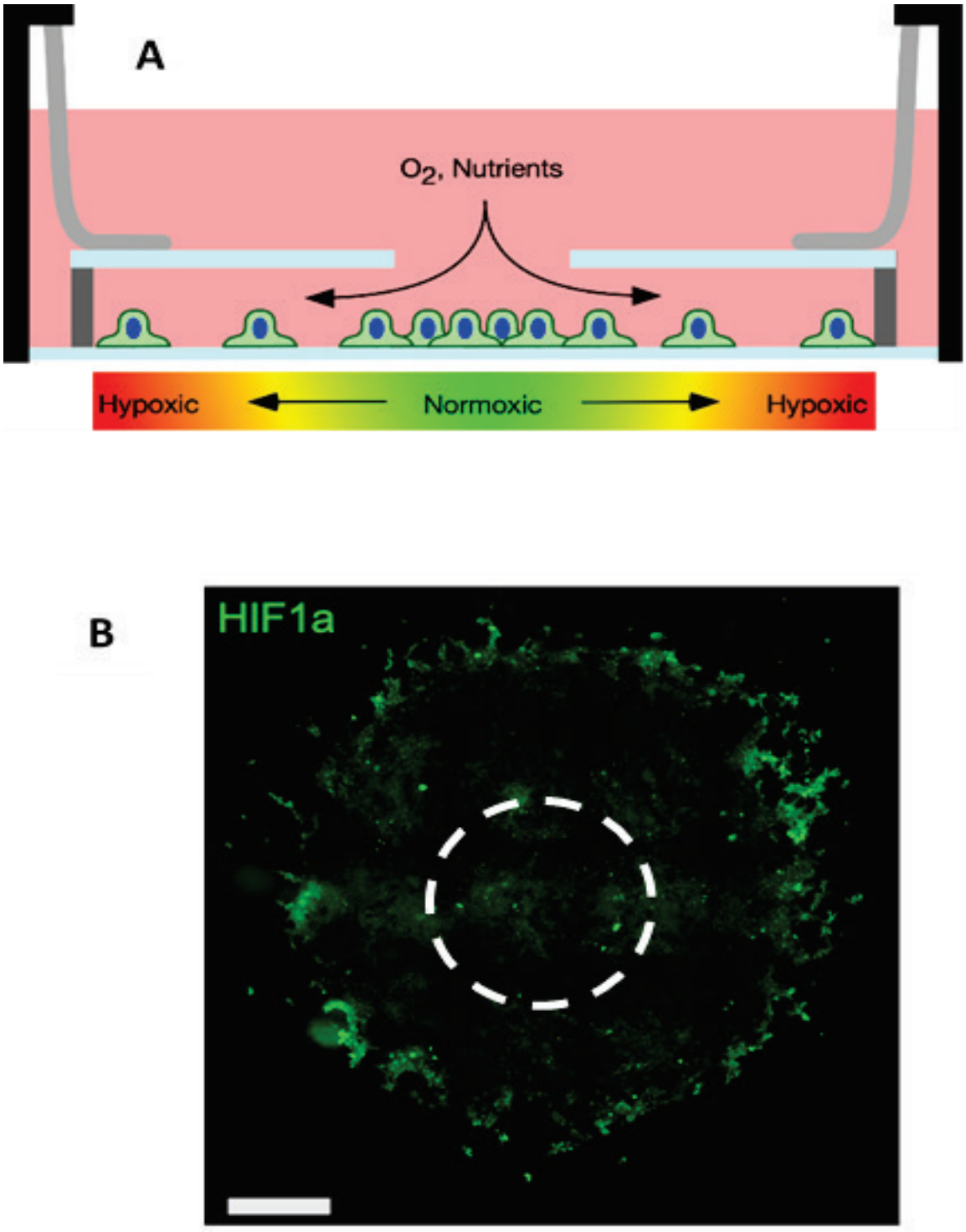


Figure 4. Tissue expansion to increase the effective spatial resolution. A Confocal microscope depth slice through 4T1 mouse tumor tissue showing Yopro-1 labeled nuclei. Scale bar 25 μm. B Equivalent slice after expansion. Scale bar 100 μm.



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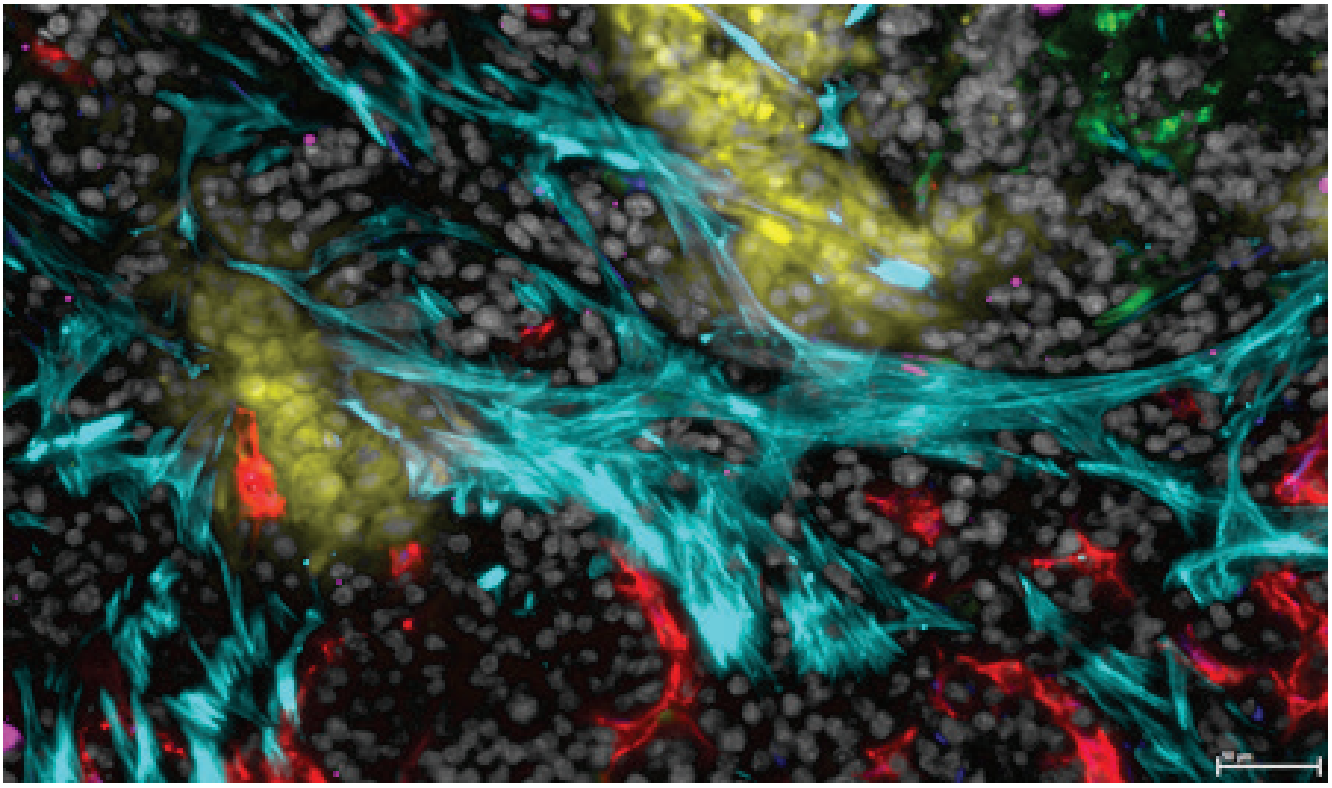

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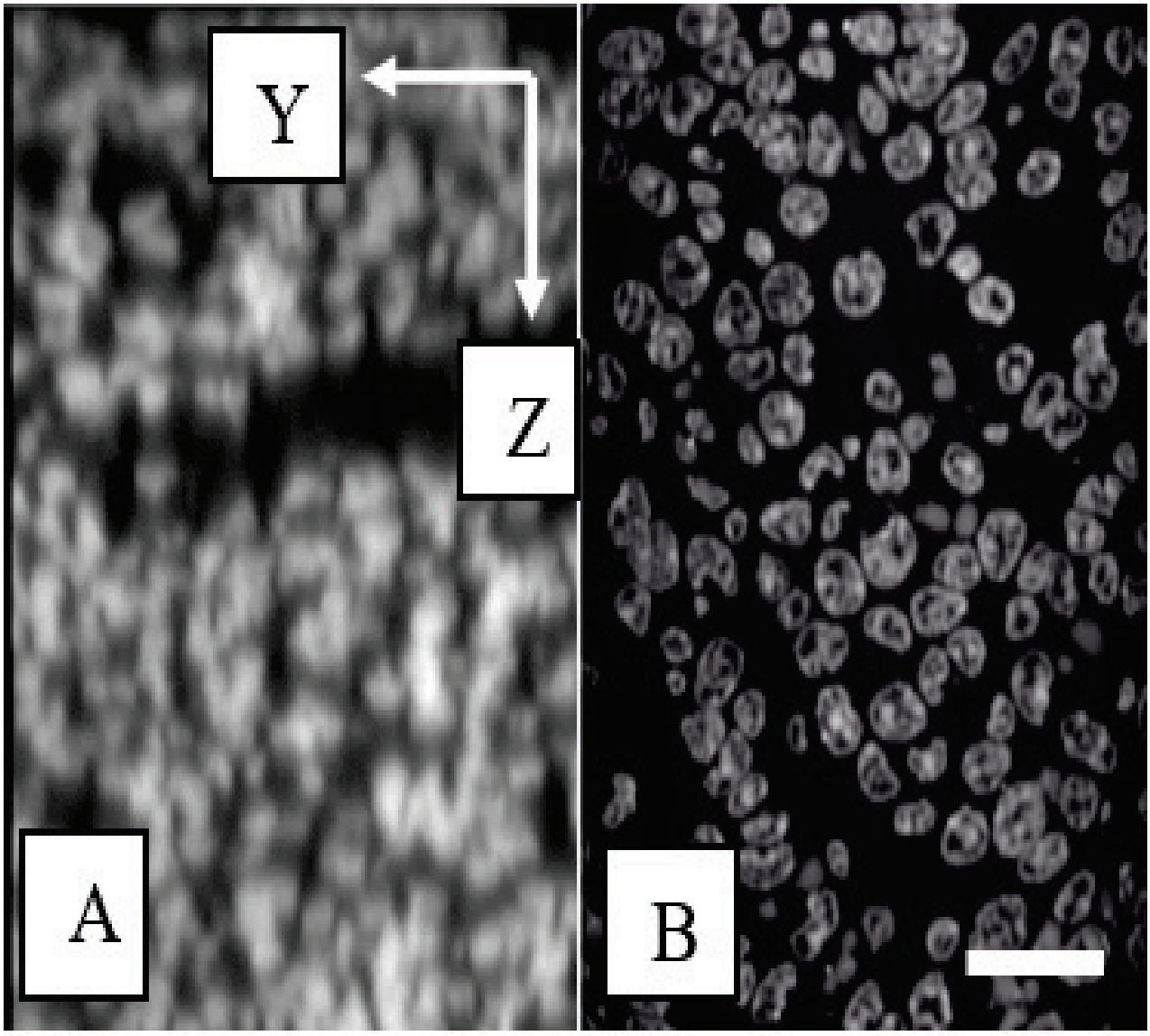

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B: Equivalent slice after expansion. Scale bar: 100 µm.

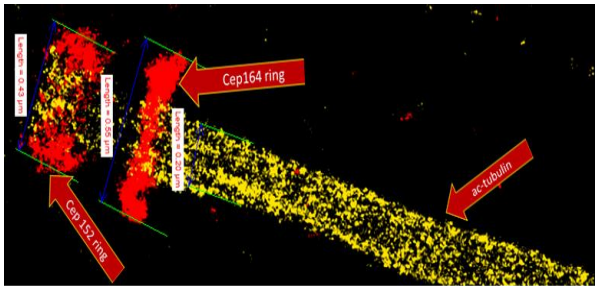

Figure 5. Multicolor DNA PAINT imaging of cilia and centrosomal proteins performed on N-STORM microscope with reagents from Massive Photonics Inc. In support of Dr. Jadranka Loncarek, CCR.

Single Cell Analysis Facility (SCAF)

The Single Cell Analysis Facility (SCAF) provides integrated support for CCR investigators to perform analysis at single-cell resolution using cutting-edge sequencing-based technologies and informatic tools. SCAF's goal is to make the broad range of existing and emerging methods in the field of single-cell biology accessible to basic and clinical research projects. This support is accomplished with six full-time employees and is based at the NIH main campus for timely processing of samples.

KEY ACCOMPLISHMENTS

In the past year, SCAF supported over 95 projects from over 30 CCR investigators, which included more than 1,200 droplet-based and more than 1,200 plate-based single cell libraries, with approximately 4 million cells analyzed. The size and complexity of projects have increased over this past year, with a considerable increase in the number of patient-associated research studies supported.

- In addition to the high-throughput characterization of single-cell gene expression profiles, T-cell receptor and B-cell receptor sequences, and chromatin accessibility at single-cell resolution, we have supported the transition to new version of these chemistries, supported increased use of combined measurement of gene expression along with cell surface proteins, and continued to improve on sample multiplexing techniques to improve experimental design and decrease cost.
- Completed evaluation of a single-cell clonal culturing system for studying cell phenotype heterogeneity and for automating aspects of TCR discovery workflows with Drs. Steve Cappell, Christian Hinrichs, and Steve Rosenberg's laboratories.
- Enabled expanded plate-based single-cell sequencing technologies with the offering of full-length transcriptomic profiling and the improved implementation with the evaluation and purchase of a flow sorter that is co-managed between SCAF and the Building 37 Flow Cytometry Core.
- Worked with the Bioinformatics and Training and Education Program and other NIH entities such as the NIH Library to provide educational workshops on experimental design and analysis.

Genomics Core Support in Bethesda

One Scientist II full-time employee is provided to support the Genomics Core located in Building 37 on the NIH Bethesda main campus. This individual provides expert molecular biology and advanced high-throughput sequencing support. In addition, this staff member works closely with the CCR Single Cell Analysis Facility team to provide additional support when extra capacity allows.

Sequencing Facility (SF)

The primary mission of the Sequencing Facility (SF) is to utilize high-throughput sequencing technologies to enrich cancer research and ensure that the NCI community can remain at the leading edge of next-generation sequencing technology. SF provides CCR, NCI, and NIAID investigators with access to two MiSeq sequencers, three NextSeq 500 sequencers, two state-of-the-art NovaSeq sequencers, one Pacific Biosciences Sequel II, Bionano Optical Mapping and 10X Single Cell Technology.

KEY ACCOMPLISHMENTS

As of July 2020, The Illumina production team has processed 6,027 samples for 108 different NIH investigators. They expanded the automation capability for both the quality control and library prep protocols this past year and collaborated with the informatics group to develop a pipeline for contamination check for the samples before they are set up for production sequencing runs. In addition to co-authoring nine manuscripts published this year, during the three months shutdown, the group worked on three different COVID-19 projects: epigenetic profiling of COVID-19 patients, identifying combinations of small molecules for targeting COVID-19 replication, and working with both NCI and NIAID investigators on a VirScan method to determine the history of viral exposure, including for COVID-19 patients. The Pacbio Production laboratory has prepared 81 library and 18 sequencing runs on Sequel I along with 10 sequencing runs on Sequel II for 12 different principal investigators.

The SF Bioinformatics Team delivered more than 133 trillion base pairs of pass quality control data to over 190 investigator laboratories from various branches across CCR/NCI and NIAID institutes. In addition, the bioinformatics team has developed new data analysis pipelines for Assay for Transposase-Accessible Chromatim (ATAC)-seq analysis, single-cell multiomics analysis, and *de novo* assembly from both short-read and long-read technologies. In collaboration with NCI investigator laboratories, the SF laboratory and

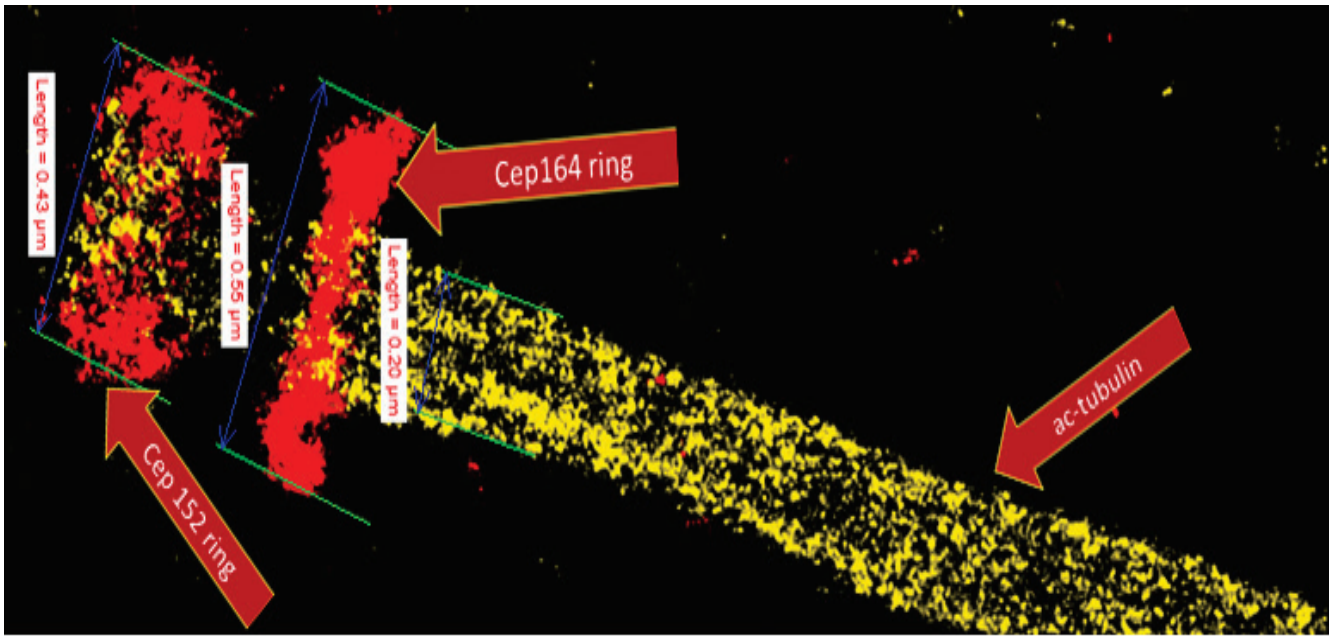

Figure 5. Multicolor DNA PAINT imaging of cilia and centrosomal proteins performed on N-STORM microscope with reagents from Massive Photonics Inc. In support of Dr. Jadranka Loncarek, CCR.

bioinformatics staff members have co-authored five published manuscripts during the review period.

Center for Molecular Microscopy (CMM)/Cryo-Electron Microscopy Group (CRYO-EM)

The Cryo-Electron Microscopy Group at the Center for Molecular Microscopy focuses on 3D electron microscopy of cellular and macromolecular assemblies. Our goal is to use emerging technologies in cryo-electron microscopy for biological and clinical research relevant to NIH's mission. With 58 active collaborations across 43 groups, primarily from CCR/NCI/NIH and the Ras Initiative, we perform high-resolution structural studies of proteins, nanomaterials, and pathogens using single-particle cryo-electron microscopy (Cryo-EM) and cryo-electron tomography (Cryo-ET). Our contributions this fiscal year resulted in several publications. (Botos et al., *Commun Biol*, 2019; Chen et al., *Stem Cell Reports*, 2019; Gao et al., *Science*, 2019; Yang et al., *Virol Sin*, 2019; Zakrevsky et al. *Nanoscale*, 2020).

The primary mission supporting this goal is maintaining a high-throughput and high-resolution cryo-EM data collection infrastructure accessible to our collaborators. The heart of this setup is our Titan Krios cryo-electron microscope, which is equipped with direct electron detectors. Our automation software, SerialEM, can generate over 3,000 images per day, while CryoSPARC Live monitors data quality and generates high-resolution models in real time.

We also assisted our collaborators with data processing, generating 15 structures from 2Å to 4Å resolution during this fiscal year. Traditionally, cryo-EM data processing is computationally expensive, requiring several days to generate a high-resolution structure. Our GPU-based CryoSPARC computer clusters generated three structures at 3Å to 4Å resolution each within hours. This exceptionally quick turnaround time enables prompt feedback to our collaborators.

To address challenging samples, we improved our robust cryo-EM sample optimization workflow. Collaborating with a CCR research lab, we optimized membrane protein samples, ultimately producing five structures at 2Å to 4Å resolution. We are expanding our setup to include specialized specimen supports such as graphene, which is superior to commercial products.

Our group has been on the front lines in the fight against COVID-19. In collaboration with six different research labs, we performed structural studies on 828 COVID-19 related samples at the early height of the pandemic, while a stay-at-home order was still in effect throughout Maryland. Our effort continues, with 90 additional samples at the end of July 2020.

As part of our dissemination efforts, we provided cryo-EM training to 17 researchers, students, and interns from CCR/NCI/NIH and Frederick National Laboratory during this fiscal year.

Center for Molecular Microscopy (CMM)– Cellular Imaging

The Cellular Imaging group at the Center for Molecular Microscopy (CMM) is a highly collaborative laboratory with cutting-edge tools for nanoscale-resolution 3-D volume electron microscopy (vEM) of cell and tissue samples. Development of focused ion beam scanning electron microscopy (FIB-SEM) and array tomography (AT), two central technologies in this nascent field, is a major focus. We have made innovations in sample preparation, image acquisition and processing, correlative methods, computational analysis, and visualization. We collaborate with intramural scientists to apply these advances to biological problems, aiming to garner high-impact co-authored publications. Our group has around 12 active and deep collaborations with CCR/NCI and other investigators.

KEY ACCOMPLISHMENTS

Collaborations

- In a *J Cell Biol* paper with Orna Cohen-Fix, newly developed high-pressure-freezing and FIB-SEM approaches captured, for the first time, fleeting intermediates during nuclear envelope breakdown in the fertilized C. elegans embryo.
- A methods paper detailing the accompanying technological advances is under review at *Methods Cell Biol*.
- As part of a consortium led by Dan Chertow (National Institute of Allergy and Infectious Diseases) and including Dr. Stephen Hewitt (NCI), we are developing methods to image SARS-CoV-2 pathology by vEM in autopsy-derived tissue samples.
- Dr. Kedar Narayan is on an international team to develop metadata recommendations for vEM and correlative images in biology. Submitted to *Nat Methods*.
- Dr. Kedar Narayan is organizing a one-day "microlab" with scientists in the field to form a vEM community, with NCI/FNL as the hub of expertise.

Technology

- A Gemini II SEM (Zeiss) and ATUMtome (RMC), were acquired to develop AT for large volume vEM imaging. A research associate was hired to operate the new tools.
- A freeze-substitution device and robotic dispensor arm were acquired to automate vEM sample preparation.
- Three short methods reports were accepted at *Microsc Microanal*:
    - A new cryo-fluorescence microscopy workflow for large samples (partly funded via a Cooperative Research and Development Agreement with Carl Zeiss GmBH).

- A neural network–based segmentation approach for 3-D reconstructions of mitochondrial architectures.
- A new module in a virtual reality (VR) environment allowing users to interact with and edit 3-D "skeletons" of mitochondria (beta test agreement collaboration with arivis AG).

Electron Microscopy Laboratory (EML)

During this reporting period, the CCR Electron Microscopy Laboratory (EML) received a total of 476 samples, which is sybstantially lower than previous reports. This is mainly due to the COVID-19 stay-at-home order in Maryland from March to July 2020.

CCR-EML provides a wide range of electron microscopy (EM) services including but not limited to legacy thin-sectioned transmission electron microscpe virus diagnosis and ultra-structural analysis, mainly for CCR users (E. Freed, A. Rein, W-S. Hu, V. Pathak, J. Acharya); pre- and post-embedding immunogold labeling (IEM) services (U. Rudloff, J. Schneider, X-F. Liu, C. Liang, C Westlake); negative stain of proteins and nanoparticles (J. Jones, Y. Hu, K. Ramamurthi, S. Chopra, R. Labid); and correlated light and electron microscopy analysis (K. Lee).

CCR-EML also provides EM analysis for users in NIAID (D. Kuhns, T. Imamichi), NCATS (T. Deng, V. Jovanovic), the National Eye Institute (A. Swaroop), and the National Institute of Neurological Disorders and Stroke (M. Holmgren). Also, EML supports the FNL Nanotechnology Characterization Laboratory (J. Clogson) and Biopharmaceutical Development Program (H. Dong).

In support of COVID-19 projects, CCR-EML received several SARS-CoV-2–infected Vero cell pellet samples for thin-sectioned EM and found both budding and mature coronavirus virions with a diameter between 70 and 90nm (presumably SARS-CoV-2). They are mostly found in cell vacuoles, with fewer on cell surfaces. This study is for the FIB-SEM group (K. Narayan) collaboration.

Another CCR-EML activity is to support the NIAID Neutrophil Monitoring Laboratory (D. Kuhns). EML is currently analyzing a COVID-19 patient's neutrophil samples by both thin-section EM and Scanning Electron Microscopy (SEM).

CCR-Genomics Laboratory (GTL)

The CCR Genomics Technology Laboratory (GTL) is funded by a Yellow Task with 6.25 full-time-equivalent employees to provide dedicated genomics technology core services to NCI CCR laboratories. GTL provides a broad range of genomics services based on next-generation sequencing (NGS) and other cutting-edge genomics technology platforms. NGS-based services include single-cell sequencing, whole-exome sequencing (in conjunction with the CCR Sequencing Facility), targeted gene panel sequencing, CRISPR-Cas9 high-throughput screening and validation, retroviral integration site analysis, and ImmunoSeq T-cell clonality analysis. Other genomics technology services include gene expression microarray, Illumina methylation array, drug metabolizing enzymes and transporters array, OncoScan array, quantitative polymerase chain reaction, droplet digital polymerase chain reaction (ddPCR), NanoString, and HTG EdgeSeq.

KEY ACCOMPLISHMENTS

- During the past year, GTL received 53 projects through the NCI at Frederick Accessioning System, including whole-exome sequencing, targeted exome sequencing, DNA methylation, ddPCR, ImmunoSeq, CRISPR-Cas9 screening, and nanostring. We processed thousands of samples for CCR investigators.
- In addition to the standard core services, GTL worked on many technology development projects involving collaborations with NCI investigators. GTL worked with Dr. Xin Wei Wang on the development of a viral exposure signature detection system called VirScan on liver cancer patients. This study was recently published (Liu et al., *Cell*, 2020).
- GTL has worked with Dr. Thomas Ried's laboratory to develop protocols using dissociated cells from formalin-fixed, paraffin-embedded (FFPE) tumor samples for whole-exome sequencing.
- GTL has witnessed many projects got delayed or canceled with the challenging FFPE samples. GTL teamed with Purigen to develop a new FFPE DNA extraction method for exome sequencing. GTL worked with Dr. Brid Ryan of NCI on a pilot project. All previously failed FFPE samples were successfully extracted and sequenced by whole-exome sequencing. This technology will greatly benefit other NCI laboratories.
- GTL has supported many CCR clinical laboratories, particularly for NCI CAR T-cell therapies. These include clinical trial support to Dr. Nirali Shah of Pediatric Oncology Branch, Dr. Javed Khan of the Genetics Branch, and Dr. Mitchell Ho of the Laboratory of Molecular Biology. We designed specific ddPCR assays or integration site assays for CAR T-cell copy number analysis and CAR T-cell integration.

Support Provided by the Clinical Monitoring Research Program Directorate and Clinical Research Directorate

Clinical Research Support

KEY ACCOMPLISHMENTS

- Contributed to approximately 50 publications related to research conducted in the Center for Cancer Research's (CCR) Molecular Imaging Program

- Completed quality control and third-party audits of the Vector Production Facility and the Cell Production Facility
- Supported investigators with writing six COVID-19 protocols and submitting them to the Institutional Review Board (IRB)
- Expanded access to care for study participants in Brain Tumor Trials Collaborative and NCI-Comprehensive Oncology Network Evaluating Rare CNS Tumors trials during the COVID-19 pandemic through partnerships with local physicians to provide study drug and offer telemedicine appointments
- Initiated development of humanized antibody with optimized epitope binding

Clinical Operations

FNL teams in the Clinical Monitoring Research Program Directorate (CMRPD) and the Clinical Research Directorate (CRD) provided clinical, administrative, and regulatory support to various groups within CCR. FNL supports CCR's mission to improve the lives of cancer patients by solving cancer research problems and translating them to clinical applications and patient care.

FNL clinical professionals (e.g., physicians, nurse practitioners) provide direct patient care to clinical trial participants; scientists and technologists conduct preclinical experiments, analyze data, operate positron emission tomography/computed tomography (PET/CT) and magnetic resonance imaging (MRI) scanners, and support manuscript development; clinical nurse administrators, patient care coordinators, and patient liaisons support activities such as managing the transplant program, coordinating donor searches, enrolling research participants, scheduling treatment visits for active clinical trial participants, and coordinating participants' complex care needs. These FNL staff members streamline operations, increase patient accrual, support high-quality patient care, and assist with dissemination of research results.

An FNL staff member in CRD helped lead the National Institutes of Health (NIH) Hematopoietic Stem Cell Therapy Program, overseeing a multi-institute program and providing operational and clinical leadership in the selection and procurement of unrelated donor stem cell therapy products. Activities included coordinating the NIH Matched Unrelated Donor (MUD) Hematopoietic Stem Cell Transplant Program, a cross-institute program that facilitates searches for and procurement of volunteer unrelated donor and cord blood grafts for all applicable intramural protocols. As part of selecting and procuring the unrelated donor stem cell therapy products, the FNL staff member reviewed approximately 150 searches for 30 transplants, liaised with the donor registry for donor research activities, managed financial activities for program contracts and subcontracts, and facilitated accreditation. In addition, FNL supported intramural and extramural collaborations for the CCR Experimental Transplantation and Immunotherapy Branch, Pediatric Oncology Branch, Surgery Branch, and Immune Deficiency Cellular Therapy Program by providing donor search and selection services through MUD, coordinating education and support, and managing federally mandated data reporting. Approximately 15 active intramural cellular therapy protocols used the MUD program, as did two protocols for facilitating unlicensed cord blood transplants and four other protocols for data and repository sample submission to the Center for International Blood and Marrow Transplant Research (CIBMTR). To support activities with CIBMTR, which collaborates with the global scientific community to advance hematopoietic cell transplantation and cellular therapy, specifically CIMBTR's audit process improvement team, FNL created audit guidebooks, database fields, and quality assurance tools to ensure team members, CIMBTR monitors, and data managers understood the relevance and processes for recording accurate and complete data. As a result, the team received a Clinical Center CEO award for quality and patient care.

Two publications (Goklemez S, et al., *Am J Hematol*, 2020; Dimitrova D, et al., *Biol Blood Marrow Transplant*, 2020), released this year, discuss results from recent transplantation clinical trials supported through these efforts. FNL coordinated the referral and care of patients on the studies, researched and selected related and unrelated donor matches in accordance with protocol criteria, and assisted in manuscript development.

For the CCR Molecular Imaging Program (MIP), FNL provided a range of project management services and staff positions to support preclinical and clinical research operations and the program's mission to discover and develop targeted imaging methods that accelerate the translation of clinical research to first-in-human testing. FNL scientists were instrumental in providing input on therapeutic drugs and in developing the new MIP artificial intelligence program, AI Resource, by researching new imaging and diagnostic techniques. Advances using artificial intelligence on prostate MRI scans may overcome some of the current limitations; FNL contributed to two recent publications (Sanford T, et al., *J Magn Reson Imaging*, 2020; Harmon S, et al., *JCO Clin Cancer Inform*, 2020) on this topic. The cover of the February issue of *Health Phys* featured a manuscript about the contamination effects of 223Ra (Adler S, Kwamena BK, et al.). In addition, the FNL scientists are currently pioneering new instrument technology to push the envelope of sensitivity and accuracy in radiation detection measurement.

The FNL clinical research nurse and technologists supported MIP clinical trials by managing patient care, scheduling imaging scans, and operating the PET/CT and MRI scanners. A laboratory technician managed day-to-day operation of the preclinical laboratory. This employee also maintained cell cultures and other *in vitro* elements needed to test new molecular probes for MIP trials studying new PET radioligands, single-photon emission computed tomography, and other radionuclide animal imaging methodologies, primarily for the study and treatment of prostate cancer. FNL provided pathology

results, data analysis, and publication support to several recent manuscripts, including advances in prostate cancer imaging research (Harmon SA, et al., *Quant Imaging Med Surg*, 2020; Mena E, et al., *J Nucl Med*, 2020; Kobayashi H, et al., *Bioconjug Chem*, 2020); the basic mechanism of the effects of photoimmunotherapy on regulatory T cells in carefully crafted animal models (Sato N, et al., *Clin Cancer Res*, 2020); and the use of cell labeling and PET imaging for *in vivo* tracking of adoptively transferred natural killer cells in rhesus macaques, along with dosimetry analysis to demonstrate safety for clinical translation (Sato N, et al., *Clin Cancer Res*, 2020).

On the preclinical side, MIP's research progress supported by FNL scientists also included: showing that rapid depletion of intratumoral regulatory T cells induces synchronized CD8 T and natural killer cell activation and IFN-γ-dependent tumor vessel regression; demonstrating, for the first time, the mechanism for glucocorticoid-induced eosinophilia is *CXCR4*-mediated bone marrow homing of eosinophils; establishing a preclinical model for evaluating hepatocellular carcinoma (HCC)-selective imaging and therapeutic agents; testing the first humanized glypican-3 (GPC3) immunoPET sensor; assessing a pilot study's efficacy of alpha-particle-emitting isotopes coupled to an antibody targeting GPC3 in a human liver cancer cell line model of HCC; and evaluating a novel somatostatin-receptor-binding peptide optimized to chelate the alpha-particle-emitting isotope, actinium-225.

Quality Assurance/Quality Control and Regulatory Compliance

FNL provides quality assurance and regulatory compliance activities for the manufacturing of, and clinical research with, investigational drugs, biologics, and devices used in clinical trials. These functions support the manufacture of retroviral vectors and cell therapies under current Good Manufacturing Practice (cGMP) and Good Tissue Practice regulations applicable to Phase I/II clinical trials.

FNL staff members supported the following activities:

- Transition from current MediaLab to new MasterControl electronic documentation system
- Completion of third-party audits of quality control, the Vector Production Facility, and the Cell Production Facility, as well as initiation of corrective and preventative action plans for continual improvement
- Production of first lot of vector in a renovated Vector Production Facility suite
- Quality assurance review of all documents and plans, from beginning of planning through commissioning remediation, of a mobile cleanroom facility that is capable of functioning as a GMP space
- Substantial completion of qualification of the tumor-infiltrating lymphocytes cell processing facility
- Qualification of Cooperative Research and Development Agreement partner, Kite Pharma, as vector supplier

In addition, FNL supported CCR's recently established Office of Sponsor and Regulatory Oversight, which ensures regulatory compliance with drug sponsor obligations for Investigational New Drugs (INDs) and Investigational Device Exemptions, by assisting with writing protocols, preparing safety review committee and IRB submissions, writing CCR meeting summaries, and administering scientific reviews.

Protocol Development Support

FNL medical writers and protocol coordinators in CMRPD support CCR's Protocol Support Office by streamlining protocol development timelines, filing regulatory documents with internal and external authorities, ensuring quality administrative coordination for regulatory activities, providing protocol navigation services, assisting with new proposal/protocol and progress report preparation for scientific review committee (SRC) and IRB meetings, responding to IRB stipulations, reviewing protocol amendments and other related documents, helping to support all ancillary review committees (e.g., safety monitoring, institutional biosafety, radiation safety committees), and maintaining required regulatory documents per local and federal regulations so they are available for internal and external audit.

The FNL staff members also track training requirements for CCR investigators to ensure compliance, work with investigators and study teams to develop and write new studies prior to regulatory submissions, maintain active Investigational Device Exemption and IND applications that require U.S. Food and Drug Administration (FDA) review, and provide ongoing administrative and regulatory coordination support.

During FY2020, FNL staff in CMRPD supported the submission of five new studies using INDs to the FDA, provided protocol writing and navigation services to investigators for IRB submission of six COVID-19 projects, processed over 130 amendment submissions of existing IND studies, coordinated more than 25 scientific review meetings, navigated more than 270 protocols, helped develop nearly 90 new protocols, and coordinated more than 230 monitoring visits.

Clinical Program/Project Management

FNL staff in CMRPD provided technical project management of several research subcontracts in support of CCR. A subcontractor completed its task order studying clinical workflow and operations of the Pediatric Oncology Branch, and the analysis, conclusions, and suggestions were shared with CCR leadership. Two subcontracts provide the Molecular Imaging Program with individuals who have expertise on PET/single-photon emission computed tomography and other radionuclide animal imaging methodologies, imaging optimization, artifact determination, data quantification,

and data analysis. Another subcontract supported the Medical Oncology Service by providing hospitalists to cover increased patient volumes at Suburban Hospital, and FNL staff managed an agreement with the National Marrow Donor Program (NMDP) to allow the NMDP-maintained database of donors to be searched for potential matches, supporting the cross-institute initiative (described earlier in this report) to conduct donor testing, search and procure graft products, and obtain other related services for participants enrolled in NIH protocols that require a matched, unrelated donor product.

Brain Tumor Trials Collaborative

The Neuro-Oncology Branch is a trans-institutional initiative sponsored by both NCI and the National Institute of Neurological Disorders and Stroke. The branch works to develop novel diagnostic and therapeutic agents for patients with primary central nervous system (CNS) tumors. FNL staff in CMRPD support NCI's role as the lead institution for the Brain Tumor Trials Collaborative (BTTC), which is a network of 32 medical institutions with the expertise and desire to participate in state-of-the-art clinical trials investigating new treatments for malignant brain tumors. The FNL team provides comprehensive administrative infrastructure, project operations, and subcontract management support for the BTTC Coordinating Center; coordinates data analysis and database activities; supports the development of research concepts into protocols; assists with IRB submissions and regulatory document filings; and coordinates communications to support a cohesive network infrastructure.

The collaborative, funded in part by the philanthropic organization Head for the Cure Foundation, was created in 2003 to: (i) provide patients access to innovative treatments through the BTTC clinical trials network, (ii) accelerate the pace of translating laboratory findings into hypothesis-based clinical trials, (iii) perform state-of-the-art clinical trials emphasizing innovation and mandating meticulous attention to protocol compliance and data quality, and (iv) evaluate the impact of therapy by integrating measures of cancer response and patient outcomes.

The BTTC network also serves as the infrastructure for NCI-Comprehensive Oncology Network Evaluating Rare CNS Tumors (NCI-CONNECT) clinical studies, which focus on rare adult brain and spine tumors. NCI-CONNECT is the Neuro-Oncology Branch program within the Rare Tumor Patient Engagement Network. NCI-CONNECT aims to partner with patients, advocacy organizations, and BTTC providers to advance understanding about these rare cancers to improve approaches to care and treatment.

During FY2020, FNL staff in CMRPD activated and facilitated patient enrollment at six non-NIH sites in the randomized, double-blind Pembro trial, a Phase II trial of surgery, radiation therapy, and temozolomide and pembrolizumab in glioblastoma patients. This is the first trial to investigate an autologous vaccine in combination with immunotherapy for patients with newly diagnosed glioblastoma.

FNL also continued to support five BTTC legacy trials on ependymomas and recurrent high-grade gliomas, facilitating data sweeps, coordinating site close-outs, and collating feedback from BTTC investigators for the publication of a primary manuscript for one of the trials.

The FNL team facilitated trial planning/logistics discussions for ongoing and proposed BTTC & NCI-CONNECT trials among NCI BTTC & NCI-CONNECT leadership, NCI's Technology Transfer Center, the pharmaceutical partner, and collaborating BTTC investigators. The FNL staff also facilitated three single-center NCI-CONNECT trials, two that are currently enrolling participants at NIH and one that was submitted to the NIH IRB in June 2020. The tissue outcomes and pregnancy substudy launched across the NCI-CONNECT consortium in March 2020, and the NIH IRB approved making the nivolumab rare CNS study multicenter.

During the COVID-19 pandemic, FNL supported NCI's efforts to partner with local physicians to provide study drug and telemedicine appointments, reducing potential virus exposure risks for BTTC and NCI-CONNECT study participants who typically traveled to NIH or another participating site. FNL collaborated with many internal and external stakeholders to prepare time-sensitive single-patient deviation submissions to the NCI CCR Office of Sponsor and Regulatory Oversight and the NIH IRB. Adding telemedicine for certain study visits to BTTC and NCI-CONNECT protocols is expected to continue, to allow study participants to be seen without risking potential exposure to COVID-19 for site visits.

To support a coordinated approach to network communications, FNL staff in CMRPD facilitated BTTC & NCI-CONNECT meetings; helped to expand the virtual, biweekly NIH Neuro-Oncology Tumor Board to include BTTC investigators and to add a journal club component; and coordinated the well-attended BTTC & NCI-CONNECT investigator meeting at the Society for Neuro-Oncology Annual Meeting in November 2019, at which NCI-CONNECT leadership presented information about the tissue outcomes study and the nivolumab rare CNS trial.

Drug Discovery and Development Program

As a part of CCR's Drug Discovery Committee, the FNL Drug Discovery and Development Program initiated the development of photoimmunotherapy, beginning with the humanization of a murine antibody and *in silico* modeling to improve antibody binding to targeted regulatory T cells. Future efforts for this project will include cGMP production of the antibody and conjugation with the therapeutic agent, efficacy and toxicity studies, and other preclinical development milestones leading to an IND application.

Support Provided by the Laboratory Animal Sciences Program

LASP: CCR Support

Genome Modification Core

KEY ACCOMPLISHMENTS

- Four published journal articles featuring the reagents made by the Genome Modification Core
- Initiation of work with the Division of Cancer Epidemiology and Genetics, which included the hiring of a new research associate who onboarded in April 2020
- More than a dozen transgenic mouse models, made in collaboration with the Transgenic Mouse Model core, achieving germline transmission
- Procurement of two automated liquid handling machines to facilitate higher-scale and higher-throughput experiments
- Pilot project of large-scale production and distribution of genome-wide CRISPR library viruses to 22 different CCR investigators

The Genome Modification Core (GMC) has successfully completed its second full year in operation. Compared to the previous year, we observed almost double the requests, including investigators making multiple requests and new requests. A summary of various metrics comparing this year and the previous year are shown in Table 1.

Table 1: Usage Comparison Between CY2019 and CY2020

Metric	CY2019	CY2020*
NCI Accessioning System requests	61	111
Number of unique principal investigators	41	65
Number of edited alleles	181	293
Transgenic mice	27	24
sgRNAs tested	742	877
Cas9/sgRNA constructs made	189	389
Donor constructs made	116	87
Custom sgRNA libraries	34	29
Published articles	0	4

*Up to July 10, 2020

OUTLOOK

Four key areas were outlined last year in regard to development for this year: clonal cell line generation, *in vivo* CRISPR screening, pooled CRISPR library screens, and RNA editing. Substantial progress has been made in all four areas.

In terms of clonal cell line generation, the interest in this service has increased steadily. We have developed a strategy for the rapid generation and screening of hundreds of clonal populations and have piloted this with a few NCI CCR investigators at the Frederick campus. We hope to finalize a robust protocol for this service, which will be published and subsequently deployed for generation of cell lines for other National Cancer Institute (NCI) Center for Cancer Research (CCR) investigators.

With respect to *in vivo* screening, we have initiated a collaborative project with Dr. Nyall London who is attempting to perform an *in vivo* screen to study the development of Esthesioneuroblastoma in a mouse model. We have generated some pilot constructs for Dr. London to test to better understand the feasibility and limitations of this approach. We anticipate completing this screen sometime this year.

For pooled CRISPR screens, we had very robust interest in this, with more than 20 investigators wanting to perform such experiments. To meet demands in a timely fashion and work with limited personnel, we initiated a plan for large-scale virus production of the most commonly used genome-wide CRISPR libraries and provided single-experiment aliquots to investigators. Overall, this approach worked well, and we are awaiting feedback from users to decide if this initiative is worth continuing this year.

Finally, we have received funding through the FNL to recruit a trainee for the fall to develop the area of RNA editing. We are collaborating with Dr. Jordan Meier, who has developed a new method to quantify RNA cytidine acetylation, to understand the impact of RNA acetylation and potentially engineer site-specific RNA acetylation enzymes.

Center for Advanced Preclinical Research

KEY ACCOMPLISHMENTS

- Working through the CCR Scientific Oversight Committee, the Center for Advanced Preclinical Research (CAPR) advanced several ongoing collaborative research projects with CCR investigators and initiated six new projects selected in late 2019 from our latest request for applications. Work has begun in a variety of malignancies, including Kaposi's sarcoma, small-cell lung cancer, pancreatic neuroendocrine tumors, pancreatic adenocarcinoma, and pancreatic acinar cancer.
- CAPR has completed a collaboration with Drs. Jack Shern and Carol Thiele evaluating a bromodomain and extra-terminal domain (BET) inhibitor in combination with a topoisomerase-1 inhibitor. Both patient-derived xenograft (PDX) rhabdomyosarcoma and neuroblastoma genetically engineered mouse-derived (GEM) allograft models were established and characterized, tolerability studies were completed, and single-agent and combination-efficacy studies were carried out. Results indicated an increased

benefit to the combination versus single agents, both in delaying tumor progression and promoting tumor regression. Importantly, BETi addition may allow lower topoisomerase-inhibitor dosing, a goal of clinical treatment. These results were pivotal for initiating a clinical trial that will test this combination therapy in pediatric patients.

- PDX models and cell lines from RAS-driven pediatric rhabdomyosarcoma and neuroblastoma tumors were established for our collaboration with Dr. Marielle Yohe. Pharmacokinetics and efficacy studies to evaluate the combination of a dual BRD4/PI3K inhibitor with histone-deacetylase inhibitors in these difficult-to-treat tumor types are complete.
- CAPR's collaboration with Dr. Glenn Merlino to model adjuvant versus neoadjuvant immune-checkpoint blockade therapies for metastatic melanoma is underway. Multiple melanoma models have been characterized for metastatic spread post-resection.
- In collaboration with Dr. Christine Heske, we evaluated indenoisoquinolines (IIQs) versus irinotecan in periosteal PDX models of Ewing sarcoma, including PK, administration route, tolerability, and maximum tolerated dose for each of the IIQs and completed an efficacy study comparing three IIQs to irinotecan. We have processed 14 PDXs and established six, which we are expanding for additional efficacy studies.
- Preliminary work for our study of epigenetic modulation of PD-L1 and antigen presentation machinery in small cell lung cancer (SCLC) with Dr. Azam Ghafoor is complete. Two GEM SCLC models (RP and RPM) were characterized by histopathology for immune markers and MRI. A combination immune-checkpoint inhibitor/targeted drug study in the RPM model is underway. With Dr. Christina Annunziata we evaluated a combination of second mitochondrial activator of caspases mimetic with taxanes in orthotopic allograft mouse models for ovarian cancer and completed tolerability, PD, and efficacy studies comparing two taxanes and standard of care to treatment with second mitochondrial activator of caspases mimetic, birinapant, and its combination with taxanes.
- CAPR has successfully generated and characterized several PDX models from platinum-sensitive and -resistant SCLC patients as part of a request-for-applications collaboration with Dr. Anish Thomas to identify predictive biomarkers of response and resistance mechanisms to ataxia telangiectasia and Rad3-related protein and topoisomerase-1 inhibition in relapsed SCLC upon progression. Efficacy studies are underway.
- Our continuing collaboration with Dr. Christine Alewine investigating mesothelin's role in pancreatic ductal adenocarcinoma (PDAC) yielded important insights into the role of this molecule in metastasis and produced an allelic series of humanized models expressing human mesothelin (hMSLN) from the endogenous murine locus. These models, documented on NCI Employee Invention Reports, will enable preclinical efficacy-versus-toxicity evaluation of MSLN-targeting immunotoxins.
- Results from our previous collaboration with Dr. George Pavlakis suggested hetIL15 can increase immune-cell infiltration into "cold" PDAC tumors. These observations, combined with our $KRAS^{G12D}$/p53/PDX1-Cre PDAC models expressing hMSLN, led to an ongoing collaboration investigating efficacy of hetIL15 and immunotoxins in this model.
- Promising results from our collaboration with Dr. Udo Rudloff to favorably alter the PDAC tumor microenvironment by inhibiting tumor-promoting macrophages using host-defense peptide RP-182 resulted in a high-level publication and ongoing follow-up study using a RP182-biomimetic small molecule targeting the same populations of infiltrating immune cells.

CAPR develops strategies for predictive preclinical research using genetically and biologically engineered murine cancer models and facilitates their routine application in clinical research to achieve optimal outcomes in cancer disease management.

Internal highlights and investigator-initiated pilot projects:

- Developed intra-adrenal orthotopic models from PDX and GEM neuroblastoma tumors, characterized by *in vivo* imaging.
- Brca1- and Brca2-deficient and Brca-WT models for human breast cancer developed with Dr. Robert Shoemaker from the Division of Cancer Prevention.
- A publication with Dr. Merlino characterizing four GEM models of melanoma that respond differently to checkpoint inhibitors. Models should prove useful for understanding variability in clinical response to these beneficial therapies.
- Generation/characterization of GEM models for ovarian cancer originating in fallopian tubes.
- Characterization of innate immunologic signatures in pancreatic models carrying different disease-driver mutations by single-cell RNA sequencing.
- Development/characterization of two histopathologic subtypes of thyroid cancer models—papillary and anaplastic.
- Following earlier work and in support of an ongoing metarrestin PDAC clinical trial, experiments in genome-edited $KRAS^{G12D}$/p53/PDX1-Cre model expressing humanized eEF1A2—a key molecular target of metarrestin.

Building 567: Animal Facility

KEY ACCOMPLISHMENTS

- An animal procedure room was converted into an animal holding room to increase animal housing capability and provide flexibility to allow much-needed floor repairs in the building to meet Association for Assessment and Accreditation of Laboratory Animal Care standards.
- The Laboratory Animal Sciences Program (LASP) provided technical support for more than 40 animal research studies. LASP maintained greater than 6,000 cages of complex rodent-breeding colonies to support the research efforts, even during the pandemic. There was less than five percent reduction in the cage census during the pandemic.
- LASP continued to provide courier service with shipping greater than 7,000 mice to Bethesda, MD, and driving more than 23,000 miles.
- The autoclaves are getting new control panels to provide consistent and uninterrupted operations, which is very important for maintaining the health status of animal colonies.

Building 567 is a dedicated animal facility to support the research efforts of CCR's Experimental Immunology Branch, Laboratory of Genome Integrity, and Experimental Transplantation and Immunotherapy Branch.

Division of Cancer Biology

Support Provided by the Cancer Research Technology Program

Repository

The Tumor Microenvironment Network (TMEN) was a program launched by NCI in 2006 and reissued in 2010 that funded research in basic cancer biology to study the mechanisms of tumor-host interactions in human cancer. TMEN was designed to provide infrastructure that established repositories of critical reagent resources in order to promote and facilitate progress towards understanding the role of the tumor microenvironment in sustaining human cancers. Although the TMEN research program has officially ended, CRTP continues to store and distribute the resultant TMEN reagents in support of ongoing tumor microenvironment studies at academic and clinical research laboratories.

Support Provided by the Clinical Research Directorate

Human Tumor Atlas Pilot

KEY ACCOMPLISHMENTS

- The Human Tumor Atlas Pilot Project (HTAPP) successfully shared data and resources, such as standard operating procedures (SOPs), with the Human Tumor Atlas Network (HTAN) and with the broader scientific community. HTAPP also published multiple articles in high-profile journals, which include *Nat Med* and *Cell*.
- HTAPP achieved 98 percent of the collection and processing of biospecimens project goal.
- Single-cell/single-nucleus RNA-sequencing (sc/snRNA-seq) methods and the majority of the spatial/imaging-based assay protocols were optimized, and sc/snRNA-seq data generation has been successfully completed for all samples.
- Site visits for SOP knowledge transfer were successfully completed for four clinical sites.

The goal of HTAPP is to develop and contribute pilot-scale tumor atlases from adult (metastatic breast) and pediatric (neuroblastoma) cancers and to develop, validate, and share SOPs detailing tissue procurement and data generation with the HTAN and wider scientific community.

The Frederick National Laboratory for Cancer Research (FNL) project team (in continuous collaborative discussions with the National Cancer Institute and subcontracted stakeholders) made tremendous progress on HTAPP during FY2020. Key accomplishments of HTAPP include multiple high-profile publications: an article describing sc/snRNA-seq methods optimization published in *Nat Med* (Slyper M, et al. *Nat Med*, 26(5):792-802, 2020), the HTAN marker paper published in *Cell* (Rozenblatt-Rosen O, et al. *Cell*, 181(2), 236-249, 2020), and a manuscript describing spatial assay method optimization published on bioRxiv (Alon S, et al. bioRxiv, doi: 10.1101/2020.05.13.094268, 2020). HTAPP also made excellent progress toward the goal of developing and sharing of resources generated under HTAPP with the wider HTAN. HTAPP resources shared with HTAN to date include 31 SOPs, a computational pipeline package, basic clinical data and biospecimen metadata for 79 samples, raw sc/snRNA-seq data for 82 samples, and HTAPP clinical data elements and metadata elements (of these, three SOPs and all pipelines are also publicly accessible). Biospecimen procurement, as well as clinical data and biospecimen metadata collection, has progressed very well. Collection and processing of samples for sc/snRNA-seq have been completed for all seven tumor types (219 samples total), and 38 samples have passed pathology quality control for downstream spatial/imaging-based assays (additional samples are pending quality control as of July 2020). This

marks 98 percent completion of the biospecimen collection for this project. Collection of clinical data and biospecimen metadata was completed for 90 percent of the samples as of July 2020. The experimental method optimization and data generation also progressed toward completion. The team completed optimization of sc/snRNA-seq method and data generation for all seven tumor types. Laboratory method optimization, which includes development and validation of cancer-specific probe panels for the assays, was completed for several spatial/imaging-based assays. HTAPP has also conducted successful site visits for knowledge transfer via on-site SOP training for sc/snRNA-seq at four clinical sites, which include two sites serving underrepresented populations.

The FNL HTAPP team will continue to provide technical and project management oversight to ensure that all experimental and computational analyses are being conducted in accordance with the approved plan and, moreover, that generated data and resources are being shared (in a timely fashion) with the HTAN and, ultimately, with the wider scientific community in compliance with the Beau Biden Cancer Moonshot Open Access Policy and the HTAN's established guidelines and policies.

Support Provided by the Laboratory Animal Sciences Program

LASP: Maintenance and Distribution of Cryoarchive Mouse Models

NCI Mouse Repository

KEY ACCOMPLISHMENTS

- The repository currently maintains 154 strains as cryoarchived stock, an increase from the 148 available for distribution in FY2019. An additional strain is currently being rederived for cryopreservation and ultimately public distribution. Ten strains are pending importation, and 23 strains that were accepted are pending completion of paperwork (importation papers or material transfer agreement) for inclusion in the repository's collection of cancer models.
- During this period, 40 orders have been received for mouse germplasm, a decrease from the 69 orders processed during FY2019. Among them, 29 have been fulfilled and 11 are pending shipment to the requesting organizations. Among the completed shipments thus far, 17 (59 percent) are domestic orders while 12 (41 percent) are international requests. Ninety-seven percent of all requests have been completed in support of extramural requestors.

Mouse models of human cancer have had a profound impact on our current understanding of the mechanisms of tumorigenesis and the pathways regulated by cancer-related genes. These models hold the promise of serving as critical tools in discovering and testing novel therapeutics to be used in cancer prevention and treatment. The NCI Mouse Repository is funded by the National Cancer Institute (NCI), Division of Cancer Biology (DCB) for maintaining and distributing mouse cancer models and associated tool strains, in addition to housing a unique collection of more than 1,500 mouse embryonic stem (ES) cell clones bearing conditionally-activated microRNA (miR) transgenes to facilitate *in vivo* exploration of miR functions. The collection of cryoarchived mouse models includes strains bearing conditional and point-mutant alleles in cancer-related genes.

The NCI Mouse Repository makes its unique collection of mouse strains, managed by FNL staff in LASP, available to all members of the scientific community (academic, non-profit, and commercial). These models are cryoarchived and distributed as frozen germplasm (embryos and/or sperm) worldwide. Detailed information about this valuable resource may be accessed through the Repository webpage: https://frederick.cancer.gov/science/technology/mouserepository/MouseModels/Default.aspx. Researchers are encouraged to submit their cancer models to the NCI Mouse Repository for archiving and distribution and to be reviewed and evaluated by the LASP director and imported by the NCI Mouse Repository administrative office once approved.

miRs are small, non-coding RNA molecules that play important roles in fundamental cellular processes by post-transcriptionally regulating gene expression. In an effort to address the role that miRs play in human cancer, their use as diagnostic tools, and their potential function as new targets for therapeutic intervention in the treatment of cancer, DCB has supported the generation of mouse embryonic stem cells (mESCs) harboring mouse miRs. As a result, 1,501 miR-ESC lines were generated at the Cold Spring Harbor Laboratory using high-throughput technology to produce clones that conditionally express each known murine miR. Mice generated from these ES clones will contain a tet-inducible miR transgenes to facilitate investigation of the role of each miR *in vivo*, providing a powerful tool for the study of miR biology. Reversible transgene expression for each mESC clone is controlled by either the tetracycline response element (TRE) or TRE-tight promoters, to accommodate a wide range of experimental designs. TRE-driven expression permits the study of miR in a variety of tissues, while TRE-tight promoters reduce leakiness, result in more restricted expression, suited for tissue specificity, and are especially useful for the study of miRs for which even the smallest level of expression may cause sterility or lethality.

The entire collection of miRs was made available for distribution to the scientific community in July 2013. The NCI Mouse Repository poster presented at the 2019 RNA Biology Symposium emphasized the value of this resource for the scientific community throughout the United States. Detailed information is available on the NCI Mouse Repository web page: https://frederick.cancer.gov/science/technology/mouserepository. The website also includes validation documentation for each mESC clone and sequencing data for each miR.

Appendices including all protocols used in the generation, care, manipulation, and use of the available clones are also provided. Although there has been only one order submitted to date in FY2020, future presentations are planned to further communicate the availability of the valuable miR resource at the NCI Mouse Repository. The miR ES cell resource is distributed to the scientific community according to the following organizational affiliations and prioritized in the following order: (i) NCI-funded investigators; (ii) NIH-funded investigators; (iii) government-funded researchers, other than those funded by NCI or NIH; and (iv) investigators from non-profit organizations without government funding.

Support Provided by the Science and Technology Group

Admin Support & Research Tool Delivery

Cancer Systems Biology Consortium/Physical Sciences – Oncology Network Summer Undergraduate Research Program

Each year, NCI convenes the Cancer Systems Biology Consortium/Physical Sciences – Oncology Network Summer Undergraduate Research Program to provide unique opportunities for students to engage in innovative and interdisciplinary approaches to cancer research. Each year, NCI matches undergraduate applicants with participating principal investigators across the country to provide an immersive research experience. CRTP provides administrative support for this initiative, including maintaining the program website and providing student housing, stipend support, and assistance with planning the annual in-person meeting for the selected students.

This year, 16 undergraduate students were selected to participate in the program. However, due to the COVID-19 pandemic and resultant quarantines at participating research institutions, each participating research institution withdrew from the program in summer 2020. A full-time interactive online course was offered as an alternative, in which all 16 selected students chose to participate. CRTP has provided administrative support, stipends, and access to Labster laboratory simulations in coordination with the NCI as part of the virtual program.

STG: Pediatric Moonshot Core for the Division of Cancer Biology

NCI Cancer Moonshot

NCI convened the Blue Ribbon Panel (BRP) in 2016 to provide recommendations for achieving the Cancer Moonshot's goal of accelerating progress in cancer research, now called the Beau Biden Cancer Moonshot Initiative. The BRP was charged with assessing the state of the science in specific areas and identifying major research opportunities that could uniquely benefit from the support of the Cancer Moonshot and could lead to significant advances in our understanding of cancer and how to intervene in its initiation and progression. The recommendations focused on areas in which a coordinated effort could profoundly accelerate the pace of progress in the fight against cancer and were not intended to replace existing cancer programs, initiatives, and policies already underway. The BRP final report was approved by the National Cancer Advisory Board and included a recommendation for the development of a pediatric immunotherapy translational science network that would facilitate the testing of new immunotherapy approaches in childhood cancer and establish a robust research pipeline to help further advance this field of study. The 21st Century Cures Act was signed into law in December 2016 to dedicate new funds to support efforts associated with the Beau Biden Cancer Moonshot Initiative. In coordination with the Division of Cancer Biology, the FNL has enlisted the support of Cherie Nichols, an authority on NCI long-term strategy, to monitor Moonshot efforts and assist in identifying scientific opportunities.

Moonshot Pediatric Core

The Moonshot Pediatric Core (MPC) was established as a dedicated resource to support the research teams from the Pediatric Immunotherapy Discovery and Development Network (PI-DDN) and the Fusion Oncoproteins in Childhood Cancers (FusOnC2) Network. The two networks were established to intensify research on the major drivers of childhood cancers and to develop immunotherapeutic approaches for children and adolescents with cancer. The awards were provided by the National Cancer Institute as part of the Blue Ribbon Panel recommendations. The MPC works to accelerate the development of new novel immunotherapies and improve pediatric cancer treatments by providing FNL resources and capabilities to the network groups.

The FusOnC2 Network advances understanding of the biology of fusion oncoproteins in childhood cancers to inform the development of targeted treatments for pediatric cancer patients. The network brings together researchers with expertise in structural biology, proteomics, genomics, medicinal chemistry, pharmacology, and cancer biology who are teaming up to gain insights into the molecular drivers of childhood cancers. The network is specifically focusing on improving the knowledge of pediatric cancers that are at high risk for treatment failure or for which there are currently no known effective targeted therapies. This network is moving the field of childhood fusion oncoproteins forward towards new, more effective treatments with fewer side effects for pediatric cancer patients. As participants in the FusOnC2 Network, FNL's Science and Technology Group helps NCI plan and coordinate fusion oncoprotein research initiatives, including recruiting researchers to participate in FusOnC2 events in order to improve the quality of research produced by the FusOnC2 consortium.

The PI-DDN identifies and advances translational immunotherapy research for children and adolescents with cancer. This network is working to discover and characterize new targets for immunotherapies, design experimental models to test the effectiveness of pediatric immunotherapies, develop new immunotherapy treatments, and improve the understanding of tumor immunity in pediatric cancer patients. This network is also working to overcome major barriers in developing effective immunotherapies for children, such as lower expression of proteins that can be recognized by immune cells and the immunosuppressive environments of tumors in some pediatric cancers. Investigators in the PI-DDN work together on multicomponent research studies centered around a pediatric cancer research area and individual pediatric immunotherapy projects. This network is striving to advance pediatric immunotherapies towards the treatment of children and adolescents with high-risk cancers. As participants in PI-DDN, FNL's Science and Technology Group helps NCI plan and coordinate pediatric immunotherapy research initiatives and recruit outside researchers and clinicians to participate in PI-DDN discussions in order to enhance the impact of the PI-DDN consortium.

Division of Cancer Control and Population Sciences

Support Provided by the Clinical Monitoring Research Program Directorate

Clinical Program/Project Management

KEY ACCOMPLISHMENTS

- Completed two subcontracts supporting the Surveillance, Epidemiology, and End Results (SEER) Program's data release and quality audit plan pilot, with recommendations to the Division of Cancer Control and Population Sciences

To support the Surveillance Research Program, which is a leadering entity in the science of cancer surveillance and disseminates reliable population-based cancer statistics, Frederick National Laboratory for Cancer Research (FNL) staff managed research subcontracts with a subcontractor that supported the SEER Program within the Surveillance Research Program.

For SEER's data release effort, the subcontractor developed a process map with key recommendations for data release after identifying test cases, product lines, potential release options, and a release prioritization schedule. The subcontractor also completed eight pre-quality-assessment-plan evaluations, developed programmatic metrics for data collection reporting metrics across SEER registries, and recommended triggers for implementing and evolving a full quality assessment plan.

FNL's subcontractor supported SEER's aim to provide information on cancer statistics in an effort to reduce the cancer burden among the U.S. population.

Support Provided by the Clinical Research Directorate

SEER Program VTR Breast Cancer Technical Pilot

KEY ACCOMPLISHMENTS

- FNL subcontracted with a vendor to perform whole-genome sequencing (WGS), whole-exome sequencing (WES), Transcriptome Capture (TCap), and the multigene PanCan Panel.
- FNL purchased services from a vendor to perform the NanoString PanCancer Pathway Panel.

The National Cancer Institute (NCI) Surveillance Epidemiology and End Results (SEER) Program consists of population-based central cancer registries that cover nearly 30 percent of the U.S. population. The Virtual Tissue Repository (VTR) aims to establish the infrastructure for using community-based tissue specimens for biomedical research. When implemented, researchers will be able to search de-identified SEER abstracts along with de-identified pathology reports and request residual tissue for research. Involving six of the SEER cancer registries, the VTR Breast Cancer (BC) and Pancreatic Ductal Adenocarcinoma (PDAC) Genomics Studies will compare genomic and transcriptomic profiles from BC and PDAC patients.

The technical pilots involve samples from case-control pairs collected from patients: DNA extracted from archival tumor; DNA extracted from normal formalin-fixed, paraffin-embedded (FFPE) tissue; and RNA extracted from archival FFPE tumor tissue. The PDAC technical pilot will perform TCap and the NanoString PanCancer Pathways Panel on tumor RNA specimens extracted from 24 patients. The BC technical pilot will perform WES and WGS on both tumor and normal DNA extracted from 12 patients and will perform the multigene PanCan Panel on tumor and normal DNA extracted from eight patients. The scope also includes performing TCap and the NanoString PanCancer Pathways Panel on tumor RNA extracted from 32 patients. FNL is supporting the NCI Division of Cancer Control and Population Sciences by managing subcontractors to perform WGS, WES, TCap, the multigene PanCan Panel, and the NanoString PanCancer Pathway Panel.

Division of Cancer Epidemiology and Genetics

Support Provided by the Applied and Developmental Research Directorate

ADRD: Repository Management

KEY ACCOMPLISHMENTS

- The NCI at Frederick Central Repository (Repository) continued to provide support for sample management, shipments, receipts, inventory, tracking, withdrawals, distributions, and storage of the Division of Cancer Epidemiology and Genetics (DCEG)'s 370 active studies, which come from 28 investigators and represent 10 branches. There are currently approximately 11.3 million DCEG samples in storage (equivalent to 15.1 million samples when normalized for 2 ml vials occupying storage space). DCEG currently owns 315 fridges and freezers, including the Bahnson -80ºC, which houses 1.8 million samples. The Frederick National Laboratory for Cancer Research (FNL) oversight team and American Type Culture Collection subcontractor staff continued working closely with Dr. Amanda Black and Ms. Marianne Henderson to optimize freezer utilization and to streamline and standardize sample data records for all DCEG studies.
- Freezer optimization with plans to refresh the freezer fleet and minimize the total number of units with a continuous concerted effort to consolidate samples are at full speed. The overall approach was identified in FY2018 with a five-year plan to replace aging mechanical units with high-energy-efficiency Varios -80ºC liquid nitrogen (LN2) freezers. The Vario freezers are optimally designed to ensure sample integrity, lower operational costs, greater capacity per unit, and longer lifespan than mechanical freezers.
- To address the projected higher need for LN2, the NCI Office of the Director (NCI-OD), Office of Scientific Operations, provided support for a large infrastructure renovation project to expand the number of LN2 drops. The expansion was completed in February 2020, resulting in 86 additional multi-use drops. The purchase and delivery of new Varios freezers was tightly coordinated with the challenging completion date of the LN2 expansion project. The concerted approaches allowed for a faster pace of the replacement units in FY2020. As a result, 39 new units were delivered in February 2020, with all units expected to be fully installed, qualified, and storing samples by the end of FY2020. Furthermore, 25 additional units are expected to be purchased and installed within the first two quarters of FY2021.
- In addition to the freezer replacement plan, consolidation opportunities and floor plan organizations have been following a continuous improvement plan with constant evaluation and implementations. As samples continue to be transferred from aging freezers to the new Vario units, the percentage of DCEG's mechanical freezers that are older than 15 years continues to drop, as the older freezers are surplus. Overall, the replacement, consolidation, and organization plans allowed for more free space and lower charges for DCEG stakeholders.
- The Repository also continued to provide support and technical input to the large initiative directed by Dr. Amanda Black to clean, standardize, and streamline sample data records in the Biological Specimen Inventory (BSI) for all DCEG studies. To achieve the goals of DCEG's "BSI clean-up project," the representatives of the Repository Quality Board agreed to transfer all studies from the Center for Cancer Research, NCI-OD, and small users from the BSI NCI database to the BSI Cenrep database. These efforts are nearing completion, with the transfer of a large number of sample records to be completed by September 2020.

ADRD: BioProcessing Laboratory

KEY ACCOMPLISHMENTS

- Provided rapid response to a COVID-19 project that required initiating a BSI study, developing laboratory and shipping solutions, and integrating processes and informatic system with an outside institute. The clinical study is underway and successfully submitting specimens to the laboratory for processing with minimal changes to original project design.
- Continued to expand the utilization of FluidX automation-ready cryovials with embedded 2D barcodes into other DCEG projects. The manual manipulation of these tubes, meant for automation, requires creative workflow redesign in laboratory space but allows for improved usability in DCEG laboratories and improved long-term storage capabilities.
- Continued to participate in discussions on updates to the NCI instance of the BSI database to align new DCEG studies with the new system structure, clean up data from existing studies in the collection, and modify existing relational tables to account for previously unknown variables.
- Provided guidance to intramural investigators for planned studies to integrate cost-effective best practices for specimen collection, specimen transport, integration of FluidX tubes into new projects, and the re-design of an aliquoting project for samples received from an international laboratory. These projects require extensive integration of different functional areas such as international and domestic transportation regulations, data management

activities in three external databases in addition to the BSI database, specimen collection kit design, kit production, specimen processing, and specimen distribution.

- Participated in weekly meetings with DCEG committees to incorporate prior project knowledge into the larger Connect for Cancer Prevention Cohort Study planning and to update study design to scale up the laboratory's service. These planning meetings include providing feedback on label design, database design, collection tube options, transport influence, courier options, kit design, and application for permission to use the NIH mail permit.
- Met with intramural investigators to discuss their study needs and the application of knowledge from prior studies for potential new collections. These include the parameters involving multi-specimen collections at multi-collection centers, questionnaire design, specimen array, specimen distributions, data management, and short and long-term specimen storage (principal investigator (PI): Katherine McGlynn) and new specimen acquisition (buccal-mouthwash) as a pilot for a larger project (PI: Emily Vogtmann).

Study	PI	Material	# Vials
Hormone Receptor Testing	Britton Trabert	Serum	69
American Cancer Society QC specimen aliquot and batching	Britton Trabert	Plasma	2,026
Buccal-Scope Pilot	Emily Vogtmann	Buccal cells	80
CONNECT pilot	Amanda Black	Plasma, Serum, Buffy coat, RBC	13,645

Support Provided by the Cancer Research Technology Program

Hormone Anaylsis

PCL focused on analyzing steroid hormones (estrogen, progesterone, and androgen) for DCEG during FY2020. Approximately 2,700 production runs were performed using the isotopic dilution mass spectrometry assay developed by PCL analyzing both for specific steroid hormones and their derivatives and a panel of different steroid hormone (parent hormone assay).

In addition to the steroid hormone analysis, PCL carried out proteomics analysis for the Laboratory of Translational Genetics, primarily focusing on identifying allele-specific binding proteins.

While laboratory work was suspended due to the coronavirus pandemic, the mass spectrometers were kept up and running, allowing us to quickly start performing quality control runs during the week leading up to the reopening of the laboratory. This allowed us to start analyzing samples from the first week we were allowed back. Secondly, preventative maintenance was arranged for the immunoanalyser in preparation for analyzing 682 samples for sex hormone binding globulin quantification.

During FY2019, PCL laboratory members were co-authors on several peer-reviewed articles.

Support Provided by the Clinical Research Directorate

Cancer Genomics Research Laboratory

KEY ACCOMPLISHMENTS

- Supported more than 250 genomics and pathology projects
- Updated laboratory procedures to support a COVID-19 genome-wide association study and the COVIDcode National Human Genome Research Institute protocol
- Implemented a new automated sample store
- Used and developed cloud-based technologies
- Relocated all operations to new facility

The Cancer Genomics Research Laboratory (CGR) investigates how germline and somatic genetic variation contribute to cancer susceptibility and outcomes, in support of the National Cancer Institute's (NCI) Division of Cancer Epidemiology and Genetics (DCEG) research. Working in concert with epidemiologists, biostatisticians, and basic research scientists in DCEG's intramural research program, CGR provides the capacity to conduct genome-wide discovery studies and targeted regional approaches to identify the heritable determinants of various forms of cancer. CGR operates as a high-throughput genomics laboratory with a focus on automation and cutting-edge technology and innovation, providing comprehensive genomics laboratory and scientific research support from project inception through specimen preparation, data generation, analysis, and publication of findings. Publications include: an article on the impact of fecal microbiome extraction (Chill S, et al. *J Biomol Tech*, S21-S22, 2019), an article analyzing prostate cancer (Darst BF, et al. *Eur Urol*, doi: 10.1016/j.euro.2020.04.060, 2020), a publication discussing the gene pool in the Americas (Grouveia MH, et al. *Mol Biol Evol*, doi:10.1093/molbev/msaa033, 2020), an article on the genetic architecture of cutaneous melanoma (Landi MT, et al. *Nat Genet*, doi: 10.1038/s41588-020-0611-8, 2020), an article on germline variant analysis

(Mirabello L, et al. *JAMA Oncol*, doi: 10.1001/jamaoncol.2020.0197, 2020), an article discussing blood cell counts (Pinheiro M, et al. *Sci Rep*, 10(1):3655, 2020), an analysis of severe aplastic anemia (Savage SA, et al. *Am J Hum Genet*, S0002-9297(20)30004-5, 2020), a case-control study (Vogtmann E, et al. *Cancer Med*, doi: 10.1002/cam4.2660, 2019), a pediatric HIV/AIDS study (Wang Y, et al. *AIDS*, 33(13):2091-2096, 2019), a publication on aplastic anemia (Wang Y, et al. *Br J Haematol*, doi: 10.1111/bjh.16153, 2019), a study on gene expression (Zhu B, et al. *Breast Cancer Res*, 21(1):147, 2019), and a publication on mutations in the human papillomavirus (HPV)-16 genome (Zhu B, et al. *Nat Commun*, 11(1):886, 2020).

Currently, the Frederick National Laboratory for Cancer Research staff in CGR uses a range of technology platforms to assess human genetic variation. Platforms and technologies include: (i) Illumina bead-based single-nucleotide polymorphism array multiplexing technologies, both for genome-wide discovery and targeted custom analysis; (ii) Illumina genome-wide methylation arrays; (iii) relative telomere length analysis; (iv) 16s rRNA microbiome sequencing; (v) HPV typing and whole-genome sequencing; (vi) sequencing of human whole genomes, whole exomes, and whole transcriptomes, as well as targeted sequencing and analysis using five platforms (Illumina NovaSeq 6000, Ion Torrent S5™, Illumina MiSeq®, Applied Biosystems 3730xl, and Pacific Biosciences Sequel) in conjunction with a wide range of sequence capture and targeting techniques/protocols; (vii) transcriptome and miRNA sequencing; and (viii) expression analysis via NanoString nCounter®. The most recent addition to the CGR catalog includes molecular and digital pathology applications for support of tissue research.

Due to the breadth and diversity of the work completed, it would have been impossible to manage the laboratory work, project planning, quality control, assay validation, sample extraction and handling, and data analysis without the support of members from each of CGR's functional groups: DNA Extraction and Staging Laboratory, Production Genotyping and Sequencing Laboratory, Scientific Research Group, Functional Laboratory, HPV Genomics Unit, Molecular & Digital Pathology, Technology Development and Implementation, Quality Assurance, Laboratory Information Management System (LIMS) Development, Bioinformatics, IT Core Services, Project Management, and Administration. CGR has once again set high goals and accomplished them with attention to the generation of high-quality data and a focus on customer support and detailed project management.

Outside of the normal high-throughput sample processing (more than 50,000 specimens), genotyping (more than 40,000 samples scanned), and sequencing research projects (more than 3,000 samples sequenced), CGR focused on several significant projects over the past year. While laboratory tasks were limited in the second half of the year due to the operational shutdown of the facility due to the SARS-CoV-2 pandemic and a laboratory relocation, several items were completed this year, highlighting the breadth of research support conducted by CGR.

Sample Store Integration

Managing the processing, storage, and downstream use of over a million specimens is no small feat. More samples are received and processed every year through CGR's extraction and staging (nucleic acid quality control) pipelines. These specimens must be appropriately tracked and stored and must be available for ongoing research projects at a moment's notice. To accomplish this, CGR has used an automated sample store for the last 10 years. Having outgrown the existing system and facing the opportunity to expand in a new facility, CGR purchased the Brooks Sample Store II to facilitate sample management goals.

The system is currently installed in the new facility and integrated with two liquid-handling robots for automated plating of selected samples. The refrigerated store can store up to two million specimens in a variety of holder types. CGR's LIMS interacts directly with the store to send sample orders for plating. Samples are arrayed in the desired configuration for downstream assay processing.

CGR's technical techniques and automation support teams designed the system over several years. CGR's LIMS development team made significant and truly remarkable efforts to develop the LIMS interfaces and new sample pipelines and integrate them with existing workflows to fully use the system's capabilities. The system will increase the laboratory's throughput and enhance sample management.

Cloud Genomics

CGR is rapidly expanding into new areas of research in support of DCEG. This requires expansion of analytical capabilities, specifically, developing expertise in migrating pipelines and analyses into cloud environments; using the cloud models to improve pipelines' performance; and establishing the capacity to integrate various types of genomic, proteomic, and metabolomic data in a systems biology approach to cancer research. In order to enable advanced computational biology for large multi-omic projects centered on investigating the etiology and progression of cancer, CGR has expanded subcontract support in this area.

Work with one subcontractor has focused on two main areas. The first area reviews existing pipelines (germline, somatic, structural variation, copy-number variations/copy-number alterations, RNA) for adherence to best practices and performance. This group provided guidance and technical support to CGR for using containers to support the environment-agnostic execution of pipelines, including transition to Biowulf (National Institutes of Health Linux cluster) and cloud micro-environments such as Amazon Web Services and Google Cloud Platform. Use of publicly available tools for

benchmarking (Global Alliance for Genomics and Health, Genome in a Bottle, etc.) is also planned. The second area identifies, prioritizes, and implements new approaches for genomic analyses, including, but not limited to, new alignment strategies and use of artificial intelligence for variant calling and functional annotation. Several projects have used these tools to date, with more planned in the coming months.

In addition, further cloud resources are being explored for use in sharing and analyzing slide image data. Using the HALO slide image management and analysis tool, CGR is moving image access to the cloud to enable the development of machine learning techniques and to share images easily with collaborators.

These advanced computing technologies are crucial for ongoing research, and progress in this area has been marked this year.

COVID-19 Projects

CGR is involved in two large initiatives related to the COVID-19 pandemic. The first is a collaboration between DCEG, National Human Genome Research Institute Home, and the National Institute of Allergy and Infectious Diseases to study the extreme phenotypes seen with SARS-CoV-2 infection, ranging from asymptomatic and mild illness to severe illness or even death. Under an approved Institutional Review Board protocol, samples are being collected from patients at the National Institutes of Health Clinical Center in Bethesda, referred patients from the National Institutes of Health Occupational Medical Service, and participating hospital centers throughout the United States and Canada. The goal of this work is a genome-wide association study of up to 40,000 SARS-CoV-2-positive cases. A subset of cases may also be selected for whole-genome sequencing.

To support this work, a comprehensive risk assessment was performed to ensure staff could safely work with biospecimens from patients infected with SARS-CoV-2 causing COVID-19. In addition, some laboratory equipment was reconfigured to address safety and throughput requirements. A series of pipeline modifications was finalized to facilitate the expected high number of specimens for this project.

CGR Relocation

While not a scientific accomplishment, the relocation of all CGR operations (and those of two other DCEG laboratories) from the Advanced Technology Center in Gaithersburg, MD, to the new Cancer Research Laboratory in Rockville, MD, was no small feat and required many staff hours to plan and execute. The new building, located at 9615 Medical Center Drive in Rockville, is adjacent to the NCI Shady Grove building that houses DCEG. This collocation will facilitate collaboration and support for ongoing research initiatives. The Cancer Research Laboratory is a new building and boasts state-of-the-art laboratory spaces.

Working in conjunction with NCI Office of Space and Facilities Management staff, the planning and construction took place over more than three years. After several delays (expected and unexpected), CGR began the relocation in mid-July 2020. Considerable effort by CGR staff resulted in a relatively smooth transition over an eight-week, four-phase move. With more than 500 pieces of equipment, including more than two dozen large liquid-handling automation systems, sequencers, and array scanning equipment, the relocation was a large effort. The move was completed in August. CGR is looking forward to this new chapter.

Automated Evaluation of Biomarker Slides

Cervical Cancer Biomarkers Research

KEY ACCOMPLISHMENTS

- Slide scanning is significantly ahead of schedule.
- Data for more than 42,000 slide images have been delivered.

This DCEG project focuses on the development of new biomarker-enhanced cytology strategies to support automated image analyses with the potential to guide diagnosis and treatment of cervical precancers. This subcontract, sponsored by CGR on behalf of DCEG, supports validating whether staining specimens with p16 tumor suppressor protein along with the proliferation marker known as Ki67 is an effective method for detecting cervical precancer, and it aims to develop automated image evaluation of biomarker-stained cervical cells.

The project will generate automated screening data from approximately 50,000 biomarker-stained cervical cytology slides. This data will subsequently be used to develop, train, and examine a machine learning algorithm for automated detection of dual-stained cells in digital cytology.

The scanning of slides for this work is significantly ahead of schedule. The subcontractor is nearly two years ahead on milestone deliverables as outlined in the statement of work for this partnership and has already provided data for more than 40,000 slides (84 percent completion of overall project). This is a considerably faster rate than anticipated and ensures the rapid advancement of research and scientific goals for detecting and preventing cervical cancer.

Accelerating Cervical Cancer Control

HPV Genomics Support for DCEG Title

KEY ACCOMPLISHMENTS

- Pipeline finalized for new extraction-free HPV typing assay (TypeSeqv2)
- HPV methylation assay development
- Automation validation for Project Itoju (Nigeria)

HPV research is an ongoing endeavor for NCI and DCEG. Areas of research include vaccine efficacy, screening/testing, genetics, epigenetics, and carcinogenesis. As part of this, in 2015, CGR developed a novel high-throughput HPV typing assay that transformed HPV genetics research. This assay, TypeSeqv1, was transferred to the Centers for Disease Control and Prevention and to DCEG-collaborating laboratories in Costa Rica this year to streamline sample processing and reduce turnaround times needed for analysis of research specimens. CGR has trained both groups, which are now working on standing up the assays in their local laboratories.

Major tasks defined under this project include HPV genotyping for up to 200,000 samples and development of virus and human methylation assays for application in up to 50,000 samples. All samples are from a variety of studies supported by the cervical cancer/HPV project.

Development continues for both the extraction-free HPV typing assay (TypeSeqv2) and methylation. Progress on the extraction-free assay was, overall, slower than planned due to the pandemic; however, a final workflow is in place for TypeSeqv2, and build-out of CGR's LIMS to track and support this new workflow is in progress.

Plans for the methylation assay include protocols to support work in both high-resource and low-resource settings. When available, protocols will be made available via publication to address access to cervical cancer screening all over the world. The low-resource assay (which is being designed entirely in-house) is in the primary and validation phase.

The assay primer design was completed this year for the high-resource methylation assay (which is based on commercially available technology). Several rounds of testing have been completed for this assay. The next round of testing will focus on locking down the assay's specificity.

Technical Planning Level 2: Epidemiologic and Molecular Features of Cervical Cancer in Nigeria – Project Itoju (Care) Phase 3

Planned work includes:

- DNA extraction of 10,000 cervicovaginal specimens received in specimen transport medium (STM)
- HPV typing of 10,000 samples post-DNA-extraction from cervicovaginal specimens
- DNA extraction of 2,000 cervical specimens in STM
- HPV typing of 2,000 samples post-DNA-extraction from cervical specimens
- Return of residual STM samples to the NCI Central Repository for long-term storage, organized in 96-well American National Standards Institute/Society for Laboratory Automation and Screening (ANSI/SLAS) format racks

This project has been significantly delayed, and samples have not yet been received from Nigeria. However, development of automation programs has been completed this year. This will support the processing when samples begin to arrive from Nigeria later in 2020.

Division of Cancer Prevention

Support Provided by the Applied and Developmental Research Directorate

ADRD: Cancer Immunoprevention Laboratory

KEY ACCOMPLISHMENTS

- Cancer vaccine support – A major ongoing project at the Cancer Immunoprevention Laboratory (CIPL) is the study of new cancer prevention vaccines targeting a number of different tumor antigens. CIPL provided preclinical/discovery support for the development of five novel cancer vaccines that target the tumor antigens TERT, RAS, MSLN, Lynch Syndrome-specific neoantigens, and colorectal cancer-causing Fusobacterium nucleatum. CIPL performed six *in vivo* mouse studies using 553 mice that generated samples for T-cell analysis by enzyme-linked immunospot (ELISpot) and flow cytometry. Findings included identification of optimal mouse strain and route of vaccine administration for the mesothelin peptide vaccine, confirmation of Fuso-derived vesicles and Fuso antigen and alum as immunogenic vaccines in mice, exploration of adjuvant activity of novel PD-1-inhibitor Microtides, comparing lots of Hiltonol adjuvant in performance with the frameshift peptide (FSP) Lynch Syndrome peptide vaccine, and the testing of the RAS and TERT peptide vaccines in mouse syngraft models for tumor efficacy. CIPL also characterized the 4T1-HER2 tumor cell line *in vitro* and *in vivo* and successfully developed an orthotopic mammary gland tumor model for vaccine testing.
- RG1-VLP vaccine support – Next-generation HPV vaccines are being developed as potentially lower-cost alternatives to more broadly respond to the diversity of HPV strains and avoid adding more varieties of virus-like particles (VLPs) for every new strain targeted. CIPL continued working on the novel RG1-VLP vaccine, which is composed of a single VLP species that targets an HPV L2 target conserved across numerous strains and should therefore have broader coverage. During FY2020, CIPL provided development support for the novel RG1-VLP in collaboration with the Laboratory Animal Sciences Program (LASP). CIPL performed four *in vivo* mouse studies utilizing 310 mice that generated sera samples analyzed for humoral anti-HPV responses via ELISA and neutralization assays. Findings included the establishment of a long-term memory antibody response to RG1-VLPs promoted by the

novel adjuvants BECC470 and PCEP and protection against vaginal HPV39 inoculation in the challenge model by RG1-VLP adjuvanted with alum, BECC470, or PCEP. CIPL further demonstrated optimization of the *in vivo* pseudovirus (PsV) challenge model by addition of Tween 80 to PsVs and protection from HPV39 by passive sera transfer from RG1-VLP-vaccinated mice.

- Lynch Syndrome (LS) support – LS is one of the most common hereditary cancer syndromes. It is an autosomal dominant condition that predisposes to various cancers including colorectal cancer (also known as hereditary non-polyposis colorectal cancer), endometrial cancer, and stomach, breast, ovarian, small intestinal, pancreatic, prostate, urinary tract, liver, kidney, and bile duct cancers. Germline mutations in several genes involved in DNA mismatch repair have been linked to LS (e.g., MLH1, MSH2, MSH6, PMS2, and EPCAM). Mismatch repair deficiency causes sporadic mutations in mononucleotide repeat regions of many genes, which promotes a phenotype of high microsatellite instability and the appearance of neoantigens with frameshift mutations (FSM). CIPL delineated the tumor initiation and progression in LS genetically engineered mouse models (GEMMs) and confirmed the FSM in intestinal tumors in collaboration with the Clinical Laboratory Improvement Amendments (CLIA) Molecular Diagnostics Laboratory at the Advanced Technology Research Facility. The Laboratory determined that the FSMs arise in relatively late stage during tumor development. During FY2020, the Laboratory generated 128 organoid lines from *de novo* tumors developed in LS GEMMs and assessed FSM status in 112 organoid lines by fragment analysis. In addition, the Laboratory generated 2D cultures from two tumor organoid lines. The tumorigenicity of four organoid lines and their serial transplantability *in vivo* was assessed. Efficacy studies of FSPs vaccine adjuvanted with either Hiltonol or Poly(I:C) in LS GEMMs and organoid syngraft models were evaluated during FY2020.
- Biomarker Project – CIPL continued to develop plasma exosomal biomarkers, comparing exosomal DNA and RNA isolation or co-isolation methods using different kits and protocols. The Laboratory confirmed that exoDNA and exoRNA from culture supernatant and normal plasma contain DNA and RNA fragments with FSMs of interest. FSM status in *de novo* tumors and organoids on fragment data was evaluated using reframe analysis (R script). The Laboratory tested the utility of duplex-specific nuclease for enrichment of FSM alleles in culture supernatant and evaluated the FSM detection sensitivity on the Ion Torrent system. The utility of the Mi-Seq platform using ArcherDx reagents was also evaluated. The preliminary results showed that Mi-Seq is promising. The Laboratory established a collaboration with ECS Progastrin, Inc. to assess whether plasma progastrin can be used as a universal marker for cancer detection.
- FSP-specific antibody screening – During FY2020, CIPL screened six hybridoma clones (mouse monoclonal) developed against mouse Senp6 FSP via immunohistochemistry (IHC). The Laboratory validated the purified clones by IHC staining and fragment analysis. In addition, the Laboratory validated one rabbitized clone FSAI204-6H7 by IHC. This work was done in collaboration with the Antibody Characterization Laboratory at the Frederick National Laboratory. Four hybridoma Senp6 clones have been deposited into the Developmental Studies Hybridoma Bank (DSHB) housed at the University of Iowa and listed at Antibody Portal at NCI Center for Strategic Scientific Initiatives (https://antibodies.cancer.gov/detail/CPTC-Senp6+derived+frame+shift+peptide+%28mouse%29-1#CPTC-Senp6+derived+frame+shift+peptide+%28mouse%29-1)
- Laboratory Directed Exploratory Research project – This project investigates the role of the gut microbiome on vaccine responses to HPV VLP vaccination. *In vivo* mouse studies were conducted to examine the effects of antibiotic depletion of host microbiota cohorts as well as oral infusion of selected bacterial strains of interest on vaccine responses to the RG1-VLP vaccine. A manuscript containing results from this project is currently under preparation.
- External collaborations – CIPL has maintained several collaborations with external institutions that utilize CIPL biological models to determine the *in vivo* activity of novel vaccine/adjuvant technologies.
    - The laboratory of Dr. Bob Ernst at The University of Maryland, Baltimore has provided CIPL with novel TLR4 agonist compounds (BECC) that have been tested by CIPL with RG1-VLPs to determine immune response and vaccine efficacy. A manuscript was submitted for publication.
    - The laboratory of Dr. Alexander Andrianov at the University of Maryland Institute for Bioscience and Biotechnology Research has provided CIPL with novel polyphosphazene polymer adjuvants (PCEP) that have also been tested by CIPL with RG1-VLPs to determine equivalence or superiority to aluminum salts. Two manuscripts containing the results of this collaboration are under preparation.
    - Oncovir (Washington, DC) has supplied CIPL with the novel TLR3 agonist adjuvant Hiltonol for potential use in peptide-based cancer vaccines. This adjuvant has been tested for use in our vaccine model systems with the RAS,

MSLN, and FSP peptide vaccines and has been chosen for inclusion in the clinical FSP vaccine.

- Explorations of Global Health (Leidos) has provided CIPL with PD-1-inhibiting Microtides, and CIPL tested them for adjuvant activity in the context of various tumor peptide vaccines.
- Frederick National Laboratory's Nanotechnology Characterization Laboratory has provided CIPL with OVA-peptide-conjugated Polyplex nanoparticles, with which CIPL is conducting testing for adjuvant activity.

- COVID-19 support – CIPL provided support in the development of the ACE2 inhibitor molecule, APN01, for clinical investigation. As this biologic needs to be administered as an inhalant for exposure to the upper respiratory tract, CIPL examined the effects of nebulization on reception binding and functional activity of APN01 by developing ELISA-based and enzymatic-based activity assays.

ADRD: BioProcessing Laboratory

KEY ACCOMPLISHMENTS

- Initiated the design of a new laboratory information system, LabVantage, for the management of Division of Cancer Prevention (DCP)-led projects and the 2.3 million clinical specimens in storage. The new system, a relational database, will function as the system of record for the laboratory's project activity, specimen management, quality assurance, kit-making, and logistics of supply/specimen transfers. Additionally, participated in a week-long familiarization training, a week-long utilization workshop, presentations on activity workflows, and the design of module build-requirements.
- Initiated a project to integrate automation into the workflow. This project has launched with the acquisition of a liquid hander, Opentron OT-2, for the automated creation of aliquots from urine, serum, plasma, and DNA. This process will maintain specimen integrity by creating aliquots in a chilled environment or at room temperature. The ability to program *ad hoc* using open source programming and manage specimens in batches will improve turnaround time for large aliquoting projects.
- Continued to participate in discussions on updates to the NCI instance of the BSI database to standardize data associated with existing studies and active studies with the new system structure. Additionally, the new structures are being reviewed for incorporation into the new LabVantage system tables that are currently being developed. Additionally, in preparation for specimen selection in an upcoming DNA extraction project, historic Prostate Cancer Prevention Trial study data was reviewed and updated to annotations for more than 16,000 records to align them with current standardization methods.
- Participated in meetings for the integration of DCP-funded project management with the newly formed Cancer Prevention Clinical Trials Network's Data Management, Auditing, and Coordinating Center. This new network will, in part, assist in the management of the group of protocols formerly known as Consortia 2003 and Consortia 2012, of which 42 protocols will house specimens long-term at the NCI at Frederick Central Repository.
- Participated in monthly teleconferences with the Early Detection Research Network (EDRN) Data Management and Coordinating Center for the management of kit shipments, specimen receipt, and SOP updates for the 17 EDRN collections currently in long-term storage, to include the New Onset Diabetes and Uterine Lavage projects.
- Participated in the selection of 26,576 specimens from two different clinical studies for inclusion in a Global Screening Array conducted in collaboration with NCI's Division of Cancer Epidemiology and Genetics, the Southwest Oncology Group, and the Cancer Genomics Research Laboratory. These specimens had previously been processed, with specimen properties codified for ease of selection. This fit-for-use approach shortened the window of specimen selection.
- Reviewed program needs with DCP and began working with project officers and their external support clinical research organizations to initiate cost-saving changes for the division. One example is the communication to extramural sites through SOP revisions, updated collection worksheets, updated manifest information, and revised shipping supplies, for the direct shipment of all DCP specimens to the laboratory for specimen inventory, vial relabeling, and data standardization prior to being transferred to long-term storage at the NCI at Frederick Central Repository. The first direct-ship project is in the process of being coordinated and is expected to arrive in first quarter FY2021 with the receipt of serum, plasma, bronchial brushings, nasal epithelium, and buffy coat samples from patients enrolled in a lung cancer clinical study.

Study	Material	# Vials	Activity
GLNE	Stool	2,784	Distribute to extramural institute
PLCO	Buccal Cells	12	Receipt, process, aliquot, store
PLCO	Serum & Plasma	14,999	Aliquot, store; ship subset to investigator
PLCO	DNA	9,678	Re-quantification, relabeling, and creation of sister vials for historic collection
Total		**27,473**	

Medicinal Chemistry Support for Chemopreventive Agent Development

KEY ACCOMPLISHMENTS

- Support optimization efforts of two chemopreventive agents including STAT3 inhibitors and gp130 inhibitors. The results of gp130 inhibitor optimization produced a handful of compounds with improved physicochemical properties and solubility suitable for oral formulation. The improved characteristics of gp130 inhibitors met the milestone requirements and warrant further *in vivo* study efforts in the coming year including pharmacokinetics/pharmacodynamics, efficacy, and toxicology studies. The results of *in vivo* studies will critically inform and position the gp130 inhibitor(s) into potential clinical development.
- Gp130 compounds with improved potency, selectivity, metabolic liability, and aqueous solubility were selected to move forward further with *in vivo* studies. This task order involves subcontracts with two collaborators, Dr. Nouri Neamati at the University of Michigan and Dr. James Turkson at Cedars Sinai Medical Center.
- The notable progress can be attributed to innovative approaches in medicinal chemistry and a highly integrated and effective project team to achieve project objectives:
    - Innovative approaches in medicinal chemistry: Making new compounds with improved potency, physicochemical properties, and aqueous solubility required ingenious design and laborious synthesis processes. Dr. Neamati's group at the University of Michigan has made more than 100 compounds, and they are highly effective in hitting targets by systematic, innovative approaches and by their deep knowledge in medicinal chemistry. The work performed by our collaborators at Michigan produced a number of promising targets with the potential of further development.
    - Highly integrated and effective project team: The technical team has been very productive in advancing the project goals. The project consultant, Dr. Cristopher Self, is an expert in drug development and medicinal chemistry with more than 30 years of industry experience. He provided advice that resulted in a high success rate for the compounds. The cohesive team dynamic safeguarded the development pathway every step of the way and achieved a high level of performance.

Support Provided by the Biopharmaceutical Development Program

Nicotine Antibodies

Current Good Manufacturing Practice Production of ATI-1013 Antibody

In support of the Division of Cancer Prevention, the Biopharmaceutical Development Program initiated the generation of a stable Chinese hamster ovary (DG44) cell expression of the monoclonal antibody ATI-1013 at a contract research organization. The stable cell line has been transferred to the Biopharmaceutical Development Program, and associated development efforts are scheduled to start in FY2021.

Support Provided by the Cancer Research Technology Program

Advanced Technology Support

Advanced Technology Support, Division of Cancer Prevention (DCP)

Dr. Bob Shoemaker (DCP) is establishing an anti-peptide vaccine clinical trial for multiple mutated genes in Lynch syndrome patients. He has engaged the Clinical Laboratory Improvement Amendments (CLIA) Molecular Diagnostics Laboratory (CMDL) to establish a set of CLIA-validated assays to detect mutations in a panel of genes using exosomal DNA from Lynch syndrome patients. The clinical trial will seek to monitor a decrease in the occurrence of deletion mutations in exosomal DNA of targeted anti-peptide vaccine genes from less than 1 ml of whole blood. While the required sensitivity is not known, an initial goal is to create an assay that can detect mutant genes present at 0.1 percent to 1.0 percent prevalence in isolated DNA. The assay seeks to identify deletion mutations in homopolymer runs from a panel of targets (TAF1B, AIM2, ASTE1, TGFBR2, ACVR2, MSH3, DAMS, MARCKS, and LMAN1 (single nucleotide deletion) and FLJ20378 (dinucleotide deletion)). FY2020 objectives were to validate assays developed in prior years; however, these assays have not yet met the criteria for moving forward with CLIA

validation. Activity in FY2020 has been directed at establishing assays that meet the requirements of specificity and sensitivity that would warrant validation for a CLIA acceptable test.

The development of CLIA assays for Lynch syndrome mutations is a technically challenging problem, as it requires an assay that functions with low DNA inputs, can detect small changes in rare mutant alleles against a high wild-type DNA background, and can identify single deletion mutations in moderately long homopolymer runs. Initial attempts to validate published assays that rely on polymerase chain reaction (PCR) followed by Sanger sequencing or fragment analysis yielded unacceptably high error rates on the order of 10 percent from exosomal samples of mismatch repair–deficient compared to wild-type cell lines. These errors are believed to arise from deletions created during PCR amplification of the target DNA. To provide a more suitable substrate for testing, we created synthetic genes of wild-type and mutant sequences that are more than 99.999 percent correct and used these to evaluate assay methodologies. We have evaluated multiple polymerases and amplification conditions using Sanger sequencing and fragment analysis but have only achieved error rates at approximately 5 percent. Alternative data analysis methods did not improve the detection threshold. We tested the Ion Torrent next-generation sequencing platform with ampliseq chemistry, but this platform yielded variable results and was unable to meet a 1 percent mutation threshold. We have tested a custom design method from ArcherDx, Inc. that uses error-correcting barcoding followed by sequencing on the Illumina MiSeqDX. In our initial test, this methodology approaches 0.1 percent sensitivity for multiple target genes. We are in the process of verifying the initial data and working on optimization of the protocol to determine if this approach will meet the initial assay requirements. We are also evaluating input DNA requirements (currently 500 ng) to see if this can be routinely met with current exosomal DNA isolation methods from small volumes of blood. We will complete testing on this technology by assessing its performance in exosomal DNA isolated from cell lines and in exosomal DNA isolated from Lynch syndrome or normal volunteer whole blood.

CMDL is also providing Illumina MiSeqDX sequencing for the detection of methylated promoter regions in a target gene in colorectal cancer.

Support Provided by the Clinical Monitoring Research Program Directorate

Clinical Program/Project Management

Human Research Protections

Frederick National Laboratory staff in the Clinical Monitoring Research Program Directorate managed a consulting agreement with a human research protections expert who has extensive knowledge of federal regulations and regulatory compliance as applicable to federally funded clinical trials conducted in large, complex organizations across a variety of cohorts and trial designs.

The independent consultant reviewed Central Institutional Review Board protocols and provided feedback, provided regulatory guidance regarding secondary research on data and/or biospecimens, and presented "Reconsent or Notification of Subjects: What, When, Who & How" at the October 2019 Central Institutional Review Board Operations Meeting. Frederick National Laboratory completed agreement close-out activities in July 2020.

Division of Cancer Treatment and Diagnosis

Support Provided by the Applied and Developmental Research Directorate

Drug Development and Evaluation

Natural Products

KEY ACCOMPLISHMENTS

- The automation laboratory delivered more than 175,000 natural product fractions and plated more than 1.4 million samples in 384-well plates.
- The chemistry laboratory published a rapid second-stage compound purification and identification methodology in *ACS Chem Biol*, one of the leading journals in the field. The work was highlighted as the June 2020 cover article.
- The microbial laboratory developed methodology for high-throughput, high-capacity fermentation of fungal cultures in the new facility in Building 433 and initiated production.

Molecular Pharmacology and High-Throughput Screening

KEY ACCOMPLISHMENTS

- Pharmacological profiling of 12 drugs was performed using 16 patient-derived organoid (PDOrg) and 10 patient-derived cell line (PDC) models from the NCI Patient-Derived Models Repository with independent replicate experiments. Of these models, the drug sensitivity profiles of 10 paired PDC and PDOrg sets were compared to each other as well as to patient-derived xenograft (PDX) drug responses. It was observed that six of the paired models had significantly correlated drug responses. Additionally, both PDOrg and PDC models partially recapitulated PDX responses to the drugs paclitaxel and gemcitabine.
- The generation of matching normal and cancer colon organoid cultures from surgical specimens is ongoing. Since September 2019, seven paired

samples were received from the Cooperative Human Tissue Network at the Vanderbilt University Medical Center. Organoid models have been successfully generated from six of the seven malignant samples. We successfully generated organoid models from four of the normal samples.

- Evaluated the feasibility of developing patient-derived organoid models from needle biopsies. To date, five needle biopsy samples have been received, and organoid models were successfully developed from four.
- Transferred all the *in vitro* pharmacology data for patient-derived or PDX-derived tumor cell lines and cancer organoids into an Oracle database so that drug sensitivities can be evaluated in the context of genetic and transcriptomic data.
- Performed testing of 1,056 drug plates containing either two five-dose samples or 10 one-dose samples for a total of 7,352 compounds.
- Performed one-dose testing on 4,032 new synthetic compounds and 1,863 natural products. These assays also included 655 tests of the internal drug standard that is included in each one-dose testing plate.
- Tested 504 synthetic compounds, 45 natural products, 13 combination compounds, and 22 internal drug standards in the five-dose screen. Tested 218 compounds in the confirmatory five-dose screen.
- Provided 44 cell line cultures to Developmental Therapeutics Program support laboratories.
- Prepared 562 samples of cell lines from the NCI-60 panel that are available for testing to verify the identity of cells in the NCI-60 screen.
- Shipped 110 cell lines for the Target Validation and Screening Laboratory for cell line authentication and mycoplasma testing.
- Integrated new technologies for counting all NCI-60 cell lines. The Nexcelom Bioscience Cellometer K2 Fluorescent Viability Cell Counter provides a highly accurate quantification of live cells, dead cells, and total cells.
- For 10 years, NCI has sought to modernize the production screen, a worldwide resource. The NCI-60 team supported NCI in reaching a decision on the new assay technology (CellTiter-Glo in a 384-well format) replacing the current assay that has been in place more than 30 years.
- Providing the new CellTiter-Glo assay in a 384-well format to the community as a screening service requires the development of an information technologies pipeline capable of integrating data from the Drug Prep Lab and Screening Lab and facilitating quality control and reporting of the data.

Pharmacodynamic Biomarkers

KEY ACCOMPLISHMENTS

- Developed, validated, and successfully completed interlaboratory transfer of the Luminex multiplex (eight biomarkers) assay to measure isoform-specific total and key phosphorylated sites on ERK1/2 and MEK1/2.
- Demonstrated clinical utility of the 'MEK/ERK multiplex' by application to paired patient biopsies collected in a Phase II trial of selumetinib to treat neurofibromatosis type I (NCT02407405). Results demonstrated significant on-target pharmacodynamic effects, including reduced ERK1/2 phosphorylation in 15 out of 17 patients (60 percent median reduction) and increased feedback signaling through higher MEK1/2 phosphorylation in 10 out of 17 patients. Our study provided the first clinical evidence of target engagement by selumetinib in patients with NF1. Selumetinib has shown significant promise in treating NF1 and was recently approved by the FDA for the treatment of asymptomatic pediatric patients with NF1 and inoperable plexiform neurofibromas.
- Supported the development and clinical implementation of a biopsy processing method customized for pharmacodynamic (PD) evaluations of immuno-oncology agents.
- Supported the clinical implementation of a T-cell activation assay in whole-blood specimens from immuno-oncology trials, including laboratory certification for a specialized specimen preservation process and establishing a critical reagent supply chain for a large panel of phenotypic and PD biomarkers.
- Developed an advanced custom image analysis software algorithm with automated segmentation of individual nuclei and segmentation of tumor tissue from normal tissue that has technically advanced our ability to develop new quantitative PD marker assays in tumor and stroma compartments for use on clinical biopsy tissue. The custom algorithm also reduces user-to-user variability and allows assays to be transferred faster.
- Discovered PMS1 as a relevant protein binding partner of MLH1 in the Mismatch Repair protein complex in dogs and humans. As a result of our findings, efforts are now underway to develop a custom monoclonal antibody to PMS1 for use in a quantitative PD marker assay.
- Conducting first-ever PD marker characterization and mechanism-of-action studies of the DS-8201a antibody-drug conjugate (anti-Her2 monoclonal antibody linked to a Deruxtecan topoisomerase inhibitor payload). PD marker evaluation includes assays to measure induction of Topoisomerase I cleavable complex and total topoisomerase 1 biomarker target engagement as well as downstream DNA repair response markers in preclinical xenograft models varying in Her2 expression.

Pharmacokinetics and Drug Analysis

KEY ACCOMPLISHMENTS

- Analyzed samples from the 17 patients on the AzaTdC clinical protocol and presented results to the working group. In addition, the analytical methods have been improved and the major biological metabolite is being measured. Levels of this metabolite have not been previously reported for this class of compounds and have been used to further the pharmacokinetic disposition on AzaTdC.
- Developed a reliable and novel method for the analysis of 8-oxo-deoxyguanosine in DNA. This method meets the accepted guidelines from the current literature for use in studies examining DNA oxidation by reactive oxygen species. This method is in use in studies to determine the extent of DNA oxidation caused by a select agent's ability to generate reactive oxygen species as a biomarker in mechanism-of-action determinations.
- Improved and expanded methods for analyzing the active metabolites of IPdR and have used these methods in IPdR combination studies. In collaboration with the Mayo Clinic, the lab was able to successfully measure plasma metabolite as well as the levels of incorporation of the active metabolite into the DNA of brain tumors in mice treated with IPdR.

Drug Toxicology and Safety Pharmacology

KEY ACCOMPLISHMENTS

- Evaluated human-induced pluripotent stem cell (hiPSC)-derived peripheral neuronal cells (Peri.4U™, Ncardia) as an *in vitro* model of chemotherapy-induced peripheral neuropathy (CIPN). Qualification of the assay system included testing several coating substrates, cell plating densities, and drug treatment schema on multiparametric endpoint measurements. An experimental design was established to quantify inhibition of neurite outgrowth and degeneration of established neurites. Sensitivity and specificity of the model system were assessed using positive control compounds (bortezomib, cisplatin, paclitaxel, and vincristine) and negative control agents (DMSO and hydroxyurea). Reproducibility and robustness of the system were demonstrated between different operators and in triplicate experiments.
- Identified at least two different populations of cells in the hiPSC-Peri4U™ model system. Neuronal cells and neurites stain positive for βIII-tubulin (tuj-1) whereas a non-neuronal cell population with processes stain positive for vimentin. A third population of "orphan" cells do not stain positive for either marker. Continuing to characterize the Peri.4U™ cells.
- Exploring alternative source(s) of a hiPSC-dorsal root ganglia sensory neuronal cell model that would provide a more reliable and scalable cell system to model CIPN and ultimately search for potential modulator(s) of CIPN.

Information Systems, Information Technology, and Data Science Support

KEY ACCOMPLISHMENTS

- The Patient-Derived Models Repository (PDMR) is adding new biomarkers like microsatellite instability to existing data analysis to make it easier to identify appropriate PDX models for preclinical studies.
- For PDX tumors that are metastatic when implanted subcutaneously in NSG mice, PDMR added related observational data such as the metastases location and timing and the penetrance among implanted NSG hosts at indicated times.
- The Molecular Pharmacology Branch has a PerkinElmer Opera Phenix High Content Screening System for 3D high-resolution multicolor imaging of cells. The branch is actively using complex spheroids and patient-derived organoid 3D imaging for multiparametric analyses to better characterize the biological properties, chemosensitivities, and pharmacodynamics of these advanced models. Image acquisition and analysis was, initially, only performed using one computer, substantially impacting productivity. The PerkinElmer Columbus software provides a powerful tool for quickly accessing, processing, and analyzing the enormous data files that are generated from the Phoenix imager without impacting image acquisition processes and therefore allowing full use of the PerkinElmer Opera Phenix High Content Screening System.

NCI Experimental Therapeutics (NExT) Chemical Biology Consortium

KEY ACCOMPLISHMENTS

- Managed 33 subcontracts to support all the research activities for nine drug discovery projects.
- Investigational New Drug application was filed for the p97 inhibitor CB-5339 by the Cleave Therapeutics members of the project team. The NCI also filed an IND for CB-5339 for use in solid tumors.
- The institutions on the lactate dehydrogenase-A gene (LDHA) project team entered into a partnership with a biotech company. Experimental evaluation of the LDHA team assets over the subsequent eight months has led to the selection of a development candidate for a rare disease indication.

- The WDR5 gene project team has optimized the *in vivo* anti-tumor activity of the lead compounds, assessed their pharmacokinetics in rats and dogs, and conducted a rodent toxicology study. These results will enable the team to select a clinical candidate within the next six months.
- Joined three other companies in a Compound Library Consortium that provides its members access to a proprietary set of novel compounds designed by the members. This investment provides NCI Experimental Therapeutics (NExT) Chemical Biology Consortium project teams with access to over 85,000 high-purity, novel, drug-like scaffolds, and the collection can be expanded as other members join.
- The p97 project team obtained the first *in vivo* efficacy results with an allosteric p97 inhibitor.
- The Nicotinamide N-methyl Transferase project, which was initiated in the last year, improved the potency of its inhibitors by 2,000-fold, guided by multiple crystal structures.

Radiochemistry Research, Radiopharmaceutical Production, and Imaging Archive Operations

KEY ACCOMPLISHMENTS

- Initiated multiple outreach activities to support collecting and sharing COVID-19 datasets on The Cancer Imaging Archive (TCIA) as an NCI-supported short-term urgent response. Released a dataset from the University of Arkansas for Medical Sciences 80 subjects). Currently developing agreements to share 10,000 subjects via the Radiological Society of North America and another agreement with the NCI Clinical Center to share 5,000 subjects. Participating in discussions with the Alliance for Digital Pathology and World Health Organization to collect and curate digitized pathology data collected from autopsies of COVID-19 patients.
- Released or updated more than 35 research-focused imaging data sets on TCIA consisting of 5,817 subjects, including a one-terabyte low-dose computed tomography projection data set that has been an ongoing collaboration with Mayo Clinic to develop and share a new image modality for capturing raw projection data prior to 3D reconstruction.
- Since September 2019, monthly TCIA active users have grown by 25 percent, and the volume of data downloaded on a weekly basis has increased by 100 percent. Over 912 publications have been written based on TCIA datasets, 215 of which were written in FY2020. That represents an increase of 35 percent over the previous year.

Medical/Scientific Writing

KEY ACCOMPLISHMENTS

- "Clinical Evolution of Epithelial–Mesenchymal Transition in Human Carcinomas" by Navas et al. was published in *Clin Cancer Res* and won first place in the 2019 Leidos (corporate) Technical Publications Competition, LBR technical publication category. This highly collaborative and clinically relevant work demonstrates how the phenotypic plasticity conferred by epithelial-mesenchymal transition in tumors enables rapid adaptive response to therapy, which may lead to acquired drug resistance.
- Currently supporting 35 Phase I and II clinical protocols for the Developmental Therapeutics Clinic, many of which include correlative pharmacodynamic assays developed with the Pharmacodynamic Assay Development and Implementation Section (PADIS) and genomic analysis in collaboration with the Molecular Characterization Laboratory (MoCha). One of the five new clinical trials opened this year was "Rapid Analysis and Response Evaluation of Combination Anti-neoplastic Agents in Rare Tumors (RARE CANCER) Trial: RARE 1 Nilotinib and Paclitaxel." Rare tumors constitute a heterogeneous group of cancers associated with limited treatment options and poor outcomes. Based on preclinical activity in rare cancer models, several drug combinations will be tested in patients with rare cancers in a series of connected Phase II clinical trials. Clinical responses may trigger further evaluation of a treatment in that rare cancer type to further evaluate response and mechanism of action.

Tumor Modeling and Drug Evaluation

KEY ACCOMPLISHMENTS

- New models developed and submitted to PDMR for quality control: 85 PDOrgs, 65 PDCs, and 90 cancer-associated fibroblasts (CAFs) *in vitro*; and 96 PDXs *in vivo*.
- 578 new specimens from 276 unique patients implanted into mice bringing the total implanted to 8,595 from 5,333 unique patients.
- Exploratory Rare PDX Tumor Studies: all 39 PDX tumor models have been implanted into mice. Four new models this period will be tested against 56 novel therapeutic combinations. To date, 128 drug combination studies have been completed or are in progress, each evaluating between six and eight drug combinations (total number of drug studies completed or in progress = 187). Numerous single-agent studies have been initiated due to the promising response observed in greater than 50 percent of the models for multiple combinations.
- Completed (i) 19 xenograft drug studies in support of the NExT Program, Natural Products Branch, PADIS,

and others; (ii) 18 toxicity evaluations for new single and combination drug protocols; (iii) 17 studies determining the ability of PDXs to grow in athymic rats; (iv) three studies to determine methods to reduce/eliminate estrogen-related toxicity in PDXs; (v) six studies to determine the mechanisms underlying a PDX model with tumor-associated coagulopathy; (vi) 73 PDOrg and 83 cell-line tumorigenicity studies; (vii) five proof-of-principle experiments using distinct tumor models completing the Department of Energy initial set of 10 PDX models for evaluation.

- As of June 2020, 376 PDX models, 95 PDC cultures, 188 CAF cultures, and 76 PDOrg cultures are publicly available for distribution from the PDMR, and over 1,000 vials of material have been distributed to PIs from academic and commercial institutions for research purposes.
- As part of the PDXNet consortium, the first pilot project to establish standards for preclinical study set-up and response evaluation across five institutions has been published. Additional efforts are underway using data from PDMR models in the *in vivo* lab and PDXNet members to push for standardization of *in vivo* response reporting.

Tumor and Natural Products Repository Operations

KEY ACCOMPLISHMENTS

- Successfully provided necessary services to NCI, including: completing approximately 5,900 slow-rate freezes consisting of over 67,000 vials, sending over 1,100 shipments totaling approximately 32,000 samples, receiving over 65,000 tumor and over 45,000 natural products samples for storage, and shipping almost 700 patient-derived model (PDM) samples in 55 shipments.

Computational Drug Development

KEY ACCOMPLISHMENTS

- Investigating computational chemistry techniques to work with empirical ultraviolet/electronic circular dichroism mobile electronic ionization phase mass spectrometry to provide computational support for the Natural Products Branch.
- P19892 (extracts from C25995) that are fully reduced hydroquinone congeners of Coenzyme Q10 that are cellular leads. Aspects of the research span a range of methods, from super-molecular assembly modeling (aggregation) through to molecular docking studies in Complex III binding sites. This effort is in collaboration with the Natural Products Branch.
- Development and testing of PRISM (Pattern Recognition Integrated with Structural Med-chem), a second-generation, web-based tool that organizes cellular leads by patterns of NCI-60 differential cytotoxicity into 3D pharmacophoric constructs (structure-based gas phase pharmacophore alignments). Extensions to the initial protocol use the resulting pharmacophore/spatial models to search the Protein Data Bank for potential targets or target homologs, producing a report with extensive links to relevant entries in structural and proteomic databases. Scoring functions derived from *a priori* confirmed test sets in crystallographic data are being developed.

Drug Synthesis of Combination Studies

KEY ACCOMPLISHMENTS

- The treatment regimens for many solid tumors include combination chemotherapy. Physicians within the NCI's Cancer Therapy Evaluation Program (CTEP) are evaluating new combinations of anticancer agents for future clinical trials. The aim is to perform *in vitro* pharmacologic profiling of these new drug combinations using patient-derived tumor cells to provide CTEP physicians with preclinical data to support decision making. The patient-derived cells are selected for the drug combination studies based on their histologies and known mutations. The patient cells are combined with stromal cells (HUVEC and hMSC) and allowed to develop for three days as complex spheroids prior to *in vitro* testing. Drugs are initially evaluated individually at nine concentrations up to their C_{max}, and concentrations for screening are selected for optimal sensitivity to drug synergies. The complex spheroids are exposed to combinations of drug(s) and/or investigational agent(s) for seven days before viability is measured using a sensitive luminescence-based readout (CellTiter-Glo 3D).
- During September, the team worked on a screen that evaluated combinations of either Trabectedin, Temozolomide, or Topotecan with one of 10 other anticancer agents for a total of 30 drug combinations. Both drugs tested in combination were evaluated at a range of doses resulting in a total of 900 drug concentrations being tested per complex spheroid model. The growth assay, single agent nine-dose assay, and combination screening were all completed by December for the entire set of 27 cell lines and the data have been provided to the NCI.
- Beginning in January, the team started working on a screen that evaluated combinations of either TAK-243 or Ipatasertib with one of 10 other anticancer agents for a total of 20 drug combinations. Both drugs tested in combination were evaluated at a range of doses resulting in a total of 600 drug concentrations being tested per complex spheroid model. The growth assay, single agent nine-dose assay, and combination screening were all completed by March for the entire set of 20 cell lines and the data have been provided to the NCI.

- A new combination project was approved to begin in March but is on hold due to COVID-19 and will resume after all staff are allowed to return to work. KRAS has been a challenging cancer target, and CTEP has an interest in obtaining new agents that target KRAS. The Drug Synthesis and Chemistry Branch has acquired both AMG-510 and MRTX-1257, which are selective for KRAS-G12C and have entered clinical trials. Currently, the molecular and cellular context that results in drug response or resistance to these agents is not well understood. The Molecular Pharmacology Branch will perform single-agent and drug combination studies using complex spheroid models that have been well-characterized for the status of KRAS and related targets. After single-agent activity has been evaluated with the RAS-directed agents and other drugs and investigational agents, the magnitude of additional cytotoxic activity with combination therapies will be determined with drugs and investigational agents chosen for relevance to KRAS, such as modulation of parallel or sequential pathways. This will help to define tumors that may be most responsive to the KRAS-directed agents as well as combination regimens that may warrant clinical trials. The screen will evaluate combinations of either AMG-510, MRTX-1257, or Tipifarnib with one of 13 other anticancer agents for a total of 39 drug combinations. Both drugs tested in combination will be evaluated at a range of doses resulting in a total of 1,170 drug concentrations being tested per complex spheroid model. A total of 20 patient-derived cell lines have been selected for growth as complex spheroids.

Identification and Delivery of Clinical Drug Candidates

KEY ACCOMPLISHMENTS

- Provided support for two NExT Chemical Biology Consortium projects in late stage lead optimization to more quickly generate the results needed to select a clinical candidate and for one project in early clinical studies for which additional preclinical mechanistic information was needed.
- The WDR5 project is seeking high-potency inhibitors that disrupt the interaction between the WDR5 protein and a histone methyltransferase subunit, MLL (mixed-lineage leukemia). These two proteins assemble into a larger complex with the RbBP5, ASH2L, and DPY30 proteins to form an active methyltransferase enzyme producing di- and tri-methylation of the lysine 4 site of histone H3, thereby exerting effects on epigenetic regulation and expression of numerous genes. The interaction between WDR5 and MLL has been shown by the project team to be essential for association of the complex with chromatin and seems to be particularly important for regulating expression of a subset of the ribosomal protein genes.
- The initial work to support WDR5 began in August 2018 with synthesis of lead compounds at Albany Molecular Research, Inc. in sufficient quantities to support *in vivo* studies. This work continued throughout this year, with quantities of lead compounds for *in vivo* studies ranging from 0.5 to 10 g being delivered each month. At the beginning of this reporting period, compounds were being tested in disseminated tumor models and in solid tumor xenografts. Also supported WDR5 project activities related to medicinal chemistry, binding assays, cell-based testing and mechanistic biology studies.
- The lead WDR5 compounds are tested in mouse pharmacokinetic (PK) studies following intravenous (IV) and oral dosing, and the analogs with the best properties are evaluated in solid tumor xenograft models for efficacy. Initial testing and signs of tumor growth inhibition had been observed in xenografts with the acute myelogenous leukemia (AML) cell line MV4;11. To expand the range of cancers that might respond to WDR5 inhibition, *in vivo* models with cell lines that were particularly sensitive to WDR5 inhibitors *in vitro* were established as xenografts. These cell lines represented Burkitt's Lymphoma (Ramos), follicular lymphoma (DoHH-2), mantle cell lymphoma (JeKo-1) and diffuse large B-cell lymphoma (OCI-LY10, SU-DHL-6). Two lead compounds were tested in each model. A good response was seen in the JeKo-1 study (60–70 percent tumor growth inhibition [TGI]), a moderate response of approximately 50 percent TGI was seen in the DoHH-2, SU-DHL-6, and OCI-LY10 xenografts, and a weaker response in the Ramos xenograft (less than 30 percent TGI). To examine if another AML cell line xenograft was similarly sensitive, the team assessed inhibition of Molm-13 cell growth as both a solid tumor and as a luciferase-expressing disseminated model. Only modest inhibition was observed in either experiment, and a small two-day survival advantage was realized in the mice harboring the disseminated Molm-13 cells. Following these studies, the team has settled on MV4;11 as the primary efficacy model, where TGI of 90 percent was observed, for evaluation of new lead compounds and additional testing of different dose levels.
- In addition to the studies in mice, the WDR5 team has examined the PK of the best lead compounds in rats to examine whether the good oral bioavailability and reasonable plasma half-life seen in mice were also observed in a second species. For some compounds this was not true. However, of the compounds where it was true, the PK of a select few has been measured in dogs to assess their potential for providing good exposure in larger, non-rodent species. Synthesis of the most promising compound to date has been prepared on a 10 g scale, and a 20 g batch is now being synthesized at AMRI. That compound was evaluated in a seven-day exploratory

rat toxicology study, with the histology results still pending. If it continues to fare well in subsequent studies, it will likely be declared a clinical candidate.

- Support has also been provided to the p97 AAA ATPase project. The AAA ATPase p97 is a master regulator of the unfolded protein response, in which it utilizes a suite of adapter proteins to extract proteins designated for degradation and transports them to the proteasome. Thus, inhibition of p97 leads to an increase in undegraded, ubiquitinated proteins that triggers a stress response, and when unresolved, results in apoptosis of the cells. Inhibitors of p97 that compete with ATP for binding to the enzyme have entered the clinic in the form of CB-5083, a first-generation inhibitor, and, within the last six months, as a second-generation compound CB-5339 that has an improved off-target profile (i.e., reduced inhibition of PDE6). The p97 project team is optimizing a series of lead compounds that inhibit p97 through a different, allosteric mechanism. These compounds bind in a crevice between the D1 and D2 domains of the protein to inhibit its activity.
- The p97 project activities encompass *in vivo* studies (PK, PD/PK, and efficacy) and the synthesis of lead compounds. The team implements an *in vivo* screening paradigm that evaluates the pharmacokinetic properties of lead compounds in mice following IV and oral administration, assesses the tolerability of the compound after five daily doses, and then conducts either a PD/PK assessment and/or tumor growth inhibition studies to determine efficacy. Xenografts with RPMI8226, AMO-1, and HL-60 cell lines have been tested with the lead allosteric inhibitor 801512. The best results were observed in the multiple myeloma–derived cell line RPMI8226, which has become the primary model to assess *in vivo* activity of the allosteric inhibitors. Compound 801512, the best lead so far, was evaluated in rats, where its PK was found to be comparable to that observed in mice. Additional assays to compare compound concentrations in whole blood to those typically measured in plasma indicated that no significant differences were observed, and thus measurements of compound in plasma would continue to be used. The activity of 801512 *in vivo* is not as good as that observed for CB-5083, but the recent tumor PK measurements for 821190 suggest that its sustained exposure over 24 hours may result in greater tumor growth inhibition than previously observed for any other allosteric p97 inhibitor. Additionally, two more sets of PD/PK studies are scheduled for the remainder of this reporting period to determine if any additional analogs are superior to CB-5083 and 801512.
- For the Thiodeoxycytidine (TdCyd) project, cellular mechanism-of-action studies and xenograft studies are underway. These studies have compared the potency of TdCyd and aza-TdCyd in U937 and MV-4-11 cells to the potency in derivatives of these cell lines in which production of the DNA methyltransferase 1 protein (DNMT1) has been blocked using CRISPR Cas9 technology to knock out the gene. The DNMT1 knockout derivative of the MV-4-11 cells was prepared and shown to have reduced sensitivity to both TdCyd and aza-TdCyd, similar to the previous observations with the U937 cells. Xenograft studies with the MV-4-11–derived lines showed the tumors of DNMT-1 KO cells grew slightly faster than the parental line and their growth was completely resistant to dosing with aza-TdCyd. Meanwhile, parental MV-4-11 xenografts failed to grow in the presence of aza-TdCyd, confirming the hypothesis that inhibition of DNMT1 is a requisite part of the mechanism of action for these compounds.
- These cell lines also have been tested for their sensitivity to fluorinated versions of TdCyd and aza-TdCyd. Similar conclusions were derived from these experiments, that loss of DNMT1 causes a reduced sensitivity to the fluorinated nucleoside analogs. Cellular viability studies with these inhibitors in the presence of Venetoclax have shown an increase in the amount of apoptosis. Because viability studies with these cells in the combined presence of the DNMT1 inhibitors and Venetoclax showed an increase in apoptosis, studies are now in progress to assess this combination *ex vivo* on leukemia cells isolated from patients.

Tumor Tissue Acquisition – Clinical Specimens

KEY ACCOMPLISHMENTS

- Due to COVID-19, accrual to the clinical trial this project supports has slowed.
- Theradex has completed Task Order 1 under its IDIQ contract. The Medidata Rave and the Web Reporting System builds for the P10231 clinical study have been completed and validated, and training of the clinical sites is underway.
- Training of the selected NCI Community Oncology Research Program clinical sites has been completed.
- Using a Prime Contractor, the clinical study has been operationalized using the IDIQ subcontract with Theradex. Theradex is currently completing Task 1 and 2 of this Task Order. Task Order 3 has been modified to accommodate a change in biopsy reimbursement level and a change in the medidata rave program to facilitate nearly automatic payments. The accrual to the clinical study is no longer on hold and is underway.
- The trial is currently under way. Patients have been biopsied and Theradex has been invoicing regularly. The individual sites have been receiving payments regularly, as well.

- A direct bill FedEx account to pay for the transfer of biopsy specimens from the clinical sites to Nationwide Columbus was established.

Canine Immuno-oncology

KEY ACCOMPLISHMENTS

- Canine cancers resemble human cancers both clinically and biologically. This provides an opportunity to evaluate many immuno-oncology combinations in dogs. One such target combination consists of two immune checkpoint modulators, CTLA-4 and PD1. To establish a canine model analogous to humans requires therapeutic antibodies for canine PD1 and canine CTLA-4. While canine PD1 therapeutic antibody is available, no such antibody targeting canine CTLA-4 (cfCTLA-4) is available. The Biopharmaceutical Development Program has generated mouse hybridoma clone 45H1 capable of blocking cfCTLA-4 binding to cfCD80. A chimeric 45H1 (murine-canine) antibody was created using the 45H1 antibody variable sequence and dog IgG B constant regions. A transient expression system was developed to produce chimeric 45H1 antibody molecule in bioreactors for production of clinical trial material.
- The Protein Expression Laboratory (PEL) continued to support protein production efforts throughout FY2020. In addition to providing productions of human and canine CTLA4 proteins for antibody production support, PEL also generated fragments of canine mismatch repair proteins to help identify antibody epitopes. PEL also produced soluble ICOS and PDL1 proteins as well as several monoclonal antibodies for use in the project.
- Plasmid vectors for production of ch45H1-B and ch45H1-D chimera constructs were made. However, efforts were focused on ch45H1-B as the ch45H1-D did not show CD80 blocking in PD studies.
- Small-scale transient transfection studies were performed to evaluate various methods for antibody expression. These studies indicated that the ExpiCHO system showed better antibody expression.
- Two-liter scale-up studies in the bioreactor were performed. Two bioreactor production runs at 2–3 L scale were performed. Various parameters of the bioreactor are now under study.
- A purification process is under development. Bioreactor harvests are used for purification development. Approximately 100 mg of ch45H1-B antibody was purified. The quality of the antibody is under study now.
- Assay development for estimation of bioreactor product is in progress. ELISA- and high-performance liquid chromatography–based methods are under study.
- Screening for antibodies that recognize canine PDL1 is ongoing. To date, hybridoma supernatants and subclone screening is underway.
- PEL has produced a series of protein fragments of canine MSH2 and MLH1 in order to support efforts to identify specific antibody epitopes of these proteins.
- Discovered PMS1 as a relevant protein binding partner of MLH1 in the mismatch repair protein complex in dogs and humans. Previously, very little had been reported about the role of PMS1 in mismatch repair. As a result of our findings, efforts are now underway to develop a custom monoclonal antibody to PMS1 for use in a quantitative PD marker assay. These studies will reveal its prevalence in mismatch repair deficiency and microsatellite instability and its underlying role in contributing to the sensitivity to immune checkpoint inhibitors in MMR-deficient cancer models and cancer patients.

A National Clinical Laboratory Network for NCI Precision Oncology

KEY ACCOMPLISHMENTS

- Two subcontractor laboratories have been established based on a competitive request for proposal process to support pharmacodynamic assays for the National Clinical Laboratory Network (NCLN). A Luminex laboratory to support tissue extraction–based sandwich immunoassays has been established at the Molecular Pathology Laboratory Network (MPLN) in Maryville, TN, and a multiplex immunofluorescence assay laboratory to support quantitative slide-based pharmacodynamic evaluations has been established at the University of Texas MD Anderson Cancer Center (MDACC) within the Division of Pathology and Laboratory Medicine. The National Clinical Target Validation Laboratory (NCTVL), within the Clinical Pharmacodynamic Program (CPP), serves as the clinical hub laboratory for the NCLN PD assays.
- During FY2020, MPLN has successfully supported the analysis of several preclinical specimens for ongoing critical CPP projects. Besides their scientific value, these studies have allowed MPLN to demonstrate continued proficiency on the required complex tissue fractionation and multiplex Luminex assay procedures.
- Transfer of the first priority assay to MDACC, DDR3: γH2AX, pNBS1 IFA with β-Catenin Segmentation has been an ongoing focus for the team during FY2020. Although laboratory harmonization efforts began in FY2019, we have faced significant personnel turnover in the laboratory and other technical and procedural challenges in establishing the required IT infrastructure at MDACC to support the image database, specialized analysis software and engines. As of this report, the reorganization and

restaffing of the laboratory have been completed and our project is moving forward at the expected rate. Operator certification has been completed for one operator on the quantitative image analysis process. Operator/laboratory certification for the slide staining and scanning procedures are underway. Task order modification to add tissue processing to the MDACC subcontract was executed. Critical equipment for the tissue processing procedures were installed at MDACC during FY2020. Initial evaluations to confirm satisfactory tissue processing at this laboratory are underway.

- NCTVL is the hub lab to support NCLN assay harmonization activities and clinically implemented validated PD assays for ongoing DTC and select external trials, that are not yet available within the NCLN laboratories. During FY2020, NCTVL supported clinical trial biopsy analyses for DDR3: γH2AX, pNBS1 IFA with β-Catenin Segmentation, EMT Panel IFA and Rad51 IFA with β-Catenin Segmentation for several Developmental Therapeutics Clinic and Experimental Therapeutics Clinical Trials Network (ETCTN) clinical trials.
- Several assays are undergoing a "harmonization exercise" between PADIS and NCTVL to establish streamlined procedures. PADIS has completed the transfer of two Luminex-based multiplex immunoassays to NCTVL. PADIS and NCTVL are also preparing for the transfer of three additional signaling multiplex panels that have completed validation by preparing SOPs and appropriate proficiency samples. Additionally, PADIS has completed work to validate the use of cell pellets prepared from blood and bone marrow aspirate samples for use with the apoptosis multiplex required tissue fractionation procedure to extend the use of this important assays to support clinical evaluations of patients with hematologic malignancies. For the immunofluorescence assay (IFA) efforts, during FY2020, the Rad51 IFA with β-Catenin Segmentation assay was transferred to NCTVL and a triplex assay for pHH3, pY15cdk, and β-Catenin is being finalized for transfer to NCTVL and MDACC. A new Definiens analysis algorithm build has been completed that will allow rapid transfer of additional DNA damage response marker IFA assays to NCTVL and MDACC. The Internal Quality Control Laboratory group is supporting the finalization of SOPs for assays planned for the near-term transfer in addition to establishing a supply chain and quality control plan for all required assay critical reagents.
- The clinical PD's internal quality control laboratory has continued support for numerous clinical validated PD assays that are in active use, including those that are active within the NCLN and NCTVL hub laboratory. This includes managing the procurement or production, analytical, and performance quality control testing and inventory and distribution management for a broad range of commercial research reagents, custom reagents including conjugated antibodies, and custom calibrator and control materials.
- Due to the PD group's recent Epithelial Mesenchymal Transition (EMT) assay *Cancer Res* paper (https://cancerres.aacrjournals.org/content/early/2019/11/15/0008-5472.CAN-18-3539), the full set of EMT SOPs for the validated PD assay have been prepared for posting to the Division of Cancer Treatment and Diagnosis (DCTD) website for inclusion in the growing library of detailed procedural documents provided by the group to support the clinical research community. Additionally, the DTCD Biomarker website has been updated to make the biomarker information for NCLN PD assays more accessible to clinical investigators.
- Work has begun with CTEP and Theradex to coordinate the lab data management and integration with the clinical trial data within Medidata RAVE (Theradex). Data reporting templates for the PD assays have been finalized and work to integrate them within RAVE has begun.
- The task order with Nationwide Children's Hospital (NCH) for biospecimen support for NCLN studies was fully executed. We have been working with NCH to operationalize all required workflows and data tracking procedures to provide the required support for the sample accrual, shipment, tracking, and distribution to the NCLN PD laboratories.
- Work continues with CTEP and ETCTN investigators to support the review and approval of letters of interest and protocols for NCLN assay support. Currently, we have nine clinical trial protocols provisionally or fully approved to receive support for NCLN PD assays.
- The Molecular Characterization Laboratory continued NGS assay development for TSO500 ctDNA, and validation began in February 2020. The validation has been delayed due to COVID-19, and now completion is expected in October 2020. MoCha is developing a streamlined whole-exome sequencing (WES) protocol using Illumina Nextera library prep with Twist exome enrichment, which performed well in early feasibility testing. Development testing and planning is in progress. Validation for Nextera/Twist is expected to start in August and be completed in late 2020. These assays will be the NCLN-approved liquid biopsy and WES assays used for ETCTN studies.
- Samples have been acquired from MD Anderson and Discovery Life Sciences for use in WES and RNAseq NCLN harmonization. Two harmonization runs will be done at each laboratory, and performance metrics are being developed for acceptance criteria. Sample collections for liquid biopsy NCLN harmonization are in progress.

- With the return of some staff from the COVID-19 suspension, TSO500 validation and Nextera/Twist development are the top priority projects in the R&D lab. Reinitialization of equipment has proceeded successfully, and library preparation and validation/development activities have been restarted.
- Nationwide Children's Hospital has aligned their sample receipt, accessioning, histological processing, extraction, and quality control protocols with MoCha's as of February 2020. Nationwide did not shutdown during the COVID-19 pandemic and has continually received samples for NCLN assays from ETCTN study sites.
- MD Anderson's NCLN NGS Lab experienced a major period of turnover, but all NCLN-associated vacancies have been filled as of May 2020. Proficiency testing and harmonization training by two technicians was completed. A third, part-time, technician shadowed refresher and proficiency trainings and completed her training. The MDACC NCLN immunohistochemistry (IHC) lab is working to develop a hybrid IHC protocol for detection of HER2 in osteosarcoma patients for an upcoming ETCTN study.

High-plex Pharmacodynamic Biomarker Assay

KEY ACCOMPLISHMENTS

- The PADIS Zeiss Axio Scan was updated with new filters. The new single-pass filters were installed so the Axio Scan could produce equivalent images to Ultivue, which was necessary because Ultivue will be developing our custom Ultimapper kits for the IO-PD Multiplex IFA panel with their DNA barcode technology. Through quantitative image analysis, it was demonstrated that both Axio Scans produced equivalent images and are harmonized. Also established scanning profiles for the Axio Scan for use on biopsy samples and locked down the layout (biopsies and tissue fixation controls) of clinical specimen slides.
- Paired ASPS biopsies from eight patients in CTEP 10005 were sectioned and paraffin dipped slides were sent to Ultivue for staining with the PD-L1 and PD-1 Ultimapper kit. Normally slides would be stained by Alizee and scanned by us but, due to COVID-19 operating restrictions, we had Ultivue stain and scan the slides. Quantitative image analysis is currently being performed.
- Currently working with Ultivue to produce two custom kits for our immuno-oncology multiplex IFA panel. One kit is specifically developed for ASPS biopsies. It will consist of CD8, PD-L1, CD3ζ pY142, and TFE3. The other kit will be CD8, PD-L1, CD3ζ pY142, and β-Catenin and is specifically developed for carcinoma biopsies. Once the kits are validated by Ultivue and PADIS, we will stain our first biopsies with our IO-PD Multiplex IFA panel and begin development of other immuno-oncology multiplex IFA panels.
- PEL continued to support protein production efforts throughout FY2020. Major efforts included production of CP110 protein fragments that were analyzed for phosphorylation of various residues and were then co-expressed with CDK2 kinase to improve phosphorylation of those sites. Similarly, DLC1 protein fragments were produced and tested for phosphorylation, and a number of these proteins were successfully generated and delivered for assay optimization. Additional proteins were generated in the RIP kinase/MLKL1 family including new heterodimers of RIPK1-MLKL1 and RIPK3-MLKL1, and the Gasdermin family (GSDMD and GSDME). Additionally, a large effort was carried out to generate useful RPS6 protein. This involved more than a dozen purifications under different conditions to find those that optimized stability and assay performance of this vital reagent.
- Development of Topoisomerase I cleavable complex and total topoisomerase 1 assays and implementation of the first-ever PD marker characterization and mechanism-of-action studies of the DS-8201a antibody-drug conjugate (anti-Her2 monoclonal antibody linked to a Deruxtecan topoisomerase inhibitor payload). PD marker evaluation includes assays to measure induction of Topoisomerase I cleavable complex and total topoisomerase 1 biomarker target engagement as well as downstream DNA repair response markers in preclinical xenograft models varying in Her2 expression. To date, we are the only lab currently able to do these immuno-fluorescent assays as proof of mechanism in tissue.

PDX Panels from Common Tumors

KEY ACCOMPLISHMENTS

- Expansion of the current approach in PDX modeling to generate fully characterized and quality control of approximately 560 new PDX models to add to the PDMR collection continues. Once added to the approximately 440 models estimated to be in the PDMR repository, the collection will reach its goal of 1,000 well-characterized, clinical annotated PDX models.
- During the past quarter, the following *in vitro* cultures were submitted to the repository: 37 PDOrgs, 20 PDCs, and 41 CAFs. This brings the total number of models that are either public or going through final QC to 172 PDOrgs, 189 PDCs, and 257 CAFs. For *in vivo* models, the total number of samples implanted into mice increased by 163 (current total: 8,531 samples). The number of models with more than 200 viably cryopreserved vials increased by 30 to a total of 616 models in the repository.

- Analyzed apoptosis multiplex data from MPLN (NCI's ETCTN Clinical Network Laboratory), processed by DCTD SOP341401 and SOP341402 (apoptosis panels 1-3). Also integrated additional data generated in-house on apoptosis biomarkers, BIM and BAK multiplex, apoptosis panels 4-5.
- Started compiling c-MET inhibitor data from 44 PDX models that were earlier analyzed with cMET IFA and ELISA (DCTD SOP341201, SOP3412023 and SOP341206). This work is ongoing and would lead to potential models with aberrant MET signaling for follow-up analysis.
- The status of the patient-derived model (PDM) tumor characterization and metastasis search is shown in Table 1. Tumor characterization includes: modalities and number of sessions per mouse; PET ([18F]FLT for cell proliferation and [18F]FDG for metabolism); Ultrasound (3D volumes and microbubbles for tumor perfusion) and MRI (non-contrast to evaluate tumor morphology and search for metastasis)]. [18F]FSPG (glutamate analog) will also be utilized if warranted.

Table 1. Overview of PDM Models for Tumor Characterization and Metastatic Search

PDX Model	Diagnosis	Gender	Imaging Status	LASP/Implants (Passage #)	PET SUVmax	Modalities (# sessions/mouse)
BL0293-F563	Bladder Cancer	F	Closed	10 (NA+10)	FDG (3.4 ± 0.8)	MRI (2); FDG (1)
144126-210-T	Neuroendocrine	M	In Progress	10 (3) 40 (4)	FLT (0.5 ± 0.2) FDG (1.2 ± 0.5)	MRI (3); FLT (1) MRI (3); FDG (1)
146476-26-R	Urothelial/Bladder Cancer	M	In Progress	5 (3) 20 (4)	FDG (1.3 ± 0.2) FLT (0.8 ± 0.3) FSPG (0.6 ± 0.2)	MRI (6) MRI (4); FDG (1); FLT (1); FSPG (1)
833975-119-R	Adenocarcinoma pancreas	F	Closed	5 (1) 20 (2)	FDG (1.8 ± 0.2) FLT (3.5 ± 0.3) FSPG (0.9 ± 0.2)	MRI (7) MRI (4); FDG (1); FLT (1); FSPG (1)
779769-127-R	Adenocarcinoma-rectum	F	In Progress	5 (4) 20 (5)	FDG (1.7 ± 0.2)	MRI (3) MRI (3); FDG (1)

- A summary of the PDM models that were observed to have metastasis by MRI and confirmed by the Pathology/Histotechnology Laboratory (PHL) was tabulated (location of metastasis; # animals in the group with confirmed metastasis, days to metastasis, and # of PDM models that metastasize without tumor excision) (Table 2).

Table 2. Recently Received PDM Models

Tumor ID (Model)	Oracle Diagnosis	Mice	Gender	Fragment Repository
144126-210-T	Neuroendocrine	Donor (10)	M	X

- The PDM characterization scheme was modified to improve the denominator for calculating the percent of tumor bearing mice with metastasis. Twenty fragment-implanted mice will be enrolled into a group, either resected or non-resected. Mice placed into the resected group will have their tumors resected when tumors reach a volume of 200–300 mm^3. Mice in the non-resected group will allow their tumors to grow until they reach Animal Care and Use Committee limits or until metastasis is resolved in an MRI scan and the mouse is transported to PHL for confirmation of human tissue metastasis.

Histology/pathology lab progress for the reporting period includes: histological processing and pathology review of 7,237 formalin-fixed, paraffin-embedded (FFPE) PDX samples; hand microdissection and pathology review of 807 fresh frozen PDX samples; laser capture microdissection and pathology review of 65 fresh frozen PDX samples; IHC assays completed for 447 FFPE samples; five IHC assays (AR, PR, ERG, CDX2, and CD34) developed and optimized (for a total of 44 assays to date); three additional IHC assays (p53, PAX8, and p16) are currently being developed and optimized; 109 PADIS clinical samples from 42 patients underwent tumor processing; and tissue processing was completed for five MPACT samples.

- Next-generation sequencing assay progress for the reporting period includes: nucleic acid extraction, quantitation, and quality assurance for all sequencing queue samples prior to sequencing; whole-exome sequencing of 1,142 PDX samples; RNA sequencing of 1,166 PDX samples; whole-exome sequencing of 219 patient germline samples matched to PDX samples for identification of somatic mutations; whole exome sequencing of 70 fibroblast samples used as patient germline specimen 'surrogates' for models with no available germline samples. MoCha also completes molecular characterization of companion *in vitro* samples such as organoids and cell culture lines. Whole-exome sequencing and RNA sequencing of 38 cell culture samples derived from PDXs have been completed.
- In addition, low-pass whole-genome sequencing is completed on select pre-clinical drug study samples to determine genomic stability from passage to passage. For each PDX model, MoCha performs molecular characterization of at least four specimens. To date, MoCha has delivered molecular characterization data to the PDMR database for 540 models for internal use and 386 models for public use, with whole-exome sequencing and RNA sequencing of an additional 200 PDX samples underway.
- Technological progress includes completing the transition of sequencing assays to Illumina's NovaSeq 6000 sequencing platform. Samples were previously sequenced on Illumina's HiSeq 2500 sequencing platform. Bridging experiments were conducted to ensure that results from both sequencing platforms are comparable. Utilizing the NovaSeq platforms has greatly increased sample throughput and decreased per-sample costs. MoCha also investigated and evaluated multiple methods to identify the preferred method to detect gene fusions in samples for PDX-related applications.
- Several new lab staff have been onboarded in the MoCha R&D NGS lab. These staff were trained and have demonstrated proficiency in the whole-exome sequencing and RNA sequencing workflows. These

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Tumor ID (Model)	Oracle Diagnosis	Mice	Gender	Fragment Repository
144126-210-T	Neuroendocrine	Donor (10)	M	X

Table 2. Recently received PDM models.

additional trained staff provide staffing redundancy to maintain continuity of operations and increase NGS sample throughput for the PDM project.

- MoCha's bioinformatics team continues to routinely complete tasks that are essential to characterize PDM project samples, such as: sequencing data quality assurance; data processing; management, storage, and archiving of data; pipeline creation and optimization; data analysis; and uploading of analyzed data to the PDMR database. Standard data analysis includes variant detection, copy number alteration assessment, fusion calling, gene expression analysis, and signaling pathway investigation. Recent data pipeline additions and improvements by the MoCha bioinformatics team include: implementing microsatellite instability (MSI) assessment; consensus variant calling; developing an HLA typing algorithm; and OncoKB variant annotation. SOPs were drafted for these additions to the pipelines. Data analysis and visualization support was also provided for American Association for Cancer Research and American Society of Clinical Oncology abstracts, posters, and manuscripts.

The Cancer Imaging Archive (TCIA) Support

KEY ACCOMPLISHMENTS

- Imaging data from clinical trials provides the opportunity to link imaging characteristics to clinical trial analyses, associated clinical data and patient outcomes. Beginning in 2019, the NCI Cancer Imaging Program Informatics Lab began supporting an additional The Cancer Imaging Archive (TCIA) image data collection center focused specifically on clinical trial data. Many TCIA clinical trial data sets will originate from NCTN, which is a collection of organizations and clinicians that coordinates and supports cancer clinical trials at more than 3,000 sites across the United States and Canada. NCTN provides the infrastructure for NCI-funded treatment, screening, and diagnosis trials to improve the lives of patients with cancer. NCTN explicitly requires data sharing of the patient-level clinical data, and TCIA serves as the imaging repository for that archive.
- The Frederick National Laboratory for Cancer Research (FNL) Cancer Imaging Informatics Laboratory collaborated with the NCI Cancer Imaging Program (CIP) to complete the SOP that will be followed for obtaining permission to process NCTN trials. Seven trials have been selected for the processing queue by CIP federal staff. The scope of work for a new TCIA data collection center contract was also drafted, and FNL contracting finalized the award with the NCI Imaging and Radiology Core (IROC). The Cancer Imaging Informatics Lab also coordinated with NCI CIP to form the TCIA Clinical Trial Stakeholder Committee, which will facilitate communication between key stakeholders at CIP, CTEP, and NCTN to keep them updated on accrual progress.
- Deployed IT infrastructure and trained IROC on TCIA submission processes. A fully operational satellite TCIA curation center at IROC now operates a pipeline for de-identification and hosting of imaging data from NCTN clinical trials, resulting in successful processing of the CALGB-50303 trial and substantial progress on the S0819 and AHEP0731 trials. In addition to NCTN trials, this activity also supported data collection from two sites as part of the Integrated Canine Data Commons (ICDC) pilot, which seeks to augment the ICDC system with imaging data from a glioblastoma study.

The Use of Rare and Recalcitrant Cancers for Establishing the Predictive Power of Patient-Derived Xenografts

KEY ACCOMPLISHMENTS

- For the Exploratory Rare and Recalcitrant PDX Tumor Model Rolling Drug Studies, all 39 tumor models to be tested have now been implanted. For the drug combination studies, 141 studies representing 6-8 drug combinations per study have been completed. Additionally, for 13 drug combination sets that showed activity, 48 studies evaluating the single agents versus drug combinations are either in progress (20 studies) or have been completed (28 studies).

Underlying Pharmacology of Brain Tumor Drug Treatment Response and Failure

KEY ACCOMPLISHMENTS

- Placed an order with Indivumed for 20 brain tumor biopsy specimens collected under controlled conditions during surgeries. The brain tumor biopsy specimens have been received.
- CDC diagnostic lab testing revealed that the brain tumor specimens ordered from Indivumed are prion free. Some of the specimens have been submitted for paraffin sectioning and analysis of cell death pathways and cell signaling pathways using validated assays from PADIS.
- A subset of biopsy specimens was analyzed successfully using IFA without interference from background fluorescence. Another subset of biopsy specimens was analyzed successfully for extractable biomarkers, enabling the future analysis with multiple validated assays.
- ADRD and MoCha met with DCTD and NIH's Neuro-Oncology Branch to begin a collaboration to collect brain tumor specimens for research and to leverage each group's knowledge about specimen analysis.

- Project staffing has been affected by the change of delivery of the new building from August 2019 to fall 2020.

MDNet

KEY ACCOMPLISHMENTS

- For the three MDNet initiatives (myeloMATCH, ComboMATCH, and ImmunoMATCH), scientific leaders have taken active roles planning for the initiatives, providing expertise for biomarker and assay selection decisions, and participating in various working group activities. Work is under way to convert the required clinical test portfolio into subcontracts to establish and operationalize the testing network.
- MoCha continues to investigate assays for use in the myeloMATCH trial. Specimens have been sourced for a pilot experiment to evaluate and compare myeloid-specific ThermoFisher and Illumina NGS sequencing assays. Genexus instruments, the ThermoFisher platform to be used for the myeloid NGS assay, have been purchased and delivered to the MoCha lab. Installation of the Genexi as well as training, feasibility, optimization, and validation of the myeloid assay were delayed due to COVID-19–related restrictions on non-essential lab activities.
- A first source request for proposal\was issued for a subcontract to provide clinical assay support to myeloMATCH. The response was received, and a source evaluation group meeting was held to evaluate the response to the proposal.
- The focus of the ComboMATCH initiative is adapting the current MATCH laboratory network to the MDNet focus. MoCha participates in discussions of relevant biomarkers, patient identification strategies, and specimen management at the biorepository. Representatives also attend meetings to provide feedback on proposals for treatment combinations. Two combinations have been approved thus far, both with HER2 as a biomarker. The Molecular Biomarkers and Specimen Management Working Group continues to discuss relevant biomarkers and patient identification strategies, as well as specimen management at the biorepository.
- The focus of the iMATCH initiative has been to define the required clinical tests; a pilot iMATCH study with a smaller cohort of patients is being planned and may launch at the end of this year.
- MoCha is in the process of developing a clinical whole-exome sequencing assay for use in the initiative. Development of the assay has required designing a customized probe set to meet specific project requirements. Feasibility testing, optimization, and analytical validation of the assay were delayed due to COVID-19–related restrictions on non-essential lab activities.
- Methods/assays to measure tumor mutational burden and generate tumor inflammation signature(s) using gene expression data are being investigated.

Imaging Support for Apollo 5

KEY ACCOMPLISHMENTS

- The purpose of this task order is to collect, curate, and archive APOLLO-associated clinical images (both *in vivo* and histology images) in TCIA— an existing public resource supported by NCI and implemented by FNL's Cancer Imaging Informatics Lab—and make it available along with generated feature data for correlation with the genomic, proteomic, and clinical data in all APOLLO analyses, including secondary public use after applicable embargoes. Building on a successful pilot with the Veterans Administration, The APOLLO imaging team continued its collaboration with the VA rePOP technical team to set up an internal image processing infrastructure and workflow that will feed TCIA. With final VA IT approval, the full on-site system will be operational and queued rePOP APOLLO data will be processed and collected. Full integration with APOLLO crosswalk identifiers was completed. The VA system that will pull imaging from VA Veterans Integrated Service Networks integration was tested. Discussions continued on organization of imaging data that comes through rePOP and APOLLO protocols as well as plans to use APOLLO external IDs to the existing data on TCIA/GDC.
- To facilitate data flow from APOLLO civilian site members, a pilot to collect and post radiology and pathology data from University of Virginia (APOLLO 1 lung cancer data) was completed and a workflow was established to collect lung data under APOLLO 5 from University of Virginia.
- The APOLLO2 Ovarian proteogenomic-imaging collaboration team, facilitated by this task order, completed a hypothesis-generating analyses of non-APOLLO image data set as a model for the ongoing analysis of the 52 APOLLO2, which was published in *Eur Radiol*.
- A histopathology imaging data workflow was established to collect imaging from the Joint Pathology Center and Research Pathology Center, and a pilot that will be applied to the prospective APOLLO-5 data was completed with the APOLLO 2 data set.
- Initial development of the requirements of an Imaging Characterization Center service that will perform image quality review and analysis of the APOLLO imaging data collected on TCIA in Task 1 of this Project is continuing along with planning in coordination with APOLLO program for informatics extracted quantitative data workflow to the APOLLO data tracking system.

- The project completed an extensive review of APOLLO-wide data flow scenarios, has developed a draft data collection point strategy document, participates in relevant APOLLO working groups (e.g., Clinical, Data Analysis, Data Repository, and Informatics Task Force), and leads the Clinical Imaging Subgroup (coordination of APOLLO imaging), VA Synchronization working group (resolve remaining workflow policy issues), and VA APOLLO Imaging De-identification Workflow Task Force (deployment of imaging workflow at the Veterans Administration). The Department of Defense Military Health System Capability Assessment Request form was completed and is awaiting approval by Walter Reed National Military Medical Center and APOLLO in order to obtain approval for imaging collection workflow at Walter Reed.

Natural Products Discovery

KEY ACCOMPLISHMENTS

- The first goal is to generate 185,000 pre-fractionated high-throughput screening–suitable samples per year. This goal involves pre-fractionation of natural product extracts based on reversed-phase C_8 solid-phase extraction chromatography, generation of 55 copies of the prefractionated library in 384-well plates, and storage of the fraction library in a dedicated sample repository. In January 2020, the program delivered 55 copies of 176,000 prefractionated natural product samples in 384-well plates, totaling 1,408,000 individual wells. Prefractionation production increased from four to six runs per week and at peak capacity produced over 15,000 fractions per month. Before suspension of laboratory work, new fraction production totaled 84,728 fractions (Figure 1). The total number of fractions produced and stored in the Brooks Repository in Building 433 to date is 414,259 samples.

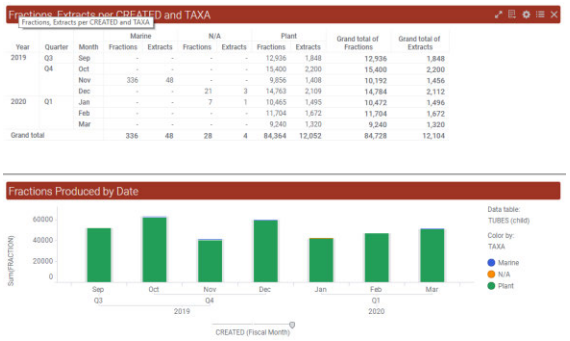


Figure 1. FY2020 fraction production metrics.

- The program has continued to improve performance and reduce costs. A tip washer for the 384-head robotic arm was installed in the Hamilton liquid handler, which has resulted in significant savings for the purchase of plastic tips. In addition, a new quality control measure was implemented, and ultraviolet-visible spectroscopy (UV-VIS) spectra of each fraction are now recorded and stored in a database. This required the generation of an additional (56th) 384-well plate, integration of a UV-VIS spectrometer into the Hamilton liquid handler workflow, and development of scripts and software to process the data (Figure 2).

Figure 2. UV-VIS analysis of the fraction library.

- The second goal of this task order is the use of second-stage rapid purification and scaleup workflow for follow-up high-throughput screening. Second-stage rapid compound purification was designed as a follow-up to initial screening of the NCI Program for Natural Products Discovery (NPNPD) fraction library and provides purified or semi-purified natural products in a 96 deep-well plate format for further secondary screening. In FY2020, the program processed 777 hit fractions from four different HTS campaigns and generated 17,871 secondary fractions for follow-up screening.
- Development and validation of the second-stage purification methodology was presented at the Gordon Research Conference on Marine Natural Products, Ventura, CA, February 2020), and

published in one of the leading journals in the field (Grkovic et al., *ACS Chem Biol*, 2020). The work was highlighted as the August 2020 cover article.

- The third goal of this task order is the addition of fungal extracts and fractions to the NCI repository collection. This goal is focused on the expansion of the NPB microbial culture collection with geographically and taxonomically diverse microorganisms. This involves processing and storing Citizen Science Soil Collection Program–sourced microbes from the University of Oklahoma as well as 1L-scale fermentation, harvest, and extraction of 100 cultures per week.
- The program grew 2,134 isolates and analyzed the cultures for viability and contamination. A total of 12,804 cryo-vials containing pure fungal cultures were deposited in the repository. In addition, methods development for the high-throughput, high-capacity liquid fermentations and extractions was completed and validated, and the production in the new laboratory in Building 433 was initiated.
- The program continued to build a database of microbial isolates that included information on the origin of samples, genomic information, growth conditions, and description of physical characteristics of microbial culture (Figure 3).

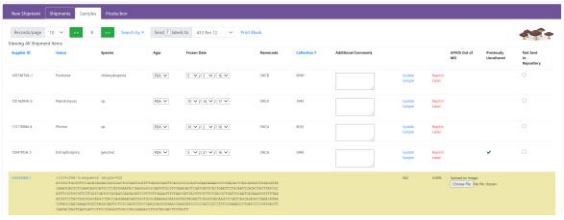


Figure 3. NPNPD fungal database.

- The fourth and final goal of this task order is the development of a bioinformatics platform capable of integrating genomic data, taxonomy, biological activity, and chemical structure. The program continued working on the following databases: Prefractionation – a database able to capture information on the generation of fractions, weight of each fraction, and plating of fractions on 96- and 384-well plates; Second Step Tools – a database containing a record of hit fractions and subfractions generated and sent to screening labs; and Fungal Shipments – a database containing information on new Oklahoma shipments received in the lab, samples processed on agar plates, as well as samples grown in large scale 1L flasks (Figure 3). In addition, new data analysis software, TIBCO Spotfire, was purchased and implemented as an accessible, user-friendly data visualization tool (Figures 1 and 2).

Support Provided by the Biomedical Informatics and Data Science Directorate

Integrated Canine Data Commons

KEY ACCOMPLISHMENTS

- Produced the minimum viable product for the Integrated Canine Data Commons (ICDC)
- Engaged the community by establishing an active steering committee and subcommittees

NCI's Division of Cancer Treatment and Diagnosis, with the Center for Biomedical Informatics and Information Technology's (CBIIT) guidance and close coordination, tasked FNL with developing the ICDC. In collaboration with FNL's Applied and Developmental Research Directorate, the Biomedical Informatics and Data Science (BIDS) Directorate's CBIIT Technical Operations Support team has developed a prototype of this new Cancer Research Data Commons (CRDC) node, which is the first that is not data-type-specific.

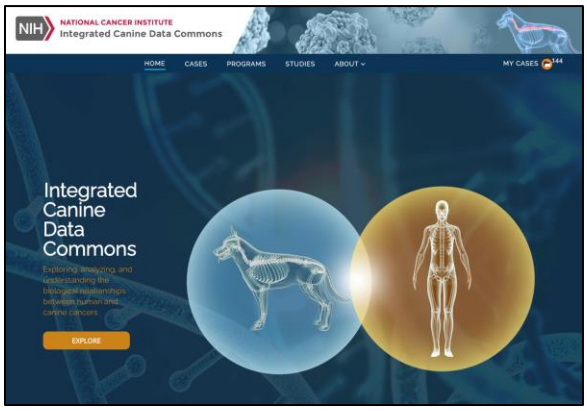


Figure 1. Home page of the ICDC.

In developing the ICDC to be flexible and expandable, the team developed a data model that can accommodate practically any study type and be expanded as required by the community. The system currently contains data for two pilot studies: a longitudinal study consisting of mainly clinical metadata, and a tumor-normal study with genomic data. The ICDC has five additional studies in the data pipeline and will soon expand to include whole-genome sequencing, whole-exome sequencing, methylation sequencing data, RNA-Seq data, and imaging data such as magnetic resonance imaging and quantative slides. A steering committee for ICDC was formed and has been actively advising NCI and FNL on topics such as types of data for ICDC, priorities for specific study ingestion, and standards for the community to use when submitting and collecting data. The steering committee is composed primarily of non-NIH members to ensure the voice of the community is driving the project. The ICDC is currently in production mode and has been "soft

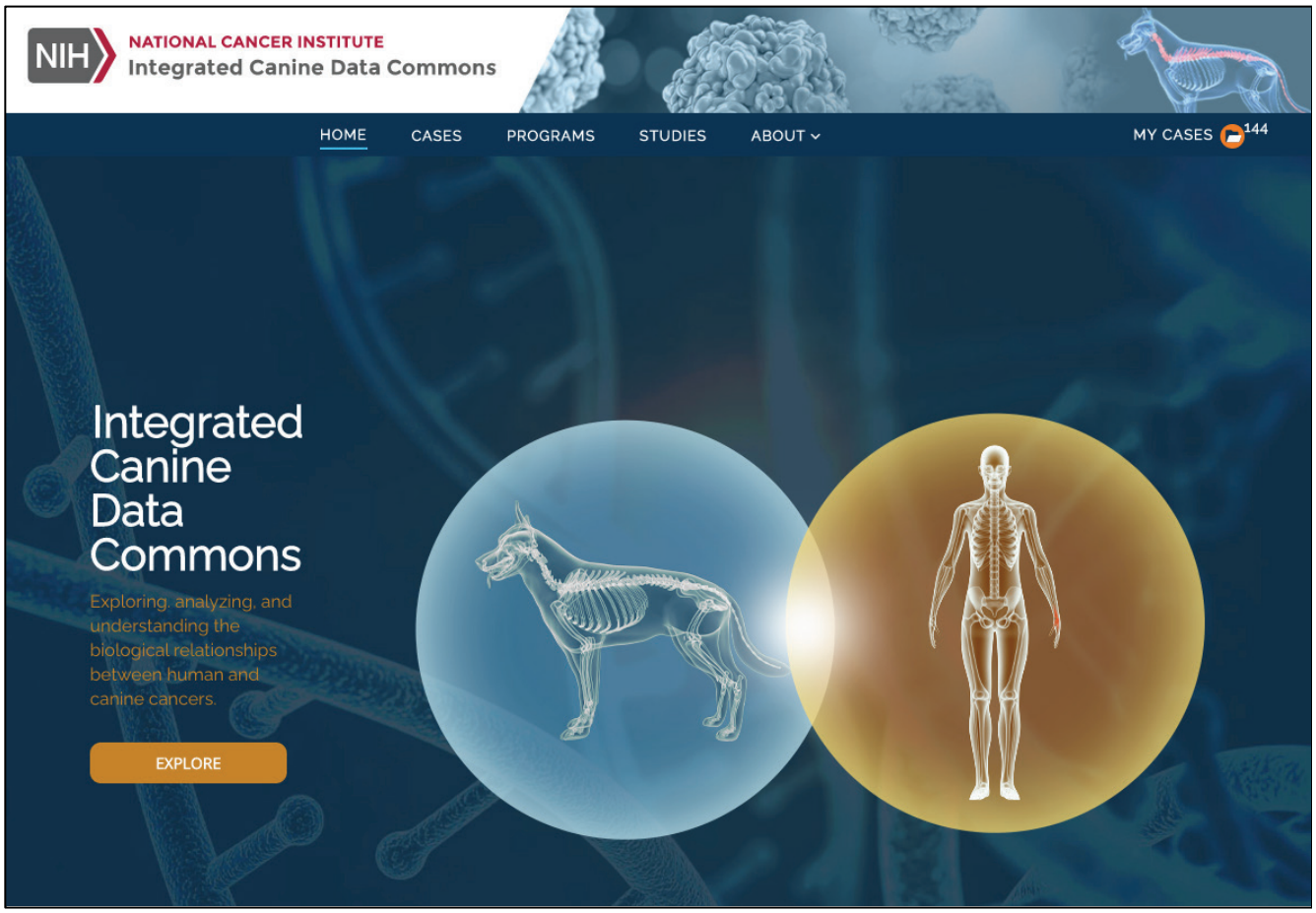


Figure 1. Home page of the ICDC.

launched" to the ICDC team until such time as we have data of sufficient quality and quantity.

ICDC expects to have that data by mid-to-late July 2020 and then work with the CBIIT Communications Team to more broadly announce the platform. As part of the CRDC, the ICDC enables users to find data files to be used in analysis and connects those files in one of the CRDC's analytical resources, Seven Bridges Cancer Genomics Cloud. As ICDC moves into the next phase of this project, the team will focus on automating data ingestion, gathering user feedback, delivering updated and new functionality, and allowing interoperability with the Center for Cancer Data Harmonization and Cancer Data Aggregator portions of the CRDC.

Figure 2. An example Cases page of the ICDC.

Support Provided by the Biopharmaceutical Development Program

KEY ACCOMPLISHMENTS

The Biopharmaceutical Development Program has been tasked with clinical production of two monoclonal antibodies: (1) hAnnA1-based immunoconjugates for cancer detection and treatment, and (2) hJAA-F11, a humanized anti-Thompson Friedenreich antibody for cancer therapy. Two contract research organizations have been engaged to generate a Chinese hamster ovary suspension cell–based expression system. The work is currently in progress, and the stable cell lines would be available at the Biopharmaceutical Development Program in mid FY2021.

Epstein-Barr Virus gp350-Ferritin

The final product EBV gp350-Ferritin virus-like particle was tested and released according to the approved master specifications. A diluent lot was also manufactured and released. Final product vials were also provided for a toxicological study. A report on the syringe stability study was provided to the principal investigator. Manufacturing reports; chemistry, manufacturing, and controls; and regulatory support were provided to enable the National Institute of Allergy and Infectious Diseases (NIAID) to file an Investigational New Drug application. NIAID successfully filed the application on the July 16, 2020.

Cell-Based Immunotherapy Products

CD33 CAR T-Cell Therapy

The CD33 Chimeric Antigen Receptor (CAR) T-cell therapy Investigational New Drug (IND) application was cleared to start in 2019 with two subjects enrolled in the first half of 2020. The Biopharmaceutical Development Program manufactured and released the first two products in 2020, and the two subjects were treated. Two IND chemistry, manufacturing, and control amendments were also provided to the IND sponsor for updates to quality control procedures and specifications.

CD2 CAR T-Cell Therapy

Technology transfer and process development for the GD2 CAR T-cell therapy product was completed. Assays were established and assay qualifications will be completed prior to the start of the clinical trial. The IND Module 3 quality section was completed, and the IND was filed to the FDA in the third quarter of 2020.

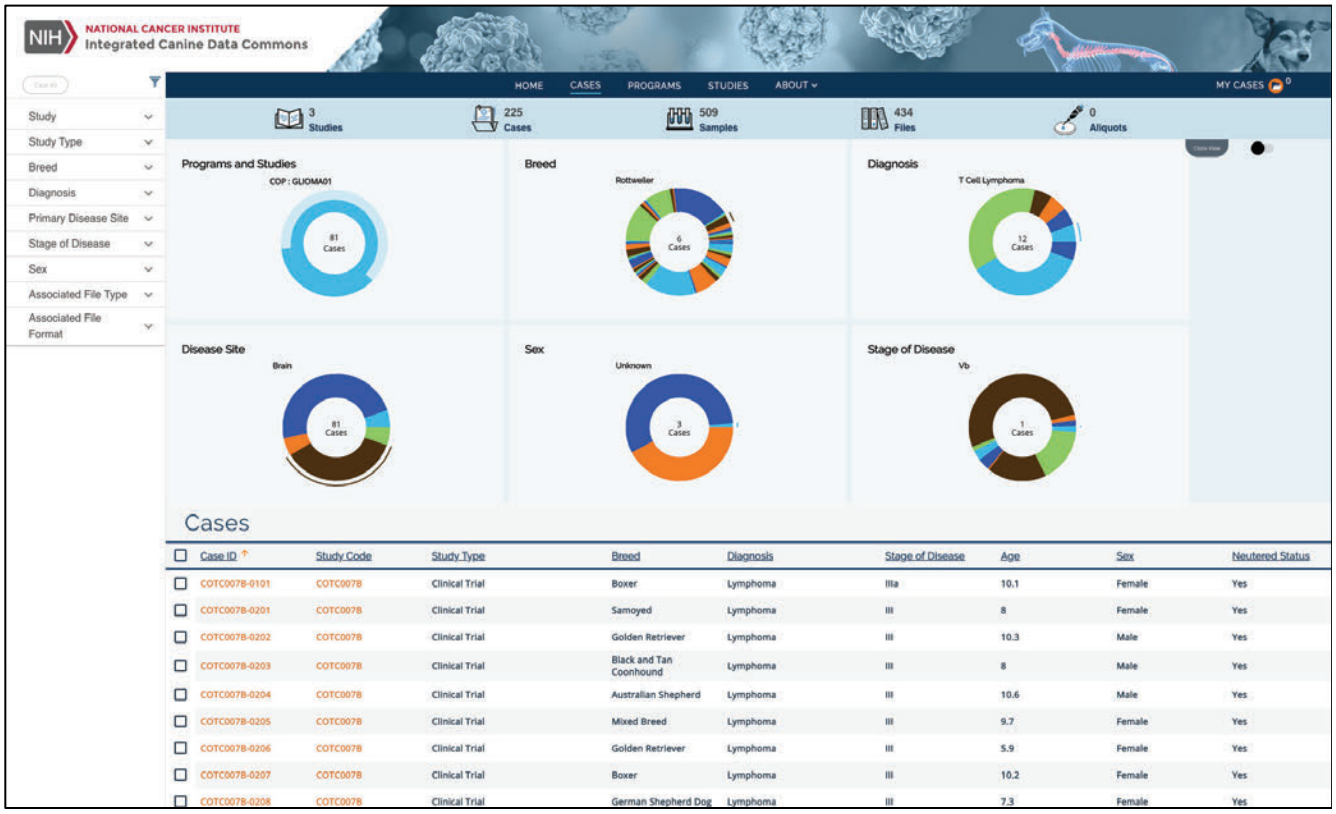
Support Provided by the Cancer Research Technology Program

Clinical Research Support to DCTD

Nanotechnology Characterization Laboratory

The Nanotechnology Characterization Laboratory (NCL) is an internationally recognized, unique resource dedicated to providing characterization, testing, and formulation services to the global nanomedicine community in order to advance the fundamental understanding and implementation of nanotechnology for medical applications. Nanotechnology is currently at the forefront of addressing global public health threats, as exemplified by the ongoing effort to develop a COVID-19 vaccine. The NCL assay cascade entails a comprehensive battery of tests that evaluate nanomaterials' sterility, physicochemical properties, immunology, and pharmacology and toxicology. The assay cascade is dedicated to cancer nanotechnology. Projects are accepted on a rolling basis over the course of the year, and on average NCL has 20 ongoing assay cascade collaborations at any given time. During the last year, NCL received 32 applications, advanced 21 of them to phase two of the evaluation processes, and accepted 14 into the program.

Upon receipt of the nanomaterials, NCL first performs a prescreen that involves sterility assessment (endotoxin and microbial contamination) and basic physicochemical characterization (e.g., size distribution, zeta potential,



Case ID	Study Code	Study Type	Breed	Diagnosis	Stage of Disease	Age	Sex	Neutered Status
COTC007B-0101	COTC007B	Clinical Trial	Boxer	Lymphoma	IIIa	10.1	Female	Yes
COTC007B-0201	COTC007B	Clinical Trial	Samoyed	Lymphoma	III	8	Female	Yes
COTC007B-0202	COTC007B	Clinical Trial	Golden Retriever	Lymphoma	III	10.3	Male	Yes
COTC007B-0203	COTC007B	Clinical Trial	Black and Tan Coonhound	Lymphoma	III	8	Male	Yes
COTC007B-0204	COTC007B	Clinical Trial	Australian Shepherd	Lymphoma	III	10.6	Male	Yes
COTC007B-0205	COTC007B	Clinical Trial	Mixed Breed	Lymphoma	III	9.7	Female	Yes
COTC007B-0206	COTC007B	Clinical Trial	Golden Retriever	Lymphoma	III	5.9	Female	Yes
COTC007B-0207	COTC007B	Clinical Trial	Boxer	Lymphoma	III	10.2	Female	Yes
COTC007B-0208	COTC007B	Clinical Trial	German Shepherd Dog	Lymphoma	III	7.3	Female	Yes

Figure 2. An example Cases page of the ICDC.

drug loading). A total of 14 nanoparticles were processed through NCL's prescreen characterization before they were subjected to rigorous physicochemical characterization and *in vitro* and *in vivo* immunology and pharmacology and toxicology studies. Advanced physicochemical characterization for these concepts included techniques such as cryo-transmission electron microscopy, compositional analysis (lipid, polymer, metal, and excipient concentrations), molecular weight, purity, size, and drug distribution by asymmetric-flow field-flow fractionation (AF4), and drug release and stability by AF4. Nanoparticle compatibility with blood as well as effects on immune cell function were assessed *in vitro* using a battery of specialized hematological and immune function tests. *In vitro* toxicology, as well as *in vitro* and *in vivo* drug metabolism and pharmacokinetic studies, were also applied. In total, NCL had 13 active assay cascade projects over the last year. Of note, NCL characterization data generated this past year for the biotech company AADi Bioscience, Inc. is being used in the Chemistry, Manufacturing, and Control section to support their Investigational New Drug application, currently under review with the U.S. Food and Drug Administration (FDA). At the request of AADi, NCL staff participated in FDA meetings with the company to address questions on the NCL-generated data.

To address the characterization gaps and needs of the nanomedicine community and to expand and improve the assay cascade, NCL continually works to establish new methods for nanoparticle characterization. For example, NCL developed a protocol for the detection of β-(1,3)-D-glucan contamination, which commonly results from fungal contamination. This assay has now become a part of our standard sterility prescreen for all new nanomaterials. Several new physicochemical characterization assays were also established, including a fluorescence-based assay to detect both free and total RNA in therapeutic nucleic acid nanoparticles, liquid chromatography-mass spectrometry methods to examine polymer molecular weight and impurities, a CHNOS elemental analyzer–based method to measure the drug content in prodrugs, and AF4-based methods to assess drug stability in nanoformulations as well as nanoparticle interactions with human plasma proteins. In collaboration with ACEA Biosciences, we developed two new in vitro assays (chemotaxis and migration/invasion) that utilize label-free xCELLigence real-time cell technology. To meet the growing needs of the cancer immunotherapy community, NCL's immunology section is aiding collaborators by investigating the risk of autoimmunity, a common side-effect of immunotherapeutics. The NCL *in vivo* immunology assay cascade has been expanded to assess the induction of anti-nuclear and anti-dsDNA antibodies in the SJL/J mouse model.

NCL makes all protocols freely available on our website and now has 70 protocols available for download (https://ncl.cancer.gov/resources/assay-cascade-protocols). Website tracking analytics show that NCL protocols were downloaded nearly 2,500 times over the last year and are reaching scientists in more than 100 countries around the world. Many protocols, including the CHNOS, AF4, and β-(1,3)-D-glucan methods developed last year are also published as part of peer-reviewed articles.

The NCL's pharmacology/toxicology section has received tremendous interest in the stable isotope tracer ultrafiltration assay to evaluate *in vitro* drug release and *in vivo* pharmacokinetics. The assay was developed and published by NCL in 2015. Since then, it has become highly sought after and has been made available as two separate Technical Service Agreements (TSA) through the Frederick National Laboratory. There were four pharmacokinetic TSAs purchased over the last year for nanoformulations developed by S.N. Biosciences (South Korea) and MegaPro Biomedical Co., Ltd. (Taiwan). NCL is currently discussing these services with other companies and expects to execute additional TSAs in the near term.

In addition to characterization, NCL also provides nanoformulation services to both the intramural and extramural communities. Highlights of NCL's formulation work this past year include the liposomal formulation of brefeldin/breflate and aclacinomycin natural products for the Division of Cancer Treatment and Diagnosis (DCTD) (Figure 1). Initial breflate/brefeldin toxicity studies in rats confirmed API-dependent neurotoxicity that was independent of excipient. This brefeldin systemic toxicity necessitated a targeted drug delivery approach to reduce systemic exposure, similar to aclacinomycin. Both brefeldin and aclacinomycin liposomal formulations underwent efficacy evaluation in murine cancer models, and ongoing studies include optimization of both liposome stability and dosing regimen.

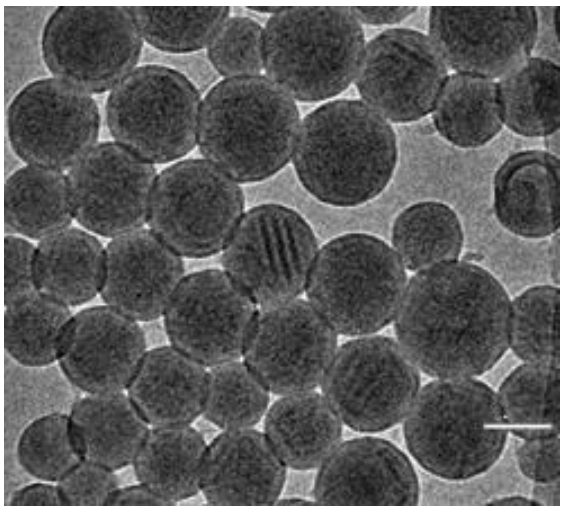


Figure 1. Cryo-electron microscopy image of a liposomal formulation of breflate that NCL prepared for DCTD.

To assist in the commercial development of NIH technologies, with funding from the NIH Technology Transfer Invention Development Fund (IDF), NCL developed a micellar anti-K-Ras lipopeptide for NCI

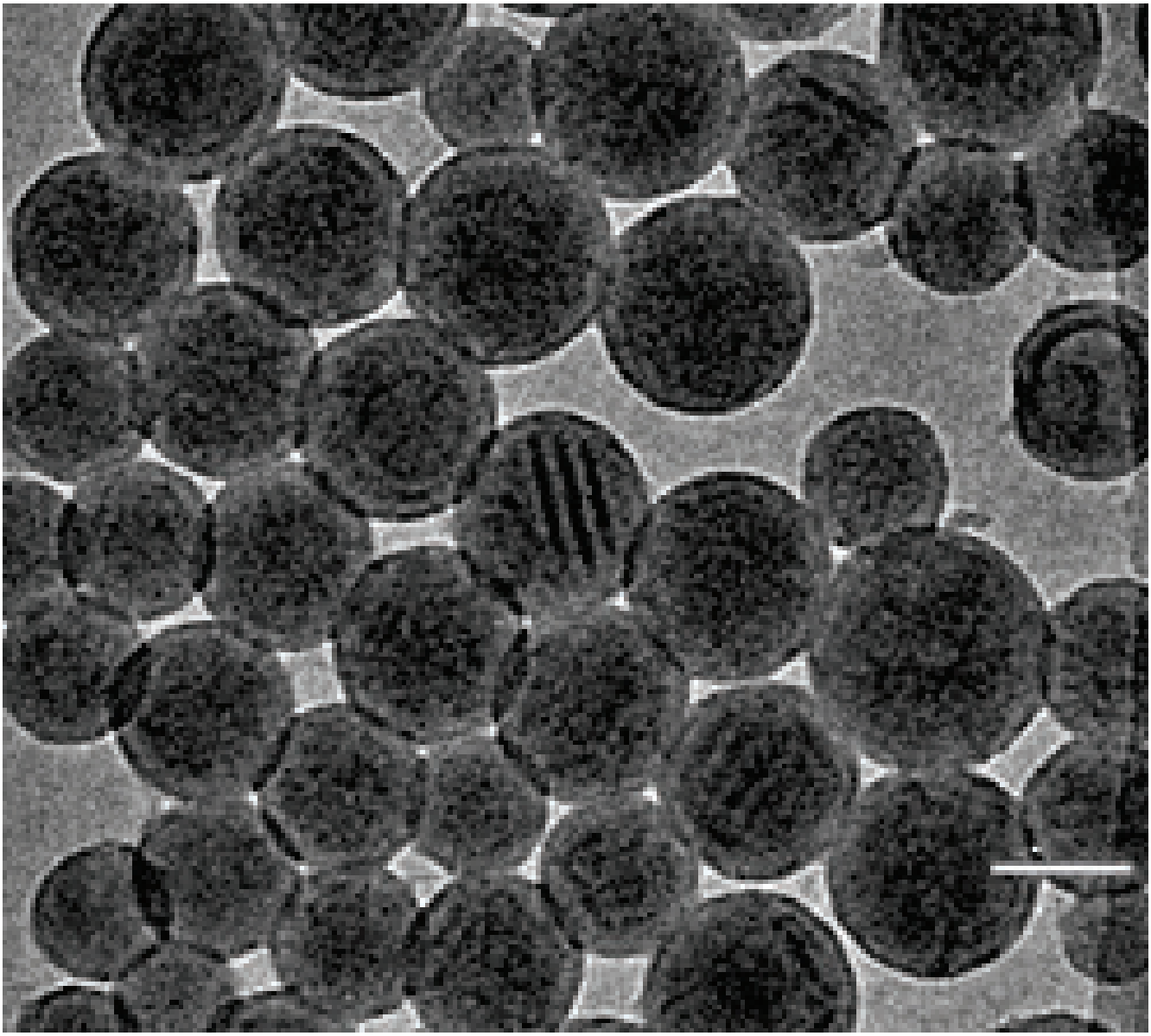


Figure 1. Cryo-electron microscopy image of a liposomal formulation of breflate that NCL prepared for DCTD.

Center for Cancer Research (CCR) scientist Dr. Tarasova with excellent *in vitro* activity. The formulation is currently undergoing pharmacokinetic and efficacy evaluation. In another IDF-funded project, NCL is formulating a polymeric nanoparticle version of an anti-HIV small molecule therapeutic for NCI CCR scientist Dr. Burke. NCL also synthesized a lymphatic targeting macromolecular prodrug version of the PD-1 inhibitor peptide LD10 for Leidos Life Sciences (LLS). The LD10 prodrug is now undergoing *in vivo* evaluation for cancer immunotherapy by LLS.

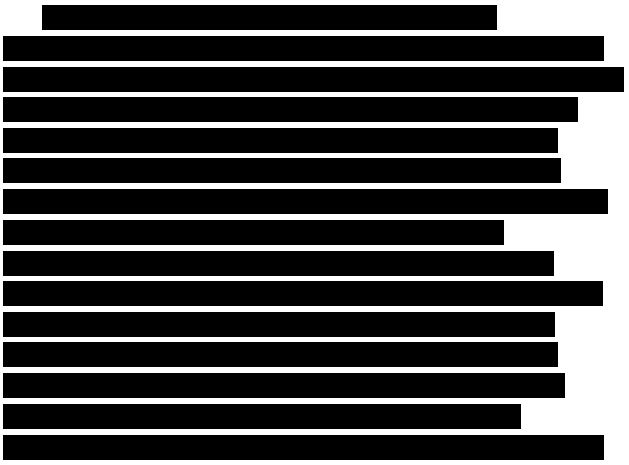
To provide access to NCL's unique services and knowledgebase to developers of non-cancer nanomedicines, we established several partnerships via contractor Cooperative Research and Development Agreements (cCRADAs), Interagency Agreements (IAA), Collaboration Agreements, and Material Transfer Agreements. In total, there were 17 projects through these mechanisms in the last year. The LLS formulation work, mentioned above, was performed under a cCRADA. NCL's physicochemical characterization section led three cCRADAs with the pharmaceutical companies Pfizer, Eli Lilly, and GlaxoSmithKline. The Pfizer cCRADA involves the evaluation of several orthogonal techniques to measure both size and particle concentration. The Eli Lilly and GlaxoSmithKline cCRADAs involve AF4 method development to evaluate their lipid nanoparticle formulations.

Conducted under an IAA, the NCL had two separate research projects in collaboration with the FDA. NCL's Pharmacology and Toxicology section published the results of a two-year FDA IAA entitled "Novel Method to Determine Bioequivalence of Nanomedicines" in *ACS Pharmacol Transl Sci*. The Immunology section completed the first year of the IAA project evaluating *in vitro* assays for the assessment of responses to innate immunity modulating impurities in reference-listed drug products and their generic versions. The methodology identified by NCL is under consideration for being included in the guidance for industry. The results of these collaborations are contributing to the ongoing global effort in accelerating the approval of generic drug products.

In other collaborative efforts, the NCL's physicochemical characterization section has several collaborations with industry and government agencies to help advance nanomedicine physicochemical characterization. NCL is participating in a Collaboratory Release team consisting of the FDA, National Institute of Standards and Technology (NIST), Joint Research Centre (Italy), SINTEF (Norway), National Physical Laboratory (United Kingdom), and AstraZeneca to standardize the methodology for nanoparticle drug release. A collaboration with Pfizer, AstraZeneca, NIST, and Trinity College Dublin published a perspective on sizing measurements in Pharmaceutical Research. In collaboration with Imperial College London, NCL is evaluating their new Raman-based technology for the analysis of single nanoparticles, which, if successful, could have significant impacts in the nanomedicine characterization field. Finally, an intramural collaboration with the National Institute of Neurological Disorders and Stroke is focused on the optimization of an imaging contrast agent.

To assist the global nanomedicine community in overcoming infusion reactions to systemically administered nanomedicines, NCL's immunology section is conducting an *in vitro-in vivo* correlation study in collaboration with Dr. Szebeni (University of Semmelweis, Hungary). Complement activation and cytokine responses measured using the NCL assay cascade protocols are being compared with the *in vivo* data obtained by Dr. Szebeni's team in a pig model. In another collaborative effort, we assisted Dr. Ghandehari (University of Utah) with understanding the immunotoxicity of nanomaterials after chronic exposure, a study of paramount importance for silica-based vaccines. Finally, as a result of multiple requests from the extramural community and the recommendation of NCL's Scientific Oversight Committee (SOC), NCL validated *in vitro* protocols for assessing the immunotoxicity of programmed nucleic acid structures. This work was performed in collaboration with Drs. Bathe (Massachusetts Institute of Technology) and Afonin (University of North Carolina, Charlotte). The lessons learned from these collaborations were disclosed to the public via a workshop and review articles.

External scientific collaborations this past year for NCL's pharmacology/toxicology section included a collaboration with VeriSIM Life, Inc. and UNC-Chapel Hill Eshelman School of Pharmacy to apply machine learning to nanomedicine physiologically based pharmacokinetic modeling. This collaboration was initiated in response to a request from NCL's SOC to explore the potential of machine learning to extrapolate trends and make predictions about nanomedicine pharmacokinetics using the more than 15 years of data acquired by NCL's characterization over 400 nanoparticles. A separate collaboration with UNC-Chapel Hill Eshelman School of Pharmacy analyzed historic nanomedicine tumor uptake data. This work has been published in *Sci Adv*. Finally, a collaboration with Georgetown Medical School is evaluating a novel gastrin-targeted polyplex for early diagnosis of pancreatic cancer.



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As part of other external outreach efforts, NCL scientists acted as expert reviewers to EuroNanoMed III and Medical Research Council funding programs. We taught two online translational immunology courses with more than 170 registrants from more than a dozen countries and presented a mini workshop on nanocharacterization at the World Molecular Imaging Congress. NCL immunology and pharmacology /toxicology teams also began an online series of training sessions for SINTEF to train them in these disciplines, with emphasis on the *in vivo* assays commonly conducted for nanomedicines.

Support Provided by the Clinical Monitoring Research Program Directorate and Clinical Research Directorate

Clinical Research Support to DCTD

Clinical Program/Project Management to Support Correlative Studies for National Trials

KEY ACCOMPLISHMENTS

- Negotiated and implemented the use of a unique administrative agreement to best reflect the National Cancer Institute (NCI) Cancer Therapy Evaluation Program (CTEP) Cooperative Research and Development Agreement (CRADA)-supported non-acquisition model representing NCI's program needs

The CRADA-supported correlative science program provides funding to CTEP's correlative studies within NCI-sponsored clinical trials that use CTEP Investigational New Drug (IND) agents. This serves the extramural community by supporting critical NCI-sponsored clinical trials conducted under separate agreements between NCI and the clinical trial site. NCI authorized the use of its CRADA funds to support research agreements that cover the cost of the approved correlative studies.

A clinical trial site may receive CRADA funding to cover the costs of conducting correlative study activities, such as assays and investigational imaging; direct patient care; specimen collection, processing, and shipping; indirect institutional costs; and protocol-mandated patient evaluations and/or sample acquisitions.

FNL staff provided project management support for the complex correlative science agreements, including blanket purchase agreements, basic order agreements, indefinite delivery/indefinite quantity agreements, task orders, research subcontracts, and administrative agreements that are sponsored by multiple drug companies. This support also included monitoring protocol progress, preparing financial and technical reports, ensuring alignment with the agreements' research objectives, and documenting and tracking CRADA-supported assays and biomarker-development activities—as well as diagnostic, correlative studies and non-standard-of-care patient care services—for the portfolio of early drug development studies for the CTEP Investigational Drug Branch and in support of the Regulatory Affairs Branch's activities.

Support shifted from an acquisition model to a novel non-acquisition administrative agreement model after NCI/CTEP and FNL staff jointly undertook a four-month effort to review, disseminate, and negotiate an agreement that better represents the unique CRADA-supported program, resulting in a more streamlined and efficient process. This first-in-kind administrative agreement template has also been used by other FNL programs supporting high-priority clinical trials.

Support to CTEP institutions and vendors demonstrated a sustained growth of approximately $2.3 million—a $700,000 increase over the past year.

Clinical Operations/Nursing Support

The Developmental Therapeutics Clinic (DTC) evaluates innovative anticancer compounds in early phase clinical trials and provides clinical care for patients with different types of cancer. An important focus is first-in-human clinical trials, particularly those that incorporate pharmacodynamic and pharmacokinetic endpoints, with the goal of informing subsequent clinical development.

FNL supported the initiation and implementation of two new DTC protocols: a Phase IB study of nivolumab in patients with autoimmune disorders and advanced malignancies, and a collaboration with the Pediatric Oncology Branch for a Phase II trial of the MEK inhibitor selumetinib in combination with the mTOR inhibitor sirolimus for patients with unresectable or metastatic malignant peripheral nerve sheath tumors. In addition, staff screened patients for protocol enrollment, completed enrollment processes for various DTC protocols, and medically managed patients while they were on protocol.

Additional support included participation with DTC team members in weekly patient care conferences to discuss issues with incoming patients, existing patients, and workflows; collaboration with other departments, such as social work and pharmacy; and facilitation of weekly conferences with interventional radiology to review computed tomography scans for patients who could be safely biopsied. This support has helped to enhance patient care delivery and streamline the trial enrollment process.

An FNL nurse practitioner supported a study and co-authored a publication in *Cancer Chemother Pharmacol* (O'Sullivan Coyne G, Wang L, et al.) about DTC's Phase II study using a novel method for detecting expression of tumor suppressor protein p16/INK4A in circulating tumor cells; further studies were recommended.

Develop and Implement Capacity to Monitor Circulating Tumor Nucleic Acid

FNL Molecular Characterization Laboratory

KEY ACCOMPLISHMENTS

- Assessed several circulating tumor DNA (ctDNA) pan-cancer next-generation sequencing (NGS) assays
- Evaluated extraction methods for circulating cell-free total nucleic acid (cfTNA)
- Made substantial progress evaluating, optimizing, and implementing the ctDNA analysis workflow

The FNL Molecular Characterization (MoCha) Laboratory made substantial progress evaluating, optimizing, and implementing the ctDNA analysis workflow over the past year. The complete workflow includes sample collection; sample extraction; NGS library preparation; sample sequencing; and data processing, analysis, and reporting.

FNL staff in MoCha have completed evaluations of extraction methods for cfTNA and selected MagMAX as the preferred method. A benefit of the MagMAX method is that it allows the isolation of both ctDNA and circulating tumor RNA (ctRNA). Another benefit is the availability of an automated workflow. The staff has evaluated, optimized, and implemented the automated version of the MagMAX workflow on the KingFisher platform. The automated workflow minimizes potential human errors, sample mix-ups, cross-contamination, and hands-on time while increasing sample throughput. The staff also completed a bridging experiment between manual and automated MagMAX extractions. Following evaluations of commercially available methods, the staff selected the Qubit, a fluorometric assay, and TapeStation, an automated electrophoresis platform, as the preferred methods for ctDNA quantitation and quality assessment, respectively. Feasibility testing of cerebrospinal fluid as a ctDNA source for NGS analysis is underway.

FNL staff in MoCha conducted preliminary assessments of several ctDNA pan-cancer NGS assays (ArcherDX, Thermo Fisher Scientific, and Illumina) and selected the Illumina TSO500 ctDNA as the production assay to implement.

FNL staff in MoCha worked closely with the technical and bioinformatics teams at Illumina to fully develop and optimize the TSO500 ctDNA assay. After initial assay feasibility testing, staff evaluated a modified library preparation standard operating procedure (SOP) provided by Illumina. Results using the new, modified SOP were better than those using the original SOP. The new SOP was implemented. The workflow using the improved SOP is more streamlined and costs less than the original workflow.

In-depth analyses of sequencing data to improve analysis pipelines and identify optimal filtering thresholds, etc. have been conducted. Changes were made to pipeline thresholds and baselines to minimize false positives and improve overall data quality. MoCha implemented Illumina's contamination workflows and error-correction pipelines to minimize sample-to-sample contamination. The FNL bioinformatics team in MoCha has been evaluating tumor fraction analysis using low-pass whole-genome sequencing of prehybridization TSO500 libraries. This method may provide a quick, inexpensive way to determine when ctDNA material is insufficient for analysis and a tumor biopsy is necessary.

The staff completed beta testing of the new Illumina DRAGEN server that significantly decreases processing and analysis time of TSO500 ctDNA assay data, thus increasing sample throughput. Installation of DRAGEN servers on site is expected by August 2020.

Matched non-small cell lung cancer blood and tumor samples possessing epidermal growth factor receptor (EGFR) mutations were received from Clovis Oncology. The samples have existing beads, emulsion, amplification, and magnetics (BEAMing) polymerase chain reaction (PCR) variant allele frequency data for EGFR mutations. FNL staff in MoCha used the existing data and histology assessment of tumor samples to select the most appropriate samples for use during the testing and initial analytical validation of the Illumina TSO500 ctDNA assay. Select tumor samples also underwent whole-exome sequencing as well as analysis using the TSO500 tumor assay for concordance analysis with ctDNA assay results. Sequencing metrics indicate high-quality data. Comparison to existing BEAMing PCR data was used to further optimize bioinformatics pipelines.

Additional staff completed training for ctDNA extractions and TSO500 ctDNA assay to further increase capacity.

An analytical validation plan for the TSO500 ctDNA assay has been created. Analytical validation of the TSO500 ctDNA assay is underway. ctDNA extractions of validation samples are underway. Library preparation and sequencing of validation samples are also ongoing. The TSO500 ctDNA assay analytical validation is expected to be complete by early fall 2020.

The FNL staff in MoCha has discussed the best course of action for implementing the TSO500 ctDNA assay as a Clinical Laboratory Improvement Amendments (CLIA) assay. The staff is also finalizing the guidelines for clinical reporting of results from the assay. Considerations include reporting structure and what, specifically, to report—especially for potential clonal hematopoiesis genes.

The Foundation for NIH quality controls material testing project activities are underway. The project will assess the performance of contrived ctDNA reference materials across multiple platforms in multiple laboratories. The findings are valuable because they will provide limit of detection and other performance data for the quality controls materials and ctDNA assays, which will benefit MoCha and the larger research and clinical community. Samples have been unblinded, and preliminary data analysis comparing results from all sites has been completed. FNL has ongoing discussions with the Food and Drug Administration (FDA) to discuss the

functional characterization (a.k.a. "commutability") phase of the project. FDA has provided feedback on study details.

Restrictions on laboratory work from mid-March to mid-July 2020 due to COVID-19 negatively impacted MoCha's ability to complete analytical validation of the TSO500 ctDNA assay, feasibility testing of cerebrospinal fluid as a ctDNA source, and optimization of the KingFisher automated extraction workflow. During the COVID-19 laboratory shutdown, staff continued to support the project by analyzing data, working in the bioinformatics pipeline, revising the validation plan and schedule, reviewing areas that would improve efficiency and increase throughput in the laboratory for the workflow, and conducting a variety of other support activities while waiting for the laboratory to reopen.

Develop and Implement Capacity for Expanded Histotechnology Efforts

KEY ACCOMPLISHMENTS

- Developed 44 immunohistochemistry (IHC) assays
- Performed analytical validation of six immunofluorescence assays
- Received, accessioned, and processed for downstream analysis thousands of samples from multiple projects and trials

The FNL MoCha histology team is a CLIA-certified laboratory capable of accepting samples from various clinical trials. SOPs are in place to receive clinical samples, accession them, trim/gross them, embed them, section them, stain them with hematoxylin and eosin, evaluate their histopathology, enrich them for tumor content, and transfer them for sequencing. Samples are currently accepted for the National Cancer Institute's (NCI) Molecular Profiling-Based Assignment of Cancer Therapy (MPACT) trial in the form of formalin-fixed specimens; stained and unstained slides; or formalin-fixed, paraffin-embedded (FFPE) blocks. Clinical samples are processed and/or stained for pathology evaluation of tumor content and enrichment and then transferred to MoCha's CLIA NGS laboratory for nucleic acid extraction and sequencing.

The FNL staff in the MoCha histology laboratory also supports the Pharmacodynamic Assay Development and Implementation Section's (PADIS) clinical and patient-derived model (PDM) histology needs.

The FNL staff in MoCha continues to receive samples from seven Developmental Therapeutics Clinic (DTC) clinical trials from PADIS for histological processing, biomarker staining, and image capture. All clinical samples are processed with established SOPs and tracked with Labmatrix, a web-based tracking system, to facilitate the transfer, generation, labeling, tracking, and inventory of all clinical samples.

Computer systems have been developed to accurately and efficiently archive and track all the patient-derived xenograft (PDX) and PADIS samples generated, as well as relevant sample information. Besides routine PDX samples, the MoCha histology laboratory also receives other samples for processing. These samples are from PDX or *in vivo* PDX animal or drug studies; PADIS preclinical drug studies; and assay development, experiments, and/or harmonization tests with other laboratories within DTC. To date, MoCha has archived more than 38,000 slides, blocks, and frozen tissue materials.

FNL continues to provide histological processing and histopathological diagnosis confirmation for the PDX model program using established SOPs and workflows. These samples' high-priority status necessitates rapid histopathological processing within a two-week turnaround time to meet PDM needs.

To date, the FNL staff in MoCha has developed, optimized, and routinely performs IHC assays for 44 protein markers to support the PDM project. Appropriate control blocks consisting of normal organ tissues or cell lines embedded in the same block have been generated to run with each IHC assay to ensure specific, accurate staining patterns. For example, estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) IHC assays have been optimized for use in PDX breast cancer models. These assays have been used to characterize hormone receptor status of PDX models in preparation for public distribution to evaluate the possibility of using castrated male mice in the development of gynecological and breast cancer PDX models and to assess the ER status of PDX tumors grown in the absence of estrogen supplement. Likewise, IHC assays with the prostate markers prostate-specific antigen (PSA), ETS-related gene (ERG), and androgen receptor (AR) have been optimized and are ready for use to characterize prostate models. MoCha develops additional IHC protocols as required by the program. Up to 70 IHC assays will be developed based on PDX program needs. As new markers have become available, existing models have been reassayed to confirm diagnoses and facilitate transfer of models to the public domain. Completing distribution lot analysis to make more PDX models available for public distribution is a priority.

MoCha is developing MGMT IHC- and PCR-based promoter methylation assays to support several current and planned temozolomide combination studies in the Division of Cancer Treatment and Diagnosis (DCTD). MGMT is a marker of temozolomide sensitivity. The MGMT promoter methylation assay will be used in conjunction with the IHC assay to determine MGMT status of samples. The IHC assay has been analytically validated and may be used in retrospective research studies. Planning for the clinical validation of the IHC assay is in progress. Development and optimization of the MGMT methylation assay is also underway. The MGMT methylation assay is a real-time-PCR-based assay that uses primers and probes designed for the methylated *MGMT* and *ACTB* genes.

MoCha has analytically validated six immunofluorescence assays—pHH3, CDK, pY15, RAD51, DDR3, and EMT—that can be used on clinical samples. MoCha continues to work on the generation of

calibrator control block and slide sets, staining evaluation and qualification of control slide sets, antibody lot-to-lot validation, and new antibody screening.

The MoCha staff has led harmonization efforts with other laboratories outside NCI so they can help process PADIS clinical samples by following the same SOPs and clinical protocols as MoCha. On-site trainings at MoCha and remote training using Webex have taken place for these technical procedures to ensure consistent high-quality results from multiple laboratory sites.

The overall number and types of specimens have increased over the last 12 months. The number of IHC samples from PDX has nearly doubled compared to FY2019 as the number of assays has increased.

Progress during FY2020:

- Total samples processed: more than 9,500
- PDX samples for routine histology: more than 8,500
- PDX sample hand macrodissection and laser capture microdissection: more than 880
- IHC on FFPE PDX samples: more than 550
- NCI-MPACT patient samples for tissue processing: 5
- PADIS samples (blocking and/or staining and/or sectioning): 109 biopsies over 42 patients

Due to COVID-19, the MoCha histology laboratory operated in a reduced capacity from March 23 to June 19, 2020. During this time, FFPE samples were received and processed to paraffin block only. Formalin-fixed samples were received and stored. All IHC assays and assay development activities were put on hold. No PADIS work was performed during this time of reduced capacity.

Support for NCI-MATCH Clinical Trial and Assay

KEY ACCOMPLISHMENTS

- Development and harmonization of rapid, targeted NGS assay
- Collection of additional clinical samples

FNL has seen continued progress in supporting the NCI Molecular Analysis for Therapy Choice (NCI-MATCH) clinical trial and the associated NGS assay. In FY2020, this task order supported the Eastern Cooperative Oncology Group–American College of Radiology Imaging Network (ECOG-ACRIN), Yale University, and Massachusetts General Hospital (MGH).

FNL executed and managed a subcontract to facilitate the collection and invoicing of biopsies and kits to support the MATCH studies, with a total of 2,118 biopsies collected and invoiced to this project. Some biopsies related to the MATCH study were not invoiced to FNL and, subsequently, NCI due to a variety of reasons, most notably third-party reimbursement for the collection. In these instances, the biopsy costs were either refunded to FNL or not invoiced by ECOG-ACRIN to FNL. To date, 41 progression kits have been invoiced to FNL.

NCI-MATCH is a 600-sample correlative study focused on assessing potential low-quality archival samples by comparing previous sequencing results to updated sequencing data with archival material. In this study, the focus is comparing pass/failure rates of both old and new sequencing runs. At the end of the study, data compilation will indicate whether archival material can be submitted for reliable genetic sequencing using Thermo Fisher Scientific's Oncomine Comprehensive Assay version 3 (OCAv3). In the past year, Yale University was funded through a partnership to perform OCAv3 sequencing. To date, 324 archival kits and 658 archival samples have been invoiced to FNL.

A second correlative study, the MATCH-Confirmation study, receives patient samples from outside sites to compare variants detected by OCAv3 to what other sites have previously detected on their assay platform. Data compilation has shown how OCAv3 variant detection compares with assays developed using other chemistry and platforms.

A third correlative study was approved to examine samples from one sub-arm (C2) of NCI-MATCH in collaboration with Massachusetts General Hospital. MGH proposed to use an orthogonal assay to confirm the presence of a particular mutation (*MET* exon 14 skipping) in patients enrolled to the C2 sub-arm. However, challenges with amendment development, review at ECOG-ACRIN, approval at the Cancer Therapy Evaluation Program (CTEP) and the NCI central Institutional Review Board (IRB), and activation severely delayed getting samples from the MD Anderson Cancer Center biorepository to MGH. The hospital received the first batch of samples in March 2020 but could not process them due to a higher priority for performing COVID-19 testing.

Support for ECOG-ACRIN (kits, biopsies), Yale (archival samples), and MGH (arm C2) will continue.

Molecular Analysis of Tumors for Treatment Selection – Phase III

KEY ACCOMPLISHMENTS

- Accrued Pediatric MATCH target of 1,000 patients and expanded the goal to 1,500
- Led the NCI-MATCH archival study
- Led the NCI-MATCH Designated Laboratory Network

The FNL staff in MoCha has continued to make progress in supporting multiple clinical trial efforts and leading the cross-platform assessment of reference standards for NGS assays. MoCha encountered numerous issues with obtaining sufficient numbers of samples from smaller clinical trials, and the COVID-19 pandemic affected numerous research efforts in this task order.

NCI Pediatric MATCH has remained steady, averaging approximately 30 patients accrued per month. As of the end of July 2020, there were 1,038 registered patients and 918 confirmed reports performed by MoCha,

MD Anderson Cancer Center, or Dartmouth College, which are both supported by FNL partnerships. NCI and MATCH approved the expansion of Pediatric MATCH to 1,500 patients. Nationwide Children's Hospital, an FNL partner, was instrumental in minimizing turnaround times and providing sufficient material for OCAv3 testing. All Pediatric MATCH laboratories were able to continue testing during the COVID-19 pandemic with significantly fewer staff present in each laboratory.

The NCI-MATCH archival study completed 205 sequencing assays as of June 2020, but the COVID-19 pandemic stalled further testing of samples since the study is considered "research" and not an "essential operation." MD Anderson focused efforts during the minimal maintenance period on studies directly affecting patient care. The study has transitioned to sequencing all primary and metastatic samples received at MD Anderson Cancer Center that meet quantity and quality standards. There are roughly 600 additional samples to be extracted, sequenced, and tested to identify single-nucleotide polymorphisms. To account for RNA failures, MoCha tested different reaction conditions for RNA sequencing but determined that additional efforts are needed to reduce failures. Additional bioinformatics analyses have been tested to determine whether quality control parameters can be adjusted, but the COVID-19 pandemic stalled further analyses.

The NCI-MATCH Designated Laboratory Network comprises approximately 30 laboratories that are cleared to identify cases. Samples from the network have been tested for confirmation using the NCI-MATCH NGS assay at MoCha, MD Anderson Cancer Center, or Dartmouth on a monthly rotating basis. As of June 2020, designated laboratories had enrolled 597 patients, with 426 enrolled on treatment arms. MoCha has led the concordance testing between the NCI-MATCH NGS assay and designated laboratory assays. This effort has been successful and will be used for future "basket" trials sponsored by NCI, including "ComboMATCH" and "iMATCH."

NCI, FNL, and Thermo Fisher Scientific were able to execute a CRADA and a material transfer agreement covering the early access to the Oncomine Plus assay (and the Oncomine Myeloid assay). The Oncomine Plus assay will support Pediatric MATCH sequencing and future planned arms. The Oncomine Myeloid assay will support the "MyeloMATCH" trial. MoCha acquired two new sequencers from Thermo Fisher Scientific, the Genexus, which will decrease sequencing turnaround time and support the new assays. The COVID-19 pandemic prevented MoCha and Thermo Fisher Scientific from installing and training on the new Genexus systems.

MoCha is working with NCI and Friends of Cancer Research (FOCR) to harmonize assessments of tumor mutational burden (TMB). Phase 2A, assessment of a common set of cell lines, has been completed. In Phase 2B, clinical specimens are being assessed for TMB, with the objective of identifying a range of TMB levels that can be assessed in 15 other laboratories in the consortium. The initial tests using 27 paired tumor-germline clinical samples from the Cooperative Human Tissue Network biorepository failed due to poor sample quality. MoCha is using these samples to implement robust quality metrics for identifying samples unsuitable for NGS. An additional set of FFPE tumor tissue and matched blood germline specimens from 34 patients was acquired from an alternative vendor, iSpecimen. Specimen and DNA quality were carefully evaluated by the MoCha histology and research and development teams, and whole-exome sequencing data was generated from 25 tumor/germline sample pairs. TMB data derived from whole-exome sequencing was shared with the FOCR TMB consortium, and aliquots of DNA were distributed from MoCha to the 15 consortium laboratories.

MoCha is implementing the 10x Genomics single-cell sequencing and expression profiling platform for DCTD studies. MoCha has confirmed feasibility and developed protocols for single-cell RNA sequencing of fresh and cryopreserved tissue samples, including tumor resection and biopsy samples, as well as peripheral blood mononuclear cell isolates from blood. Using single-cell RNA sequencing of ovarian cancer cell lines, MoCha identified several transcripts as potential pharmaco-dynamic biomarkers for triapine treatment, and discussions are underway to use single-cell sequencing in a triapine window of opportunity study for endometrial carcinoma. Several other pilot studies are ongoing, including comparative single-cell transcriptome profiling of primary tumor, circulating tumor cell, and metastatic cell populations in a bladder cancer PDX model and single-cell transcriptome assessment of heterogeneity in PDX models of uterine carcinosarcoma. Feasibility studies of other single-cell sequencing assays, including T-cell repertoire profiling and single-cell DNA copy number analysis, are being planned. Applications to Experimental Therapeutics Clinical Trials Network and DTC trials could include biomarker discovery, genomic profiling of circulating tumor cells, and response–resistance expression signatures in tumor cells and infiltrating immune cells.

FNL continued to support clinical sequencing for the NCI-MPACT study, which closed to accrual in July 2020. MoCha performed Oncomine sequencing for the NCI Community Oncology Research Program (NCORP) Tissue Procurement Protocol (NTPP #10231) study for 17 patients, with an average turnaround time (receipt to upload of report to Rave) of 11 days.

MoCha started a PCR-based MGMT promoter methylation assay but did not finalize it due to COVID-19 restrictions on operations. The assay will be used in DTC studies with temozolomide. Validation with clinical samples would have concluded in April 2020 but was delayed indefinitely due to COVID-19.

Cancer Moonshot Biobank

KEY ACCOMPLISHMENTS

- Received NCI/DCTD/CTEP and IRB approval of the Cancer Moonshot Biobank protocol
- Subcontracted a biospecimen source site coordinator (BSSC) for specimen payments and agreements
- Identified 81 clinical sites to enroll participants and initiated agreements with most sites
- Worked with a subcontractor to develop a Patient and Provider Engagement (PPE) website with Federal Information Security Management Act (FISMA) Moderate Authorization to Operate (ATO)
- Worked with the partner to develop an electronic informed consent (eConsent) application
- Subcontracted a biorepository

Overall, the Cancer Moonshot Biobank project has made significant progress, even with the COVID-19 pandemic affecting clinical trials. Progress included receiving CTEP and IRB approval of the protocol, partnering with a clinical site coordinator, identifying the initial NCORP sites, initiating agreements with most NCORP sites, fully developing the PPE website, developing the eConsent application, developing the Medidata Rave study build, partnering with a biorepository, and initiating biorepository activities.

Even so, the pandemic had some effect on the project. FNL staff in MoCha and staff in the Biorepositories and Biospecimen Research Branch (BBRB) teleworked, and many NCORP affiliates focused local efforts on responding to COVID-19. Agreements and site-specific submissions to the external IRB were slowed down during the early months of the pandemic.

The protocol was further developed in August 2019 and submitted to CTEP for review and approval. Comments were received in October 2019, and the protocol was revised and then submitted to CTEP for additional review in January 2020. It was quickly granted "Approval on Hold" status with the stipulation of IRB approval and Medidata Rave development. The protocol was submitted to the external IRB for review and granted approval in March 2020 with the stipulation that the eConsent application would be submitted for full review.

An FNL subcontractor serves as the BSSC to execute clinical study agreements with NCORP sites and facilitate payment for training sessions and biospecimen-associated costs. BBRB, MoCha, DCTD, and the Division of Cancer Prevention collaboratively developed an application to identify NCORP sites (and subsites) to enroll participants for the Biobank study. A total of 21 NCORP sites and 81 subsites were selected. The BSSC used prior experience with the NCORP Tissue Procurement Protocol (NTPP #10231) to execute agreements with 14 of 21 NCORP sites.

The FNL staff in MoCha worked with a subcontractor to develop the PPE website through two phases. The website was designed and developed as a cloud-based application hosted by NCI's CloudOne system. The PPE will serve as a gateway portal for patients and providers to get patient-specific clinical reports and consent forms, which necessitates higher security controls and review. Importantly, the website obtained the first FISMA Moderate ATO from NCI's Center for Biomedical Informatics and Information Technology based on the website being developed and deployed in NCI's CloudOne system. Collectively, the team successfully integrated with the Oncology Patient Enrollment Network registration system and the new NIH Login system. Incorporation of Login.gov credentialing represented another first for the Biobank project. Phase one was completed in December 2019, and phase two was completed in July 2020. The PPE website will represent a model patient/provider portal for NCI in other projects.

Development of the eConsent application and video only commenced following CTEP and IRB approval of the protocol and informed consent documents. Two subcontractors were placed on work stoppages during protocol development and review; another obtained a FISMA Moderate ATO for the eConsent application in February 2020. Video development was more extensive than originally proposed and budgeted, which drastically delayed development. A video was developed in June 2020, but additional changes were requested by BBRB leading to a delay in eConsent application development. Integration between eConsent, Oncology Patient Enrollment Network registration, and the partner clinical site's Medidata Rave instance began in July 2020 and represents another "first."

MoCha and BBRB worked with the clinical site on Medidata Rave case report form (CRF) development. Rave CRF development for the Biobank study only commenced following (i) NCI/DCTD/CTEP-directed development of Clinical Data Acquisition Standards Harmonization (CDASH)-compliant forms (March 2020), (ii) development of two COVID-19 protocols of higher priority (June 2020), and (iii) the Biobank protocol approval by CTEP and IRB. Work commenced in July 2020 and was based on the CDASH forms and the NTPP #10231 forms.

FNL encountered numerous issues with identifying a biorepository for the Biobank project. In FY2019 (July 2019), FNL was close to subcontracting, yet BBRB initiated a task order statement of work modification that transferred various activities from FNL (Medidata Rave, biorepository, etc.) back to NCI control. However, NCI was not able to engage the preferred vendor. FNL responded to another task order statement of work modification in December 2019 that added biorepository control to FNL. MoCha engaged a new subcontractor to join the Biobank team in June 2020. The subcontractor accelerated activities in July, including kit development, SOP generation, and IT integration with Medidata Rave.

Progress on providing a hematological NGS assay was delayed. FNL worked to engage separate sole-source subcontract procurements; however, one vendor would not agree to the subcontract terms and conditions to provide NGS assay services for patients with acute

myeloid leukemia and multiple myeloma. [redacted].

Even in the absence of an outside hematological NGS assay vendor, MoCha undertook a separate validation of a myeloid assay in early 2020. However, validation was put on hold per the NCI/DCTD directive in response to the COVID-19 pandemic. This development led to removal of acute myeloid leukemia from the protocol; it will be added to the protocol following full analytical validation of the assay.

Support Provided by the Clinical Research Directorate

GCC for NCI MATCH

Comprehensive Genomic Characterization of Residual Patient Samples Enrolled in NCI-MATCH

KEY ACCOMPLISHMENTS

- FNL executed an integrated project management plan involving four collaborator groups, key NCI collaborators, and external stakeholders involved in this project.
- FNL provided key coordination between the NCI Program Office, the National Center for Biotechnology Information Database of Genotypes and Phenotypes (dbGaP), and the Clinical Data Center collaborator team to establish workflows for uploading controlled-access clinical data and registering 16 NCI-MATCH arms, prioritized for this project, in dbGaP.
- FNL managed the successful distribution (both tumor DNA and RNA) of just over 200 cases to Genomic Characterization Centers (GCC) across six NCI-MATCH arms, upload of biospecimen and clinical data to the NCI Genomic Data Commons (GDC), and submission of sequencing data to the GDC.

FNL recommended and evaluated improvements to processes related to sample aliquoting, biospecimen metadata, and clinical data submissions for the 200 cases shipped so far. The staff managed aliquoting of pre-extracted tumor DNA and RNA from patients enrolled in the NCI-MATCH clinical trial and DNA extraction and aliquoting from paired germline samples (when available at the source site). Samples were distributed to the GCCs.

FNL coordinated communications between relevant groups to finalize clinical data elements and standard terms to be used for the project, establish requirements for content on the project site, and define the structure of real-time reporting of information. The staff managed transfer of biospecimen metadata and clinical data from the source site to the Clinical Data Center for processing and upload to the NCI GDC.

The FNL team provided NCI and the GCCs key technical guidance to review sequencing data of almost 200 cases generated by the GCCs and ensured that the GCCs adhered to quality requirements when submitting the molecular characterization data to the GDC, which was important given the risks related to using minimally recommended starting material and the other known limitations associated with these residual project samples. The staff managed activities at GCCs, including comprehensive molecular characterization of the DNA (whole-exome sequencing) and RNA (mRNA sequencing). In addition, FNL facilitated communications between the NCI Program Office and the GDC team on issues related to data dictionary updates, the harmonization process for 82 samples, and access to results for NCI's review.

Retrospective Tumor Characterization Analysis

KEY ACCOMPLISHMENTS

- For the two studies selected in the first round of proposal solicitation, FNL helped the study investigators to complete several project-initiation tasks, including registering the study at dbGaP, creating the project at the GDC, and executing necessary material transfer agreements.
- For these studies, FNL managed the biospecimen bank subcontractor to process all of the samples for the Wilms tumor study and approximately 40 percent of the samples for the acute lymphoblastic leukemia (ALL) study.
- For the two studies selected in the second round of proposal solicitation, FNL helped NCI solicit and review the proposals. In addition, FNL has awarded subcontracts to support the study teams and the biospecimen banks for these studies.

NCI created the Molecular Profiling to Predict Response to Treatment (MP2PRT) program, which invited funded National Clinical Trials Network Groups and NCORP Research Bases to submit proposals requesting characterization of biospecimens collected from NCI-sponsored National Clinical Trials Network or NCORP clinical trials in which clinical data have already been presented or published. The MP2PRT solicited proposals that are hypothesis-driven, hypothesis-generating, or exploratory and that propose studies wherein comprehensive molecular analyses could answer a key clinical question. After the first round of proposal solicitation and review, the NCI selected the following two pediatric cancer studies: (i) "Comprehensive Genomic Profiling to Identify Alterations Associated with Relapse for NCI Standard Risk B-lineage ALL and NCI High Risk B-lineage ALL with Favorable Genetic Features," and (ii) "Identification of Genetic Changes Associated with Relapse and/or Adaptive Resistance in Patients Registered as Favorable Histology Wilms Tumor

on AREN03B2." After the second round of proposal solicitation and review, NCI selected the following two adult cancer studies: (i) "Identifying Novel Molecular Markers of Response to Radiotherapy in Meningiomas Using Samples from NRG-RTOG-0539," and (ii) "Genomic and Molecular Characterization of Biomarkers Associated with Tumor Angiogenesis, DNA repair, and Immunologic Tolerance Among Exceptional Responders and Long-term survivors in NRG-GOG-240 (a clinical trial on cervical cancer)." NCI intends to select at least one more study through a third round of proposal solicitation and review.

FNL provides multifaceted support for the MP2PRT program, helping NCI during each round of proposal solicitation and review. Once NCI selects the studies, FNL provides project management support toward successful study completion. The FNL staff contracts with the study team to help ensure that they meet NCI requirements while completing their study. NCI requirements for the MP2PRT program include submitting relevant clinical data to the GDC; registering the study with dbGaP; depositing the underlying genomic data to an NCI repository such as the GDC; and complying with applicable laws, policies, and regulations such as the NCI Cancer Moonshot℠ Public Access and Data Sharing Policy.

The selected studies characterize and analyze biospecimens collected in clinical trials that are now closed. FNL contracts with the biospecimen banks that house the biospecimens to provide the necessary processing services for completing the study. These services include performing pathology review on tumor tissues, extracting DNA/RNA analytes from tumor and normal tissues, and distributing the analytes to other laboratories for characterization. Each study has its own set of characterization requirements. FNL manages several contracted laboratories (the GCCs) to fulfill each study's characterization needs. The molecular characterization platforms provided by the contracted laboratories include whole-genome sequencing, whole-exome sequencing, transcriptome sequencing (RNA-Seq), DNA methylation arrays, and other molecular assays. FNL also contracts with Genomic Data Analysis Centers to provide any additional genomic data analysis support that the study investigators may need.

Support Provided by the Laboratory Animal Sciences Program

Animal Program Frederick

KEY ACCOMPLISHMENTS

- Old and aging bulk autoclaves in buildings 1032 and 1038 were upgraded to maintain the health status of the breeding colonies of immuno-compromised non-obese diabetic (NOD) severe combined immunodeficiency (SCID) IL-2Rg^{null} (NSG) and athymic nude mice.
- The breeding operations of immunocompromised NSG and athymic nude mice transitioned from semirigid flexible film isolators to Lab Products, Inc. ventilated rodent racks. This transition has helped increase the efficiency of breeding operations.
- In building 1029, Thoren static caging racks were switched to Lab Products, Inc. ventilated racks to increase housing capacity, reduce occupational hazards (allergens), and improve working conditions.
- The majority of Division of Cancer Treatment and Diagnosis (DCTD) animal buildings do not have automatic backup generator and HVAC to maintain proper environmental conditions for the animals in case of power outages. FNL staff must rely on manual generators, coolers, and heaters in case of power outages. LASP conducted training for more than 25 staff members for safe operations of manual generators, coolers, and heaters. LASP initiated the process of designing and installing an automatic generator with assistance from the Facilities Maintenance and Engineering Directorate, Office of the Director, NCI at Frederick, and the Office of Space and Facilities Management.
- LASP has played a significant role in designing the DCTD modular animal facility and laboratory that will meet AAALAC standards. This building will have a large autoclave that can sterilize a rodent rack, which will be very beneficial for DCTD. Currently, DCTD does not have any autoclaves big enough to fit a rodent rack. The modular facility will have thimble-connected ventilated rodent racks, which will reduce the allergen load and ammonia smell.
- During the pandemic, LASP continued to provide essential husbandry services for DCTD animal facilities with only a five to ten percent reduction in the animal census.
- We obtained a Hydropac® bagger from LASP Bethesda, installed and decontaminated it in Building 1021, and tested it to ensure no unwanted microbial contaminants were present. This water bagger is automatic and less labor-intensive for providing drinking-water bags for animals housed in buildings 1032, 1029, and 1038.
- LASP relocated animals, offices, equipment, and personnel to support the installation of a new HVAC unit in building 1023.

Animal Research Technical Support and the Small Animal Imaging Program

KEY ACCOMPLISHMENTS

- LASP developed the techniques and animal study protocols and facilitated the collaboration with Charles River Laboratories to conduct the ^{89}Zr-BetaSphere project using the intrahepatic arterial

catheterization surgical procedure. Unfortunately, due to COVID-19, all non-essential studies were halted and placed on hold.

NCI Experimental Therapeutics Program (NExT)'s mission is to advance clinical practice and bring improved therapies to patients with cancer by supporting the most promising new drug discovery and development projects. NCI partners with successful applicants to facilitate the milestone-driven progression of new anticancer drugs and imaging agents toward clinical evaluation and registration. As part of this mission, the LASP Animal Research Technical Support Group and the Small Animal Imaging Program assist the NExT Program in performing validation experiments.

Immediate Office of the Director, Coordinating Center for Clinical Trials

Support Provided by the Clinical Monitoring Research Program Directorate

Clinical Program/Project Management

KEY ACCOMPLISHMENTS

- Collaborated with the National Cancer Institute (NCI) Clinical Trials Reporting Program (CTRP) engineering team and NCI ServiceNow to transition CTRP to a new communications management application
- Supported an NCI-sponsored research study awarded to The MITRE Corporation to study CTRP's organizational effectiveness and business processes
- Published the 2020 Biomarker, Imaging, and Quality-of-Life Studies Funding Program (BIQSFP) Guidelines and their 10 supporting documents

Frederick National Laboratory for Cancer Research (FNL) provides project management and program analysis as part of the NCI Coordinating Center for Clinical Trials (CCCT) team, supporting the CTRP, NCI steering committees, and BIQSFP and providing logistical meeting planning and travel support for a variety of CCCT-sponsored meetings. FNL provides travel services for employees and nonemployees (nongovernment personnel), which includes making travel plans and paying for trips as requested in support of program operations (e.g., program operations and/or projects).

CCCT requested expanded supervisory support, and in June 2020, an FNL staff member in the Clinical Monitoring Research Program Directorate (CMRPD) transitioned to a new role that provides overarching leadership, strategic advice, and program management to ensure seamless support to CCCT. The staff member collaborates with CCCT leadership, CCCT program directors, and other government staff to ensure clear communications and processes are in place to meet program goals; share program metrics; identify opportunities, risks, and gaps within and across the programs; and make suggestions for addressing them. This new program management position oversees the FNL teams in CMRPD that support the following programs and activities for CCCT.

NCI Clinical Trials Reporting Program

The FNL Clinical Trials Reporting Office (CTRO) in CMRPD, which supports NCI's CTRP, includes both protocol and scientific data analysts who review clinical trial protocol documents submitted to CTRP and create clinical trial summary records. These records serve NCI's portfolio analysis purposes and are the source for the NCI Office of Cancer Centers Data Table 4 reports to support the cancer research grants review process. Some of the clinical trial information that CTRO abstracts is shared on public clinical trial search websites, including Cancer.gov and ClinicalTrials.gov. The team is responsible for maintaining the CTRP clinical trial records and continually updates their content based on change notifications and protocol amendment submissions.

FNL staff members manage NCI's ClinicalTrials.gov account for NCI-sponsored trials registered in CTRP, ensuring that the information about the trials displayed on the site is accurate and managing compliance with results reporting to ClinicalTrials.gov. FNL staff members also work with trial owners to ensure timely and accurate submission of accrual data to CTRP. User support specialists respond to queries from the external and internal CTRP user community and provide training presentations for CTRP registration and accrual reporting upon request.

NCI leadership engaged The MITRE Corporation to run an NCI-sponsored research study of CTRP's organizational effectiveness and business processes to identify ways to improve CTRP operations. The FNL team led meetings to facilitate MITRE's understanding of CTRO operations. Staff subsequently participated in brainstorming sessions, interviews, and focus groups. These efforts resulted in a detailed list of suggestions for ways to enhance CTRP and prepare for programmatic updates, expansion, and strategic directions in the coming years.

As a follow-up to the MITRE-CTRP study, the FNL team collaborated with CTRP engineering staff to design and review improvements for CTRP applications, including protocol abstraction, persons and organizations curation, and registry and accrual reporting. In addition, the team collaborated with CTRP engineering staff and NCI ServiceNow to transition CTRP to a new communications management application built from the ServiceNow platform. Migration efforts included reviewing the new application design, performing user acceptance testing, transferring legacy data, and training users.

CTRO streamlined the project management process by discontinuing dependence on a subcontract that supplied staff. Effective August 31, 2020, all CTRO staff are FNL

employees and directly managed by FNL's operations director for the office. External subcontract management activities are no longer needed.

NCI Steering Committees

In support of the NCI steering committees, FNL provides project management, analyzes programs, and manages quarterly invoicing and payments based on meeting attendance for vendors that participate in clinical research trials. FNL manages project planning, implementation, performance, and alignment across the current 18 steering committees comprising more than 550 steering committee and task force members and ensures a cost-effective and efficient support mechanism for reimbursing the members.

Biomarker, Imaging, and Quality of Life Studies Funding Program

In support of BIQSFP, FNL facilitates the evaluation of proposals and related programmatic activities that require project management and/or program analysis. FNL provides comprehensive clinical program/project management to the funding program by supporting essential biomarker, imaging, and quality of life studies associated with clinical trial concepts and primary symptom management trial concepts.

FNL staff in CMRPD supported the review and revision process that led to updating BIQSFP's 2018 Guidelines and publishing the 2020 BIQSFP Guidelines and its 10 supporting documents. The process included collaboration with CCCT, the Cancer Therapy Evaluation Program, the Division of Cancer Prevention, Cancer Imaging Program, the Cancer Diagnosis Program, the Division of Cancer Control and Population Sciences, NCI's Biometric Research Program, and internal and external stakeholders and culminated in publication to the BIQSFP website. The guidelines provide direction to principal investigators, NCI's National Clinical Trials Network groups, NCI steering committees and task forces, and the NCI Community Oncology Research Program (NCORP) research bases for considering the design and submission of a biomarker, imaging, or quality of life study for BIQSFP funding.

All revised supporting documents for application and evaluation of the studies, including FAQs, were posted on the BIQSFP website, which receives several hundred visits every month.

FNL staff also supported the planning and rollout of the BIQSFP End-of-Year Non-Real-Time Integrated Study Funding Opportunity.

Meeting Planning/Travel Support

FNL staff members in CMRPD provided logistical meeting planning and travel support for 10 in-person meetings and seven webinars, outlined below. (Due to the COVID-19 pandemic, nine in-person meetings were cancelled.) The meetings support CCCT's role in integrating NCI's clinical and translational research programs to advance science and patient care by encouraging the exchange of information about NCI's clinical trials programs and associated translational research. FNL engaged the extramural community and provided transparency regarding NCI's clinical trials enterprise while supporting various meetings, including: (i) steering committee face-to-face meetings, (ii) clinical trial planning meetings, (iii) CCCT-supported American Society of Clinical Oncology meetings, (iv) Clinical Trials and Translational Research Advisory Committee working group meetings, (v) Office of the Director special meetings, and (vi) NCI-requested workshops.

Minimal Residual Disease Testing in a Clinical Trial for Acute Leukemia

CCCT enhances the scientific quality of large Phase II and Phase III trials conducted by NCI's National Clinical Trials Network (NCTN) and the NCORP by improving prioritization, funding, and standardization of associated imaging, biomarker, and quality-of-life studies. This task order supports research on a new treatment for acute leukemia in children. NCI is seeking a sensitive assay to identify children who are most likely to relapse following initial treatment and, thus, are eligible to receive additional treatment, while sparing children unlikely to relapse from unnecessary treatment. This project aims to use an ultrasensitive assay to identify patients whose disease has returned following treatment in a clinical trial of a new therapy for acute leukemia.

An FNL partner provides assay tracking, reconciliation with the respective NCTN group receiving the results, and a payment process for satisfactory completion of the assays. The protocol continues to accrue participants well, with the integral assays supported by this partnership determining pediatric patients' eligibility for enrollment.

Immediate Office of the Director, Center for Cancer Genomics

Support Provided by the Biomedical Informatics and Data Science Directorate

Informatics Operations Support

Data Coordinating Center

KEY ACCOMPLISHMENTS

- Completed transfer of all Therapeutically Applicable Research to Generate Effective Treatments (TARGET) data to the Genomic Data Commons
- Successfully updated file transfer technology, where submitters provide files, replacing secure file transfer protocol with Globus

- Transitioned a new staff member without any interruption of service

Within the Center for Cancer Genomics, the Office of Cancer Genomics (OCG) runs several cancer genomics and translation projects, such as:

- TARGET, which is focused on identifying therapeutic targets as well as prognostic and diagnostic markers in multiple childhood cancers. The initiative includes the study of:
  - High-risk acute lymphoblastic leukemia
  - Neuroblastoma
  - High-risk and treatment-refractory acute myeloid leukemia
  - Osteosarcoma
  - Kidney tumors (including the high-risk Wilms tumor)
- The Cancer Genome Characterization Initiative, which supports cutting-edge genomics research on rare cancers. Researchers develop and apply advanced sequencing and other genome-based methods to identify novel genetic abnormalities in tumors. The extensive genetic profiles generated by the initiative may inform better cancer diagnosis and treatment. Researchers use molecular characterization to focus on cancers such as Burkitt lymphoma and HIV-positive tumors.
- The Cancer Target Discovery and Development (CTD^2) Program, which works to functionally validate discoveries from large-scale genomic initiatives and advance them toward precision medicine through the efforts of the 13 OCG-supported research teams—called "Centers"—and open-access data sharing. Through cross-network collaborations, CTD^2 uses innovative bioinformatics and functional biology to mine data to perform the following tasks:
  - Find alterations that potentially influence tumor biology
  - Characterize the functional roles of candidate alterations in cancers
  - Identify novel approaches that directly or indirectly target causative alterations

These programs all contribute to OCG's mission to help identify genomic alterations that offer pathways to novel therapeutic interventions that may lead to more effective cancer treatments.

A Data Coordinating Center (DCC), managed by Frederick National Laboratory for Cancer Research (FNL) staff in the Biomedical Informatics and Data Science Directorate, was established to accept all the data generated by these programs. The DCC has been in place for several years and has updated its functionalities to meet OCG's evolving needs. At a high level of task description, the DCC accepts, quality-controls, inventories, processes, stores, and manages data availability. Data includes clinical, genomic, and pathology images. Data availability for public access and controlled access is managed by the DCC and uses a web interface specific to each program. The following two figures show the interfaces to access TARGET and CTD^2 data.

Figure 1. The TARGET Data Matrix is a data availability matrix for one subset of the programs the DCC manages. The data range that the DCC handles in the matrix is extensive, from metadata, exome sequencing, and DNA methylation to mRNA sequencing and clinical data.

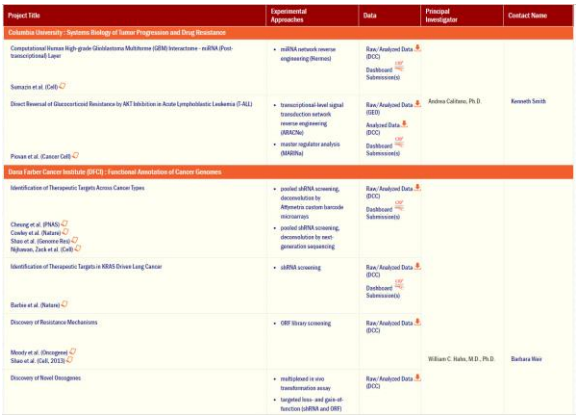


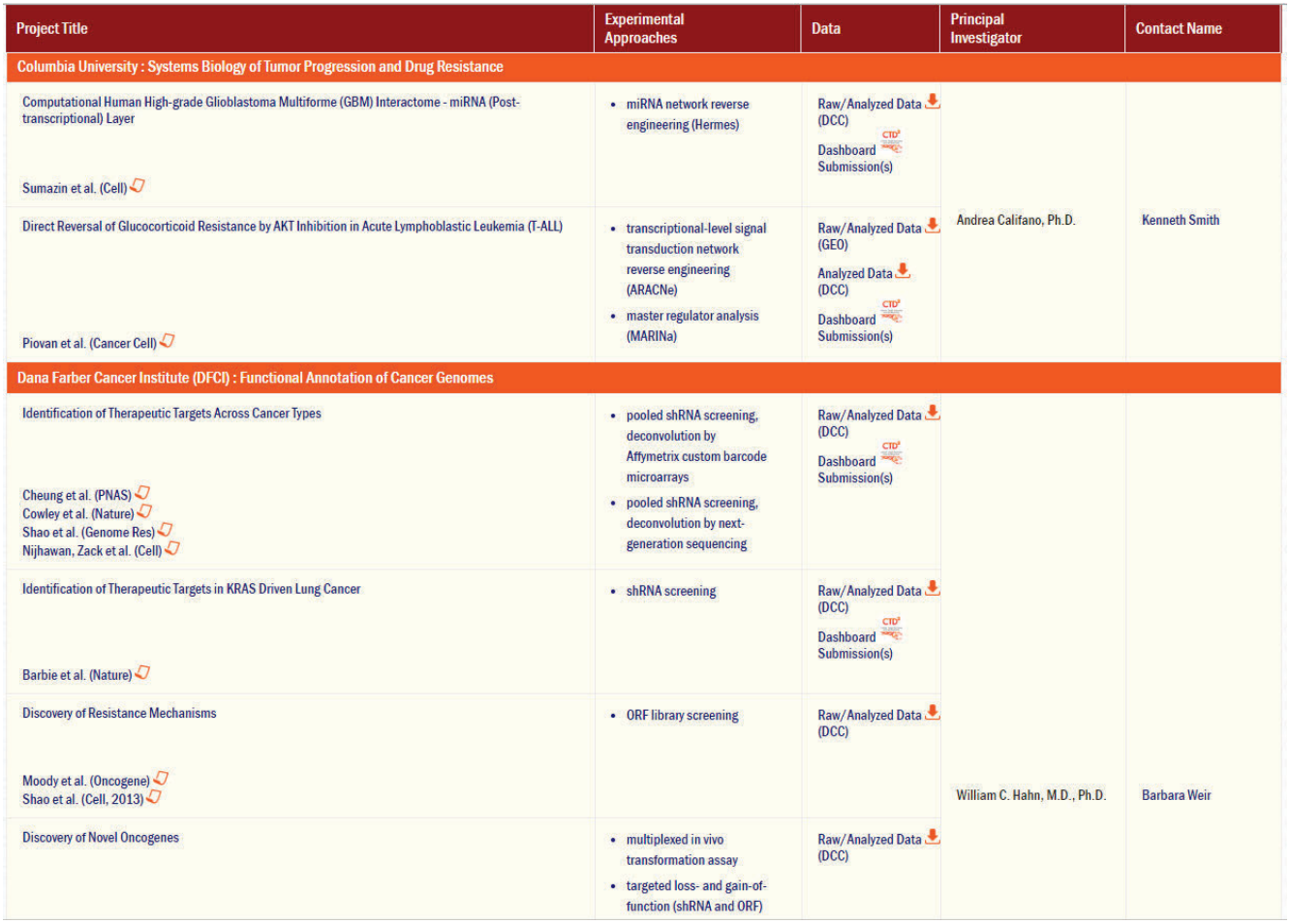
Figure 2. The CTD^2 Dashboard shows the data available for the CTD^2 Program's output.

Unlike many such programs, the submission groups did not have to adhere to any submission standards for data format. Therefore, the DCC has been very flexible. For example, the team handles the same data type generated on different platforms. The team also manages those instances when groups present the same data types but in different formats. The DCC has built a range of experience, capabilities, procedures, and tools to help process the data. Considerable work has also been devoted to managing the logistical elements of the project:

- Tracking the status of sample submission across many different groups

Disease	Patient Data	Gene Expression	Copy Number	Methylation	miRNA	Sequence				Other
Acute Lymphoblastic Leukemia (ALL)										
ALL Phase I		Affymetrix U133 Plus 2	Affymetrix SNP 500k			Whole Genome	Whole Genome Lite	mRNA-seq	Targeted Resequencing	Kinome
	Clinical File	DCC Open*	DCC Open* DCC Controlled†			View cases* FASTQ/BAM† DCC Controlled†	View cases* FASTQ/BAM†	FASTQ/BAM† DCC Open* DCC Controlled†	Sequence Trace Linking Table† 125 Genes*	Kinome Linking Table† 113 genes*
	Sample Matrix									
ALL Phase II		Affymetrix U133 Plus 2	Affymetrix SNP 6.0	NimbleGen HELP	miRNA-seq	Whole Genome	Whole Exome	mRNA-seq		
	Clinical File				FASTQ/BAM†	FASTQ/BAM†	DCC Controlled† FASTQ/BAM† DCC Open*	FASTQ/BAM†		
	Sample Matrix									
Acute Myeloid Leukemia (AML)										
AML		Affymetrix Gene ST	Affymetrix SNP 6.0	Illumina Infinium 27k	miRNA-seq	Whole Genome	Whole Exome	mRNA-seq		
	Clinical File				FASTQ/BAM†	FASTQ/BAM†	DCC Controlled† FASTQ/BAM† DCC Open*	FASTQ/BAM†		
	Sample Matrix									
AML Induction Failure (AML-IF)		mRNA-seq	Whole Genome		miRNA-seq	Whole Genome		mRNA-seq		
	Clinical File	FASTQ/BAM†	FASTQ/BAM†		FASTQ/BAM†	FASTQ/BAM†		FASTQ/BAM†		
	Sample Matrix									
Kidney Tumors										
Wilms Tumor (WT)		Affymetrix U133 Plus 2	Affymetrix SNP 6.0	Illumina Infinium 450k	miRNA-seq	Whole Genome	Whole Exome	mRNA-seq		
	Clinical File	DCC Open*	DCC Open* DCC Controlled†		FASTQ/BAM†	FASTQ/BAM† DCC Open*	FASTQ/BAM†	DCC Controlled† FASTQ/BAM† DCC Open*		
	Sample Matrix									

Figure 1. The TARGET Data Matrix is a data availability matrix for one subset of the programs the DCC manages. The data range that the DCC handles in the matrix is extensive, from metadata, exome sequencing, and DNA methylation to mRNA sequencing and clinical data.



Project Title	Experimental Approaches	Data	Principal Investigator	Contact Name
Columbia University : Systems Biology of Tumor Progression and Drug Resistance				
Computational Human High-grade Glioblastoma Multiforme (GBM) Interactome - miRNA (Post-transcriptional) Layer Sumazin et al. (Cell)	• miRNA network reverse engineering (Hermes)	Raw/Analyzed Data (DCC) Dashboard Submission(s)		
Direct Reversal of Glucocorticoid Resistance by AKT Inhibition in Acute Lymphoblastic Leukemia (T-ALL) Piovan et al. (Cancer Cell)	• transcriptional-level signal transduction network reverse engineering (ARACNe) • master regulator analysis (MARINa)	Raw/Analyzed Data (GEO) Analyzed Data (DCC) Dashboard Submission(s)	Andrea Califano, Ph.D.	Kenneth Smith
Dana Farber Cancer Institute (DFCI) : Functional Annotation of Cancer Genomes				
Identification of Therapeutic Targets Across Cancer Types Cheung et al. (PNAS) Cowley et al. (Nature) Shao et al. (Genome Res) Nijhawan, Zack et al. (Cell)	• pooled shRNA screening, deconvolution by Affymetrix custom barcode microarrays • pooled shRNA screening, deconvolution by next-generation sequencing	Raw/Analyzed Data (DCC) Dashboard Submission(s)		
Identification of Therapeutic Targets in KRAS Driven Lung Cancer Barbie et al. (Nature)	• shRNA screening	Raw/Analyzed Data (DCC) Dashboard Submission(s)		
Discovery of Resistance Mechanisms Moody et al. (Oncogene) Shao et al. (Cell, 2013)	• ORF library screening	Raw/Analyzed Data (DCC)	William C. Hahn, M.D., Ph.D.	Barbara Weir
Discovery of Novel Oncogenes	• multiplexed in vivo transformation assay • targeted loss- and gain-of-function (shRNA and ORF)	Raw/Analyzed Data (DCC)		

Figure 2. The CTD^2 Dashboard shows the data available for the CTD^2 Program's output.

- Handling sample updates
- Updating processes and formats to handle new types of information and assays
- Communicating with the submission groups regarding schedule and data definitions

Data are also processed to ensure consistency, quality, and adherence to privacy protection regulations (for example, correct handling of germline variations). Once the data have been thoroughly processed, they are made available using National Cancer Institute (NCI)-hosted systems that enable access control; some data are available only to the program's members, whereas data that meet OCG's data-release parameters are publicly available. In addition, data prepared by the DCC have already been submitted to the International Cancer Genome Consortium.

In addition to all the logistics and bioinformatics activities involved in handling the data that flows into the DCC, the team also supports the website for the CTD^2 Dashboard. The CTD^2 Dashboard hosts analyzed data and other evidence generated by the CTD^2 Network. It is a web interface for the research community to browse and search for CTD^2 Network data related to genes, proteins, and compounds or to read stories that summarize key findings from completed projects associated with publications.

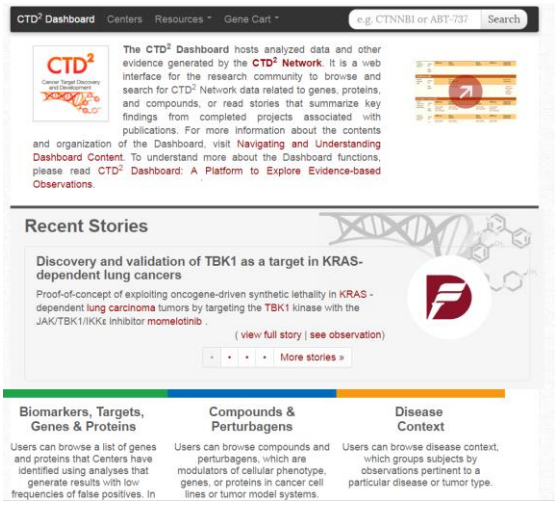


Figure 3. CTD^2 Dashboard.

From a DCC perspective, the TARGET project had been nearing completion, and the team processed small amounts of new data. The team has been preparing TARGET data for inclusion in the Genomic Data Commons (GDC). The GDC is providing a subset of the TARGET data to the cancer community, along with the other projects that GDC supports. The DCC has been actively supporting the GDC in their understanding of the data and helping to review the processing data from a quality-control perspective. All the TARGET data can still be found on the TARGET website, but the GDC is actively updating their repository of the data, with the goal of having all the TARGET data in the GDC and available to the community. Although support for TARGET by the DCC is ending, there is no finalized end date yet.

The DCC has continued to support the Gabriella Miller Kids First Pediatric Research Program, a pediatric-focused project funded by the National Institutes of Health Common Fund. As this project matures, the DCC is providing support, such as guidance on submitting data to the Sequence Read Archive and the database of Genotypes and Phenotypes. The team has also supported the Pediatric Genomic Data Inventory project. This project provides a simple view of various non-NCI-based resources of pediatric genomic data. Users can browse and filter a list of data sets that describe the number of cases, cancer type, and type of genomics data. If users find a set of interest, the relevant contact information is displayed for them to use.

Figure 4. Pediatric Genomic Data Inventory Overview.

The DCC's future activities are to maintain current operations supporting the ongoing activities of the OCG's genomic data generation programs. In addition, the DCC will maintain its flexibility in adapting to new data and data types needed by the community.

Support Provided by the Clinical Research Directorate

Genomic Data Commons

KEY ACCOMPLISHMENTS

- Deployed several data and software releases of the Genomic Data Commons (GDC) Data Portal, the GDC Data Submission Portal, the GDC Data Transfer Tool, and the GDC Application Programming Interface (API)

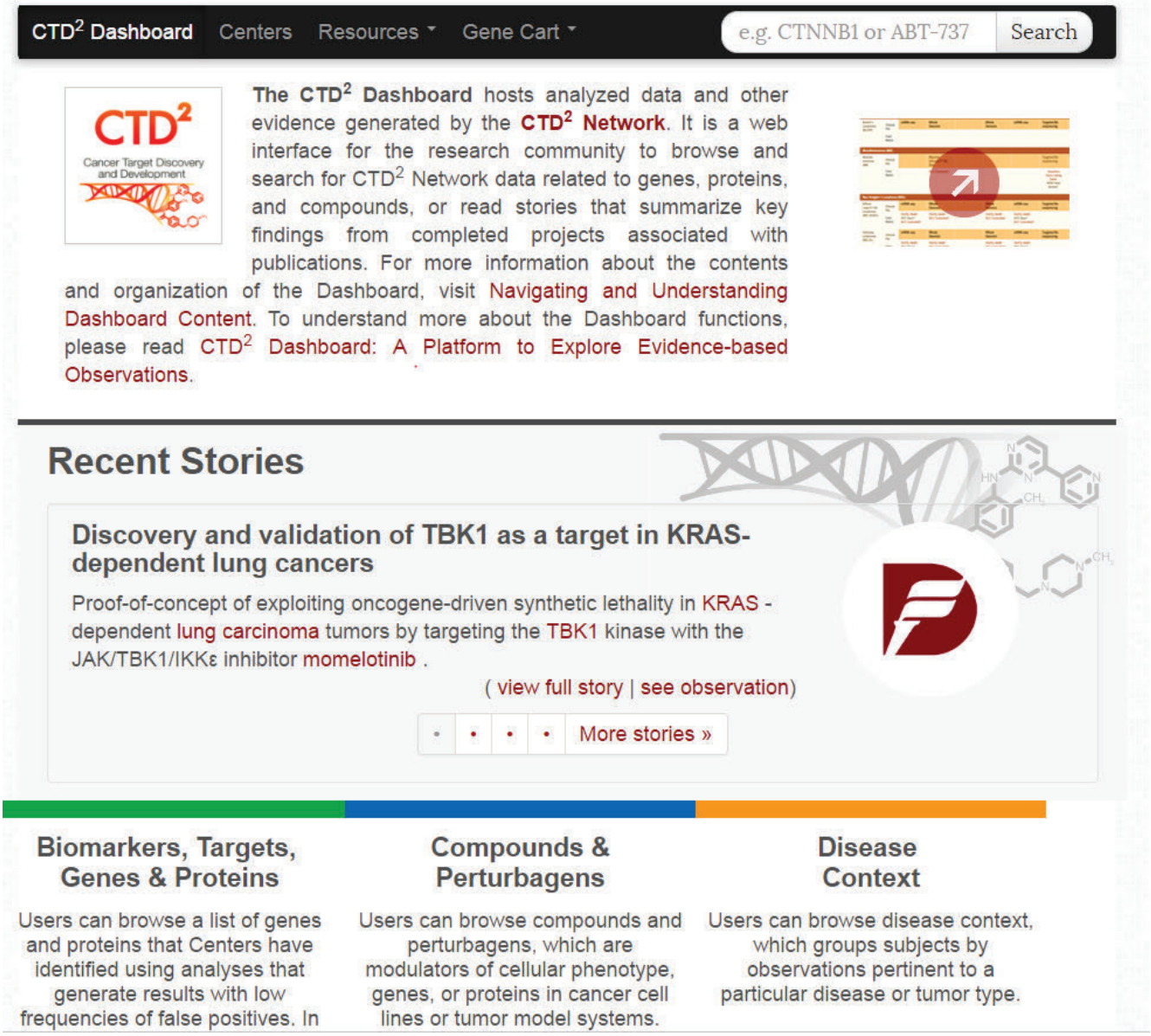


Figure 3. CTD² Dashboard.

49 projects available for review in the PGDI Display

About

Pediatric cancer is a genetic disease that can largely differ from similar malignancies in an adult population. To fuel new discoveries and treatments specific to pediatric oncologies, the NCI Office of Cancer Genomics has developed a dynamic resource known as the Pediatric Genomic Data Inventory to allow investigators to more easily locate genomic datasets. This resource lists known ongoing and completed sequencing projects of pediatric cancer cohorts from the United States and other countries, along with some basic details and reference metadata.

This inventory is an evolving list that will be continually updated as new information is deposited by the research community. The success of this resource requires ongoing participation from the research community, and feedback is both welcomed and encouraged. To get involved and deposit information about a pediatric cancer sequencing cohort or project, click the link below to become a data submitter.

Figure 4. Pediatric Genomic Data Inventory Overview.

- Supported National Cancer Institute (NCI) Analysis Working Groups (AWG) by deploying releases of GDC processed data in the GDC AWG Portal for analysis and review
- Maintained and optimized GDC bioinformatics pipelines
- Hosted monthly GDC webinars for the research community and provided help desk support
- Performed monthly maintenance activities on equipment hosted in the GDC Data Center
- Completed security updates in support of the annual security assessment
- Provided project management support for all GDC functional areas and developed a road map for achieving GDC milestones

New Data Visualizations

New data visualizations focused on GDC Data Analysis, Visualization, and Exploration (DAVE) tools for RNA sequencing (RNA-Seq) data. This included implementing the necessary visualizations, data transformations, and optimizations for enhanced performance.

- Deployed single-project selection DAVE for controlled access data for user acceptance testing
- Designed and developed a gene expression visualization tool

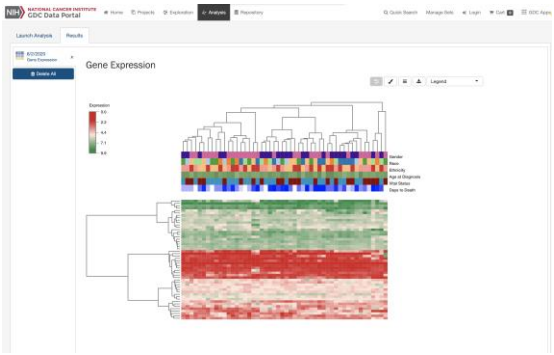


Figure 1. Gene expression visualization tool.

- Designed and developed a tool for visualizing single-cell RNA-Seq data

Figure 2. Single-cell RNA-Seq visualization tool.

- Began design of a proof-of-concept for using ProteinPaint from St. Jude Children's Research Hospital in the GDC to visualize mutations for protein-coding regions

Data Access Enhancements

GDC data access tools were enhanced to enable bench scientists to perform analysis more efficiently. This included providing researchers with access to reference genome annotations and additional faceted searches.

- Incorporated the reference genome annotations that the GDC uses to generate/process data as a link in the GDC Data Portal
- Enabled users to add a custom facet for searching the GDC Data Portal Repository by microsatellite instability and associated microsatellite instability counts

Data Submission Enhancements

The GDC Submission System was enhanced to provide greater flexibility to GDC submitters. This included support for new processes supporting batch data submission and additional automated review/quality control (QC) tools. The GDC Submission System was also updated to support new data types and platforms for single-cell RNA-Seq data submission.

- Established new processes supporting data submission and tracking, including new project setup forms, methods for tracking batch data submission, and increased communication with data submitters
- Deployed automated submission review/QC checks for read group verification associated with sequence data submission

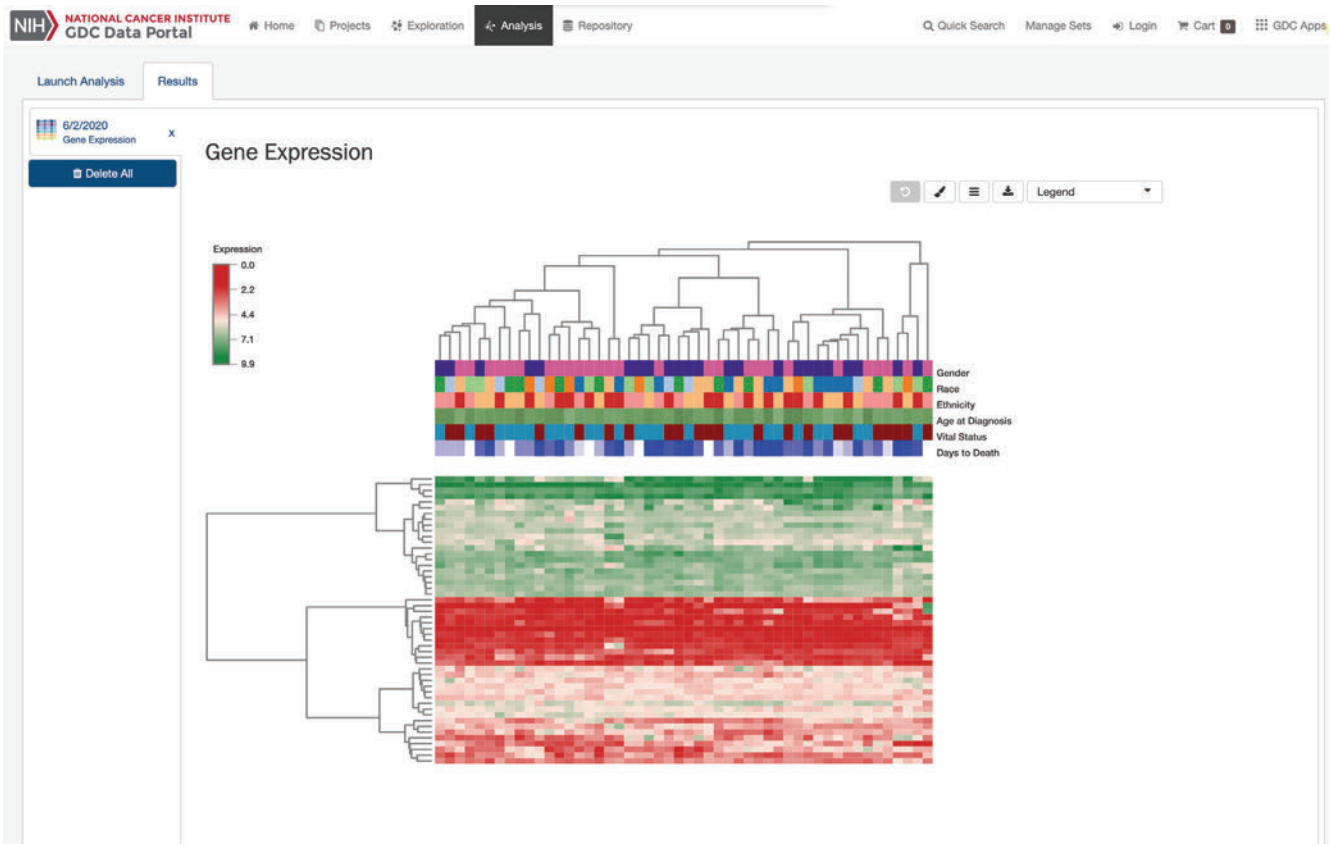


Figure 1. Gene expression visualization tool.

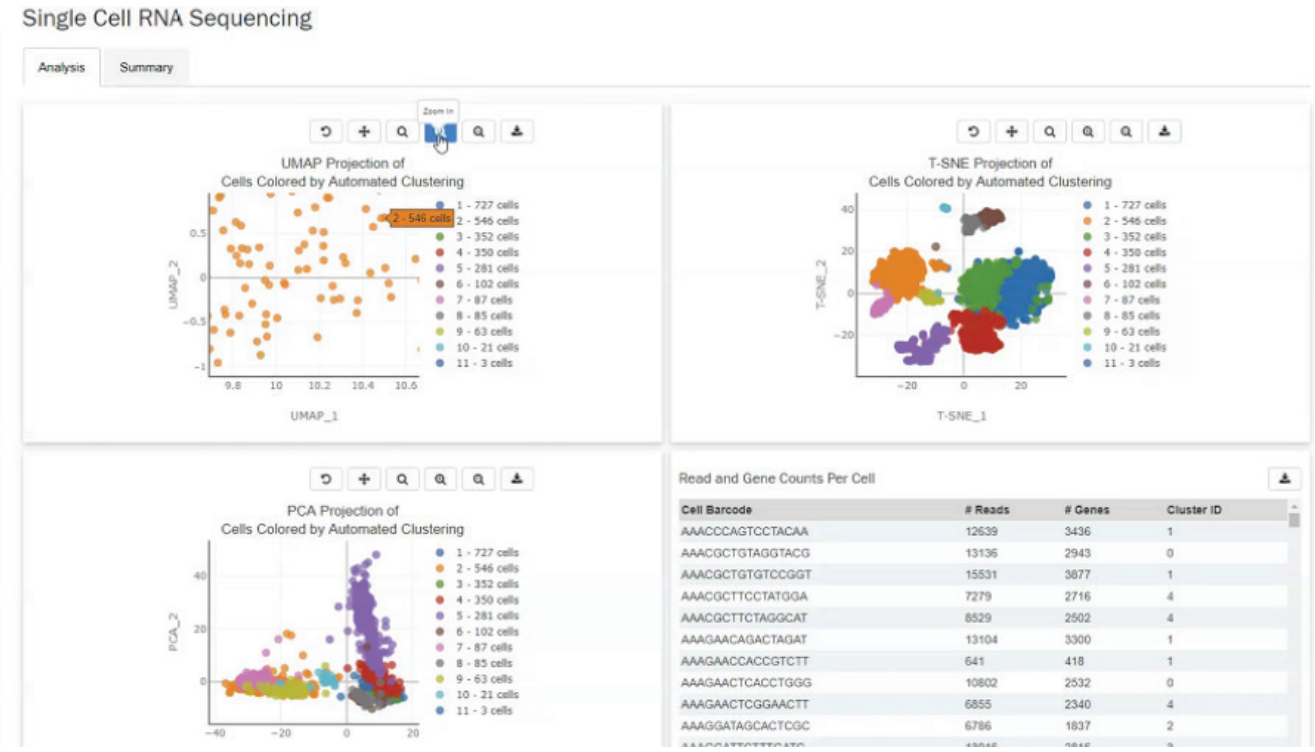


Figure 2. Single-cell RNA-Seq visualization tool.

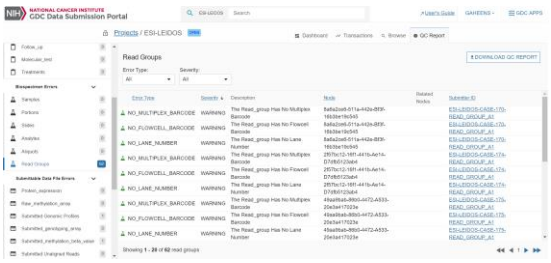


Figure 3. Automated submission review/QC tool.

- Enabled support for the submission of single-cell RNA-Seq data from the 10x Genomics and Smart-seq2 platforms. Moreover, the system was extended to support single-nuclei RNA-Seq data submission.
- Deployed several GDC Data Dictionary changes, such as standard terminology for therapeutic agents, NCI Thesaurus codes for diagnostic properties, a new pathology details node, additional gene and antigen properties in molecular tests, new data elements for the RNA expression workflow, and several data elements supporting project-specific needs

Harmonization of New and Existing Data

GDC pipelines were developed to support the harmonization of new single-cell RNA-Seq data from the 10x Genomics and Smart-seq2 platforms. New and existing pipelines were also designed, developed, enhanced, and optimized to process new data sets from several programs.

- Completed implementation of single-cell RNA-Seq harmonization pipelines supporting the 10x Genomics and Smart-seq2 platforms

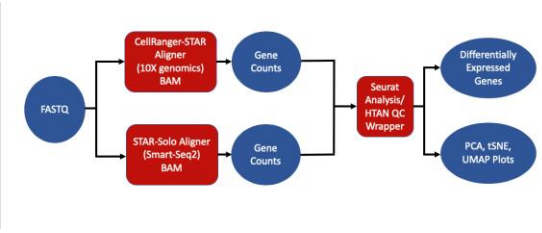


Figure 4. Single-cell RNA-Seq workflow..

- Analyzed and evaluated tools supporting RNA-Seq variant calling
- Implemented the Pindel variant calling pipeline to detect insertions and deletions and generated Pindel Variant Call Format (VCF) files
- Deployed the Sanger whole-genome sequencing (WGS) somatic variant calling and copy-number variation pipeline for generating copy-number segments using the allele-specific copy-number analysis of tumors
- Implemented an aliquot-level Mutation Annotation Format (MAF) pipeline for whole-exome sequencing data and began investigating a mutation annotation format pipeline for WGS data
- Processed and released new data sets for the following programs: The Cancer Genome Atlas (TCGA), Therapeutically Applicable Research to Generate Effective Treatments (TARGET), Clinical Proteomic Tumor Analysis Consortium (CPTAC), Genomics Evidence Neoplasia Information Exchange (GENIE), Human Cancer Models Initiative (HCMI), Cancer Genome Characterization Initiatives (CGCI), and the Functional Genomic Landscape for Acute Myeloid Leukemia (Beat AML)

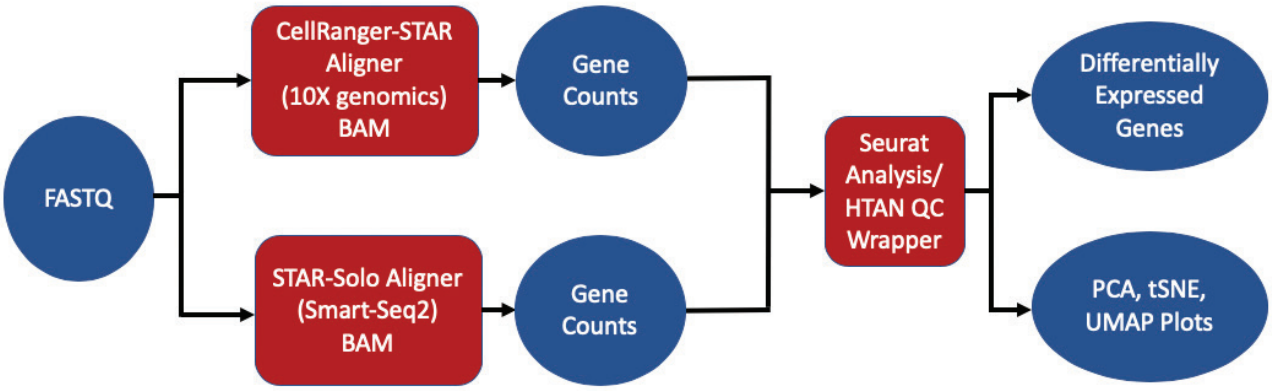
Project ID	Project Name	Processed and Released Data
All TCGA Projects	The Cancer Genome Atlas	SNP6 ASCAT Copy Number Segments
BEATAML1.0-COHORT	Functional Genomic Landscape of Acute	WGS, RNA-Seq
CGCI-BLGSP	Burkitt Lymphoma Genome Sequencing	WGS, RNA-Seq, miRNA-Seq
CGCI-HTMCP-CC	HIV+ Tumor Molecular Characterization Project - Cervical Cancer	RNA-Seq, miRNA-Seq, WGS, Targeted Sequencing
CPTAC-2	CPTAC Proteogenomic Confirmatory Study	WXS, RNA-Seq, miRNA-Seq
CPTAC-3	CPTAC Proteogenomic Confirmatory Study	Pindel VCFs
GENIE	AACR Project Genomics Evidence Neoplasia Information Exchange	Targeted Sequencing, Transcript Fusion, Copy Number Estimate
HCMI-CMDC	NCI Cancer Model Development for the Human Cancer Model Initiative	Pindel VCFs, Aliquot-level MAFs
MMRF-COMMPASS	Multiple Myeloma CoMMpass Study	WGS, WXS, RNA-Seq
OHSU-CNL	Genomic landscape of Neutrophilic Leukemias of Ambiguous Diagnosis	WXS, RNA-Seq
ORGANOID-PANCREATIC	Pancreas Cancer Organoid Profiling	WGS, WXS, RNA-Seq
TARGET-ALL-P1	Acute Lymphoblastic Leukemia - Phase I	WGS, RNA-Seq
TARGET-ALL-P2	Acute Lymphoblastic Leukemia - Phase II	WGS,RNA-Seq, WXS, miRNA-Seq, Pindel VCFs, Aliquot-level MAFs, SNP6 ASCAT Copy Number Segments
TARGET-ALL-P3	Acute Lymphoblastic Leukemia - Phase III	RNA-Seq, miRNA-Seq, Pindel VCFs, Aliquot-level MAFs
TARGET-AML	Acute Myeloid Leukemia	WGS, WXS, miRNA-Seq, miRNA, Pindel VCFs, Aliquot-level MAFs, SNP6 ASCAT Copy Number Segments
TARGET-CCSK	Clear Cell Sarcoma of the Kidney	RNA-Seq, Pindel VCFs
TARGET-NBL	Neuroblastoma	WXS,RNA-Seq, WGS, Pindel VCFs, Aliquot-level MAFs
TARGET-OS	Osteosarcoma	RNA-Seq, WGS, WXS, Aliquot-level MAFs
TARGET-RT	Rhabdoid Tumor	WGS, RNA-Seq
TARGET-WT	High-Risk Wilms Tumor	WGS, WXS, RNA-Seq, Aliquot-level MAFs

Figure 5. Data processed and released in the GDC.

System Scalability

The GDC designed and implemented scalability improvements for the GDC Data Portal, the GDC Data Submission Portal, and the GDC API to scale to more than 100,000 cases.

- Implemented scalability improvements for building cohorts and visualizing data through GDC DAVE tools, with notable performance enhancements in rendering survival plots
- Upgraded the GDC indexing system (ElasticSearch) and refactored the mutation indexer for improved performance



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TARGET-ALL-P3	Acute Lymphoblastic Leukemia - Phase III	RNA-Seq, miRNA-Seq, Pindel VCFs, Aliquot-level MAFs
TARGET-AML	Acute Myeloid Leukemia	WGS, WXS, miRNA-Seq, miRNA, Pindel VCFs, Aliquot-level MAFs, SNP6 ASCAT Copy Number Segments
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TARGET-RT	Rhabdoid Tumor	WGS, RNA-Seq
TARGET-WT	High-Risk Wilms Tumor	WGS, WXS, RNA-Seq, Aliquot-level MAFs

Figure 5. Data processed and released in the GDC.

- Increased the number of cases supported in the GDC to more than 84,000
- Supported a GDC user base, which expanded from 50,000 users per month to 70,000 during COVID-19

Development, Operations, and Maintenance

Development, operations, and maintenance for GDC included support for several functional areas, such as software development/quality assurance, bioinformatics, user services, operations, security, and management.

CCG-GDC Hardware Refresh

Hardware Refresh and Planning

The GDC hardware refresh involved replacing hardware that expired, leveraging a four-year refresh cycle, and planning for future refreshes.

KEY ACCOMPLISHMENTS

- Completed updates to the five-year hardware refresh plan
- Performed a storage refresh, which included installing and configuring new hardware, migrating existing data to the new storage rack, and decommissioning expired equipment.
- Designed the network and compute rack refresh and performed equipment-procurement activities

GPAS Expansion

The GDC Pipeline Automation System (GPAS) expansion aimed to increase data processing throughput and reduce time required to process large data sets. This involved expanding on-premises compute and adding the ability to burst data processing in the cloud.

- Completed support for the expansion of on-premise compute, which resulted in a notable increase in data processing throughput and reduction in data processing time
- Developed a proof-of-concept for cloud bursting the GDC WGS alignment pipeline to Amazon Web Services and enhanced the cloud bursting proof-of-concept to make it production ready

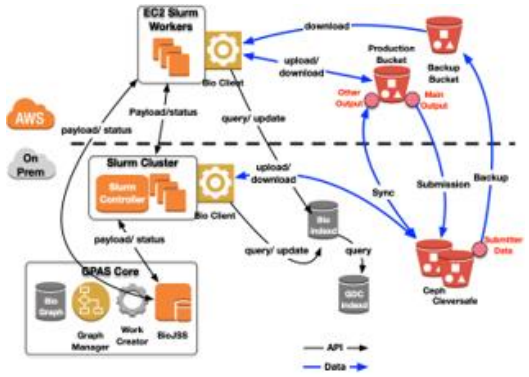


Figure 6. GDC cloud bursting design.

- Optimized the GDC WGS alignment pipeline to run efficiently in Amazon Web Services for cost effectiveness

Genomic Characterization Centers

KEY ACCOMPLISHMENTS

- For FY2020, FNL managed a subcontractor to increase reverse phase protein array (RPPA) coverage on more than 2,000 TCGA samples.
- The American Association of Cancer Research recently recognized the TCGA founding members and current project team with the 2020 Team Science Awards. For nearly a decade, FNL has supported the NCI Center for Cancer Genomics (CCG) on numerous cancer genomics projects, including TCGA.

FNL continued to support the CCG by managing Genomic Characterization Centers (GCCs). These GCCs generate various types of molecular data from analytes provided by a biospecimen processing center and deposit the molecular data at the GDC.
In FY2020, FNL continued to manage a subcontractor, first brought in in FY2019, that is working to increase RPPA antibody coverage on more than 4,000 TCGA samples. This effort is the ongoing response to NCI CCG's February 2019 request for help in increasing the coverage from approximately 220 antibodies to approximately 500 antibodies.

Human Cancer Models Initiative

KEY ACCOMPLISHMENTS

- FNL managed Cancer Model Development Centers (CMDCs), which have submitted more than 300 cancer models from a variety of tumor types.
- The FNL team worked with the two newly established CMDCs to complete the regulatory requirements for the CMDC project.

- Many of the cancer models that have been established (under FNL management) are now available to the research community via the HCMI model distributor.

HCMI is generating a community resource of large numbers of next-generation *in vitro* cancer models. These cancer models, along with the clinical and molecular characterization data, will be made accessible to scientists from academic, industry, and/or nonprofit organizations. FNL has awarded four subcontracts to function as NCI-sponsored CMDCs for the HCMI. FNL, collaborating closely with all project stakeholders, has ensured that the CMDCs are complying with the regulatory requirements for the project (such as Institutional Review Board–approved protocols and consents, intellectual property requirements, and other data and material deposit/transfer agreements).

During FY2020, the CMDCs continued to successfully establish the cancer models from patient tissues, verify that the cancer models contain predominantly cancer cells (by using internal quality control measures such as targeted sequencing, immunohistochemistry, etc.), and proceed with the next steps per the HCMI pipeline for the models and associated clinical and molecular data. The CMDCs have thus far shipped more than 300 cancer models to the Biospecimen Processing Center and to the HCMI distributor. The CMDCs are continuing to establish the cancer models from a variety of tumor types, many of which have been generated and are at different stages of expansion and QC verification. A number of these cancer models (generated from diverse tumor types, such as gastroesophageal cancer, breast cancer, pediatric cancers, glioblastomas, and lung cancer) are now available to the research community via the HCMI model distributor.

The FNL project team (in collaborative discussions with all project stakeholders as needed) will continue to closely monitor, track, and manage the CMDC project deliverables, moving toward the goal of submitting up to 673 models under this effort.

HCMI/CMDC Sequencing and HCMI Searchable Catalog

Comprehensively Characterizing Cancer Models' Genomes and Establishing an Online Searchable Catalog for the Models

KEY ACCOMPLISHMENTS

- The GCCs completed comprehensive sequencing of more than 300 cancer models and additional paired tumors and germline samples.
- For the Searchable Catalog component, FNL managed deliverables, provided technical oversight, and coordinated discussions with project stakeholders to successfully launch the Catalog for use by the scientific community. Link to website: HCMI Searchable Catalog

FNL actively managed the two GCCs subcontracted to perform high-throughput next-generation sequencing of cancer models and ancillary samples. The FNL staff provided technical oversight to the GCCs, facilitated key communications between project stakeholders, and managed end-to-end tracking on the status of samples selected for sequencing. FNL's management of expenses and adoption of the best available sequencing technologies allowed more work to be performed.

In parallel, FNL reached the key milestone of launching an online database called the HCMI Searchable Catalog. The FNL staff provides ongoing oversight to improve the site, which captures key details and displays rich content for, and allows query of, a selected set of cancer models. FNL facilitated the process of executing security documentation required for the website and provided technical inputs to enhance the visual, interactive, user-friendly features embedded in the catalog.

HIV+ Tumor Molecular Characterization Project

Milestone 1: Tissue and Data for 60 Cases Procured

KEY ACCOMPLISHMENTS

- Reinstated the acquisition portion of the milestone through the Impact Assessment Report process
- Engaged two potential vendors for tissue acquisition

In concert with the Program Office, FNL staff identified the need for subcontracting additional tissue source sites. FNL staff formally requested and obtained approval to identify and solicit potential vendors and commit funding to support the work.

FNL staff pursued the Program Office's recommended vendor through a sole-source subcontract mechanism. While the vendor was considering participation, COVID-19 introduced delays and staff unavailability. After some months, the vendor ultimately decided to pursue participation via another mechanism. The FNL staff remains poised to continue the subcontract process should it ultimately be needed to procure cases from this vendor.

The second vendor was identified through the request for information previously performed under Milestone 1. Their feasibility analysis has been delayed due to COVID-19. They remain interested in participating and optimistic that they will ultimately be able to provide cases to the program. The FNL staff continues to work with the vendor to understand their concerns and timelines toward active participation in the HIV+ Tumor Molecular Characterization Project (HTMCP).

Milestone 2: Discovery (est. 50 Cases) and Validation (est. 50 Cases) Cohorts Processed, Characterized, and Analyzed

KEY ACCOMPLISHMENTS

- Contributed to a cervical HTMCP manuscript publication in *Nat Genet*
- Included cervical cases as part of Milestone 2 through the Impact Assessment Report process

FNL staff reviewed the manuscript (Gagliardi et al., *Nat Genet*, 2020) at various stages, from early drafts to the final submission. Staff ensured that funding was appropriately acknowledged.

Additional sequencing and analysis were needed to address reviewers' comments. FNL staff formally requested and obtained approval to determine human papillomavirus status and characterize cervical cases within the study. Staff also performed the follow-on work to modify the subcontractors' statements of work and funding to complete the necessary research to finalize the manuscript.

Refractory Cancer Biospecimen Acquisition and Genomic Characterization

KEY ACCOMPLISHMENTS

- FNL executed three tissue source site subcontracts to acquire 440 (285 retrospective and 155 prospective) peripheral T-cell lymphoma cases against the goal of 500 cases.
- All regulatory- and subcontract-related stages were passed for the first site to submit clinical data.

The subcontractors are at varying stages of completing regulatory documents (Institutional Review Board approval, material transfer agreements, data use agreements, and Genomic Data Sharing certification). FNL organized an introductory data-submission call to review the data-submission process and was able to start the data submission from two domestic sites. The staff is in the process of scheduling an introductory sample-submission call as the sites are getting closer to the start of the biospecimen submission.

FNL overcame several issues in the initial phase of the project, such as the international site not being registered in the System for Award Management, that delayed the subcontract award. Initially there was no clarity regarding who the lead hub site would be for all domestic sites. It took some time for sites to decide on the hub site and subsite structure and submit proposals. Although the subcontract award process was delayed due to the subcontractor's slow responses and other initial obstacles, the project is progressing well. FNL anticipates completing the project goals.

Immediate Office of the Director, Center for Global Health

Support Provided by the Clinical Monitoring Research Program Directorate

Clinical Project/Project Management

Data Analysis/Program Evaluation and Support to CGH Regional Activities

KEY ACCOMPLISHMENTS

- Published findings from the 2018–2019 Global Oncology Survey of National Cancer Institute (NCI)-Designated Cancer Centers in *J Glob Oncol*
- Convened a series of knowledge exchange sessions in response to the COVID-19 pandemic for the Africa Cancer Research and Control Extension for Community Healthcare Outcomes (ECHO) Program

The Frederick National Laboratory for Cancer Research (FNL) staff supports NCI's Center for Global Health (CGH) by managing meetings and analyzing and disseminating data to help CGH with its primary goal: supporting NCI's mission by advancing global cancer research and coordinating NCI's engagement in global cancer control.

The objectives of FNL's support are to:

- Conduct high-quality and timely data analyses that highlight NCI's portfolio in global cancer research for use in decision-making by NCI, the U.S. government, and external global cancer research stakeholders
- Support the dissemination and use of relevant evidence and research within CGH and NCI global cancer research and control networks and actively support the maintenance and development of partnerships to advance CGH's work
- Provide quality and efficient meeting and travel support to allow CGH to achieve its aim of convening the global cancer research community to address gaps in global cancer research and control capacity and scientific knowledge

Data Analysis

FNL staff in the Clinical Monitoring Research Program Directorate (CMRPD) supported CGH's conduct of data analyses that highlight NCI's portfolio in global cancer research for use in NCI's, the U.S. government's, and external global cancer research stakeholders' decision-making processes. In addition to routine data-analysis activities such as analyzing country-level portfolios, responding to data requests, and conducting the annual NCI International Projects Data Call, FNL staff led the analysis of the 2018–2019 Global Oncology Survey of NCI-Designated Cancer Centers, published in

July 2019, and authored the subsequent manuscript, published in the *J Glob Oncol* in November 2019 (Abudu R, et al., JCO 2019). FNL staff also prepared several materials for the arrival of the new CGH director in February 2020, including a retrospective portfolio analysis of CGH's past key achievements. Upcoming work includes providing analytical support to new initiatives from the CGH director and preparing for the 2021 iteration of the Global Oncology Survey of NCI-Designated Cancer Centers.

Dissemination

FNL staff in CMRPD served in a liaison or representational capacity on behalf of CGH to foster partnerships to advance CGH's global cancer research and control mandate. This role included: serving as coordinator of the Scientific Steering Committee for the Eighth Annual Symposium on Global Cancer Research, which entailed facilitating a series of planning meetings in preparation for the symposium scheduled for April 2020; serving as technical advisor to the Steering Committee of the Africa Cancer Research and Control ECHO, which entailed making relevant researcher and expert speaker suggestions and arranging for introductory meetings; serving as CGH representative to the Breast Health Global Initiative Steering Group and Union for International Cancer Control Breast Cancer Consultative Group, which entailed sharing CGH and NCI's global cancer research and control initiatives and providing input on proposed activities; and coordinating the 2020 Academic Global Oncology Meeting in partnership with the American Society of Clinical Oncology, which entailed holding planning meetings and setting the agenda for this virtual meeting to convene more than 100 members of the academic global oncology community. These activities and other similar representational activities have strengthened CGH's network for disseminating relevant research, research training, and funding opportunities and have provided a platform to discuss gaps in global cancer research where NCI can provide additional support.

On behalf of CGH, staff collaborated in the International Cancer Control Partnership's telementoring program for national cancer control plan implementation, connecting global cancer research stakeholders to relevant NCI-Designated Cancer Center contacts based on outcomes of the above-referenced Global Oncology Survey, and provided recommendations for global cancer research and control experts for the Africa Cancer Research and Control ECHO.

FNL staff also worked with the Africa ECHO Steering Committee to develop a dedicated series of weekly sessions on cancer and COVID-19 that brought together NCI, U.S.-based, Africa-based, and international colleagues in global cancer research and control. These discussions have contributed to NCI's and NCI-Designated Cancer Centers' understanding of the challenges and lessons that can be applied for cancer control in the pandemic, particularly in low-resource settings. These activities have supported establishing CGH as connector and knowledge hub in the global cancer research and control community.

Meeting Support

FNL staff in CMRPD continued to plan and coordinate meeting and travel activities related to domestic and international workshops, conferences, and training events for both government and nongovernment attendees who are collaborating on many initiatives and programs. Services included (i) providing comprehensive logistical support, (ii) preparing and monitoring meeting and travel budgets, and (iii) establishing formal processes and procedures to streamline planning while ensuring that all meeting and travel policies and directives are followed.

During the COVID-19 pandemic, FNL staff facilitated an increased number of virtual meetings on the Zoom platform due to decreased travel and meeting cancellations.

Subject-Matter Experts

FNL managed three subcontract agreements with subject-matter experts in support of the CGH portfolio of work in the U.S. and abroad, including Africa, Europe, Asia, Latin America, and the Caribbean.

Immediate Office of the Director, Cancer Research Technology Program

Support Provided by the Cancer Research Technology Program

RAS Initiative

1) Targeting KRAS as a treatment for KRAS-driven tumors

a. Small-molecule covalent inhibitors of KRAS

We previously discovered histidine 95 (H95) in KRAS as a potential site for covalent modification. H95 is unique for KRAS (Q in HRAS and L in NRAS), and H95 is conserved in both splice variants (KRAS4a and 4b).

In our efforts to develop direct KRAS inhibitors targeting H95, we explored hits identified in a disulfide tethering screen at the University of California, San Francisco. We partnered with TheRas, Inc. in 2017 with a contractor Cooperative Research and Development Agreement (cCRADA). TheRas expanded the cCRADA to 12 FTEs in 2019, allowing the recruitment personnel to support in-house chemistry, crystallography, and NMR needs for the project. TheRas also established a cCRADA with Lawrence Livermore National Laboratory that brought two computational scientists to the project for computer-aided drug design (CADD) support to help guide structure–activity relationship (SAR) studies.

TheRas also provides the 12 FTEs additional medicinal chemistry support through the contract research organization mechanism.

Currently, we are optimizing hits to improve noncovalent binding to the pocket using surface plasmon resonance spectroscopy as a screening method. Co-crystal structures of our best binders with RAS are guiding computer-aided drug design and medicinal chemistry.

n an attempt to identify histidine-reactive electrophilic groups, we have generated covalent analogues of our best binders, which are screened for H95 covalent engagement using MALDI-TOF mass spectrometry. In addition, we screened a library of 1,500 covalent fragments to identify histidine covalent modifiers. We pursued 105 of these compounds with a MALDI-TOF MS–based screen. Hits from this screen will be considered for future chemistry development to combine the best noncovalent fragment with the histidine-targeted covalent warhead.

b. Characterize and disrupt KRAS complexes in live cells and develop assays for screening

To date, we have developed protein–protein interaction assays using bioluminescence resonance energy transfer (BRET) as a platform. This platform can give a readout of interactions of less than 10 nm between RAS and other proteins in the membrane of live cells. We have designed and tested probes that allow us to measure interactions between mutant and wild-type KRAS4b and its effectors, including RAF1 and B-RAF, RALGDS, and PI3K. We have also developed assays for interrogating the interactions between SHOC2 and MRAS and for measuring KRAS/KRAS interactions. For example, using the RAS inhibitor BI-2852, a compound that induces a non-functional RAS dimer, we can show using these assays that the compound increases interaction between full-length KRAS in cells. Correspondingly, the same compound inhibits interactions of KRAS4b with RAF1.

Using BRET as a platform, we have screened 160,000 compounds in collaboration with Eli Lilly. We are currently in the process of selecting hits for lead optimization. Further activities in the lab include developing biosensors for MAPk activation and studying the interactions of PI3k and RAF with the membrane using single-molecule and super-resolution techniques.

c. PROTAC approaches to targeting KRAS

The concept of redirecting protein degradation by artificially recruiting an E3 ligase, coined Proteolysis Targeted Chimeras (PROTACs), was demonstrated nearly 20 years ago. With the resurgence of successful cell-effective PROTACs in recent years, the field of drug discovery is now experiencing a paradigm shift. PROTACs can completely remove a target of choice by degradation rather than inhibition. This approach could be especially effective for targets such as KRAS, whose primary function is scaffolding of signaling transduction through protein-protein interactions with effectors. The degradation team is actively designing various approaches to specifically degrade KRAS wild-type or mutant alleles.

In order to generate KRAS binders that could be used for PROTAC design, a collaboration agreement was established with Griffith University. Using a native mass spectrometry screening process on fully processed KRAS G12D, 38 potential binders were identified.

Covalent PROTAC approaches are also being investigated as a viable approach to degrade KRAS. Using disulfide trapping to generate new fragment binders, several potential covalent handles are being evaluated for PROTAC design.

To expand our ability to screen and characterize potential degraders, we designed an innovative cell-based platform for potential KRAS degrader discovery. So far, a positive control compound has been identified that enabled the platform proof-of-concept to be established. This effort, based on engineering in-cell ubiquitination detection, is now being developed in collaboration with the NCI Molecular Targets Program.

d. Disulfide tethering

Disulfide tethering is a site-directed method of fragment-based drug-discovery. It allows screening of low-affinity disulfide containing fragments against cysteine residues in a target protein. Fragment binding is reversible and can be tuned to favor detection of only the strongest bound fragments. This binding is not purely driven by reactivity; it is strongly dependent on protein/ligand interactions. Bound fragments are detected by mass spectrometry and provide a lead into the drug discovery process.

Disulfide tethering could help reveal cryptic drug binding sites related to the intrinsic conformational plasticity of the protein target. We decided to apply this technology to search for cryptic pockets in the KRAS oncogenic protein, which lacks obvious binding pockets in which a small molecule could dock. However, it is highly allosteric, so a pocket could form upon ligand binding.

Using bioinformatic and structural biology approaches, we predicted 95 surface-accessible amino acids predicted to lie on the surface of KRAS4b and developed a protein library of cysteine mutants of these residues to enable drug discovery and structural biology efforts. This library was fully validated with a variety of quality control measures. Purified proteins were tested for their ability to be labeled on the unique cysteine sites by multiple methods prior to initiation of tethering screens.

We created and validated the disulfide tethering library containing 1,200 chemically diverse fragments. We optimized a screening protocol for MALDI-TOF MS to increase throughput and reduce use of reagents. Screening of our cysteine mutant library is in progress. The first 10 targets have been completed successfully and used to validate the disulfide library performance using bioinformatics methods. Screening is currently ongoing with follow-up experiments planned for promising hits.

2) Characterization of KRAS-KRAS interactions on membran

The objective of the RAS structure and dynamics in cellular membranes project, a component of the Joint Design of Advanced Computing Solutions for Cancer (JDACS4C) initiative, is to create a multiscale computational framework that uses experimental input parameters, including diffusional data from single particle tracking of RAS in the membranes of live cells (Goswami et al., *eLife*, 2020). The framework can be used to explore the conformation and dynamics of the RAS protein alone or in complex with effectors at length and time scales than cannot be interrogated by experimental approaches alone. The first phase of this project focused on the behavior of RAS on lipid membranes of varying complexity. Three discrete conformations of RAS on a simple bilayer consisting of anionic and neutral lipids were characterized using a variety of biophysical measurements. There was good correlation between these experimental results and coarse-grained molecular dynamics (CG MD) simulations performed by Los Alamos National Laboratory (Van et al., *Proc Natl Acad Sci USA*, 2020, manuscript in press).

Macroscale simulations of RAS on a membrane containing eight lipids from the Multiscale Machine-learned Modeling Infrastructure (MuMMi) were performed, selecting 120,000 unique membrane patches that resulted in 200 ms of aggregated CG MD simulations. Distinctive patterns of local lipid composition correlate with interfacially promiscuous RAS multimerization and lateral diffusion (Ingolfsson et al., *Nat Struct Mol Bio*, 2020, manuscript under review). Simulations predicted the presence of higher concentrations of phosphatidylinositol bisphosphate (PIP2), which was associated with higher RAS co-localization and confined diffusional behavior. These predictions were confirmed by surface plasmon resonance and single particle tracking experiments on supported lipid bilayers. The supported lipid bilayers composed of eight lipids were systematically varied to correspond to the compositions that favored (high PIP2) or disfavored (low PIP2) RAS multimerization. Under these varying conditions, we found that the diffusion behavior of RAS and lipids were well correlated with the simulations. This highlights the value of the project: the simulation provides a wealth of testable hypotheses that can now be tested experimentally. New parameters derived experimentally can then be used to parameterize subsequent MD simulations to refine the model.

Phase 2 of this project focuses on MuMMI simulations between membrane-bound RAS and RAF kinase. The X-ray crystal structure of the RAS-binding domain/cysteine-rich domain (RBD-CRD) bound to KRAS has been solved (Tran et al., *Nature Commun*, 2020, manuscript under review) and will be used to initiate CG MD that results from the MuMMI simulations. This structure was solved in the absence of a membrane, so nuclear magnetic resonance (NMR) experiments were conducted to solve the structure of RBD-CRD bound to a membrane mimetic. The NMR data indicates that leucine 149, phenylalanine 158, and leucine 160 from the CRD penetrate the acyl chains of the lipid bilayer with the RBD, making transient membrane interactions. In cell-based experiments, we are measuring the membrane residence time of RDB-CRD. When we mutate these key residues, we see a decrease in the residence time and decreased interactions with RAS (as measured in protein–protein interaction assays). We are also measuring the diffusion behavior of fluorescently labeled RBD-CRD and KRAS on supported lipid bilayers using simultaneous two-color, single-particle tracking experiments. Using these innovative experimental approaches, we can test the hypotheses generated from the next round of MuMMI simulations.

3) RAS Mass Sepctrometry Proteomics

a. Mass spec and Ras proteoforms

During FY2020, the RAS Initiative recruited a new hire and obtained substantial upgrades in instrumentation, equipment, and data processing capabilities for proteomic analyses. As a result, the RAS Initiative now possesses a state-of-the-art proteomics facility (RAS Mass Spectrometry, RMS) on par with other leading institutions. Moreover, the RAS Initiative can now perform top-down proteomic analysis, a method by which intact proteins and their modified forms (proteoforms) can be precisely characterized with mutation linkages and post-translational modification (PTM) stoichiometry preserved, providing a level of detail inaccessible by standard proteomic approaches. This technology has been employed to develop tailored methods for analyzing each RAS isoform (KRAS4A, KRAS4B, HRAS, and NRAS) with parameters optimized to maximize detection, sequence coverage, and mutation or PTM localization. These optimized methods were first used to verify correct binding of covalent KRAS4B-targeting compounds under study at the RAS Initiative and validate the targeting capabilities of a series of Cys mutants to be used in future compound tethering assays. Subsequently, these methods were combined with immunoprecipitation to facilitate the targeted analysis of endogenous proteoforms (RAS proteoform assay) present in a panel of malignant cell lines under study at the RAS Initiative. Comprising three tissue backgrounds (colon, lung, and pancreas) and representing five RAS mutation backgrounds (KRAS: G12D, G12C, G12V, G13D; NRAS: Q61K), these cell lines were found to contain a wealth of novel RAS proteoforms, indicating that the complexity of RAS-dependent signaling may be greater than originally anticipated. Efforts are currently underway to obtain improved PTM characterization of these novel proteoforms and better understand the roles of these modifications in RAS and cancer biology.

b. Proteomic Support for RAS Initiative Projects

In FY2020, RMS established new workflows and standard operating procedures on the instrument platform primarily employed for RAS reagent quality control by the Protein Expression Laboratory (PEL) and Protein Characterization Laboratory (PCL). Results included the successful characterization of the RAF kinase domain, intact RAF, intact PI3Kα, and NF1 subdomains, all of which had previously presented significant technical challenges. Complementary proteomic analyses of these protein populations resulted in up to an 80 percent improvement in sequence and PTM coverage over prior analyses, providing much-needed details of the modifications and biochemical properties of each protein population. RMS also performed a broad range of standard proteomic analyses for RAS Initiative collaborators, including but not limited to identification of differential KRAS4B binding partners between treatments with covalent or noncovalent inhibitors, identification of RAS signaling pathway components responsible for phenotypes observed in RAS Initiative reference reagent cell lines, and detection of endogenous RAS proteoform-specific differences upon treatment of cell lines with a KRAS4B G12V-targeting compound (Tosk collaboration). Most recently, RMS developed new proteomic workflows to validate and characterize COVID-19 proteins produced by the RAS Reagents Core Protein Expression Laboratory to support a broad range of projects (e.g., Ragon and Kramer RAS-binding domain protein, COVID-19 spike protein, and COVID-19 serology assay reagents). RMS is currently developing methods to detect and quantify nucleotide-bound KRAS4B proteoforms by native mass spectrometry, with potential applications to an array of RAS Initiative projects, particularly localization and target validation of noncovalent inhibitors.

4) Structure-based drug discovery and new insights into KRAS Biology

The RAS structural biology group within the RAS Initiative continues to carry out new target identification by solving KRAS structures in complex with effectors and regulatory proteins. A major focus of this group is carrying out structure determination of hit or lead compounds with the target proteins to facilitate their optimization via structure-activity relationships (SAR). Ongoing drug discovery efforts are focused on targeting KRAS-PI3K interaction, KRAS-RAF1 interaction, and the oncogenic G13D mutant of KRAS. These drug discovery projects are being carried out in collaboration with external partners under cCRADAs. Besides these drug discovery projects, the structural work done by this group on KRAS in complex with partner proteins (described below) have provided novel insights in RAS biology.

a. A bedside-to-bench analysis uncovers the molecular mechanism underlying a case of highly aggressive RAS-driven colorectal cancer

In a collaborative study between the RAS Initiative and the University of Minnesota, we carried out an in-depth bedside-to-bench analysis of internal tandem duplication (ITD) in NRAS from a patient with an extremely aggressive colorectal carcinoma. Results of whole-exome DNA sequencing of primary and metastatic tumors indicated that this mutation was present in all analyzed metastases and excluded the presence of any other apparent oncogenic driver mutations. Biochemical analysis done at FNLCR revealed a strengthened interaction of the RAS ITD with Raf kinase, leading to increased phosphorylation of two downstream kinases, MEK and ERK. At FNLCR, we solved the first crystal structures of NRAS and KRAS ITD at 1.65 and 1.75 Å resolutions, respectively, providing insight into the physical interactions of this class of RAS variants with its regulatory and effector proteins. The ITD prevented interaction with neurofibromin 1 (NF1)-GTPase-activating protein, providing a mechanism for sustained activity of the RAS ITD protein. This collaborative effort also illustrated the importance of robust biochemical and biophysical approaches in the implementation of individualized medicine.

b. Does SPRED1 interact with neurofibromin and regulates active KRAS levels in normal and pathologic conditions?

The NF1 tumor suppressor gene encodes the cytoplasmic protein neurofibromin. It acts as a RAS-specific GTPase-activating protein (GAP) and promotes the conversion of an active RAS-GTP-bound form to an inactive RAS-GDP form, thereby downregulating its biological activity. Like neurofibromin, SPRED proteins have been shown to negatively regulate RAS/MAPK signaling following growth factor stimulation. This inhibition of RAS is thought to occur primarily through SPRED1 binding and recruitment of neurofibromin to the plasma membrane. SPRED1 and neurofibromin loss-of-function mutations occur across multiple cancer types and developmental diseases such as neurofibromatosis type 1 and Legius syndrome. We recently reported the crystal structure of neurofibromin (GAP-related domain) complexed with SPRED1 (EVH1 domain) and active KRAS. The structure of the ternary complex shows that SPRED1 and KRAS bind to the neurofibromin via two distinct interfaces. The structural analysis provided insight into how the membrane recruitment of neurofibromin by SPRED1 allows simultaneous interaction with activated KRAS. Analysis of the neurofibromin-SPRED1 interface provides a rationale for mutations observed in Legius syndrome. We also showed that oncogenic EGFR(L858R) signaling leads to the phosphorylation of SPRED1 on serine 105, disrupting the SPRED1-neurofibromin complex. Our structural, biochemical, and biological results provided new

mechanistic insights about how SPRED1 interacts with neurofibromin and regulates active KRAS levels in normal and pathologic conditions.

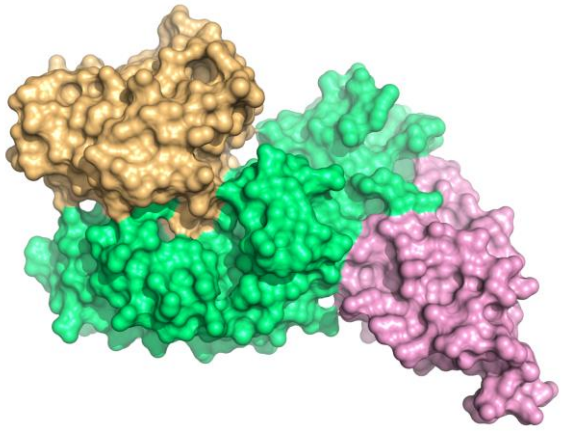


Figure 1. Surface representation of the crystal structure of the protein–protein complex formed by KRAS (beige), neurofibromin (green), and SPRED1 (pink). This crystal structure showed how SPRED1 interacts with neurofibromin and regulates KRAS activity in normal and pathological conditions.

c. Biochemical and structural studies of Nerofibromin

We produced highly purified full-length neurofibromin (NF1) protein using an insect cell system and discovered that the protein formed extremely high-affinity dimers in solution at concentrations in the nM range. Using a collection of truncated proteins and a variety of advanced biophysical and structural techniques, we identified regions of the protein responsible for dimerization and ascertained the three-dimensional structure of the protein using negative stain transmission electron microscopy. The 20 Å resolution structure clearly highlighted the dimeric nature of the protein and provided additional information on the potential function of various subdomains. Activity assays both *in vitro* and *in vivo* helped to better elucidate the role of dimerization in the RAS-GAP function of the protein. Further work to improve the resolution of the structure using cryo-electron microscopy is underway.

5) RAS Reagents Core

a. Protein Expression

The RAS Reagents Core (RRC) has the two-fold goal of generating reagents to support all projects within the FNLCR RAS Initiative and producing reagents that will assist external RAS scientists with their research. The group's focus is on generating new DNA clones, cell lines, and proteins. In FY2020, RRC generated numerous materials and developed new protein production technologies to support these efforts.

- The RRC cloning group produced 840 new DNA constructs in support of RAS Initiative efforts. Fifty percent of these constructs were designed for protein expression in support of biochemistry, biophysics, and structural biology of RAS and RAS effector proteins. An additional 25 percent were generated in support of assay development and drug screening studies, while the remaining constructs were used for RAS biology efforts, including DNAs designed for delivery of RAS to cell lines.
- The RRC cell line development group produced 300+ new cell line clones in support of RAS Initiative projects and continued to improve quality control procedures on newly generated cell lines including the RAS-dependent mouse embryonic fibroblasts (MEFs). A significant focus entailed generating cell lines co-expressing multiple fluorescently labelled RAS and RAF proteins for imaging work and cell lines designed for PROTAC degradation studies.
- The RRC protein production group generated over 59 grams of protein from more than 600 large-scale protein production experiments. Over 1,200 liters of bacterial expression materials and 500 liters of eukaryotic expression materials were generated to support these productions using E. coli (55 percent), insect (40 percent), or mammalian (5 percent) expression systems. More than 500 small-scale production scouting experiments were also carried out to identify optimal conditions for protein expression. Proteins purified in this period of time included processed KRAS-FMe, full-length and various domains of the RAF family kinases, full-length and truncated domains of NF1, RAS effectors including Calmodulin, MAPKAP1, RALGDS, PIK3CA, and over 350 different mutants of KRAS4b.
- Support of intramural and external collaborators: More than 125 DNA constructs were generated in support of collaborations with Center for Cancer Research (CCR) investigators as well as external collaborators and scientists. Additional lentiviral and E. coli clones were made for collaborations with researchers at numerous universities around the world.
- RAS reagent distribution: In FY2020, 215 individual clones and six collections of clones generated by the RAS Reagents Group were distributed by Addgene to researchers around the world. Another 104 clones were distributed directly from FNLCR to researchers, and DNA clones and baculovirus reagents for the production of processed KRAS protein were distributed to five additional investigators at nonprofit institutions and were licensed to two companies for drug screening efforts. Eighty-three RAS-dependent MEF cell lines were distributed to 18 different researchers in six countries and new licenses are being generated with five companies.
- Technology development: [redacted]

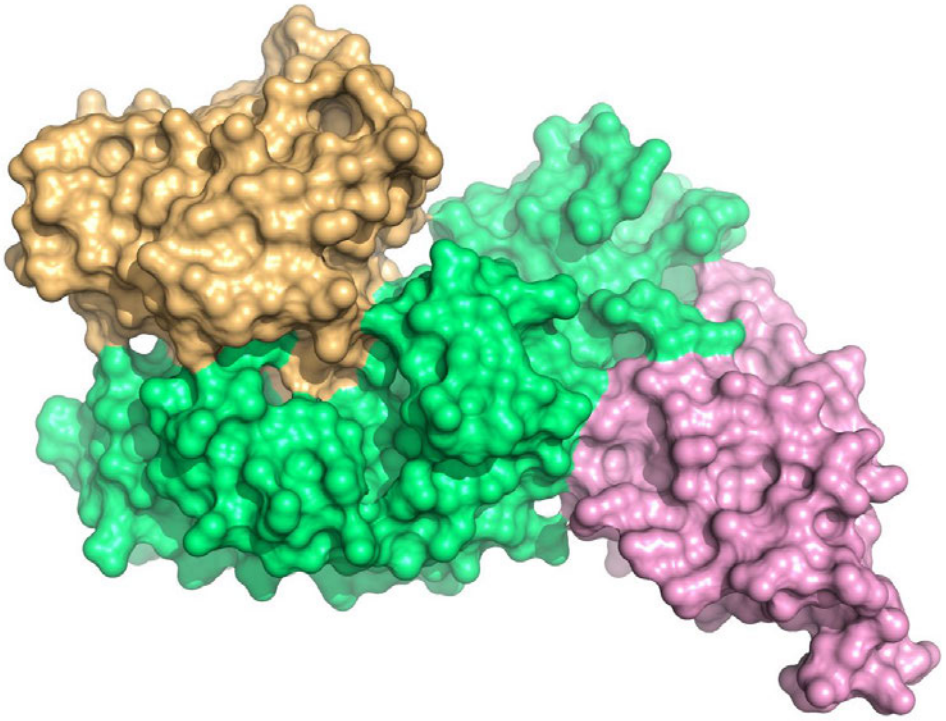


Figure 1. Surface representation of the crystal structure of the protein–protein complex formed by KRAS (beige), neurofibromin (green), and SPRED1 (pink). This crystal structure showed how SPRED1 interacts with neurofibromin and regulates KRAS activity in normal and pathological conditions.

c. Support for COVID-19 efforts

i. Support for NIH SeroSurvey

RAS Initiative staff carried out protein production work in support of a trans-institute/center NIH initiative led by Drs. Kaitlyn Sadtler (National Institute of Biomedical Imaging and Bioengineering), Matthew Hall (National Center for Advancing Translational Sciences (NCATS)), and Matthew Memoli (National Institute of Allergy and Infectious Diseases) to survey 10,000 people nationwide for SARS-CoV-2 antibodies using ELISA-based serological assays. RRC staff rapidly optimized the production of multiple forms of SARS-CoV-2 spike and RBD proteins, in some cases improving published protocol yields by 10-fold. Over 100 mgs of spike and 250 mg of RBD were generated to directly support serology assay development and deployment of the first phase of the SeroSurvey. In addition, a series of other coronavirus spike proteins were generated for this project, which were also distributed to other laboratories for additional assay development work. Three preprints were published on this work and are currently under review in various journals, and one peer-reviewed paper on the purification optimization was published in June in *Protein Expr Purif*.

ii. Support for NCI Serology Efforts

RRC efforts in production of spike and RBD proteins also aided the initial startup of the NCI serology effort for SARS-CoV-2, performed in the laboratory of Dr. Ligia Pinto. Multiple batches of spike, RBD, and nucleocapsid proteins were provided to Dr. Pinto's laboratory, along with purified monoclonal antibodies from NIAID Vaccine Research Center clones. RRC continues to support the NCI serology laboratory with additional protein as needed.

iii. Production of SARS-CoV-2 proteases for covalent tethering approaches

RRC staff produced SARS-CoV-2 main (3CL) and papain-like (PLP) proteases for use in covalent tethering screens using the FNL RAS Initiative covalent tethering library. These proteins were challenging to produce at high yield, and significant optimization was required to generate high-quality proteins of both wild-type and catalytically inactive proteases.

iv. Production of nanobodies to inhibit SARS-CoV-2 entry

In collaboration with Drs. Ying Fu and Matthew Hall at NCATS, RRC produced large quantities of 10 different single chain nanobody proteins developed from phage panning at NCATS. These proteins were low yielding in NCATS' standard E. coli production methods, but RRC was able to generate high yields of all 10 proteins using a combination of high-density E. coli growth or growth in the novel organism, Vibrio natriegens. These proteins were used by NCATS for assay development and cryo-electron microscopy. A manuscript on this work has been submitted for publication.

v. Support for NIAID Moderna SARS-CoV-2 vaccine efforts

Multiple batches of high-quality spike and RBD proteins generated by RRC were sent to NIAID for use in serology work on Phase I clinical trial samples in the NIAID/Moderna trials.

6) RAS Bioinformatics

The RAS Initiative bioinformatics group provides data processing, management, and analytical support to the RAS Initiative through three main areas: 1) databases and data storage infrastructure, 2) direct initiative project support, and 3) data mining, integration, and analysis to enhance our understanding of RAS biology.

a. RAS Initiative data sharing, storage, and analysis

The RAS Initiative database system provides electronic laboratory notebook, project tracking, and data warehouse system functionalities. More RAS Initiative teams have been able to share data and track projects using these updated resources, enhancing communication across groups.

The bioinformatics group has continued to analyze data produced by drug development, assay, and screening efforts underway within the RAS Initiative. We applied a set of versatile applications for drug dose-response curve fitting to a diverse set of screening assays (image/bioluminescence resonance energy transfer, tethering, homogeneous time-resolved fluorescence, surface plasmon resonance, etc), to help promote drug discovery and reveal Ras oncogenic mechanisms. This set of analyses provides visualizations that aid in assay evaluation and facilitate hit identification and comparison. Analyses based on multiple fitting models enhanced our single drug screening abilities, which are further enhanced with Bliss model or derivative-based analyses to enable combination screening assays.

The RAS Initiative has applied single-cell sequencing to develop a signature profile for the effects of KRAS inhibition in cancer cell lines at the single-cell level to account for heterogeneity, which could reveal drug resistance mechanisms. The in-house Pathway Pattern Extraction Pipeline (PPEP) analysis method has been adapted to help interpret the single-cell sequencing transcriptome data at both pathway level and gene level. The PPEP method has also been adapted to many projects outside the RAS Initiative, becoming a core application for interpretation of underlying biology.

Recently, many large cancer research databases have become available to the public, including the AACR Project Genomics, Evidence, Neoplasia, Information, Exchange (GENIE) and cBioPortal. Data mining in these resources enhances our understanding of RAS biology. Evaluation of the commonality and significance of

mutation rates of KRAS gene mutations between cancer patients of various tumor types in combination with the annotation of oncogenic driver mutations (Bailey et al. 2018, Cell,173:371) could provide the group with a comprehensive understanding of the biological implication of RAS mutation on oncogenesis (Figure RB1). We have also assessed the global distribution and frequency of RAS genes (KRAS, HRAS, NRAS) across various tumor types in the Cancer Cell Line Encyclopedia (CCLE), COSMIC, GENIE databases. The bioinformatics group has reviewed the importance of oncogenic RAS in cancer metabolism for Initiative members and updated RAS co-mutation analysis in GENIE and cBioPortal for the RAS Dialogue.

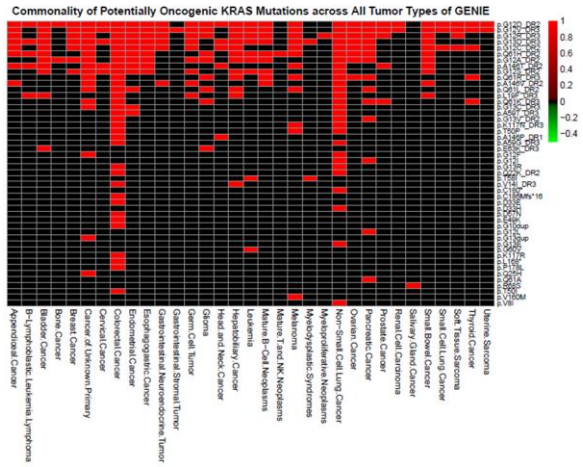


Figure RB1. The commonality of KRAS mutations as driver mutations across multiple tumor types. Data from the GENIE database for various tumor types were used to assess the mutation rates of all detected mutations for each tumor type. The KRAS mutations that occurred at rates higher than expected by random chance for each corresponding tumor type were selected and combined into a heatmap showing their commonality across tumor types, with higher commonality on the top. "DR1", "DR2", or "DR3" denotes the corresponding mutations at rows that were flagged as driver mutations with one, two, or three of the evaluation tools, respectively, in a previous study on oncogenic driver mutations in The Cancer Gengome Atlas (TCGA) data (Bailey et al., Cell, 2018).

and driver mutations across multiple tumor types as described earlier (Figure RB1). We also assessed other potential oncogenes from the Ras pathway. Based on the annotation of driver genes from several computational studies, including an International Cancer Genome Consortium (ICGC) Pan-Cancer Atlas of Whole Genomes (PCAWG)–based study (Figure RB2) and TCGA-based study data (Bailey et al., *Cell*, 2018), we found that the Ras pathway has several oncogenic driver genes that may play critical oncogenic roles in a wide variety of nearly all tumor types similar to KRAS. This is also supported by "dark matter" analysis that assessed the impact of mutations, copy number alterations, and elevated gene expression of RAS pathway genes on individual patients across all TCGA tumor types. We found that the vast majority, if not all, of the TCGA cancer patients were affected by RAS pathway genes (unpublished observations).

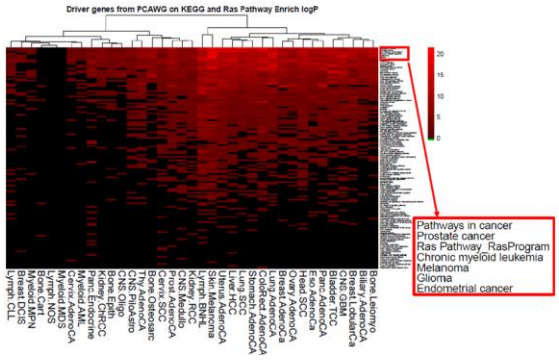


*Figure RB2. The Ras pathway is commonly enriched by oncogenic driver genes across tumor types. Genes in the Ras pathway were mixed with KEGG pathways, all of which were subjected to enrichment analysis for oncogenic driver gene lists of each tumor type that was defined by ICGC PCAWG study (Campbell et al., Nature, 2020). This pathway-level heatmap shows enrichment levels as the transformed enrichment p-values (-1*log10(p-values)), with red for enriched pathways and black for not-enriched pathways. The pathway rows are ordered based on the commonality of the enrichment of pathways across tumor types. The Ras pathway, as annotated by the Ras Initiative, was shown as one of the top three pathways commonly enriched across the majority of the tumor types.*

b. Database mining and analysis for Ras biology

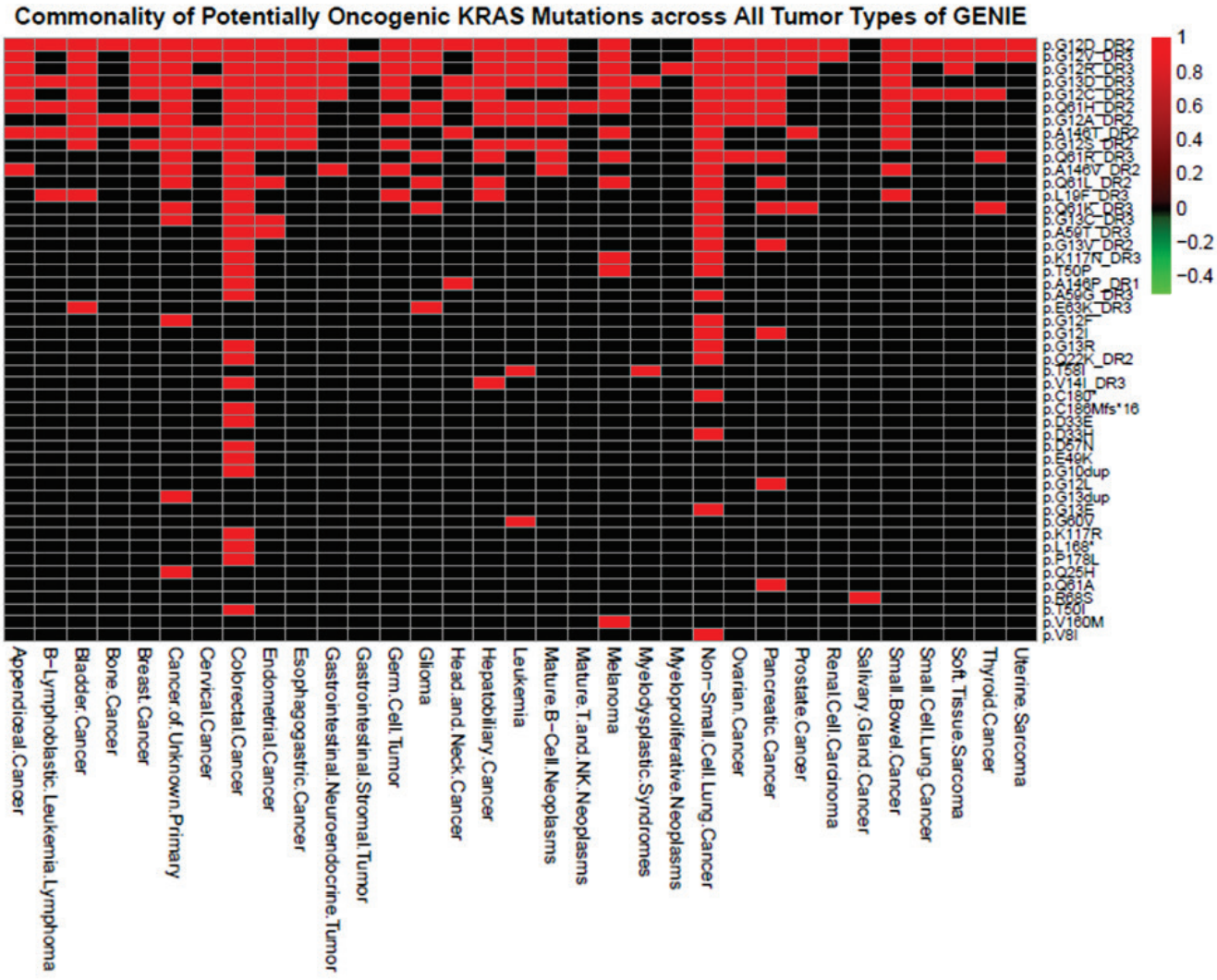
Combining existing databases such as TCGA and Pan-Cancer databases, GENIE, and cBioPortal (https://www.cbioportal.org/datasets) with coming databases such as the All of Us Research Program, we anticipate a rich future in genomic data mining. Combining the large genomic datasets with oncogenic driver genes/mutation studies, the bioinformatics group addressed RAS biology questions in alignment with the Initiative's priorities.

To further enrich our comprehensive biochemical analysis of KRAS oncogenic mutants, we sought to understand the impact of these mutations on the conformation and structure of KRAS and its regulators. We first assessed the commonality of KRAS mutations

c. Biological themes associated with RAS dependency

Previously, our group derived a method that permitted the computational assignment of a RAS-dependency index (RDI) score across the full panel of cell lines of the CCLE. In the last year, we successfully expanded our study on Ras dependency by uncovering many new findings. In addition to the initial finding of agreement with the experimentally derived RDI values, we also found that the computationally derived RDIs across CCLE cell lines show high correlation with a previous in-house siRNA effector node (siREN) study (Yuan et al., *Cell Rep*, 2018) and other external studies.

Initially, using EMT signature-derived RDIs and data from cell lines representing the extremes in RAS dependency, we identified the Fas signaling pathway and



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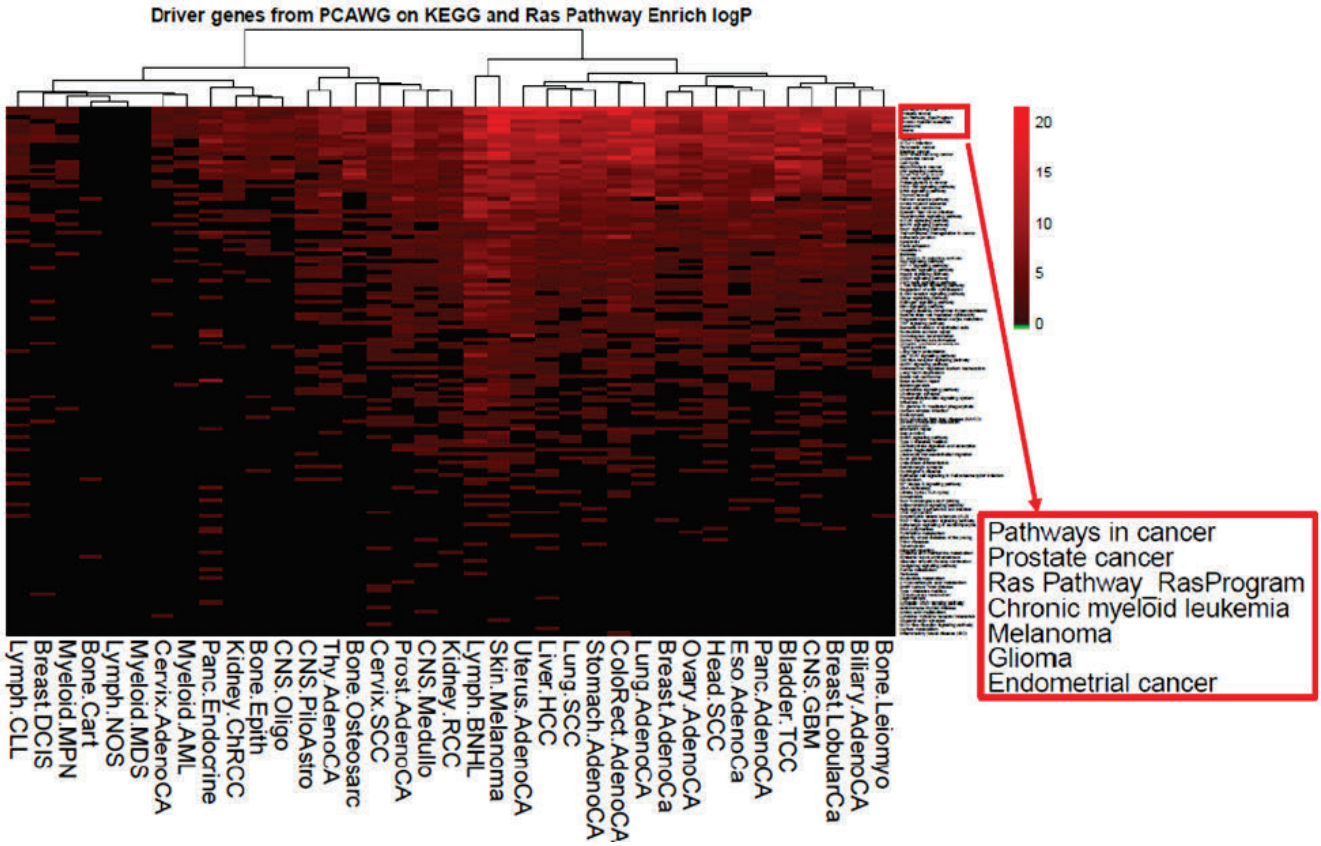

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a putative Ras-independent pathway initially identified in NK cells. We also extended the analysis from cell lines and TCGA patient samples, which demonstrated the same consensus differential expression patterns for these two pathways across multiple tissue types. Furthermore, our observation that this Ras-independent signaling pathway includes SYK, PAK1, and PI3K as the critical genes in the context of Ras independency (Figure RB3) provides insights into the role of PI3K in the Ras independency.

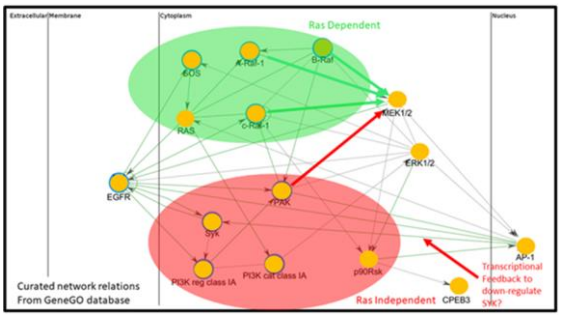
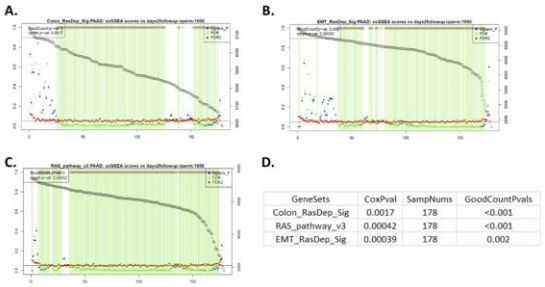


Figure RB3. Accumulated evidence led to the postulated global presence of both Ras-dependent and Ras-independent cascades that lead to context-dependent activation of the downstream MAPK signaling pathway. The network view shows the potential global presence of both Ras-dependent and Ras-independent cascades that lead to context-dependent activation of the downstream MAPK signaling pathway. Network relations were retrieved for relevant genes from the curation databases of GeneGO (https //portal.genego.com/cgi/data_manager.cgi#) using knowledge from a protein–protein interaction database, canonical pathways, and literature mining. There are two signaling cascades highlighted either as conventional Ras-dependent context (green oval) or Ras-independent context (red oval) involving SYK, PAK1, and PI3K converged into MEK1/2 for the downstream MAPK signaling pathway. The RSK gene, a critical gene/node in the internal siREN study (Yuan et al., Cell Rep, 2018), crosstalks with many components of this network.

We also observed complementary differential expression patterns of the RAC1 and PREX1 genes, which are among the critical upstream driving components in the putative Ras-independent pathway and its GEF, respectively. This is highly consistent with the uncovered synthetic lethality scheme of RAC1/PREX1 and Ras genes as well as the postulated PI3K/VAV-triggered and RAC1/PREX1-mediated MAPK signaling in a Ras-independent context from a previous essentiality profiling study. This provides additional support for MAPK signaling in Ras-independent contexts that were defined by our ssGSEA score-based computational Ras dependency index. Finally, the single-sample Gene Set Enrichment Analysis (ssGSEA) scores of RAS dependency–related signatures and Ras pathway genes showed a significant association with cancer patients' survival outcomes by our in-house survival analysis method GradientScanSurv (Figure RB4). Together, these observations indicate that computationally derived ssGSEA scores faithfully represent the levels of Ras dependency of cancer cell lines and cancer patient samples. To our knowledge, this is the first report of a computational method that uses genome-wide gene expression profiling to represent RDIs, and we assert that these findings constitute an opportunity to revitalize the RAS dependency discussion. This work has been published recently (Yi et al., *Sci Rep*, 2020)



GeneSets	CoxPval	SampNums	GoodCountPvals
Colon_RasDep_Sig	0.0017	178	<0.001
RAS_pathway_v3	0.00042	178	<0.001
EMT_RasDep_Sig	0.00039	178	0.002

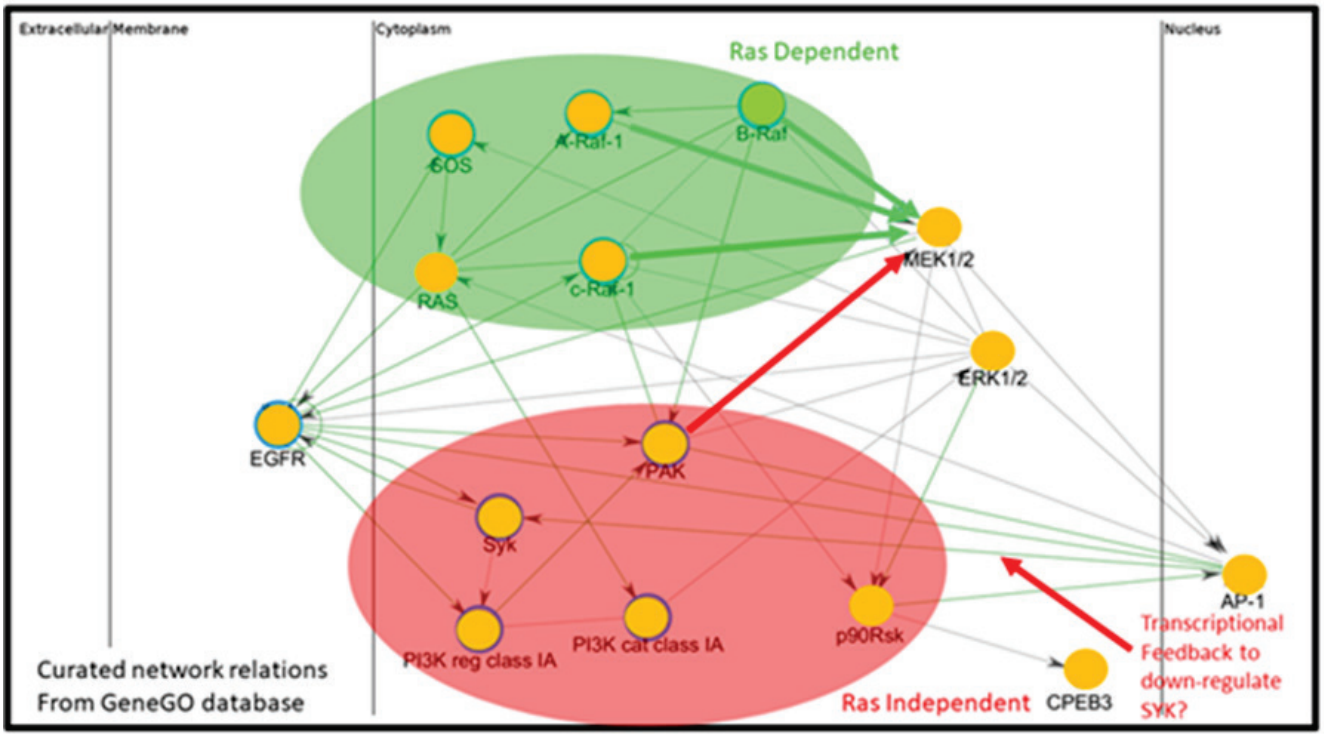
Figure RB4. GradientScanSurv results in TCGA pancreatic adenocarcinoma data using ssGSEA scores of RDI gene signatures and the RAS pathway. Survival-gradient plots derived from the GradientScanSurv method with gradients of selected gene signatures (A) Colon_RasDep_Sig colon signature; (B) EMT_RasDep_Sig EMT signatures; (C) RAS_pathway_v3 (Ras pathway genes annotated from Ras Central). (D) Statistical summary in the table, where GoodCountPvals were derived from the GradientScanSurv method, showing that all three signatures' ssGSEA scores have a significant association with patients' survival outcomes. SampNums numbers of samples in the dataset; CoxPval Cox regression model-derived p-values.

d. In-house survival analysis method assesses Ras association with survival outcome

In the last report, we described an in-house survival analysis method that we developed to help evaluate the possible association between RAS gene expression levels and survival in TCGA, with consistently better performance in comparison with other relevant tools to avoid analysis pitfalls that many similar tools encountered. This analysis method GradientScanSurv was published and has been applied to the Ras dependency project to help provide association of Ras dependency with cancer patients' outcome (Figure RB4). GradientScanSurv was applied to TCGA data to analyze all Ras pathway genes for their association with cancer patients' survival outcomes in all tumor types, and the results will be validated with larger datasets such as GENIE. A similar strategy can be applied to computational Ras Dependency Indexes once both gene expression and survival datasets become available for other cancer databases.

7) RAS Initiative Outreach

The RAS Synthetic Lethality Network (RSLN), a group a six investigators stemming from FNLCR's KRAS Synthetic Lethality Workshop, exchanges information via quarterly WebEx conference and convenes annually for a full day to hear presentations from the teams. RSLN members have published over 60 studies.



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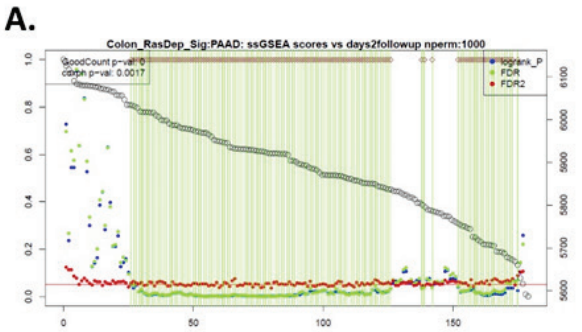

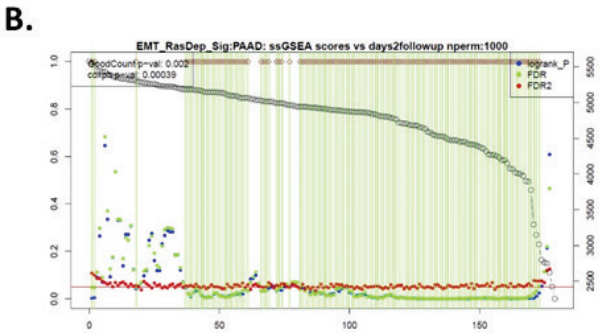

D.

GeneSets	CoxPval	SampNums	GoodCountPvals
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EMT_RasDep_Sig	0.00039	178	0.002

Figure RB4. GradientScanSurv results in TCGA pancreatic adenocarcinoma data using ssGSEA scores of RDI gene signatures and the RAS pathway. Survival-gradient plots derived from the GradientScanSurv method with gradients of selected gene signatures: (A) Colon_RasDep_Sig: colon signature; (B) EMT_RasDep_Sig: EMT signatures; (C) RAS_pathway_v3 (Ras pathway genes annotated from Ras Central). (D) Statistical summary in the table, where GoodCountPvals were derived from the GradientScanSurv method, showing that all three signatures' ssGSEA scores have a significant association with patients' survival outcomes. SampNums: numbers of samples in the dataset; CoxPval: Cox regression model-derived p-values.

Two websites to facilitate communications with the RAS community have been established. Cancer.gov/RAS is primarily an outward-facing, publicly accessible site managed by FNL in coordination with the NCI Office of Communications and Public Liaison. It has 4,000 to 8,000 unique visitors each month. RAS Lab is a private, NCI-approved site that is accessible by invitation only. As of July 2020, RAS Lab has over 1,000 members who have participated in over 350 discussions. New private web sites with unlimited membership and large sharing capacities can be established in just a few minutes through the RAS Initiative's Basecamp account.

The Third RAS Initiative Symposium will be organized in 2021 at the Frederick National Laboratory. It was "postponed" due to the SARS-CoV-2 pandemic.

National Cryo-EM Facility

The National Cryo-EM Facility (NCEF) provided the extramural cancer research community in the United States with access to high-end cryo-EM imaging services. During the COVID pandemic NCEF also added service for research projects impacting directly the development of treatments and vaccine against SARS-CoV-2.

During most of FY2020 one Titan Krios microscope was used for imaging samples of cancer and COVID researchers while the other one needed to be relocated to the newly renovated room. Six FTE were supporting NCEF during most of FY2020 and an additional microscopist joined the team in August 2020. Over 160 data collection runs for cancer and COVID researchers were performed. The resulting data enabled many users to reconstruct high resolution structures. Based on user feedback there are at least 15 structures in a range of 2.2 Å to 4.0 Å resolution. During FY2020, users were able to use NCEF data to publish 17 high impact papers in journals like Science, Nature, Cell, PNAS, and eLife. Microscope usage and efficiency was very high in the reporting period with fewer than 4% of downtime days in the reporting period. This is partly due to NCEF's rigorous operation and preventive maintenance schedule. As in the previous reporting period NCEF routinely exceeds the goal of 10 data collections per month per microscope. Data monitoring is available to extramural users and on-the-fly image analysis includes ice thickness measurements and 2D classification to judge particle quality. All of the QC results are provided to users as feedback to allow them to optimize sample preparation conditions more effectively.

Molecular Pharmacology Program

This laboratory uses retinoic acid receptor (RAR, retinoid) and retinoid X receptor (RXR, rexinoid) agonists or antagonists and other agents as discovery tools to uncover critical antineoplastic pathways. Currently, they are exploring the retinoid-regulated deubiquitinase USP18 and how retinoids cooperate in immune-based therapy. Recently, they discovered a pathway called anaphase catastrophe that can target aneuploid cancers (a hallmark of cancer) while sparing normal cells. Signals that trigger anaphase catastrophe are now under intensive study.

Targeting chromosomal instability in lung cancer

Our team seeks to reverse deregulated cancer cell growth by exploiting genomic instability (aneuploidy) from cancer-driving oncogenes like KRAS. We identified a mechanism designated as anaphase catastrophe that targets aneuploid cancer cells for death by inhibiting cyclin-dependent kinase 2 (Cdk2) activity. We reported that the centrosome protein CP110, a direct Cdk2 target, regulates anaphase catastrophe. Anaphase catastrophe is active in lung cancer cells with KRAS mutations (an unmet clinical need). The roles of regulators of chromosome stability (like CP110) in mediating anaphase catastrophe were previously reported by our team in the Journal of the National Cancer Institute, Clinical Cancer Research and Molecular Cancer Therapeutics. We reported in the past year (as part of a collaboration with Dr. Tak Mak) in the Proceedings of the National Academy of Sciences (USA) that polo-like kinase 4 (PLK4) inhibition can promote centrosome reduplication and mitotic catastrophe.

Recently, our manuscript titled "A Novel CDK2/9 Inhibitor CYC065 Causes Anaphase Catastrophe and Represses Proliferation, Tumorigenesis and Metastasis in Aneuploid Cancers" was submitted to and favorably reviewed in Molecular Cancer Therapeutics. In this work we found that Cdk2 antagonism can broadly induce anaphase catastrophe and apoptosis in aneuploid cancers whether or not these tumors express the KRAS oncoprotein.

USP18-ISG15 protein degradation pathway as a pharmacological target for lung cancer

Our prior work reported that expression of the ubiquitin-specific peptidase 18 (USP18) was augmented in diverse cancers and USP18 knock-down suppressed lung cancers. This deubiquitinase stabilizes target proteins by removing interferon-stimulated gene 15 (ISG15) from substrate proteins. Our recent study found USP18 promotes lung cancer metastasis by stabilizing a key protein 14-3-3 zeta. Knockdown of USP18 reduced lung cancer cellular proliferation, migration and invasion. Syngeneic lung cancer metastasis models were established and confirm this mechanism. Reverse Phase Protein Array (RPPA) analysis and Ingenuity pathway analysis highlighted 14-3-3 zeta as a potential USP18 target that regulated lung cancer metastasis. Functional studies confirmed the function of 14-3-3 zeta in lung cancer metastasis through USP18 regulation. These findings provide a strong rationale for targeting the deubiquitinase USP18 to reduce lung cancer metastasis and combat lung cancers.

Combination Therapy using Retinoids and Immune Checkpoint Inhibitors

Immune checkpoint inhibitors, including PD-1/PD-L1 inhibitors, improved overall survival with an acceptable safety profile in non-small cell lung cancer (NSCLC) patients. However, more than 70% of patients remain unresponsive. We explore a novel strategy to improve the efficacy of immune checkpoint inhibitors.

Retinoic Acid Receptors (RARs) are nuclear receptors and transcription factors activated by both all-trans-retinoic acid (ATRA) and other retinoids. The set of transcriptionally-activated species are highly tissue and cell-type dependent as well as RAR subtype-dependent. RAR subtype-specific agonists and antagonists can thus activate or inactivate distinct gene expression profiles and exert substantial biological effects. Our recent publication in Cancer Discovery uncovered an RARα transcriptional activated gene, CD38, which mediated immunosuppression as a mechanism of tumor cell escape from PD-1/PD-L1 blockade. ATRA suppresses murine syngeneic lung cancer growth via specific T cells. We sought to explore pharmacological activation and/or inhibition of specific RARs in distinct T cell populations augments anti-tumor immunity in combination with immune checkpoint inhibitor in NSCLC. We use ATRA and a unique set of RAR subtype specific ligands to test the hypothesis that retinoids promote checkpoint blockade and potentially translate thee findings into future clinical trials. The goal of this study is to uncover a novel pharmacological approach for combating NSCLC through immunotherapy. This would have major translational implications to advance our understanding of lung cancer biology and therapy.

CLIA Laboratory

The CLIA Molecular Diagnostics Laboratory (CMDL) establishes CLIA validated assays supporting both NCI and NIH investigators. During FY2020 the laboratory supported work for DCP, NIAID and the Clinical Center. In addition, the laboratory supported the development of assays for potential validation as CLIA laboratory developed tests to be used for NCI investigators in clinical trials or in-patient management. These are:

- An RNA signature assay to be used to stratify patients in Dr. Lou Staudt's MasterLymph clinical trial. (The MasterLymph trial has been placed on hold.
- An RNA histology assay for enrollment of patients expressing CT83 , the target of a CART cell directed clinical trial, in lung cancer for Dr. Chrisian Hinrichs.
- A Mass Spectrometry based biomarker for monitoring patients and for selection of therapy in Lung Cancer for Dr. Curtis Harris.
- An immuno-MRM Mass Spectrometry CLIA registered assay for Thyroglobulin for the CPTAC program (Dr. Henry Rodriguez).

Immediate Office of the Director, Center for Strategic Scientific Initiatives

Support Provided by the Applied and Developmental Research Directorate

ADRD: HPV Serology Laboratory

HPV Serology Laboratory

- Key Received blood from 61 vaccinated recipients and 22 unvaccinated donors through Occupational Health Services and other commercial sources. Enzyme-linked immunosorbent assays (ELISAs) were performed to screen samples and to categorize the antibody response of each sample.
- One laboratory goal is the generation of well-qualified HPV L1 virus-like particles (VLPs) to serve as assay reference reagents to the HPV serology scientific community involved in the immune monitoring of samples from clinical trials. Plasmids were produced and sequences were verified. VLP production of additional lots of HPV VLPs was completed (53.1 mg of VLPs for nine different HPV types). VLPs were qualified using electron microscopy as well as binding specificity. The laboratory completed validation of a 9-plex Luminex-based assay for the detection of antibodies against nine different types of HPV. A total of 258 assay runs (4,386 samples) were tested using the Luminex instrument.
- Given its expertise, the HPV Serology Laboratory started working on COVID-19 serology assay development and validation. The laboratory optimized and implemented quantitative ELISAs for SARS-CoV-2 antibody testing (IgG and IgM assays) in serum/plasma. In collaboration with FDA, the laboratory evaluated the performance of over 75 serology tests, including lateral flow devices and ELISAs. In addition, in collaboration with the National Cancer Institute (NCI) and the National Institute of Allergy and Infectious Diseases (NIAID), the laboratory developed performance/validation panels using samples from SARS-CoV-2 infected patients and uninfected blood donors. More than 37,000 serum/plasma sample aliquots into 0.5 mL vials were generated and more than 3,000 SARS-CoV-2 ELISAs were performed, including SARS-CoV-2 Receptor Binding Domain, Spike, and nucleocapsid.
- [redacted]

[redacted]

- The HPV Serology Laboratory is evaluating the participants' immune response to one, two, and three doses of the two HPV vaccines by determining serum HPV-16/18-specific antibody levels and antibody avidity at various time points after vaccination. The laboratory created 18,448 sample aliquots into 0.5 mL vials. The laboratory tested 5,760 samples for HPV-16 ELISA and 2,592 samples for HPV-18 ELISA. These numbers included the assay validation testing. Given the public health burden of HPV and cervical cancer in Africa, this research is critical in providing data necessary to support future recommendations for reduced vaccine schedules in Africa.

HPV Immunology Laboratory

KEY ACCOMPLISHMENTS

- Evaluation of antibody avidity following one or more doses of the bivalent HPV vaccine in the Costa Rica HPV Vaccine Trial. A single dose of the bivalent HPV vaccine elicits durable anti-HPV-16/18 antibody levels, which confer protection against HPV types included in the vaccine for over a decade following initial vaccination. In addition to evaluating anti-HPV-16/18 antibody levels, the HPV laboratory has developed and validated assays to assess antibody avidity, which is a measure of the strength of the binding of the antibody to the antigen. In previous studies, the HPV laboratory demonstrated that HPV-16 VLP antibody avidity increased steadily between year 1 and year 4 after vaccination in women receiving three doses of the bivalent HPV vaccine. Marginal difference in HPV-16 antibody avidity was observed between women who received one or three doses of the HPV bivalent vaccine at year 4 after vaccination, and levels stabilized for both dose groups at year 7 following vaccination.
- Continued evaluations of HPV-16 antibody avidity out to 11 years post-vaccination in HPV-naïve women that either received the standard three-dose HPV vaccine regimen or only a single dose of the bivalent vaccine. A total of 2,440 samples were tested for HPV-16 antibody avidity.

Support Provided by the Biomedical Informatics and Data Science Directorate

Scientific Infrastructure/Support Development

KEY ACCOMPLISHMENTS

- Deployed a local instance of cBioPortal (mocha-cbioportal) for the National Clinical Laboratory Network (NCLN)
- Maintained and enhanced the antibody data portal
- Improved authentication and integrated CSSI-DCC data sets into Google data set search

Office of Cancer Clinical Proteomics Research

The Reagents Antibody Data Portal

The Frederick National Laboratory for Cancer Research (FNL) staff maintained, performed required security patches for, and enhanced the Reagents Antibody Data Portal (http://antibodies.cancer.gov/) for the Office of Cancer Clinical Proteomics Research (OCCPR) initiative to support protein/peptide measurement and analysis efforts for the scientific community.

Protein Capture

The Protein Capture Reagents Program (http://proteincapture.org/) was a collaboration between OCCPR and the National Institutes of Health (NIH) Common Fund to provide low-cost, high-quality renewable affinity reagents for human proteins as a resource for the scientific community. FNL staff provide limited support using cloud hosting as the site infrastructure. Support includes general system monitoring, security patching, and activity monitoring to verify systems are operating as expected and to meet all NIH requirements. The program is currently scheduled for migration to the CentOS operating system. This project was scheduled for retirement in late summer of 2018 but has been extended throughout FY2020 as its resources are used by the scientific community.

Data Coordinating Center for the Center for Strategic Scientific Initiatives

The FNL staff supports a Data Coordinating Center (DCC) that it developed for the Center for Strategic Scientific Initiatives (CSSI). The web portal, accessible at https://cssi-dcc.nci nih.gov, houses data sets generated through projects funded by CSSI.

The FNL staff added new features that include: (i) authentication using CILogon and (ii) integration of the linked data schema JSON for indexing by the Google data set search tool. Development began on a metadata wizard feature to simplify creating metadata for scientific data sets. Ontologies for biobanking and biomedical investigations were incorporated for seamless integration of standardized concepts and terms into the metadata. Templates and architectural framework are being implemented for reading file directories, understanding file extensions, and suggesting probable data flow and the processes required for file conversions. Graphical interfaces for presenting the results and displaying study design also have been implemented. In addition, the team performed demos for the Human Cell Atlas Project and data commons teams in the Center for Biomedical Informatics and Information Technology. The portal is currently being designated as the default data sharing resource for experiments that lack a data commons or

other data sharing resource, and plans are underway for integrating CSSI-DCC into the National Cancer Institute (NCI) data sharing ecosystem.

National Clinical Laboratory Network for NCI Precision Oncology – Data Solutions and Systems Biology Group

FNL staff provide automation, integration, and analysis support to NCLN for precision oncology conducted by the Molecular Characterization Laboratory (MoCha), which is leading the effort.

FNL staff have deployed a local instance of cBioPortal (mocha-cbioportal) by customizing the instance to support both Cancer Therapy Evaluation Program and NIH authentications. DevOps pipelines were also integrated to streamline deployment of future versions and the integrated customizations.

Efforts were also focused on automating and integrating the sequencing data deposition, analysis, and loading of the resulting files to mocha-cbioportal. Extensive validation, auditing, and process integration now allow for data uploaded from both the MoCha and MD Anderson Cancer Center sequencing teams to be transferred into appropriate directories and for analyzed results to be made available to approved users through the mocha-cbioportal web interface.

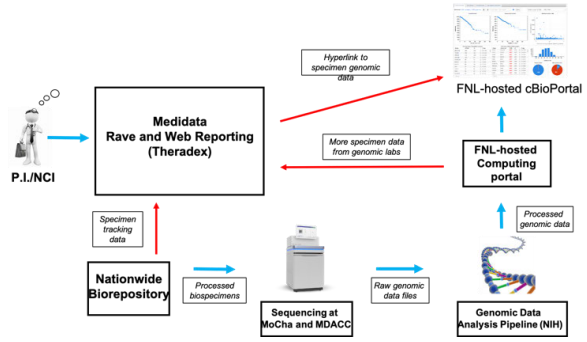


Figure 1. Schematic showing data flow and automated analysis integration for the NCLN precision oncology effort.

Support Provided by the Cancer Research Technology Program

Antibody Characterization Laboratory

Antibodies are among the most commonly used tools in the biological sciences, put to work in many experiments to identify and isolate other molecules. Approximately once per year, the NCI Office of Cancer Clinical Proteomics Research (OCCPR) initiates a callout for cancer relevant antibodies to the scientific research community, and awards to scientists from intramural, academic, and international sites. Targets typically are geared towards peptides and proteins, but more exotic targets such as small molecules and nucleic acids have also been awarded. OCCPR funds these antibody projects, coordinated by the Antibody Characterization Laboratory (ACL). Rigorous antibody validation is performed on these antibodies at the ACL, which acts as an unbiased intramural reference laboratory to validate antibody specificity and reproducibility. Antigens and antibodies are expressed, purified, and characterized using standard operating procedures, with accompanying protocols and data made available to the public.

The ACL has produced 619 antibodies to 342 antigens to date and has qualified data as requested by the OCCPR. The awardees of the OCCPR callout, now ACL collaborators, participate in the screening of targets and experimental validation of the final antibodies by a wide array of relevant techniques. The number of current investigators collaborating with the ACL have increased to approximately 30 in the last year. To complement more traditional antibody characterization approaches such as Western blot and immunoprecipitation, ACL has introduced immunofluorescence (IF) or immuno-cytochemistry and Single Cell Western blot (SCWB). IF and SCWB have been successfully applied in the characterization of 39 antibodies raised against DNA damage protein targets, with or without post-translational modification (phosphorylation). ACL is also applying, wherever feasible, kinetics studies with Biolayer Interferometry, a label-free technology for measuring biomolecular interactions. All antibodies have been characterized, and the data has been posted on the ACL web portal (http://antibodies.cancer.gov).

The focus on the production of antibodies for use in immunoaffinity peptide-enrichment multiple reaction monitoring (immuno-MRM) assays has been a major initiative at ACL, and ACL has collaborated with multiple external sites (Fred Hutchinson Cancer Research Center, Broad Institute, Moffit Cancer Research Center) to help produce antibodies for a series of assay panels of interest to biomedical researchers, such as the RAS pathway and DNA damage repair pathways. Recently, ACL has purchased an in vitro diagnostic (IVD) liquid chromatography/mass spectrometry (LC/MS) instrument to explore the use of the antibodies developed at the ACL in immuno-MRM format clinical assays. Future plans include collaboration with the FNL CMDL laboratory in order to produce and validate CMDL immuno-MRM assays in order to demonstrate the potential of the immuno-MRM technique in clinical diagnostics

Imaging Mass Cytometry Lab

As part of the primary goal to explore the suitability of highly multiplexed imaging for use in translational and clinical research, the Strategic Pilots Incubator (SPI) established and fully operates a laboratory to evaluate the new Fluidigm Hyperion Imaging Mass Cytometry (IMC) platform for addressing key scientific questions that require highly multiplexed analysis of tissues samples. IMC is an expansion of mass cytometry, using a laser to ablate tissues labeled with antibodies carrying high mass

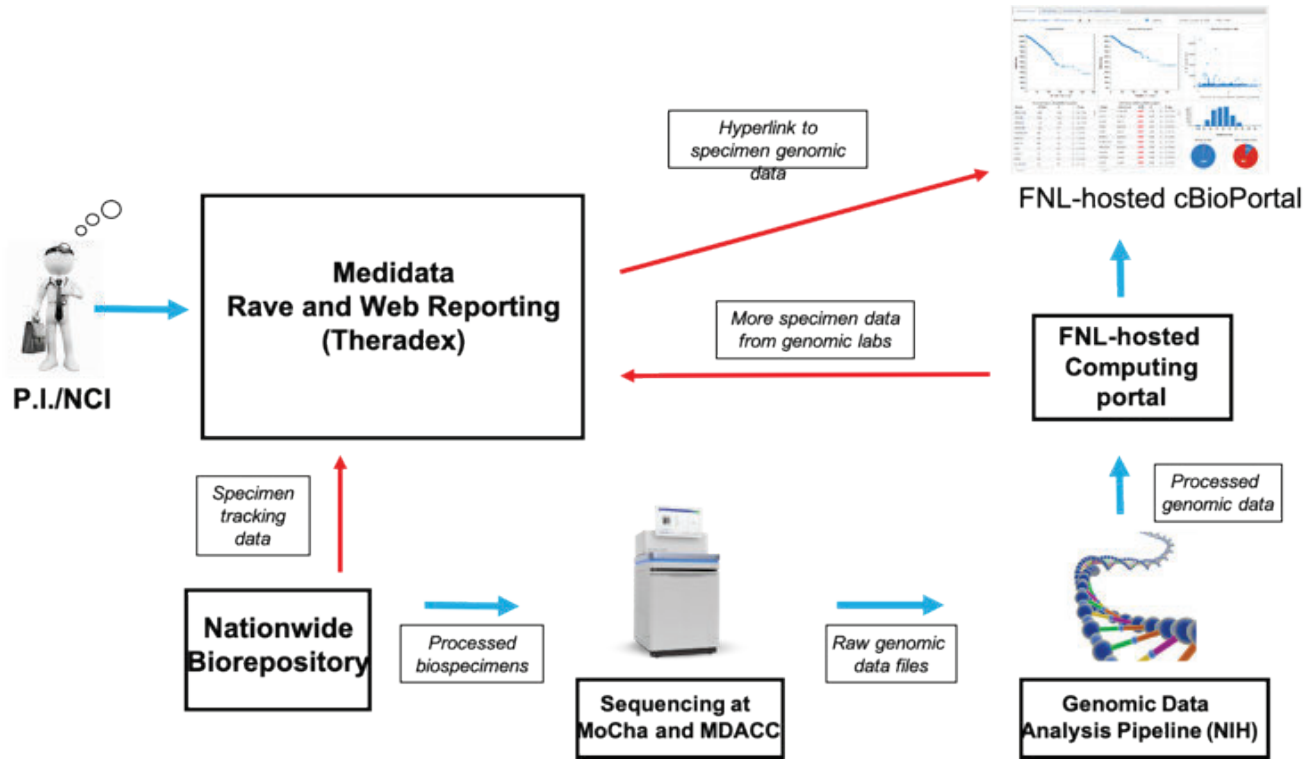


Figure 1. Schematic showing data flow and automated analysis integration for the NCLN precision oncology effort.

metal tags. The particles are carried to the mass cytometer, with the ions from each ablated spot are measured by time of flight (TOF) mass spectrometry. The use of rare earth metals allows for multiplexing up to around 40 antibodies per sample. The laboratory can enable the development of new assays focusing on functional tissue phenomics to investigate tumor heterogeneity and understand how tissue microenvironments affect the behavior and function of the resident cell populations. These new assays will extend cell typing and biomarker analysis to include the proximal location of tumor, stromal, and immune cells of interest, thus allowing the quantitation of both specific drug responses (pharmacodynamics and toxicology) and the subset of cells in which they are occurring, either in biopsy materials or cells isolated from blood.

SPI's first IMC project has evaluated IMC with a validated clinical biomarker assay from the Clinical Pharmacodynamics Program, which supports the NCI's Division of Cancer Treatment and Diagnosis (DCTD). The same panel is planned to also be used to examine the epithelial–mesenchymal transition at the tumor margins in triple-negative breast cancer in collaboration with a team in the Center for Cancer Research (CCR). A second antibody panel is currently under development that includes cancer metabolism, cell cycle regulation, and hypoxia related targets. These projects have also addressed the impact of the Hyperion's one-micron pixel size on image quality and begun to explore the compatibility of IMC image files with downstream image analysis algorithms currently in use for quantifying and visualizing histological quality images. Current work is focusing on refining the antibody panels in development, testing a new mouse immune panel in collaboration with CCR, optimizing the workflow by integrating sample prep automation, and bridging the high-definition single-cell analysis platform with IMC analysis.

STG: Scientific Initiative Development

Strategic Pilots Incubator

The Strategic Pilots Incubator (SPI) was developed in collaboration between the Frederick National Laboratory's Science and Technology Group (STG) and the NCI's Center for Strategic Scientific Initiatives (CSSI) in order to plan and implement both NCI-CSSI and trans-NCI pilot projects in order to address strategic gap areas in cancer research. As part of this initiative, the Frederick National Laboratory provides readily deployable support to CSSI activities.

Data Coordination Center

The Data Coordination Center (DCC) provides integrated management of datasets across all deposited projects, making its data more accessible and easily reusable by the cancer research community. DCC stores and manages access to data, enabling researchers or data depositors to grant controlled access only to specific collaborators while maintaining a user-specified embargo on deposited datasets. DCC enables a data access and sharing capability aimed to facilitate the development of new biological insights. DCC hosts a wide variety of data, including but not limited to cellular and histopathological imaging, cell mechanical property measurements, genomics, transcriptomics and proteomics profiling, focused ion-beam scanning electron microscopy (FIB-SEM) imaging, and traditional and contemporary enzyme-linked immunosorbent assays (ELISAs). DCC implements the Investigation-Study-Assay (ISA) paradigm, whose framework provides a rich description of experimental metadata that is agnostic and irrespective of sample characteristic, technology or measurement type. It provides clear and simple sample-to-data relationships that enables resulting data and discoveries to be reproducible and reusable. The DCC portal is a public repository of experiment-related information describing cancer and biomedical research investigations. The portal can be used to browse, search, and access data from uploaded datasets. Through its stand-alone toolset (Metadata Designer plus Validator), DCC offers data depositors and researchers a simple interface to create and validate metadata associated with data generated in their research. Deriving new insights from aggregates of datasets and the need for reproducible research has never been more apparent. However, a fundamental requirement for these is a comprehensive metadata annotation and documentation of the research processes—an art that is elusive to researchers. This work is performed with the expertise of Uma Mudunuri and her developers and validation engineers. The DCC portal is located at https://cssi-dcc.nci nih.gov/cssiportal/.

HD-SCA Laboratory

The high-definition single-cell analysis (HD-SCA) laboratory was initiated and is fully operated by SPI. The focus of the lab is to help refine technology and facilitate the broader use and adoption of the HD-SCA platform developed by Peter Kuhn's lab at the University of Southern California (USC). HD-SCA is an enrichment-free, high-throughput approach for rare cell detection to revolutionize how researchers capture and analyze cancer cells circulating in patients' blood. The platform combines immunofluorescent staining and automated digital microscopy to identify individual circulating tumor cells (CTCs). This assay has potential advantages over other CTC capture technologies because it analyzes all nucleated cells in blood samples, ensuring a non-biased approach to CTC isolation and allowing a comprehensive analysis of all cell populations. Following the identification of CTCs, single cells of interest may be picked for downstream analysis by genomic sequencing or subsequently stained for proteomic analysis by imaging mass cytometry (IMC).

The FNL acquired the technology from USC and collaborated extensively with the Kuhn lab for the development of the computing architecture, building and deployment of the scanner system, full integration of the

software and hardware, and training of the FNL staff scientists in CTC sample processing, staining and imaging, data analysis/reporting, and cell picking. Validation experiments were carried out to test the reproducibility of the FNL system in direct comparison to the USC system. A validation scheme was developed and executed to compare the blood processing, staining, scanning, and candidate report generation between the two laboratories using healthy donor blood spiked with tumor cell lines at varying concentrations. Current work at FNL is focusing on optimizing inter- and intra-technician reproducibility of the assays, validating the platform concordance between the two laboratories using cancer patient samples, and preparing to accept and process patient samples from collaborators at Thomas Jefferson University. Additional studies will be carried out through January 2021 to continue assessing and optimizing the platform including improving sample preparation for the characterization of CTC clusters, non-epithelial cancer associated cells, and additional tumor specific epithelial cells.

Lung Cancer Multiple Reaction Monitoring Assay Project

The NCI Office of the Director's Center for Strategic Scientific Initiatives Office of Cancer Clinical Proteomics Research envisioned a project to produce a series of assay panels for multiple reaction monitoring (MRM) and immuno-multiple reaction monitoring (immuno-MRM) to identify and quantify clinical proteomic biomarkers. Scientists from the Antibody Characterization Laboratory (ACL) were recruited to contribute. ACL in turn enlisted Dr. Amanda Paulovich of the Fred Hutchinson Cancer Center to develop these assays due to her extensive expertise in MRM and immuno-MRM assay development. Dr. Paulovich convened a panel of experts from academic research institutions, clinical research institutions, and pharmacology corporations to select targets for assay development, and the focus of the assay panels shifted from lung cancer to immuno-oncology in order to target the most meaningful biomarkers. Approximately 60 of a planned 87 antibodies have been selected for immuno-MRM assay development. A series of panels of both immuno-MRM and direct MRM assays are underway at Fred Hutchinson. All antibodies developed for these panels will be shared with ACL and included on the ACL antibody portal (http://antibodies.cancer.gov).

Support Provided by the Clinical Research Directorate

Biospecimen Collection and Analysis

KEY ACCOMPLISHMENTS

- The team met the goal of collecting 352 new cases (out of the 375 originally planned) with accompanying clinical data by July.
- The team also contracted with six new tissue source sites (TSS) that were targeted to minimize the bias in the biospecimen collection cohort and increase samples from underserved and minority populations.
- The Program Management team supported the publication of five manuscripts in *Cell* during FY2020.

Program Management

The FNL team provided program management, quality management, and scientific support to the Clinical Proteomic Tumor Analysis Consortium (CPTAC) program through several activities, including developing and executing a project management plan; executing a project schedule; facilitating technical, scientific, and management interactions between the different CPTAC components; representing FNL at steering committee, data analysis, and disease working group meetings; presenting results in meetings; contributing to manuscripts; managing quality system essentials for the program; tracking finances and budget; interfacing with the government customer; and meeting the goals, milestones, and deliverables as specified in the statement of work.

FNL's past CPTAC collection activities supported the research and analysis for five publications appearing in *Cell* during FY2020: articles describing therapeutic vulnerabilities in lung adenocarcinoma (Gillette MA, et al. *Cell*, 182(1):200-225, 2020), proteogenomic characterization of ovarian tumors (McDermott JE, et al. *Cell*, 1(1):100004, 2020), proteogenomic characterization of endometrial carcinoma (Dou Y, et al. *Cell*, 180(4):729-748, 2020), the integrated proteogenomic characterization of clear cell renal cell carcinoma (Clark DJ, et al. *Cell*, 179(4):964-983, 2019), and new therapeutic opportunities for colon cancer (Vasaikar S, et al. *Cell*, 177(4):1035-1049, 2019).

Biospecimen Accrual

The FNL team managed 24 active domestic and international TSS in FY2020 to prospectively collect biospecimens from 17 tumor types. The team also contracted with six new TSS that were targeted to minimize the bias in the biospecimen collection cohort and increase samples from underserved and minority populations in the upcoming years. The team met the goal of collecting 352 new cases (out of the 375 originally planned) by July. A shift in priorities due to COVID-related closures for four months contributed to the selection of cases for the program. A separate effort is underway for the acquisition of 120 additional retrospective cases to enhance the collection of head and neck squamous cell carcinoma (HNSCC). Out of the 352 cases, 125 were from 10 new tumor types that are in the first year of pilot collection. The remaining 227 were from the existing phase that helped close the cohort for downstream analysis. The team specifically went after

extremely hard-to-accrue glioblastoma multiforme cases from young adults aged 18–39 to complete the collection critical to a publication. The team also recruited sites specific to HNSCC cases from the oropharyngeal site that were either HPV+ or HPV- (confirmed through immunohistochemistry) in order to provide key insights into molecular characterization. The team handled several critical responsibilities such as qualifying samples, ensuring sample quality and managing the accompanying data, tracking invoices, completing change requests, executing safe shipments, navigating customs regulations from different countries due to travel restrictions, and shifting priorities due to COVID closures. The team kept the customer and the subcontractors well informed of status to resume operations when sites reopened. In addition, the team met the program goals for the year without any interruptions, and all samples were shipped safely to the Biospecimen Core Resource (BCR) facility on time.

Biospecimen Processing and Shipment for Omics Characterization

The TSS have shipped 305 unique cases so far and a total of 725 samples to the central BCR in FY2020. The BCR facility (managed by FNL) was under lockdown from March 16 to June 12, 2020, due to COVID-19; however, shipments resumed May 29, and all goals for safe shipments were met. BCR processed the samples on time for a subsequent shipment to the genomic and proteomic characterization laboratories. There were nine shipments to the characterization laboratories in FY2020. All shipments were paused between March and June but resumed when the centers reopened and could receive the samples. The BCR processed samples, starting with pathology qualification of slides—a shared task between the BCR pathologist and FNL pathologist as part of the Pathology Resource Center—followed by molecular qualification, which included cryopulverization or, occasionally, coring of samples prior to aliquoting them for proteomics and nucleic acid extraction. The BCR also processed 50 cases through laser microdissection to study tumor heterogeneity. In addition, the staff scanned all slides used for pathology qualification for an upload into The Cancer Imaging Archive for dissemination of images.

Clinical Data Review and Submission

The FNL team of expert clinical data reviewers review clinical data that are collected with the cases from the source sites. The team also oversees the development and maintenance of the case report forms that are used to collect data. The program collects 250 data points through 13 case report forms for each case. The team reviewed 817 cases in FY2020 and cleared a significant backlog of cases using automated and manual methods that enabled the use of data for downstream analysis. In FY2020, 626 aliquots were registered at the Database of Genotypes and Phenotypes, and clinical data from 313 cases were submitted to the Genomic Data Commons. The team also worked with the Center for Biomedical Informatics and Information Technology (CBIIT) to generate National Cancer Institute (NCI) Thesaurus code and clinical data element (CDE) code for all fields from the case report forms. A total of 7,674 CPTAC-specific NCI Thesaurus codes and 406 CPTAC-specific CDEs were assigned this year. The team worked extensively to revise the case report forms for all tumor types.

Comprehensive Data Resource Development, Releases, and User support

The comprehensive data resource (CDR) is an online web portal that supports the complete CPTAC workflow. All components of CPTAC, including subcontractors, use CDR to enter or review data. CDR also provides analytics and reporting capabilities for the program. Key developments this year include:

- A dashboard for the project managers and the TSS to track their work and monitor case status, including payment information
- New case report forms to collect pediatric data for the program
- Extension of alternative normal collections (without tumor) for other cancer types
- Ability for an expert pathologist from the consortium to review cases when needed
- Definitions for each field as a mouse-over option was added
- CDE and NCI Thesaurus codes for half the fields
- Case report form consistency checks
- Omics status information in the portal
- Protein aliquot shipment information and additional automations for data integrity

The CDR team also continues to work on security compliance tasks required by NCI CBIIT to maintain Federal Information Security Management Act compliance, in addition to plan of actions and milestones tasks towards obtaining Authorization to Operate for the next three years.

Immediate Office of the Director, The Cancer Genome Atlas

Support Provided by the Clinical Research Directorate

The Cancer Genome Atlas Clinical Data Update

KEY ACCOMPLISHMENTS

- The subcontractor obtained their Authorization to Operate (ATO).
- The subcontractor managed communications and outreach activities to sites for case data collection.

FNL worked closely with the subcontractor to interpret and develop key artifacts to successfully accomplish the ATO. This involved significant oversight by FNL. The ATO was required to be in place prior to the receipt of clinical follow-up data. The subcontractor had limited knowledge of the process and requirements, and with FNL's assistance, they were able to successfully accomplish this task.

In addition, FNL oversaw the project for outreach activities to individual sites. This included overseeing subcontracting, as well as regulatory and logistical elements. FNL spent considerable time and effort working with the site to prioritize outreach activities to best optimize their efforts. To meet the requirements for case numbers, the subcontractor will need to put agreements in place with 8–12 sites, of which four are currently in place. FNL mediated relations with Information Management Services and the National Cancer Institute (NCI) to fulfill artifacts.

In addition, FNL requested an impact analysis and mitigation plan from the subcontractor, which included key assumptions considering the COVID-19 pandemic. The pandemic significantly affected key staff's availability and limited operational capacities at the site level. FNL, in concert with NCI, made decisions based on this analysis and other key factors to reduce the scope of work by reducing the number of cases for the period of performance with de-obligation of the related funds on the contract.

Immediate Office of the Director, Office of Cancer Genomics

Support Provided by the Clinical Research Directorate

Burkitt Lymphoma Genomic Sequencing Project

KEY ACCOMPLISHMENTS

- Released Request for Proposal (RFP) for tissue acquisition
- Expanded possible approaches to acquire cases from a vendor who had reduced resources to respond to an RFP
- Recommended consideration of additional research on currently held cases to mitigate the lack of new cases due to COVID-19 delays
- Ensured the central pathology review could support a new electronic-only type of case review

A statement of work was developed, and an RFP was issued as a limited competition. Both vendors were receptive to participating but encountered difficulties and delays due to COVID-19. The timeline for responding was extended to accommodate the delays. Both vendors are still being pursued and are provided the same follow-up and support for participation in the RFP.

Through conversations with the Program Office, the vendor, Subcontracts, and other management colleagues, a series of four different pathways to attempt procurement were outlined. Reimbursement via a material transfer agreement has been declined, and acquisition of discrete case packages via a purchase order is currently being pursued. Other options include a standard subcontract or case donation.

FNL staff regularly monitored expenses and identified potential funds that would not be used because new acquisitions were delayed due to COVID-19. The staff engaged the Program Office and vendor to consider what additional research could be performed that would enrich the Burkitt Lymphoma Genome Sequencing Project knowledge portfolio. Additional methylation work using the Nanopore PromethION sequencing technology was performed on 18 cases of interest.

FNL staff communicated and coordinated with the Program Office and the vendor to ensure the standard pathology-review process was adapted so that cases already having stained slides could be accepted for review and the vendor would be allowed to be reimbursed for this new type of work.

Immediate Office of the Director, Technology Transfer Center

Support Provided by the Partnership Development Office

Invention Development Program

KEY ACCOMPLISHMENTS

In FY2020, the Invention Development Program (IDP) funded three new projects:

- Dr. Choung Hoang, Nanoparticle-Hydrogel Composite for Nucleic-Acid-Molecule Delivery
- Dr. Brad St. Croix, Development of Next-Generation Antibody Drug Conjugates Against CD276
- Dr. Udo Rudloff, Use and Preparation of CD206 Small-Molecule Modulators as Therapeutics for CD206 Expressing Cancers

In December 2019, IDP was moved into the Science and Technology Group within the Partnership Development Office. IDP aims to accelerate the development timeline of NCI intramural inventions by advancing the technologies through critical, early stages of validation. The Partnership Development Office provides project management support for IDP and coordinates the preclinical validation work to be performed using Frederick National Laboratory for Cancer Research resources including the Nanotechnology Characterization Laboratory, Cancer Research Technology Program, and Laboratory Animal Sciences Program, and external resources when necessary.

Immediate Office of the Director, Office of the Director, NCI at Frederick

Support Provided by the AIDS and Cancer Virus Program Directorate

AIDS and Cancer Virus Program–Base and Office of AIDS Research Units of Work

The AIDS and Cancer Virus Program (ACVP) is an integrated, multidisciplinary program that pursues basic and applied studies aimed at improving the understanding of AIDS-associated viruses, including studies intended to facilitate the improved diagnosis, prevention, and treatment of HIV infection and AIDS and AIDS-related tumors, particularly those associated with cancer viruses such as Kaposi sarcoma–associated herpesvirus (KSHV). ACVP has five principal investigator–headed Research Sections and eight Research Support Cores providing innovative and often unique capabilities in support of ACVP, National Institutes of Health (NIH), and extramural investigators.

ACVP Key Accomplishments

- ACVP and collaborators published 51 scientific articles in peer reviewed journals (see Appendix B).
- COVID-19 research:
  - Participation in a nationwide clinical study examining the effects of SARS-CoV-2 infection on HIV-1 viral loads in dually infected patients
  - Development of RNAScope, DNAScope, and immunofluorescent/immunohistochemistry approaches for detecting SARS-CoV-2 nucleic acids and proteins and other COVID-19–relevant molecules in tissues from infected individuals to localize and identify the cells harboring SARS-CoV-2 vRNA and/or vDNA to better understand the pathogenesis of SARS-CoV-2 infection and COVID-19
  - Technical consultation, precision flow cytometry, and cell sorting instrumentation for Frederick National Laboratory for Cancer Research (FNL) users performing COVID-19–related research throughout the outbreak
  - Technical support for the NCI HIV Dynamics and Replication Program (HIV-DRP)'s study of SARS-CoV-2 entry into target cells
  - Collaboration with the Uganda Virus Research Institute on a large SARS-CoV-2 population-based monitoring study investigating the effect of SARS-CoV-2 infection on herpesvirus reactivation
- Contributions to the public partnership/stewardship of FNL:
  - ACVP received 35 technical service agreement requests from 21 collaborators at 15 institutions.
  - ACVP continued with three ongoing contractor collaborative research and development agreements, with two additional agreements signed during the period and two others nearing execution.

Retroviral Pathogenesis Section

The Retroviral Pathogenesis Section (RPS) conducts basic and applied research, both *in vitro* and *in vivo*, in nonhuman primate (NHP) models on AIDS virus/host interactions to better understand key disease mechanisms. Current NHP models are focused on studying the virus that persists despite extended combination antiretroviral drug treatment (cART) and gives rise to recrudescent infection when treatment is stopped ("the viral reservoir").

Key Accomplishments

In collaboration with Dr. Stephen Hughes (HIV-DRP), we demonstrated that simian immunodeficiency virus (SIV) integration into rhesus macaque CD4+ T cells *in vitro* and *in vivo* recapitulates key features of HIV integration in humans (Ferris et al., *PLoS Pathog*, 2019), including that expanded clones of CD4+ T cells harboring clonally integrated proviruses can arise within the first few weeks of infection and persist despite extended suppressive cART. An ongoing NHP study is evaluating the role of antigen-driven proliferation in the establishment and persistence of expanded clones, a key component of the viral reservoir that persists despite cART.

Ongoing collaborative studies with the Retrovirus–Cell Interaction Section (RCIS) are investigating the effect of recombinant rhesus heterodimeric IL-15 in supporting adoptively transferred autologous T cells engineered *ex vivo* for expression of SIV-specific T-cell receptors, antiviral activity, and tissue preferential trafficking. The NIH Office of AIDS Research (OAR)–supported portion of the project demonstrated preferential small intestine homing and persistence of CD8 T cells in rhesus macaques achieved by molecularly engineered expression of CCR9 and reduced *ex vivo* manipulation (Trivett et al., *J Virol*, 2019).

Ongoing collaborative studies with the Viral Persistence Section (VPS) have evaluated cART administration to simian-tropic HIV (stHIV)–infected pigtail macaques to further develop this model that incorporates a more HIV-like virus, including studies of HIV-specific chemoprevention approaches for which SIV is not an optimal target. Along with the VPS, the RPS is also evaluating the role of ligands for Toll-Like Receptors 7 and 8 as potential inducers of latent proviruses and immunomodulators in SIV-infected rhesus macaques receiving cART.

Dr. Jeffrey Lifson was elected a Fellow of the American Society for Microbiology and named a "Highly Cited Researcher" in microbiology by Clavirate Analytics/Web of Science, indicative of the top 1% of impactful researchers based on citation analysis over the preceding decade.

Retrovirus–Cell Interaction Section

The RCIS studies interactions between AIDS viruses and their host cells, employing both *in vitro* and *in vivo* approaches in NHP models to study T-cell-mediated anti-AIDS virus immunity.

Key Accomplishments

Adoptive transfer of engineered antiviral CD8 T cells prior to virus inoculation impacts viral transmission and early viral dynamics. In collaboration with the RPS, the RCIS continued experiments in which rhesus macaques receive adoptive transfer of SIV-specific T-cell-receptor–transduced autologous antiviral T cells administered recombinant rhesus heterodimeric IL-15 (rRhHet-IL-15; Watson et al., *PLoS Pathog*, 2018) three days prior to intravenous inoculation with the barcoded virus SIVmac239M (Fennessey et al., *PLoS Pathog*, 2017), followed by a second dose of rRhHet-IL-15 on the day of inoculation. Each experiment is conducted in pairs of animals, with one receiving major histocompatibility complex (MHC)-matched engineered cells and the other receiving MHC-mismatched (and thus inactive) engineered cells in a protocol designed to control for all aspects of the apheresis, *ex vivo* manipulation, infusion, and inoculation procedures as well as the effects of rRhHet-IL-15. Therefore, any difference in outcome between the animals in each pair can be attributed to the antiviral activity of the infused cells. This study is currently ongoing. Three pairs of animals have already undergone adoptive transfer and inoculation, and an additional two pairs are planned for subsequent replicates. In all three pairs of animals studied to date, we have seen an approximately two-log reduction in peak plasma viral load and an approximately 66% reduction in the number of transmitted founder variants in the active vs. paired inactive engineered cell recipients, suggesting that the infused cells are able to effectively limit the initial foci of infection from spreading systemically.

We are also evaluating the persistence and tissue distribution of engineered CD8+ T cells in adoptive cell transfer experiments using heterodimeric recombinant rhesus IL-15 cytokine (rRh-Het-IL-15) and cells sourced from autologous lymph nodes. As part of our ongoing efforts to improve adoptive T-cell transfer methods, in collaboration with the RPS, the RCIS evaluated the impact of serial dosing with recombinant rhesus IL-15 cytokine (rRhHet-IL15), known to enhance proliferation, cytotoxic potential, and B-cell follicular localization of CD8+ T cells on infused cell trafficking and persistence (Trivett et al., *J Virol*, 2019). Additionally, the RCIS has evaluated differences in persistence and trafficking to lymphoid tissue when using infused cells originating from autologous blood or lymph node tissue. Analyses of these studies are currently underway.

Retroviral Evolution Section

The Retroviral Evolution Section (RES) uses molecular biology approaches to develop and employ novel viral systems to take full advantage of the unique benefits afforded by NHP models to address critical questions in key areas of AIDS research including transmission, treatment, HIV persistence and "cure," and viral adaptation, pursuing studies suited to FNL's unique research environment.

Key Accomplishments

RES personnel authored/coauthored 16 papers, many involving validation and application of the barcoded virus approach that RES developed, including studies in 120 rhesus macaques, validation of the barcoded approach for "HIV cure" research, and the addition of six distinct barcoded virus models for use in NHP (Khanal et al., *J Virol*, 2019). With Miles Davenport, University of New South Wales, (Pinkevych et al., *eLife*, 2019) RES showed that later initiation of combination anti-retroviral therapy (cART) following infection increased the size of the rebound competent reservoir. Interestingly, a 100-fold increase in total viral DNA was associated with only a two-fold increase in viral reactivation rates, which implies that the paradigms for assessing the rebound competent viral reservoir may need to be revised. The barcoded virus model was also used to examine, in unprecedented depth, the dynamics of immune escape based on host cytotoxic T-lymphocyte selection (Immonen et al., *Proc Natl Acad Sci USA*, 2019).

RES has initiated multiple high-impact long-term NHP studies including one to model the safety of taking HIV-infected humans off cART and thereby causing a period or recrudescent viral replication before reinstituting cART. Preliminary data from four rhesus macaques show that if the rebound is sufficiently high or long in duration, the viral reservoir can change and expand. An OAR-supported study currently being analyzed assesses potential residual virus replication during cART, a point of continuing controversy in HIV research. SIV-infected macaques were maintained on cART for up to three years, and the full viral genome was sequenced for evidence of ongoing virus replication. In a second OAR-funded project, novel chimeric stHIV (Schmidt et al., *Proc Natl Acad Sci*, 2019) and SHIV clones (O'Brien et al., *PLoS Pathog*, 2019) were developed for use in NHPs. Several of these clones were recently barcoded (Khanal et al., *J Virol*, 2019) and are being made available to the research community.

The expertise and technological advances of the RES have led to the establishment of multiple collaborative projects. The use of the barcoded virus model alone has prompted collaborations for 20 new additional research projects across 10 different institutions, many of which

are supported in part by contractor Cooperative Research and Development Agreements. These include studies of the viral reservoir, post-rebound control, chimeric-antigen receptor (CAR)-T cells, novel latency reversing agents, and other intervention strategies intended to eliminate the viral reservoir.

[redacted] was recognized as a "highly cited researcher" in microbiology by Clavirate Analytics/Web of Science, which includes the top 1% of impactful researchers based on citation analysis over the preceding decade. [redacted]. The barcoded virus model will be used to define the anatomic origins of seminal virus, determine the cell type of productively infected cells in semen, and correlate seminal virus to blood plasma virus. This study will provide unprecedented depth of analysis of how the semen collects infectious virus, providing insights for novel intervention strategies to block virus accumulation in the semen and prevent viral transmission via this most epidemiologic significant route.

Viral Persistence Section

The newest research section within the ACVP (established in 2018), the Viral Persistence Section (VPS) develops and applies novel *in vitro* methods and *in vivo* NHP models to better understand the establishment, maintenance, and consequences of persistent lentiviral and AIDS-related non-lentiviral infections in order to develop and evaluate preventions and treatments.

Key Accomplishments

To evaluate the potential of antibody-mediated deletion of infected cells that persist despite cART, VPS conducted a NHP study using a depleting mAb to CD4 in SIV-infected rhesus macaques receiving cART. Targeting CD4, expressed on both persistent, infected T cells and uninfected T cells, avoided the limitations of virus-specific mAbs. Meanwhile, allowing post-depletion recovery of target CD4 cells under continued cART provided permissive target cells to assess viral rebound when cART was stopped. Despite profound CD4+ cell depletion in the blood, no significant impact on viral rebound parameters was observed, likely due to incomplete depletion in key tissue sites, even using CD4 as a "best case" depletion target. These findings underscore challenges in the use of antiviral antibodies as a reservoir reduction approach.

[redacted]

[redacted].

To develop much-needed improved NHP models of central nervous system HIV-1 infection, VPS completed several pilot experiments examining direct intrathecal inoculation of infectious barcoded SIVmac239M into the cerebrospinal fluid of rhesus macaques, observing unprecedented levels of acute viral replication in the central nervous system and evidence of early virus sequence compartmentalization, supporting further utilization of this approach as a key component of our model development efforts.

With the Viral Oncology Section (VOS) and the Tissue Analysis Core (TAC), VPS continued efforts to identify tissue sources of gamma herpesviruses shed in saliva, using samples from rhesus macaques. Samples from 40 macaques were studied, with quantification of viral DNA for each of the three rhesus γ-herpesviruses in oral fluid and multiple naso-oral tissues from each animal, along with identification of sites, specific cell types, and distribution of γ-herpesvirus-infected cells in tissues using immunohistochemistry and novel *in situ* hybridization approaches.

Viral Oncology Section

VOS studies the role of viruses in cancer, focusing on three major areas: KSHV epidemiology and transmission, KSHV immunity and pathogenesis, and viral and host genetics in KSHV infection and disease.

Key Accomplishments

In the area of KSHV epidemiology and transmission, with the Uganda Virus Research Institute (UVRI; Entebbe, Uganda), VOS completed OAR-supported studies of risk factors for KSHV infection (Nalwoga et al, *PLoS Negl Trop Dis*, 2019) and Kaposi sarcoma in rural Uganda (Nalwoga, et al, *Clin Infect Dis*, 2019), with two additional manuscripts in preparation. VOS also established a new infant cohort to study KSHV transmission. VOS is also collaborating with UVRI on a large SARS-CoV-2 population-based monitoring study in which VOS will measure KSHV and Epstein-Barr Virus viral load to investigate the effect of SARS-CoV-2 on herpesvirus reactivation. Additional epidemiologic studies in Africa include case-controlled studies of KSHV and Epstein-Barr Virus blood and salivary viral load in Cameroon (Labo et al, *Int J Cancer*, 2019) and studies of cohorts of mothers and children in Kenya. Samples from a mother-child cohort have been tested at VOS using our multiplex antibody assays for IgG and IgM. With regards

to KSHV immunity and pathogenesis, VOS has developed and validated IgA and IgM multiplex assays for herpesvirus antibodies, and these are being used to test samples from Kenyan and Ugandan cohorts. T-cell responses have been studied by KSHV enzyme-linked immunospot in subjects over a wide range of ages, and KSHV-specific T-cell clones have been generated from a study participant. VOS maintains Clinical Laboratory Improvement Amendments certification and continues to collaborate with the HIV and AIDS Malignancy Branch analyzing clinical specimens to understand basic KSHV pathobiology and evaluate novel therapeutic approaches for KSHV-related disease. This support has continued during COVID-19-related restrictions (Ramaswami et al., *Blood*, 2020; Gruffaz et al., *PLoS Pathog*, 2020). During the review period, the FDA approved the use of Pomalidamide for KS treatment based in part on data from VOS. Additional studies of viral and host genetics in KSHV infection and disease have further characterized host genetic influences on KS risk (Cornejo-Castro et al., *Genes Immun*, 2019) and treatment response (Yap et al., *J Clin Imunol*, 2020) and greatly expanded the database of KSHV sequences by >100 whole viral genome sequences, including documentation of superinfections.

Biological Products Core

The Biological Products Core (BPC) provides expertise for multiscale production, purification, and characterization of AIDS-relevant virus preparations, purification and characterization of recombinant proteins of interest, and preparation and distribution of materials to enable cost-effective HIV-1 p24 antigen capture assays.

KEY ACCOMPLISHMENTS

For 2020, BPC provided 62 ml of purified, 1000x concentrated retrovirus or control microvesicles of interest, produced from 62 liters of culture supernatant, to seven different AIDS research laboratories. All preparations provided by BPC are unique products that are not available from commercial sources at any cost. BPC also produced and purified 18 lots of an HIV-1 isolate of interest based on unusually high virion Env content (HIV-1 BAL/SUPT1-CCR5 [CLN204]). Some of this purified virus was essential to enabling a high-impact cryo-electron microscopy–based publication describing the conformational features of native HIV-1 gp120 envelope trimers *in situ* on virions, for comparison with recombinant trimeric proteins under evaluation as experimental HIV/AIDS vaccine immunogens (Li et al., *Nat Struct Mol Biol*, 2020). BPC worked closely with ACVP's Retroviral Protein Chemistry Core and the laboratory of Dr. George Pavlakis (NCI) to develop, validate, and apply a method for purification of a novel heterodimeric form of the cytokine IL-15, producing in excess of 100 mg of purified cytokine for use in studies by Dr. Pavlakis and ACVP investigators. BPC also provided 1,150 HIV-1 p24 antigen capture immunoassay kit key reagents to support the work of 14 different extramural laboratories.

Retroviral Protein Chemistry Core

The Retroviral Protein Chemistry Core (RPCC) provides protein chemistry support to the ACVP and collaborating investigators by analyzing purified virus preparations and providing expertise in the purification and characterization of proteins, including recombinant proteins.

KEY ACCOMPLISHMENTS

RPCC continues to characterize purified preparations of retroviruses of interest. Analyses include: assessment of the state and amount of surface and transmembrane envelope glycoproteins and gag proteins using gel-based calibrated fluorescent staining analysis and immunoblot analysis; high-performance liquid chromatography fractionation and quantitative amino acid analysis and mass spectroscopy; and more intensive interventional analyses involving enzymatic digestions or covalent modifications. Different preparations of viral samples, cell lysate samples, and samples of monkey sera were analyzed during the period. Ninety viral samples, constituting 80 gels and 40 immunoblots (most with multiple re-probes) were performed with densitometric and other analyses when appropriate. RPCC also performed quantitative amino acid analysis on 40 samples (both for ACVP and external requests). Working with [redacted] (BPC/ACVP), RPCC developed and validated methods and purified and biochemically characterized ~60 mg of recombinant rhesus macaque FcHet-IL-15 in support of studies by Dr. George Pavlakis (NCI) and Dr. Jeff Lifson (RPS/ACVP).

Quantitative Molecular Diagnostics Core

The Quantitative Molecular Diagnostics Core (QMDC) provides expert consultation and advanced quantitative polymerase chain reaction (PCR)/reverse-transcription PCR (RT-PCR) monitoring of AIDS virus RNA and DNA species in support of studies in NHP models conducted by ACVP and collaborating investigators, including the development of customized assays.

KEY ACCOMPLISHMENTS

In support of studies by ACVP and collaborating investigators, QMDC performed a total of 19,642 standard assays for plasma SIV or SHIV RNA (15 copies/mL) and 1,146 high-sensitivity assays (1-3 copies/mL), including rapid turnaround assays to guide real-time protocol decision making. QMDC also performed 1,035 assays for cell- or tissue-associated SIV/SHIV viral RNA and DNA levels, along with discriminant assays for simultaneous quantitation of two distinct virus species present in specimens from the same co-infected animal. Notable contributions include support of studies that: demonstrated the lack of ability of antibody to the $\alpha 4\beta 7$ integrin to mediate SIV control in infected macaques (Iwamoto et al., *Science*, 2020;

Di Mascio et al., *Science*, 2020); evaluated the impact of Fc-dependent effector functions of HIV-1-specific anti-Env monoclonal antibodies (Asokan et al., *Proc Natl Acad Sci USA*, 2020); documented to role of SIV nef-mediated tetherin antagonism in viral control (Tavakoli-Tameh et al, *PLoS Pathog*, 2020); documented the ability of stimulation of non-canonical NF-kB pathways to increase plasma viremia in SIV-infected macaques receiving cART (Nixon, et al., *Nature*, 2020); established validation and application of barcoded virus approaches for studying AIDS virus biology in NHP (Khanal et al., *J Virol*, 2019; Immonen et al., *Proc Natl Acad Sci USA*, 2020); and evaluated the role of IL-15 in AIDS virus pathogenesis and treatment (McBrien et al., *Nature*, 2020; Okoye et al., *J Immunol*, 2020; Chen et al., *J Infect Dis*, 2020).

QMDC also developed two new assays, introducing one as a new service available through the Technical Services Agreement program.

Cellular Immunity Core

The Cellular Immunity Core (CIC) provides expert cellular immunology support to ACVP and collaborating investigators, chiefly through flow cytometry analyses and fluorescence activated cell sorting.

Key Accomplishments

CIC maintained and provided precision flow cytometry and cell sorting instrumentation for FNL users performing COVID-19-related and mission-critical research throughout the pandemic-related reduction in laboratory activities, including procurement of a new spectral flow cytometer to increase capabilities while reducing costs and specimen requirements. CIC supported 16 different NHP studies and numerous other projects, developing customized flow cytometry panels for immunophenotypic and functional testing and analyzing more than 25,000 samples, predominantly for AIDS-related studies but also including support of the NCI RAS Initiative and SARS-CoV-2–related projects. Specialized methods developed included: an RNA-flow method for simultaneous flow cytometric single cell detection of latent and lytic KSHV transcripts with VOS; a customized high-throughput flow cytometric cell sorting of antigen-specific cells for a project with RPS to analyze the role of antigen-driven proliferation in the establishment and persistence of SIV in expanded clones of CD4+ T cells in rhesus macaques on antiretroviral drug treatment; and development, validation, and application of numerous custom antibody reagents. CIC also organized and provided introductory and advanced flow cytometry data analysis training sessions for FNL staff and maintained the Flow Cytometry Interest Group on the Insite social network to encourage collaboration and provide flow cytometry resources, news, and upcoming meeting/training notifications to the local research community.

Nonhuman Primate Research Support Core

The Nonhuman Primate Research Support Core (NHPRSC) provides critical logistical and laboratory support and coordination for all NHP studies conducted by ACVP investigators, including collaborative studies.

Key Accomplishments

NHPRSC provided critical support for 17 unique ACVP NHP study protocols, including processing ~40,000 ml whole blood, 12 leukapheresis products, >250 lymph node samples, >140 intestinal tissue specimens, and >100 bronchoalveolar lavage and cerebrospinal fluid samples; provided processing, cryopreservation, and storage of >18,100 vials of blood plasma, ~7,300 vials of dry cell pellets, and ~7,600 vials of viably cryopreserved single-cell suspensions; and prepared 104,000 ml of sterile, co-formulated tenofovir disoproxil fumarate, emtricitabine, and dolutegravir for injection administration to NHP study animals, including 16,000 ml prepared for collaborators at the Vaccine Research Center, NIAID, NIH.

Studies supported by NHPRSC and published during FY2020 include: (i) a study further developing and advancing the utility of the barcoded synthetic viral swarms for use in NHP models of AIDS (Khanal et al., *J Virol*, 2019); (ii) a detailed examination of the viral population dynamics underlying emergence of mutations that enable escape from host immune responses (Immonen et al., *Proc Natl Acad Sci USA*, 2020); (iii) an in-depth analysis of viral genome intactness in SIV-infected rhesus macaques on suppressive antiretroviral therapy (Long et al., *J Virol*, 2019); and (iv) a study demonstrating that following adoptive cell transfer, autologous CD8+ T cells engineered *ex vivo* to express tissue homing markers preferentially home to tissue types determined by the chosen homing marker (Trivett et al., *J Virol*, 2019).

HIV Molecular Monitoring Core

The HIV Molecular Monitoring Core (HMMC) provides specialized HIV nucleic acid quantitation and sequencing expertise in support of cutting-edge clinical HIV research. Technical services provided include ultrasensitive HIV-1 viral load quantification, single-genome sequencing, and evolutionary analysis performed with culture supernatants, plasma, cerebrospinal fluid, cells, and tissues from clinical and supporting research studies.

Key Accomplishments

HMMC employs a unique ultrasensitive HIV viral load assay format with excellent coverage against diverse HIV clades. HMMC viral load testing supported studies that: evaluated the impact of an autologous dendritic cell vaccine, AGS-004, plus Vorinostat on persistent HIV-1 infection (Gay et al., *Sci Rep*, 2020); tested the safety and efficacy of Bortezomib in AIDS-associated Kaposi sarcoma (clinical trial AMC-063) (Reid et al. *Clin Cancer Res*, 2020); and evaluated white matter abnormalities

linked to interferon, stress response, and energy metabolism gene expression changes in older HIV-positive patients on ART (Solomon et al., *Mol Neurobiol*, 2020). HMMC also applied a novel droplet digital PCR assay for HIV proviral integrity to study individuals on long-term cART (Anderson et al., *Viruses*, 2020). The sequencing and virus evolution expertise of HMMC supported numerous collaborative studies on topics including: preexposure prophylaxis in humanized mice with long-acting Rilpivirine (Melody et al., *J Virol*, 2020); *in vivo* proliferation of T cells infected with HIV without expressing integrated proviral gene products (Musick et al., *Front Microbiol*, 2019); determining the principles governing the establishment versus collapse of HIV-1 cellular spread (Hataye et al., *Cell Host Microbe*, 2019); analysis of the combination of HIV-1 sequence and integration site provides details regarding viral dynamics and allows reconstruction of replicating viral ancestors (Patro et al., *Proc Natl Acad Sci USA*, 2019); and analysis to predict the susceptibility of HIV reservoirs to broadly neutralizing antibodies (Yu et al., *JCI Insight*, 2019). In the coming year, HMMC will be participating in studies of HIV-positive individuals co-infected with SARS-CoV-2.

Viral Evolution Core

The Viral Evolution Core provides specialized molecular cloning, sequencing, and viral evolution analysis expertise to support ACVP and collaborating investigators conducting a broad range of basic and applied studies in HIV/AIDS research, emphasizing work in NHP models.

KEY ACCOMPLISHMENTS

The Viral Evolution Core contributed to 44 different projects resulting in 16 high-impact publications, including three documenting the lack of impact of anti-α4β7 antibody on SIV viremia in rhesus macaques (Iwamoto et al., *Science*, 2019; Di Mascio et al., *Science*, 2019; Abbink et al., *Science*, 2019). To assess AIDS virus genomic integrity, a key question in studies of viral persistence and "HIV cure" research, VEC collaborated with HMMC and RPS to develop a novel multi-amplicon quantitative PCR assay combined with full-length viral sequencing (Long et al., *J Virol*, 2019).

The Viral Evolution Core also developed multiple novel next-generation sequencing–based assays to query specific regions of the viral genome to identify the presence of drug and immune escape mutations in a viral population. These assays are particularly important, as they provide essential insight into viral evolutionary dynamics in response to drug and immune pressure (Immonen et al., *Proc Natl Acad Sci USA*, 2020), a topic of particular importance to inform strategies for preventing viral recrudescence in HIV-suppressed persons. VEC also supported numerous collaborative projects involving key research questions in NHP models.

Tissue Analysis Core

TAC provides state-of-the-art tissue analysis capabilities including immunofluorescence, single- and double-label immunohistochemistry, *in situ* hybridization single-plex and multi-plex, quantitative image analysis, and laser capture microdissection for ACVP and collaborating investigators.

KEY ACCOMPLISHMENTS

TAC provided key contributions to seven publications, with three additional manuscripts submitted and two in preparation. Highlights include analysis of lymph node tissues from HIV-1 "elite controllers" to characterize cells harboring viral RNA and vDNA and characterize cytotoxicity-associated proteins in CD8+ T cells (Nguyen et al., *Sci Transl Med*, 2019) and immunohistochemistry and *in situ* hybridization in SIV-infected macaques treated with fingolimod (FTY720), demonstrating lymph node retention and reduction of SIV DNA *(*Pino et al., *Plos Pathog*, 2019*)*. Currently, TAC is working with Drs. Andrea Lisco and Irini Sereti (NIAID) on the development and application of RNAScope, DNAScope, and immunofluorescent/immunohistochemistry approaches for detecting SARS-CoV-2 nucleic acids and proteins and other COVID-19-relevant molecules in tissues from infected individuals. These methods will be used to localize and identify the cells harboring vRNA and/or vDNA, assess the different immune cell populations, and look at cytokine expression in lungs and other tissues in order to better understand the pathogenesis of SARS-CoV-2 infection and COVID-19 (see photomicrograph).

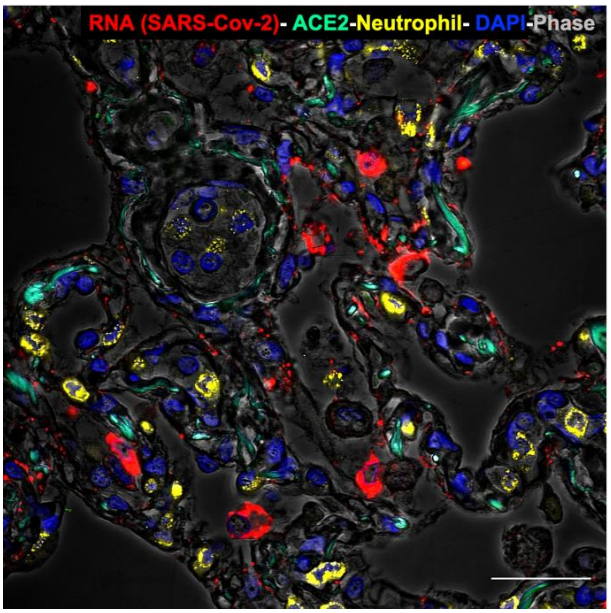


Figure 1. Representative fluorescence photomicrograph of lung tissue from COVID-19 patient illustrates visualization of infected cells by RNAscope technique targeting SARS-CoV-2 viral RNA (red) and one of the receptors for the virus, ACE2 (green). Neutrophils (yellow) were identified with anti-myeloperoxidase antibody. Nuclei are in blue (DAPI) against the phase contrast background (grey). Scale bar=100μm.

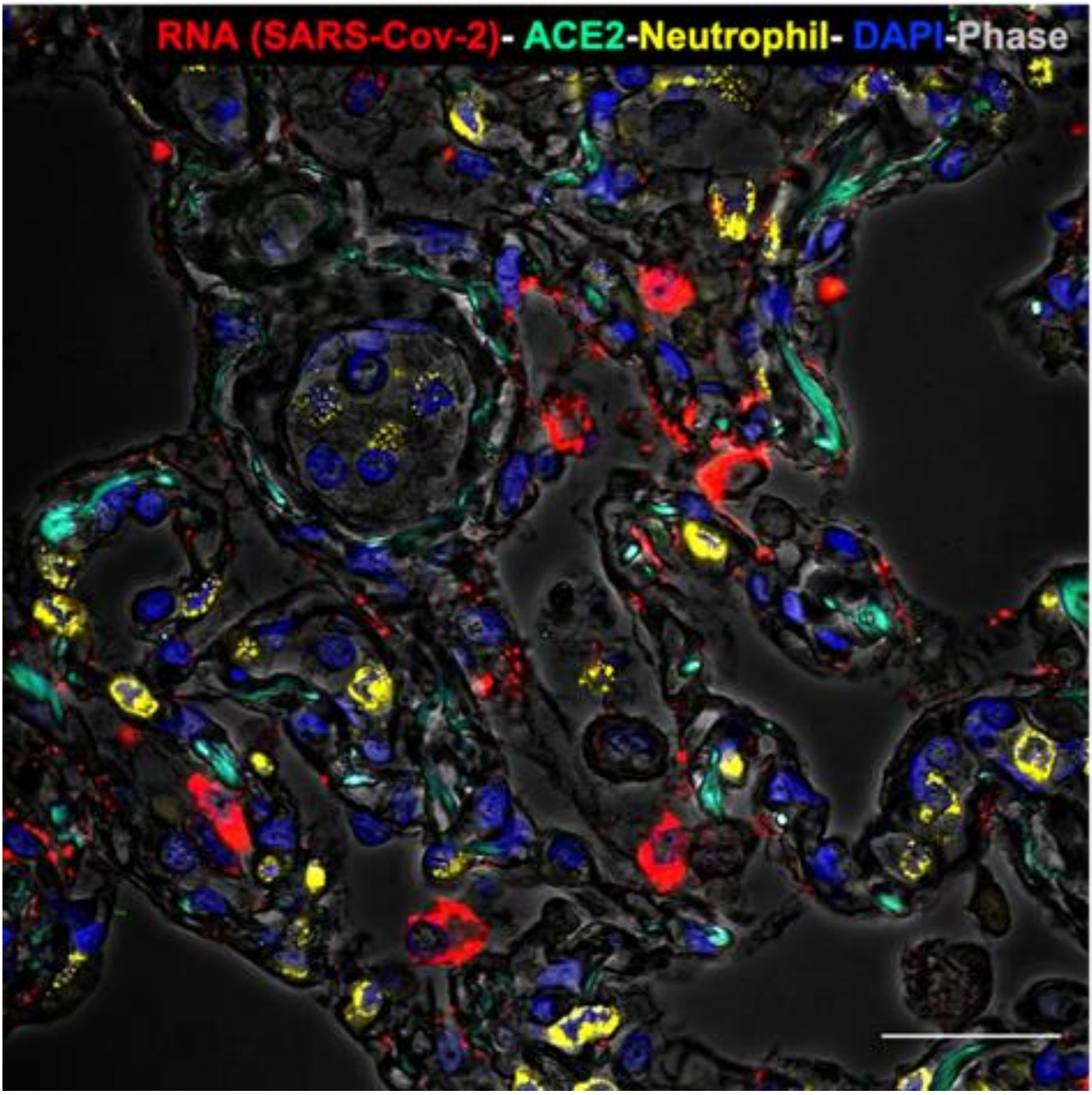


Figure 1. Representative fluorescence photomicrograph of lung tissue from COVID-19 patient illustrates visualization of infected cells by RNAscope technique targeting SARS-CoV-2 viral RNA (red) and one of the receptors for the virus, ACE2 (green). Neutrophils (yellow) were identified with anti-myeloperoxidase antibody. Nuclei are in blue (DAPI) against the phase contrast background (grey). Scale bar=100μm.

Support Provided by the Biomedical Informatics and Data Science Directorate

Bioinformatics

KEY ACCOMPLISHMENTS

- Provided statistical power analysis for the SARS-CoV-2 National Institutes of Health (NIH) "All of Us" study
- Provided structural and molecular modeling studies for hydrogel-mediated protein delivery and anti-CD19 chimeric antigen receptor (CAR) studies
- Helped design improved small-molecule imaging agents active in the biocompatible far-red and near-infrared range
- Developed artificial intelligence (AI)-based digital pathology solutions for the HIV/simian immunodeficiency virus (SIV) tissue microenvironment, rhabdomyosarcoma, liver tumor segmentation, and multi-organ nuclei segmentation
- Developed a workflow, Proviral Sequence Annotation & Intactness Test, to determine the intactness of the proviruses, which was subsequently incorporated into the database psd.cancer.gov
- Developed the Analytics and Visualization through interactive Data Dashboards and the Collaborative Data Sharing Platform to allow easy data analytics, visualization, integration, sharing, and management within scientific collaborations
- Primary operational accomplishments:
- Oversaw commencement and operations of the task order, including identifying key operational efficiencies for required approvals and tracking of contracting officer's representative concurrence requests
- Successfully filled an open position for program and administrative operations director to oversee and direct operations despite the COVID-19 response
- Managed the transition to telework status as required for the COVID-19 response

The Biomedical Informatics and Data Science (BIDS) Directorate Program and Administration Office provides program management, project management, and operational support for the BIDS Directorate.

NIH – Statistics COVID-19 Activities

FNL staff provided a statistical power analysis for the NIH "All of Us" study to determine the number of samples that need to be taken from different populations and geographical areas for SARS-CoV-2 prevalence estimation and serological validation.

Structural Biology

Hydrogel-Mediated Protein Delivery

FNL staff performed statistical analysis modeling and molecular dynamics simulations that helped correlate the experimentally observed properties of the hydrogels. Molecular dynamics studies provided a molecular-level understanding of the mechanisms that govern release and identified optimal binding zones on the gel fibrils that facilitate strong interaction domain–material interactions, which are crucial for sustained release of protein. FNL staff also used the experimentally observed release rates and an assumed first-order kinetics model to calculate the activation barrier to release, which was found to agree qualitatively with the molecular dynamics results.

For one of FNL's prominent publications on this subject, please see Miller et al., *ACS Cent Sci,* 2019, in Appendix B.

Anti-CD19 Chimeric Antigen Receptor

Anti-CD19 CAR-expressing T cells are an effective treatment for B-cell lymphoma but often cause neurologic toxicity. FNL's molecular dynamics simulations contributed to the interpretation of these CARs' experimentally observed properties. Our simulations suggest Hu19-CD828Z has remarkable differences when compared to FMC63-28Z. FMC63-28Z had a murine scFv hinge and transmembrane; costimulatory domains from CD28; and a CD3ζ activation domain. The molecular investigation of the CAR properties may help design more potent, less toxic treatments.

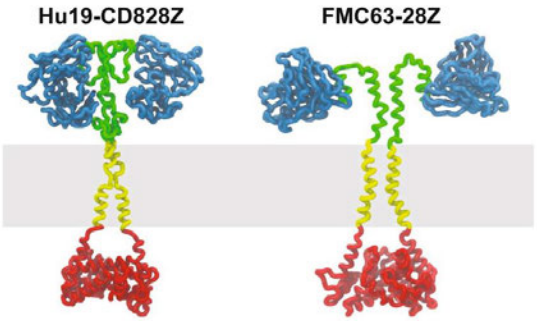


Figure 1. Models of Hu19-CD828Z and FMC63-28Z compared. Blue scFv; green hinge; yellow transmembrane domain; red intracellular domain.

Electron Microscopy Automation

In support of the Center for Molecular Microscopy/Electron Microscopy Laboratory, FNL analysts installed and maintained electron microscopy software packages and pipelines such as Leginon to automate the acquisition of negative-stain images in the FEI T12 microscope at the National Cancer Institute (NCI) at Frederick. The FNL staff modified this platform to facilitate interfacing with machine learning tools for the

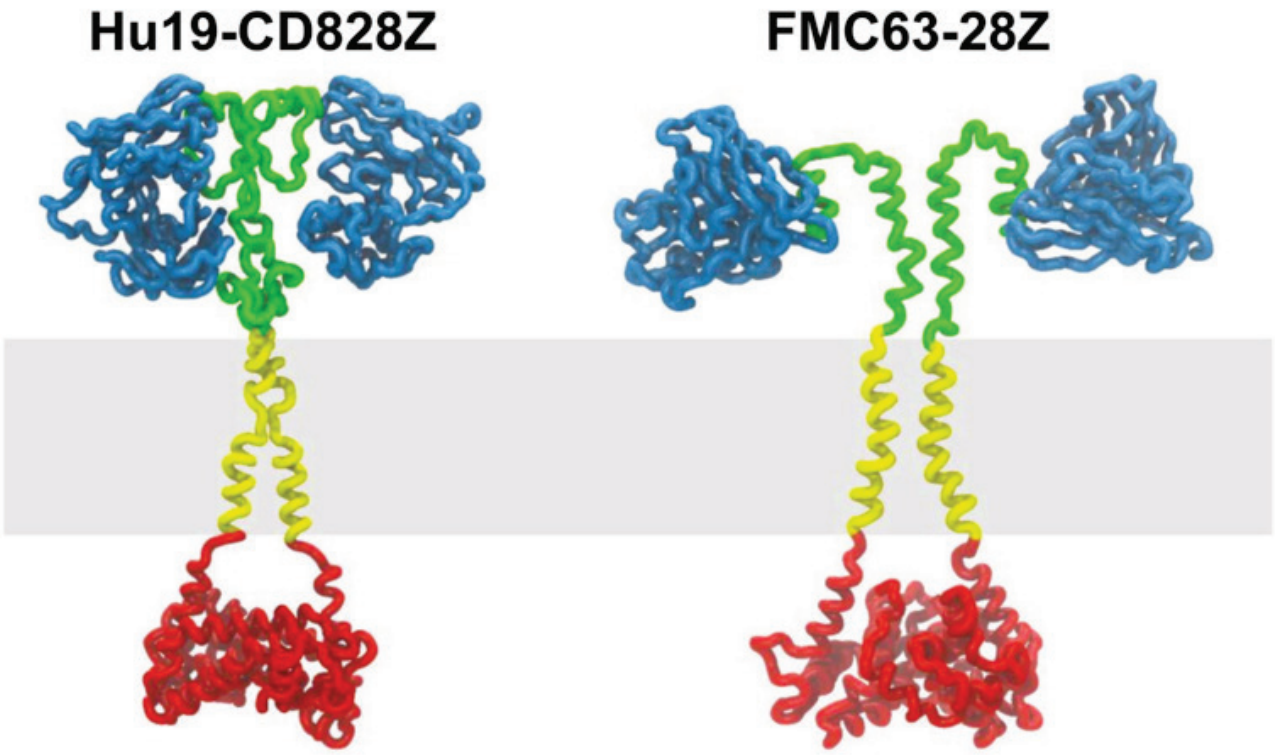


Figure 1. Models of Hu19-CD828Z and FMC63-28Z compared. Blue: scFv; green: hinge; yellow: transmembrane domain; red: intracellular domain.

rapid identification of targets in electron microcopy grids and to automate multimodal data acquisition (i.e., diffraction data concurrently with image data acquisition).

Computational Chemistry

Design of Improved Small-Molecule Imaging Agents Active in the Biocompatible Far-Red and Near-Infrared Range

Computational chemistry was used to analyze known coumarin-based fluorophores and predicted that a completely new redesign via the incorporation of a CF_2 group would result in a dramatic absorption/emission red-shift.

These results led to the preparation of a series of fluoro-coumarin dyes that are optically active in the far-red and near-infrared regions. Furthermore, as predicted, they show a 40-fold increase in emission brightness. Further work led to a plasma-membrane-binding variant that showed significant success in imaging live-cell walls or boundaries.

For one of FNL's prominent publications on this subject, please see Matikonda et al., *Chem Sci*, 2020, in Appendix B).

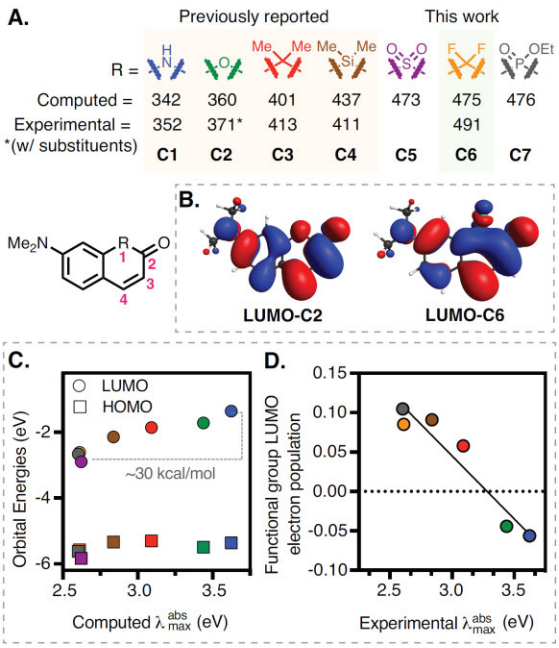


Figure 2. Computational data for modifications of the coumarin scaffold showing (A) the CF_2 (C6) variant is predicted to have a longer absorption wavelength than those previously reported; (B) the excitation orbital (LUMO) of C6 extends further onto the R group (top-right blue region); (C) excitation orbital (LUMO) energies correspond with optical properties; (D) the R-group electron "pull" explains why some systems absorb in the near-infrared region.

Image Analysis and Machine Learning/AI

AI-Based Digital Pathology Solutions for the HIV/SIV Tissue Microenvironment

FNL staff in the Imaging and Visualization Group developed a deep-learning-based workflow for CD4+ T-cell estimation on immunohistochemistry-stained tissue scans in collaboration with the AIDS and Cancer Virus Program Tissue Analysis Core. This new workflow, in conjunction with the HIV/SIV virion detection and quantification workflow previously developed by the Imaging and Visualization Group, enabled image-based virus load quantification for HIV/SIV tissue micro-environment studies. The workflow produces nuclei segmentation for CD4+ T cells and estimates T-cell abundance based on mean and standard deviation of average T-cell nuclei areas.

Estimation of CD4+ T Cells

Image Name	CD4+ T Cell (Mean)	Low	High
17111005	117,242	89,963	168,260
17110987	294,643	226,090	422,860
17111423	685,481	525,993	983,775
17110990	262,853	201,696	377,236

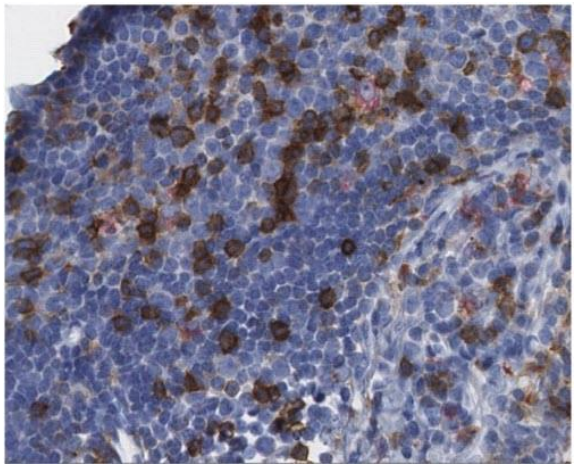
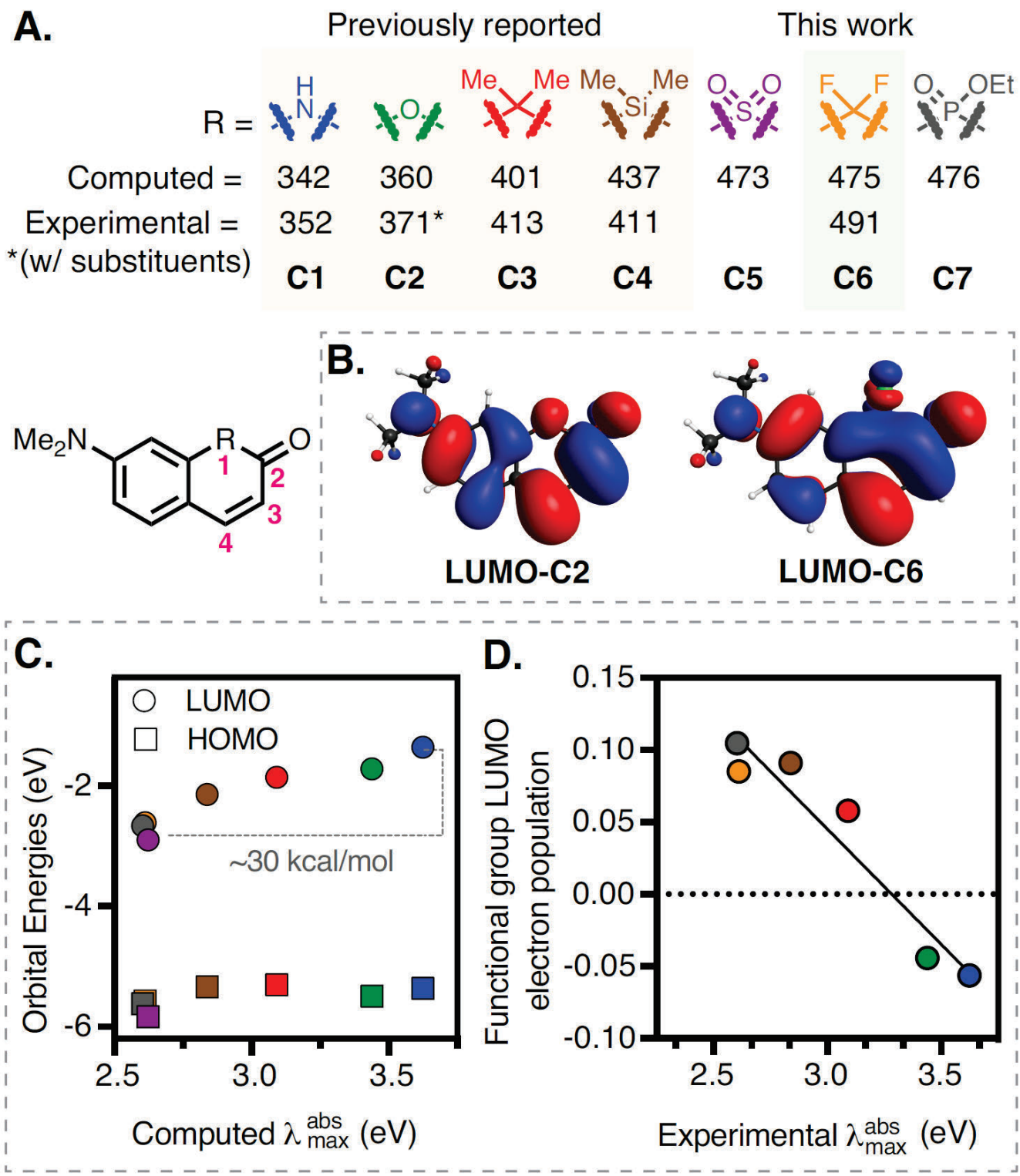


Figure 3. An example region of interest of a stained tissue section (Tissue Analysis Core, AIDS and Cancer Virus Program).



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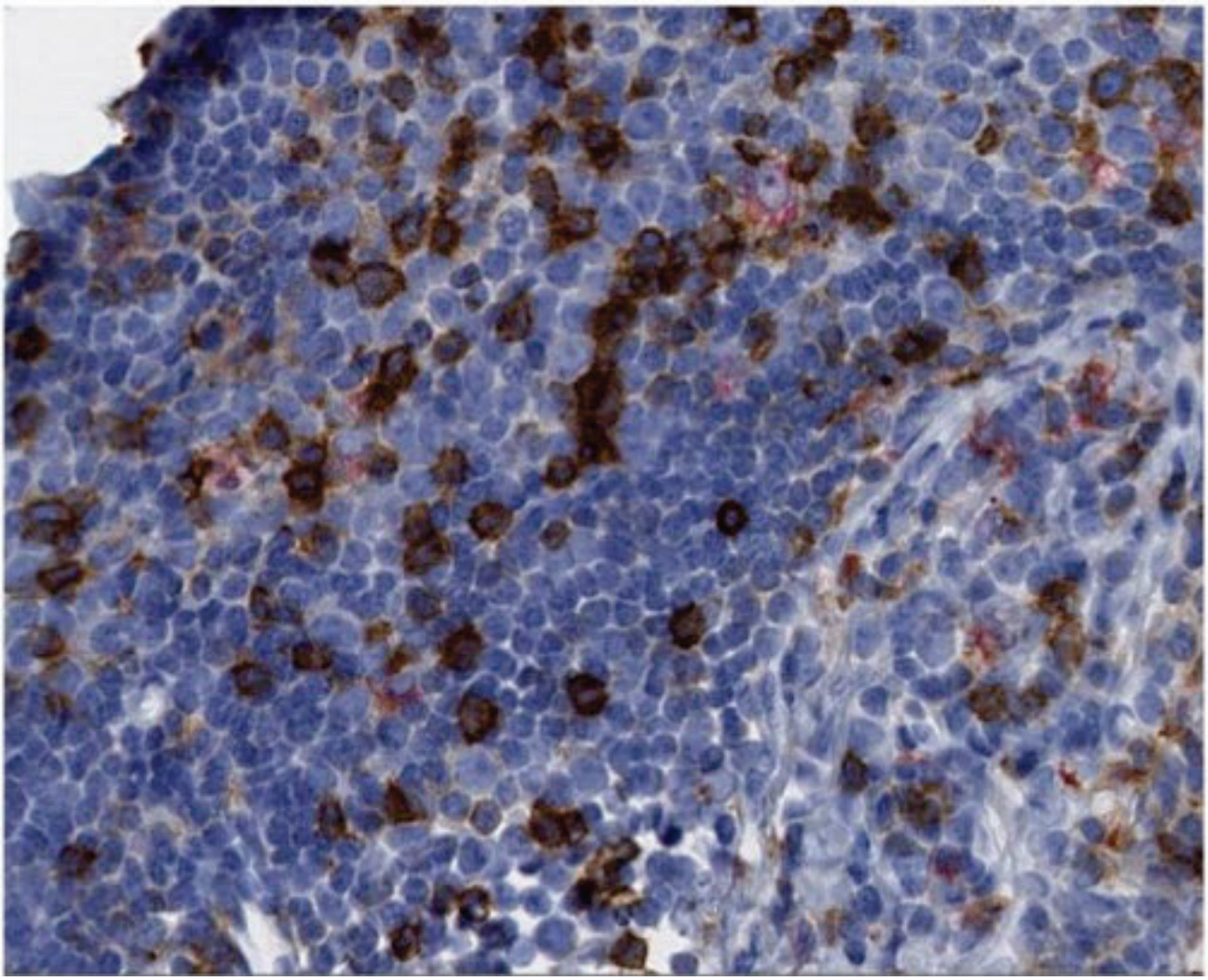

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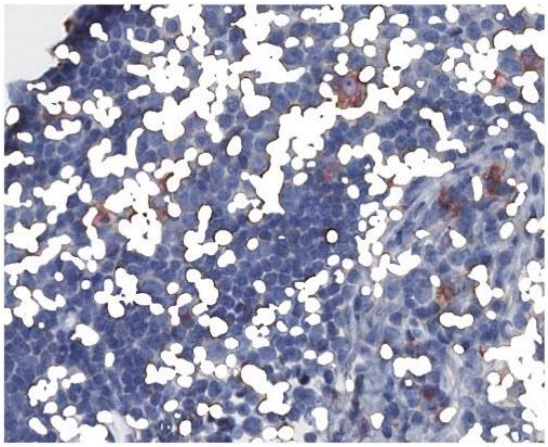

Figure 4. CD4+ T cells detected (white pixel labels) via AI models.

Benchmarking Study of Deep Neural Network Performance on Low-Magnification Pathology Region-of-Interest Images

The NCI Patient Derived Models Repository (PDMR) database hosts a catalog of low-magnification (4×) regions of interest (ROIs) of tissue histology images across a total of 60 cancer models, providing an ideal test case for evaluating deep neural networks' performance in real-life scenarios. Using five pre-trained models, we have benchmarked the NCI PDMR database ROIs on a selected set of popular deep neural network classifiers. Overall, on the binary carcinoma vs. sarcoma classification test, we have reached 89.57 percent accuracy on 4× ROIs using our downsizing models and 84.18 percent accuracy on 4× ROIs using our patch-based models. On the multi-class carcinoma classification test, we have reached 72.06 percent Top-2 accuracy on 4× ROIs using our downsizing models and 78.07 percent Top-2 accuracy on 4× ROIs using our patch-based models. Given that pathologist accuracies hover around 85 percent, our models were comparable in performance.

HIV Bioinformatics Analysis

HIV Treatment Failure Linked to Dual-Class Resistance Mutations and Replicating Viral Ancestors Deduced from HIV-1 Sequence and Integration Site Analysis

A statistical analysis by the FNL staff showed that there was a significant increase in antiretroviral therapy (ART) failure in individuals with a linked dual-class drug-resistant mutation (DRM), while single-linked DRMs showed no significant change in failure rate. It was concluded that linked dual-class DRMs present before the initiation of ART are associated with ART failure, whereas linked single-class DRMs are not. FNL staff also developed a workflow, Proviral Sequence Annotation & Intactness Test, to determine the intactness of the proviruses, which was subsequently incorporated into the database psd.cancer.gov. This method was applied to lymph node and peripheral blood mononuclear cells from five ART-treated donors to determine whether groups of identical sub-genomic sequences in the two compartments are the result of infected cells' clonal expansion or a viral genetic bottleneck.

For two of FNL's prominent publications on this subject, please Boltz et al., *JCI Insight*, 2019, and Patro et al., *Proc Natl Acad Sci USA*, 2019, in Appendix B.

Data Analytics, Integration, Visualization, and Sharing

Analytics and Visualization Dashboard

FNL staff in BIDS developed the Analytics and Visualization through interactive Data (AViD) Dashboards to provide a mechanism for dynamic interaction with clinical and research data relevant to multiple groups, such as the Division of Cancer Treatment and Diagnosis, the Center for Cancer Research (CCR), and the National Institute of Allergy and Infectious Diseases. The AViD Dashboard is being implemented for COVID-19 studies in addition to cancer and rare disease projects.

Collaborative Data Sharing Platform

The Collaborative Data Sharing Platform (CDSP) allows easy data and file management within scientific collaborations. Features include convenient uploading, browsing, file previews and downloading. Managing files in scientific collaborations spanning multiple groups and aspects of research data collection often becomes messy because of complexities associated with the number, types, and versions of files. CDSP incorporates data-aware features for custom fine-grained file-, assay-, and project-level access, and it automates and audits file-, user-, and role-management features, allowing seamless sharing. The module was initially deployed to facilitate data management in complex systems biology collaborations for the U.S. Army Medical Research and Materiel Command. This year, interest and needs in multiple research projects, including the NCI My Pediatric and Adult Rare Tumor Network, liver cancer research, and the National Institute of Allergy and Infectious Diseases' COVID-19 effort, have prioritized the customization of the platform to be deployed as independent instances.

Operational Software and Scientific Application Support

FNL staff serve as a software and database hub, thereby maximizing resources and reducing duplication of effort. The group currently maintains hundreds of Perl/Python/R modules and close to 400 bioinformatics software programs. In the past year, FNL staff migrated software programs to the Slurm cluster and installed new software, such as Longshot, AnnotSV, Minipolish, FREEC, TFEA, CRISPResso2, BUSCO, Canu, GMap, RAxML, SQANTI2, ANGEL, and many new

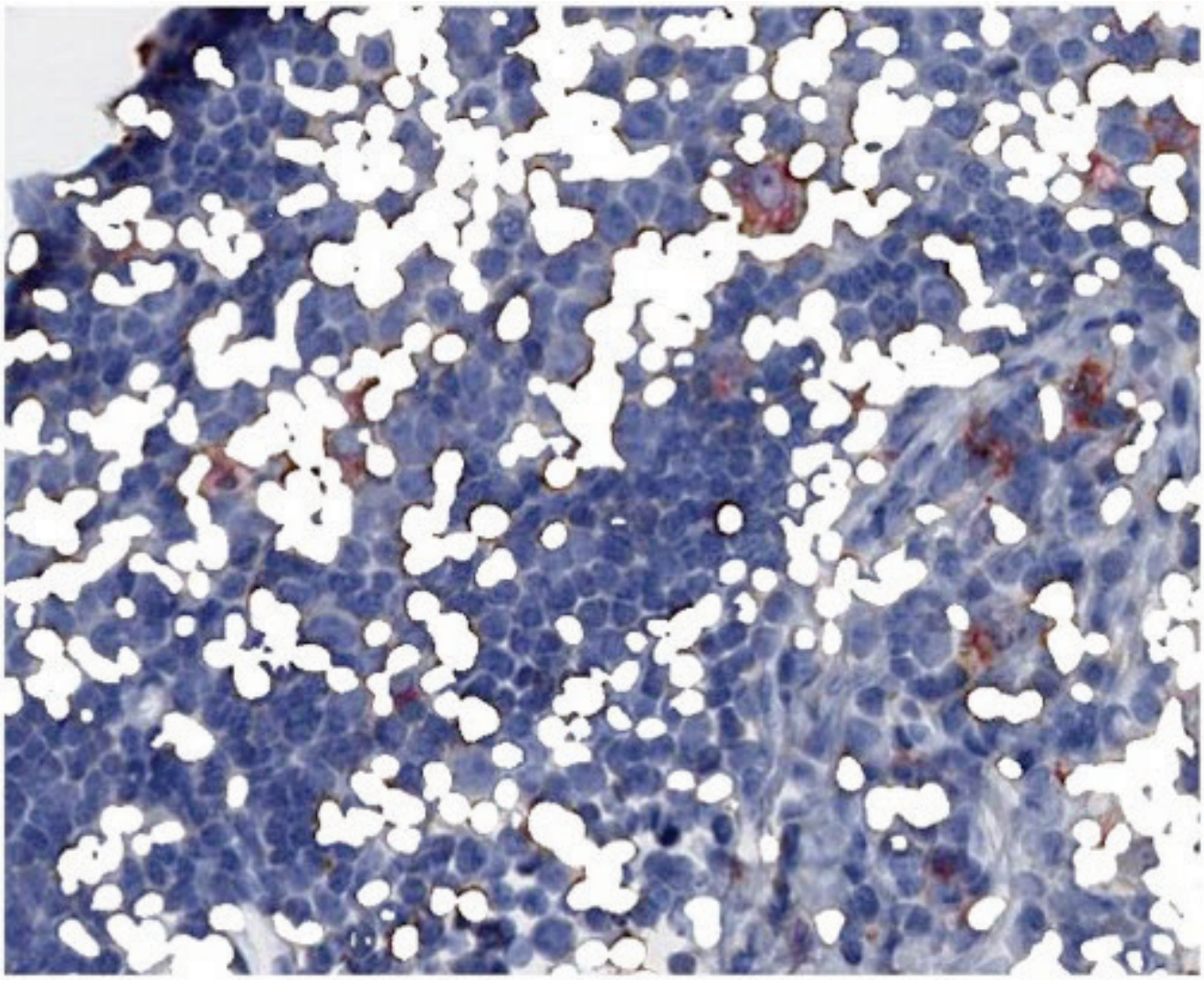

Figure 4. CD4+ T cells detected (white pixel labels) via AI models.

Perl/Python/R modules. FNL staff continue to maintain a data warehouse with more than 100 bioinformatics databases providing coverage on entities such as genes, miRNAs, proteins, drugs, diseases, pathways, species, and variant annotations. Almost all the databases are automatically updated through complex downloads, parsing, and validation scripts. In addition, the FNL staff has developed and continues to maintain the web interfaces providing automated information and details on all the available software (https://appdb.ncifcrf.gov) and databases (https://bioinfo-abcc ncifcrf.gov). This year, FNL staff incorporated several improvements into the appdb interface so the application can be used to document and detail the software installed by FNL staff in both the Advanced Biomedical Computational Science group and the Enterprise Information Technology Directorate, thereby providing documentation for all software installed on the Frederick compute cluster.

Tumor IsomiR Encyclopedia

FNL staff scientists and developers worked with the CCR RNA Biology Laboratory to develop The Tumor IsomiR Encyclopedia (https://isomir.ccr.cancer.gov/), a dynamic tool that allows researchers to query and compare isomiR expression across more than 11,000 adult and pediatric tumor samples from The Cancer Genome Atlas and the Therapeutically Applicable Research to Generate Effective Treatment data sets. The isoMIR website uses unique motifs and searches through more than 17 billion miRNA sequences and counts from The Cancer Genome Atlas obtained from 10,431 samples across 34 different cancer types. The obtained sequences are then filtered based on their similarity to the consensus sequence. This filtered list is then displayed by cancer type and by sample within each cancer type.

cCRADA with the Henry M. Jackson Foundation on Behalf of the U.S. Navy

Validation of Transcriptomics Assays from Samples Acquired from Whatman FTA Cards

In an effort to characterize gastrointestinal microbiome variability due to disruption associated with travel and/or antibiotic exposure, the Navy is planning to collect longitudinal samples on Whatman FTA filter paper cards. A validation study of these cards was conducted using frozen stool samples and with varying storage protocols. In a preliminary analysis of the data, FNL staff observed that the relative abundance of *Bacteroidetes* decreases to less than half the abundance during the first 24 hours after preparation of the Whatman cards.

Data Management Services Statistical Support

The Data Management Services Statistical Support group completed 14 projects, with seven additional ongoing projects for nine laboratories/groups/programs:

- RNA Biology Laboratory, CCR, NCI
- Center for Advanced Preclinical Research and Mouse Cancer Genetics Program, CCR, NCI
- Immunology Section, Nanotechnology Characterization Laboratory, FNL
- Rare Tumor Initiative, CCR, NCI
- HIV Dynamics and Replication Program, CCR, NCI
- Operations and Maintenance, Facilities Maintenance and Engineering Directorate, FNL
- Enterprise Information Technology Directorate, FNL
- Natural Products Branch, Division of Cancer Treatment and Diagnosis, NCI
- Laboratory of Cell and Developmental Signaling, CCR, NCI

The group also supported 21 applications for nine groups/programs:

- Mouse Cancer Genetics Program
- Retroviral Replication Laboratory
- Laboratory of Cell and Developmental Signaling
- Center for Advanced Preclinical Research
- Laboratory Animal Sciences Program
- Biological Testing Branch, Developmental Therapeutics Program
- NCI Mouse Repository
- Laboratory Information Management System, High-Throughput Animal Genotyping Laboratory
- Receiving and Quarantine Facility

The group offered the following services:

- Statistical consultation: Statistical consulting provides analytical/scientific investigative support for NCI at Frederick and the Frederick National Laboratory.
    - Analytical services: Statistical, mathematical, and computer science approaches are leveraged, along with available data repositories, as requested, to analyze scientific and other relevant data.
    - Experimental design/process consultation: Statisticians work collaboratively with scientists upon request to design analytical projects, interpret results, and provide information needed to plan follow-on investigation.
    - Manuscript, grant, reporting, etc. contributions: Upon request, statisticians provide tables, figures, and legends and contribute to writing the methods, results, discussion, and other relevant document sections.
- Scientific programming: The team develops and supports scientific-information-handling applications to meet client needs.

LASP Animal Colony Management System

CCR Support

KEY ACCOMPLISHMENTS

- Deployed the production-version Laboratory Animal Sciences Program Animal Care and Use Committee system that principal investigators across NCI are using to submit and manage Animal Study Proposals

FNL staff in the Integrated Project Team configured, tested, and deployed a new Animal Care and Use Committee system that replaced a previous approach. This system significantly reduces time required for renewals and allows digital tracking of activity related to Animal Study Proposals. Training, documentation, and support were provided for all principal investigators to ensure a smooth transition from the old system.

The objective of this work is to acquire, enhance, integrate, and implement a commercial off-the-shelf software system for use by FNL and NCI personnel to facilitate Institutional Animal Care and Use Committee activities and the tracking and maintenance of animal inventory, breeding, and health status.

Support Provided by the Partnership Development Office

Laboratory Directed Exploratory Research

The Laboratory Directed Exploratory Research (LDER) Program provides funding for investigator-driven projects that are outside the scope of current work under the Federally Funded Research and Development Center contract. The LDER Program was initiated to promote the conduct of novel research activities to enhance the innovation, creativity, originality, and quality of the research activities of the FNL; facilitate collaborations within the directorates of the FNL; engage universities and other external partners to encourage collaboration and strategic interactions; and enable the demonstration of exploratory proof-of-concept projects that could lead to follow-on funding through contract or grant mechanisms.

The Partnership Development Office (PDO) manages the LDER Program in coordination with Dr. Ligia Pinto, who serves as the LDER Scientific Lead. Together, they handle program promotion; manage proposal solicitation, review, and selection; monitor project progress; and report results. They also assist in generating high-quality proposals by holding information sessions, organizing a LDER-specific proposal-writing workshop, and creating a team of mentors to assist interested investigators in proposal development.

Support to the **National Institute of Allergy and Infectious Diseases**

Frederick National Laboratory
for Cancer Research

sponsored by the National Cancer Institute

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Division of Clinical Research

Support Provided by the Applied and Developmental Research Directorate

Clinical Research Support

AIDS Monitoring Laboratory

KEY ACCOMPLISHMENTS

- The AIDS Monitoring Laboratory (AML) Clinical Flow Cytometry Group successfully validated and brought online two new Sysmex XNL-350 hematology analyzers that were needed to perform complete blood counts (CBCs) with white blood cell differential on peripheral blood specimens. AML also studied the effects of blood storage time on hematological results because studies have documented that a variety of analytical factors, such as time from blood draw to analysis, can lead to variability in hematologic measurements. AML determined that automated CBCs with differentials performed on the XNL-350 are stable when using EDTA whole blood stored up to 30 hours post blood draw.
- The AML Functional Immunology Group performed numerous ELISAs and electrochemiluminescent multiplex assays to measure a wide range of biomarkers in support of several National Institute of Allergy and Infectious Diseases (NIAID) clinical trials and research projects. 402 biospecimens were assayed, which resulted in 3,690 biomarker measurements.
- The AML Research Flow Cytometry Group provides research flow cytometry support to Drs. Anthony Fauci and Clifford Lane, and the laboratory performed numerous immunophenotypic analyses by flow cytometry including: 39 high-speed cell sorting procedures, 108 12-color assay, 108 11-color assays, 123 nine-color assays, 340 eight-color assays, 16 six-color assays, 13 five-color assays, 5 four-color assays, 654 three-color assays, 524 two-color assays, and 100 single-color assays.
- AML continued to serve as the central biospecimen repository for several domestic and international HIV, influenza, and Ebola-Zaire vaccine clinical trials conducted by NIAID and the International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) and coordinated the shipment of laboratory supplies/packaging to clinical sites as well as the receipt, inventory, and storage of approximately 7,991 biospecimens (sera, plasma, oropharyngeal swabs, nasopharyngeal swabs, and lower respiratory tract specimens). AML coordinated the requisition and shipment of over 2,400 biospecimens to domestic and international collaborators for six new research proposals and protocol-mandated immunogenicity testing. AML represented the Frederick National Laboratory for Cancer Research (FNL) and provided logistical, repository, and protocol support on the INSIGHT Laboratory Procedures Group, FLU/IVIG Protocol Team Calls, and the PREPARE Laboratory and Merck Protocol teleconferences.
- AML continued to serve as the Technical Project Manager for a subcontract with Advanced BioMedical Laboratories, LLC (ABML) for the storage of approximately 330,000 legacy biospecimens collected from various INSIGHT studies such as ESPRIT, SMART, STALWART, and CPCRA IL-2 clinical trials. The Technical Project Manager reviewed and approved monthly invoice statements and progress reports provided by ABML and assisted with the procurement and delivery of a new -80° C freezer to ABML to replace an existing freezer that failed.
- During the COVID-19 Pandemic, Dr. Clifford Lane, Clinical Director, NIAID, requested AML to rapidly facilitate the testing of numerous blood specimens collected in support of a project between NIAID and the White House Medical Unit, Executive Office of the President. AML staff collaborated with the White House Medical Unit and swiftly developed a plan to receive, inventory and process the specimens. AML processed 964 blood specimens and coordinated the transfer of the specimens to Dr. Michael Holbrook, NIAID Integrated Research Facility, for COVID-19 testing.
- AML rapidly leveraged its resources during the COVID-19 pandemic to provide immunological monitoring for two new COVID-19 clinical trials. AML provided biospecimen processing and performed six-color and eight-color immunophenotyping on participants enrolled in the CALYPSO trial, a study about how SARS-CoV-2 affects the immune system and the RECON-19 trial, a study about long-term medical problems experienced by people who recover from COVID-19 and whether people who recover develop long-term immunity.

Virus Isolation and Serology Laboratory

KEY ACCOMPLISHMENTS

- Significant achievements this year include additions to the laboratory's Next-Generation Sequencing and serology capabilities and evaluation of COVID-19 and HIV-1 assays and technology. In the midst of the COVID-19 pandemic, the lab remained open to support the clinical trials being conducted at NIAID. That alone was an achievement, as guidelines recommended to prevent infection were adopted and work schedules were staggered to ensure the timely

delivery of results to NIH. In addition to regular samples, the lab investigated and acquired multiple SARS-CoV-2 serology assays in preparation to support trials in development and initiated a collaboration with the Palo Alto Veterans Administration and NIAID's Integrated Research Facility to test SARS-CoV-2–positive samples. We also evaluated the Abbott reverse-transcription polymerase chain reaction assay for SARS-CoV-2 detection and completed successful proficiency testing on that platform.

- Before the pandemic, the lab was evaluating different platforms to replace the aging Abbott viral load system. Once the Hologic Panther was settled upon, it was a challenge to arrange delivery and set up and validate the platform under COVID-19 restrictions, but they were accomplished, and a validation was completed that allows the platform to be used in our lab. This new platform also has an Emergency Use Authorization for COVID-19 testing and will allow continuous sample processing with SARS-CoV-2 samples as well as the usual load of HIV specimens.
- Another work in progress has been the development of the luciferase immunoprecipitation system (LIPS) assay. This liquid-phase immunoassay quantitates antigen-specific serum antibodies by measuring luminescence emitted by the reporter enzyme *Renilla* luciferase (Ruc) fused to an antigen of interest. The LIPS assay can be used as a high-throughput and sensitive serological method for profiling serum antibodies recognizing diverse antigens. It has been adapted to detect antibodies to SARS-CoV-2 as well as to HIV.
- Finally, strides were made in the creation of armored RNA (protected RNA enclosed in bacteriophage shell), which has been useful in viral load testing as well as next-generation sequencing (NGS). That and the development of 16S ribosomal NGS, which may serve as a robust one-step tool for microbiological identification and characterization of a wide range of clinically relevant bacteria in clinical samples, has broadened the lab's menu of offerings to support the important work at NIH.

Laboratory of Molecular Cell Biology

KEY ACCOMPLISHMENTS

- The Laboratory of Molecular Cell Biology (LMCB) monitors samples from three different gene therapy studies for the presence of modified markers or therapeutic genes introduced into patients. The following samples were monitored and quantitated for the gene-modified cells by polymerase chain reaction (PCR): 15 samples for the chimeric T-cell receptor (*CD4/CD3-ζ*) gene and six samples for antisense HIV transactivation response element (TAR) and/or transdominant HIV Rev. For many years, patients enrolled in the Neo/Nip protocol were monitored using a radioactive material–based assay. A new PCR assay based on fluorescent IR-Dye has been optimized to replace the radioactive material–based assay.
- Based on the analysis of autopsy samples, LMCB concluded that HIV persists in tissue samples of patients with undetectable viremia. Near-full-length HIV DNA was amplified by PCR of DNA isolated from blood, lymph nodes, spleen, intestine, brain, lung, heart, kidney, liver pancreas, and testes of eight donor samples. Single-genome PCR amplification and sequencing of HIV proviral DNA and analysis of more than 1,300 sequences showed that most of the viral DNA (greater than 95 percent) present in tissue samples were defective since they lacked different sections of the viral sequence. About 40 percent of the defective sequences were expanded clones. Most of the intact viral sequences were found in the lymph node. Based on these analyses, the laboratory concluded that there no tissue hot spots with concentrated viral DNA or RNA.
- Previous work has established that a 6.5 kb region located upstream of IL-2 receptor alpha (IL-Rα) gene transcription start site inhibits transcription from heterologous promoters. Further characterization of this region has narrowed it to about 2.4 kb. In order to validate the transcriptional regulatory activity of this region *in vivo*, CRISPR/Cas9 technology was used to selectively delete genomic regions under investigation in Jurkat cells. In order to achieve a large deletion in the genomic region of interest, a plasmid that permits simultaneous expression of two guide RNAs targeting two different loci on the genome was used. The Jurkat cells were transfected with a pDG458 plasmid expressing Cas9 enzyme and predefined two gRNAs to delete a predetermined sequence. Using the above approach, three different Jurkat mutant cell lines with varying sizes of deleted fragments (mutant A with approximately 1 kb deletion, B with approximately 1.5 kb deletion, and C with approximately 2.7 kb deletion) were created. There was no significant difference in the IL-Rα expression between wild-type and any of the mutant cell line. All the founder cell lines of mutant B and mutant C showed significantly decreased IL-2 secretion. To determine the functionality of this region, Jurkat cell extract was tested to identify protein factors that may bind to it. A 500 bp subfragment of this region specifically binds factors in the extract. Further characterization of this protein would provide insight into the regulation of the IL-Rα gene.
- Previous results from this laboratory have shown that GADD34, when overexpressed in HeLa and HEK293FT cells, inhibits HIV-1 replication by HIV-1 5' UTR- mediated translation (Ishaq et al., *Virology*, 2020). The laboratory found that HIV-1 UTR nucleotides 1–289 exhibited significant internal

ribosome entry site (IRES)-dependent translational activity. GADD34 expression had no effect on 1–289-driven IREs mediated-translation, indicating that GADD34 does not inhibit HIV-1 UTR-mediated translation by IRES-dependent mechanisms. A sensitive luminescence-based assay has been developed to test and quantitate GADD34-mediated loss of HIV-Tat protein expression.

- Several groups have shown the existence of complex interactions between long non-coding RNAs (lncRNAs) and HIV. LncRNAs from human cells play crucial roles in virus–host interactions and viral pathogenesis. To explore whether inhibition of HIV by Gaad34 involves LncRNAs, a CRISPR-mediated GADD34 knockout HeLa CD4+ cell line has been constructed to identify LncRNAs that are regulated by Gaad34 expression and use that knowledge to study the role of Gadd34 in the regulation of HIV-1 replication. The laboratory found that LncRNAs that were either up or downregulated in GAAD34 knockout Hela cells. Further studies may investigate whether any of these LncRNAs has a role in HIV.
- LMCB has shown that LZTFL1 is induced during T-cell activation and that it migrates to the contact site between T and B cells during immune synapse formation. To gain insight about the role of LZTFL1, a proteomic analysis based on liquid chromatography–tandem mass spectrometry was carried out to identify proteins that interact with LZTFL1. The β1 subunit of AP-1 and the clathrin heavy chain were among the proteins identified as LZTFL1-interacting proteins. LZTFL1 directly binds to AP-1 and AP-2 and coimmunoprecipitates AP-1 and AP-2 from cell lysates. DxxFxxLxxxR motif of LZTFL1 is essential for these bindings, suggesting LZTFL1 has roles in AP-1 and AP-2–mediated protein trafficking. Since AP-1 and AP-2 are known to be involved in transferrin receptor 1 (TfR1) trafficking, the effect of LZTFL1 on TfR1 recycling was analyzed. TfR1, AP-1, and LZTFL1 from cell lysates could be coimmunoprecipitated. However, pull-down results indicate there is no direct interaction between TfR1 and LZTFL1, suggesting that LZTFL1 interaction with TfR1 is indirect through AP-1. The colocalization of LZTFL1 and AP-1, AP-1 and TfR1, as well as LZTFL1 and TfR1 in the perinuclear region (PNR) and the cytoplasm suggested a potential complex between LZTFL1, AP-1, and TfR1. The results from the disruption of adaptin recruitment with brefeldin A treatment suggested ADP-ribosylation factor-dependent localization of LZFL1 and AP-1 in the PNR. Knockdown of AP-1 reduces the level of LZTFL1 in the PNR, suggesting that AP-1 plays a role in LZTFL1 trafficking. Knockout of LZTFL1 reduces the cell surface level and the rate of internalization of TfR1, leading to a decrease of transferrin uptake, efflux, and internalization. However, knockout of LZTFL1 did not affect the cell surface levels of epidermal growth factor receptor and cation-independent mannose 6-phosphate receptor, indicating that LZTFL1 specifically regulates the cell surface level of TfR1 (Promchan et al., *PLoS One*, 2020). These data support a novel role of LZTFL1 in regulating the cell surface TfR1 level by interacting with AP-1 and AP-2.
- The laboratory has also shown that T3 induces integrated stress response (ISR) signaling pathways and inhibits viral replication, suggesting a link between ISR and antiviral pathways. Results show that the T3 hormone induces interferon response and the expression of interferon-stimulated genes (ISGs), and this effect is significantly amplified in the presence of ds-RNA mimic Poly IC. Induction by T3 was due to non-genomic mechanisms involving integrin binding, calcium mobilization, and PI3K-AKT pathways but was independent of TLR3, RIG-I, and IFNβ1 pathways. Whereas siRNA-induced knockdown of protein kinase RNA-activated **(**PKR) was found to abrogate the T3-induced expression of select ISGs, expression of other T3-induced ISGs was strongly induced by PKR-knockdown, indicating the differential role of PKR in modulating T3 action. These results point to a novel role of T3 in modulating innate immune response and identified the importance of PKR in regulating T3-induced immune activation. These findings have important implications in the basic understanding of the mechanisms of T3 function at supraphysiological concentrations and crosstalk involved in the thyroid hormone function and the innate immune responses.

Laboratory of Human Retrovirology and Immunoinformatics

KEY ACCOMPLISHMENTS

- Accomplished genotyping of a total of 5,500 influenza virus-infected patients, 362 HIV genotype analyses, and 1,031 full-length (9.8 kbp) proviral DNA sequence analyses to support NIAID clinical research (Drs. H. Clifford Lane and Hiromi Imamichi). In addition, the laboratory completed a total of 173 T-cell receptor analyses from 53 HIV-infected patients to support the NIH Clinical center (Dr. Joseph Kovacs) and 71 samples of gene editing samples to support the National Institute of Neurological Disorders and Stroke (Dr. Avindra Nath).
- As related to innovation and creativity, the laboratory discovered that manganese ion (Mn^{2+}) enhances the DNA-repair protein mediated innate immune response and enhances efficiency of CRISPR-mediated gene editing. It also discovered 52 novel microRNAs in T cells and macrophages.
- The laboratory also updated the DAVID bioinformatics system and developed the Quasi-seq analysis pipeline using SgClustering of PacBio sequencing data and VIGILANT, a comprehensive

database to support HIV clinical diagnostic and scientific research for the Virus Isolation and Serology Laboratory.

- Finally, the laboratory started virtual screening of small compounds to identify potent SARS-CoV-2 RNA polymerase or main protease as a telework task.

Neutrophil Monitoring Laboratory

KEY ACCOMPLISHMENTS

- Investigated the use of Ella instrumentation as a "point of care" rapid turnaround cytokine determination. The technology demonstrates excellent intra- and inter-assay variability, linearity in the response, and ease of use.
- Continued to define new parameters that measure neutrophil chemotaxis. The angle of migration was used to demonstrate a chemotactic defect in patients with GNAI2 mutations.
- Developed multicolor fluorescence-activated cell sorting panel to determine the level of gp91phox expression concurrently in polymorphonuclear neutrophils, eosinophils, monocytes, and B cells.

Immunological Monitoring Laboratory

KEY ACCOMPLISHMENTS

- Continued measuring Lyme-specific antibodies in patients with Lyme disease. Over 2,400 determinations were made in the past year.
- At the request of Dr. Adriana Marques, the Immunological Monitoring Laboratory (IML) determined the level of free and bound Vitamin D in 480 sera from patients with Lyme disease.
- Compared droplet digital polymerase chain reaction (ddPCR) technology to quantitative real-time PCR (qRT-PCR) for the quantitative detection of Epstein-Barr Virus viral load in blood from humanized mice. These studies were developed in anticipation of a need in an upcoming human EBV vaccine trial by Dr. Jeff Cohen, who was surprised and grateful because of the increased sensitivity of the ddPCR. He plans to incorporate it into the monitoring of the vaccine trial.
- In March, the Neutrophil Monitoring Laboratory (NML)/IML, at the request of their NIAID investigators, pivoted from their normal clinical duties at Frederick National Laboratory. After a quick amendment to their Pathogen Registration and an expedited approval by the Institutional Biosafety Committee, the NML/IML began to receive COVID-19 samples from the NIH remdesivir trial as well as approximately 650 vials of sera/plasma from COVID-19 patients (many longitudinal samples) from the Lombardy region of Italy and from Washington state. Thus far, as part of the NIAID consortium response to COVID-19 (https://www niaid.nih.gov/research/immune-response-covid-19), NML has tested more than 1,100 samples using newly designed flow cytometry protocols and has determined that both neutrophils and monocytes from patients with COVID-19 have distinct activation signatures based on cell surface marker expression. To further understand the immunological processes involved in the cytokine storm observed in patients with COVID-19, NML/IML have analyzed COVID-19 plasma/serum longitudinally, performing over 45,000 analyses by multiplex biomarker (64 distinct analytes) testing. Many analytes reflect the severity of the disease – cytokines (IL-6, IFN-λ, IL-1Ra, IL-18, TNF-a, sTNF-RI and sTNF-RII, CXCL-9, CXCL-10), markers of endothelial activation and damage (VCAM-1, RAGE), markers of neutrophil activation (gelatinase, lactoferrin, and MPO), markers of platelet activation (thrombomodulin, u-plasminogen activator), and markers of coagulation (D-dimer). Results show that while many expected biomarkers (IL-6, IFN-λ, IL-10) are elevated, many novel biomarkers (IL-1RA, VCAM, ICAM, MPO, ST2, von Willebrand factor, and D-dimer) are also elevated, suggesting that many cells types and pathways can contribute to pathogenesis of COVID-19.

Laboratory Support to NIAID

KEY ACCOMPLISHMENTS

- The Clinical Support Laboratory (CSL) Clinical Trials Processing section supported the Laboratory of Parasitic Diseases by receiving samples from 12 clinical protocols, from 1,169 patient visits, resulting in 5,435 aliquots of clinical materials for return to NIAID investigators. On a subset of these samples, CSL performed 39 eosinophil isolations to prepare isolated sample vials.
- CSL provides dedicated support to Dr. Malcolm Martin, Laboratory of Molecular Microbiology, for processing blood samples obtained from macaques and other nonhuman primates involved in simian immunodeficiency virus (SIV) vaccine studies. The laboratory received approximately 715 blood samples for separation of mononuclear cells and plasma and generation of cell pellets for DNA extraction. Approximately 2,429 aliquots of mononuclear cells and 4,208 vials of plasma were produced for storage in the NCI at Frederick Central Repository or returned to the investigators as requested. The laboratory also prepared shipments of samples for return to Bethesda.
- In support of Dr. Michele Di Mascio, chief of the AIDS Imaging Research Section, CSL performed flow cytometry experiments using 200 blood samples processed from SIV-infected rhesus monkeys or from controls to evaluate T-lymphocyte subsets as part of a

whole-animal imaging study. Immunophenotypic analysis was performed using a five-color panel to evaluate changes in lymphocyte subsets, as well as proliferation marker and transcription factor expression. Plasma was also isolated and frozen from these samples and sent to [redacted] for SIV viral load determination. [redacted]

- Supporting [redacted], FNL staff within ADRD, specifically head of the NML, the CSL Cell-Mediated Immunity section, performed assays of [^{3}H]-Thymidine proliferation on a total of 56 patient donor samples for a total of 1,340 data points. In addition, the Cell-Mediated Immunity section continued its multiparameter flow cytometry proliferation assay that simultaneously evaluates four different immune cell types six days after they received cell-type-specific stimuli. [redacted], in close contact with NIAID's Dr. Suk See De Ravin, had CSL assess 17 patient samples in this assay, with generation of 1,180 data points.
- The Radiochemistry support laboratory continued support of the whole-body positron emission tomography (PET) imaging with Zr-89 labeled anti-CD4 antibody's fragments [Zr89-F(ab´)$_2$-CD4R1] in healthy and SIV-infected rhesus macaques. This is a continuing study to image lymphocytes, their subsets in tissues in real-time, and CD4+ cell recovery in SIV-infected rhesus macaques. PET is used to compare the imaging with SPECT by Tc99m radiolabeled Ab and to study antibody dose-effect primarily provides support to Dr. Michele Di Mascio, chief, AIDS Imaging Research Section. The following specific objectives of this study were met:
  - Fragmentation of the antibodies (CD4R1) to F(ab')2 and purification.
  - Conjugation with *p*-isothiocyanatobenzyl-desferioxamine (Df–Bz–NCS) to F(ab')2-CD4R1.
  - Radiolabeling with Zr89 to Df-F(ab')2-(CD4R1) and purification for doses for PET imaging.
  - Performing *in vivo* metabolites test and *in vitro* immunogenicity test by plasma-cell binding assay and high-performance liquid chromatography for more than 60 samples.
  - *Ex vivo* autoradiography in biopsied lymph nodes.
- The laboratory also continued support of anti-envelope antibodies studies and met the following objectives:
  - *In vivo* – conditioned and optimized methods of conjugation Df–Bz–NCS to 7D3 and radiolabeling with Zr89: Zr89-7D3 and produced the doses for PET imaging.
  - *In vitro* – several Abs were radiolabeled with Zr-89 and/or I-125 and performed infected cell binding assays.
  - *Ex vivo* – anti-env antibodies were radiolabeled with Zr-89 and performed binding assays and autoradiography with cells and tissues from lymph nodes and spleen of healthy and SIV-infected rhesus macaques.

Operational and Administrative Support

KEY ACCOMPLISHMENTS

- The Clinical Services Program Data Management Group (CSP DMG) added new modules to two existing programs, the AML 6 Color Lyric Flow Program and the AML 8 Color Lyric Flow Program, that provides enhanced data analysis selections and reporting capabilities. The Final Flow Selections module provides functionality that allows the AML Flow Group to analyze and select sample antibody data from nine different repeat and verification flow files. In addition, the 8 Color program allows 26 different 8 Color antibodies to be displayed per patient sample during the final selection process. The Edit Final File module provides the AML Flow Group with the capability to perform a final review of the flow data and flagged flow results that are non-reportable. These new modules allow a Flow Technician the capability to view all data in one location on a selection screen for verifying patient sample antibody data for final reporting. This reduces human error and reduces the number of man-hours needed for flow analysis and reporting.

Support Provided by the Clinical Research Directorate

Clinical Operations Support to the Intramural Clinical Management and Operations Branch

The National Institute of Allergy and Infectious Diseases (NIAID) Division of Clinical Research Intramural Clinical Management and Operations Branch facilitates efficient and effective clinical operations and research by providing assistance, guidance, resources, and issue resolution in support of the NIAID clinical research programs and the National Institutes of Health Clinical Center. The Frederick National Laboratory for Cancer Research staff in the Clinical Research Directorate provides clinical care professionals and clinical research support staff to meet the evolving requirements of the intramural clinical research programs that include human-subject Phase I–III studies, stored sample protocols, and natural history studies.

Support Provided by the Clinical Monitoring Research Program Directorate

Clinical Research Support

FNL staff members in the Clinical Monitoring Research Program (CMRPD) manage and conduct a broad range of clinical research, research support, and administrative support services in response to the scientific directions and priorities of NIAID Division of Clinical Research's (DCR) intramural and extramural programs. FNL supports NIAID's efforts to understand, treat, and ultimately prevent infectious, immunologic, allergic, and other emerging/re-emerging diseases threatening the health of people in the U.S. and around the world.

FNL staff members in CMRPD continued to refine a rapid-response toolkit, which is an internal resource for responding to urgent clinical research initiatives. Building on lessons learned from multiple emergency infectious disease outbreaks, such as the 2014 Ebola virus epidemic in West Africa and the 2018 outbreak in the Democratic Republic of the Congo, the team strengthened the toolkit by codifying FNL processes; collecting and banking tools; collecting job descriptions; identifying new sections for toolkit expansion (e.g., pharmacy and biostatistics); and reviewing and assessing the assumptions, constraints, and risks associated with implementing clinical research in a public health crisis.

Using these toolkit resources and leveraging lessons learned from the recent experiences supporting NIAID's Ebola clinical research in the Democratic Republic of the Congo, FNL was well positioned to rapidly respond to NIAID's efforts to address the global health threat posed by the COVID-19 pandemic. Staff members established early communication with NIAID and the NCI Management Operations Support Branch to determine the most efficient mechanisms for commencing emergency-response efforts, allowing FNL to initiate work and authorize vendors within days instead of weeks. These collaborations and well-defined rapid-response processes facilitated FNL's ability to support the rapid development/finalization of several protocols and quickly secure clinical research organization support, which allowed for rapid activation of multiple clinical sites and immediate participant enrollment.

Many of the FNL team members involved in the various rapid response initiatives also performed literature searches and contributed substantial content for a book currently being developed about the importance of clinical research as part of responding to emergency infectious disease outbreaks. Multiple collaborators, including NIAID staff, institute members, FNL staff, and representatives from a variety of organizations (e.g., global health policy experts, the World Health Organization, research and academic institutes, nonprofit organizations), have shared expertise and authored material for this book, which consists of nearly 40 chapters. FNL staff specifically co-authored sections on research-capacity-building, human resources, mobile research teams, site assessments, social mobilization/community engagement, and clinical research logistics and supplies. The book, intended to inform outbreak responders and emergency clinical research supporters, is undergoing editorial review.

FNL CMRPD's activities in support of specific DCR branches, offices, and special projects are described in the following sections.

Biostatistics Research Branch

KEY ACCOMPLISHMENTS

- Supported the Adaptive COVID-19 Treatment Trials (ACTT) by assisting with the statistical analysis plan and performing statistical analyses for data and safety monitoring board (DSMB) reports and the preliminary publication (Beigel JH, Tomashek KM, et al., *N Eng J Med*, 2020)
- Supported the AIDS Imaging Research Section (AIRS) with planning, installing, operating, and providing training on a novel positron emission tomography (PET) camera designed for use in monkeys
- Provided statistical, validation, data management, and data analysis support to the Pamoja Tulinde Maisha (PALM ["Together Save Lives"]) trial, including generating data tables and figures for the *N Eng J Med* publication (Mulangu S, Dodd LE, Davey Jr. RT, Tshiani Mbaya O, et al., PALM Consortium Study Team: "A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics," 2019, 381:2293–2303).

The Biostatistics Research Branch engages in collaborative relationships, develops new statistical methods and mathematical models, and provides advice and oversight for intramural and extramural researchers.

FNL biostatisticians within CMRPD provided statistical, mathematical, data management, programming, and data analysis support to many intramural clinical research protocols. In addition to maintaining data quality and integrity, the biostatisticians helped develop and test novel statistical methods for researchers, assisted with safety evaluations, and prepared DSMB reports.

The biostatisticians supported a variety of projects, from developing and drafting statistical analysis plans to coding and performing complex statistical analyses, writing reports, presenting findings, and co-authoring manuscripts. Among the supported studies are the Mexico Emerging Infectious Disease Clinical Research Network (La Red) Zika and dengue clinical trials; PredictTB and multidrug-resistant tuberculosis clinical trials; preclinical simian immunodeficiency virus (SIV) studies; Vaccine Research Center studies; malaria studies; HIV/SIV studies; allergy studies; Lyme disease studies; multiple sclerosis studies; Zika studies; the COVID-19 (ACTT) studies; and several vaccine studies. The data analysis support ranges from basic descriptive statistics and graphs to sophisticated statistical analyses such as generalized

estimating equation modeling, mixed-effect models, meta-analysis, competing risk analysis, time-varying Cox regression, and decision-tree and classification-tree analyses. Support also included organizing, coding, programming scripts, and executing massive and complex simulations on the National Institutes of Health (NIH) supercomputer (Biowulf cluster).

A biostatistician on this team provided significant support to AIRS in planning, installing, operating, and providing training on a novel PET camera designed for use in monkeys. With higher spatial resolution and sensitivity compared to clinical single-photon emission computed tomography (SPECT) and PET cameras, this novel PET system enhances the AIRS laboratory's *in vivo* imaging capabilities by providing a clear and well-defined delineation of whole-body lymphoid tissue. Support for the AIRS laboratory also included: (i) acquiring and analyzing data from noninvasive *in vivo* SPECT and PET imaging of nonhuman primates infected with SIV or simian/human immunodeficiency virus, (ii) testing new antibodies for potential noninvasive *in vivo* imaging, (iii) studying the changes in lymphoid tissue volumes in infection and after initiation or interruption of antiretroviral therapy, and (iv) designing *in vitro* and *in vivo* HIV studies.

FNL data management staff in CMRPD provided clinical trial support in designing data flows, validating clinical databases, creating randomization procedures, overseeing data-entry processes, managing queries, ensuring high-quality data, and performing interim and final data analyses. Data management staff supported Good Clinical Data Management practices, including case report form development and review, clinical database development and testing, data management guideline evaluation, and data management procedure implementation and oversight to ensure high-quality data. Data management staff supported ACTT by assisting with the statistical analysis plan and performing statistical analyses for DSMB reports and the preliminary publication (Beigel JH, Tomashek KM, et al., *N Eng J Med*, 2020). Staff also supported PredictTB, various Indonesia Research Partnership on Infectious Diseases and University Clinical Research Center (Mali) projects, and the COVID-19 plasma collection protocol.

FNL biostatisticians and data management staff in CMRPD supported the PALM trial through the entire data management life cycle, which included developing case report forms, developing and testing the REDCap database, randomizing participants, performing double data entry, cleaning data and managing queries, and reporting on trial operations.

One biostatistician supported the PALM trial as part of the consortium study team. Activities included generating plots and performing quality-control checks on tables for the published manuscript (Mulangu S, Dodd LE, Davey Jr. RT, Tshiani Mbaya O, Proschan M, et al., PAL Consortium Study Team: "A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics," *N Eng J Med*, 2019, 381:2293–2303); performing quality-control checks and validating daily reports, including enrollment reports and the day 28 report; and creating and maintaining an interactive online study team contact directory.

Data management staff supporting PALM were involved in designing data flow, validating the clinical database, creating the randomization procedure, overseeing the data entry process, managing data queries, and performing interim and final data analyses.

One biostatistician received a NIAID Merit Award as part of the NIAID benchmarking working group for "grant characteristic modeling." The staff member implemented a random forest model that accurately predicted the number of grants under management by various project officers.

Collaborative Clinical Research Branch

KEY ACCOMPLISHMENTS

- Provided technical and clinical expertise on seven protocols implemented under the Infectious Diseases Clinical Research Program (IDCRP) network in response to the COVID-19 pandemic

The Collaborative Clinical Research Branch (CCRB) facilitates high-quality clinical research in infectious diseases through active participation in selected domestic and international collaborations.

Since 2005, FNL staff members in CMRPD and the Clinical Research Directorate have worked with NIAID to establish and maintain a collaborative effort with the Department of Defense (DOD) through the IDCRP. The mission of this collaboration is to conduct multicenter clinical research on infectious diseases, focusing on high-impact cohort and interventional trials, to inform and improve care for military personnel.

The FNL staff supports CCRB activities by providing technical expertise for research strategy implementation and management, developing and teaching sound clinical concepts related to infectious disease research, and presenting and publishing information for the research community.

Technical and Scientific Support

FNL clinical project managers in CMRPD helped lead operations for, coordinate, and manage multiple special projects, including collaborations with the Indonesia Research Partnership on Infectious Diseases, La Red, the Partnership of Clinical Research in Guinea (PREGUI), and groups in Mali. They also provided strategic program and project management to various COVID-19 rapid-response activities, including ACTT and the Accelerating COVID-19 Therapeutic Interventions and Vaccines surveys. This support included coordinating and tracking responses to site capability/feasibility surveys/requests as part of the global effort to identify sites that could be leveraged for responding to COVID-19 and other infectious diseases, collecting and managing capability information in Smartsheet, and providing meeting support. Staff members

also supported programs as representatives to International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) studies, including early intervention and natural history studies for COVID-19.

Clinical Consulting and Scientific Support

An FNL physician in the Clinical Research Directorate provided scientific (protocol design, conduct, and analysis and help with manuscripts and presentations) and planning support to IDCRP and other CCRB networks, including La Red and INSIGHT. The physician provided technical and clinical expertise to seven protocols under the IDCRP network in response to the COVID-19 pandemic, including ACTT-1 and 2, natural history studies in DOD beneficiaries, studies in health care workers deployed to New York City and the USNS *Comfort*, and studies in health care workers at Walter Reed Medical Center and the U.S. Naval Academy. The physician served in a key leadership role as needed, which included management and oversight of all CCRB networks.

The physician worked with the IDCRP network to obtain funding outside of DCR to support the Pragmatic Assessment of Influenza Vaccine Effectiveness trial testing three influenza vaccines. This trial continues into year three and has enrolled more than 10,000 participants.

The physician served as a clinical employee attending on the inpatient infectious diseases consulting service at the NIH Clinical Center, engaging in patient care and teaching medical students, residents, and fellows.

The physician also served as a mentor and co-investigator with NIAID and NIH fellows and other medical staff on various research efforts, including: (i) evaluating natural history and outcomes of bacteremic patients in the NIH Clinical Center population, (ii) evaluating epidemiology and outcomes in DOD beneficiaries with bloodstream infections, (iii) determining the probability of inferior outcomes in non-inferiority trials, (iv) reviewing the validity of urine cultures as a surrogate endpoint for patient symptoms in urinary tract infection studies, and (v) publishing manuscripts with the NIAID Antibiotic Resistance Outcomes Research Initiative (Strich JR, Ricotta E, Warner S, Lai YL, Demirkale CY, Hohmann SF, Rhee C, Klompas M, Palmore T, Powers JH, et al., *Clin Infect Dis*, 2020).

The physician maintains the Influenza Patient-Reported Outcomes (FLU-PRO) symptom scale that is now being used in over 13 studies, including those in IDCRP and in academic institutions conducting COVID-19 studies in the U.S., Canada, the United Kingdom, Australia, and New Zealand.

The physician facilitated research capacity development by helping IDCRP staff create and implement protocols and by helping prioritize research protocols within the network's seven research areas (HIV, sexually transmitted infections, skin and soft tissue infections, trauma-related infections, deployment and travel-related infections, acute respiratory infections, and emerging infections and antimicrobial resistance).

COVID-19: Observational Study and Treatment Guidelines Website

KEY ACCOMPLISHMENTS

- Provided substantial project management support in executing a subcontract for the development of the COVID-19 Treatment Guidelines website: https://www.covid19treatmentguidelines nih.gov
- Delivered a go-live version of the COVID-19 Treatment Guidelines website within several weeks of the initial request
- Established a subcontract with a vendor to initiate the management, oversight, and execution of the international observational study of outpatients with COVID-19

In support of NIAID DCR's coronavirus clinical research, the FNL team in CMRPD supported the following COVID-19 initiatives:

COVID-19 Observational Study (INSIGHT011)

FNL, through CMRPD, was requested to initiate the management, oversight, and execution of NIAID's International Observational Study of Outpatients with SARS-CoV-2 Infection (INSIGHT 011). This is a cohort study of adults with COVID-19 who have been assessed as not requiring hospitalization (outpatients). The general aim of this study is to estimate the rate of disease progression for adults who seek testing for COVID-19 and who test positive for SARS-CoV-2. The primary study endpoint and the basis for sample size are hospitalization or death during the 28-day follow-up period. Secondary outcomes include participant-reported return to excellent or very good health status and change in severity of shortness of breath.

The study received authorization to proceed on April 20, 2020; contract full award occurred on June 11, 2020; and the first enrollment occurred on June 18, 2020.

The conducted activities focused on providing project management and programmatic oversight of a subcontract to coordinate the INSIGHT network; design and execute the protocol; and provide data management, laboratory, and biorepository support. INSIGHT is a unique network of clinical research sites around the world, led by various international coordinating centers, which taps into a host of investigators, operations, and biostatisticians who lend to the network's success.

FNL staff managed daily aspects of program planning and performance, including procurement, budget oversight, and communication with NIAID project leaders. Staff also supported initiation of enrollment and follow-up visits at study sites. Enrollment began in June. Staff worked with the subcontractor, the INSIGHT network, the Washington International Coordinating Center, and NIAID on study oversight, execution of pilot sites, and engagement of DCR special project sites in the conduct of the observational study.

FNL staff facilitated regular biweekly meetings with the Washington International Coordinating Center to review study status and coordinate the integration of DCR special project sites, including Mali and La Red.

With the challenges in identifying COVID-19 testing supplies during the outbreak, staff members were able to identify a vendor and secure nasopharyngeal swabs and viral transport media for kit production that was critical to study implementation.

NIH COVID-19 Treatment Guidelines Website

FNL staff members were requested to initiate a subcontract with a global consulting company to rapidly design and deploy a public-facing website as part of the effort to disseminate current COVID-19 therapy and treatment guidelines to health professionals and the general public.

The initiative is led by a panel consisting of representatives from federal agencies, health care and academic organizations, and professional societies. The guidelines will allow clinicians to reference best practices for COVID-19 patients and will give the general public access to the most current evidence on the treatment of COVID-19 and related conditions, treatment protocols and available clinical trials for experimental drugs, and materials used in the educational process for implementing the guidelines.

FNL's initial support for the website centered around rapidly establishing a subcontract and assembling a team of IT experts from multiple NIH institutes to guide the technical delivery of the project. Meetings were held to identify the critical pathway for implementing the website, including naming conventions, communication methods, security considerations, and compliance with Federal Information Security and Management Act requirements. Penetration testing and security simulations were coordinated to assess go-live readiness, with implementation of a temporary Authority to Operate (ATO) while the formal ATO package was being compiled and submitted for approval.

FNL, in coordination with the technical stakeholder team and members of the Treatment Guidelines Panel, delivered a go-live version of the website (https://www.covid19treatmentguidelines.nih.gov) within several weeks of the initial request. It was vetted through the White House COVID-19 Task Force and approved for delivery to the public on April 21. A comprehensive, printable, and Section 508–compliant version of the treatment guidelines was also developed to download from the website.

Overview of COVID-19 Treatment Guidelines Website Activity from 4/21/20 to 7/27/20

(Note: Data in this report reflects website activity on https://www covid19treatmentguidelines nih gov/ through July 27, 2020)

Date	Users		Sessions	Page Views
	U.S.	International		
Week 1 (4/21–4/27)	161,547	118,030	360,669	1,184,572
Week 2 (4/28–5/4)	43,892	26,928	89,145	278,947
Week 3 (5/5–5/11)	27,525	18,019	56,692	177,032
Week 4 (5/12–5/18)	27,024	22,057	60,884	151,770
Week 5 (5/19–5/25)	21,090	21,050	51,340	121,498
Week 6 (5/26–6/1)	23,798	18,826	50,949	115,213
Week 7 (6/2–6/8)	12,275	16,875	35,974	83,429
Week 8 (6/9–6/15)	17,568	21,957	48,180	108,641
Week 9 (6/16–6/22)	15,952	20,586	44,321	101,166
Week 10 (6/23–6/29)	28,195	28,128	68,094	140,008
Week 11 (6/30–7/6)	34,074	32,109	78,804	155,020
Week 12 (7/7–7/13)	35,361	31,587	80,326	165,283
Week 13 (7/14–7/20)	45,383	46,946	108,285	209,201
Week 14 (7/21–7/27)	43,762	45,165	107,011	210,533
Total	**537,446**	**468,263**	**1,240,674**	**3,202,313**

Users *– the number of new and returning people who visit the site*
Sessions *– a visit to the website. Note that if a person returns within a 30-minute timeframe, it will still count as one session. If they return after 30 minutes, it will count as a new session.*
Page Views *– total instances of any page on the website being loaded (or reloaded) in a browser*

COVID-19 Plasma Collection and Intravenous Hyperimmune Immunoglobulin Studies

KEY ACCOMPLISHMENTS

- Completed startup activities for the COVID-19 plasma collection protocol
- Created over 45 Smartsheet tabs in support of the COVID-19 program

In support of NIAID DCR's coronavirus clinical research, FNL staff in CMRPD supported the following COVID-19 initiatives:

COVID-19 Coordination Center

A research subcontract was established with a clinical research organization to set up and maintain a coordination center that provides administrative and information technology support to coordinate resources and reporting activities for a series of NIAID DCR COVID-19 research protocols. FNL staff worked with the clinical research organization to develop and release trackers, reports, and dashboards for numerous COVID-19-specific special projects and studies in less than a month. These tools provide information on project

history, financials, protocols, plasma samples (collecting, shipping, shipment tracking, and testing), agreements, and laboratory reports. FNL staff in CMRPD, NIAID DCR project managers, division directors, and other senior leadership use the executive summary dashboard as a one-stop shop for accessing COVID-related project data. FNL created more than 45 Smartsheet trackers, reports, and dashboards in support of DCR's COVID-19 program. The research subcontract agreement with the clinical research organization was allowed to expire at the end of August, and FNL began to support the coordination center tasks. Twenty-five Smartsheet tabs transitioned from the subcontractor to FNL.

COVID-19 Plasma Collection Study

The primary objective of this multicenter pilot study is to identify eligible donors from which to collect anti-SARS-CoV-2 immune plasma. This study consists of two parts: (i) screening for SARS-CoV-2 antibody titer and eligibility to donate plasma, and (ii) plasma collection by apheresis. As of July, the FNL staff in CMRPD has supported 160 shipments containing 1,675 plasma/serum samples sent from participating sites to the NIAID Integrated Research Facility for testing. Moreover, the staff has tracked the collection and transfer of more than 100 liters of plasma sent to Emergent BioSolutions for the manufacture of *in vitro* immunoglobin.

FNL staff in CMRPD worked to engage sites near COVID-19 outbreak regions (including Seattle and Miami) and worked with NIAID to develop partnerships with manufacturers, such as Grifols and Emergent BioSolutions; set up regular teleconferences in multiple time zones to help define the protocol; establish shipping mechanisms using QuickSTAT; and complete a manual of operations for use by all parties. Material transfer agreements were initiated and executed to allow for the screening and collection process to begin much quicker than through standard contracting mechanisms.

INSIGHT012

This trial of intravenous hyperimmune immunoglobulin (IVIG) in COVID-19 patients aims determine whether IVIG administration, when added to standard-of-care treatment, is superior to placebo in terms of reducing disease severity and duration. INSIGHT012 (outpatient IVIG randomized controlled trial) will serve as a platform for assessing treatments for adult outpatients in the early stages of COVID-19 before signs and symptoms of pneumonia have developed. The study population will include symptomatic patients diagnosed with COVID-19 early in the course of the disease (within 96 hours of symptom onset) who are at an increased risk of clinical disease progression but not hospitalized or in the process of being hospitalized at baseline. A shift in project priorities at the direction of NIAID DCR required INSIGHT013 to be conducted before INSIGHT012.

INSIGHT013

INSIGHT013 (inpatient IVIG randomized controlled trial), a trial of the safety and efficacy of investigational therapeutics for treating COVID-19, will serve as a platform for assessing treatments for hospitalized adult patients without related serious end-organ failure. Initially, this trial will compare IVIG with matched placebo, when added to standard of care, for preventing further COVID-19 disease progression and mortality. Standard of care will include remdesivir unless it is contraindicated. The trial's primary endpoint in hospitalized patients is an ordinal outcome based on the patient's clinical status on day seven. It includes seven mutually exclusive categories capturing the range of organ dysfunction that may be associated with progression of COVID-19, such as respiratory dysfunction and coagulation-related complications.

FNL established a sole-source research subcontract with a vendor to provide rapid deployment of scientific, technical, operational, database, and clinical research resources and expertise in establishing a coordination center and conducting the INSIGHT012 and INSIGHT013 protocols. (As noted above under INSIGHT012, INSIGHT013 will be conducted first.) Under this research subcontract, the INSIGHT network will be engaged quickly and repeatedly so it can provide urgent support, including clinical operations and data management expertise, to clinical research for emerging and re-emerging infectious diseases.

Therapeutics for Inpatients with COVID-19 (ACTIV-3)

Through NIH's Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV) program, NIAID DCR requested that FNL staff in CMRPD provide overarching clinical project management, operational, and clinical trials/regulatory/pharmacovigilance support for managing and conducting a clinical trial to test antibody treatment in hospitalized COVID-19 patients. The Phase III randomized controlled study, known as ACTIV-3, is designed to test the safety and efficacy of investigational therapeutics for hospitalized patients with COVID-19 (Therapeutics for Inpatients with COVID-19).

The government issued a letter agreement to FNL on June 30, 2020. A task order was priced, and a technical response was provided to the NCI Management Operations Support Branch and NIAID DCR contracting officer representative in July 2020.

Authorization to Proceed (ATP) was issued to a clinical research organization for overall protocol development, implementation, and management services for 90 days pending subcontract execution, and an ATP was issued to another clinical research organization for program/project management and monitoring services until subcontract execution.

The master protocol was initiated in late July. By the end of August, 12 sites at select hospitals in existing clinical trial networks around the world had been activated and over 50 participants were enrolled in the multicenter study.

Office of the Director – Clinical Consulting and Support

KEY ACCOMPLISHMENTS

- Deployed a staff member, on request of the World Health Organization (WHO) Global Outbreak Assessment Response Network, as a technical expert to the Ebola virus outbreak in the Democratic Republic of the Congo
- Spearheaded development of a first-in-kind study of combination therapeutics in a rhesus monkey model of Ebola virus
- Supported rapid stand-up of SARS-CoV-2/COVID-19 nonhuman primate (NHP) models

The Clinical Consulting and Support group in FNL's CMRPD provides project/program management, scientific and technical oversight, information technology, financial management, and administrative and programmatic support for various DCR initiatives.

Scientific and technical staff support the mission of the NIAID Integrated Research Facility (IRF) to manage, coordinate, and facilitate research on emerging infectious diseases and biodefense pathogens that aims to develop medical countermeasures and improve patients' medical outcomes. The IRF contains advanced medical imaging equipment in a biosafety level 4 laboratory environment.

FNL clinical staff collaborated with IRF staff to establish an Excel template for recording COVID-19 neutralizing antibody titer data. The data were used to provide multiple real-time graphs, charts, and statistics for NIAID leadership to reference.

FNL staff also designed an overarching coronavirus project management tool using Smartsheet. This tool includes data from various NIAID DCR coronavirus initiatives that are used to create project-specific dashboards and an executive summary for NIAID DCR leadership.

In conjunction with FNL Contracts and Acquisitions, FNL staff in CMRPD provided multiple mechanisms to allow for work to begin on urgent coronavirus initiatives prior to full execution of agreements with clinical sites and subcontractors. ATPs were issued to multiple subcontractors while provisions were added to material transfer agreements to allow work to begin while budgets and terms and conditions were negotiated.

In response to the urgent coronavirus pandemic, FNL staff in CMRPD quickly analyzed staffing resources to ensure adequate, experienced staff were made available to provide support. This included making adjustments on projects that were slowing down or stopping, rearranging project tasks/teams, and identifying gaps that needed to be filled. Continuous assessments were made as needs ebbed and flowed in different parts of the world.

Building Management

Staff provided support to a leased building in Frederick, Maryland, which houses CMRPD staff working in support of NIAID DCR. Staff members coordinated all areas of lease oversight and facility maintenance.

Information Technology Support

Information technology staff provided software systems development and computer, network, application, and backup/disaster-recovery technical support services for domestic and global clinical research initiatives.

Project/Program Management

Technical and clinical staff provided project/program management services for subcontracts and consultant agreements, site renovations, shipping, inventory, travel, meeting coordination, foreign insurance, and budgets. They also oversaw all aspects of the Department of Health and Human Services Policy on Promoting Efficient Spending as it relates to CMRPD staff supporting NIAID DCR. The staff monitors project plans and timelines, develops progress reports, assesses project issues, and develops resolutions to customer objectives. The staff also provides regulatory expertise, manages single and multisite domestic and international clinical research programs, develops infrastructure, creates and implements strategic and operational plans, drafts standard operating procedures, and supports training initiatives.

Scientific and Technical Support

Clinical project staff helped lead operations and provided technical and scientific support to NIAID and oversaw special projects and/or international clinical research initiatives. They also supported the IRF's infectious disease imaging research efforts.

One staff member received a NIAID Merit Award as part of the NIAID Division of Microbiology and Infectious Diseases (DMID) Filovirus Animal Nonclinical Group (FANG) Workshop Organizing Committee. Staff members also received a NIAID Merit Award as part of the NIAID DCR PALM trial team.

Ongoing scientific and technical support to NIAID DCR IRF included:

- Providing technical expertise to Congolese clinicians in Ebola treatment units in North Kivu, Ituri, and South Kivu provinces and providing direct operational support to PALM clinical trial sites as part of ongoing response efforts to the Democratic Republic of the Congo Ebola virus outbreak
- Continuing efforts to develop and refine animal models of high-threat emerging pathogens, with an active focus on a first-in-kind study of combination therapeutics for Ebola virus in rhesus monkeys, further development of the Lassa fever NHP model, and characterization of Ebola virus sequalae in NHP survivors

- Supporting IRF efforts toward rapid stand-up of appropriate SARS-CoV-2/COVID-19 animal models, with a focus on unique imaging readouts in NHP models, including a leadership role in a small team designing pilot and follow-on experiments, analyzing in- and post-life data, and communicating results at internal and external meetings
- Co-leading the Animal Model and Human Data subgroup of FANG, a federal interagency working group led by NIAID and the Department of Defense with particular emphasis on the interplay between emerging human data and developing animal models of filovirus disease
- Supporting ongoing WHO-led efforts to develop clinical guidelines for optimized delivery of Ebola treatment unit care and materials (built around the guidelines) designed for training efforts in the Democratic Republic of the Congo and for regional preparedness
- Serving as a subject-matter expert with the WHO to update guidelines for pregnant or breastfeeding women with suspected or confirmed Ebola virus
- Performing image analysis on eight IRF imaging studies of infectious disease derived from Ebola virus and COVID-19
- Leading the IRF artificial intelligence working group on image segmentation projects studying filovirus disease and COVID-19 disease progression, including one quantitative measure, called percent change in lung hyperdensity, that is being used at the IRF to track disease progression in the lungs of animal models infected with COVID-19
- Leading the IRF collaboration with the University of Southern California on using radiomic feature analysis of NHPs infected with Ebola virus based on computed tomography scanning and finding correlations with non-imaging biomarkers
- Serving as a co-author on a book chapter in *Global Virology III: Virology in the 21st Century* on innovative technologies for WHO risk group 4 pathogens research (Logue J, Solomon J, et al., November 2019)

Subcontract Management

CMRPD managed and renewed a research subcontract with the HIV Resistance Response Database Initiative (RDI) for modeling various antiretroviral responses. (Note: the subcontract is managed by FNL staff in CMRPD, yet the budget for it falls under the FNL Applied and Developmental Research Directorate.) RDI was formed to collect and harness "big data" using computational modeling to aid antiretroviral treatment selection in the face of virus resistance. RDI collected anonymized biological, clinical, and treatment outcome data from more than 250,000 HIV-1 patients around the world over 17 years. From these data, RDI developed models to predict HIV-1 treatment outcomes and to identify optimal, individualized therapies.

Office of Planning and Operations Support

KEY ACCOMPLISHMENTS

- Supported technology needs for NIH COVID-19 emergency preparedness efforts by participating in a trans-institute collaboration with the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) to create a COVID-19 surveillance tool
- Conducted a detailed environmental scan to allow Intramural Clinical Management and Operations Branch (ICMOB) staff to implement a more productive workplace, resulting in an organization-development initiative with interactive workshops

The mission of the Office of Planning and Operations Support (OPOS) is to provide services and innovative solutions that optimize NIAID clinical research and special projects.

The FNL staff in CMRPD provides executive leadership and management oversight for a variety of programmatic and administrative activities in support of NIAID DCR OPOS clinical research and special projects. These include strategic and operational planning, learning and professional development, technical support and project administration, financial oversight, and research subcontract management and oversight.

Research Subcontracts Management

FNL staff managed a research subcontract supporting the DCR leadership initiative and a research subcontact providing strategy management assessments of clinical research networks and recommendations regarding DCR's clinical research toolkit development initiative (READI) framework, workflow, and process mapping. Staff also managed a consulting agreement to support organizational change and help to develop, execute, implement, and evaluate DCR strategies.

Learning and Professional Development

The FNL staff in CMRPD's Learning and Professional Development (L&PD) group provides three primary areas of support to OPOS: (i) identifying/ developing training resources to address customer-identified training needs, (ii) providing training and professional development subject-matter expertise, and (iii) participating in professional development to ensure staff members maintain their subject-matter expertise.

The major focus of FNL staff in L&PD was on activities supporting the Partnership for Research on Vaccines and Infectious Diseases in Liberia (PREVAIL). These activities are described in the "Special Project – PREVAIL Liberia" section of this report.

At the request of DCR senior management, FNL staff in L&PD conducted a detailed environmental scan to allow ICMOB staff to increase workplace productivity. FNL staff conducted extensive interviews with all staff and aggregated staff input and recommendations to develop the productive workplace that ICMOB staff envisioned. The DCR director described FNL staff members' efforts and the outcome as "brilliant." This environmental scan resulted in an extensive organization-development initiative, including numerous interactive workshops over several months to address each element of the environmental scan.

Technical Solutions Group

FNL staff in CMRPD managed the transition of the administration and support for external Clinical Research Information Management System of NIAID (CRIMSON) users from DCR to the Division of Intramural Research and provided training for the Division of Intramural Research administrative officers.

Staff worked with a Program Planning and Analysis Branch senior administrative officer to verify users with multiple pieces of equipment. More than 600 pieces of equipment were reviewed, resulting in 77 going to surplus.

Staff served as the point of contact for updates and maintenance issues for DCR's SharePoint sites and completed all update requests within 48 hours.

Staff also served as the central point of contact for facilitating technical equipment ordering (e.g., phones, laptops, desktops, and monitors), supported the annual inventory of more than 600 pieces of equipment, and helped with property portal reconciliations.

Staff supported the following technology projects:

- The Chronic Granulomatous Disease Level Up Program – Staff attended bimonthly meetings to gain insight into program needs, identified inconsistencies in the program's ability to manage meeting documentation, and introduced Huddle as a documentation-management tool. The program now uses Huddle to keep track of all documentation as needed.
- Virtual brown-bag sessions – Staff worked with colleagues to create a series of virtual brown-bag sessions focused on available technologies to enhance the telework experience for DCR and FNL staff. The initial brown-bag session focused on the capabilities of Microsoft Online and OneDrive. Surveys were given before and after the session to gauge participants' knowledge of OneDrive.
- NIH COVID-19 technology needs – Staff supported technology requirements for NIH COVID-19 emergency preparedness efforts by participating in a trans-institute collaboration with NIDDK to create a COVID-19 surveillance tool. The tool was developed in NIDDK's REDCap, an open-access database system, but due to the urgency of the project, DCR and the Systex support team were leveraged to assist. Through a series of collaborative discussions over several weeks, each team was assigned a specific area on which to focus. All requirements were meet and/or exceeded the government customer's expectations.

Regulatory Compliance and Human Subjects Protection Program

KEY ACCOMPLISHMENTS

- Submitted a Breakthrough Therapy Designation request to the Food and Drug Administration (FDA) that was approved to use plerixafor to treat Warts, Hypogammaglobulinemia, Infections, and Myelokathexis (WHIM) syndrome
- Provided protocol navigation and development support to 15 new COVID-19-related protocols
- Evaluated clinical sites for participation in NIAID DMID's Adaptive COVID-19 Treatment Trials in Mexico, Japan, and Korea

FNL's Regulatory Compliance and Human Subjects Protection Program (RCHSPP) in CMRPD supports the Office of Clinical Research Policy and Regulatory Operations' (OCRPRO) mission and the NIAID DCR clinical research enterprise by providing comprehensive clinical project management and programmatic support; scientific and technical oversight; regulatory compliance; regulatory support for Clinicaltrials.gov and Investigational New Drug (IND), Investigational Device Exemption (IDE), clinical trial, and Drug Master File (DMF) applications; pharmacovigilance oversight; medical writing; clinical trials management; protocol development and navigation; information technology support; and clinical research training, both domestically and internationally.

FNL staff in CMRPD's RCHSPP provided significant support to COVID-19 studies. Activities included supporting protocol navigation and development; initiating protocol reviews and facilitating expedited reviews; reviewing the Therapeutics for Inpatients with COVID-19 (ACTIV-3) study protocol; providing study monitoring and monitoring oversight; preparing training documents; supporting site activation; coordinating pre-IND meetings; submitting information requests; and preparing, submitting, and maintaining associated IND and DMF applications. More details are in the following sections.

Clinical Safety Office

FNL staff in CMRPD's Clinical Safety Office processed approximately 230 deaths and serious adverse events, largely related to Ebola clinical research efforts in the Democratic Republic of the Congo, which is an unprecedented volume compared to previous years. The staff reviewed more than 90 clinical research protocols, including 26 new studies and 62 protocol amendments; more than 60 FDA IND annual reports; and 15 investigator brochures and brochure amendments.

The FNL staff administratively operates the NIAID intramural data and safety monitoring board and safety monitoring committees to meet increasingly complicated and voluminous safety and data-quality oversight requirements.

For information on the Clinical Safety Office's support to the PALM 1 trial in the Democratic Republic of the Congo, see the "Special Project – Ebola Clinical Research, Democratic Republic of the Congo" section.

FNL staff provided significant support to multiple studies to address the COVID-19 pandemic, including protocol review and/or monitoring for plasma collection, intravenous immunoglobulin, BTK inhibitor, antiviral, and monoclonal antibody therapeutic studies, as well as additional support related to remdesivir use in COVID-19.

FNL staff continued to work on streamlining the protocol review process to harmonize with NIH Institutional Review Board (IRB) policies, including reviewing IND safety template language and technical language for specialized products, and developing a tool/guide with leading questions for investigators.

Clinical Trials Management

The FNL staff in CMRPD's Clinical Trials Management (CTM) team manages and monitors approximately 155 active NIAID clinical research studies conducted at U.S. and international sites. The types of studies vary and include Phase I, II, and III IND studies; IDE studies; natural history studies; studies involving pediatric participants; and research studies that are minimally invasive to noninvasive and do not fall under an IND/IDE. The team conducted 14 study initiation visits, seven mini–study initiation visits, 167 interim monitoring visits, and nine study close-out visits.

FNL staff in CTM helped the contracted site management organization revise monitoring reports and letter templates for the Mexico Emerging Infectious Disease Clinical Research Network (La Red) Coordinating Center. Staff designees also reviewed and approved the monitoring visit reports and letters for visits conducted by La Red monitors, discussed issues identified by the monitors, and recommended corrective measures as applicable.

FNL staff monitored data and regulatory files for the ongoing PREVAIL studies in Liberia, Mali, and Guinea and provided guidance to site principal investigators and site personnel.

FNL staff supported various vaccine trials targeting respiratory syncytial virus, influenza, Zika, dengue, and Ebola at the Johns Hopkins University Center for Immunization Research and the University of Vermont.

For information on CTM's support to the PALM 1 trial in the Democratic Republic of the Congo, see the "Special Project – Ebola Clinical Research, Democratic Republic of the Congo" section.

COVID-19 Studies

For the Therapeutics for Inpatients with COVID-19 (ACTIV-3) study, the Clinical Trials Management team in CMRPD worked with INSIGHT to help register the international coordinating centers in the Washington, D.C., area and the Veterans Affairs, Petal, and Cardiothoracic Surgical Trials Network sites on the protocol. CTM also helped activate vanguard sites. A review of the protocol for regulatory input was completed, and an informed consent form review checklist and case report forms (CRFs) were drafted. FNL staff in CTM worked with the clinical research organization to develop a monitoring plan and tools and to draft sections of the statement of work to assist with monitoring other networks.

FNL staff supported the COVID plasma collection study by preparing training documents, a manual of operations, the monitoring plan, and CRFs. The staff also supported site activation for the three sites and scheduled the first monitoring visit in August.

FNL staff provided clinical study site-assessment support in Japan and South Korea for the NIAID DMID Adaptive COVID-19 Treatment Trials. Staff provided key documents, including the monitoring plan and updates, to DMID for the study and for the sites in Mexico and at NIH. Staff provided monitoring oversight of the Mexican sites and monitoring of the NIH site. More information on support provided to this study can be found in the NIAID DMID "Adaptive COVID-19 Treatment Trials" section.

Contract Research Organization Oversight: Southeast Asia, China, South Africa

FNL staff provided project oversight and managed activities for the subcontracted clinical research organization's monitoring functions in Thailand and Bangladesh as well as the two clinical research organizations' monitoring sites as part of the Using Biomarkers to Predict Tuberculosis (TB) Treatment Duration (PredictTB) study.

Database Systems Support

FNL staff continued to use FrameMaker/DataFax to create CRFs for studies conducted across various international sites, including a rapid response to the PALM 1 study extension phase in the Democratic Republic of the Congo and adjustments to other CRFs due to COVID-19. FNL staff worked with the NIAID Office of Cyber Infrastructure and Computational Biology to provide CRF packets that were easily transferred to DataFax or REDCap for use by the site team.

FNL staff continued to work with CRIMSON staff and the OCRPRO clinical research oversight manager to enhance the electronic monitoring function of CRIMSON for NIAID studies carried out at NIH.

Document Control

FNL staff in CMRPD's Document Control group managed files in the electronic document records management system for active INDs, IDEs, and master

files and for non-IND clinical studies. The team created more than 70 protocol records in support of FNL's clinical research and facilitated the regulatory review of various clinical protocols and amendments. FNL staff supported the NIAID OCRPRO quality internal audit inspection of CMRPD's RCHSPP. Staff members supported COVID-19 research efforts by initiating protocol records and facilitating expedited review of COVID-19-related clinical protocols.

Institutional Review Board Support

The FNL IRB support team in CMRPD collaborated with OCRPRO to process documents for IRB submission and provide administrative and programmatic support to NIAID's IRB. Support included serving as a liaison between the study teams, the NIAID IRB, the NIH Office of Protocol Services, and the Office of Human Subjects Research Protections; processing full-board and expedited protocol actions for review; preparing electronic meeting packets; tracking protocol submissions from initial submission through the Office of Protocol Services approval phase; preparing tracking reports; and maintaining protocol-specific records.

The NIAID IRB merged into the NIH intramural IRB in December 2019.

Learning and Professional Development

The FNL L&PD group within CMRPD provided learning and organizational/professional development support to OCRPRO/RCHSPP. The three primary areas of support were, (i) identifying/developing resources to address customer-identified training needs, (ii) providing training and professional development subject-matter expertise, and (iii) participating in professional development to ensure staff members maintain their subject-matter expertise.

FNL L&PD hosted a Business Writing Workshop that provided International Association for Continuing Education and Training continuing education units, updated the TrackWise all users' computer-based training to reflect the new version of TrackWise, and facilitated several webinars for various RCHSPP groups. The webinar topics included Adaptive Clinical Trials Design, Effective Oral Presentations, Informed Consent under the Revised Common Rule, Pediatric Risk Determination, and Real-World Approaches to Informed Consent.

Project Management

FNL staff in CMRPD's Project Management Team supported logistics and program operations for Ebola clinical research to ensure continuity of operations for FNL's rapid response related to the Ebola outbreak in the Democratic Republic of the Congo. As part of the rotating staff, an FNL staff member traveled to provide on-the-ground operational support to in-country shipment logistics, capacity-building, and the infrastructure needed to conduct clinical research during the outbreak.

FNL staff supported DCR's READI initiative to develop a clinical research rapid-response toolkit by assisting with the toolkit, wikis, and process mapping effort.

Protocol Navigation

FNL staff in CMRPD's Protocol Navigation/Protocol Development Program (PN/PDP) worked with principal investigators to prepare 17 new protocols and amend 13 existing protocols.

The FNL staff in PN/PDP normally supports the development of about 20 new protocols each year. In the wake of the COVID-19 pandemic, in three-and-a-half months, the FNL supported 15 COVID-19-related new protocols. These studies received top priority for development and review, significantly shortening the normal time from concept to study start, with one protocol being developed and approved in a little over two weeks. In addition, the FNL staff in PN/PDP saw a twofold increase in requests for protocol amendment writing and logistical support to harmonize documents with the new NIH IRB structure and policies and/or to include COVID-19-related research.

Regulatory Affairs

FNL staff in CMRPD's Regulatory Affairs group supported 85 INDs, three clinical trial applications (CTAs), three IDEs, and four DMFs and prepared and submitted approximately nine new IND applications. Three of these INDs were for COVID-19 treatments or vaccines and required expertise because they were published and submitted urgently. As part of the ongoing maintenance for these new and existing applications, staff developed and submitted approximately 320 IND, CTA, IDE, and DMF amendments.

FNL staff supported an NIH investigative team evaluating plerixafor as a treatment for WHIM syndrome by providing regulatory guidance, writing, reviewing, and submitting an important request to the FDA for Breakthrough Therapy Designation (BTD) for that drug and indication. A drug product that receives BTD is eligible for all the benefits of FDA's fast-track review programs, including expedited development and review of product applications, and extensive guidance from the agency on efficient drug development. The FDA granted the BTD for plerixafor in WHIM syndrome, thus providing additional important incentives to allow for continued development of the drug as a treatment for this rare disease.

FNL staff provided regulatory support for COVID-19-related studies sponsored by OCRPRO, including coordinating pre-IND meetings; submitting information requests; and preparing, submitting, and maintaining associated IND and DMF applications.

FNL management staff in Regulatory Affairs collaborated with OCRPRO and the FDA to strategize on the best process for submitting the necessary data and documentation to support planned COVID-19 studies.

Rolling pre-IND submissions were implemented for the International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) 012 trial of intravenous immunoglobulin in COVID-19 patients so the FDA could review the protocol and other documents prior to receipt of the complete IND, allowing for quicker safety review of the full application and thus a shorter time to study start.

Special Project: Ebola Clinical Research, Democratic Republic of the Congo

KEY ACCOMPLISHMENTS

- Description of work text (Normal – Completed the PALM 1 randomized controlled trial extension phase
- Provided significant clinical operations support to the PALM 1 study in the Democratic Republic of the Congo, culminating in a *New Engl J Med* publication
- Received the distinguished 2019 David Sackett Trial of the Year Award from the Society for Clinical Trials for the PALM 1 study
- Received a NIAID Merit Award for the PALM 1 study

As a foundation for NIAID DCR's emergency response to the Ebola outbreak in the Democratic Republic of the Congo, FNL's CMRPD supported the establishment of a multilateral clinical research program called PALM through a collaborative partnership with the Institut National de Recherche Biomédicale (National Institute of Biomedical Research [INRB]) and is managing a portfolio of international clinical research studies supporting NIAID's efforts to effectively respond to viral hemorrhagic fevers and other emerging and re-emerging infectious diseases through early response, disease characterization, treatment and prevention, collaboration, and flexibility.

Staff supported the extension phase of the PALM 1 trial, in which two Ebola virus disease therapeutic agents were evaluated and shown to significantly improve patient survival based on the interim review determination of the data and safety monitoring board. A total of 363 participants were enrolled across four Ebola treatment research sites in the eastern Democratic Republic of the Congo, and all follow-up visits were completed. The results of the PALM 1 trial were published in *N Engl J Med* in December 2019 (Mulangu S, Dodd LE, Davey Jr. RT, Tshiani Mbaya O, Proschan M, et al., PALM Consortium Study Team: "A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics," 381:2293–2303).

FNL staff in CMRPD's Clinical Trials Management team continued monitoring support of the trial by overseeing site monitoring visits, reviewing laboratory results and informed consents remotely, ensuring the sponsor trial master file was current, supporting site regulatory file maintenance, providing quality control of data, providing guidance to the site teams on transitioning to new consent forms for the new arms of the study, and overseeing a contract research organization (CRO) for monitoring visits. CTM assisted OCRPRO clinical research oversight manager with preparations for an FDA inspection in August 2020 following the request of one of the pharmaceutical companies for licensure of its product. CTM worked with the CRO to perform study close-out activities after the sites were declared Ebola-free in June.

FNL staff in CMRPD's Clinical Safety Office provided mission-critical daily support to the trial, which included nearly continuous email communication with colleagues in both Kinshasa and the outbreak-zone Ebola treatment units. The team provided medical monitoring, which focused on reviewing all deaths, serious adverse events, and pregnancies, as well as priority comprehensive protocol and amendment review, daily interface with safety team members on the ground, and weekly intercontinental pharmacovigilance team meetings to adjudicate safety reports.

Data management and biostatistics teams supported rigorous data collection and quality management in collaboration with Congolese research staff. A subcontractor provided ongoing support to the trial by developing and maintaining a communication portal. Activities included hosting a website for trial documentation and study reports, implementing an electronic case report form binder system, and providing systems security and access control.

The PALM 1 trial team received the distinguished 2019 David Sackett Trial of the Year Award from the Society for Clinical Trials due to the high-impact, high-quality research that was determined to improve humankind and provide the basis for a substantial, beneficial change in global health care. In addition, members of the PALM 1 trial team, including 19 FNL staff in CMRPD, received a 2019 NIAID Merit Award.

Until the COVID-19 pandemic, staff maintained a full-time presence in Kinshasa, with a rotating project management team providing essential programmatic oversight and helping to lead the local research team. The team developed local capacity and transferred research operations and project management knowledge and skills to Congolese colleagues to ensure local teams could implement the research and manage program operations requirements with minimal oversight. As part of continued in-country support, technical project managers oversaw operations and logistics through various subcontractors, who provided staffing and technical expertise for the emergency response logistics and medical management of patients in the Ebola treatment units. Staff also supported social analytics reporting through a subcontracting mechanism that allowed the social mobilization and psychosocial teams to develop messages to local communities.

FNL staff in CMRPD worked with NIAID, a subcontractor, and INRB to formalize the PALM base operations (warm base) as a priority since the program is now in transition from emergency response to steady state. Staff supports strategic planning efforts to define the vision, mission, and goals of the PALM research program and establish a governance structure and research operations infrastructure, including core staffing

requirements. A clinical research team with broad technical, scientific, and operational expertise will be designated so that the PALM research program can rapidly deploy a research response by drafting research studies, performing site assessments, and implementing new clinical research protocols in support of PALM strategic objectives.

Staff provided technical and project management oversight of cold-chain capabilities at the INRB campus in Kinshasa, which allows critical perishable research supplies and investigational product to be stored with redundant power and temperature monitoring. Staff also implemented an environmental monitoring system that allows for continuous, real-time temperature tracking for biospecimens and investigational product, and they trained local staff on proper usage of the equipment and response to temperature failures.

FNL staff in CMRPD worked with NIAID, INRB, and the U.S. State Department to establish a biorepository to track and store biomedical and research samples, which included implementing Agile biospecimen-tracking software, a Koovea cold-chain monitoring system, and a barcode labeling strategy. INRB laboratory staff were trained to use these systems and now manage them with minimal oversight from staff based in the U.S.

FNL staff in CMRPD also supported the establishment of an extensive electronic supply chain that covers pharmacy, laboratory, clinical, office, and general supplies spanning seven locations and 22 sublocations in the Democratic Republic of the Congo. Staff performed virtual and in-person trainings for U.S. and Congolese staff on managing the supply chain, conducting stock takes, uploading and adjusting current inventories, and tracking critical expiration dates. The electronic system notifies users of impending stock shortages and reorder point thresholds.

FNL staff in CMRPD established reorder points and expiration date notifications for laboratory and pharmacy supplies at field sites. Democratic Republic of the Congo staff members were trained to use Finale Inventory software with little oversight provided by staff in the U.S. The procurement team continued to implement a new shipment clearance process, in which critical shipments were pre-approved prior to shipping and perishables were included as part of the shipment. This enabled the paperwork and customs processes to begin early, allowing for timely clearance by the U.S. Embassy team upon arrival at the Kinshasa airport. FNL project managers in CMRPD established trackers that facilitated the shipment and reconciliation of more than 20 shipments from international vendors.

Staff worked creatively with subcontractor leadership to implement safety measures aimed to protect PALM research staff in the Democratic Republic of the Congo at the onset of the COVID-19 pandemic. CMRPD staff worked closely with subcontractor operations leads and INRB scientists to develop a risk reduction plan, which included returning staff to Kinshasa from the research sites in the eastern Democratic Republic of the Congo, a targeted testing strategy, decontamination of offices and laboratories, quarantining in place, and long-term telework agreements that were accompanied by any necessary equipment and work plans to ensure staff remain effectively engaged in the new remote working environment.

As a follow-on research activity to the PALM 1 trial, staff supported the planning and protocol development for PALM 2, a therapeutic trial to assess the efficacy of remdesivir in reducing Ebola virus detected in the semen of male survivors. This study was put on an indefinite hold due to the COVID-19 pandemic.

In addition, the FNL staff in CMRPD was requested to initiate protocol development and planning to implement the PALM COVID-19 Observational Study, which will enroll 2,000 adults and children in the Democratic Republic of the Congo. The study's primary objectives are to estimate the rate of and risk factors for hospitalization or death among COVID-19 outpatients during 28 days of follow-up.

CMRPD provided project management and programmatic oversight of activities conducted through a subcontract, and staff coordinated with the protocol team, which includes representatives from NIAID DCR, FNL, and INRB and collaborators at the Institute of Tropical Medicine (Belgium), to ensure overall operational research activities aligned with NIAID DCR's expectations. CMRPD staff provided scientific oversight to protocol development and supported program planning and research preparation activities. The study is expected to begin in FY2021.

Special Project: Ebola Clinical Research, Guinea

KEY ACCOMPLISHMENTS

- Completed the month 12 visits for the Partnership for Research on Ebola Vaccination (PREVAC) study

The FNL staff in CMRPD provides project management and technical oversight for the research infrastructure and operations to support clinical studies in Guinea. Support to the Research Program in Guinea (Programme de Recherche en Guinea [PREGUI]) includes managing international clinical research studies and collaborations through protocol-specific technical assistance, logistics, procurement, shipping, IT infrastructure, meeting coordination, travel support, research subcontract management, training support, and overall operations management.

The team facilitated engagement of partners, in alignment with the Guinea–U.S. governmental strategic plan to ensure that PREGUI is primed and responsive to join a diverse collaborative portfolio of research. Supported collaborations included the NIAID Laboratory of Malaria Immunology and Vaccinology; the Naval Medical Research Center; the Kirby Institute, Australia; the French National Institute of Health and Medical Research; the European and Developing Countries Clinical Trials Partnership, European Union;

and the Malaria Research and Training Center/ University Clinical Research Center, Mali.

The team facilitated the PREGUI research staff's training, including Good Clinical Practices and Good Clinical Data Management, and general and technical laboratory operations. This last area included use of the Applied Biosystems 7500 diagnostic equipment to support COVID-19 research studies. The Maférinyah research laboratory was selected as a country-wide reference laboratory for COVID-19 diagnostics in Guinea following the deployment of the Applied Biosystems 7500 equipment.

Staff supported the research operations through technical project management of research subcontracts. An important milestone was met this year: hiring for a full complement of 10 PREGUI research professionals supported under the warm base model was completed. The staff supported the ratification of a standard process to streamline the review and approval of new research proposals submitted to the PREGUI Executive Committee.

Staff provided technical and project management oversight to various research site renovations and maintenance projects, including a civil engineering initiative establishing walkways and adequate drainage and completion of renovations that transformed an old health center into a functional clinical research unit.

Staff supported completion of the month 12 visits for the PREVAC randomized controlled trial in December, concluding FNL's support to PREVAC in Guinea. The study enrolled 1,723 participants across two sites. Staff also worked on the close-out process for the PREVAIL IV randomized controlled trial, initiated the Dolutegravir and Darunavir Evaluation in Adults Failing Therapy (D^2EFT) HIV second-line treatment randomized controlled trial and the new pathogen-discovery sequencing study, and supported planning and preparation activities for two malaria studies.

The team also provided logistics and administrative support for meetings and training events hosted by PREGUI in Guinea. The PREGUI team contributed to scientific and collaborative program goals by participating in international and regional scientific conferences, including the American Society of Hygiene and Tropical Medicine annual meeting (U.S.), the Biological Threat Reduction Program meeting (Poland), the PREVAC-UP kickoff meeting (France), the EDTCP – PfTBV study kickoff meeting (Mali), the strengthening ethics in clinical trials in West Africa meeting (Guinea), and presentation of research program updates at the NIAID/DCR Research Symposium (U.S.).

Special Project: PREVAIL Liberia

KEY ACCOMPLISHMENTS

- Supported the PREVAIL fifth anniversary event in February, showcasing PREVAIL's accomplishments and presenting its five-year strategic plan
- Completed John F. Kennedy Medical Center/PREVAIL imaging suite renovations and computed tomography (CT) installation
- Supported the PREVAIL supply chain team restructuring to increase efficiencies and ownership by the Liberian team
- Successfully recompeted the multimillion-dollar statements of work for the PREVAIL warm-base activities and associated studies, ensuring a smooth transition between subcontractors

In support of viral hemorrhagic fever and other infectious disease initiatives, NIAID DCR requested FNL's CMRPD to provide significant support for infrastructure and clinical operations to conduct studies in Liberia, West Africa. The Liberia–U.S. PREVAIL was formed in 2014 at the height of the West African Ebola outbreak. Since then, PREVAIL has developed clinical research infrastructure across four sites: John F. Kennedy Medical Center (JFKMC), Redemption Hospital, C.H. Rennie Hospital, and Duport Road Clinic; a research laboratory at the Liberia Institute for Biomedical Research; and a renovated imaging suite at JFKMC, outfitted with a CT scanner to support PREVAIL III protocol research and other diagnostic purposes.

The PREVAIL clinical research portfolio includes the following studies: PREVAIL I, II, III, IV, VI, VII, VIII (activities are described in another section of this report); the Immunogenicity of Recombinant Vesicular Stomatitis Vaccine for Ebola-Zaire (rVSV[Delta]G-ZEBOV-GP) for Pre-Exposure Prophylaxis (PREP) in People at Potential Occupational Risk for Ebola Virus Exposure (PREPARE study); PREVAC (PREVAIL V); Partnership for Malaria Research Opportunities in Liberia (PROPEL-1/PREVAIL IX); and PREVAIL XI (COVID-19 observational study).

Monitoring and Execution of Subcontracts

FNL staff provided technical and clinical expertise, project management, and programmatic oversight of activities conducted through subcontracts for data management, laboratory and logistical support, clinical research staffing, and operational support.

A critical subcontract supporting the PREVAIL warm base was recompeted, with the award of the work to another subcontractor in May 2020. Successful transition to the new subcontractor was completed in August 2020 and included program coordination, human resources management, supply chain logistics, clinical research site operations, facilities site management, travel support, accounting and financial management, social mobilization and community engagement, regulatory and ethics support, information technology management, administrative support, and learning and professional development. The recompete will result in a structure that allows local Liberian operations management subcontractor staff to take a more active role in managing the clinical research network's operations.

Study Oversight

Screening, enrollment, and follow-up visits continued for PREVAIL I, III (and associated substudies), VIII, IX, and PREVAC at the PREVAIL clinical sites: JFKMC, Duport Road, C.H. Rennie, and Redemption Hospital.

A contingency plan was developed and implemented to determine how and when to perform study activities during the COVID-19 pandemic. FNL staff provided clinical project management expertise to reopen studies through a phased approach and initiated procurement of various program supplies.

The PREVAIL team began developing a COVID-19 observational protocol in Liberia and preparing for study implementation with the operational team. The study is estimated to enroll 500 participants with COVID-19 and 1,000 close-contact participants, for a total enrollment of 1,500 participants of all ages. The study will enroll over a two-year period, with three years of follow-up to better understand the disease characteristics in Liberia. The study was submitted to the local IRB on July 28, 2020, and began enrolling participants in September 2020.

Study Enrollment Table

Study Name	Recruitment Status	Enrollment Status	Date Halted	Estimated Start Date
PREVAIL I (vaccine)	Closed to enrollment, follow-up only	1,500	3/31/20	8/10/20
PREVAIL III (natural history; eye and neurology substudies)	Closed to enrollment, follow-up only	4,043	3/27/20	TBD
PREVAIL III substudy: CYTOF	Open for enrollment	265	3/27/20	TBD
PREVAIL III substudy: CT scan	Open for enrollment	157	3/27/20	TBD
PREVAIL III substudy: psychosocial sequelae	Open for enrollment	427	3/27/20	TBD
PREVAIL V (PREVAC)	Closed to enrollment, follow-up only	5,002	3/31/20	8/20
PREVAIL VIII (HONOR)	Open for enrollment	158/2,500	4/13/20	7/16/20
PREVAIL IX (PROPEL)	Open for enrollment	654	3/27/20	8/3/20

Program Oversight

Operational Meetings: Staff facilitated regular meetings with PREVAIL study teams to review operational processes to ensure efficiency and sustainability by developing standard operating procedures and proactively assessing anticipated rate-limiting steps for study protocols and working groups.

Annual Meeting: Staff helped to plan, oversee logistics for, and execute the PREVAIL fifth anniversary event held in Liberia in February 2020. This support included travel arrangements, venue coordination, and programmatic support for the agenda and supporting materials. The annual meeting showcased PREVAIL's accomplishments and presented its five-year strategic plan to key stakeholders. Staff supported the PREVAIL publications and presentations working group in developing more than 30 posters that were presented at the meeting.

Strategic Plan: In support of the PREVAIL strategic plan, staff continued to collaborate with the Liberian Ministry of Health and the National Public Health Institute of Liberia, the Centers for Disease Control and Prevention, the World Health Organization, and other international organizations to foster mutually beneficial relationships in support of PREVAIL. PREVAIL developed a new streamlined governance structure that reflects the vision for "One PREVAIL" and supports the the local Liberian management team's increased capacity to oversee all aspects of PREVAIL.

Laboratory and Clinical Site Infrastructure: Staff facilitated regular meetings with the PREVAIL study working groups and stakeholders to review operational processes to ensure efficiency and increased Liberian-led capacity and sustainability related to laboratory and clinical-site infrastructure. This included logistics for procuring, shipping, and installing a liquid nitrogen system at the PREVAIL biorepository and ensuring that the research protocol was implemented smoothly and operational research sites were safe by establishing site staffing assessments and logistics for site repairs and resource supply maintenance.

Supply Chain Management: Staff collaborated to fix the broken supply chain process in Liberia by assessing the issues; crafting an improved process with input from local site staff; and implementing a new system that connects the U.S. procurement team with Liberian clearing and receiving staff, inventory management staff, and staff managing supplies at the sites. CMRPD staff initiated and supervised the training, implementation, and use of the Finale Inventory system at all Liberian sites and laboratories to track inventory and submit supply requests.

Learning and Professional Development: The Enhancing PREVAIL Program was launched in FY2020 under the management of FNL's L&PD group in CMRPD. The program was developed to support one of the PREVAIL strategic plan goals: to establish and sustain the expertise required to conduct research by building to the staff's research, administrative, management, and foundational skills. The process flow was developed, endorsed by PREVAIL leadership, and rolled out to PREVAIL staff. FNL staff in L&PD helped manage the receipt, review, and routing of the application process for the program. Enhancing PREVAIL received 140 applications for funding opportunities. Due to the COVID-19 pandemic, all opportunities were transferred to an online format.

FNL staff in L&PD facilitated numerous instructor-led International Association for Continuing Education and Training sessions of Good Clinical Practices (GCP) for Clinical Research, configured and delivered a GCP awareness course, and qualified PREVAIL staff to deliver both GCP courses independently. FNL staff in L&PD assembled 30 hard-copy professional skills packets for PREVAIL staff members to complete so they could continue their professional development during COVID-19 restrictions.

FNL staff in L&PD also introduced a standard curriculum vitae/resume template for use by PREVAIL physicians when applying for fellowships and developed documents to support maintenance of a robust learning environment.

Special Project: FLU003/Respiratory Viruses

This project aims to establish and conduct studies to characterize the natural history of influenza, influenza-like illness, and other respiratory diseases, as well as evaluate novel therapeutic interventions for these diseases.

The FNL staff in CMRPD provides clinical research support related to study conduct, including, but not limited to, operational leadership; special project coordination and management; project strategy development, implementation, and management; and study team direction.

The International Network for Strategic Initiatives in Global HIV Trials (INSIGHT)

FLU003 Plus: This is a natural history study of participants hospitalized with confirmed or suspected influenza or influenza-like illness. In September 2019, NIAID DCR decided to continue study enrollment for an additional year. As of September 2020, 4,756 participants had been enrolled throughout the study. FNL staff provided operational, technical, and project management oversight of the study. Support included managing research subcontracts and agreements (review/approval of progress reports and invoices) as well as monitoring budgets, budget modifications, expenditures, and end-of-year forecasting.

INSIGHT004: This is a genomics substudy for the FLU002 Plus, FLU003 Plus, and INSIGHT006 studies. In September 2019, NIAID DCR decided to continue study enrollment for an additional year. As of September 2020, 6,382 participants had been enrolled. FNL staff provided operational, technical, and project management oversight of the study. Support included managing research subcontracts and agreements (review/approval of progress reports and invoices), as well as monitoring budgets, budget modifications, expenditures, and end-of-year forecasting.

The FLU003 Plus/INSIGHT004 protocols also allow for the enrollment of participants with other influenza-like illnesses (e.g., severe acute respiratory syndrome and Middle East respiratory syndrome). Enrollment of COVID-19 participants in these studies began in January 2020. It is anticipated that 500–600 participants with COVID-19 will be enrolled. As of August 31, 2020, 450 participants had been enrolled.

INSIGHT006: The INSIGHT006 (FLU-IVIG) study, which evaluated the use of anti-influenza hyperimmune intravenous immunoglobulin (IVIG) to treat participants with severe hospitalized influenza, was closed to enrollment in June 2018, and all study-related activities were completed during the summer of 2020. A total of 313 participants were enrolled, and 322 were randomized (randomization includes 16 participants from the INSIGHT FLU005 study). FNL staff provided technical and project management support for research subcontracts (including subcontract-specific close-out activities) and study site close-out. Results from the study were published in *Lancet Respir Med* in November 2019 (Davey Jr. R, Fernández-Cruz E, Markowitz N, et al., "Anti-influenza hyperimmune intravenous immunoglobulin for adults with influenza A or B infection (FLU-IVIG): a double-blind, randomised, placebo-controlled trial").

INSIGHT009: This is a randomized, double-blind, placebo-controlled trial of IVIG in adults with influenza B. Preplanning activities were conducted until early April 2020, when NIAID DCR decided to put the study on hold due to priority COVID-19 projects. Prior to the study delay, FNL staff worked with NIAID DCR on the structure of the work to be conducted. Multiple options were outlined to allow for specific regions by International Coordinating Center to commence enrollment during their active influenza seasons (for example, northern hemisphere, southern hemisphere), with a base option for study oversight, along with an option for just the IVIG manufacturing.

Special Project: Mali Clinical Research Program (University Clinical Research Center)

KEY ACCOMPLISHMENTS

- Contributed to initiation of the Epidemiology and Clinical Characteristics of Emerging and Re-emerging Infectious Diseases in Mali (ECERID) study in June
- Helped the University Clinical Research Center (UCRC) prepare for potential participation as a site in several COVID-19 DCR studies
- Supported final renovations to the UCRC biosafety level 3 (BSL-3) laboratory air handling units and mechanical system, resulting in the laboratory's selection as a COVID-19 testing facility in Mali

NIAID DCR partners with the University of Sciences, Techniques, and Technologies of Bamako (USTTB) in Mali to develop sustainable research programs in geographic areas of highly infectious disease burden and enhance clinical research capacity at African research sites in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals/Good Clinical Practice guidelines and

applicable U.S. government–mandated regulatory requirements. Through this initiative, the UCRC was established.

The FNL staff in CMRPD provides clinical research operation support services, project and program management, procurement and logistics support, and regulatory compliance support to the USTTB, UCRC.

The final renovation of the UCRC BSL-3 laboratory air handling units and mechanical system upgrade was completed. FNL staff collaborated with the subject-matter expert engineer, mechanical systems vendor, UCRC BSL-3 laboratory chief, DCR laboratory lead, and Mali service center staff to facilitate a successful renovation. The staff also coordinated with all parties to develop the scope of work for the renovation, arranged travel logistics for the engineer and mechanical systems vendor, tracked the shipment of the mechanical system equipment to ensure timely customs clearance, and monitored the renovation's progress. One outcome of these renovations was that the Malian Ministry of Health selected the laboratory as one of four COVID-19 testing facilities in the country.

The UCRC team was involved in site readiness preparation for possible participation in several COVID-19 DCR studies, including procedure development, regulatory requirement review, and facility modifications. These preparations resulted in the UCRC's selection to participate in the FLU003 Plus/Genomics Study.

The observational study ECERID began in June 2020 and is aimed at preparing for a rapid coordinated clinical investigation of acute infections of emerging and re-emerging infectious agents in Mali. This study was pivotal in the UCRC's ability to collect infectious disease data, including COVID-19 data, from hospitalized patients participating in the study. FNL staff coordinated planning, developed the budget, worked with the team to modify study requirements, purchased additional required personal protective equipment due to the COVID-19 pandemic, and ensured all logistical and monitoring requirements were in place.

The Mali UCRC team completed the PREVAC study participant month 12 visits and prepared for the start of PREVAC-UP. FNL staff worked with the UCRC team to monitor inventory and counseled the USTTB on negotiating the budget and preparing the clinical trial renewal agreement with the Center for Vaccine Development, Mali and Baltimore divisions. Filovirus Animal Nonclinical Group enzyme-linked immunosorbent assay testing began on PREVAC samples to evaluate the efficacy of the Ebola vaccine in this trial.

FNL staff worked with the Mali UCRC team and DCR leadership to establish and maintain a prioritized procurement plan, resulting in an opportunity to purchase an Applied Biosciences 3500 genetic analyzer sequencing platform with broad applications for resistance testing for HIV and other diseases. This platform will allow the UCRC to expand its capabilities and perform a broader range of testing at the laboratory.

Special Project: Mexico Emerging Infectious Disease Clinical Research Network

KEY ACCOMPLISHMENTS

- Prepared for and supported study initiation for INSIGHT011 COVID-19 study
- Supported the Mexico Emerging Infectious Disease Clinical Research Network (La Red) reorganization, leading to a more robust governance structure
- Supported expedited Federal Commission for the Protection Against Sanitary Risk (COFEPRIS; a Mexican regulatory authority) approval of the Adaptive COVID-19 Treatment Trials (ACTT)

La Red was established in September 2009 to share resources that enhance the conduct of research ranging from natural history studies to highly regulated multisite clinical trials.

The scope of work supported by La Red is aligned with NIAID DCR's primary objective of providing an effective rapid clinical research response in support of emerging disease priorities.

The FNL staff in CMRPD provides clinical research support related to study conduct, including operational leadership, coordination, and overall management of multiple special projects; contributes to the development, implementation, and management of project strategies; identifies and addresses project barriers; establishes project communication strategies; and directs study teams.

FNL staff in CMRPD supported the following La Red studies during FY2020:

An International Observational Study of Outpatients with SARS-CoV-2 Infection (INSIGHT011)

This study is anticipated to begin in early FY2021. To prepare for expedited startup, FNL staff modified an existing agreement with a qualified vendor to allow for study-specific support within La Red. FNL staff in CMRPD provided logistical support to ensure La Red could implement INSIGHT011.

Zik02

Staff provided project management and scientific support to finalize the Zik02 protocol and prepare for study initiation upon COFEPRIS approval. All activities for the Zik02 study are on hold due to the ongoing COVID-19 pandemic and other network research priorities. The study will evaluate the long-term neurocognitive performance of individuals with Zika virus infection.

Evaluating the Safety, Effectiveness, and Tolerability of Nitazoxanide in Addition to Standard Care for the Treatment of Severe Acute Respiratory Illness in People Who Are Hospitalized (NTZ-SARI)

The NTZ-SARI study, a randomized blinded treatment study comparing nitazoxanide versus standard of care for the treatment of hospitalized influenza-like illness, was closed to enrollment in March 2017. All study-related activities were completed in March 2020. FNL staff in CMRPD reviewed and approved the final task order report and worked with FNL research subcontracts to obtain a quick close-out agreement with the vendor. A total of 260 participants were enrolled, and 59 were randomized. Results from the study were published in *Clin Infect Dis* in 2019.

La Red Network Reorganization

Staff attended meetings and provided input to support the La Red reorganization initiative that led to a more robust governance structure, which included establishing a governing board, executive committee, and scientific steering committee. A network director was appointed, and a manager of the La Red Coordinating Center was hired to lead this effort. As a result, a site staff reorganization, a performance assessment, and training programs were implemented, and a new public-facing website was created. Staff worked with a vendor to align the La Red sites and coordinating center with the new organizational structure. The reorganization took approximately 18 months to complete and was completed in September 2019.

When the COVID-19 pandemic began, the leadership structure in place allowed La Red to quickly obtain the necessary approvals to participate in the ACTT study (arms ACTT-1 and ACTT-2). The new governance structure also allowed La Red to be considered a tier one site for the INSIGHT011 study.

Adaptive COVID-19 Treatment Trial

In support of NIAID Division of Microbiology and Infectious Diseases studies, staff quickly implemented processes for executing agreements and funding to expedite La Red's participation in ACTT-1 (remdesivir vs. placebo), which began in April 2020 and continued through June 2020. Having established processes and a formal communication plan also allowed for quick startup of ACTT-2 (remdesivir vs. remdesivir/baricitinib vs. placebo). FNL staff in CMRPD's Clinical Trials Management group regularly oversaw and advised on study implementation and monitoring activities. Ten participants were enrolled across La Red sites for ACTT-1. As of June 2020, 35 participants had been enrolled in ACTT-2.

Staff assisted in developing a detailed communication plan for the ACTT-1 and ACTT-2 studies that La Red used to streamline communication and obtain approvals. The plan clearly identified the lead for each functional area/group as well as who needed to be informed, which eliminated potential confusion and subsequent delays.

La Red worked with COFEPRIS leadership to obtain approval of the ACTT-1 and ACTT-2 protocols within one week. Historically, the timeline for COFEPRIS approval is three to six months. This accomplishment demonstrates La Red's ability to streamline activities for high-priority, emergency global emerging infectious disease research. Staff led numerous organizational meetings to ensure all stakeholders were aware of the integral next steps to move quickly toward study activation.

More information about the NIAID Division of Microbiology and Infectious Diseases ACTT-1 and ACTT-2 studies can be found in the "Adaptive COVID-19 Treatment Trials" section.

Special Project: The Indonesia Research Partnership on Infectious Diseases Network

KEY ACCOMPLISHMENTS

- Supported publication and acceptance for publication of multiple manuscripts in highly visible journals for the Etiology of Acute Febrile Illness Requiring Hospitalization (AFIRE) study
- Supported achievement of the primary study objective for the Tuberculosis Research of INA-RESPOND on Drug Resistance (TRIPOD) study

The Indonesia Research Partnership on Infectious Diseases (INA-RESPOND) Network was formed in 2010, together with the National Institute of Health Research and Development Ministry of Health (MoH) and several research sites in Indonesia, to build and sustain scientifically productive research infrastructure and to translate and implement research findings into mutually beneficial public health practices in Indonesia. The Indonesian MoH has prioritized malaria, avian influenza, dengue, HIV/AIDS, tuberculosis, and neglected infectious diseases, among others.

The FNL staff in CMRPD provides clinical management oversight and administrative, programmatic, scientific, and technical support to the INA-RESPOND Network to increase understanding of disease pathogenesis and prevent and treat infectious diseases that affect Indonesia and align with the MoH's priorities. Staff provided support to the following INA-RESPOND studies:

AFIRE – INA101

This study to identify the etiology of acute febrile illness cases and evaluate clinical manifestations and outcomes closed and is in the analysis phase.

Four manuscripts resulting from this study were published on the following topics:

- Dengue (Utama IMS, Lukman N, Sukmawati DD, Alisjahbana B, et al., *PLoS Negl Trop Dis.* 2019 Oct; 13(10): e0007785)

- Acute febrile illness in Indonesia (Gasem MH, Kosasih H, Tjitra E, Alisjahbana B, et al., *PLoS Negl Trop Dis.* 2020 Jan; 14(1): e0007927)
- Leptospirosis (Gasem MH, Hadi U, Alisjahbana B, Tjitra E, et al., *BMC Infect Dis.* 2020 Feb; 20(1):179)
- Rickettsia (Lokida D, Hadi U, Lau C-Y, Kosasih H, et al., *BMC Infect Dis.* 2020 May; 20[364])

Manuscripts on severe acute respiratory infections and chikungunya were accepted for publication.

TRIPOD – INA102

The primary study objective for TRIPOD was achieved: to report the rate of multidrug-resistant tuberculosis (TB) among new and re-treated TB cases. A total of 490 participants were enrolled across six sites. As of February 2020, the number of ongoing participants was 79; 186 participants completed the study. Follow-up visits were conducted and will continue through November 2020 (for multidrug-resistant TB participants).

Prospective Observational Cohort Study on HIV Infection and Risk Related Coinfections/ Comorbidities in Indonesia (INA-PROACTIVE) – INA104

The INA-PROACTIVE study aims to collect standardized baseline and longitudinal data describing the course of HIV disease in antiretroviral-naïve and treatment-experienced individuals to inform policy. Study enrollment was completed at 16 of 19 sites, and follow-up visits continued. As of March 2020, 4,277 of up to 10,000 planned participants were enrolled across 19 sites. NIAID staff, INA-RESPOND staff, and FNL staff in CMRPD finalized the study Statistical Analysis Plan in January 2020.

Implementing a Combination of Clinical Parameters (Rapid Diagnostic Tests, Biomarkers, and Standard of Care Procedures) for the Etiology Diagnoses of Pneumonia in Pediatric Patients to Improve Clinical Management in Indonesia (PEER-PePPes) – INA201

The aim of the PEER-PePPes study is to develop an algorithm for diagnosing viral and bacterial pathogens in pediatric patients with pneumonia. The study closed to enrollment in September 2019, with 189 participants enrolled. Staff ensured that close-out activities occurred as follows: preparations for a data analysis meeting that occurred in Jakarta in January 2020 and site close-out visits that occurred in April and August 2020. Preparations for database lock are also underway.

Validation of the Schistosomiasis Point-of-Care Circulating Cathodic Antigen (POC-CCA) Rapid Urine Test for Qualitative Detection of Schistosoma japonicum (Schistosomiasis) – INA105

The objective of the schistosomiasis (INA105) study is to estimate the accuracy of the schistosomiasis POC-CCA urine test for monitoring *Schistosoma japonicum* infection in settings of low prevalence such as the Lindu, Napu, and Bada regions of Central Sulawesi, Indonesia. The study closed to enrollment in February 2020, and follow-up visits are ongoing. A total of 149 participants were enrolled. The study team worked on synchronizing REDCap and completing study documents.

INA-RESPOND Observational Research on Infectious Disease Outbreaks and Difficult Cases of Unidentified Etiology in Indonesia (INA-ORCHID)

This retrospective and prospective observational study has been developed, though enrollment has not yet begun. It will investigate suspected infectious diseases of unknown etiology prospectively during outbreaks and at health care facilities as well as retrospectively through historical samples where no etiology was ever determined.

A Phase IIIB/IV Randomized Open-Label Trial to Compare Dolutegravir with Pharmaco-Enhanced Darunavir Versus Dolutegravir with Predetermined Nucleosides Versus Recommended Standard of Care Antiretroviral Regimens in Patients with HIV-1 Infection Who Have Failed Recommended First Line Therapy (D^2EFT)

This study is currently ongoing. More information can be found in the "HIV Clinical Research in West Africa" section.

Subcontract Monitoring and Program Oversight

FNL staff in CMRPD provided technical expertise, clinical project management, and programmatic oversight of activities conducted through subcontracts for laboratory and logistical support and human resource administration.

Staff managed daily aspects of INA-RESPOND program planning and performance—project management and reporting, procurement and budget oversight, communications, travel, and logistical support—and provided oversight and mentoring for data management activities.

Staff traveled to Indonesia to support: (i) quarterly Network Steering Committee meetings, (ii) study protocol meetings, (iii) study-site- and laboratory-related activities (e.g., assessments, monitoring visits, data management), and (iv) financial planning and subcontract management.

In response to the COVID-19 pandemic, the subcontractor provided periodic updates regarding the ongoing situation in Indonesia. Travel was cancelled (including conferences and monitoring visits), and all monitoring visits were conducted remotely. Participant recruitment (as applicable) and study follow-up visits were delayed for the PROACTIVE, D^2EFT, and TRIPOD studies. INA-RESPOND is working to implement COVID-related studies, including the DCR-funded INA-ORCHID protocol and the INSIGHT network–sponsored observational study.

FNL staff worked with NIAID and Indonesian partners to identify additional funding for the TRIPOD and INA-PROACTIVE studies.

Ebola Virus and Other Infectious Diseases

Funding from this task order was used to support Ebola research in Liberia and the Democratic Republic of the Congo, and activities are described in those sections.

Zika Mexico

Funding from this task order was used to support the La Red Zik02 study, the INA-RESPOND schistosomiasis study, and the Mali UCRC ECERID study. Activities are described in those sections.

Viral and Hemorrhagic Fevers: Ebola

Funding from this task order was used to support Ebola research in Liberia, and activities are described in the "Special Project: PREVAIL Liberia" section.

Respiratory Diseases

KEY ACCOMPLISHMENT

- Supported publication of two manuscripts for NIAID Influenza Research Collaboration studies IRC004 and IRC005

The NIAID Influenza Research Collaboration aims to establish and conduct studies to characterize the natural history of influenza, influenza-like illness, and other respiratory diseases, as well as evaluate novel therapeutic interventions for these diseases.

FNL staff members provide clinical research support related to study conduct, including operational leadership, special project coordination and management; project strategy development, implementation, and management; and study team direction.

The NIAID Influenza Research Collaboration

Antiviral Efficacy of Combination Antivirals in the Treatment of High-Risk Outpatient Influenza (IRC003): The study closed to enrollment in April 2016. Secondary analysis of the combined IRC003/IRC004 data for a secondary manuscript, "A Randomized Double-Blind Phase 2 Study Comparing the Efficacy, Safety, and Tolerability of Combination Antivirals (Amantadine, Ribavirin, Oseltamivir) versus Oseltamivir for the Treatment of Influenza in Adults at Risk for Complications," has been completed, but the generation of the intended manuscript has been delayed.

Antiviral Efficacy of Oseltamivir Versus Placebo in Low-Risk Outpatient Influenza (IRC004): The study was closed to enrollment in October 2017. A total of 709 participants were enrolled, and 560 were randomized. Results from the study were published in *Clin Infect Dis* (Beigel JH, Manosuthi W, Beeler J, Bao Y, Hoppers M, Ruxrungtham K, et al., 2020) and showed that oseltamivir decreased viral shedding in the low-risk population enrolled in the study but did not significantly decrease the time for clinical symptoms to resolve.

Randomized Double-Blind Phase III Study Comparing Efficacy and Safety of High-Titer Versus Low-Titer Anti-Influenza Immune Plasma for the Treatment of Severe Influenza A (IRC005): The study was closed to enrollment in August 2018. Completion of all study-related activities (final monitoring visits and study site closures), including the end of the period of performance for the research subcontract supporting this effort, occurred in December 2019. A total of 200 participants were enrolled, and 140 were randomized. Based on the study results, a manuscript was published in *Lancet Respir Med* (Beigel JH, Aga E, Elie-Turenne M-C, Cho J, Tebas P, et al., 2019) describing that high-titer anti-influenza plasma did not show a significant benefit over non-immune plasma in the treatment of patients with severe influenza A.

Funding from this task order was also used to support the INSIGHT006 and INSIGHT009 studies, and activities are described in the "Special Project: FLU003/Respiratory Viruses" section.

Ebola Virus and Other Emerging and Re-emerging Infectious Diseases

Funding from two task orders was used to support the INA-PROACTIVE study as well as Ebola research in Guinea and Liberia. Activities are described in those sections.

HIV Clinical Research in West Africa

NIAID DCR requested FNL's CMRPD services to develop and manage a portfolio of multi-year HIV/coinfection clinical research studies tailored to HIV/AIDS in West Africa. Building on the partnerships and capacity established for viral hemorrhagic fever clinical research efforts, the FNL staff supports scientific/clinical, technical, and program/project oversight for the development, rapid deployment, and management of an HIV clinical research network in West Africa.

PREVAIL VIII: A Cohort Clinical, Viral, and Immunologic Monitoring Study of People Living with Retroviral Infection in Liberia

KEY ACCOMPLISHMENTS

- Initiated enrollment and follow-up visits for the PREVAIL VIII: A Cohort Clinical, Viral, and Immunologic Monitoring Study of People Living with Retroviral Infection in Liberia (HONOR) study
- Secured funding to continue current and anticipated study operations and activities through 2024

HONOR is a five-year observational cohort study that intends to describe major social/demographic, clinical, immunological, and virological characteristics of HIV

disease in the study population at baseline and to describe the course of HIV disease in the study population as a whole or by subgroup.

Screening, enrollment, and follow-up visits continued at the John F. Kennedy Medical Center in Liberia. The HONOR study was initiated on August 26, 2019, and baseline visits began in October 2019. Month six visits began in April 2020. As of June 2020, 199 participants were screened, 99 percent of whom tested positive for HIV. Of those who tested positive, 80 percent (approximately 158) were enrolled.

Staff provided technical expertise, clinical management, and programmatic oversight of activities conducted through subcontracts to provide overarching data management, laboratory and logistical support, clinical research staffing, and administrative/infrastructure/ financial support. Multiple agreement modifications were executed to extend the period of performance, provide funding, and add option years to one of the vendors.

Staff managed daily aspects of program planning and performance, reviewed and approved all procurement, and worked with NIAID and PREVAIL leads to review operational processes to ensure efficiency and sustainability. An action plan was generated and agreed upon between FNL, NIAID, and the laboratory support vendor to establish support for equipment repair and laboratory training for all PREVAIL studies.

To address the study's funding expiration, FNL worked with NIAID leadership to track timelines, project schedule, and budget shortfalls and establish a funding contingency plan. Consequently, a task order was awarded to allow for the HONOR study's continuation and completion through 2024.

Staff worked with the laboratory support vendor to ship reagents and supplies to John F. Kennedy Medical Center so that the study could reopen during the COVID-19 pandemic. The supplies were expected to arrive at the site after mid-July 2020, but instead arrived in early June 2020. The HONOR study resumed in July 2020.

Information about other PREVAIL studies and activities can be found in the "Special Project: PREVAIL Liberia" section.

Dolutegravir and Darunavir Evaluation in Adults Failing Therapy Trial

The Dolutegravir and Darunavir Evaluation in Adults Failing Therapy (D^2EFT) trial is a Phase IIIB/IV randomized open-label trial to establish whether a simple, novel combination of antiretroviral drugs (dolutegravir and ritonavir-boosted darunavir) is as safe and effective as the currently recommended World Health Organization standard-of-care second-line regimens. It primarily evaluates whether a simplified approach of dolutegravir with pharmaco-enhanced darunavir or dolutegravir with predetermined nucleosides compares to recommended standard-of-care regimens and, secondarily, evaluates how the two experimental treatments compare against each other.

FNL staff in CMRPD provided clinical project management support, budget/timeline tracking/ monitoring, and technical oversight of subcontracting activities. Monitoring visits were completed for each lead site in Guinea, Indonesia, and Mali. Clinical and research laboratory testing and biospecimen management training were completed at all sites. Study sites were activated in February and March 2020.

Staff monitored technical progress to support expanded enrollment targets and implementation of the clinical study at new research sites in Guinea, Indonesia, and Mali to match the standard of currently activated research sites.

Cytokine Research and Development Initiatives: IL-15 and IL-27

DCR's preliminary approach to this effort was to launch an initial study involving recombinant interleukin-27 (IL-27), a heterodimeric cytokine that preferentially inhibits HIV-1 replication in monocyte-derived macrophages (MDMs), one of the suspected reservoirs for HIV infection; it also induces HIV resistance in MDMs. Studies of similar cytokines are anticipated, as promising scientific findings have been revealed in this initial study. IL-27 significantly induces interferon-related antiviral genes in MDMs and has been shown to be capable of inhibiting simian immunodeficiency virus infection in nonhuman primate MDMs.

Initial laboratory work supporting this effort began in 2014. The FNL staff continues to provide technical project management of a research subcontract to study the roles of IL-27 and IL-15 in viral immunity and their impact as a potential cytokine-based therapy in people living with HIV.

Encouraging results from the research being conducted by Georgetown University Medical Center has resulted in multiple manuscripts currently under review.

Division of Intramural Research

Support Provided by the Biomedical Informatics and Data Science Directorate

Clinical Research Support

KEY ACCOMPLISHMENTS

- Frederick National Laboratory for Cancer Research (FNL) staff processed sequencing and clinical data for approximately 8,000 COVID-19 patient germline samples from a cohort from Italy.
- FNL staff in the National Institute of Allergy and Infectious Diseases (NIAID) Collaborative Bioinformatics Resource handled 58 separate projects from 28 different principal investigators.

- The team helped transition to a new sequencing core and developed novel workflows for human leukocyte antigen typing and candidate variant prioritization.

NIAID Collaborative Bioinformatics Resource Year Two Summary

This year, the NIAID Collaborative Bioinformatics Resource (NCBR) handled 58 separate projects from 28 different principal investigators. Of these 58 projects, 15 are considered complete, 31 projects have the primary analysis completed, seven are in progress, and five projects are awaiting sequencing data.

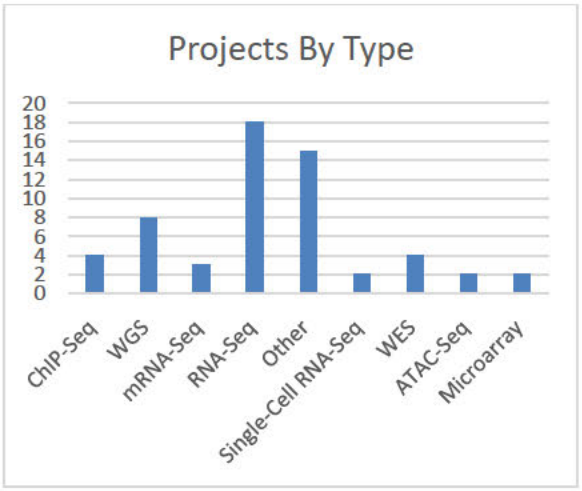

Figure 1. Total project breakdown for the past year.

Analysis Management Portal (NIAID and NCI Center for Cancer Research)

The Analysis Management Portal (AMP) (https://abcs-amp.cancer.gov/) is a collaboration software suite, supporting internal and external customers, that allows users to track and manage analysis project requests. AMP enables teams to collaborate remotely. The initial release includes management and tracking of analysis requests. AMP currently supports more than 300 users across five groups, with more than 300 analysis requests to date.

NCBR Group Project Highlights

NCBR-97 (scRNA-seq on thymic epithelial cells isolated from wild-type and *Rag1*-mutant mice)

In this project, FNL staff in NCBR used single-cell RNA sequencing (scRNA-seq) data generated with the high-throughput sequencing of RNA isolated by the crosslinking immunoprecipitation (CITE-seq) approach, and they implemented clustering, cell-type identification, differential expression, and trajectory analysis to study the heterogeneity among thymic epithelial cells (TECs) of mice at different developmental points and genetic backgrounds, including mutant mice carrying *Rag1* hypomorphic mutations observed in patients with immune deficiency and immune dysregulation.

TECs isolated from adult *Rag1*-mutant mice revealed an excess of cortical TECs (cTECs) that segregated in different clusters. The reduced medullary TEC (mTEC) compartment showed a similar distribution of previously described mTEC I–IV subsets, suggesting perturbed mTEC development, rather than differentiation into functional subsets. To address whether such abnormalities of cTEC and mTEC abundance and in *Rag1*-mutant mice may reflect defects in TEC development, the staff extended scRNA-seq analysis to TECs from wild-type mice of neonatal age. By comparing the gene expression and cellular developmental trajectory patterns in more than 30 sample- and cell-type-specific subpopulations, the staff identified potential drivers of dysregulation (in mutant mice) present in the interferon, NOTCH, Wnt, and NF-κB signaling pathways, some of which were genes associated with epithelial cell development and transcriptional regulation. These findings provide biological insights into potential mechanisms leading to immune deficiency phenotypes observed in human patients. This project is in the manuscript preparation phase.

NCBR-89 (Assessment of causative biological pathways in disease severity in multiple sclerosis)

Multiple sclerosis (MS) is a debilitating disease caused by an abnormal immune system response directed against the central nervous system. NCBR integrated SOMAscan-based proteomics data and genotype data using a decision-tree-driven machine learning approach to identify genetic variants predictive of MS severity. Potential protein expression quantitative trait loci (pQTLs) were identified using data from a healthy cohort, and their impact on the protein levels was replicated in a disease cohort. The final set of validated genetic variants was found to be present mainly in genes associated with biological processes related to the central nervous system, including remyelination and neurogenesis, paving the way for therapeutic decisions tailored to patients with different MS severity levels.

NCBR-15/NCBR-65 (RNA-Seq discovery of circulating parasite mRNA in infected host plasma as a marker of active infection)

A major challenge in treating patients infected with macroparasites is knowing when the parasite has been eradicated and treatment can be stopped. Previous attempts to measure circulating DNA from host plasma has shown that dead macroparasites can shed DNA into the host plasma for weeks after eradication, making DNA a poor marker for successful treatment. Therefore, mRNA biomarkers may work in patients with current infection of parasitic diseases, including elephantitis, tapeworm, and river blindness. Using noninfected control samples and a novel pipeline that NCBR developed, we were able to identify potential regions in the parasite genome that were biomarkers. This pipeline leveraged the power of kmer-

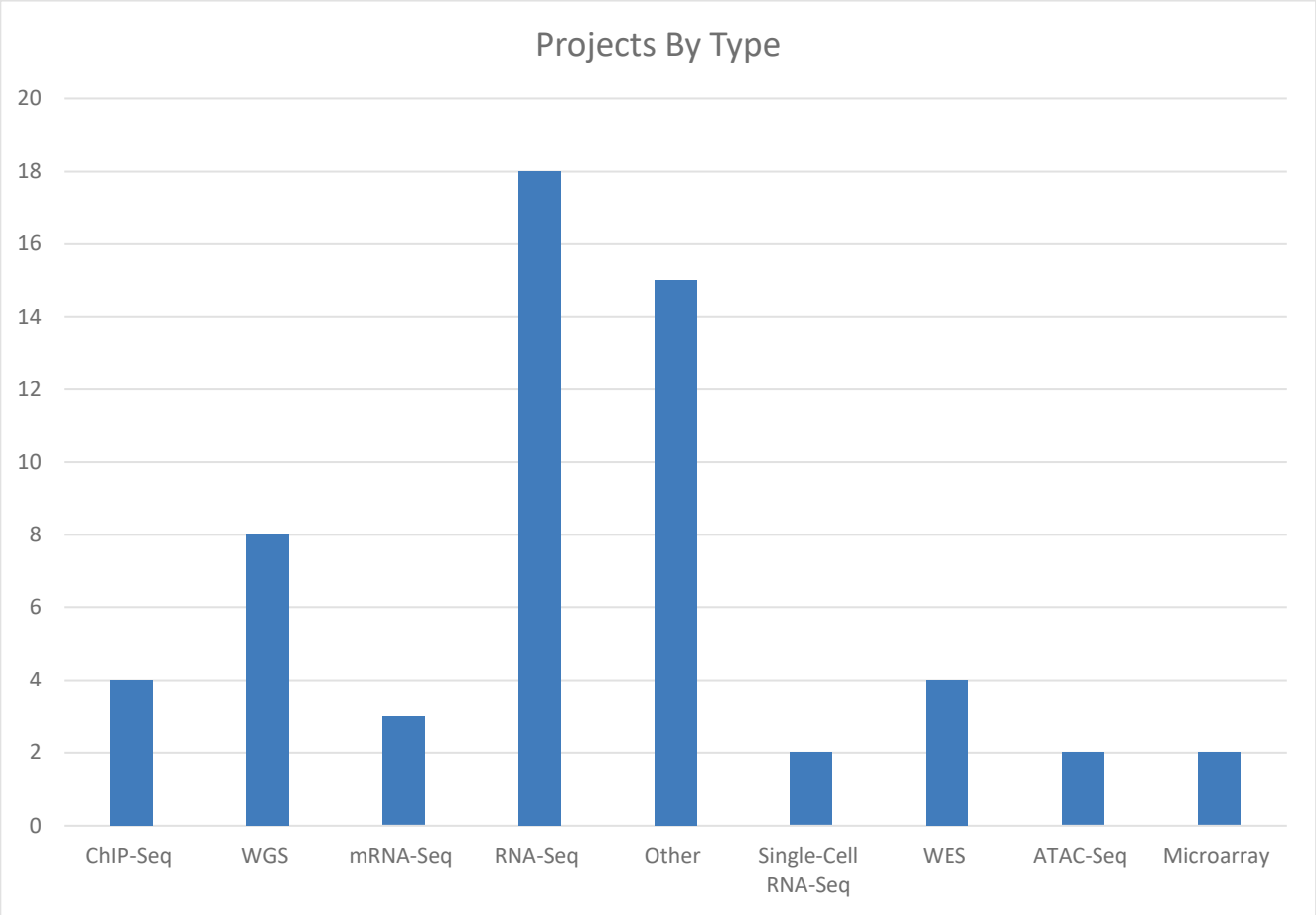

Figure 1.Total project breakdown for the past year.

based classification to eliminate all contaminating sequencing reads from both nontarget microbes and the host. This focused on those sequences that were extremely likely to be derived from the parasite of interest. Filtering was automated to eliminate those regions of off-target coverage in the control or repetitive regions. The remaining regions of coverage in the parasite genomes were compared via the Basic Local Alignment Search Tool against the nonredundant nucleotide database and found to be specific to the parasite of interest, suggesting the approach's significant potential for identifying active infection.

NCBR-75 (Genome-wide screens for lineage- and tumor-specific genes modulating MHC-I and MHC-II immunosurveillance in human lymphomas)

This project aimed to identify positive and negative regulators of major histocompatibility complex I (MHC-I) in human diffuse large B-cell lymphomas. The Cancer Genome Atlas was analyzed to determine whether there was a correlation between gene expression and CD8+ T-cell signatures. Approximately 30 positive and 10 negative MHC-I regulator genes showed statistically significant positive correlations with CD8+ signatures across more than 20 of the analyzed cancer cohorts. They are the most likely to be useful candidates for immunotherapy to manipulate MHC-I immunosurveillance in cancers, infectious diseases, and autoimmunity. This manuscript is currently in press at *Immunity*.

NCBR-16/NCBR-17/NCBR-18/NCBR-54/NCBR-55 (Epigenetic analysis of HPV genome regulation via Sp100 and the macroH2A histone variant; HiC analysis of human–HPV genome associations)

Human papillomaviruses (HPVs) like HPV-31 integrate their DNA into the host genome and can promote cancer in their human hosts. NCBR is analyzing where these integrations are likely to occur in the human genome and what might be driving those patterns. This includes comparisons of HPV-insertion breakpoints to enhancers and identification of broad FANCD2 domains (a marker of DNA damage) in various cancer lines. One particularly fruitful effort involves the analysis of Hi-C data from an HPV-31-positive keratinocyte cell line. Since it is likely that the original integration occurs in regions of the genome where the virus and the host DNA are in close proximity, the idea was to identify regions of the human genome that tended to share the same space as the HPV genome. HPV fragments were significantly enriched near highly expressed gene family clusters such as histones, 5S rRNAs, ZNF transcription factors, and keratins.

NCBR-22 (Preprocessing, quality control, and downstream analysis of the NIAID Clinical Sequencing Initiative)

NCBR has taken on the responsibility of downloading, preprocessing, quality control (QC), and downstream analysis of the NIAID Clinical Sequencing Initiative (CSI). Over the past year, the NCBR staff has helped transition to a new sequencing core and developed novel workflows for human leukocyte antigen typing and candidate variant prioritization. As of June 2020, the staff had processed whole-exome and single-nucleotide polymorphism array data from more than 2,000 patients. In addition, after they identified recurring issues with deliveries from the sequencing facility, the staff developed a batch Variant Call Format (VCF) concordance tool as an additional QC measure. This tool allows large sequencing projects to rapidly check batch-to-batch concordance at the cohort and individual sample level, thereby identifying potentially problematic samples with ease. There are no published tools available that can accomplish this multi-sample comparison, and this tool will likely be of value to large variant analysis projects.

A major challenge in clinical mutation analysis is that, even with extensive coverage and functional analyses, a large proportion of cases remain without solved germline mutations. While there is a myriad of reasons for this, one major contributor is that many potentially causative mutations are of unknown functional consequence and are therefore ignored in the clinical analysis. To overcome this, the NCBR staff is working to develop an improved candidate variant identification approach that integrates mutation and Human Phenotype Ontology (HPO) data from solved and unsolved cases. This approach uses the HPO data to cluster samples, then builds a classifier to identify candidate mutations/genes based on their proximity in pathways and protein–protein interaction networks to the causative genes from solved cases.

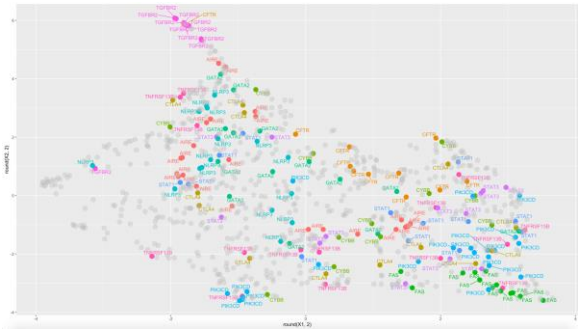

Figure 2. Uniform Manifold Approximation and Projection clustering of solved (colored) and unsolved (grey) cases based on HPO annotations. Solved cases are labeled with the causative gene.

In the early stages of development, the staff has already been able to use this tool on the NIAID CSI cohort to identify candidate mutations that were previously overlooked in patients with Loeys-Dietz syndrome. The staff is now working to extend the approach to the entire NIAID CSI cohort.

The staff is currently preparing a manuscript for the first 1,000 probands for this project and will publish the HPO classification approach in parallel.

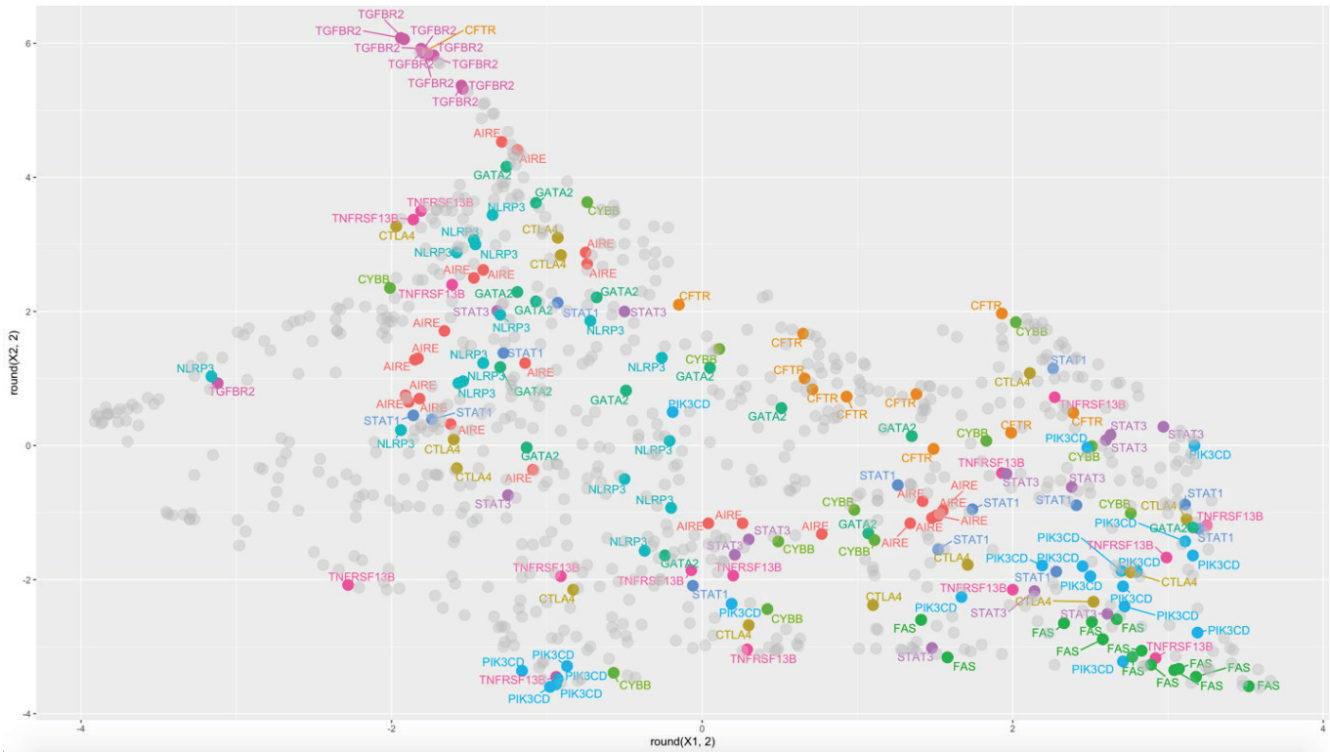

Figure 2. Uniform Manifold Approximation and Projection clustering of solved (colored) and unsolved (grey) cases based on HPO annotations. Solved cases are labeled with the causative gene.

NIAID COVID-19 Research Support

Tsang Laboratory COVID-19 sequencing

The NCBR staff has been actively supporting Dr. John Tsang's laboratory's sequencing efforts to look into single-cell CITE-seq, single-cell Assay for Transposase-Accessible Chromatin with high-throughput screening (ATAC-seq), and bulk RNA-Seq from patients infected with the novel coronavirus SARS-CoV-2. The staff has helped to coordinate the sequencing runs as well as processing, QC, and delivery of more than 10 NovaSeq flowcells. The staff has been able to process every sequencing run in less than 24 hours after sequencing is completed. In most cases, the staff has optimized the process so that it only takes a few hours to deliver the results to the Tsang laboratory.

Whole-genome sequencing and clinical analysis of approximately 8,000 COVID-19 infected patients from northern Italy

FNL staff in NCBR are providing critical support to the NIAID COVID-19 consortium through variant calling, QC, and analysis of approximately 8,000 COVID-19-infected patients from northern Italy. For this effort, they facilitate transfer of the raw data from the Center for Cancer Research Sequencing Facility, process all samples from raw reads through to analysis-ready germline variants, and then deliver the final variants to members of the consortium for analysis. For the QC and variant-calling portions of this effort, the staff developed a novel whole-genome sequencing workflow that allows them to process batches of approximately 200 samples in less than four days. In addition, the pipeline scales linearly up to and beyond the 8,000 target samples, and all variant calling is cumulative, so that each batch is genotyped jointly with all previously processed batches.

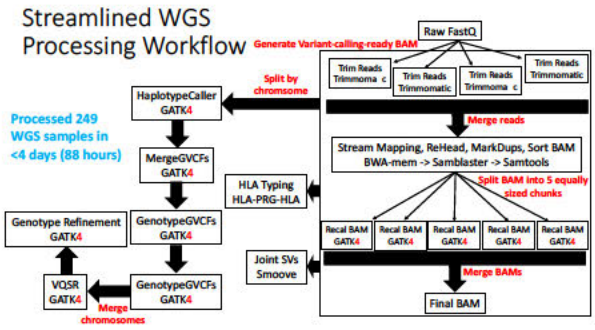

Figure 3. Streamlined whole-genome sequencing processing workflow.

Clinical and laboratory data preprocessing, normalization, and QC

Thorough data preprocessing, normalization, and QC are critical components of a large, complex analysis of genomic, clinical, and laboratory data across thousands of patients from multiple locations. FNL staff in NCBR are contributing to this effort, supporting the COVID-19 Data Management Working Group to automate the processing of incoming Italian patient raw data in multiple formats and prepare it for upload to data sharing and analysis platforms. The staff has developed a workflow based on optical character recognition and XML scraping to pull critical timecourse data (e.g., dates of symptom onset, drug treatment courses, discharge, etc.) from hospital images and generate normalized data tables. They are also working to generate an automated workflow for all raw data tables.

In addition, FNL staff are customizing and deploying independent instances of the Analytics and Visualization through interactive Data Dashboard and the Collaborative Data Sharing Platform for a large COVID-19 collaboration. The team also provided guidance on data review and automation tasks to help streamline data flow in the project and provide transparency in data validation and access tasks.

Support Provided by the Biopharmaceutical Development Program

Development of Biopharmaceuticals

Epstein-Barr Virus gHgLgp42-Ferritin

The Epstein-Barr Virus (EBV) gHgLgp42-Ferritin virus-like particle (VLP) bulk drug substance was manufactured. Three lots of Good Manufacturing Practice-Source grade plasmids needed for VLP production were subcontracted, manufactured, and released prior to use in the VLP production process. A fill/finish of the bulk drug has been tentatively scheduled for the latter half of 2020.

Conjugated Malaria Vaccine

A number of activities were completed in the ongoing statement of work in support of the Laboratory of Malaria Immunology and Vaccinology's (LMIV) malaria vaccine efforts. Purification of PVS230D1M was completed, yielding an excess of bulk purified product, the majority of which remains in storage. A small portion was used to successfully transfer the conjugation process from LMIV, after which the process was scaled up to produce bulk clinical PVS230D1-Exoprotein A (EPA) conjugate, which is presently in storage awaiting vialing. At LMIV's direction, the focus then shifted to the manufacture of EPA to provide GMP material that can be used to conjugate with multiple vaccine candidates. To that end, an engineering fermentation was performed but was unsuccessful. Further efforts have been on hold as a result of the COVID-19 pandemic.

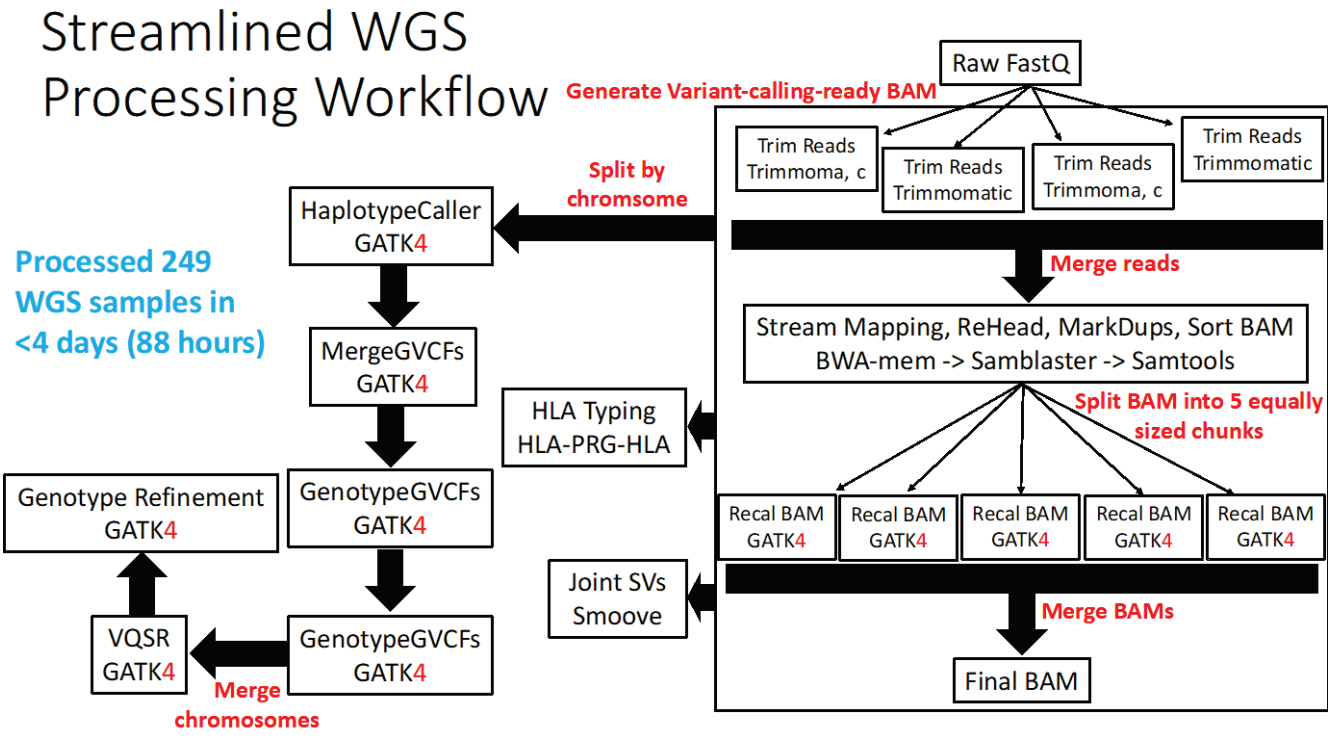

Figure 3. Streamlined whole-genome sequencing processing workflow.

Support Provided by the Cancer Research Technology Program

Clinical Research Support

Protein Expression Laboratory

In FY2020, NIAID-PEL supported the work in Dr. Jesus Valenzuela's laboratory in the Laboratory of Malaria and Vector Research (LMVR) by producing over 100 liters of mammalian cell culture expressing various vector salivary proteins for purification at LMVR. In addition, several purifications were carried out at PEL to support this project, and additional productions of tick Ixolaris protein were performed to support COVID-19 research.

The Sequencing Facility R&D group

The Sequencing Facility R&D group has been working closely with NCI and NIAID investigators to help them with their single-cell projects (single-cell gene expression or variable, diversity, and joining analysis; cellular indexing of transcriptomes and epitopes by sequencing; cell-hashing; etc.), ATACseq projects using (fresh and frozen) cells and tissues, nanopore sequencing (direct RNA and long-read DNA), optical mapping using Bionano Saphyr, etc. In addition to in-house R&D projects for testing and optimization of various new applications and protocols, the R&D group processed 150+ samples from 13 different labs for single-cell sequencing, ATACseq, direct RNA sequencing, or optical mapping in FY2020. Additionally, the R&D group tested new instruments like Mission Bio Tapestri and Celsee Genesis for single-cell library preparation, Megaruptor 3 for high-molecular weight DNA shearing, and Bionano Saphyr for optical mapping. R&D team members have so far co-authored four manuscripts published in FY2020.

Intramural Research Clinical Laboratory Improvement Amendments

In FY2020, the Clinical Laboratory Improvement Amendments (CLIA) Molecular Diagnostics Laboratory (CMDL) provided Sanger sequencing for genes known to be important in Chronic Granulomatous Disease. These include CLIA-certified assays for the coding domains of commonly mutated genes (NCF2, CXCR4 (WHIM syndrome), CYBA, CYBB, NCF4, CD18, IRAK4, CLIC1).

CMDL has designed additional assays and updates them regularly to account for new genomic information related to novel variants identified in the population. During FY2020, sequencing has been performed on more than 50 patient samples.

CMDL established and characterized the performance of a new droplet digital polymerase chain reaction assay for the detection of copy number variation at the NCF-1 locus. This is a particularly challenging problem, as the locus contains variable copies of one gene and two pseudogenes that are more than 99 percent identical.

CMDL also provides support for characterization of alterations in NCF-1, CYBB, GP91, and CTLA4 for Dr. Malech's preclinical research program.

The Genomics Technology Laboratory integrated a new NanoString assay platform into the laboratory during FY2020 and trained staff on the molecular signature assay to be used in support of the MasterLymph clinical trial. The clinical project has been placed on hold and a stop work order is currently in place. No activity is planned on this project until the stop work order is removed or modified.

Support Provided by the Clinical Research Directorate

Clinical Operations

The Clinical Research Directorate (CRD) provides clinical care professionals and clinical research support staff to meet the evolving requirements of National Institute of Allergy and Infectious Diseases (NIAID) Division of Intramural Research–sponsored clinical research programs and studies. Specifically, CRD staff provided pharmacy, team coordination, and administrative support to the National Institutes of Health COVID-19 Treatment Guidelines Panel, and they provided study coordination, patient recruitment, and screening support for multiple COVID protocols.

A protocol nurse coordinator received a 2019 NIAID Merit Award. She was a co-author on a *J Allergy Clin Immunol Pract* publication about platelet-derived growth-factor-alpha-positive myeloid neoplasm presenting as eosinophilic gastrointestinal disease (Constantine GM, et al., 2020).

Drug Discovery and Development Program

Preclinical Toxicity

KEY ACCOMPLISHMENT

- Final report for Good Laboratory Practice (GLP) study of a gp350-ferretin-based vaccine for Epstein-Barr virus

Subsequent to the Frederick National Laboratory's Biopharmaceutical Development Program's Chemistry, Manufacturing, and Controls development of a gp350-ferretin-based vaccine for Epstein-Barr virus, the CRD Drug Discovery and Development Program deployed a subcontractor to conduct a Good Laboratory Practice toxicity study, which completed *in vivo* conduct and bioanalytical testing in fall 2019. The delivery of the final GLP report in spring 2020 enabled the Laboratory of Infectious Diseases to complete an Investigational New Drug application and bring this vaccine into clinical trials.

Support Provided by the Clinical Monitoring Research Program Directorate

Clinical Research Support

KEY ACCOMPLISHMENTS

- Supported the development of five first-author scientific abstracts by junior researchers at the Rakai Health Sciences Program (RHSP), including oral presentations accepted for presentation at two conferences: the Conference on Retroviruses and Opportunistic Infections (CROI) 2020 and the 24th International Workshop on HIV and Hepatitis Observational Databases (IWHOD)
- Initiated a program to encourage junior researchers in the RHSP to develop first-author abstracts for submission to international HIV conferences and workshops

The FNL staff provides comprehensive clinical program/project management support to multiple Division of Intramural Research laboratories, facilitating and managing clinical research studies by providing technical, logistical, project management, and meeting support.

Laboratory of Immunoregulation

FNL has supported the Laboratory of Immunoregulation's (LIR) initiative with the RHSP to establish the provision of antiretroviral drugs to rural villages in Uganda's Rakai District since 2004. RHSP is a National Institute of Allergy and Infectious Diseases (NIAID) International Center for Excellence in Research (ICER) initiative, which is a laboratory-oriented grant that funds many of the laboratory studies conducted on biospecimens. The primary purpose of the ICER initiative has been to build infrastructure in Rakai for collaborative biomedical research with Ugandan scientists. LIR's International HIV and STD Section conducts this research in collaboration with Johns Hopkins University.

FNL staff aids the International HIV and STD Section and RHSP, supporting data analysis and manuscript writing for clinical research; providing operational support to clinical protocol development and implementation; providing technical and budgetary oversight of subcontracts; assisting with personnel logistics; and providing project procurement support, travel support, and overall coordination of administrative program-level functions.

FNL staff continued to provide subcontract management and administrative support to task orders established with RHSP to conduct clinical research studies. Protocol 14-I-N123, Quantitative Measurement and Correlates of the Latent HIV Reservoir in Virally Suppressed Ugandans, is in its fifth year of follow-up visits and is anticipated to close in late 2021. Protocol 17-I0072, Herpesviruses Reactivation in HIV-Infected Women Initiating ART (HERA), had enrolled 187 participants as of March 2020, 138 of whom completed the protocol. To support daily operations for the studies, FNL staff facilitated shipments as well as prepared travel packages for conferences and site visits. Both studies were paused due to the COVID-19 pandemic.

An FNL biostatistician in the Clinical Monitoring Research Program Directorate (CMRPD) supported RHSP by providing research mentorship and expertise in state-of-the-art data manipulation and statistical analyses. In addition, the biostatistician consulted with investigators on the design and analysis of clinical and observational studies; supported database management activities; and helped NIAID principal investigators write and implement protocols, design studies, and develop case report forms. The biostatistician continued to contribute to manuscripts and co-authored several publications on HIV-related research (Ssempijja V, Nason M, et al., *J. Clin Infect Dis*, 2019; Kagaayi J, et al., *Lancet HIV*, 2019; and Tibaukuu M, et al., *J Viral Hepat*, 2020).

The biostatistician also initiated a program to encourage junior researchers at RHSP to develop first-author abstracts for submission to international HIV conferences and workshops by organizing group work progress sessions for simultaneous mentorship and progress checks towards submission deadlines. As a result, eight independent abstracts were developed for the CROI 2020 conference, three of which were accepted. Another three abstracts were submitted to the 24th IWHOD conference, and two were accepted.

Laboratory of Malaria Immunology and Vaccinology

The Laboratory of Malaria Immunology and Vaccinology (LMIV) conducted collaborative projects with the Partnership for Research on Ebola Vaccines and Infectious Diseases in Liberia (PREVAIL) to foster partnership development and help expand the PREVAIL clinical research portfolio to include other infectious diseases, including malaria. To assess malaria burden in Liberia, LMIV sought to build the laboratory capacity for malaria diagnostics.

FNL staff oversaw a research partnership that provided staffing for the PREVAIL team to support the protocol titled "Cross-sectional Survey of Plasmodium and Other Parasites in Pregnant Women and Children around Margibi and Montserrado Counties, Liberia." The study was paused in March 2020 due to the COVID-19 pandemic.

Laboratory of Clinical Immunology and Microbiology/Genetic Immunotherapy Section

The mission of the Genetic Immunotherapy Section is to develop gene therapy and hematopoietic stem cell transplantation approaches to treat a variety of inherited primary immune deficiencies. Associated with that mission is the diagnosis and treatment of the infections, inflammation, autoimmunity, pulmonary dysfunction, and growth failure that may complicate management of immune deficiencies.

FNL staff supported activities to help procure graft products and services for a few National Institutes of Health protocols that required a matched unrelated donor product.

Laboratory of Clinical Infectious Diseases

The Laboratory of Clinical Infectious Diseases conducts clinical and basic studies of human infectious and immunologic diseases. One such research effort is focused on adults of Asian ethnicity without HIV infection yet with autoantibodies to interferon gamma (IFN-γ) and non-tuberculous mycobacterial disease and other opportunistic infections. An observational study was launched in 2009 to follow individuals with this syndrome. Patient accrual occurred over five years, with annual follow-up to investigate the origins of their autoantibodies and examine potential immunogenetic factors influencing the development of this disease and other intracellular opportunistic infections.

FNL staff continued to manage a collaboration with Khon Kaen University that provides clinical and operational support for the conduct of the study titled "Mycobacterial and Opportunistic Infections in HIV-Negative Thai Patients Associated with Autoantibodies to Interferon-γ." The clinical monitoring activities for the study were performed under a separate agreement with a clinical research organization.

Laboratory of Infectious Diseases and Laboratory of Viral Diseases

The Laboratory of Infectious Diseases (LID) has a long history of vaccine development and identification of new agents of viral diseases. Clinical studies complement LID's major areas of research, including testing candidate vaccines in clinical trials, a human challenge study with influenza to study pathogenesis and immune correlates for protection against the virus, and studies of severe virus infections in persons without known immune deficiency.

The Laboratory of Viral Diseases carries out investigations on the molecular biology of viruses, the interactions of viruses with host cells, the pathogenesis of viral diseases, and host defense mechanisms. The studies are designed to increase fundamental knowledge as well as to facilitate the development of new approaches to preventing and treating disease.

For both laboratories, FNL staff provided clinical project management oversight and programmatic support for a partnership that provides a repository for clinical research material storage and handles shipping of materials from the repository to the clinical research sites, the National Institutes of Health, and various laboratories.

Laboratory of Parasitic Diseases

The Laboratory of Parasitic Diseases (LPD) conducts basic and applied research on the prevention, control, and treatment of a variety of parasitic and bacterial diseases to identify immunological and molecular targets for disease intervention. Patient-centered research is conducted at the National Institutes of Health Clinical Center and in international field studies in India, Latin America, and Africa.

FNL staff oversaw project procurement, shipments, and travel for LPD efforts in Cameroon and the ICER initiatives in India and Mali. The staff supported seven service maintenance agreements for equipment located in India and Mali, and they coordinated shipments of perishable, ambient-temperature, and frozen items to India, Mali and Cameroon.

FNL staff also oversaw a research subcontract that provides a scientific director who leads research projects conducted at the National Institute for Research in Tuberculosis ICER.

Live-Attenuated ZIKA Vaccine Development

KEY ACCOMPLISHMENTS

- Executed a subcontract with a vendor to complete current Good Manufacturing Practice (cGMP) pilot production of vaccine candidates and challenge virus
- Completed cGMP production of dengue challenge virus through a subcontract with Charles River Laboratories
- Executed a collaborative partnership with Johns Hopkins University for database development and conduct of the Phase I controlled human infection model study

Following the recent emergence of Zika virus (ZIKV) in the Americas, Zika vaccine development has been accelerated to formulate a second-generation combined vaccine for dengue virus (DENV) and ZIKV. The key activities of this project include pilot production of live-attenuated ZIKV vaccine candidates/challenge strains, a Phase I ZIKV-controlled human infection model study, and Phase I clinical evaluation of up to three ZIKV vaccine candidates.

Subcontracts with vendors were executed in February. FNL staff supported the transfer of the DENV challenge virus Accession Virus Bank from NIAID to the subcontractor for production and continues to provide clinical project management and oversight of the ongoing cGMP pilot production. The staff also continues to provide clinical project management and oversight of the clinical trial preparation activities at Johns Hopkins University in anticipation of study initiation.

FNL staff in CMRPD worked with NIAID to advance the production of the DENV challenge virus, which was readily available, into the first production slot that the vendor had available, as the ZIKV vaccine candidates were not yet ready for production. This allowed FNL to take advantage of the earliest available opening in the vendor's schedule.

The COVID-19 pandemic deferred the trial's initiation, yet FNL staff in CMRPD collaborated with NIAID and Johns Hopkins University to identify project

activities, including study document development/ refinement and database development, that could advance remotely.

Division of Microbiology and Infectious Diseases

Support Provided by the Clinical Monitoring Research Program Directorate

COVID-19 Studies

The National Institute of Allergy and Infectious Diseases (NIAID) Division of Microbiology and Infectious Diseases (DMID) rapidly deploys resources to facilitate high-priority, global, collaborative clinical research. When the Wuhan Municipal Health Committee in China identified a cluster of viral pneumonia cases of unknown cause in December 2019, coronavirus RNA was identified in some patients. The novel coronavirus, SARS-CoV-2, and the disease, COVID-19, caused by this virus rapidly became a public health emergency. According to various international health reporting agencies, the number of confirmed cases quickly escalated from 59 on January 5, 2020 to more than 64,000 by February 14, 2020. Without approved therapeutic or prophylactic agents available for coronaviruses, DMID requested services from the Frederick National Laboratory for Cancer Research (FNL) to initiate the management, oversight, and conduct of COVID-19 clinical trials.

Adaptive COVID-19 Treatment Trials

KEY ACCOMPLISHMENTS

- Opened 60 sites in 10 countries and enrolled 1,063 participants in 58 days (Adaptive COVID-19 Treatment Trial 1 [ACTT-1])
- Activated 71 sites in eight countries and enrolled 1,034 participants in 54 days (ACTT-2)
- Activated 54 sites in four countries and enrolled 279 participants in 28 days (ACTT-3); continued enrollment and activation activities for new sites
- Facilitated execution of clinical trials agreements (CTAs), allowing sites to begin enrolling study participants in advance of the effective date
- Expedited the execution of more than 40 subcontracts and CTAs
- Coordinated transition of site-essential documents to an online project management platform to more efficiently support site activation, real-time reporting, document access, and update capabilities
- Based on preliminary findings published in *N Eng J Med* in May, the U.S. Food and Drug Administration (FDA) issued an emergency use authorization of remdesivir for the treatment of hospitalized COVID-19 patients.

Officially titled *A Multicenter, Adaptive, Randomized, Blinded Controlled Trial of the Safety and Efficacy of Investigational Therapeutics for the Treatment of COVID-19 in Hospitalized Adults*, this DMID-sponsored, adaptive design protocol study is more commonly referred to as ACTT. The study is comparing different investigational therapeutic agents to a control arm and introducing new arms according to scientific and public health needs.

In ACTT-1, participants were randomly assigned to receive either remdesivir or placebo for up to 10 days. ACTT-2 evaluated the combination of baricitinib and remdesivir compared to remdesivir alone. ACTT-3 evaluated the combination of interferon beta-1a and remdesivir compared to remdesivir alone.

FNL provided a broad range of clinical and technical support, administrative management services, and program-dedicated research support to conduct the ACTT study domestically and internationally, supporting 60 ACTT-1 sites and more than 80 ACTT-2 sites.

In February and March, FNL staff in the Clinical Monitoring Research Program Directorate (CMRPD) traveled to Japan and South Korea to help initiate and prepare sites for the pioneering remdesivir treatment study.

FNL helped establish and implement a comprehensive ACTT Coordinating Center, which included site activation support, collection of all site-essential documents, comprehensive review of study documents (protocol, manual of operations, frequently asked questions), development of communication and continuity of operations plans, compilation of the trial master file, administrative support for meetings with study partners, and oversight for the development and launch of a 24/7 urgent call line to streamline and triage emergency calls from sites to the DMID subject-matter expert. FNL evaluated the skill sets of existing resources and allocated experienced, dedicated staff to focus on this high-priority project. The coordinating center provided project management, site operations management, regulatory and protocol management, and publication management, and it conducted a broad range of research site support.

FNL subcontracted with a clinical research organization (CRO) to provide additional support and collaborated with DMID and the CRO to stand up the ACTT Coordinating Center. Upon execution of a task order with an established clinical trials network, FNL was able to provide clinical sites in Europe. Another subcontract was executed with a Central Institutional Review Board (CIRB) to provide regulatory review and approval services for ACTT.

FNL identified the need to support the European sponsor for the United Kingdom/European Union (EU) ACTT sites by providing site-essential documents to DMID more efficiently than through email. The FNL staff spearheaded the transition of existing DMID Excel documents to Smartsheet, an online project management platform. This allowed multiple study partners to access documents and contribute updates to the government sponsor. In addition, the Smartsheet tabs were enhanced

to help manage site activation throughout the emergency pandemic response. Reports generated from Smartsheet were provided to NIAID senior leadership, the FDA, and the DMID agreements team, and the program officer used them for daily reports to the White House. The Smartsheet tabs also allowed the European sponsor to upload documents and confirm that the documents were reviewed and approved prior to upload into the DMID Site Essential Regulatory Document Library. The creation of the Smartsheet tab also documented approval for all United Kingdom/EU sites in a single location for access by multiple parties.

To align with industry practice, FNL proposed the use of CTAs rather than subcontracts. The CTA template was finalized in March 2020. The resulting benefits to the government included: less time to negotiate and finalize; no subcontract ceilings that would limit sites to the number of participants enrolled; the ability to make quick modifications to add study arms without needing separate agreements or additional negotiations; and rapid response to the global pandemic, as the CTAs included a provision allowing sites to begin enrolling study participants up to 45 days in advance of the effective date.

FNL's collaborative and comprehensive approach contributed to the successful outcome of ACTT. DMID transitioned site activation activities directly to the FNL CMRPD team and the CRO in March 2020, which immediately expanded the ACTT study team's bandwidth to expedite site activation and participant enrollment. FNL staff also worked with study partners and the DMID quality management team to develop a study-specific quality assurance checklist for sites that did not have an existing DMID Clinical Quality Management Plan; the document was finalized and posted to the study website in early April. In May, the FDA, Gilead Sciences, and Eli Lilly began urgently requesting documents, reports, and information from DMID to support regulatory submissions. FNL staff in CMRPD managed the expedited collection of documents from the sites and coordinated with representatives from each of the networks to meet the stringent timelines.

Overarching project management activities over the course of the study included: anticipating the shift to 100 percent telework during the pandemic and migrating a DMID working document to Smartsheet in two days so multiple contributors could simultaneously make updates in real time, developing electronic tracking tools to confirm and monitor site activation requirements, streamlining communications via distribution lists, developing a process flow and plan to support eliminating the site-essential-document collection backlog, providing invoice guidance to the clinical sites, and creating a successful infrastructure to support this multifaceted program in an emergency response environment. The CMRPD staff also created a questionnaire to poll the 60 sites that participated in ACTT-1 to gauge interest in ACTT-2, compiled feedback and responses, and helped support DMID's plan for additional onboarding of sites. CMRPD staff used the ACTT-2 questionnaire, tailoring it for ACTT-3 to poll and query 65 sites. Feedback and responses were compiled once again to help support DMID's plan for onboarding sites.

The outcomes of ACTT-1 were shared at a White House press conference; in a NIAID press release on April 29, 2020; and in *N Eng J Med* (Beigel JH, Tomashek KM, Dodd LE, et al., 2020). The promise of remdesivir led to emergency use authorization, and remdesivir became the standard of care for patients critically ill with coronavirus. Remdesivir's manufacturer, Gilead Sciences, used data from the pivotal ACTT-1 for registration with the FDA.

Big Effect Trial

KEY ACCOMPLISHMENTS

- Rapidly executed subcontracts with a full-service CRO and CIRB to support study initiation target timelines
- DMID approved nine sites for study participation

In April 2020, DMID asked FNL to provide a broad range of clinical, technical, and administrative management services as well as program-dedicated research support to implement, coordinate, oversee, and conduct its Big Effect Trial (BET). BET is a multicenter platform (up to 20 domestic sites) to evaluate the clinical efficacy, as assessed by time to recovery for adults hospitalized with COVID-19, of different investigational therapeutics compared to a control arm; the trial has an initial sample size of 600.

FNL's requirements include clinical project management and overall support for all clinical-site activities, including clinical site identification, site readiness, and activities from trial start-up through close-out, to conduct a small series of studies in order to gather sufficient data to determine which agents should move into larger clinical trials.

To rapidly deploy the BET study and avoid delays in study start timelines, FNL initiated the acquisition process to execute agreements with a CRO and CIRB prior to receiving the final protocol. FNL released a limited-competition Request for Proposal for a full-service CRO and provided an intent to award notification to the selected offeror within 20 days. FNL also established an operations center to implement the trial and subcontracted with a CIRB at the beginning of June to provide regulatory review of the protocol and associated documents. DMID requested FNL's laboratory support for virology testing. FNL identified potential laboratories and initiated the acquisition process to issue a request for information to solicit capabilities and pricing in order to engage and subcontract with a laboratory capable of processing the virologic endpoints of the protocol.

FNL staff evaluated available resources; assigned experienced, qualified staff to oversee the day-to-day operations of the subcontractors; and collaborated with DMID and the CRO subcontractor at the BET kick-off meeting in early July. FNL staff also provided high-level

medical writing support to help DMID complete protocol and informed consent forms for the BET program; the final protocol was submitted to the CIRB at the end of August, and the first patient enrolled in mid-August. In addition, several Smartsheet tabs were developed to give all study partners centralized access to project management tools for BET.

Vaccine Research Center

Support Provided by the Biomedical Informatics and Data Science Directorate

NIAID Data Ecosystem Framework Pilot

The National Institute of Allergy and Infectious Diseases (NIAID) had initiated the Data Ecosystem Framework pilot, a project to inform its strategy for increasing the accessibility and utility of its databases and knowledgebases. The pilot was started without the Frederick National Laboratory for Cancer Research's (FNL) participation and involved the University of Chicago. However, NIAID wanted to leverage the FNL staff's unique Cancer Research Data Commons experience and have the them manage this project. The FNL staff worked closely with the partner and NIAID. In addition to developing a user interface to help navigate and browse the selected NIAID data sets, the team wrote a report to document recommendations for a production system. A final presentation was made to NIAID leadership at the end of the pilot in February 2020.

Support Provided by the Cancer Research Technology Program

Electron Microscopy: Research and Development: Support

Vaccine Research Center

The Vaccine Research Center (VRC) electron microscopy (EM) unit, currently consisting of two full-time employees, provides support to VRC and the Vaccine Production Program Laboratory (VPPL) in the general area of electron microscopy analysis. The development and production of modern vaccines rely heavily on visualization and structural analysis of the immunogens. We perform EM services and computational processing necessary for the research, development, and production of recombinant vaccines. There are three principle component activities: 1) analysis of immunogens and related specimens by negative-stain EM, which is usually performed for quality-control purposes and for low-resolution structure determination; 2) analysis of immunogens and related specimens by cryogenic EM (cryo-EM), which provides high-resolution structures of proteins and virus-like particles; and 3) detection and quantification of virus-like particles in cell cultures necessary for vaccine production. During the reporting period, we analyzed over 1,000 samples using negative-stain EM. Additionally, we solved 12 cryo-EM structures of diverse potential immunogens at resolutions between 2.4 and 5 Å. Eight of these structures are of the spike protein of SARS-CoV-2, the pathogen responsible for COVID-19. Cumulatively, these efforts resulted in six publications in peer-reviewed journals and two preprints describing the current COVID-19 work.

VRC-EM has been involved in multiple research projects pertaining to the design of vaccines against such pathogens as HIV, various human paramyxoviruses, influenza, noroviruses, and hepatitis B. HIV remains a major focus of our research, with four manuscripts published or accepted for publication in the reporting period (Gorman et al., *Immunity*, 2019; Chuang et al., *J Virol*, 2020; Ou et al., *Sci Rep*, 2020; Cheng et al., C*ell Rep*, accepted). We contributed cryo-EM structures of virus-like particles carrying hemagglutinin to a manuscript describing the role of glycans in the elicitation of protective cross-group antibody responses to influenza (Boyoglu-Barnum et al., *Nat Commun*, 2020). Our results were also instrumental in the development of a generalizable approach to paramyxovirus immunogen development (Loomis et al., *Front Immunol*, 2020), expanding on the previous work on human parainfluenza virus and human metapneumovirus. Additionally, we solved a number of high-resolution cryo-EM structures of hepatitis B virus-like particles, alone and in complexes with patient-derived antibodies, which shed new light on the mechanisms of acute liver failure caused by this virus. Both negative-stain EM and cryo-EM are being used to create new, more stable norovirus immunogens. We also utilized negative-stain EM to study antibody binding to Ebola virus proteins.

Research and work related to COVID-19

VRC-EM has undertaken extensive efforts to support the development and production of potential COVID-19 immunogens as well as to facilitate research related to SARS-CoV-2. In the period between March 19 and July 24, we analyzed over 300 diverse samples consisting of recombinant proteins of SARS-CoV-2, virus-like particles displaying such proteins, pseudoviruses, and cell cultures. The SARS-CoV-2 spike protein, the main target of future vaccines, is known for its fragility and highly dynamic nature, which makes creating stable immunogens complicated. A major task of the VRC-EM unit has been to screen the spike protein samples produced at VRC using negative-stain EM to verify their proper conformation and stability. One manuscript utilizing the results of this work is available as a preprint (Zhou et al., *bioRxiv*, 2020). Additional work is being conducted to visualize virus-like particles displaying the spike protein in the cells that express them.

VRC-EM's main contribution in the reporting period, however, has been to the research of SARS-CoV-2 spike interactions with its receptor and potentially neutralizing

antibodies and of the mechanisms by which the virus evades antibody recognition and neutralization. We showed that the SARS-CoV-2 spike is not very stable at serological pH and is much more stable at endosomal pH, where we observed it shedding bound antibodies. We partnered with NCI's National Cryo-Electron Microscopy Facility to obtain high-quality cryo-EM data of the SARS-CoV-2 spike using state-of-the-art instrumentation. Using these datasets, we determined a number of structures of the SARS-CoV-2 spike at various conditions, achieving the highest resolution available as of the time of this writing (2.4 Å). This enabled us to develop a new concept of pH-dependent changes in spike conformation and dynamics and in the availability of its receptor-binding domains (RBDs), which are the prime targets of neutralizing antibodies. Additionally, the differences in the availability of the RBDs that we observed for the spike protein of SARS-CoV-2 D614G strain provided a molecular explanation for its enhanced infectivity. The results of this high-resolution analysis are summarized in the manuscript available as a preprint (Zhou et al., *bioRxiv*, 2020).

Support Provided by the Clinical Monitoring Research Program Directorate

Clinical Research Support

KEY ACCOMPLISHMENTS

- Contributed to Vaccine Research Center (VRC) investigational product safety reporting efforts
- Contributed to data evaluation for Investigational New Drug (IND) monoclonal antibody 114 (mAb114) for Ebola virus disease clinical trials

FNL staff provided pharmacovigilance support to oversee safety surveillance of current VRC protocols by participating in safety team calls and reviewing and following up on IND annual reports, monthly and weekly safety reports, and adverse event/serious adverse event reports. FNL staff contributed to VRC investigational product safety reporting efforts, which included HIV broadly neutralizing antibodies, a mAb for Ebola virus disease, HIV trimers and vaccines, and safety issue communication related to VRC INDs. Safety reports were also reviewed for VRC-sponsored clinical trials, Division of AIDS trials, and other VRC collaborators using the VRC HIV mAb products to identify safety signals and trends.

FNL staff also contributed to data evaluation for IND mAb114 for Ebola virus disease clinical trials and established safety communication with collaborators for this IND.

Advance Vaccine Candidates for Clinical Trials

KEY ACCOMPLISHMENTS

- Supported approval of study closure in April 2020 by the Emory University Institutional Review Board (IRB)
- Supported final database lock procedures completed by the data management, biostatistical, and regulatory subcontractor

The revised scope of this task order, "A Phase 1 Open Label, Dose-Escalation Clinical Trial to Evaluate the Safety and Immunogenicity of a Trivalent Virus-Like Particle (VLP) Encephalitis Vaccine, VRC-WEVVLP073-00-VP, in Healthy Adults," was to provide regulatory services, clinical testing support, and execution of a clinical study of universal alphavirus vaccine using VLP platform technology successfully developed for chikungunya. The overarching objective was to conduct a Phase I clinical trial of the vaccine in a single domestic research site, randomizing up to 40 healthy adult research participants (with a minimum target of 30) for approximately nine months of study follow-up. The primary objectives were to evaluate the safety and tolerability of the vaccine at three dose levels administered alone or with adjuvant in healthy adults. Secondary objectives were related to immunogenicity of the investigational vaccine and dosing regimen.

FNL managed three research subcontracts to support a domestic clinical trial site; a research laboratory; and regulatory, data management, and biostatistical services and managed several subcontract modifications to accommodate NIAID VRC requirements. Following an aggressive timeline, FNL staff effectively oversaw projects, completing all clinical project management and monitoring requirements in order to meet the September 2020 closure timeline for this task order.

The Emory University IRB approved study closure in April 2020. At study closure, 30 participants had been randomized.

All study samples were sent from the clinical research site to the research laboratory subcontractor in March for immunological testing and storage. The research laboratory subcontractor transferred all remaining samples to the VRC biorepository by May, after completing the testing requirements. The data management, biostatistical, and regulatory subcontractor performed an additional programmatic quality control comparison between the uploaded data sets from the research laboratory subcontractor and the raw research laboratory testing data. Final immunological testing results were provided to the data management, biostatistical, and regulatory subcontractor per sponsor specifications. The data management, biostatistical, and regulatory subcontractor provided statistical support to manuscript development.

The data management, biostatistical, and regulatory subcontractor completed final database lock procedures; ensured data was compliant with Clinical Data Interchange Standards Consortium standards; generated

final tables, figures, and listings; transferred the electronic trial master file to the sponsor; and provided the necessary updates to Clinicaltrials.gov.

All subcontractor activities and deliverables were completed for this study.

Zika – VRC 705

KEY ACCOMPLISHMENTS

- Developed a process for providing all participants with information regarding their treatment assignment (unblinding) following the last subject visit in early October 2019
- Managed the completion of all site management visits by the subcontractor, with the final visit completed in February 2020

In 2016, the NIAID VRC sought support from FNL to plan, coordinate, and manage clinical research activities to assess VRC's Zika DNA vaccine candidate in a placebo-controlled study. Parts A and B of the Phase II/IIb study enrolled 2,490 healthy adult and adolescent participants at 17 U.S. and international study sites.

FNL staff supported clinical and business operations oversight for VRC 705, a Phase II/IIb randomized trial to evaluate the safety, immunogenicity, and efficacy of a Zika virus DNA vaccine in healthy adults and adolescents. This included managing domestic and international clinical study sites; managing project timelines; providing logistical and laboratory support, regulatory oversight, financial management, and procurement support; facilitating the shipment of materials and capital equipment; coordinating equipment calibration; managing subcontracts; and reviewing and editing study documents.

Due to the low possibility of additional Zika infections, participant follow-up for the study ended in October 2019. The last diagnostic specimen shipment was received at the Primary Diagnostic Laboratory/University of Washington (PDL) on November 1, 2019. All PDL-retained Zika-, dengue-, and chikungunya-positive serum samples were subsequently shipped to the repository on December 4, 2019. The last research specimen shipment from VRC 705 sites was received at the repository on October 28, 2019. This completed all specimen processing and shipping activities for the VRC 705 sites.

FNL staff collaborated with VRC and the subcontracted clinical research organization (CRO), focusing on site-management efforts at the study sites. All sites underwent final site management close-out visits that emphasized readiness for the monitoring close-out visit and ultimate closure.

FNL staff continued to manage and administer the CRO agreement, five clinical study site agreements, and three laboratory services agreements. The staff facilitated and managed the close-out budgets for the subcontracts to align with study close-out conduct. In addition, the staff worked with the CRO subcontractor to streamline its process for negotiating costs related to early study termination, conduct, and unblinding of participants and to identify inconsistencies related to participant visit fees from study sites, resulting in a significant project cost savings. The staff worked closely with the subcontractor to finalize and complete the post-activation laboratory visit items.

Restrictions related to the COVID-19 pandemic affected the VRC 705 study sites, leading to temporary site closures and limits on participants' planned visits to the sites to receive their unblinding letter. FNL staff oversaw the necessary interactions between each site, the subcontractor, and VRC regarding revisions to the unblinding letter process and related regulatory authority approvals. The Clinical Monitoring Research Program Directorate directed the development of a weekly tracker that identified the impact local restrictions were having on the study sites. The weekly tracker supported the government sponsor and study partners in anticipating and planning for timeline shifts and making key decisions leading up to study closure.

Support Provided by the Vaccine Clinical Materials Program Directorate

Research and Development Support

The Vaccine Clinical Materials Program (VCMP) supports the Vaccine Research Center (VRC) by overseeing scientific projects executed by subcontractors and supporting purchasing and procurement for the Vaccine Production Program (VPP) and VRC investigators (non-VPP investigators). VCMP supports various activities including basic research, candidate discovery and optimization, process development, clinical manufacturing, regulatory affairs, and preclinical and clinical testing.

KEY ACCOMPLISHMENTS

- We made eleven combined subcontract awards or modifications to existing to subcontracts in order to facilitate research and development projects for VRC investigators.
- COVID-19 research was rapidly initiated using an existing subcontractor agreement.
- Six pieces of capital equipment were procured for the VPP to improve capabilities and replace aging equipment.
- Two hundred thirteen orders were completed to support VRC investigators and the Vaccine Immunology Program.

The VCMP and Subcontracts groups collaborated to modify existing subcontracts for ongoing research projects supporting VRC investigators under the new contract. In addition, multiple new indefinite delivery/indefinite quantity contracts were issued to enable multiyear collaborations related to vaccine research and development at various institutions.

Service was able to continue without disruption for critical research projects during the transition to the new contract. VCMP provided technical oversight for the following research and development projects performed via subcontract:

- Genotyping samples to determine IgG allotype of patients participating in VRC antibody clinical trials
- Optimizing antibodies by high-throughput mutagenesis and screening of heavy- and light-chain yeast display libraries
- Biophysical characterization of antibody and vaccine candidates
- Structure-function studies of coronavirus (CoV) and novel anti-CoV antibodies identified by VRC
- Pharmacokinetic design for VRC antibody clinical trials and analysis of clinical trial pharmacokinetic data
- Investigation of combination antibody therapies using AAV8-vectored anti-HIV antibodies in a humanized mouse model of HIV-1 infection
- Consultant support for regulatory and preclinical strategy for VRC products
- Analysis of the serum antibody repertoire from patients administered an experimental respiratory syncytial virus vaccine
- Single-molecule interrogation of HIV-1 envelope binding to neutralizing antibodies by fluorescence resonance energy transfer
- Establishment of yeast cell lines for vaccine target expression
- Filovirus challenge studies to investigate Ebola and Marburg virus vaccine and antibody efficacy

In conjunction with Purchasing at the FNL, VCMP consistently provided procurement support to the VPP, Vaccine Immunology Program, and other programs within the VRC. We purchased equipment necessary for new technologies and maintained existing equipment at VPP and procured services and supplies for other programs at the VRC.

Due to rapid scientific advancement, occasionally the VPP adopts new technology platforms that may require new equipment. This year, the VPP pursued a new adjuvant technology platform to facilitate future HIV vaccine strategies. The new technology required a Microfluidizer unit to support critical development work. In addition, the VPP needed to replace several key pieces of equipment to continue day-to-day operations for process and method development. No equipment was estimated in the initial budget. As a result, the VCMP followed change-management processes to assess impact to the budget, requested appropriate changes in baseline cost elements, and received contracting officer approval for the unplanned expenditures.

Due to personnel restrictions and changes in research priorities resulting from the COVID-19 pandemic, several subcontract-based projects were paused or redirected. VCMP worked with subcontractors and VRC investigators to identify impacted projects, establish contingency plans, and facilitate restarting projects when appropriate in instances where institutions were required to delay or pause activities. VCMP oversaw an existing subcontract project with a primary research focus on coronaviruses, including severe acute respiratory syndrome–associated coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus. In response to the COVID-19 pandemic, VRC investigators and the subcontractor quickly shifted focus to SARS-CoV-2-related work. Due to the broad research objectives in the existing subcontract agreement, minimal changes were necessary at the subcontract level to facilitate this pivot in research priorities.

Product Support

As a contractor supporting NIAID/VRC, FNL is responsible for operating VCMP, including a pilot plant in Frederick, MD, and all necessary activities for the manufacture and disposition of investigational clinical products for evaluation in human clinical trials. The pilot plant is a leased facility that includes NIH-furnished equipment and is operated by VCMP. Major responsibilities for the program include:

- Supported all aspects of current Good Manufacturing Practice (cGMP) product development, manufacturing, testing, and release in compliance with Title 21, Part 210 and 211 of the *Code of Federal Regulations* and U.S. Food and Drug Administration (FDA) and International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines for industry governing Phase I and Phase II clinical trials. Routine activities and/or activities conducted on request are summarized below.

Routine:

  - Provided robust scale-up and bulk drug-manufacturing capabilities and corollary infrastructure to generate filled, vialed drug product (DP) and to store/stockpile vaccine materials as required
  - Supported warehousing (materials management), facility maintenance, quality control, and quality assurance
  - Provided regulatory support in the form of Chemistry, Manufacturing, and Controls content input for Investigational New Drug (IND) applications, filed domestically and/or internationally

Upon Request:

  - Manufactured clinical lots of candidate products according to cGMP standards, not already covered under current task orders; products

include adjuvants, plasmid DNA vaccines, nucleic acid vaccines, recombinant protein subunit vaccines, nanoparticle vaccines, virus-like particle vaccines, and monoclonal antibodies

- Conducted long-term stability of clinical agents manufactured at VCMP or received under cooperative agreements from other VRC collaborators while following International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines in the maintenance of long-term stability
- Established quality systems to support manufacturing of candidate vaccines by other VRC contractors
- Facilitated technology transfer (manufacturing processes and qualified/validated analytical methods) to external VRC partners for late-phase product development and/or commercialization

- Supported pilot-plant facility operations and maintenance on systems including electricity, water, gas, and dark fiber; provided trash removal, snow removal, landscaping, pest control, cleaning supplies, facility improvement, and equipment and equipment replacement; maintained software; provided equipment calibrations, validation services, office supplies, offsite storage, work clothing and uniform services, spare parts and gases, cellular service, and paper recycling; facilitated EHS-protective services; and provided training and labor for administrative, quality control and assurance, manufacturing operations support, facility maintenance, manufacturing operations, and cleaning staff

KEY ACCOMPLISHMENTS

- Successfully completed the annual facility shutdown for 2019–2020 with a two-week compression; this enabled earlier return-to-service for planned cGMP production, which turned out to be beneficial when the VCMP used a two-week period in April 2020 to pause clinical product manufacturing and redirect to hand-sanitizer production to support the COVID-19 pandemic response.
- Procured/received 100 percent of capital equipment requested to improve VCMP infrastructure and capabilities. These included several computerized systems upgrades (Quality Control: laboratory information management system, Warehouse: Sage X3, Microbial Fermentation Group: Uniflux Unicorn and ViCell cell counter), one new systems addition (Validation: Val Genesis), a chiller building controls upgrade, new analytical instrumentation (Protein Simple iCE-3), environmental monitoring air samplers, select freezers/refrigerators, uninterrupted power supply, benchtop meters for buffer preparation, a Wave rocker bag for mammalian cell culture, clean room HEPA filter changeouts, a disinfection efficacy study (for rotating disinfectants), a hydrogen generator for the Quality Control General Support System, a K-Sep cell separator (Flu program),an Adjuplex™ microfluidizer (non-alum adjuvant program), a new purification AKTA ready system, and a small-scale filling machine rebuild.

As part of the ongoing goal of upgrading the pilot plant facility to ensure sustainability and business continuity, the VCMP Facilities group upgraded the controls package for both the glycol and HVAC chilled water systems. The existing control package, a local control system, was no longer supported by the manufacturer, making some of the system components unavailable. This system had to be accessed at each chiller module (six total modules). Personnel could only view one of 47 variables at a time due to a four-inch LCD. It also maintained a supply setpoint that caused the compressor to run in idle mode with no demand, resulting in unnecessary runtime and electricity consumption.

The new control package is web-based, allowing access through the existing Building Automation System, and graphic-based, providing access to all 47 variables of operation from one screen view. Compressor runtimes are now monitored, resulting in compressor rotation based on units with lowest usage. All setpoints and alarm settings are now adjustable for better control of the system during extreme weather conditions.

The primary benefit of implementing the new system is not needing to run compressors in idle mode when a lower demand of chilled water occurs. By running on demand rather than setpoint control, there will be times when multiple compressors can shut down, reducing wear and tear on the system, and most importantly, reducing the facility's electrical consumption.

HIV Vaccines and Passive Therapies

HIV Membrane Proximal Expression Region Broadly Neutralizing Antibodies for Prevention, Therapy, or Cure

VRC's highest priorities in the quest to prevent HIV in humans are developing vaccines or immune modulators. Several novel, broadly neutralizing antibodies (bNAbs) that are directed against the membrane proximal expression region (MPER) and other, non-CD4 binding site neutralization epitopes on the HIV envelope have been identified and characterized. Production and clinical testing of long half-life bNAbs targeting multiple neutralization epitopes may improve the coverage and likely success of bNAbs for prophylaxis, therapy, and cure strategies. The scope of this task order included two bNAbs: 10E8VLS, directed against MPER, and CAP256V2LS, directed against the V1V2-region of the HIV envelope. While the MPER program was discontinued by VRC due to clinical pause with 10E8VLS, the CAP256V2LS program continues to progress as summarized below in related anti-HIV bNAb

task orders. In the final year of this five-year task order, work focused on generation of the final report and initiating project closeout.

KEY ACCOMPLISHMENTS

- A final report was issued for this task order.

Research, Development, Production and Support for Clinical Trials – Broadly Neutralizing Antibodies

The VRC has developed several bNAbs directed against the CD4 binding site of the HIV envelope protein, including VRC01, VRC01LS, VRC07-523LS, and N6LS.

VRC01, a monoclonal antibody expressed in Chinese hamster ovary (CHO) mammalian cells and manufactured at the 2,000-liter bioreactor scale for VRC by the VCMP in a multiyear campaign spanning 2016 through 2018, continues to be evaluated by NIAID in large trials with thousands of patients. These include a global Phase II Antibody-Mediated Prevention clinical trial in the Americas and in Africa, as well as Phase I, II, and I/II trials supported by the International Maternal Pediatric Adolescent AIDS Clinical Trials Network. The scope of this task order was heavily focused on VRC01 clinical production and supply and associated product-stability testing; while drug-substance and vialed-drug-product manufacturing was completed prior to this reporting period, product-stability studies and clinical shipments of vialed DP continue. Approximately 23,000 vials of VRC01 DP remain in released clinical inventory of the more than 150,000 vials produced under this task order.

In addition to supporting VRC with the ongoing clinical supply of VRC01, VCMP remains engaged in a crucial external technology transfer of N6LS, a more potent anti-CD4 binding site bNAb being transferred to ViiV Healthcare, a subsidiary of GlaxoSmithKline, for additional clinical development and potential commercialization. N6LS was produced at the VCMP in 2018 using CHO mammalian cell culture at the 1,000-liter single-use bioreactor scale. This past year, VCMP addressed and documented several risk assessments and multiple corrective and preventive actions (CAPAs) that arose from a facility and quality systems audit led by ViiV Healthcare in January 2019; the audit was a prerequisite needed to justify the transfer of N6LS from NIAID/VRC to ViiV Healthcare.

KEY ACCOMPLISHMENTS

- N6LS External Transfer to ViiV Healthcare
  - Seven CAPAs completed to support transfer of clinical materials to ViiV Healthcare; one final CAPA targeted for completion in late 2020
  - Preliminary transfers of master cell bank (MCB) and reference standard completed to support development work
- VRC01: Long-Term (Frozen) Stability Studies
  - Fifteen active stability studies on vialed DP lots
  - One active stability study on one drug substance lot (unfilled)
  - Several stability interim reports (SIR) issued to support VRC regulatory filings
- Clinical Supply/Shipping of VRC01 DP
  - Shipped 122 vials to NIH Clinical Center for clinical testing
  - Shipped 4 vials to Duke University Medical Center for clinical testing

Integrated long-range planning is integral to advancing this and other task orders in partnership with the VRC. Long-range planning meetings occur monthly and focus on integrated schedules (six-month to two-year horizon) for multiple projects so we can use plant capacity optimally and build capability planning into workplans. These meetings also serve as a forum to recalibrate the current plan (6-month overview) as necessary to accommodate priority VRC programs.

ViiV Healthcare's VCMP pilot plant audit in January 2019 led to several ongoing improvement opportunities. In addition to addressing several CAPAs, the audit incentivized us to embed risk assessments and risk-based principles in several facets of our operations (change control management, environmental monitoring, facility operations, and GMP clinical manufacturing). These improvements influenced the successful outcome of two other facility/quality systems audits also conducted in this reporting period—a one-day audit by Ridgeback Biotherapeutics LP (Ridgeback) and a three-day audit by the NIH Office of Research Support and Compliance—which both occurred in October 2019 and reported no critical observations.

Research, Development, and Production to Support Clinical Trials for HIV Vaccines

We focused on moving forward with developing, producing, and supplying two novel, structure-based recombinant glycoprotein "trimer" subunit vaccines, designed by VRC for HIV prevention. The first trimer (HIV Trimer 4571) was manufactured at VCMP in 2018 and is currently being administered to healthy volunteers with alum-based adjuvant under an IND application submitted to the FDA in November 2018, supporting ongoing Phase I clinical trial VRC-018, a dose, safety, tolerability, and immunogenicity study. The second trimer (HIV Trimer 6931) was manufactured at the VCMP in the current reporting period (refer to key accomplishments below). Along with a fusion peptide conjugate vaccine (refer to additional task order summary below) and a proprietary non-alum-based adjuvant (Adjuplex™) to be manufactured in late 2020 at VCMP, both HIV Trimer vaccines will be evaluated under a new IND to support planned 2021 clinical trial VRC-019. Additionally, all immunogens will be shared across NIAID-funded groups via the Collaborative HIV Immunogen Project initiative.

KEY ACCOMPLISHMENTS

- HIV Trimer 6931: Completed technology transfer (drug substance (DS) cell culture and purification; DP formulation and fill/finish) and method transfer and qualification in this period, enabling clinical production and testing
- HIV Trimer 6931: reference standard qualification report in final review (QP.00.R36)
- HIV Trimer 6931 clinical production:
  - MCB L/N 19-381: produced 300 vials on September 17, 2019; quality assurance released on January 31, 2020
  - DS L/N 20-146: bulk (DS yield: 3.7 grams) completed June 16, 2020
  - DP L/N 20-296: filled 2,649 vials (1.2 ml in 3-ml vials) DP yield on July 8, 2020
- HIV Trimer 6931 regulatory support: Chemistry, Manufacturing, and Controls content inputs assembled and on target for new Drug Master File submission in 2020
- HIV Trimers 4571 and 6931: long-term stability studies
  - SIR issued for Trimer 4571 DS (suspended after 19.5 months)
  - SIR issued for Trimer 4571 DP (active; 18-month time point reached)
  - Trimer 6931 stability studies for DS and DP initiated
- Clinical Supply/Shipping HIV Trimer 4571
  - Shipped 95 vials to Illinois Institute of Technology Research Institute for the Good Laboratory Practice repeat-dose toxicity study on May 6, 2020
  - Shipped 75 vials to NIH Clinical Center for clinical studies (VRC-018 and 19-I-0069)

Early partnering and collaboration with VPP concerning the technology transfer of Adjuplex™ under license to NIAID led to significant advancements: (i) identifying raw material vendors, (ii) purchasing new equipment for adjuvant-components emulsification, and (iii) aligning on bulk-versus-terminal sterilization strategies for planned clinical manufacturing later in 2020. This collaboration involved innovation and creativity in streamlining considerations before the product was manufactured and supplied.

Broadly Neutralizing Monoclonal Antibodies

We supported VPP in developing a manufacturing process, producing a compliant MCB, and characterizing a reference standard in preparation for the future cGMP clinical production of CAP256V2LS, a bNAb directed against the V1V2 binding site of the HIV envelope protein.

The CAP256V2LS-expressing CHO-cell-derived MCB was previously manufactured by VCMP (September 2018; 250-vial bank) and was released on January 31, 2019 for future clinical manufacturing use. The reference standard, produced by VPP, was tested and demonstrated to be "fit for purpose" by VCMP to support the analytical release testing of production batches and long-term stability studies. Analytical tests included Octet potency assay; reducing and non-reducing microfluidic electrophoresis; isoelectric focusing; purity profiling with size-exclusion chromatography; and analyses of neutralization, appearance, pH, osmolality, residual Protein A, residual host cell protein, and residual DNA. All results were documented and summarized in a reference standard report approved by VPP during this reporting period.

KEY ACCOMPLISHMENTS

- Reference standard qualification report (QP.00.R26) was completed on October 28, 2019
- The MCB remains in inventory storage at the VCMP for future use in clinical manufacturing
- A final report for this task order was submitted on February 28, 2020

Development and Manufacturing of HIV-1 Fusion Peptide Conjugate Vaccine

Conjugated vaccines are created by covalently attaching a poor antigen to a strong antigen, thereby eliciting a stronger immunological response. Large inactivated toxins such as KLH, recombinant tetanus toxoid heavy chain (rTTHC), and diphtheria toxoid CRM197 can be used as carrier proteins to elicit stronger responses when conjugated to a target of interest. Conjugation technology historically has been used in bacterial vaccines to induce the generation of T cell–mediated immune responses. Conjugated vaccines can improve priming, elicit an immunogenic memory response (production of long-lived memory B cells), boost effect upon new contact with the specific antigen (revaccination), lead to affinity maturation of the antibody response, and generate mucosal immune response (secretory IgA and mucosally active IgG) to elicit long-lasting immunity.

The VRC aims to quickly advance a promising eight–amino acid HIV-1 fusion peptide (antigen) conjugated via small molecule (chemical linker) to rTTHC (carrier protein) for evaluation in human clinical trials. This project includes preclinical support, development support, technology transfer of production processes, analytical method transfer/qualification, and clinical manufacturing/supply of a peptide conjugate vaccine. The ultimate goal is to use the peptide conjugate vaccine in planned Phase I studies that include HIV trimer vaccines (described above) and the proprietary non-alum adjuvant Adjuplex™ (described above).

We have produced critical starting materials for this project using subcontracted GMP production (FP8v1 synthetic peptide and a small-molecule chemical linker)

and in-house GMP manufacturing (rTTHC carrier protein). VCMP will perform chemical conjugation of the three components into a vaccine conjugate (FP8v1-rTTHC). Production of the rTTHC carrier protein was completed at the VCMP in August 2019 using a bacterial fermentation process that yielded 140 grams of purified protein. We have developed the peptide-linker-carrier conjugation process is complete, and the technology transfer from VRC's VPP to VCMP is planned. Manufacturing the FP8v1-rTTHC conjugate vaccine DS and vialed DP is scheduled for late 2020 or early 2021. The vialed DP will be released to VRC in early 2021 for Phase I clinical trial VRC-019.

KEY ACCOMPLISHMENTS

- The rTTHC (carrier) intermediate DS was released on December 26, 2019 for use in planned conjugate vaccine manufacturing.
- The FP8v1 synthetic peptide was received from the subcontractor in September 2019; its identity was confirmed with quality control in June 2020.
- Manufacturing of the chemical linker is ongoing.
  - Subcontract activities initiated in March 2020
  - Feasibility batch delivered in August 2020
  - GMP batch manufacture and delivery planned for December 2020
- Repeat-dose toxicity studies initiated via subcontract; studies involve HIV trimer vaccines (4571, 6931), FP8v1-rTTHC conjugate vaccine and Adjuplex™ adjuvant

As a result of a VCMP-led quality systems audit of the initial supplier of development-grade linker material, we have changed course to identify a new subcontractor capable of GMP-grade manufacturing. FNL followed an established, rigorous source evaluation group (SEG) process to select a qualified subcontractor to develop and produce a GMP-grade chemical linker. Additionally, integrated planning in close collaboration with VRC was required to redefine the production timeframe for GMP manufacturing and releasing the peptide conjugate vaccine in preparation for its clinical evaluation in early 2021.

Anti-HIV Broadly Neutralizing Monoclonal Antibodies for AMP Trials

We filled vialed DP for VRC01LS, VRC07-523LS, and 10E8VLS derived from previously produced DS batches. Additionally, we manufactured DS (2,000-liter bioreactor scale) and filled vialed DP of CAP256V2LS, and filled vialed DP of PGT121.414.LS, a novel anti-HIV bNAb directed at the N332 glycan region of the HIV envelope. A third-party contractor to the Division of Acquired Immunodeficiency Syndrome (DAIDS) at NIAID supplied the DS for this final bNAb.

All vialed DP lots (CAP256V2LS, VRC01LS, VRC07-523LS, and PGT121.414.LS) were released to VRC for clinical use in this reporting period as outlined below.

KEY ACCOMPLISHMENTS

- Product Release Notifications
  - VRC07-523LS: L/N 19-218, 5,770 vials (6.25 ml in 10-ml vials), September 10, 2019
  - VRC01LS: L/N 19-150, 3,300 vials (6.25 ml in 10-ml vials), September 10, 2019
  - PGT121.414.LS: L/N 19-125, 3,570 vials (4.75 ml in 10-ml vials), October 10, 2019
  - CAP256V2LS: L/N 19-321, 1,405 vials (6.25 ml in 10-ml vials), December 11, 2019
- CAP256V2LS was determined "safe to proceed" by the FDA on March 24, 2020
  - The first patient was administered CAP256V2LS by Center for the AIDS Programme of Research in South Africa (CAPRISA) in July 2020.
- SIRs issued:
  - CAP256V2LS: three SIRs were issued (up to six months stability data collected to date for DS and DP)
  - PGT121.414.LS: three SIRs were issued (up to 12 months stability data collected to date for DP)
- Clinical Shipments of bNAbs: PGT121.414.LS, VRC07-523LS, and CAP256V2LS
  - CAP256V2LS
    - Shipped 100 vials to CAPRISA for CAP012B
  - VRC07-523LS
    - Shipped 1,200 vials to the Clinical Research Products Management Center (CRPMC) for ACTG5357
    - Shipped 160 vials to CRPMC for HVTN 136/HPTN 092
    - Shipped 40 vials to CAPRISA for CAP012B
  - PGT121.414.LS
    - Shipped 240 vials to CRPMC for HVTN 136/HPTN 092

Innovation and creativity were required to support the PGT121.414.LS program, in which DS was manufactured by a third party contracted under DAIDS, and the corresponding vialed DP was filled at VCMP. This required a complex collaboration and coordination involving multiple government agencies and their respective contractors and subcontractors.

Pandemic Response and Reemerging Infectious Disease

Malaria Vaccine Evaluation

The VCMP supports Malaria vaccine and therapeutic development for the VRC. The scope of work includes analysis of samples from patients receiving experimental Malaria vaccines to improve vaccine design and dosing regimens, understand immune correlates of protection, and identify bNAbs for potential development as prophylactic therapies. In the final year of this five-year task order, work focused on completing animal studies, advancing a GMP product into Phase I clinical trials, and initiating project closeout.

KEY ACCOMPLISHMENTS

- More than 40 anti-Malaria antibody candidates screened by a subcontractor in a mouse model of Malaria infection
- Product release notification: Anti-Malaria antibody CIS43LS DP L/N 19-237, 1,504 vials (5.5 ml in 10-ml vials), September 10, 2019
- IND application for CIS43LS was submitted and received a "safe-to-proceed" letter from the FDA
- Received the Special Achievement Award for a Scientific Team in FNL's 22nd Annual Achievement Awards in response to efforts for the CIS43LS anti-Malaria antibody
- Issued six SIRs (up to nine months stability data collected to date for DS and DP)
- Shipped 105 vials in three shipments to the NIH Clinical Center Pharmacy to support a Phase I clinical trial (VRC 612)
- Issued final report for this task order

The VCMP successfully concluded this project by submitting an IND application for the CIS43LS anti-Malaria antibody, which was manufactured by VCMP in the previous contract year. An FDA "safe-to-proceed" letter enabled the VRC clinical team to initiate Phase I clinical trials in early 2020. The VCMP regulatory affairs team provided critical technical support to the VRC Office of Regulatory Sciences both for the initial IND submission as well as for responses to questions received from the FDA following the submission.

In recognition of the VCMP team's innovation and ability to quickly adapt to project changes in the previous year, the VCMP received a Special Achievement Award, capping off a successful five-year project that resulted in new therapeutic candidates for Malaria prevention and treatment and the initiation of a first-in-human clinical trial.

Tuberculosis Vaccine Development

The VCMP supports Tuberculosis (TB) vaccine research and development for the VRC. The scope of work includes research focused on improving existing vaccine approaches, such as identifying the optimal route of vaccine administration and generating new vaccines with improved efficacy. In the final year of this five-year task order, work focused on completing existing animal studies, generating a final report, and initiating project *closeout.*

KEY ACCOMPLISHMENTS

- TB challenge study in nonhuman primates (NHPs) completed under subcontract to assess efficacy of bacillus Calmette-Guérin (BCG) vaccine candidates administered at increasing dose levels via intravenous route
- TB challenge study in NHPs initiated under subcontract to compare BCG vaccine candidates administered via intravenous route

The VCMP was able to complete one NHP study and initiate work on one additional study to advance development of animal models for TB vaccine evaluation. The COVID-19 pandemic impacted the studies, which required coordination with the government and subcontractors to establish mitigation plans. In one instance, the study plan was altered to enable the subcontractor to continue the study with limited staffing. Initiation of an additional study has been delayed until the next year due to commitment of the subcontractor's BSL3 animal facilities to COVID-19 research. Through close coordination with the government, we have maximized the amount of work that can be completed on this expiring task order, as well as identified a successor task order to continue providing support for the TB vaccine development project at VRC.

Research, Development, and Production of mRNA Vaccine Antigens

The VCMP supports research and development for novel vaccine platforms being explored at the VRC. One approach that has been of major interest over the past four years is the development of vaccines based on messenger RNA (mRNA), which, if successful, would offer a rapid and scalable approach to vaccine production. The scope of work for this task order includes the development of up to 30 mRNA-based vaccine candidates encompassing various disease targets. The resulting candidates will enable the VRC to compare mRNA-based vaccines with conventional protein-based vaccines in animal models to assess immunogenicity and *efficacy.*

KEY ACCOMPLISHMENTS

- Six mRNA vaccines were synthesized, formulated into lipid nanoparticles, and delivered to VRC for testing.

Research, Development, Production, and Support of Filovirus for Clinical Trials

There is only one licensed vaccine and no licensed monoclonal antibodies available to prevent or treat Ebola or Marburg virus infection. In support of VRC's Filovirus Program, VCMP advances promising candidates into the clinic by overseeing preclinical studies where vaccines or monoclonal antibodies are tested for efficacy in animal models, supporting process development at the VPP, and transferring technologies to the VCMP pilot plant for execution of GMP DS and DP manufacturing. In previous years under this task order, VRC and VCMP collaborated to advance an experimental antibody initially isolated from an Ebola survivor in the Democratic Republic of Congo (DRC) in 1995. The antibody, identified as mAb114, has been manufactured by VCMP and has proven to be one of the most promising and effective Ebola therapies in decades. Additional support is provided by managing stability programs for products manufactured at contract manufacturing organizations and filled by VCMP, including investigational vaccines utilizing a chimpanzee adenoviral vector (ChAd) platform.

KEY ACCOMPLISHMENTS

- Four preclinical studies were completed via subcontract to examine the efficacy and durability of vaccine candidates and identify protective monoclonal antibodies.
- Five SIRs were issued for mAb114 (up to 12 months stability data collected to date for two DP lots).
- Two SIRs were issued for ChAd-Ebola and ChAd-Marburg vaccine products.
- Nine shipments totaling 2,400 vials of mAb114 were shipped to the Institut National de la Recherche Biomédicale in the DRC to support the Pamoja Tulinde Maisha (PALM) randomized controlled trial (RCT) and expanded access protocols for compassionate use.
- We shipped 833 kits and assembled more than 200 additional kits ready for shipment to field hospitals to support the ongoing trials in DRC.
- Sixty vials of mAb114 were shipped to the Royal Free Hospital in the United Kingdom for individual authorization.
- The combined efforts of VCMP and the Clinical Monitoring Research Program Directorate led to the PALM trial being selected by the Society for Clinical Trials for the 2019 David Sackett Trial of the Year Award.
- We received Leidos, Inc.'s Scientific Innovation Award in the 2019 Achievement Awards.

The activities performed by the VCMP during this period have had a tremendous impact on public health and long-term potential for an effective, licensed Ebola therapy. Over the course of this project, VCMP has manufactured over 4.2 kg of mAb114 DS resulting in more than 10,000 filled DP vials for clinical use. VCMP has shipped more than 6,000 vials of mAb114 DP in total to clinical sites, including a Phase I clinical trial overseen by the VRC and, most importantly, an RCT being conducted in the DRC, where an Ebola outbreak has persisted since 2018. Due to early results from the RCT study, which originally included four separate experimental treatment groups, the data safety and monitoring board recommended no further enrollment in two of the experimental groups, with all patients going forward being randomized into only two groups. One of the groups that will continue to enroll patients is the mAb114 group. This decision was due to interim safety and efficacy data, indicating superiority of mAb114 relative to two of the other experimental groups. This marked a tremendous moment for the FNL and the VRC. As a follow-on effort to this project, VCMP has produced additional lots of mAb114 DS and DP under a successor task order, described in more detail elsewhere in this report. As part of this follow-on task, VCMP has supported an external technology transfer to Ridgeback, who has licensed the mAb114 process and is seeking a Biologics License Application from the FDA to support future needs for this public health initiative.

Pandemic and Vector-Borne Diseases

The VRC mission and scope includes responding to infectious disease outbreaks through research and development of clinical candidates targeted against a variety of public health threats. Such efforts include responses to pandemics, such as the pandemic in 2016 caused by the Zika virus and, most recently, the COVID-19 pandemic caused by the SARS-CoV-2 virus. In addition, there is a need to prepare for future pandemics, such as those caused by influenza, as well as to protect against vector-borne diseases, such as Malaria, which continue to affect large proportions of the global population. This project requires support from VCMP for a wide array of research, development, and clinical material production to enable maximum flexibility and adaptability for the VRC in its mission to respond to public health threats.

KEY ACCOMPLISHMENTS

COVID-19-related support:

- VCMP was granted an FDA registration for production of hand sanitizer.

- VCMP has manufactured more than 900 liters of hand sanitizer, resulting in 2,065 bottles available for healthcare use at the NIH Clinical Center, FNL, and other US government-affiliated facilities. Approximately 1,000 bottles have been shipped to the various organizations.
- Cell-line development efforts have been initiated under subcontract for a novel fungal-based expression platform for SARS-CoV-2 vaccine candidates.
- A subcontract has been issued to evaluate immunoglobulin isolated from vaccinated animals or convalescent serum in a SARS-CoV-2 challenge model.

In order to facilitate a rapid change in priorities for VRC and pivot efforts to the COVID-19 pandemic as well as other disease agents, the VCMP responded to a revised statement of work received from the government through an impact analysis report. This resulted in the reallocation of funds and effort toward unique aspects of the overarching VRC mission for pandemic response as well as responses to emerging and other vector-borne diseases.

One of the first requests related to the pandemic response efforts led by NIH was to determine if VCMP could rapidly and efficiently refocus manufacturing efforts toward production of hand sanitizer for the NIH campuses, FNL, and area hospitals. The VCMP received an FDA registration and put in place key manufacturing and quality measures to facilitate this request. These efforts resulted in production of over 900 liters of hand sanitizer with shipments to multiple destinations within NIH and FNL, as well as area hospitals/clinics, including the NIH Clinical Center, Suburban Hospital, Frederick Health Hospital, Children's National Hospital, Adventist Health Hospital, and University of Maryland Laurel Medical Center. The VCMP team demonstrated innovation and creativity in fulfilling this unique request, which has showcased FNL's ability to partner locally in service of public health.

Ebola mAb114 Drug Production, Labeling, Storage, and Distribution

There is only one licensed vaccine and no licensed monoclonal antibodies available to prevent or treat Ebola virus or Marburg virus infection. VCMP supports the VRC by advancing promising candidates into the clinic and assisting with external partnerships to advance products to late-phase and commercial development. Under a previous contract, VCMP manufactured and supplied a novel anti-Ebola monoclonal antibody, mAb114, in support of Phase I clinical trials at NIH and an RCT in the DRC, where an Ebola outbreak has persisted since 2018. In December 2018, NIAID entered into a license agreement with Ridgeback for the mAb114 product. VCMP is tasked with generating additional GMP materials and providing technology transfer support to facilitate additional manufacturing of mAb114 and a Biologics License Application by the licensee. Promising interim results from the RCT have increased the importance of this overarching program objective to advance mAb114, which is one of two treatments recommended for continued use by the data and safety monitoring board overseeing the RCT.

KEY ACCOMPLISHMENTS

- Two SIRs were issued for mAb114 (up to three months stability data collected for two DS lots)
- More than 3.0 kg of released mAb114 DS (2 total lots)
- Successful completion of a GMP facility audit for the VCMP pilot plant in October 2019 with no observations; audit was performed on behalf of Ridgeback to support Ridgeback's use of GMP mAb114 materials produced by VCMP
- One mAb114 DS lot transferred to Ridgeback to support license-enabling activities
    - Product release notification: mAb114 DS lot 19-399, January 8, 2020
    - Transferred 2.9 kg (83 containers totaling 59 liters of bulk at 50 mg/ml)

This period provided a unique opportunity for VCMP to support a mission-critical objective for the VRC, which includes transferring products to external licensees for advancement to late-phase and commercial development. For this project, the success of mAb114 as demonstrated by results in Phase I clinical trials and the RCT has led to a license agreement between Ridgeback and NIAID. In order to support the process transfer outlined in the license agreement, VCMP was required to provide manufacturing and regulatory documentation and technical guidance; submit to a facility audit; and transfer GMP DS. Each of these activities required extensive and unique engagement with the government and Ridgeback. Our success in this endeavor and the lessons learned will be critical to future external transfers for other products that show promise in the clinic and to NIAID's long-term objective of commercial availability of infectious disease therapeutics.

Tuberculosis Vaccine Candidate Testing and Development

The VCMP supports TB vaccine research and development for the VRC. The scope of work includes research focused on improvement of existing vaccine approaches, such as generating and testing new vaccines with improved safety and efficacy. This project succeeds a prior contract with similar objectives and builds off findings generated during the performance of the prior work.

KEY ACCOMPLISHMENTS

- TB challenge study in NHPs continued under subcontract to compare BCG vaccine candidates administered via intravenous route

The VCMP continued to oversee subcontractor institutions performing NHP TB challenge studies aimed at investigating new TB vaccine approaches. Future studies planned for the VRC will exceed the current capacity at existing subcontractor locations. As a result, subcontracts are being negotiated with additional institutions with expertise and capacity for NHP TB challenges.

Universal Influenza

Advance Universal Flu Ferritin Nanoparticle Candidates

Structural and immunological studies of the hemagglutinin (HA) and neuraminidase (NA) proteins support the concept of attaining increased immunological potency and breadth by novel presentation of the HA or NA proteins to the immune system. The VRC and its collaborators have developed traditional vaccines based on plasmid DNA (pDNA) and novel, self-assembling recombinant nanoparticle-based vaccines. These achievements have provided a framework for strategies to improve the potency and breadth of influenza virus immunity and provide a foundation for building broader vaccine protection against emerging influenza viruses, including the potential for a universal influenza vaccine with cross-strain protection. To date, this task has advanced influenza vaccines targeting influenza A Group 1, Group 2, and pan-group, including strains with both seasonal and pandemic potential. Three clinical products produced by VCMP are under evaluation in Phase I trials. In the final year of this task order, work has focused on continued stability monitoring of vaccines produced in prior years, evaluation of new clinical candidates in animal studies, and advancing a Group 2-directed vaccine targeted against the stem region of the HA protein into Phase I clinical trials. In addition, we have initiated project closeout activities, and new influenza work has transitioned to a successor task order to continue efforts toward a universal influenza vaccine.

KEY ACCOMPLISHMENTS

- H10ssF-6473 (Group 2 Influenza A vaccine)
  - Successful release of DS (42 g)
  - Product release notification: DP L/N 19-411, 4,463 vials (0.7 ml fill in 3-ml vials), May 2020
  - Received "safe to proceed" letter from FDA for a Phase I clinical trial on June 12, 2020
  - Three SIRs issued (up to six months stability data collected to date for one DS and DP lot)
  - IND-enabling repeat-dose toxicity and viral-clearance studies completed under subcontract
- H1ssF_3928 (Group 1 Influenza A vaccine)
  - Nine SIRs issued (up to 18 months stability data collected to date for two lots of DS and DP)
- pDNA NA vaccine
  - Three SIRs issued (up to 24 months stability data collected to date for one DS and DP lot)
- pDNA HA vaccine
  - Three SIRs issued (up to 24 months stability data collected to date for one DS and one DP lot)
- Completed 10 preclinical studies under subcontract to assess potential candidates in animal models of infection

The VCMP successfully concluded this project with the release of a Group 2 Influenza A vaccine (H10ssF-6473) for clinical use and submission of an IND application. We received an FDA "safe-to-proceed" letter, which will enable the VRC clinical team to initiate Phase I clinical trials in 2020. The H10ssF-6473 product marked the first successful production of a ferritin-based nanoparticle vaccine using a CHO-based expression platform. Previous efforts using the ferritin-based nanoparticle technology relied on transient transfection of cells in order to generate product, which can lead to inconsistencies when moving from batch to batch. Through engagement with the VPP, the VCMP executed a manufacturing process using the more stable CHO-based expression system, which provides higher titers of vaccine in addition to more consistent expression of the target. This new technology required innovation and collaboration by both teams in order to be successful.

Advance Vaccine Platforms for Universal Influenza Vaccine

Through prior efforts, VCMP and VRC have made promising advances toward universal influenza vaccines that are broadly protective. Furthermore, an executive order issued on September 19, 2019, for modernizing influenza vaccines has led to increased focus and determination by the VRC and VCMP in producing a universal influenza vaccine. New technologies have been requested to reduce the need for annual vaccinations, which are currently based on predictions of circulating influenza strains. Successful universal influenza vaccines will not only target seasonal influenza but also pandemic influenza strains. Starting in 2019, and continuing through 2020 and beyond, the VRC and VCMP have collaborated to advance a novel, mosaic influenza vaccine candidate. The top candidate uses a self-assembling nanoparticle that can co-assemble with targets of interest, allowing for a single vaccine molecule to display multiple target epitopes. In this case, VRC investigators have used HA molecules encoded by varying influenza strains. Once assembled, the nanoparticle vaccine displays a mixture of HA targets from its surface. The VCMP is tasked with advancing the lead candidate (FluMos-v1) through GMP manufacturing to Phase I clinical trials.

KEY ACCOMPLISHMENTS

- H10ssF_6473 (Group 2 Influenza A vaccine)-readiness for potential transient transfection process
  - Development cell stock and MCB completed

  - Raw material GMP pDNA completed
- Progress toward a mosaic flu nanoparticle vaccine (FluMos-v1)
  - Five MCBs completed for individual components including four HA targets and a nanoparticle-scaffold recombinant protein
  - Four raw material pDNA GMP batches for HA's
    - Strain 1: Lot# 20-111; Date of manufacture (DOM): March 13, 2020 (5.7g)
    - Strain 2: Lot# 20-085; DOM: February 29, 2020 (5.7g)
    - Strain 3: Lot# 20-124; DOM: March 27, 2020 (5.4g)
    - Strain 4: Lot# 20-386; DOM: August 25, 2020 (2.4g)
  - One GMP DS campaign for HA
    - Strain 1: Lot# 20-292; DOM: August 4, 2020 (340 mg)

The VCMP continued to provide support for the VRC universal flu program by completing starting material production (such as cell banks and other GMP raw materials) and has produced one DS component for the first mosaic flu vaccine product (FluMos-v1). In order to support this undertaking, the VCMP had to alter the approach outlined in the original request for proposal, which assumed an alternate platform would be used for the vaccine molecule. The VCMP collaborated with the process development team from the VRC to identify the critical technologies and processes needed to accurately reprice and adjust the project plan accordingly. With support from FNL's Finance, Project Management, and Contracts teams, these details were captured in an impact analysis report which was ultimately approved by NCI in June, enabling critical work to continue on this project.

Support to **Other Institutes**

Frederick National Laboratory
for Cancer Research

sponsored by the National Cancer Institute

OTHER INSTITUTES

Office of the Director, National Institute of Arthritis and Musculoskeletal and Skin Diseases

Support Provided by the Clinical Monitoring Research Program Directorate

Clinical Protocol/Regulatory Support

The mission of the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) is to support research that will lead to increased knowledge and understanding of the causes, treatment, and prevention of arthritis and musculoskeletal and skin diseases. Toward this effort, the NIAMS Intramural Research Program conducts studies in natural history and treatment, as well as basic investigations of the etiology and/or pathophysiology of rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, vasculitis, scleroderma, myositis, osteoarthritis, and other inflammatory/rheumatic diseases.

Frederick National Laboratory for Cancer Research (FNL) staff in the Clinical Monitoring Research Program Directorate (CMRPD) supported eight active NIAMS studies that varied from screening and training to natural history and Phase I and II Investigational New Drug (IND) clinical trials.

Staff provided protocol review services and regulatory and clinical trials management support to allow NIAMS to streamline protocol development time, provide flexibility for emerging/fluctuating needs, eliminate costly time delays, and ensure the success of their clinical mission. The staff supported the writing of protocols and informed consent forms, offered regulatory guidance, ensured regulatory compliance, and provided training and clinical trials management services, including case report form review and monitoring activities. They also prepared and submitted an IND annual report and assisted with document creation, data collection, and compilation for regulatory submissions to the Food and Drug Administration and other regulatory authorities.

To improve efficiencies, FNL staff in CMRPD developed an internal tool that tracks FNL's performed site visits and monitoring activities to help track overall project status and monitoring status. The online tool allows team members to quickly determine each project's status on a protocol, site, and monitoring level and improves the efficiency of site monitoring visits and communication about project deliverables.

National Institute of General Medical Sciences

Support Provided by the Business Services Directorate

G08 – NIGMS Government LBR Support

The Business Services Directorate provided technical project management on one research subcontract.

Office of the Director, National Institute of Environmental Health Sciences

Support Provided by the Clinical Research Directorate

Clinical Operations Support

A Frederick National Laboratory for Cancer Research nurse practitioner supported the evolving requirements of the National Institute of Environmental Health Sciences clinical research programs. Activities included supporting patient scheduling, evaluation, and treatment; providing clinical trials support; gathering complete and accurate clinical data; and ensuring all regulations for the protection of human subjects were followed.

Office of the Director, National Institute of Mental Health

Support Provided by the Clinical Monitoring Research Program Directorate

Clinical Protocol/Regulatory Support

KEY ACCOMPLISHMENTS

- Facilitated approvals of three new COVID-related research protocols and amended four protocols to add a COVID-19 research task
- Audited informed consent forms for actively enrolling protocols on behalf of the National Institute of Mental Health (NIMH) Office of the Clinical Director

FNL staff in the Clinical Monitoring Research Program Directorate (CMRPD) provide protocol development/navigation, regulatory guidance/support, clinical trials management, and data safety monitoring for intramural clinical research protocols conducted by the NIMH. Specialized staff manage approximately 80 protocols per year. The NIMH protocols consist of Investigational New Drug (IND) and non-IND clinical research studies.

Clinical Trials Management

The CMRPD Clinical Trials Management team monitored more than 60 protocols, approximately half of which are considered more than a minimal risk and 18 of which are under Food and Drug Administration (FDA) INDs/Investigational Device Exemptions (IDEs). The team developed four and revised six monitoring plans, reviewed new protocols prior to initial Institutional Review Board (IRB) submission, helped the NIMH Office of the Clinical Director develop two Division of Intramural Research Program guidance documents and a standard operating procedure on the use of laboratories and tests for protocols, maintained the Access monitoring database to keep track of the monitoring activities at NIMH, kept track of independent safety monitor reporting and annual IND reporting deadlines, developed training videos for incoming NIMH intramural research award trainees, and verified that all credentialed NIMH investigators listed on the FDA 1572 forms of the NIMH-sponsored protocols had valid and up-to-date medical licenses.

These FNL staff members in CMRPD also represented the Office of the Clinical Director at 11 meetings with quality assurance managers and personnel from different National Institute of Health (NIH) institutes/centers; helped to facilitate 12 protocol coordinators' meetings; ensured the accuracy and integrity of collected study data, as well as compliance with IRB-approved protocols and International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use/Good Clinical Practices (ICH/GCP) guidelines; resolved study documentation discrepancies; and communicated all site monitoring reviews and observations to principal investigators (PIs) and clinical research oversight managers.

To ensure protocol compliance and study data validation, the Clinical Trials Management team conducted three site initiation visits, 20 interim monitoring visits, and one study close-out visit. Additional support activities included training clinical research staff, preparing and distributing study documents, tracking study agents in NIH required pharmacies, and ensuring that the study agent(s) and devices were maintained in compliance with study protocols and FDA regulations that are under an IND or IDE.

FNL staff in CMRPD also participated in the NIH Quality Assurance Intramural Research Auditing Committee monthly meetings to discuss the different institutes'/centers' quality assurance procedures and practices and to ensure consistency among overall standards for activities such as monitoring trials, consenting participants, and implementing corrective and preventative action plans.

To support the streamlining of essential document maintenance within the institute, FNL staff developed and presented an executive summary to the NIMH Office of the Clinical Director to promote the use of the Clinical Trial Database electronic regulatory binder and library feature. The database will ultimately help investigators stay compliant with ICH/GCP guidelines, FDA regulations, and NIH policies with regard to record-keeping and maintaining essential study files. FNL staff also conducted an audit of informed consent forms for 29 NIMH protocols actively enrolling across 13 branches and confirmed that the original paper consent forms could be destroyed by the Health Information Management Department, given that the certified forms had been appropriately scanned into the medical records.

Protocol Navigation

The FNL Protocol Navigation Team (PNT) in CMRPD worked with NIMH PIs and clinical study staff to ensure protocols and informed consent forms aligned with policies that govern human subject research and helped to prepare submissions to the appropriate regulatory bodies and the Scientific Review Committee. The transition to the new NIH centralized IRB brought about new policies that affected the investigators, including policy 201 (training) and policy 801 (problem reporting). Although the IRB provided information to investigators, FNL staff managed the changes and helped the PIs understand how the policy revisions affected their individual protocols. This required frequent communication to ensure PIs understood updated training and reporting requirements. PNT completely revised the NIMH training database and expanded the training spreadsheet to include a full roster of on-site and off-site investigators and any special agreements, improving the communication of training needs to research teams.

The PNT helped investigators navigate the NIH IRB's protocol submission process with the new protocol tracking system (iRIS), directed PIs to training and tutorial information, hosted instruction sessions, and answered iRIS questions at the monthly PI/study coordinator meetings. The PNT also met with investigators and coordinators in one-on-one settings to provide additional support and answer more complicated questions.

In response to the coronavirus pandemic, PNT helped NIMH PIs write three new COVID-19-specific protocols and amend four exiting protocols to add COVID-19 research-related questions. PNT helped PIs prepare the new protocols using the new Office of IRB Operations (IRBO) template and guided them through the submission process. Most of the NIMH PIs had not yet written a protocol or a consent on the new IRBO template and needed assistance with sample language and completion of new protocol sections. PNT also assisted with the document submission process, ensuring that forms were completed properly and the correct materials were submitted to the IRB to avoid delays.

To help amend existing protocols with the addition of electronic research questions aimed at understanding the mental health impact of COVID-19, PNT reviewed the draft amendments and offered suggestions to clarify the language and ensure alignment with the original protocol focus.

PNT also managed, tracked, and coordinated associated regulatory activities for the 67 protocols under the navigation program (56 active protocols, 12 in data analysis only). Each protocol required the coordination of yearly regulatory oversight/continuing review between PNT, the research staff, and the IRB. PNT also provided writing/editing support in all aspects of the protocol life cycle, consent forms, recruitment materials, and associated forms.

To facilitate scientific committee reviews, protocol amendments, and applicable data and safety monitoring board (DSMB) reviews, FNL staff completed all necessary paperwork for the clinical and scientific directors' review, served as the executive secretary for the DSMB, and provided technical and operational management for all DSMB activities (e.g., prepared DSMB meeting documents, coordinated conference calls, prepared meeting minutes). There were two DSMB meetings, at which approximately 20 protocols were reviewed.

FNL staff also provided oversight and support to the PIs for IRB initial reviews, continuing reviews, informed consent forms, and responses to stipulations, as well as for regulatory documentation, including clinical report writing, FDA IDE/IND serial submissions, research-related training oversight, safety reporting, and annual FDA reporting. The team manager regularly presented at bimonthly meetings with the NIMH team members, providing updated status reports on clinical/regulatory activities, data quality, and FDA/IRB and DSMB submission timelines; additionally, a CMRPD member served on the data sharing team, which oversees the implementation of genomic and human data sharing for all applicable protocols.

Office of the Director, National Institute of Neurological Disorders and Stroke

Support Provided by the Clinical Monitoring Research Program Directorate and Clinical Research Directorate

Clinical Research Support

Clinical Protocol/Regulatory Support

The National Institute of Neurological Disorders and Stroke (NINDS) conducts and supports research on brain and nervous system disorders. The FNL supports NINDS' mission to reduce the burden of neurological disease by providing regulatory and clinical trials support for basic, translational, and clinical research on the normal and diseased nervous system.

NINDS clinical research applies directly to the mechanisms of nervous system diseases, which can then be translated into disease detection, prevention, and treatment, including studies of brain imaging techniques; trials to test new drugs; and novel therapy development, such as stem cell implants and gene transfer. Some key areas of this research include the neurological consequences of AIDS, Alzheimer's disease, brain tumors, developmental disorders, epilepsy, motor neuron diseases, muscular dystrophies, multiple sclerosis, neurogenetic disorders, pain, Parkinson's disease and other neurodegenerative disorders, sleep disorders, spinal cord injury, stroke, and traumatic brain injury.

FNL staff members in the Clinical Monitoring Research Program Directorate (CMRPD) regulatory group serve as liaisons on regulatory issues with sponsors, the Food and Drug Administration (FDA), and other stakeholders. The staff develops, assembles, submits, and maintains Investigational New Drug (IND) applications; writes and edits protocols; and prepares protocol amendments, continuing review applications, and reportable events reports.

The regulatory team supported NINDS' expanding portfolio of IND products, including treatments for gene therapy of giant axonal neuropathy, recurrent glioblastoma, and Pompe disease. The regulatory staff submitted several protocol amendments, information amendments, and IND annual reports for these projects, and they regularly provided strategic advice and guidance to the study investigators on best options for submitting IND materials or obtaining feedback from the FDA regarding study issues.

An FNL protocol navigator facilitated the following processes for NINDS studies: closure of one study; change of status for another; and development of: five initial studies, eight ethics clearances, three continuing reviews, nine protocol amendments, and one site initiation visit.

Due to the COVID-19 pandemic, most clinical protocols were unable to recruit participants. FNL staff helped the research teams pivot to protocols that would allow volunteers to participate from home, including facilitating protocol amendments and initial reviews of new protocols so that research could continue.

Clinical Operations

A nurse practitioner in FNL's Clinical Research Directorate supported the NINDS Human Cortical Physiology Section (HCPS), which aims to further understand motor learning, motor function, and motor control to help improve the lives of chronic stroke patients through noninvasive stimulation and neurorehabilitation. The nurse practitioner helped HCPS recruit and enroll participants (including healthy volunteers and traumatic brain injury and stroke patients) in protocols, providing coverage for a high volume of experimental sessions and improving the quality of the section's research activities.

The nurse practitioner also provided clinical coverage, served as an additional medical consultant to HCPS, supported inventory management and medical supplies

acquisitions, tracked training requirements for fellows and investigators, and served as the primary point of contact for for a laboratory postbaccalaureate fellow.

Collaborating with HCPS investigators and fellows, FNL supported continued enrollment in a protocol that examines the brain phase timing of magnetic stimulation on motor learning/motor signal in stroke patients and healthy volunteers. Additional support included working closely with a protocol navigator to make protocol amendments to improve recruitment and clinical operations, integrate new templates, add telehealth provisions, and reimagine an existing study to better serve patients in the surrounding communities. FNL helped the NINDS Clinical Trials Unit improve the adverse events log in the CiSTAR database and was instrumental in getting each new protocol's database and data management operations/operating procedures up and running.

FNL collaborated on a study seeking to address gaps in knowledge about the accuracy of delivering phase-dependent transcranial magnetic stimulation (TMS) to chronic stroke patients. The study showed promising results (Hussain SJ, Hayward W, et al., *Brain Stimul*, 2020) that TMS can be accurately delivered during predefined oscillatory phases in the lesioned brain.

Office of the Director, Walter Reed Army Institute of Research

Support Provided by the Biomedical Informatics and Data Science Directorate

SysBioCube

FNL staff have developed and maintained the SysBioCube data platform for the U.S. Army Medical Research and Materiel Command, U.S. Army Center for Environmental Health Research (USACEHR). SysBioCube serves as a central portal for data collection, integration, analysis, mining, and knowledge-sharing by collaborators in the army, academia, and private institutions.

In FY2020, USACEHR was deactivated, and funded research was transitioned to the Walter Reed Army Institute of Research. Owing to these organizational changes, during the initial months, FNL staff were requested to support transitioning SysBioCube to a third party. However, in the latter months, transition efforts were shelved and FNL staff were requested to continue supporting the platform and team members from the Coagulopathy of Trauma (CoT) project.

Owing to the team's exceptional knowledge of the platform, FNL staff were well positioned to support this fluidity in requirements during FY2020. The team initially focused on creating documentation, deployable code bases, and multiple data packages. To ease migration efforts, the code was also customized to support both MySQL and Oracle relational databases. In the latter months, the team expertly handled, collaborated, and supported CoT users. Clinical data from all sites was reviewed, and reports were generated with detailed notes on validation issues. Expertise in the team was immediately recognized, and the team is now being requested to lead and validate all clinical and assay data generated by the CoT collaboration.

Office of the Director, NIH Clinical Center

Support Provided by the Cancer Research Technology Program

Clinical Center

The Clinical Laboratory Improvement Amendments (CLIA) Molecular Diagnostics Laboratory (CMDL) provides support to the NIH Clinical Center for pharmacogenomics assays. The laboratory has established a CLIA-verified array-based assay to identify more than 1,900 variations in a panel of drug metabolism, excretion, and transporter (DMET) genes. The CLIA-validated DMET assay is performed on DNA extracted from whole blood for patients undergoing treatment at the NIH Clinical Center. Assay results are reviewed by the CLIA group, and a CLIA report is generated for a subset of targeted genes with all data and the report submitted to the Clinical Research Information System.

During FY2020, we have integrated and validated a new array-based platform, PharmacoScan, to replace the DMET assay, which will not be supported by Thermo Fisher Scientific after December 1, 2020. The PharmacoScan assay contains more than 4,000 variants, including those currently on the DMET platform. The PharmacoScan assay provides comparable performance and is capable of handling 24 samples per assay, albeit at a higher cost for samples submitted individually.

UGT1A1 is a gene of clinical interest that displays population variants in the length of a dinucleotide repeat in the 5 prime promoter region. The UGT1A1 variants are not well identified on the DMET or PharmacoScan platforms. We have collaborated with Dr. Tristan Sissung (Center for Clinical Research) to evaluate several methods for detecting length variants in the UGT1A1 promoter region and have published those comparative studies (Sissung et al. *Int J Mol Sci*, 2020).

National Center for Advancing Translational Sciences

Support Provided by the Biomedical Informatics and Data Science Directorate

Therapeutics for Rare and Neglected Diseases program – NCATS

KEY ACCOMPLISHMENTS

- Developed an integrated web interface for displaying known genotype and phenotype details for rare diseases, specifically creatine transporter deficiency (CTD) and Farber disease (FD)
- Manually curated *SLC6A8* variants for CTD and *ASAH1* variants for FD
- Harmonized curated variants and integrated presentation with protein structural annotations

Rare Disease Informatics Portal

The Therapeutics for Rare and Neglected Diseases (TRND) program, part of the National Center for Advancing Translational Sciences (NCATS), supports the preclinical development of therapeutic candidates intended to treat rare or neglected disorders. There are more than 7,000 rare and neglected diseases, yet only about 250 treatments are available. The majority of rare diseases have a genetic cause, and because of the low incidence, the syndrome may not be recognized for months or years. Correctly diagnosing the cause in a timely manner is necessary to facilitate potential treatment options.

FNL staff piloted a research effort to determine incidence and prevalence, focusing on two rare diseases: CTD and FD. The team approached the daunting task by creating an integrated environment containing engineering and research components. They created a web interface by integrating gene, variant, and literature modules previously developed by FNL staff and disease modules developed by NCATS. Extensive literature curation was conducted, and all published variants for *SLC6A8* and *ASAH1*, genes known to cause CTD and FD, respectively, were documented along with all relevant clinical details. The manually curated variant list was then compared with known variants in widely accessed variant resources such as ClinVar and the Single Nucleotide Polymorphism Database (dbSNP), and overlaps and differences were documented. The web interface further incorporated components for visualizing the manually curated variants and all known variants derived from FNL's internal Annotation, Visualization, and Impact Analysis (AVIA) application. Custom modules for displaying variants on protein 2D and 3D structures were also integrated.

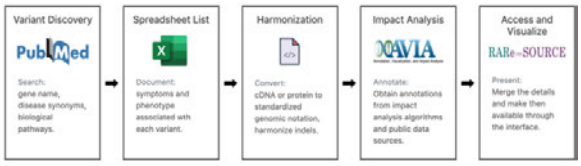

Figure 1. Workflow for manual curation, harmonization, annotation, and integration.

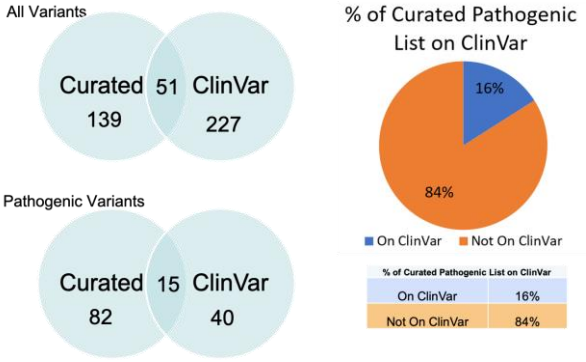

Figure 2. Venn diagrams and pie charts comparing the percent overlap of SLC6A8 *variants in literature and ClinVar.*

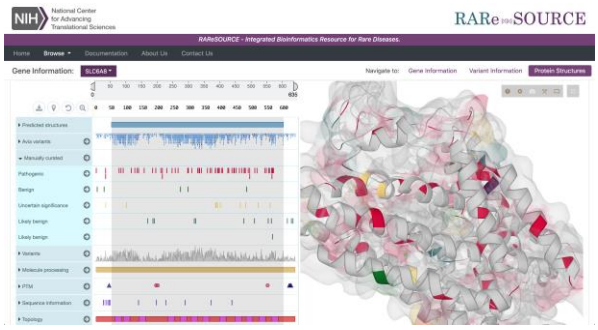

Figure 3. Variant visualization on protein structures.

Niemann–Pick disease type C

In a preliminary analysis of two studies and additional surveillance data collected on Niemann–Pick disease type C (NPC) patients, FNL staff observed a statistically significant decrease in the rate of progression after treatment with 2-hydroxypropyl-β-cyclodextrins (VTS-270).

NPC is a rare genetic disorder with childhood onset that results in the inability of the body to transport cholesterol and other lipids within the cell. The accumulation of these lipids in the brain and other tissues

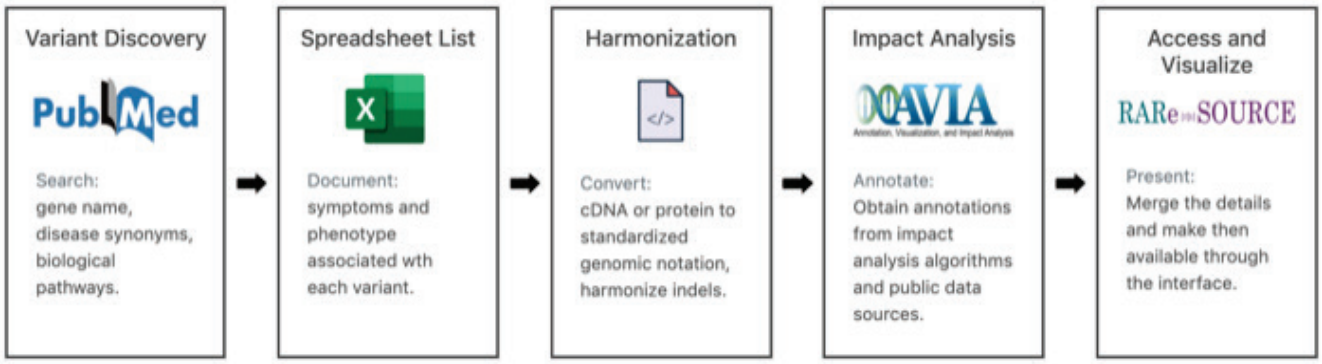

Figure 1. Workflow for manual curation, harmonization, annotation, and integration.

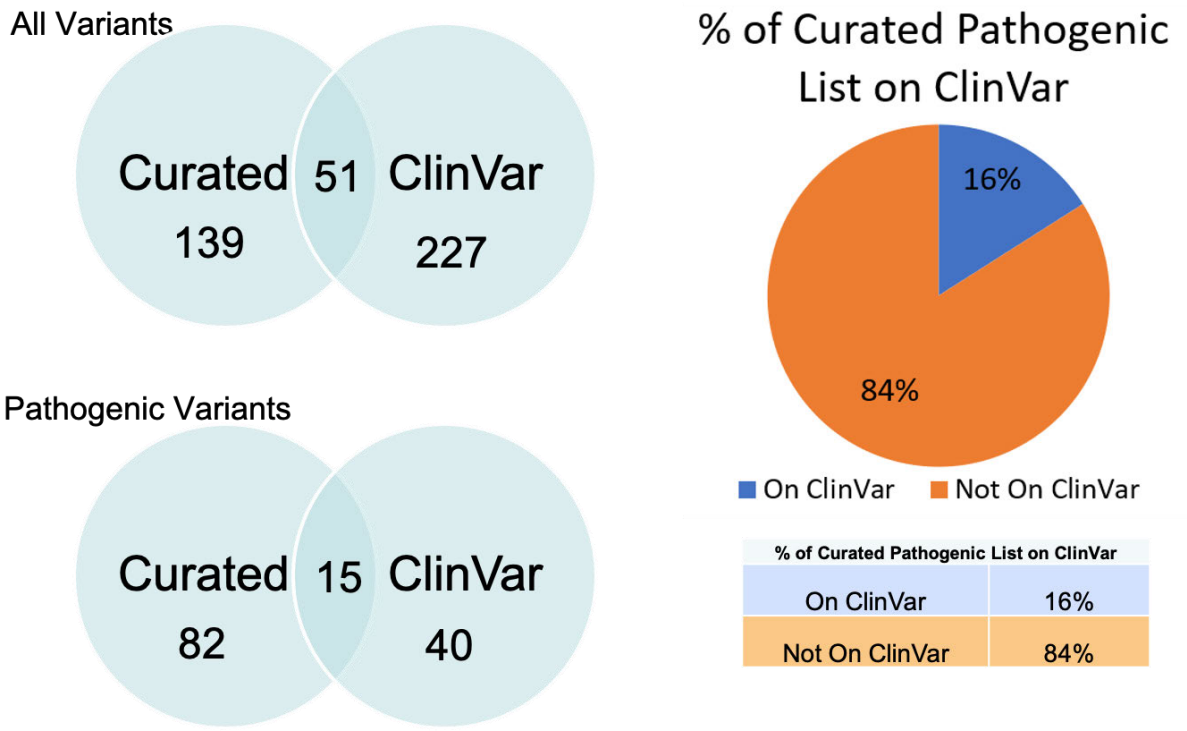

Figure 2. Venn diagrams and pie charts comparing the percent overlap of SLC6A8 *variants in literature and ClinVar.*

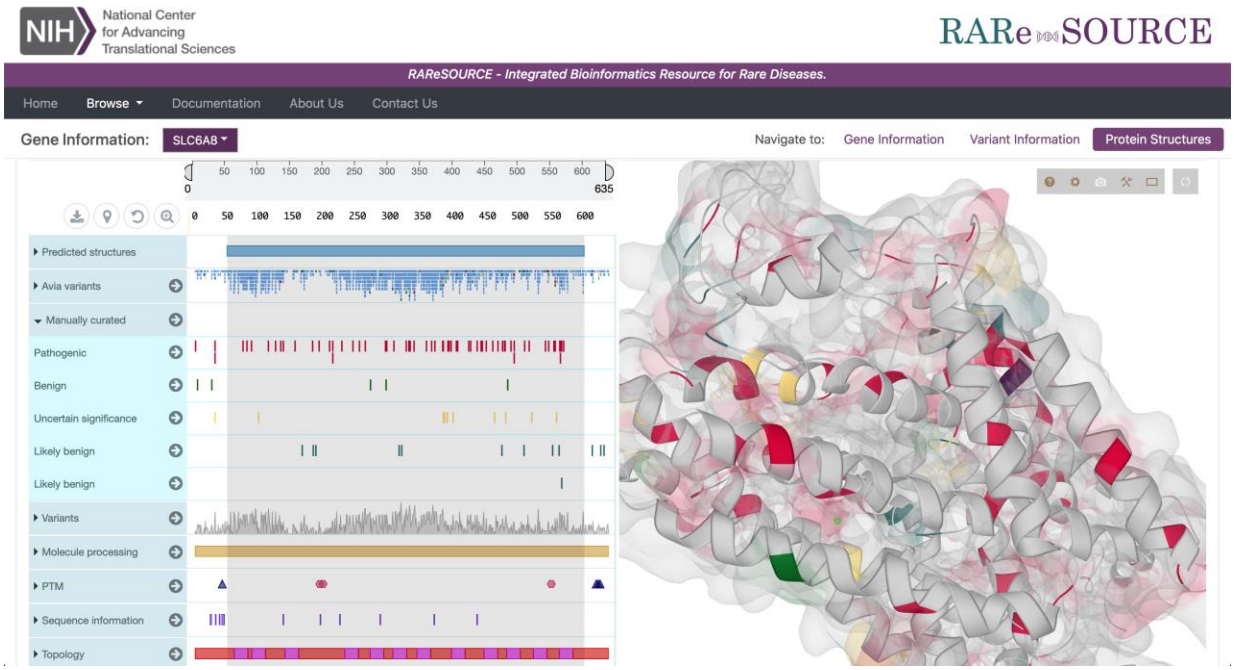

Figure 3. Variant visualization on protein structures.

results in damage to the affected areas and typically presents as a chronic progressive disorder that eventually causes life-threatening complications. Disease onset and progression vary significantly, with some fatalities occurring a few months after birth and other patients experiencing severe complications into adulthood.

The use of 2-hydroxypropyl-β-cyclodextrins (VTS-270) has been shown to decrease symptoms and increase lifespan in mouse models and has been tested previously in Phase I and II trials over 18 months (Ory et al., *Lancet*, 2017). However, the results from these trials indicate that a shorter trial of one year is unlikely to yield conclusive results. Given the debilitative nature and often-rapid progression of the disease, it will be difficult to enroll enough patients affected with this rare disease in a placebo-controlled trial spanning multiple years. Data from two studies as well as continued surveillance of study participants are being combined and analyzed to establish whether existing evidence is sufficient to obtain Food and Drug Administration approval for use of VTS-270 without the need of a Phase III trial. These studies include a natural history study following the progression of 18 individuals, concluded in 2009 (Yanjanin et al., *Am J Med Genet B Neuropsychiatr Genet*, 2009); 14 individuals from the Phase I/II trial (Ory, 2017); and an additional 75 individuals who have been treated with VTS-270 under expanded access.

Preliminary analysis of the data shows a significant decrease in the rate of progression after beginning treatment with VTS-270. When comparing the rate of progression after treatment with progression prior to treatment in the 94 individuals who spent at least one year on VTS-270, FNL staff observed a significant decrease in the rate of progression. In this comparison, FNL staff measured progression prior to treatment as if NPC were progressing linearly from birth until the time treatment began. In a linear mixed regression model comparing the observed rate of progression before treatment with the rate of progression of after treatment, we also observed a statistically significant decrease in progression. This is an indication of efficacy, which is required for Food and Drug Administration approval.

Support Provided by the Biopharmaceutical Development Program

Chemistry, Manufacturing, and Controls Support for the National Center for Advancing Translational Sciences

TTHX1114

The Biopharmaceutical Development Program had initiated TTHX114, recombinant protein for treatment of Fuchs Dystrophy. Feasibility, development, scale-up/Good Manufacturing Practice production, and testing and product release have been completed. An Investigational New Drug application was submitted and approved in July/August 2020. This is the first pharmacologic therapy for patients who have endothelial disease, mitigating the need for a corneal transplant for many patients.

Support Provided by the Clinical Research Directorate

Drug Discovery and Development Program

KEY ACCOMPLISHMENTS

- Eight projects supported by the Drug Discovery and Development Program (DDD) received authorization to proceed on their Investigational New Drug (IND) applications from the Food and Drug Administration (FDA) in FY2020, allowing those therapeutics to begin clinical trials.

DDD in the Clinical Research Directorate supports drug development candidates from a variety of National Institutes of Health (NIH) programs by leveraging the Frederick National Laboratory's capabilities and resources to bring NIH clients' therapeutics to the clinic. DDD and its forerunners have supported the Therapeutics for Rare and Neglected Diseases (TRND) and Bridging Interventional Development Gaps (BrIDGs) programs within the National Center for Advancing Translational Sciences Division of Preclinical Innovation since 2009 by providing a broad range of technical support and program management. To date, 31 IND applications received approval from the FDA. In FY2020, DDD added support to the National Center for Advancing Translational Sciences for one project for ocular wound healing, and it continued support for one development project for spinal cord injury with the BrIDGs program, one development project for gene therapies in collaboration with the Biopharmaceutical Development Program for the TRND program, and maintenance for 12 more projects entering the clinical phase, collectively providing $3.1 million of research and development for client therapeutics.

Office of the Director, National Heart, Lung, and Blood Institute

Support Provided by the Clinical Monitoring Research Program Directorate

Clinical Research Support

KEY ACCOMPLISHMENTS

- Submitted Program Management and Support Services Concept of Operations plan to the National Heart, Lung, and Blood Institute (NHLBI)
- Supported NHLBI investigators during the COVID-19 health emergency by facilitating the submission and approval processes of several new protocols, protocol amendments, and external collaborations

- Received appropriate approvals within 10 days to implement an expanded access protocol allowing use of convalescent plasma for treatment of COVID-19 patients at the National Institutes of Health (NIH)
- Completed multiple key updates to NHLBI protocols based on new Institutional Review Board (IRB) requirements, including extensive changes to Radiation Safety Review submission, donor changes to allogeneic transplant protocols, and changes to the new IRB consent template

Support to NHLBI Program Management and Support Services

FNL staff in the Clinical Monitoring Research Program Directorate (CMRPD) provide leadership and comprehensive program/administrative support to NHLBI for its new Program Management Support Services (PMSS) framework and infrastructure, the NHLBI Program Management Office. The Program Management Office will be responsible for directly supporting major NHLBI initiatives and implementing robust project management processes and procedures across the institute through training efforts, policy development, and change-management strategies. To date, the focus for FNL's support has been directed at three large-scale complex initiatives: Cure Sickle Cell, Catalyze, and BioData Catalyst.

The Cure Sickle Cell initiative is an NHLBI-led collaborative research effort that aims to accelerate the development of genetic therapies to cure sickle cell disease and to transform the lives of people who have the disease by creating a collaborative, patient-focused research environment. The Catalyze initiative offers funding to support product definition studies; preclinical research; and the development of cutting-edge platform technologies, technical support, and mentorship to help transform basic scientific discoveries into viable therapeutics, devices, and diagnostics to treat heart, lung, blood, and sleep diseases and disorders. The BioData Catalyst initiative is a cloud-based platform for tools, applications, and workflows that provides secure workspaces to share, store, cross-link, and analyze large sets of data generated from biomedical and behavioral research while also ensuring patient privacy.

The FNL staff member in CMRPD who serves as the PMSS operations director leads, in concert with NHLBI leadership, the PMSS effort to offer direction and oversight, manage awards, analyze business and budgets, manage projects, facilitate and administrate meetings, and coordinate programs for the extramural community. A mix of staff supports the work/research being conducted in the extramural community funded by NHLBI for each of the initiatives. This central team allows for the initiatives and other activities to receive consistent support and cross-coverage.

FNL hired a new staff member to serve as the PMSS operations director. The final draft for the PMSS Concept of Operations plan to begin documentation of the optimal structures, processes, training, communications, design frameworks, and methodologies that meet the objectives of the PMSS concept was submitted to NHLBI in July 2020.

FNL staff in CMRPD also provided administrative support to NHLBI's activities related to COVID-19, collaborated with the FNL Drug Discovery and Development Program to consider solutions for supporting the Catalyze Coordinating Center, and collaborated with the FNL Bioinformatics and Data Sciences Directorate to potentially support the BioData Catalyst initiative.

Clinical Protocol/Regulatory Support

FNL support to the NHLBI Office of the Clinical Director resulted in the rapid deployment of clinical services for time-sensitive, critical clinical research. FNL staff in CMRPD helped streamline protocol development times, provide flexibility for fluctuating needs, and ensure the success of the NHLBI clinical mission. The staff supporting the NHLBI Protocol Navigation Team (PNT) manages more than 200 clinical protocols and ensures proper submission of all protocols to the NIH IRB and the Food and Drug Administration, as applicable.

Program activities centered on updating various protocols to comply with new regulatory requirements imposed by the new NIH IRB. Staff also assisted several NHLBI clinical research teams with the submission of COVID-19-based clinical protocols or protocol amendments, as well as COVID-19-based collaborations with other institutions. This included technical review and report preparation, protocol navigation support, administrative coordination, and general logistical support for regulatory activities.

In order to comply with new NIH IRB requirements, the team consolidated several standard-of-care-based protocols into a single protocol supporting data collection of standard procedures for NHLBI by reviewing the protocols for common elements, assisting with regulatory language changes, working with the research nurses to ensure proper personnel were included, and facilitating protocol start-up by training all NHLBI Division of Intramural Research investigators. PNT assisted with major updates to the NHLBI screening protocol and tissue and sample collection protocols, with the goal of centralizing resources for better accountability.

PNT worked with NHLBI leadership, the NIH Transplant Group, the NIH Department of Transfusion Medicine, and the IRB to update seven NHLBI stem cell transplant protocols with new requirements (remove transplant donors from the protocols and incorporate them into standard-of-care protocols). Protocol navigators helped develop new protocol and consent language and trained nurses how to use these new protocols.

PNT helped to develop and implement several new intramural NHLBI COVID-19 protocols as well as protocol amendments to support COVID-19 research. Staff members helped quickly implement a Mayo Clinic–sponsored expanded access protocol at NIH for the

treatment of COVID-19 patients with convalescent plasma. While the Mayo Clinic requirements for approval of a site were minimal, the internal NIH requirements were far more stringent and required effective collaboration and communication across various NIH groups. FNL worked with the NIH and Mayo Clinic IRBs, the Office of Human Subjects and Research Protection, and the Ethics Office to ensure proper internal approvals were quickly granted and that documents were approvable per Mayo requirements. This process was completed in approximately 10 days, and the protocol became the only mechanism at NIH for the use of convalescent plasma.

PNT also worked on the development of several natural history studies to better define genetic markers of COVID-19 in patients with rare diseases and in patients who have undergone heart and/or lung transplants. The staff also collaborated with external sites for exchange of COVID-19-positive samples for additional research studies. These included two natural history studies evaluating genetic associations between outcome and genetic mutations in participants with rare diseases, as well as the development of one treatment study.

PNT members participated in beta-testing several new templates and processes for the IRB, identifying areas for improvement with electronic forms (e.g., ethics clearance, radiation safety), and providing feedback to the database developers.

Support to
NCI at Frederick Infrastructure

Frederick National Laboratory
for Cancer Research

sponsored by the National Cancer Institute

NCI AT FREDERICK INFRASTRUCTURE

Office of the Director, NCI at Frederick

Applied and Developmental Research Directorate

ADRD: ODF Support

ADRD Clinical Courier Services

KEY ACCOMPLISHMENTS

- Clinical Courier Services provides a dedicated clinical courier service for the transport of time-critical clinical samples and associated data between the NCI Campus at Frederick laboratories, the Advanced Technology Research Facility, and the NIH Bethesda campus as well as other locations within the Washington, D.C. metro area in support of NCI clinical trials. The primary responsibility of the Clinical Services drivers (driver/courier) is to transport patient samples from the NIH Clinical Center in Bethesda to the NCI at Frederick campus. In addition, our drivers also transport samples to and from the following: Georgetown University; Johns Hopkins University; Navy Medical Center; and the Poolesville Non-Human Primate Facility. Deliveries made within the Frederick National Laboratory for Cancer Research (FNL) include the Advanced Technology Research Facility, Wedgewood campus, etc.
- This transportation is required for all work performed by laboratories within the Clinical Services Program, including Clinical Laboratory Improvement Amendments–regulated diagnostic testing support. In addition, this service has been extended to include transporting clinical samples for Division of Cancer Treatment and Diagnosis Developmental Therapeutics Program support, specifically the Laboratory of Human Toxicology and Pharmacology, with the support effort extending over more than 100 clinical trials. The success of clinical monitoring and patient data analysis is dependent upon the timely and safe transport of potentially hazardous biological samples from patients enrolled on the various NCI and National Institute of Allergy and Infectious Diseases (NIAID) clinical protocols. The couriers perform this function daily, and each make two to three trips per day.
- With the approval of different Institutional Biosafety Committee registrations, the CSP Couriers are also involved in the pickup of critical COVID-19 patient samples from various labs within the NIH Clinical Center for delivery to the Integrated Research Facility as well as labs within the Applied and Developmental Research Directorate. Future sample pickup from other facilities outside of the normal routine, (i.e., Johns Hopkins) is anticipated.
- Due to the COVID-19 shutdown of research activities and many of the outpatients not being as active in their specific protocols, the number of FY2020 courier runs is down approximately 280, or 3,922 total runs, from last year.

ADRD Clinical Data Management

KEY ACCOMPLISHMENTS

- The Clinical Services Program Data Management Group (CSP DMG) Network Office was part of a Frederick IT Operations team that addressed high and critical vulnerabilities across 21 servers and 120 workstations on the Frederick NIAID network. The project consisted of performing a rapid evaluation of the public and private IP virtual local area network subnet on the Frederick NIAID network, which included monitoring security vulnerability assessments to determine the number and the severity of the vulnerabilities. The project required all critical vulnerabilities to be remediated within 30 days of discovery and all high vulnerabilities to be remediated within 60 days. The CSP DMG Network Office successfully completed this project by remediating over 120 critical vulnerabilities and 310 high vulnerabilities within the allocated time. In addition, the CSP DMG Network Office was extremely instrumental in troubleshooting and finding an innovative solution to resolve vulnerabilities related to embedded versions of Java within software applications used by the CSP laboratories.
- The CSP DMG Network Office performed an upgrade to replace and enhance the Production and Development/QA SQL Server environment on the Frederick NIAID network. A newer SQL server application platform was instituted to replace existing standalone SQL servers housing critical SQL server databases for the CSP which consisted of consolidating two physical servers to one virtual server. The upgraded server platform now provides increased manageability, efficiency and a more secure application server environment for the CSP.

Business Services Directorate

Other Support and Logistics Services

Central Glassware Services

Central Glassware Services (CGS) consists of five processing kitchens and a daily van run that provides satellite services to 12 buildings.

During FY2020, Central Glassware provided glassware processing services to 239 laboratories at the Frederick National Laboratory for Cancer Research. Services include the daily pick-up and processing of

soiled glassware and restocking of sterilized glassware. Central Glassware also provides special services on request, such as preparing bell units, preparing pipettes, processing laboratory spatulas and stir bars, and transporting and delivering media. All used media bottles and caps are recycled.

KEY ACCOMPLISHMENTS

- During this reporting period, Central Glassware added support to four new laboratories throughout the Frederick National Laboratory for Cancer Research. Central Glassware provided services (via the van run) to Buildings 321, 376, 426 (OHS), 429, 431, 434, 469, 535, 538, 550, 560, 562, 567, 1036, 1047, 1071, and the Advanced Technology Research Facility. Centralized ordering and deliveries are accomplished through the CGS office via the van run.
- Central Glassware continues to provide media transport services to and from Building 560 (via van pickups) to Buildings 376, 469, 535, 538, 560, and 567.
- The Building 539 glassware kitchen was closed permanently in August 2019. Relocation of labs in Building 539 to Buildings 376, 469, 538, and 560 was completed in July 2020.
- Central Glassware Services assisted with packing and unpacking all glassware-related materials for labs in transit.

Material Processed Annually
(Noted reductions of material processed is due to the COVID-19 pandemic)

Building	Material Processed
ATRF	98,820
535	64,540
538	55,890
560	155,200
567	25,200
Van Run Pickups	
321 (from 560)	6,100
376 (from 560)	51,200
426 OHS (from 560)	15
429 (from 560)	70
431 (from 560)	720
434 (from 560)	160
469 (from 560)	15,750
550 (from 560)	1,060
562 (from 560)	970
1036 (from 560)	2,100
1047 (from 560)	2,300
1071 (from 560)	1,980
Total material processed	**482,165**

Clinical Monitoring Research Program Directorate

Other Support and Logistics Services, Support to the Office of the Director

KEY ACCOMPLISHMENTS

- Provided oversight to several COVID-19 clinical research support activities
- Served on the International Association for Continuing Education and Training (IACET) Council on Standards Development (ICSD)

The FNL Clinical Monitoring Research Program Directorate (CMRPD) represents a comprehensive resource for many intramural clinical research programs. The directorate's management oversight facilitates NIH researchers' ability to provide the highest-quality clinical research that is compliant with applicable regulations and guidelines (e.g., Food and Drug Administration, Good Clinical Practice). CMRPD provides management and administrative support to regulatory, clinical, and programmatic efforts for government customer initiatives. It also hires, trains, and manages program personnel; develops and maintains IT infrastructures; develops and monitors clinical protocol/project budgets; provides medical and technical writing services; ensures compliance with all contract report requirements; and provides program-specific courier services.

Staff helped to maintain and operate the IT infrastructure within the Building 430 Data Center at the National Cancer Institute Campus at Frederick, provided continuity of IT operations and infrastructures, managed IT equipment for the directorate, and ensured technical support.

Staff also coordinated and tracked all required trainings and maintained the training documentation as required in a regulatory environment, ensuring Food and Drug Administration and Good Clinical Practice inspection readiness. A staff member also participated in the selection and configuration of a new learning management system for FNL and was selected to serve on the IACET ICSD, enabling best practices to be reflected in the revision of FNL training standards.

Staff members ensured the integrity of CMRPD's financial management, provided financial reports, and served as property accountability officers to ensure that all capital and sensitive equipment aligned with clinical research operations.

Medical and technical writers facilitated the development of publications, supported contract reporting requirements, and provided meeting summaries to document decisions for high-level meetings. In addition, a courier supported clinical research activities by transporting interoffice mail, regulatory documents, and medical records between government and contractor offices; by picking up clinical patient samples from various clinical sites; and

by delivering the samples to the FNL Applied and Developmental Research Directorate's clinical laboratories.

Contracts and Acquisitions Directorate

Alignment to Responsive Mission Support by Leveraging Mission-aligned and Engaged Employees

The Contracts and Acquisitions Directorate (C&A) is responsible for all FNL prime contract administrative functions required in performance of an indefinite delivery/indefinite quantity (IDIQ) TO (TO) contract awarded in fiscal year (FY) 2015 and the National Cancer Institute (NCI) Federally Funded Research and Development Center (FFRDC) IDIQ TO contract awarded in August 2019.

These contracts require similar but independent contract administration, purchasing, research and construction subcontracts, intellectual property and strategic agreements, and small business support by C&A.

As described within each of C&A's areas of responsibility, team members provide significant support and contributions to ensure the FFRDC's successful performance of the scientific work attributable to the FNL, including small business outreach activities.

These responsibilities include a direct interface with NCI contracting clients to ensure the contractual commitments of both parties are met and issues are addressed appropriately for existing TOs awarded to the FNL; early intellectual property identification and handling; and development of strategic agreements in support of the FNL.

C&A's responsibilities include purchasing all goods and services for FNL and subcontracting portions of the performance requirements to fully qualified and vetted third parties.

Representing the FNL, C&A has a very proactive small-business outreach program to ensure FNL's use of diverse supplier businesses including women-owned, historically underutilized business zone (HUBZone), veteran-owned, and service-disabled veteran-owned businesses. Often, FNL small business outreach is done in collaboration with our government small business offices and local Frederick organizations, such as the Chamber of Commerce.

Environment, Health, and Safety Directorate

Security

KEY ACCOMPLISHMENTS

The Emergency Management Program established a 68-member Full Emergency Operations Center (EOC) that has been instrumental in the COVID-19 preparedness, response, and recovery stages of the FNL and the National Cancer Institute (NCI) at Frederick's emergency plan. The EOC has operated virtually, with many subgroups established to coordinate multiple tasks from Command. The emergency manager coordinates and consults with the Fort Detrick emergency manager, fire department officers, and installation antiterrorism officer; the Frederick County emergency manager; the Frederick County Health Department; and Frederick and Montgomery Counties' Local Emergency Planning Committees.

Security/Access Control is responsible for all Homeland Security Presidential Directive 12 identification badge and cardkey transactions. FNL staff in Environment, Health, and Safety (EHS) designed and implemented substantial changes to visitor-management and key-control programs in response to a directive from National Institutes of Health (NIH) Security to eliminate the use of access cards for the majority of visitors. In addition, EHS informed employees of the need to register their personal identity verification (PIV) cards at the Fort Detrick Visitor Center so that the cards could be used for automated entry at the installation gates; moreover, EHS supported the coordination of employees' enrollment in this system.

Security closely monitors scientific equipment located at the NCI Campus at Frederick, the Advanced Technology Research Facility (ATRF), and the Vaccine Pilot Plant. Evaluations of fire extinguishers, automated external defibrillators, exit lights, fire sprinklers, water meters, electric meters, emergency lights, and exterior lights occur monthly. The Security staff monitors and grants access to irradiator room users. Security officers greet and register NCI at Frederick and FNL visitors. The staff also:

- Performed more than 900 PIV badge transactions for FNL and NCI at Frederick employees.
- Conducted more than 200,000 foot patrols, resulting in:
  - Investigation of 12,000 scientific and utility alarms.
  - Discovery of more than 2,000 security violations.
  - Discovery of more than 275 fire safety violations (such as portable heaters or coffee pots left on).
- Granted 200 employees access to secure irradiator rooms.
- Issued more than 1,600 visitor passes.
- Handled more than 1,800 escorts and door unlocks.

The Security staff has played an instrumental role in the COVID-19 pandemic response by maintaining its services uninterrupted and by handing out NIH-provided face coverings and distributing hand sanitizer to other employees who requested them.

Research Donor Program

The FNL staff in Occupational Health Services (OHS) administers the NCI at Frederick Research Donor Program (RDP), a research protocol approved by the NIH Institutional Review Board. The RDP supplies anonymous

donor blood and other human samples to researchers at NCI at Frederick. The RDP donations helped research in more than 25 different programs during FY2020. Currently, there are 258 donors in the program. Services to the RDP continued uninterrupted through the pandemic.

Waste Management

The FNL staff in Waste Management continued to improve operations in several ways:

- Deployed a recycling, neutralization, decay-in-storage, and surplus chemical program that saved $96,000 in costs
- Developed and implemented a program to collect animal-feed waters containing pharmaceutical compounds to prevent direct drain disposal of materials that are difficult to treat by a municipal wastewater treatment plant
- Implemented a new satellite accumulation area training and inspection program in support of hazardous waste accumulation compliance
- Implemented a program to retrofit medical waste cart lids to prevent water intrusion
- Implemented recommendations from the Biennial NIH Environmental Compliance Audit and the newly issued NIH Drain Disposal Guidelines, which were received late in the 2018–2019 contract year

General Safety

Administration

The FNL staff in EHS had several achievements during FY2020, including those with strategic significance and long-term impact.

KEY ACCOMPLISHMENTS

- FNL has successfully responded to the COVID-19 pandemic by implementing critical processes, establishing a high-performing cross-organizational leadership team, and monitoring and managing activities through the emergency management organizational structure.
- EHS has implemented the Job Profile Questionnaire (JPQ), which involved developing the questionnaire and establishing an online tool for employees to take the questionnaire. The tool gives employees and supervisors tailored reports that enumerate the proper training and other requirements they need to complete work safely and compliantly. This process provides the necessary foundation for moving toward EHS services tailored to employees and work groups. Moreover, this work, along with EHS's critical participation in the implementation of the new Empowering Development, Growth, and Excellence (EDGE) training management system, will allow for the retirement of the Job and Safety Profile Educational Requirements web application developed by Data Management Services (DMS).
- FNL implemented the TOPAZ enterprise system's Institutional Biosafety Committee Registration module, which, for the first time, allows for integration with the Animal Care and Use Committee (ACUC) Animal Study Protocol processes. This new system will also allow for the retirement of the Institutional Biosafety Committee (IBC) web application developed by DMS.

Response to COVID-19 Pandemic

Beginning in early January 2020, EHS staff began tracking the virus now known as SARS-CoV-2 that caused the COVID-19 pandemic. Early identification of the pandemic emergency through participation in local, state, and federal communications meetings and briefings allowed EHS to rapidly establish processes to respond to the pandemic early on. Among its activities and accomplishments, the staff:

- Activated the EOC to coordinate the pandemic response efforts across FNL and NCI at Frederick and interface with external public health groups and other stakeholder organizations.
- Established leadership team meetings to brief managers, to allow for discussion and decision-making on key actions and processes, and to ensure that all parties were aligned. In addition, EHS established a daily tracker that was published for several months, moving to a weekly tracker upon the return of the first wave of staff during Return to Work operations.
- Prepared and delivered required communications, such as mission-essential personnel lists, to the U.S. Army at Fort Detrick.
- Reviewed surface disinfectants currently in use to determine whether the Environmental Protection Agency (EPA) deemed them effective against human coronavirus.
- Developed and implemented a tool for screening possible COVID-19 cases in the workplace and supported recommendations for FNL's and NCI at Frederick's subsequent action.
- Developed and implemented the EHS Public Health Emergency Infectious Agent Cleaning and Disinfection Enhanced Cleaning & Disinfection standard operating procedure with an appendix for SARS-CoV-2. The appendix included a calculator for supplies and effort based on square feet of affected facilities. To date, 15 enhanced cleaning and disinfection operations have occurred.
- Developed a critical system "quick look" resource to underpin decision-making with respect to resource allocation.

- Facilitated rapid approval of COVID-19-related IBC registrations, as well as the development of additional laboratory space and resources.
- Procured face masks and set up a distribution operation to employees who requested them.
- Donated N95 respirators to the NIH Clinical Center, Johns Hopkins University Hospital, and Frederick Memorial Hospital.
- Performed a literature review of Return to Work recommendations and developed recommendations specific to FNL.
- Established processes and tracking procedures for employees concerned about COVID symptoms, which included ordering COVID testing when needed; tracking test results and consultations; and reporting activities to parties including NIH, NCI, Fort Detrick, FNL Human Resources, and Leidos Corporate.
- Performed contact tracing for positive COVID-19 cases in Frederick facilities. All OHS nursing staff members are certified contact tracers.
- Organized a cross-functional team to develop and implement Return to Work web resources, which include guidance; signage; training; checklists; in-depth questions and answers; and links to NIH, Centers for Disease Control and Prevention (CDC), and EPA COVID-19 information.
- Coordinated Return to Work preparedness, including:
    - Coordinating FNL employee lists for Return to Work groups.
    - Procuring and setting up a request, dispensing, and distribution operation for small bottles of hand sanitizer.
    - Helping the warehouse procure COVID-19 supplies, including reviewing disinfectants for efficacy against SARS-CoV-2.
    - Flushing water coolers/filter systems to remove stagnated water.
    - Placing outdoor waste receptacles to provide additional, convenient disposal for face coverings and other trash generated while outdoors.
    - Placing signs throughout the campus and setting up cleaning and disinfection stations in common areas.
    - Establishing an FNL-specific COVID-19 symptom self-assessment process used by persons reporting to the physical workplace.

Industrial Hygiene

As part of its COVID-19 pandemic response, EHS played a vital role in defining safe policy, Return to Work guidance, procedures for cleaning facilities, appropriate personal protective equipment use, and emergency operations support to advance FNL's and NCI at Frederick's mission. EHS conducted considerable outreach and education on the topics of respirators and face coverings. A policy on face coverings was created and communicated, and approval processes were instituted to ensure that employees purchased correct and compliant respiratory protection and face coverings. EHS developed and communicated training on proper N95 respirator use, along with advisories about new safety concerns of imported non-approved respirators flooding the market (KN95). Controls were instituted to prevent the inadvertent purchase of inappropriate masks and respirators, saving money and resources and ensuring compliance.

EHS helped FNL employees test and acquire a new type of powered air-purifying respirator that will be more comfortable and a safer alternative to traditional N95 respirators that are now scarce and required for health care workers dealing with COVID-19.

EHS conducted more than 2,200 comprehensive JPQ reviews for employees as subject-matter experts in multiple areas. This enormous undertaking provides each employee with a customized list of requirements for proper job training based on their documented job hazards. In addition, EHS reviewed all new-hire JPQs to ensure the correct training courses and surveillances were assigned at the start of employment.

EHS acquired expert consulting services to evaluate and make recommendations for the safe design and use of a laboratory apparatus to synthesize highly toxic nitric oxide compounds. The comprehensive report verified EHS's initial risk assessment and design, as well as assisted in the communication and development of written safety and compliance procedures for this task. These risk assessment methodology tools will be used on other future risk assessments.

EHS sampled 44 employees for exposure to noise, formaldehyde, isoflurane, formamide, methylene chloride, peracetic acid, and xylene, as appropriate, to ensure compliance with limits delineated by Occupational Safety and Health Administration, American Conference of Governmental Industrial Hygienists, National Institute for Occupational Safety and Health, and NIH policy.

Nine comprehensive risk assessment reports for other various laboratory work processes involving hazards such as ethane, hydrogen, benzidine, and nitric oxide were also completed, resulting in the mitigation of these hazards through engineering controls, administrative controls, and refinements to policy.

EHS was instrumental in the acquisition process for a new EHS enterprise system that will ultimately streamline, consolidate, and improve the efficiency of multiple EHS areas. EHS has begun to thoroughly document workflows and identify requirements to streamline integration into the new platform.

EHS conducted a security assessment of all critical infrastructure on the main campus (generators, transformers, steam and chilled water lines, etc.) to determine necessary measures/improvements to prevent damage and disruption to essential operations.

EHS reviewed 360 ACUC documents to evaluate chemical and biological hazards associated with animal research. In support of ACUC protocols and occupational safety, EHS continues to research, create, and update detailed recommendations for work involving hazardous agents. During FY2020, risk assessments were performed or updated for approximately 70 chemicals, drugs, and toxins, and appropriate work practice guidance has been issued for each hazard. More than 750 nonbiological agents are now approved for use in research animals with EHS guidance.

EHS led an effort to audit and make improvements to the asbestos management plan as a result of a contractor inadvertently disturbing asbestos-containing building materials in the Building 560 Wing 2 refurbishment. The corrective action plan has resulted in ongoing meetings to improve both EHS and Facilities Maintenance and Engineering (FME) processes and training regarding surveying, inventorying, communicating about, and removing hazardous materials. Improvements include emergency-response standard operating procedures for EHS, a mechanism to document hazardous materials surveys that may be required for shop work, a plan for incorporating hazardous materials drawings into all planned work orders, updated specifications for all abatement activities including blanket order and general contractors, additional specific training, and approval responsibilities for designated and better-defined job roles in EHS and FME. Meetings will continue to create and implement processes to improve construction designs and communications to all workers and employees.

Occupational Health Services

OHS provides occupational health services for all employees at NCI at Frederick and FNL.

All OHS staff members have completed certification in contact tracing by Johns Hopkins Hospital. OHS provides contact tracing services for employees in the workplace who test positive for COVID-19.

OHS aims to focus on proactive interventions aligned with CDC's Total Worker Health approach. This approach integrates protection from work-related safety and health hazards with the promotion of injury- and illness-prevention efforts by advancing the employee's well-being.

OHS provides international-travel consultations to support FNL's mission for employees working and traveling off-site. OHS continued to partner with a CDC-established yellow fever clinic to bridge the current shortage of yellow fever vaccines and provide an uninterrupted continuity of care.

The OHS team has developed collaborative relationships with experts at CDC, NIH, the National Institute of Allergy and Infectious Diseases, the local scientific communities, and the local emergency medical services team. OHS works to ensure that FNL employees receive the benefit of every work-health-related discipline in managing occupational medical concerns and issues specific to any or all potential or sustained occupational injuries and illnesses.

OHS coordinates an animal-allergy screening program for all NCI at Frederick and FNL employees who enter an animal facility and/or work with live animals or tissue. This screening identifies those employees who may present with allergy signs and symptoms, and it aims to prevent occupation-related asthma. There are 768 employees enrolled in the animal exposure program.

OHS is diligent in teaching employees the Emergency 1-2-3 approach to first aid for biological exposures. OHS used the Emergency 1-2-3 posters to promote awareness and ensure that employees know and understand measures to take in case of an exposure or injury. Professional staff remain on call 24 hours a day, seven days a week, to respond to after-hours biological emergencies. Efforts to augment responses to biological exposures include providing support to the HIV production laboratory on the NCI Campus at Frederick and establishing a lockbox with post-exposure medications at an ATRF laboratory working with viral vectors.

Work-related injuries and illnesses are treated in the Occupational Health Clinic by OHS clinicians. This in-house treatment enables OHS to help reduce employees' medical expenses and lost time, as well as workers' compensation costs. Most importantly, it enables a continuity of medical care that is the best practice for the health and welfare of NCI at Frederick and FNL employees. The OHS staff includes a certified workers' compensation nurse case manager who is knowledgeable in the unique risks and job requirements of working in a biomedical research facility. The case manager and clinicians work with employees; their supervisors; Human Resources; and, if required, outside medical providers to enable employees to return safely to work following injury or illness.

With the support of senior management and Human Resources, OHS submits an annual application for Leidos Biomedical Research, Inc., in Healthiest Maryland Businesses, which scores our organization's commitment to improving employees' health and well-being. The scoring tool is based on the CDC Worksite Health Scorecard. OHS met with the CDC liaison assigned to the Frederick region to review the scores and discuss additional resources to support our wellness efforts.

Biosafety

The COVID-19 pandemic caused a surge in IBC registration submissions, requiring expedited review and approval processes to meet the demands for supporting high-priority research. Over four months, 26 IBC registrations (including new and amendment registrations) were submitted and/or approved to conduct work with SARS-CoV-2.

EHS developed and implemented three computer-based training modules to encourage employee engagement in best safe practices for dry ice, viral vectors, and biosafety basics in animal research.

EHS conducted 111 audits to verify that research programs were complying with Alcohol and Tobacco Tax and Trade Bureau requirements for the use of tax-free alcohol for research purposes.

EHS reviews and classifies all outbound shipments for regulatory compliance requirements, including classifications for dangerous goods as well as import and export compliance, to support NCI at Frederick's and FNL's mission and to aid with obtaining necessary regulatory permits to comply with Export Administration Regulations. The EHS staff performs approximately 600 shipment reviews per month.

EHS collaborated with the TOPAZ Integrated Project Team to evaluate, develop, and implement the TOPAZ Elements IBC module. The TOPAZ module provides an advanced technology to support the research mission by streamlining completion, submission, and approval of IBC registries. In addition, the system makes the IBC administrative process for recordkeeping more efficient and enables IBC members to perform lead reviews and track their comments electronically within the system. The IBC module has also been successfully linked with the ACUC TOPAZ Animal Study Proposal module to integrate working relationships for both *in vivo* and *in vitro* research collaborations. To promote continuous improvement of the TOPAZ modules, EHS continues to collaborate with the Laboratory Animal Sciences Program and the Enterprise Information Technology Directorate to validate the functionality of the modules as scheduled system updates from the vendor take place and as process improvements are made in response to user feedback.

The Biosafety staff continue to serve on the ACUC to support the research mission while ensuring collaboration with the IBC. EHS reviewed an average of 28 Animal Study Proposal documents per month, providing a risk assessment and recommendations for best practices based on the hazards posed by the research scope.

Construction Safety

The Construction Safety Program helped ensure that construction subcontractors, maintenance subcontractors, and service vendors complied with federal safety and health regulations when working on FNL properties, which reduced the possibility of personal injury, property damage, and liability losses during their activities. More than 825 subcontracted workers completed the subcontractor safety orientation hosted on the subcontractor safety website.

The Construction Safety Program staff inspected job sites daily to determine whether subcontractors were working within the bounds of their accepted safety plan and federal safety and health regulations. As of early August 2020, more than 400 individual job site visits were made, and approximately 60 safety issues were identified and corrected. Four incident reports and three letters of concern were issued based on safety issues.

The Construction Safety Program staff remained instrumental in the review of subcontractors' safety plan elements by evaluating the planned sequences of work, the specific hazards anticipated at each step, and the control measures to be implemented to eliminate or reduce each hazard to an acceptable level of risk. Equipment to be used, inspection criteria, personnel, and training requirements were also taken into consideration. Overall, evaluation of the subcontractors' safety plan helped ensure that the subcontractor intended to work safely and in compliance with federal safety regulations. Approximately 1,000 safety plan elements were reviewed and accepted under the Construction Safety Program this year.

Complex projects requiring substantial EHS support completed during FY2020 include the planning and execution of major critical lifts for the Division of Cancer Treatment and Diagnosis modules, chiller upgrades at the ATRF, completion of the Building 560 Wing 2 refurbishment and moves, and work on Building 539 refurbishment projects. In addition, substantial improvements in the erosion and sediment control programs have been effective in partnership with FME.

Environmental Protection

Environmental Protection manages NCI at Frederick's environmental permits; tracks the emissions of air pollutants; ensures stormwater, erosion, and sediment control and sewer discharge permit compliance; conducts training; assists in emergency operations planning; and performs National Environmental Policy Act review of projects.

EHS obtained a reclassification of the ATRF wastewater permit from a Significant Industrial User to a High Strength User. This will provide $10,000 per year in cost savings.

EHS performed waste determinations for lead paint debris and concrete truck washout pit water that allowed both to be disposed as nonregulated waste. Lead debris was sent for metal recycling, and water was discharged to the Fort Detrick sanitary sewer with, permission from the U.S. Army Garrison (USAG) Department of Public Works. These saved approximately $15,000 in disposal costs.

A white paper discussing the need to address Maryland Department of the Environment Municipal Separate Storm Sewer regulation requirements via a specific agreement with the USAG was written and submitted to NCI at Frederick.

EHS performed an in-depth inspection of all stormwater management structures and submitted an annual report to the USAG for use in their Municipal Separate Storm Sewer System reporting. The EHS staff also added stormwater management structures to the Safety Inspection and Issue Management System database for issue tracking.

Radiation Safety

The possession and use of radioactive materials at the NCI Campus at Frederick and ATRF are controlled by the regulations of and licenses issued by the U.S. Nuclear Regulatory Commission (for licensed activities at the NCI

Campus at Frederick) and the Maryland Department of the Environment (for licensed activities at the ATRF). The Radiation Safety Office supports multiple open-source programs associated with the manipulation of microcurie and millicurie amounts of isotopes, including a positron emission tomography production facility and radiochemistry operations. Radiation Safety also supports programs such as X-ray-producing machines, electron microscopes, and sealed source programs. In addition, the Radiation Safety Office supports two gamma-cell irradiator programs.

EHS successfully obtained a radioactive materials license amendment from the Nuclear Regulatory Commission to add the isotope radium-223 in support of a clinical study on prostate cancer.

EHS amended protocols to provide relief from certain administrative requirements on radioactive materials programs during the COVID-19 pandemic.

Enterprise Information Technology Directorate

The Enterprise Information Technology Directorate (EIT) provides supporting IT services to the FNL and the National Cancer Institute (NCI) at Frederick scientific community. This support includes core infrastructure as well as conferencing audio/video, information security, web hosting, software solutions, end user support, and business systems. EIT data centers house the backbone of networking, compute, and storage across the enterprise and provide the scientific community with high-performance computing and tiered-storage capabilities. EIT software solutions, custom and commercial off-the-shelf (COTS), provide the scientific community with web and logistical automation solutions, as well as the back-office backbone, the enterprise resource planning (ERP) solutions. End user services provide personal computing equipment, deskside support, and help desk activities for handling service requests and resolving issues.

Key Accomplishments

During FY2020, FNL staff in EIT accomplished many operational initiatives and projects in support of the scientific community and operational organizations. Every area within EIT delivered on projects, which range from software solution upgrades, to new automation capabilities, to high-performance computing refresh, to modernization of networking infrastructure. In all, EIT completed over 75 projects, small to large, including:

- Supported rapid shift to telework for all staff as part of the COVID-19 response. This work included a significant spike for end user support, including remote laptop deployments and at-home configuration help calls, and required a surge in support from the information security team and infrastructure team to ensure connectivity and safe computing.
- Supported deployment of several new National Institutes of Health (NIH) enterprise desktop tools: Box.com, Microsoft SharePoint Online, Microsoft Teams, and Microsoft OneDrive. FNL staff in EIT also configured 100+ new teams and sites.
- Upgraded Facilities Maintenance and Engineering's (FME) enterprise asset management and work order system, Maximo, from an end-of-life version to the latest version. This effort included a data migration and functional changes to extricate nonstandard add-ons and move toward a best-practice implementation that can be upgraded in a regular cadence. Supporting capabilities were replaced with standard enterprise tools. This effort was completed in the fall of 2019.
- Completed and maintained the General Support System (GSS) Authorization to Operate, ensuring sound and compliant information security for continued operation of the core IT facilities, data center, network, storage, and compute
- Implemented a modern software solution to support the Institutional Biosafety Committee operations, replacing a legacy application with insufficient capabilities
- Upgraded the Frederick Research Compute Environment (FRCE) with modern high-performance-computing central processing unit (CPU) and graphics processing unit (GPU) nodes. The effort was completed in the first quarter of 2020, with additional compute nodes added in the second quarter.
- Completed two large milestones, in alignment with the NCI chief information officer, in movement toward an Information Technology Infrastructure Library service delivery model. In the first quarter of 2020, FNL staff in EIT began using the help and service ticketing capability of the NCI IT Service Management (ITSM) tool, allowing for the decommission of two legacy ticketing systems. In addition, EIT is using the Configuration Management Database capability of the ITSM tool to maintain IT items such as end user workstations, servers, databases, and applications.
- Provided a COTS-based shipping support solution to the Laboratory Animal Sciences Program (LASP), allowing for the decommission of a limited-capability legacy application
- Deployed several SharePoint workflow solutions, including a redesign of the FME Work Induction System
- Replaced the legacy FME "trends" database, deploying its replacement in the second quarter of 2020
- Consolidated disparate file shares to an enterprise network-attached storage (NAS) with encryption
- Consolidated the voice over Internet protocol (VoIP) servers with NCI IT

- Completed a Federal Information Security Management Act system survey to ensure software systems across FNL and NCI at Frederick are secure. This will be used to prioritize information security initiatives and improvement plans.

Continuous Improvement

Formed approximately two years ago, EIT has matured as a service-delivery organization comprising functional teams. Each team performs a functional area of EIT's efforts, working together as an integrated group to provide the IT solutions and services required to operate FNL and NCI at Frederick. EIT's goals are to continuously improve its service delivery, provide robust and modern IT infrastructure to the scientific community, replace technical debt with enterprise capabilities, and improve the information security posture.

An important part of EIT's effort to drive efficiency is having an enterprise architecture perspective that unifies solutions when possible, replacing legacy, point solutions with modern applications that provide capabilities applicable across the enterprise. The NCI at Frederick IT Governance process and EIT's work intake funnel, both of which were instituted and improved over the past two years by EIT's Program Management Office (PMO), allow EIT to review needs from an enterprise architecture vantage.

COVID-19 Support

During FY2020, FNL staff in EIT supported the COVID response with multiple efforts. The maximum telework effort was supported with a major spike in laptop deployments, virtual private network configurations, and infrastructure adjustments. In addition, EIT supported IT and information security for laboratories that pivoted to COVID work. Since the maximum telework policy was instituted, EIT has successfully continued and initiated IT project work without interruption.

Program Management Office

FNL staff in the EIT PMO continued centralizing and standardizing work intake and project delivery. The office works with NCI to ensure that IT projects comply with the Department of Health and Human Services project management framework, the Enterprise Performance Life Cycle. Working with the Office of Scientific Operations and the Center for Biomedical Informatics and Information Technology (CBIIT), the team has established common processes and governance principles for successfully delivering and implementating EIT projects. The EIT PMO team comprises project managers and other staff who help with project management, oversight, and governance.

KEY ACCOMPLISHMENTS

PMO's accomplishments in FY2020 include the following:

- Continued efforts to leverage cloud solutions and reinitiated cloud infrastructure as a service/platform as a service initiatives. EIT has deployed three significant software as a service product and continues to adopt a "cloud-smart" approach to new requests. EIT is currently working with CBIIT to set up infrastructure and application support in AWS Cloud One.
- Rapidly implemented (less than one month) a tool to support LASP laboratories' return to the workplace after maximum telework. The tool enabled principal investigators and other workers to schedule their time in the laboratory in accordance with post-COVID-19 guidelines.
- Completed a critical project to restructure the Costpoint ERP to support the new task order framework
- Deployed the new Maximo Asset Management and Work Order system for FME. This will act as a foundation for modernizing FME processes and making use of some of the latest capabilities of the tool moving forward.
- Managed several operational projects to support key EIT modernization efforts for infrastructure technology, including storage, networking, and servers
- Delivered on several key initiatives for business process improvements for FME (work induction and intake process), the Human Resources Directorate (automating integrations with external vendors), Finance (FocusPoint, Costpoint, and reporting improvements)

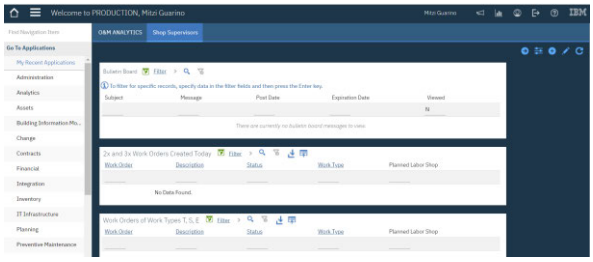

Figure 1. Maximo 7 production screenshot.

Continuous Improvement

- Continued to enhance and update the SharePoint site that is common across EIT and integrated documents from the IT Operations Group (ITOG), the Information Security and Compliance Office (ISCO), Business Enterprise Systems (BES), and Data

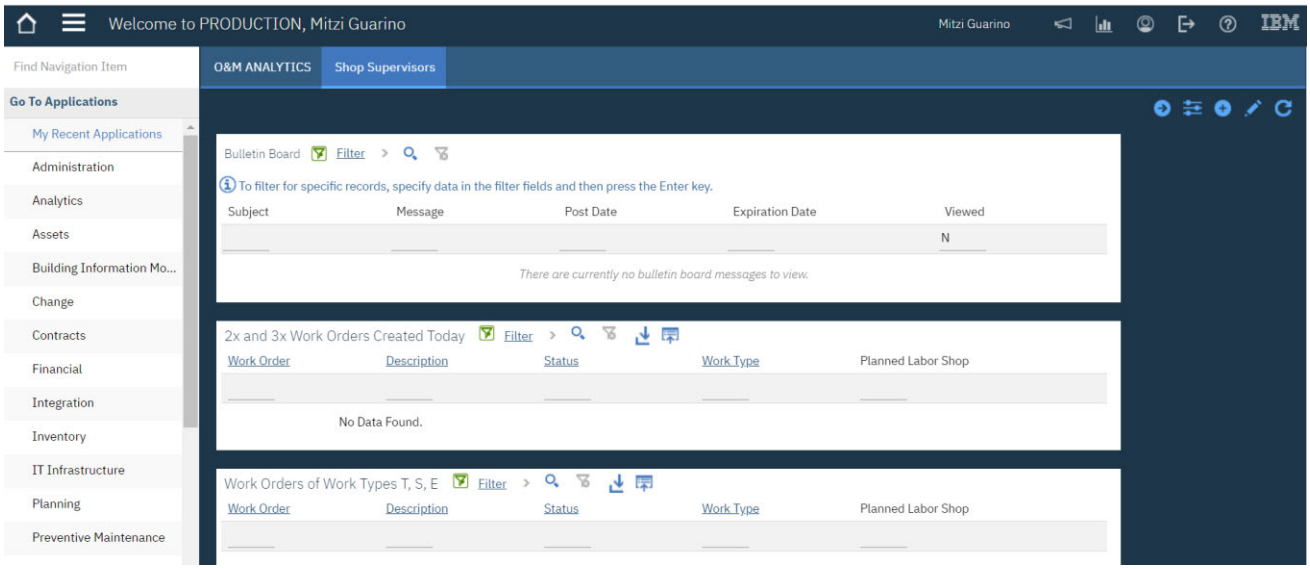

Figure 1. Maximo 7 production screenshot.

Management Services. These follow a periodic review process for accuracy and relevancy.

- Continued to make progress toward standardizing project and program portfolio management for all of EIT, in accordance with FNL and government leadership. This includes working with our enterprise architect to apply key The Open Group Architecture Framework principles.
- Worked on engaging key business stakeholders and set up a process for maintaining and managing their portfolios.
- Identified processes and services for improvement through customer feedback. FNL staff in EIT are now working to simplify and automate service requests across the community.

Infrastructure Technology Operations Group

ITOG is responsible for data center operations and the associated network, compute, and storage infrastructure that provide the basis for scientific and business operations. In alignment with the NCI chief information officer, ITOG maintains redundant NCI at Frederick data centers that host mission-critical enterprise storage; core networking capabilities; and a virtual, hyper-converged compute environment.

Figure 2. FNL/NCI at Frederick Data Center facilities.

KEY ACCOMPLISHMENTS

Key accomplishments specific to ITOG include the following:

- FNL staff in ITOG performed and supported the annual disaster recovery functional exercise to test the NAS infrastructure failover process. This exercise satisfied an EIT and NIH security requirement.
- The network team, jointly with CBIIT, continued the design and prototyping phase of the Network Modernization Project. This complex project involves information security and multiple vendors to design and implement the next-generation NCI network. The next-generation Fabric network of Core and Data Center switches uses dual 40-gigabit (Gb) connections for interconnections between Core switches, spine switches, and leaf switches, providing 80 Gb of aggregate bandwidth. These 40-Gb connections can be easily upgraded to 100 Gb when required.
- The network team upgraded 28 local area network (LAN) closets with 88 next-generation access switches; installed six new Core switches; and installed 12 new Data Center switches, six of which are chassis-based switches offering 10Gb, 40Gb, and 100Gb connections, and six of which are stackable switches that provide 1 Gb connectivity at the Advanced Technology Research Facility (ATRF) Data Center.
- The High-Performance Computing systems team upgraded the batch compute/high-performance computing hardware and software during FY2020, adding two high-memory nodes (3 TB ram per node) designed for graph-database use. The team also added 10 GPU nodes, each with eight NVIDIA v100 GPU cards, to meet the additional demands from image analysis (e.g., cryo-electron microscopy) and machine learning.
- The High-Performance Computing team maintained the FRCE compute system, including the 111 installed applications, and provided support for the 59 users who submitted 2,016,387 jobs that consumed 5,145,008 CPU hours.
- The systems team upgraded the capacity of the virtual machine infrastructure to meet the current and future demands for server resources. The team also completed the Windows server 2008 to Windows 2012 migration, began a migration of servers from Ubuntu to CentOS 7, and continued to migrate server and storage resources from the Building 430 Data Center to the ATRF Data Center as part of the Building 430 Data Center shutdown project.
- The storage team continued to increase storage capacity, with 3 PB of additional Isilon, 832 TB of Qumulo NAS platform, and 14.5 PB of Cloudian S3 archive storage deployed. Storage demands continue to increase significantly due to advancements in scientific equipment, such as genomic sequencers and imaging devices. The total storage capacity across all platforms is approximately 43 PB, an increase of 74 percent from this time last year.
- The Unified Communications (UC) team, working as a combined NCI UC team, completed the project to unify VoIP equipment and services. Working the CBIIT UC team, FNL staff in EIT merged all the NCI at Frederick resources into a single NCI cluster supporting all NCI locations. The merging of the VoIP systems will improve fault tolerance, improve capacity, and allow new features and functionality for all of NCI.

Figure 2. FNL/NCI at Frederick Data Center facilities.

- ITOG supported NCI at Frederick's ongoing building renovations project during FY2020. The network team was involved with planning LAN closets, preserving network connectivity, and assisting with moving users to temporary locations as buildings were renovated. The network team is currently monitoring the project status and collaborating with FME project managers to plan and execute future phases of these renovations.
- FNL staff in ITOG migrated the SQL server backups from the legacy Microsoft Data Protection Manager to an enterprise-grade solution, Commvault, improving security and reliability via encryption and multisite replication.
- The FNL staff in ITOG migrated data from legacy, disparate storage platforms to the enterprise storage solution.
- The FNL staff in ITOG has nearly completed the Windows Server 2008 migration project, successfully working with various stakeholders to migrate more than 100 Windows Server 2008 systems to newer Windows Server versions to meet the Windows Server 2008 end-of-life date of January 2020.
- FNL staff in ITOG helped Center for Information Technology (CIT) teams migrate CIT-hosted Active Directory domain controllers from Windows Server 2008 to 2016.
- The staff worked with various stakeholders to migrate 10 TB of Aperio archive data, followed by a 40 TB archive and a consolidation to NetApp. The staff also implemented an Aperio Accelerator solution to improve overall Aperio functionality.
- The staff worked with various stakeholders to move, convert to virtual machine, or retire systems housed in the Building 430 Data Center. This resulted in approximately 11 physical systems being surplused.
- The staff is migrating approximately 200 Ubuntu servers to CentOS before the Ubuntu 16.04 end-of-life date of April 2021. Thirty-six have been completed so far.
- The NCI at Frederick Conference Center staff is responsible for planning, testing, setting up, and executing conferences and meetings at both the ATRF and the NCI at Frederick campus. The Conference Center staff maintains audiovisual (AV) equipment (such as video teleconferencing units, projectors, laptop computers, flat-screen TVs, microphones, and speaker systems) in 75 conference rooms at the ATRF and the NCI at Frederick campus. The Conference Center staff also manages major AV technical refreshes of all conference rooms and maintains an AV and video teleconferencing strategic plan that lays out the time frame and plan for recurring upgrade efforts.
    - For FY2020, seven rooms received AV upgrades. Equipment was purchased for the installation of 16 digital signage stations that will expand communications across campus. The previous scheduling tool, Meeting Room Manager, was phased out and replaced by NCI's AgilQuest. The rooms managed by the Conference Center staff (22) were transitioned on February 24 and the remainder of conference rooms (27) on June 1. The Conference Center successfully supported many meetings, including the RNA Biology Laboratory site visit and the Distinguished Scientist Lecture Series.

Application of Advanced Technology

The FNL staff in the networking team is a key partner of the NCI network modernization team, providing engineering design, prototyping, and deployment for the future NCI backbone. Using advanced technology in computer communications, such as software-defined networking and dense-wavelength optics, the team is maintaining the current operations while engineering the future.

The FRCE team completed a modernization of the FNL/NCI at Frederick batch compute environment. The team has upgraded the research compute nodes to modern high-performance computing nodes with increased CPU density and GPU capabilities. These updates will increase throughput and support the increasing demand from FNL workloads.

Continuous Improvement

- Consolidated Windows Patching to BigFix
- Made substantial progress on transitioning the Linux platform support and hyper-converged virtual infrastructure support under EIT to the platform management team, consolidating and standardizing support for server-based OS platforms
- Re-engineering and modernizing the Commvault backup system. Tasks include re-architecting the current system to meet the growing enterprise management requirements, standing up new hardware to support this new architecture, and ensuring the capability is in place to support disaster recovery requirements.
- Restructuring the Active Directory for both Windows and Linux to enable managed, customer-managed, and unmanaged/hosted support models

Information Security and Compliance Office

ISCO serves as the point of contact for NIH security compliance requirements, risk assessments, and responses to security incidents. ISCO is responsible for security assessments, waivers, IT risk assessment, and security incident handling. Its staff also works with NCI at Frederick IT groups to integrate best practices into IT planning and implementation. The assessment team conducts security assessments related to the Federal

Information Security Management Act each year. In addition to assessing new systems, the FNL staff in ISCO conducts annual assessments each year to confirm that authorized systems are up to date on security requirements.

KEY ACCOMPLISHMENTS

For FY2020, ISCO has successfully completed several initiatives, including the following:

- FNL staff in the ISCO Operations team continued configuring Splunk, an enterprise software tool, to increase the NCI at Frederick security posture, providing a log analysis tool for the many systems and applications across the enterprise. Phase 1 implementation has been successful, and logs are being collected from all centrally managed systems. The team has completed the initial phase and is beginning the next phase to increase adoption and insight with applications.
- ISCO continues to provide a key role as NCI readies for the Office of the Inspector General audit, working as a liaison between NIH, NCI, and NCI at Frederick. ISCO operated as the point for data calls related to the audit.
- ISCO completed and maintained the Authorization to Operate for the Frederick GSS. The GSS includes LAN, storage, and VoIP technologies, processes, and infrastructure for the NCI at Frederick campus and the ATRF. ISCO worked with ITOG; FME; and the Environment, Health, and Safety Directorate to mitigate Plans of Action and Milestones (POA&Ms) for the GSS and performed the annual reassessment of the Frederick GSS and added the findings to the POA&M.
- ISCO worked with various groups of centrally managed platforms to write a system security plan for this security boundary. Once completed, the system security plan will allow ISCO to audit the controls and build control inheritance for all of the systems that use these controls.
- ISCO performed a full assessment of the Biospecimen Resource Group after they modified their security boundary. The Biospecimen Research Group's IT system consists of two applications: (i) the Comprehensive Data Resource, a collection of applications supporting data collection, data cleaning, data correlation, and reporting of biospecimen sample collections, and (ii) the Biospecimen Research Database, a free online database containing curated peer-reviewed articles, standard operating procedures, and a tissue image library in the field of human biospecimen science.
- ISCO continues to review existing waivers and firewall exceptions to confirm exceptions are still needed and systems are meeting compliance requirements.
- ISCO worked with system administrators across the enterprise to remediate vulnerabilities discovered during the annual NIH penetration testing.

Continuous Improvement

ISCO has added a member to the NCI at Frederick IT Governance Board, ensuring that cybersecurity concerns are addressed early during projects and throughout the project phases.

Business Enterprise Systems/Enterprise Solutions

Enterprise Solutions/Business Enterprise Systems (BES), provides enterprise application life cycle management and business intelligence to FNL. One of BES's key missions is supporting the ERP system, critical financial reporting, Human Resources systems, and other useful systems and services for business operations and science. Comprising enterprise architects, business analysts, and workflow system developers, the BES team is focused on building an enterprise-wide view of the data sources and business applications currently deployed. The FNL staff in BES is supporting an EIT-wide initiative to create an enterprise architecture and IT road map that is used to modernize, consolidate, and secure applications that support the operational directorates within FNL.

The BES team provides application support, user setup, and security for FNL business systems, as well as application administration and help desk support for FNL personnel. In addition, the BES team creates, develops, and administers SharePoint sites and provides OnBase document management and workflow applications. The resulting 200-plus sites are used to share content and provide custom workflows, many for approvals across FNL.

KEY ACCOMPLISHMENTS

- Successfully deployed and launched a new Maximo system, completing the first phase of the FME Maximo re-implementation project
- Supported NCI's rollout of major new Office 365 capabilities, configuring more than 100 Microsoft Teams sites and supporting new SharePoint Online sites and other Office 365 features
- Redesigned and rebuilt the FME work order SharePoint submission site to address the latest contract requirements and need
- Retired several legacy FME planned work order systems for budgeting and reporting and provided updated solutions with Cognos
- Deployed and launched the LASP Request for Animal Shipment system for LASP mice couriers and delivery requests
- Deployed and launched a new learning management system in partnership with the FNL Human Resources department
- Created a laboratory inventory solution using SharePoint for the Chemical Biology Laboratory in the Center for Cancer Research

- Implemented and deployed TM1 Planning Analytics Workspace and created FNL financial tracking dashboards. The planning analytics replaced a number of legacy manual Excel reports and optimized the estimate at completion and interim contract reporting.
- Implemented an OnBase work authorization process for FNL compliance
- Automated multiple ERP processes to eliminate human intervention and errors:
  - Costpoint to Unanet project propagation and auto-closing of projects
  - Vanguard integration automations
  - Simplified wireless billing processes
- Started a design phase to upgrade to Deltek T&E 10, which will significantly reduce the ERP server need by combining two applications into one
- Started implementation of the contractor Cooperative Research and Development Agreement (cCRADA) SharePoint solution to track and aid new and existing cCRADA agreements and proposals

Web Development and Support

Enterprise Solutions includes a team providing website design, hosting, and maintenance, along with custom application development. The team supports many applications, including the NCI Accessioning System, and many NCI at Frederick and Center for Cancer Research websites. Some solutions of interest include:

- The Center for Cancer Research website of approximately 2,200 pages that provides information on the research areas to the public
- More than 80 sites/applications that provide form and database logistical support to the NCI at Frederick and FNL community
- Modernization of legacy applications across the enterprise

COVID-19 Support

- Supported Occupational Health Services' transition to telephonic medicine due to COVID-19 quarantine restrictions and COVID-19-related business processes
- Helped develop and publish COVID-19-related communications for new and existing employees
- Quickly modified business and accessioning systems to address new requirements induced by COVID-19-related contractual changes
- Supported the transition to remote work by addressing impacts of virtual private network and firewall configurations, group security settings, etc.

Continuous Improvement

- Continued to mature EIT's support processes by transitioning all of the enterprise applications into the Configuration Management Database
- Initiated an effort to consolidate database-hosting and application-hosting services, forming EIT teams, and engaged Shady Grove IT (CBIIT) on a number of joint efforts
- Implemented MotioCI for Cognos to take control of Cognos report versioning, perform report automatic testing, and streamline Cognos upgrades and patches
- Enhanced security within FNL's enterprise footprint
- Implemented ERP additional audit and review processes
- Upgraded and patched all of the Transport Layer Security One cyphers and upgraded all Windows 2008 to 2012 and 2018. This includes continuous monitoring and resolution of the items noted in the security scans.
- Matured capabilities and processes to a systematic approach to define, validate, and deliver on reporting requirements. In addition, the Enterprise Reporting team provided many new reports for Finance and Contracts, expanded reporting to support FME and work order data/Maximo data, and expanded reporting to support the TOPAZ application.

End User Services

End User Services comprises two main teams that provide support to the NCI at Frederick and FNL: Helpdesk and Deskside Support. The Helpdesk team supports and coordinates IT service requests and issue resolution via phone calls and the online portal. The team is familiar with the enterprise customer base and EIT technical teams, so it can respond and escalate optimally. The Deskside Support team performs frontline services to ensure that customers are equipped with standard end-user compute machinery, whether it is Windows, Mac, or Linux.

KEY ACCOMPLISHMENTS

The End User group's accomplishments in FY2020 include the following:

- Through the Technology Refresh Program, FNL staff in the Desktop Support team deployed NCI-provided end-user compute devices in an ongoing manner within the refresh cycle period.
- The Desktop Support team worked with scientists and the infrastructure team to ensure that laboratory equipment can be operated with IT security and a fast connection to meet scientific needs. This effort included managing a large number of virtual LANs that enable the use of nonstandard equipment at NCI at Frederick.
- The End User Services teams, along with ISCO, provided Active Directory maintenance for NCI at Frederick staff and service accounts.

- As part of the ITSM project, the End User group continued to work many hours with CBIIT to align incident, service, and knowledge management processes

COVID-19 Support

- Set up over 100 loaner laptops for staff to use for telework during COVID-19 restrictions
- Transitioned to primarily remote support using the Bomgar remote support tool during initial COVID-19 restrictions
- Modified work schedules to provide on-site technicians for essential, Group A, and Group B Return to Work phases
- Initiated a walk-up window service for teleworking customers who require an in-person visit
- Deployed "jump" boxes to facilitate remote access by laboratory staff, enabling telework to include data analysis

Computer Software Training

The End User group also provided computer software training for standard applications. This training was offered by request, and program areas continued to use it for custom training specific to their missions

Facilities Maintenance and Engineering Directorate

Building 538 Refurbishment and Infrastructure Upgrades

As part of the Building 538 refurbishment/ infrastructure program, a work order was issued to refurbish the entire second floor, a work order was issued to upgrade the infrastructure for the second floor, and two work orders were issued for the infrastructure and refurbishment upgrades on the first floor. The FNL managed asbestos abatement and demolition activities for Building 538 under a separate work order.

Building 469 Second Floor Refurbishment & Infrastructure

Building 469 is a two-story masonry and steel frame structure built in 1952. The second floor refurbishment included upgrades to approximately 11,000 net square feet of Center for Cancer Resarch (CCR) laboratories and supporting rooms. Programs that relocated into the completed space include the Molecular Histopathology Laboratory (formerly the Pathology/Histotechnology Laboratory), the Center for Advanced Preclinical Research, and the Animal Health Diagnostic Laboratory/ High-Throughput Animal Genotyping Laboratory. The building around the construction area remained occupied and operational for ongoing CCR and National Institute of Allergy and Infectious Diseases work efforts; the design and construction efforts were heavily phased to ensure that the critical work ongoing in these areas was not affected by noise, vibrations, or the installation of new air handlers.

The design efforts were completed under separate funding mechanisms. All new work is compliant with national codes and the Design Requirements Manual (DRM) that was in effect at the time of design. An architect/engineering firm completed the design, and representatives from the programs; the National Cancer Institute (NCI); Environment, Health, and Safety (EHS); and Facilities Maintenance and Engineering (FME) were actively involved in the layout of the new areas and in design reviews throughout the design effort.

Demolition was completed under a separate funding mechanism.

All construction efforts under the original base contract have been completed. Utility upgrades to the project area include raising a substantial portion of the Building 469 roof to accommodate the installations of new supply and exhaust air handlers, new electrical panels, new sprinkler lines, and an upgraded IT room. Refurbishment efforts included installation, testing, and insulation of all ductwork, water, steam, and gas lines; installation of new floors, walls, and ceilings; procurement and testing of new A2 and B2 biosafety cabinets, chemical fume hoods, and necropsy hoods; and installation of casework and a cold room. The entire project area, to cover both utility and refurbishment upgrades, was successfully commissioned, and air flows within the project area and the first floor area were balanced.

FNL successfully completed the original task order effort under budget. Because of this accomplishment, NCI and FNL were able to collaborate on different work efforts to continue supporting the task order's intent. New scope items were added to the task order, including procuring a new chemical fume stand for the Molecular Histopathology Laboratory; re-caulking the exterior of the second-floor windows; replacing the roof and gutter system; and upgrading the existing fire alarm system to an addressable, Class A system. This scope has been completed; however, the National Institutes of Health fire marshal has requested that additional fire alarm devices be installed on the second floor to extend notification coverage to other areas. The FNL staff in FME is working through these design changes and will add to the new fire alarm system accordingly.

At the time of this report, the project effort is complete. Occupants are fully operational in the refurbishment area, and FNL completed all work related to task order close-out.

Building 560, Wing 2, First Floor Refurbishment

Building 560 is a 183,548-square-foot masonry and steel structure constructed in 1956. This task order will upgrade approximately 12,500 square feet of the first

floor of Wing 2 in support of the NCI Cancer and Developmental Biology Laboratory, RNA Biology Laboratory, and HIV Dynamics and Replication Program. The project will enhance the space while maintaining as much of the existing layout as feasible. The work will include new finishes; reconfigured and/or upgraded plumbing, piping, and mechanical systems; reconfigured and/or upgraded electrical systems; and modifications to fire protection systems as required

At the beginning of FY2020, the subcontractor had already completed all major construction activities related to this project, including demolition activities and the installation of new ductwork, new piping and plumbing, electrical panels and wiring to outlets and switches, casework, and flooring. During FY2020, the subcontractor completed the extent of the construction activities, tested and balanced the mechanical systems, and completed commissioning activities to ensure systems and building equipment were operating as intended. Substantial completion was achieved on February 28, 2020, and the area was turned over to NCI to allow for scientific programs to occupy the laboratory spaces.

When the subcontractor was connecting the ductwork for the first floor of Wing 2 to the building's HVAC systems in the attic, they discovered an issue that required additional ductwork and planning to minimize the impact to the overall project schedule. The Wing 2, first floor project was designed with the understanding that the Building 560 roof exhaust stack project would be constructed in parallel. As such, the exhaust ductwork in the attic specific to four chemical fume hoods and one ducted biosafety cabinet was designed to tie into new manifolds to be constructed under the roof exhaust stack project. After design was complete and construction started, the roof exhaust stack project was placed on hold due to the subcontractor bids being submitted at a higher cost (approximately $6 million) than the estimate in the budget (approximately $2.3 million) and NCI not having funding available to cover the difference. However, at that time, it was not known how long the project would be placed on hold, so this issue was not identified as an impact to the Wing 2, first floor project. Despite rebidding the project in an effort to solicit more competition, the bids submitted were still higher (approximately $5.2 million) than the estimate in the budget (approximately $2.3 million). Therefore, additional ductwork was required in the Building 560 attic to connect the chemical fume hoods to the existing general building exhaust. In addition, two new exhaust fans and ductwork were required to provide exhaust for one ducted biosafety cabinet. Although the roof exhaust stack project was released to proceed with work in Wing 2 on October 31, 2019, the work on the first floor of Wing 2 was completed in advance of that project, requiring this adjustment to the project schedule and use of the remaining project contingency to support this change.

The construction subcontract was awarded over the estimated cost but within the project budget. However, after discussions and with NCI's authorization, the project contingency was reduced to 7 percent of the overall budget to cover the difference between the estimate and award cost. At that time, NCI did not wish to add additional funds to the budget but wished to wait and determine the overall impact of construction change orders. However, the project contingency was used, with NCI's authorization, to cover the cost of identified construction change orders during the execution of the project—most notably the change to the attic ductwork, as noted above. FNL submitted an Impact Assessment Report (IAR), which NCI approved, for additional funds to the budget to replenish enough of the project contingency to cover any potential change orders during final completion of the construction effort and to rebalance the labor requirements for the project.

The subcontractor continued to work on punch list and close-out items of the construction subcontract and completed the project by the end of July 2020. At that time, FNL closed out the project.

Building 539 Phase One Refurbishment and Infrastructure Upgrades

Building 539 is a 138,047-square-foot, partially two-story masonry and steel structure built in 1955. The refurbishment effort on this building was broken into two phases to allow for concurrent construction on the first and second floors of the building's two-story portion. This project will support the construction efforts of phase one, which will renovate approximately 5,000 square feet of laboratory space in the single-story portion of the building, creating a new animal facility. Animals housed on the second floor of the building will be relocated to the new facilities at the completion of phase one, supporting the vacating of the two-story portion for the start of the hase wo refurbishment.

The design efforts were completed under separate funding mechanisms. All new work is compliant with national codes, Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) requirements, and the DRM that was in effect at the time of design. The same architect/engineering firm designed both phase one and phase two. Representatives from the programs, NCI, EHS, and FME were actively involved in the layout of the new areas and in design reviews throughout the design effort.

The timeline of the phase one refurbishment was sequenced with the Building 538 refurbishment (which was designed and constructed under a separate funding mechanism). Occupants in the area being refurbished under phase one moved into the completed Building 538, which freed up the area to allow construction efforts to begin. During phase one, only the 5,000 square feet associated with the refurbishment effort were vacated; the remainder of the building remained occupied and operational during construction, supporting both CCR laboratories and Laboratory Animal Sciences Program (LASP) animal facilities. To minimize noise and vibration impacts to the occupants or animals, sound blankets were

installed around the construction footprint. Demolition of the phase one area was completed under a separate funding mechanism.

For FY2020, all construction efforts have been completed. Utility upgrades to the project area include installations of a new exterior supply air handler and interior exhaust air handlers, new electrical panels, new sprinkler lines, and two new IT rooms. A new sidewalk was installed, and the exterior area around the building was graded to ensure proper drainage. Refurbishment efforts included installation, testing, and insulation of all ductwork, water, steam, and gas lines, as well as a new animal watering system loop; installation of new epoxy floors and AAALAC-compliant wall coverings and ceilings; procurement and testing of new B2 biosafety cabinets, necropsy hoods, and an autoclave; and installation of casework. The entire area, covering both utility and refurbishment upgrades, was successfully commissioned, and air flows within the project area were successfully balanced.

The phase one project incorporated several new industry innovations. These items include:

- Use of cutting-edge noise- and vibration-monitoring equipment. This equipment included both stationary and mobile monitors. The stationary monitors were placed in the existing animal facilities at areas agreed to by both LASP and the equipment vendor. The equipment was tested for a week in order to establish "baseline" noise levels for the facilities. Once calibrated, the equipment sent alarms to designated LASP personnel if noise or vibration levels exceeded the normal amounts. An LASP representative would take a mobile station to animal rooms, or even into animal cages, to see if the mice were affected. If they were, LASP would contact FME, which would stop the work associated with noise or vibrations immediately, and a solution to complete the work without disruptions to the animals was found.
    - Use of this equipment allowed for fewer construction stoppages, as work was only stopped based on an actual impact to the animals, versus the previous approach, which was to stop work based on a qualitative impact assumed by nearby personnel.
    - Note that this equipment was procured under a separate funding mechanism.
- Application of a new wall covering in animal areas. Walls in animal areas need to be durable to withstand wash-downs and damage caused by carts and/or animal racks. Animal facilities on the campus use a standard epoxy-based Sherwin-Williams paint, which is washable but not especially durable against cart or rack damage. At LASP's request, Phase One incorporated the use of a new epoxy material, which was mixed and applied on-site by qualified vendors. This material proved to be substantially more durable than regular epoxy paint and was well received by the end users. In future projects, LASP will work with FME during the design process to determine whether this material should be required in their animal facilities. The cost of the material is many times greater than the cost of the epoxy paint, and the material can only be applied by a certified vendor, meaning a damaged wall cannot be repaired immediately by the FME shops; however, the durability of the material may outweigh these concerns.
- Installation of state-of-the-art room monitors. During the design of phase one, LASP noted historical issues with easily tracking critical set points in each of their animal rooms. To address this, state-of-the-art room monitors were installed outside of each animal room. These monitors are connected to the building automation system and track temperature, humidity, and pressurization, keeping a historical log of the data for future reference. Alarms will sound for individual rooms and individual issues (temperature, humidity, or pressure) as soon as the set points are exceeded. The monitors are set both to give a warning alarm if a variable starts to trend outside of the "acceptable threshold" and to give an alarm if the variables have exceeded threshold limits. Thresholds were established by LASP, FME, and the architect/engineer. These monitors were tested holistically during the commissioning process.

To achieve the project objectives, FME integrated key LASP personnel into the construction phase. The following activities allowed full transparency with the program throughout the construction effort and kept open lines of communication between the project stakeholders:

- Key NCI stakeholders attended a weekly meeting to review project progress and/or issues and received a semi-annual progress report on the project. Stakeholders were also encouraged to coordinate a tour of the project at any time throughout the duration of the contract effort.
- Key LASP stakeholders attended a biweekly update meeting focused specifically on LASP projects. This is a smaller group and allows the LASP team a more open forum to ask FME project managers questions about funding, outages, project concerns, etc.
- LASP representatives attended the weekly construction progress meeting, during which the construction schedule was reviewed, with specific focus on outages, "noisy" work, coordinating access into the animal facilities, etc. This is also a forum that allows LASP to ask to the FME construction team and/or engineers specific questions about upcoming work.
- Knowing that LASP has far more knowledge of AAALAC expectations in functional animal facilities, FME asked LASP to complete inspections at the following points: wall close-in, ceiling close-in, and final inspections. The LASP team inspections were focused only on the

AAALAC requirements and so proved invaluable in ensuring that the final space was ready for certification.

- LASP programs were given the opportunity to walk through the project area at three different points: after wall installation, after casework installation, and in parallel to final inspections. Their input, questions, or concerns were always captured and addressed. Additional walkthroughs were given for second-shift workers and personnel who wanted an update on the project's status. FME coordinated appropriate personal protective equipment for LASP employees during these walks to ensure the safety of all participants.

The project effort is complete. Occupants and animals moved into the building in June, and FNL completed all work related to task order close-out. The phase one area of Building 539 is now a completely functional, operational animal facility.

Building 539 Phase Two Refurbishment

This project will support the construction efforts of phase two, which will renovate approximately 60,000 square feet of Building 539 to create new animal facilities on the first floor and new laboratory space in the second floor. The project will also provide new infrastructure to support these areas.

The design efforts were completed under separate funding mechanisms. All new work is compliant with national codes, AAALAC requirements, and the DRM that was in effect at the time of design. The same architect/engineering firm designed both phase one and phase two. Representatives of the programs, NCI, EHS, and FME were actively involved in the layout of the new areas and in design reviews throughout the design effort.

The timeline of phase two was sequenced with the Building 539 phase one and the Building 560 Wing 2 refurbishments, both of which were completed under separate funding mechanisms. Animals on the second floor of the phase two footprint moved into the phase one areas, and four laboratory programs moved to Building 560. During phase two, only the 60,000 square feet associated with the refurbishment effort were vacated; the remainder of the building will be occupied and operational during construction, supporting LASP animal facilities. To minimize noise and vibration impacts to the occupants or animals, sound blankets were installed around the construction footprint.

The following progress was made in FY2020:

- NCI gave the FME team permission to use Building 536 for the phase two field trailer. The FME team relocated from Building 539 to Building 536 to further support the vacating of the phase two footprint.
- The architecture and engineering firm (AE) provided Issue for Construction drawings to support the refurbishment effort.
- A contract was issued to complete the construction assessment at the end of the refurbishment effort and grant the Green Globes certification.
- A contract was issued for the third-party commissioning agent for the duration of the construction effort. A commissioning kickoff meeting was held with the general contractor. The third-party commission agent is currently reviewing applicable submissions and collaborating on any design-related questions with the AE.
- A contract was issued to disconnect the animal water system that supported the animal facility previously located on the second floor.
- The replacement of the 1,600 A breaker in the east attic has been completed, and the new breaker has been successfully tested. This contract effort is complete.
- A substantial part of the construction effort involves separating the fire notification system between the occupied portion of the building and the construction area. FNL removed this work from the main refurbishment effort to reduce the construction contract duration and overcome some of the delays to the start of the refurbishment construction effort. A subcontract to separate these systems has been awarded, and the work is 95 percent complete.
- Contracts have been issued for the large-scale abatement effort, which has been completed, and related industrial hygiene support throughout this effort.
- A contract was issued for part of the east attic abatement effort. Substantial amounts of asbestos-containing insulation were removed from utility lines in the east attic. This contractor will reinsulate the lines that will remain throughout the refurbishment effort.
- A contract was issued for the main general contractor refurbishment effort. Their contract consists of both the demolition and abatement and the refurbishment.
- The phase two refurbishment effort is underway, including establishment of the general contractor laydown areas, installation of the fire-rated barrier walls, completion of fire alarm separation (by the general contractor), and start of demolition and abatement.
- To mitigate the schedule risk associated with the funding expiration, FNL and NCI had several meetings to review possible acceleration of the construction efforts or possible descoping of the post-construction efforts. In July, NCI notified FNL to proceed with the descoping efforts, and FNL provided a list of "new work" activities that could be added to the project to offset the cost impact associated with removing post-construction activities. An official contracting officer approval for a project modification will be submitted once new work activities are agreed upon.

To achieve the project objectives, FME has started to integrate key stakeholder personnel into the construction phase. The following activities will allow full transparency with the stakeholders throughout the construction effort and will keep an open line of communication:

- Key NCI stakeholders attend a weekly meeting to review project progress and/or issues and receive a quarterly progress report on the project.
- Key LASP stakeholders attend a biweekly update meeting, focused specifically on LASP projects. This is a smaller group and allows the LASP team a more open forum to ask FME project managers questions about funding, outages, project concerns, etc.
- LASP representatives attend the weekly construction progress meeting, during which the construction schedule is reviewed, with specific focus on outages, "noisy" work, coordinating access into the animal facilities, etc.

Building 560, Roof Exhaust Stacks

In the years since Building 560 was constructed in 1956, numerous exhaust stacks have been installed as needed to vent chemical fume hoods, Type B biosafety cabinets, and general laboratory exhaust. Many of these stacks do not comply with the NIH DRM requirements for stack placement, construction, and height. This project will bring all the laboratory exhaust stacks in Building 560 into compliance with the DRM guidelines on separation, height, and velocity.

Building 560, Building Generator/Automatic Transfer Switches

This project's purpose is to provide a generator and automatic transfer switches to support all of Building 560. The new generator is sized per the NIH DRM requirements, specifically addressing life safety, legally required standby loads, and optional standby loads applicable to Building 560.

All significant work on the new building generator and automatic transfer switches has been completed, the old generator has been removed, and the building is being supported on the new generator. Construction subcontract substantial completion was granted on April 7, 2020. The subcontractor is working through construction contract close-out documentation. Project acceptance was granted in July 2020.

Due to the new generator capacity being much larger than the old generator, the program occupying Building 560 (CCR) has requested that additional scientific equipment loads be placed on the generator. CCR has provided a list of these requests. The FME Engineering team is working to develop design documents to identify the scope and prepare an estimate and schedule. Once complete, an IAR will be prepared and submitted for NCI to authorize the additional scope, cost, and schedule. There is a significant surplus in the task order budget due to the original construction bids received being substantially lower than the project estimate. It is anticipated that the additional work under this IAR will be funded by this budget surplus and not require additional funds.

Buildings 434, 560, 567 Exterior Ladders Phase Two

Statement of Work

The attic egress ladders serving Buildings 434, 560, and 567 were evaluated as part of a prior campus-wide fixed ladder upgrades initiative to bring deficient campus ladders into compliance with National Fire Protection Association 101 Life Safety Code and Occupational Safety and Health Administration standards, and they were found to require a staircase. Building 560, by size, was found to require two staircases. The design for the stairs was completed under a separate work order with separate funding. This project installed the required exterior stairs on these buildings.

The construction process for each of the stairs required the detailed execution of the following major phases of work: the contractor's development of the design/shop drawings, the project team and fire marshal's review and approval of the design/shop drawings, the completion of supporting site work and installation of the stair foundations, the completion of the building modifications in support of each stair, the contractor fabrication and delivery of the structural steel, the erection of the structural steel stairs, the painting of each stair structure, and the final inspection by the fire marshal to place the stairs into service.

The installation of each stair also required close coordination with various end users and programs in all three buildings, including, most notably, the Building 434 Repository. The Building 434 stair had to serve not only the attic but also the Repository second-floor mezzanine, as the mezzanine was also lacking a code-complaint second means of egress. Thus, the Building 434 stair was designed as an over/under stair, with one stair serving the attic and the other serving the Repository second-floor mezzanine.

Objectives for Statement of Work

Beneficial occupancy was achieved for the project on January 21, 2020, following the fire marshal's successful final inspection of the four stair structures that day, which released the stairs to be placed into service. Being an exterior project, weather affected construction throughout all phases of the work. Significant rainy periods during the completion of the site work, foundations, and stair installation resulted in the final painting of the stairs to be included as punch list work to be completed following the end of the cold winter months. It was anticipated that the weather would improve in early April, allowing the work to be completed with no impact on the period of performance (POP) date of June 23, but the weather

during April continued to remain cold and wet, preventing the contractor from completing this work. The work couldn't begin until May 4, and with four stair structures to paint, was not able to be completed until June 8 for the contractor to achieve project acceptance. Thus, the POP date required an extension from June 23 to August 28 to accommodate the time required to complete project close-out activities. The NCI at Frederick Management Operations Support Branch (MOSB) approved the requested extension of the POP date. The project is on schedule to be closed out by August 28.

Project Accomplishments

The project's execution included several strategic initiatives to minimize unforeseen costs and to execute the schedule as efficiently as possible.

First, to minimize the potential for interference with existing underground utilities, reinforced concrete mat slabs were used in lieu of the concrete pier foundations typically used for these types of structures. This initiative proved to be very beneficial during the installation of the foundation for the Building 567 stair, where the subcontractor encountered an abandoned concrete and steel foundation from former Building 527, which once enclosed the Eight-Ball Test Sphere. Rather than having to remove a major portion of the below-grade old building foundation to install concrete piers deep into the ground, the subcontractor only had to remove a much smaller portion of the foundation to provide six inches of clearance from the bottom of the new stair mat slab to install compacted crushed stone before the mat slab foundation could be installed. Thus, the mat slab for the stair was able to be installed without a major cost and schedule impact to the project.

Second, the installation of the stairs required all exterior windows within ten feet of each stair structure to be replaced with fire-rated windows to meet life safety requirements for safely exiting the building. The project team foresaw that the installation of these windows would be difficult due to their size and weight, especially at Building 560, where the windows are very large. Typically, commercial fixed-pane windows are installed from the outside of a building and glazed (glass installed) from inside of a building; however, this would be difficult, time-consuming, and very disruptive to the occupied offices and laboratories affected by the work. Thus, the windows were specified to be externally glazed for easier installation and less effect on the occupied building.

Third, since the project required the installation of four stairs on three separate buildings, the project schedule was carefully sequenced and phased to efficiently execute the work to provide the shortest time to completion. Construction was intentionally planned not to start until approximately two months after the construction award due to the time required for the contractor to develop the first set of stair design/shop drawings, the time required for the review and approval of the design/shop drawings, and the fabrication lead time for the delivery of the first of the stairs. The preparation of each site and the installation of each stair foundation were completed sequentially, one immediately following the other, so that each concrete stair foundation would be properly cured just prior to the scheduled arrival and setting of the stair structural steel. This enabled the subcontractors involved in this project to continuously work so the project was completed efficiently.

At the time of this report, the stairs have been in service for approximately six months. They have been well designed and well built and will provide decades of continuous service to these buildings.

Building 560 Replace Reheat Water Heat Exchangers and Condensate Pumps

This project is intended to replace the existing reheat water heat exchangers and associated equipment, the flash tank, two condensate recovery pumps, and the condensate return piping to the main. The equipment is beyond its useful life. The reheat piping to the building was replaced recently, so it will not be a part of this scope of work.

The subcontractor continues to work in the Building 560 basement, making final connections and working on testing and balancing the new systems. Several shutdowns are currently in the planning phase for the installation of the new exterior piping and demolition of the existing piping.

Per an agreement with NCI, the subcontractor was directed to perform all excavation and backfill operations during off-hours. The installation of the new piping, demolition of the existing piping, and restoration of the site will be performed during normal working hours.

Unforeseen conditions have been encountered during excavation activities, namely piping in the path of the planned new pipe installation. Plans are being developed to resolve these conflicts. Current projections estimate that the schedule adjustment required to resolve these conflicts will delay substantial completion by approximately one month. Once the plan to resolve the conflicts is agreed upon, the project schedule can be accurately updated to reflect the impact.

Currently, and with the potential impact of the conflict resolution, the target substantial completion date is mid-September 2020. The project is targeted to complete within the budget. However, this depends on the final agreed-upon plan for resolution of the pipe-routing conflicts.

Expand Liquid Nitrogen Piping Distribution, Wedgewood Suite K

The NCI at Frederick Central Repository has nearly run out of liquid nitrogen freezer capacity. This project expanded the liquid nitrogen freezer capacity at Wedgewood Suite K to add 86 liquid nitrogen freezers. These additional liquid nitrogen drops will supply liquid nitrogen to the growing number of liquid-nitrogen-fueled freezers.

Accomplishments Directly Related to the Statement of Work

The project achieved substantial completion on April 21, 2020, and project acceptance on June 16, 2020. The project is on schedule for the task order close-out date of September 30, 2020. In addition to the construction contract with the general contractor, FNL also managed three other subcontracts under this task order to provide a complete and usable facility. All subcontracts are complete:

1. Liquid nitrogen pipe expansion
2. GfG oxygen monitoring system
3. Scientific alarm expansion

Expansion on Accomplishments

The Division of Cancer Epidemiology and Genetics purchased 20 liquid nitrogen freezers, to arrive at the end of January 2020, to meet the demand of several critical studies that would be shipping samples to Wedgewood from January–August 2020. To meet this schedule, the liquid nitrogen expansion project was re-sequenced to receive beneficial occupancy of the renovated interior space on February 3, 2020. This early beneficial occupancy milestone ahead of the overall project completion in April 2020 allowed the Repository staff and American Type Culture Collection (the Repository's operating contractor) to use the space for the new freezers and the upcoming critical studies.

Other Support and Logistics Services – Cafeteria

The Discovery Café is located in Building 549 on the NCI Campus at Frederick. The café is designed and outfitted as a cafeteria capable of seating up to approximately 200 people. The "Grab 'n Go" is a satellite facility located at the Advanced Technology Research Facility (ATRF). Cafeteria services are provided Monday through Friday, except for federal holidays observed by FNL. The space and utilities are provided by NCI and maintained by FNL. Catering services are also available for on-site conferences, meetings, and training sessions. Online ordering is available through the NCI website. The café is operated by a subcontractor. FME provides the facility support service for and preventative maintenance on all café equipment.

FME is currently working with the NCI at Frederick Office of Scientific Operations on an enhancement project to develop a 95 percent design that will provide coffee-bar amenities to NCI and Fort Detrick personnel. The design consists of a flowing egress layout, complete with coffee preparation, pastry selection, and dine-in areas. New work will include revisions to the existing mechanical, electrical, and plumbing infrastructure to accommodate the necessary utilities for the space. New interior floor, wall, and ceiling finishes will be installed throughout to contribute to the "coffee shop" atmosphere while maintaining accessibility to existing café services. Security devices will be installed to prevent unauthorized access during nonoperational hours. Once the design is finalized and funding is approved, FME will proceed with solicitation and construction services for performance of project scope.

In addition to the coffee bar, the following improvements are planned in support of the café: installing handicap-accessible doors, expanding the outdoor eating area, replacing the carpet with laminated vinyl flooring, repairing/repainting walls in the food service area, and renovating the café restrooms for accessibility compliance and to modernize the aged restroom facilities. FME has coordinated with NCI to provide a fresh, new look for these restrooms. In addition to making them fully handicap accessible, the project will install hands-free, hard-wired restroom fixtures and accessories in response to the COVID-19 pandemic.

The FME custodial staff supports the café by vacuuming the seating area and shampooing the carpet. In addition, the custodial staff strips and waxes the floor in front of the food service area quarterly. In early 2019, a deep-cleaning subcontractor was hired to steam-clean the kitchen, equipment, and food preparation area twice yearly.

The Discovery Café and the Grab 'n Go were temporarily closed on March 30, 2020, due to the COVID-19 pandemic. During the closure and Return to Work preparations, FNL monitored the facility alarms and has subcontracted a disinfecting cleaning company to sanitize the kitchen and food preparation area; installed sneeze guards at the registers and food preparation area; subcontracted a fire suppressions systems inspection, which led to additional cleaning to remove grease buildup on the duct filters and stainless steel backsplash; monitored the café fleet vehicle's battery for proper charge maintenance; and provided weekly walkthrough checks during the COVID-19 closure.

Facilities Maintenance and Engineering, Core Services

FNL staff in FME provided continuous custodial service to facilities during the COVID-19-related minimal maintenance period. This included increased sanitation of high-touch surfaces and flexibility and responsiveness in satisfying occupant needs and changing requirements.

FME prepared dozens of complete planned work order designs, which were developed as part of the necessary predecessor activities that enabled NCI to advance the same work through the task order process. The process entailed initial scoping and programming with requestors, then detailed design through various steps, typically including conceptual design, 35 percent design, 65 percent design, and 95 percent desgn. This process also included numerous reviews and approvals as well as adjustments and responses to customers and entities within NCI.

Staff in FME also coordinated with NCI to map IT needs within FME. These efforts are providing a baseline and the road map for future IT components of FME's operations.

FME periodically met with NCI to review actions related to the FY2020 statement of work. The staff worked closely with its contracting officer representative (COR) and MOSB to identify and resolve open items with the statement of work (post-negotiation and award) that would consequently enable the enterprise to advance successfully against the new statement of work's expectations.

Staff also led and organized the weekly Plan of the Week meeting, which is a key component of both FME and NCI operations management of the portfolio of certain work. This meeting, typically attended by more than 30 principals at NCI and FNL, gathers decision-makers on a weekly basis to review critical details of all work in the portfolio. FME creates and distributes timely data on each project under development and in progress.

The FME team delivered many *ad hoc* requests related to project status and costs for the FME COR, developing, updating, and adjusting unique data products that the COR requested throughout the year. These data products entailed multiple iterations and investigations to satisfy the requests.

FME also coordinated with its COR to identify a cost-saving staffing reorganization at the ATRF that presented itself with the unexpected retirement of a supervisor. FME closely coordinated with the COR on the proposed plan and, with the COR's concurrence, built this into the FY2021 proposal, making the review and approval faster.

Staff developed and provided a custom quarterly resource assessment for FME that includes both actuals to date and forecasts. This was periodically provided to the COR as the data changed appreciably. FME provided breakdowns by specific disciplines to identify areas of attention, if required.

On the software side, FME coordinated closely with the NCI COR, MOSB, and the FNL Enterprise Information Technology Directorate to expand the SharePoint FME package upload/review/approval capabilities. This complicated and labor-intensive effort included engagement by key parties within FNL and NCI and, ultimately, led to the activation of a significantly expanded capability within the existing SharePoint site that successfully subsumed the actions that formerly were executed through Federal Contracting Award System.

FME identified and implemented several improvements to the new statement of work process for project submissions, reviews, and approval. Staff in FME and Contracts and Acquisitions jointly examined and refined a set of changes and improvements that significantly affected project timelines and, thereby, advanced against NCI's interest in expediting certain projects. This included changes to the acquisition strategy and contracting mechanisms, recommendations from FME for "skipping" certain design packages for straightforward projects, and reduction of cycle times for reviews among FNL and NCI.

FME updated and improved the content of its entire portfolio of procedures as necessary, performing a complete review of all existing procedures and ensuring that material was current and relevant. These procedures were appended to the latest version of the continuously updated Facility Operations Manual.

FME's operations and maintenance managers at the ATRF and the NCI Campus at Frederick held frequent (typically monthly) face-to-face discussions with the FME COR. The meetings enhanced communication on key maintenance issues at the two facilities, providing the COR with direct access to the leaders of maintenance operations.

FME's Project Delivery Group coordinated closely with MOSB to deliver materials necessary for planning certain activities. The Project Delivery team of FME project managers carefully crafted, through multiple interactions, detailed scopes of work associated with the design effects within a task order so that MOSB could apply the concepts to the development of the task orders for other construction on these same projects.

FME closely coordinated with the FME COR to develop and tune a message for the programs to request that they submit their projects for FY2021 and to direct them to the improved FME website for activation and prioritization of their requests. This effort also included an update to the FME website to account for changes to the process, which now has a self-reported prioritization field.

FME staff continued to examine and assess customer survey data for all work orders conducted as part of certain activities. The comprehensive work order survey system that was jointly conceived several years ago with the FME COR has provided valuable information about customer expectations and satisfaction. FME continues to receive remarkably high ratings for services rendered, with 99 percent of the respondents rating FME at least a 3 out of 4 for overall satisfaction. FME is delighted to report that thousands of surveys are sent and responded to each year, and the results are consistently exceptional. As part of FME's continuous improvement efforts, all customers who provide low scores are contacted, and every effort is made to ensure that their concerns are heard and addressed to the extent possible and that, if necessary, changes are made for future responses. These efforts have ensured that the 99 percent approval rate has remained consistent for many years.

The Operations and Maintenance teams at both facilities adjusted to the never-before-experienced conditions presented by COVID-19 and continued to maintain the ATRF and the NCI Campus at Frederick despite the challenges. Both teams continuously assessed staffing levels and service demands to ensure that the campus was properly maintained while minimizing staff presence on-site. These actions required careful assessment of changing levels of personnel across the campuses and recognition of non-deferrable maintenance. FME worked closely with the FME COR and CCR to identify and recognize the risks associated with broad-

scale deferral of recertification of chemical fume hoods. FME successfully demonstrated the value in continuing some maintenance while deferring others.

In conjunction with that effort, FME crafted responsive staffing plans and provided NCI with updates that identified an approach for rotating FME staff off-site (using discretionary funds) to minimize its staff levels on campus. This enabled FME to reduce on-site levels in light of COVID concerns, while maximizing telecommuting and partial on-site activity. The result has been a highly responsive organization that has met all expectations of customer service and long-term operational readiness.

Despite the new challenges in FY2020, FME continued to rapidly respond to requests, employing both in-house resources and external contractors. FME has successfully maintained a mixed model of service providers to respond rapidly to and guarantee quality for routine and unusual tasks. The blanket order contracting system that was jointly developed by FME and Contracts and Acquisitions has allowed the organization to focus in-house talent on routine maintenance while applying various outside specialty contractors to the unique needs that arise within the portfolio.

Staff in FME also coordinated closely with NCI at Bethesda on the new software rollout for space planning while continuing to respond to contractual requirements for space planning deliverables as the source software for the work is undergoing a somewhat lengthy conversion process at NCI at Bethesda.

FME collaborated with the FME COR to examine metrics that would be suitable for NCI and are also currently available within the FME operating approach. FNL and FME have reviewed operational metrics for maintenance multiple times so that FME can upload these data to the Cognos system such that the FME COR can access these materials. This allows the COR to independently review and analyze trends.

FME also collaborated with the FME COR to identify project delivery information that is currently available within the FME operating approach and can satisfy COR and Office of Scientific Operations data needs. FME worked with NCI and the Enterprise Information Technology Directorate to plan and scope for what ultimately will be new reports within Cognos that can provide a multitude of data fields for every project. These new reports are being crafted to respond to various data calls that the FME COR generated and/or advanced to FNL.

Laboratory Animal Sciences Program

Animal Research Facilities Operations

LASP manages 29 animal research vivaria: 24 at the NCI at Frederick and five at the NCI at Bethesda. Both the Frederick and Bethesda facilities are accredited (separately) by the AAALAC International. Collectively, these facilities support 224 investigators (90 in Frederick and 134 in Bethesda) and 563 active animal study proposals (ASPs) (180 in Frederick and 383 in Bethesda). They are currently maintaining 332,028 animals (180,363 in Frederick and 151,665 in Bethesda) occupying 46,178 cages (33,143 in Frederick and 13,035 in Bethesda). LASP's animal program support areas include NCI animal facilities, the Animal Health Diagnostic Laboratory (AHDL), Receiving and Quarantine, and Laboratory Animal Medicine (LAM). LASP serves as a comprehensive resource for animal-based research for the NCI scientific community. LASP also significantly supports other institutes and entities, including the National Institute of Allergy and Infectious Diseases (NIAID); Leidos Biomedical Research, Inc. (Leidos Biomed); and other agencies approved by NCI. In addition to facility operations, LASP supports several areas, including colony management, ad-hoc technical support, requested dedicated technical support, and performance and/or training in advanced surgical procedures. The current animal population is distributed as follows:

Species	Animals
Mice	330,747
Rats	287
Frogs	342
Non-human primates	272
Fish	380
Total	**332,028**

Bethesda

KEY ACCOMPLISHMENTS

- LASP provided high-quality animal care, research support, and technical support despite challenges faced during the COVID-19 pandemic. In response to the pandemic, LASP implemented an alternate work schedule for staff, promoted social distancing in facilities, secured additional personal protective equipment and cleaning supplies, and implemented a room-reservation system for all animal facility users. We developed algorithms to guide testing of animals potentially exposed to SARS-CoV-2, which served as a guide for other institutes at NIH.
- While the pandemic resulted in an overall slowing of research activities for many months, FNL staff in LASP still performed greater than 1,000 non-human-primate minor surgical procedures in support of the AIDS and Cancer Virus Program and Vaccine Branch.
- Rodent technical staff provided more than 1,900 hours of technical support to NCI researchers, including procedures ranging from weaning mice to advanced orthotopic tumor injection surgeries.

- We made several facility upgrades. To enhance the safety of staff and limit exposure to allergens and other risks, we procured new respirators for cage wash staff and are exploring further expansion of their use within the program. We procured a water-bottle filling station to improve workflow and delivery of acidified water to several facilities.
- The Bethesda Animal Care and Use Committee processed approximately 128 new and renewal animal study proposals and more than 600 modifications. The designated member review process implemented last year has helped to speed approvals and turnaround time for both new protocols and modifications.
- Dedicated LASP technical staff provided high-quality technical support to various laboratories. Significant accomplishments included: (i) leading canine clinical trials to determine ideal dosing for a melanoma treatment; (ii) generating spontaneous metastatic canine melanoma xenograft models to test the efficacy of the treatment in preventing/treating tumor metastases; (iii) generating drug-resistant cell lines to study the mechanism governing the acquired drug resistance; and (iv) managing the Neuro-oncology Branch Pre-clinical Translational Research Facility for *in vivo* and *in vitro* experimental design, scheduling, and data analysis.
- Dr. Sarvesh Kumar was awarded an NIH Summer Mentor Award in 2019 and 2020.
- Drs. Josh Kramer and Matt Breed contributed to the development of antibiotic stewardship guidelines for the Association of Primate Veterinarians and were instructors for an endoscopy workshop at the American Association for Laboratory Animal Science National Meeting. Dr. Breed was named to the editorial board for the *Journal of Medical Primatology*.
- LASP Bethesda was part of an unannounced, comprehensive audit of the NIH intramural research program by the Office of Laboratory Animal Welfare, which praised our complex program, specifically that it "incorporates numerous well-developed policies into a comprehensive program of oversight that assists investigators."

Frederick

KEY ACCOMPLISHMENTS

- After Building 539 phase I refurbishments were completed, LASP conducted decontamination, health surveillance, and relocation of more than 3,000 rodent cages and laboratory equipment into the new space. Relocation occurred during the pandemic by taking proper safety precautions in order to provide uninterrupted support for CAPR and CCR research activities.
- LASP completed the transition to Lab Products, Inc.'s ventilated rodent racks and Allentown Inc.'s cage-changing stations in more than 15 animal holding rooms (with more than 5,000 rodent cages) in Building 571's second and third floor to reduce occupational hazards (allergens) and improve working conditions.
- During the pandemic, LASP continued to provide essential husbandry and technical services in all the animal facilities with only a five to ten percent reduction in the animal census. FNL staff followed split-shift schedules, frequently decontaminated common areas, wore proper personal protective equipment, and followed social-distancing rules to safely provide uninterrupted services.
- A SteraMist™ fogging device has been procured for decontamination of large areas and equipment that cannot be autoclaved. LASP building managers and supervisors attended a WebEx and in-person training to learn how to use the equipment safely and effectively. This equipment will help save costs as LASP will not have to not rely on outside vendors for decontamination of the areas within animal facilities after various renovation projects.
- In preparation for Building 539 phase II refurbishments, three animal holding rooms were converted into animal procedure rooms (with necropsy stations, biosafety cabinets, and an anesthesia and euthanasia station) for uninterrupted animal studies.

Laboratory Animal Medicine

KEY ACCOMPLISHMENTS

- We created a one-year postdoctoral fellowship program in LAM to provide a training opportunity for freshly graduated laboratory animal veterinarians. Fellows who complete the program will earn an FNL "certificate of training" and gain a better understanding of the principles and practices of laboratory animal medicine. This training will also prepare the fellow for specialty boards exams (e.g., American College of Laboratory Animal Medicine). Dr. Stella Spears started as a first fellow in July 2020.
- The LAM and Animal Care and Use Committee (ACUC) coordinator reviewed and processed 56 ASPs and 250 modifications for the FNL ACUC. LAM and Quality Assurance reviewed and updated 50 standard operating procedures.
- LAM conducted more than 150 technique training certifications, American Association for Laboratory Animal Science certification classes, and post-approval monitoring sessions. LAM continued quarterly all-hands training sessions for the husbandry and technical staff. During the COVID-19 pandemic, FNL staff have provided resources for online training opportunities, as well as resources for individuals without internet access.

- LAM supported a technical development project titled "Determination of Pathogenesis (Especially in Immunocompetent Mice) and the Best Diagnostic Sample for Mouse Kidney Parvovirus."

LAM provides research, training, and technical support to NCI investigators; ensures the health and welfare of NCI animals; and ensures compliance with all local, state, and federal regulations that govern the ethical use of animals in biomedical research, with the continuing objective of maintaining full compliance with the AAALAC.

Animal Research Technical Support Group

KEY ACCOMPLISHMENTS

- Animal Research Technical Support (ARTS) members manage approximately 2,500 cages of complex breeding colonies comprising 163 strains of genetically modified mouse models and rats. Colony management support is provided to investigators from the NCI, CCR, Division of Cancer Prevention (DCP), National Center for Advancing Translational Science (NCATS), NIH, and U.S. Army Center for Environmental Health Research (USACEHR).
- In technology development, projects aimed at establishing new animal models were completed, including an orthotopic preclinical model for lung cancer and a model for leptomeningeal metastases. The work was performed in collaboration with the Mouse Model and Cryopreservation core (MMC), the Small Animal Imaging Program (SAIP), and the Molecular Histopathology Laboratory (MHL). FNL staff performed animal procedures to help characterize patient-derived xenograft (PDX) models for the Cancer Imaging Program, and in support of NExT. Furthermore, ARTS is implementing procedures for collecting bone marrow aspirates from mice in preparation for new studies sponsored by NCATS.
- Under the NCI Accessioning System, ARTS received 66 requests, including from CCR-Frederick (16), CCR-Bethesda (26), DCP (14), NCATS (4), Invention Development Program (3), DCTD (1), Office of Translational Resources (1), National Institute of Arthritis and Muskuloskeletal and Skin Diseases (1), and USACEHR (1). ARTS conducted more than 220 experimental studies in mice and rats comprising survival surgeries; tumor-cell and PDX implantations, including orthotopic routes; preparation and dosing of at least 120 chemical compounds, plus medicated water and special diets; blood and tissue collections for pharmacokinetics, pharmacodynamics (PD), and toxicity studies; vaccine administration, including oral/vaginal inoculation; and tumor growth kinetics and efficacy studies. Some of this work was included in two publications (Gril et al., *Neuro Oncol*, 2020; Marzi et al., *Mol Cancer Ther*, 2020).
- In addition, the group provides technical assistance for projects managed by other LASP core facilities such as SAIP. FNL staff in ARTS also provide experimental study support within the Gnotobiotics Facility (GF).

The ARTS Group provides customized technical support for basic and translational animal-based research to the NCI scientific community. Services range from expert colony management to the performance and development of technical procedures aimed at disease induction, characterization, and treatment of animal models. FNL staff in ARTS work closely with principal investigators to provide assistance with animal study proposals and Institutional Biosafety Committee registrations; develop highly detailed study plans; develop, coordinate, and perform all aspects of studies; provide progress reports; and deliver unbiased, detailed statistical analysis of study results. All tasks are conducted in strict accordance with regulatory and safety protocols (ACUC and Institutional Biosafety Committee) and in coordination with other LASP facilities to ensure safe, timely completion of work.

Gnotobiotics Core Facility

KEY ACCOMPLISHMENTS

- The core received 12 NCI Accessioning System requests from CCR-Bethesda (7), CCR-Frederick (1), DCP (1), National Institute of Environmental Health Sciences (1), National Institute of Diabetes and Digestive and Kidney Diseases (1), and NIAID (1). Using 10 breeder isolators, 40 experimental isolators, one extra-large breeder isolator, and four Allentown, Inc. biocontainment racks, the group has conducted three rederivation projects and 40 experimental studies involving 737 germ-free mice.
- Complex breeding strategies are ongoing to maintain nine strains of germ-free mice in the colony.
- FNL staff prepared and dosed animals with 11 drug compounds, tailored cocktails of bacteria made from 35 microbial strains, and numerous innocula from the gut microbiome and virome.

The Gnotobiotics Facility supports research focused on the role of microbiota in inflammation, pathogenesis, and anti-tumor response. Offered services resemble those of ARTS, though all work is conducted on germ-free and gnotobiotic mice. An important additional service is rederivation of wild-type and genetically modified mouse strains.

Animal Diagnostic Laboratory

KEY ACCOMPLISHMENTS

- The Animal Diagnostic Laboratory (ADL) performed 12,717 polymerase chain reaction (PCR) assays for 792 cell line Molecular Testing of Biological

Materials tests; 20,578 regular mouse genotyping; and 344 mycoplasma assays for NCI/NIH investigators.

- In April, ADL diagnosed pinworm contamination in one of the rederivation mouse colonies, ensuring timely elimination of the pinworm outbreak.
- ADL continues to develop new assays and techniques to increase the capability of the laboratory including completion of human pathogen panel development and validation, PCR testing of *Hafnia Alevi* for animal colonies and facilities, and revising of genotyping protocols for performance robustness in the lab.
- During the pandemic, ADL developed SARS-CoV-2 nucleic acid tests and implemented COVID-19 antibody tests for Non-human primates. The nucleic acid tests have been used to evaluate certain animals and environmental samples to ensure there is no contamination of the viral RNA. The serology tests have been used to assess the antibody status of certain NHP to be used in COVID-19 studies.
- ADL obtained a new SeqStudio instrument to continue support of speed congenic projects.
- Four genomic scans were performed in support of three investigators. The supported projects are detailed in the following table.
- The High-Throughput Animal Genotyping Laboratory (HTAGL) has processed and determined 35,500 genotypes.
- HTAGL continues to support new NCI investigators while maintaining major support to CAPR.

Principal Investigator	Affiliation	Number of Projects
Dr. Angela DeVico (Thornton)	NIAID	2
Dr. Woo Yong Park	NCI, CCR	1
Dr. Karen Laky	NIAID	1

The major focus of ADL's diagnostic services includes microbiology, molecular biology, parasitology, serology, and health-monitoring necropsies. ADL also serves as the genetic quality-control laboratory for the NCI Mouse Repository, the LASP Cryopreservation Program, and NIH investigators. In addition, the laboratory conducts mycoplasma assays and speed congenics genome scans for NIH investigators.

The LASP Speed Congenics Service, including genetic testing and colony management, derives congenic strains of mice for NIH intramural investigators by marker-assisted backcrossing. Through polymorphic microsatellite marker analysis and optimal breeder selection, congenic mice can usually be obtained in 12 months, whereas they may take as long as two and a half years to produce by conventional backcrossing.

HTAGL provides genotyping in a high-throughput format using both real-time PCR and end-point PCR with the LabChip detection platform. Enhanced workflows in conjunction with a Laboratory Information Management System are used to automate the whole genotyping process from sample submission to final reports.

Animal Health Monitoring

KEY ACCOMPLISHMENTS

- FNL staff in ADL performed and managed 1,790 necropsies; 12,214 bacteriological tests; 5,004 ectoparasite tests; 9,241 endoparasite tests; 32,030 molecular diagnostic tests; and 54,376 serological tests.

The ADL monitors the health of laboratory animals at the NCI-Frederick and NCI-Bethesda animal facilities and provides diagnostic resources to several other NIH facilities.

Receiving and Quarantine

KEY ACCOMPLISHMENTS

- Approximately 38 animal importations were processed, including 10 from modified-approved sources, 23 from non-approved sources, and five from cross-fostering.
- PCR-based testing has been instituted, which has reduced the quarantine period by 25 to 50 percent. It has helped principal investigators receive their animals faster, thus speeding up their research.
- Standard operating procedures were updated to streamline and improve the Receiving and Quarantine operations.

Receiving and Quarantine is a vital program for safeguarding and protecting the health status of animal colonies at NCI Frederick and Bethesda.

Scientific Support Programs

Mouse Modeling Core Technology Laboratory

KEY ACCOMPLISHMENTS

- Generation of 69 genetically engineered mouse models in support of 25 NIH investigators, 40 of which used CRISPR/Cas9 technology.
- Mouse germplasm (sperm and embryos) cryopreserved from 118 valuable mouse models; 100 strains archived by sperm isolation and freezing; and 18 embryos with more complex genetics cryopreserved.
- Reconstitution of 19 mouse models from cryopreserved germplasm.
- Rederivation of 16 lines to specific pathogen-free status.

- Establishment of a new cell-culture space with full capabilities and staffing to address requests for cell-line expansion and tumor dissociation.
- Establishment of five cell lines from spontaneous mouse renal tumors in support of Dr. L. Schmidt (NCI).
- Generation of stable leptomeningeal metastatic tumor cells in collaboration with ARTS and Small Animal Imaging Program SAIP.

The MMC program provides expert services to the NIH scientific community for production of genetically engineered mouse models as well as cryopreservation of germplasm from valuable mouse strains. The MMC comprises TMM, Cryopreservation and Assisted Reproduction, and Cell Culture. The program has continued to receive positive feedback regarding the outstanding quality and timeliness of services fulfilled in support of NIH investigators.

TMM primarily generates genetically engineered mice by pronuclear microinjection, which has been instrumental in the study of *in vivo* gene function through the use of genetically engineered mice to model human diseases, specifically cancer. TMM offers a complete array of services aimed at successfully generating transgenic and gene-targeted mice. The laboratory has vast expertise in performing Southern blot hybridization used to identify transgenic founders and clones of embryonic stem cell transfected by electroporation. TMM also performs microinjection of mutant embryonic stem cells obtained from other repositories such as the European Conditional Mouse Mutagenesis Program and the microRNA resource available through the NCI Mouse Repository. The laboratory also has the capability to generate mouse embryonic stem cells *de novo*.

The Cryopreservation Laboratory offers a complete array of services to the NIH community to ensure valuable mouse models are protected against genetic drift or loss due to unforeseen events (disease outbreaks or natural disasters). Mouse embryos and sperm can both be cryoarchived. Colony expansion and strain rescue by *in vitro* fertilization, and rederivation of mouse strains to specific pathogen-free status are among other services that the program offers to the NIH community.

Cell Culture Laboratory

MMC's cell culture capabilities have been expanded to accommodate the significant increase in demand for its services and fulfilling 72 requests in CY2019 in support of NCI investigators. Among them, 56 tumor cell lines were processed by culturing, expansion, and processing for injection, and 16 requests for tumor dissociation in preparation for injection into host mice were completed. The addition of a new staff member has allowed the Cell Culture Laboratory to continue to address requests in a timely manner and fulfill requests requiring large cell numbers for drug efficacy studies. Cell types processed by the laboratory include leptomeningeal metastatic cells generated in house and diffuse intrinsic pontine glioma cells that form neurospheres and demand expert handling.

Small Animal Imaging Program

KEY ACCOMPLISHMENTS

- Major technology development projects included the hyperpolarizer [^{13}C] magnetic resonance spectroscopy imaging (MRSI) for metabolic imaging and development of the short-wave infrared (SWIR) fluorescence scanner. SAIP performed MRSI drug challenge studies to evaluate modulation of the lactate signal (technique validation) and implemented an MRSI dynamic imaging sequence to evaluate metabolic kinetics. SAIP is collaborating with Dr. Schnermann (Chemical Biology Laboratory) in developing the SWIR scanner, which will provide enhanced fluorescence imaging (improved image contrast and spatial resolution) and will be available to CCR investigators.
- SAIP collaborated with several LASP programs (ARTS, MMC, MHL, CAPR, and facility staff) to conduct CCR investigator studies characterizing animal models and efficacy studies (listed below). SAIP also provides training to CCR/NCI investigators requesting self-service optical bioluminescence imaging.
- The Dr. Schneider group (Chemical Biology Laboratory) designs and characterizes novel peptide and protein-based hydrogels for use in tissue regenerative therapy, parenteral delivery of therapeutics, and antibacterial therapy. Yamada et al. (*ACS Appl Mater Interfaces*, 2019) developed a negatively charged hydrogel for direct encapsulation of 3-D cells, where *in vivo* fluorescence and tissue resection experiments demonstrated that the gel supports long-term engraftment of cells.

SAIP performs quantitative preclinical non-invasive oncologic imaging: characterizing mouse models; evaluating molecular imaging probes; *in vivo* monitoring of tumors for testing new therapies; developing standards; and performing validation experiments for the NCI Technology Transfer Center and the NCI Experimental Therapeutics Program. These validation studies provide the necessary information for NCI to proceed with patent applications and FDA submissions.

SAIP Modalities

Modality	Process
3T Magnetic Resonance Imaging	Anatomic; Permeability
^{13}C MRI-Spectroscopy (MRSI)	Metabolics
Ultrasound	Anatomic; Perfusion
Optical: Bioluminescence	Cell trafficking; Metastasis
Optical: Fluorescence	Drug Biodistribution

SAIP Modalities	
Multi-modality	**Process**
Positron Emission Tomography (PET)/X-ray CT	Glucose metabolism Cell Proliferation
PET/MRI (1T)	Metastasis (Glucose)
Single Photon Emission Computed Tomography/CT	Molecular imaging

SAIP conducted 83 imaging projects in support of the following programs/divisions:

NCI Division/Program	Projects
NCI Intramural	
Center for Cancer Research (CCR)	**43**
Chemical Biology Laboratory	7
Laboratory of Cancer Biology and Genetics	3
Laboratory of Cancer Immunometabolism	2
Laboratory of Cellular and Molecular Biology	1
Laboratory of Human Carcinogenesis	4
Laboratory of Molecular Biology	2
Mouse Cancer Genetics Program	1
Technology Transfer Center/NCI	1
Thoracic and GI Malignancies Branch	5
Urologic Oncology Branch	10
Vaccine Branch	1
Women's Malignancies Branch	6
NCI Extramural	**30**
Division of Cancer Treatment and Diagnosis (DCTD)	**29**
Biologic Testing Branch	1
PDX_TCIA Subcontract (DICOM)	5
Cancer Imaging Program PDX Models:	
Donor Models—Common Tumors	4
Donor Models—Rare Tumors	1
Characterization-Common Tumors	7
Characterization-Rare Tumors	1
Rapid Evaluation—Common Tumors	1
Rapid Evaluation—Rare Tumors	9
NCI Experimental Therapeutics Program (NExT)	**1**
^{89}Zr-BetaSpheres	1
Leidos Biomedical Research—FNL	**10**
Advanced Development Research Directorate	
Cancer Immunoprevention Laboratory	2
Laboratory Animal Sciences Program	
Center for Advanced Preclinical Research	4
LASP/NCI-Office of Scientific Operations	
Technology Development:	4
TD21: Leptomeningeal Project (Completed)	
TD24: ^{13}C-Modulation MRSI (Ongoing)	
TD25: Orthotopic Lung Model	
TD27: Short-Wave Infrared Fluorescence (Ongoing)	

Major Studies: DCTD PDX Models

KEY ACCOMPLISHMENTS

- DCTD PDX provides extramural investigators with a resource of characterized patient-derived xenografts for drug discovery. The publication Tatum et al. (*J Transl Med*, 2019) and images in The Cancer Imaging Archive (TCIA) provide the standard operating procedures for characterization of a PDX spontaneous metastatic model in a drug challenge study. These resources provide the extramural community characterized PDX models for testing new therapies. Presently, four characterized PDX models of metastasis are available in TCIA for extramural investigators (adenocarcinoma pancreas: https://doi.org/10.7937/TCIA.2020.PCAK-8Z10; adenocarcinoma colon: https://doi.org/10.7937/TCIA.2020.BRY9-4N29; melanoma: https://doi.org/10.7937/TCIA.2020.7YRS-7J97; bladder: https://doi.org/10.7937/tcia.2019.b6u7wmqw)

SAIP characterized 23 PDX models of primary tumors and metastasis using contrast and non-contrast MRI and PET scans.

Statistics (images: 9/1/2020–6/15/2020):	
MRI primary tumor and metastasis	757
PET [^{18}F]FDG metabolism (glucose)	45
PET [^{18}F]FLT cell proliferation	27
PET [^{18}F]FSPG metabolism (glutamine)	11

ARTS provided tumor implant/excision and the MHL provided pathological confirmation of human metastasis in models imaged by MRI.

Molecular Histopathology Laboratory

KEY ACCOMPLISHMENTS

- **LEIDOS 2020 Achievement Award for Team Safety.** MHL was honored for a "hands-on" approach to laboratory design that included engineering controls for chemical, biological, and physical/ergonomic risks.
- **NCI Laboratory of Molecular Biology, Dr. Ira Pastan.** MHL demonstrated that l-lysine administration to mice prevents SS1P-mediated kidney damage.
- **NCI Laboratory of Cell and Developmental Signaling (LCDS), Dr. John Brognard.** MHL identified TNIK as a potential therapeutic target in lung squamous-cell carcinoma.
- **NCI laboratory of Allergic Diseases, Dr. Ana Olivera.** MHL used digital image analysis to diagnose osteoporosis in systemic mastocytosis.
- **NCI Exceptional Responders Initiative.** MHL quantified tumor infiltrating lymphocytes and developed assays to investigate cell-of-origin.
- **NCI Division of Cancer Epidemiology and Genetics, Clinical Genetics Branch, Dr. Anil Chaturvedi.** MHL provided support for the Taiwan oral cancer project, the goal of which is to guide screening and early detection of head and neck cancer.
- **NCI Validation of PDX models of metastasis.** MHL provided pathology characterization and validation for TCIA, a DCTD subcontract, which provides the extramural research community MRI images, time to metastasis, and pathology validation of PDX models of metastasis.
- **NICHD/DIR/OSD Reproductive Endocrinology and Infertility Branch**. MHL began work to establish a virtual slide repository of more than 2,000 ovary samples within an onco-fertility study.

INFRASTRUCTURE AND OPERATIONAL ENHANCEMENTS

- **Zoetis Piccolo express chemistry analyzer** for clinical chemistry analysis.
- **Two Oxford Genesis multispecies analyzers** for veterinary hematology testing.
- **NCI HALO Client and HALO Link cloud upgrade.** MHL has transitioned access for Indica laboratory HALO Client WSI analysis, HALO AI, HALO AP, and HALO Link from a single-user, on-premises workstation to a multi-user (n=10), and cloud-based version (3.1), which included new support for high-plex fluorescent images.
- **FNL MHL Collaborative Study Module.** MHL and DMS are working with the NCI to develop a suite of integrated study and work management tools/modules. A new study-design and pricing-estimation module went into testing in July 2019. A final release went live in July 2020. Release of a second module for request submission is anticipated in November 2020.

MHL supports animal study design and immune, anatomic, and toxicological pathology. Cross-trained staff are experts in rodent necropsy, hematology and clinical chemistry, tissue processing, tissue microarrays, staining, immunohistochemistry, RNAscope® *in situ* hybridization, and goal-appropriate target enrichment workflows, including yield and quality assessment. MHL also offers bright-field or fluorescent digital whole-slide imaging as advanced digital image analyses via Indica HALO Client and QuPath including machine learning via HALO AI.

Logistics Support and Property Compliance

Other Support and Logistics Services

Logistics and Warehouse

KEY ACCOMPLISHMENTS

- Processed 459 domestic hazardous and 188 international hazardous shipments (includes chemicals/biologicals and/or bloodborne pathogens that require special handling and packaging)
- Processed 2,922 domestic non-hazardous and 643 international non-hazardous shipments
- Processed 641 shipments using contracted couriers (special couriers) and more than 9,703 hand-carry packages
- Applied postage to more than 14,930 pieces for issuance to the U.S. Postal Service for delivery
- Recorded 46,742 perishable deliveries and 111,089 non-perishable deliveries
- Recorded 1,732 liquid nitrogen cylinder deliveries, 655 specialty gas deliveries, and 15,867 boxes of dry ice
- Recorded 121,425 parcels entering the receiving warehouse
- Recorded 27,290 parcels entering the Advanced Technology Research Facility (ATRF) receiving warehouse
- Distributed 8,221 parcels from the supply warehouse to various sites at the ATRF
- Received 8,785 requisitions containing over 21,085 lines for materials and supplies, valued at approximately $2.18 million, for distribution to the facility
- Issued 24,215 bags of animal bedding, valued at $204,616.75 and 7,519 bags of animal feed, valued at $93,614.04
- Issued 5,867 boxes of dry ice valued at $139,629.60, along with 86,600 gallons of liquid nitrogen, valued at $37,238

- The annual physical inventory for the Central Supply Warehouse was conducted this year and involved the inventory of more than 292 products, valued at more than $523,300, with a net adjustment of -$1,543.24.
- The Maintenance Supply Warehouse received 4,234 requisitions containing 8,559 lines for materials and supplies, valued at approximately $438,100, for issuance to Facilities Maintenance and Engineering Directorate craftsmen.
- The annual physical inventory for the Maintenance Supply Warehouse was conducted this year, and involved the inventory of over 3,962 products, valued at more than $813,600.00, with a net adjustment of +$958.05.
- Facilities Maintenance and Engineering Directorate management transferred the shop stock items that were in various shops to the Maintenance Supply Warehouse along with new items being added on a regular basis. To date, over 44 items valued at slightly more than $14,400 have been added.

Logistics Warehouse offers centralized receipt for all materials, supplies, and equipment; inspections; delivery services; risk management; freight forwarding; and operation of a Central Supply Warehouse and Maintenance Supply Warehouse.

Property Compliance

KEY ACCOMPLISHMENTS

- Managed 41,682 items of accountable government property valued at $545,752,791.60
- Received and processed 3,888 items of accountable property valued at more than $35.7 million into the property system
- Maintained management of 2,444 items at various offsite subcontract locations valued at $29,615,073.18
- Transferred 93 items of accountable property valued at $182,926.60 to other federal agencies
- Arranged for the donation of 72 items valued at $127,052 to educational institutions
- Reissued 30 items of accountable property valued at $246,338.19 from surplus to various NCI at Frederick programs

The Property Compliance Department supports personal property management for the FNL. The Property Accountability program encompasses a wide range of functions such as a biennial inventory and accountability report, property disposal, advertisement of good surplus for reutilization, surplus monitoring, educational donations, property sales, and accountable equipment acquisition from other institutions and agencies. The program also oversees inventory and policies regarding government-owned equipment purchased and used by subcontractors.

COVID-19-RELATED ACCOMPLISHMENTS

- Implemented process for off-boarding employees to return computer equipment to the Advanced Technology Research Facility
- Tag and issue large volumes of portable equipment for teleworking
- Process temporary loans and property passes to facilitate teleworkers
- Implemented automated property pass form
- Implemented delivery schedules for teleworking laboratory staff to come in and obtain materials
- Added several new stock items related to COVID-19 protection and cleaning
- Served on the Emergency Operations Committee to help coordinate, order, and distribute critical supply needs
- Delivered critical, COVID-19-related supplies to many different locations on the Bethesda campus
- Stored and managed undeliverable packages and perishables until laboratories reopen for business
- Safely coordinated and conducted inventories of laboratories and offices to account for government property

Scientific Publications, Graphics and Media

SPGM & Publications

The Frederick National Laboratory for Cancer Research staff in Scientific Publications, Graphics and Media (SPGM) provides resources; expertise; instruction; and, where necessary, services for publishing and presenting scientific and other information, including illustration; graphics; writing; editing; coordination of posters; training in scientific graphics, publications, and presentations; and audiovisual support.

KEY ACCOMPLISHMENTS

- Hired a science communications and training specialist and launched SPGM's first full-length training in less than two months
- Hosted multiple lunchtime webinars for scientific staff on transitioning to a digital poster presentation to support the increase in virtual scientific meetings during the pandemic. Each participant received an educational PDF handout to accompany the training. Current and signed-up participants total 69. Those who responded to the post-webinar survey have reported 100 percent satisfaction with the training.
- Nearly matched its previous annual high in manuscript editing projects (52 vs. 55) despite being short-staffed for most of the period
- Transitioned to teleworking for half of the contract year in response to COVID-19, with no impact on responsiveness to job requests or quality of work

- Engaged the community at large to determine ongoing needs and topics for training while avoiding duplication with other professional development offerings at the facility
- Designed the 2019–2020 *CCR Milestones* magazine, providing design and layout, illustrations, and photography
- Provided major support for the Return to the Workplace website on the NCI at Frederick intranet, including an immediately recognizable and well-received graphic identity package for the website, LISTSERV dispatches, and on-site signage
- Published weekly LISTSERV articles on relevant topics (508 compliance, writing tips)
- Produced videos (1–3 minutes) on relevant training and creative services topics (improving presentations, taking video on a phone, etc.)
- Conducted a video shoot for cryo-electron microscopy laboratories in the Center for Molecular Microscopy (Dr. Natalia de Val)
- Conducted another video shoot for the Center for Molecular Microscopy (Dr. Kedar Narayan)
- Produced a training video, *Being a More Dynamic Presenter*, featuring Public Affairs intern Julia Lizmi
- Produced a training video, *Using PowerPoint to Build Posters*
- Produced three training videos on the TOPAZ Elements system for the Laboratory Animal Sciences Program (Travis Sheets)
- Produced six Protein Mass Spectrometry Program videos (Dr. Mariam Malik/Dr. Lisa Jenkins)
- Produced the following published scientific journal covers:
    - *Mol Cell Biol*, July 2020 (Dr. Kylie Walters)
    - *Molecules*, vol. 25, issue 12 (June 2, 2020) (https://www mdpi.com/1420-3049/25/12) (Dr. Terry Burke/Dr. Xue Zhi Zhao)

Operational Support

Frederick National Laboratory
for Cancer Research

sponsored by the National Cancer Institute

OPERATIONAL SUPPORT

Partnership Development Office and Technology Transfer Center

The Partnership Development Office (PDO) spearheads the Frederick National Laboratory for Cancer Research's (FNL) strategic partnering efforts. The PDO manages contractor Cooperative Research and Development Agreements (cCRADAs), Technical Service Agreements (TSAs), and formal collaborations for the FNL. During fiscal year (FY) 2020, the PDO continued expanding its outreach and business development initiatives, which yielded a robust pipeline of strategic partnership opportunities.

The PDO also fronted a variety of additional efforts, including coleading the 2020 Laboratory Directed Exploratory Research (LDER) Program and 2021 LDER award cycle, supporting the NCI Technology Transfer Center (TTC) Invention Development Program (IDP), and assisting The Edge business accelerator.

The Intellectual Property and Strategic Agreements Office (IPSA) works closely with the PDO in establishing and handling strategic agreements. These agreements with collaborators allow FNL research teams to evaluate new and innovative technologies and seek out ways to remain ahead of the curve in delivering state-of-the-art scientific methods in response to the national laboratory mission. The IPSA also works closely with researchers to develop and evaluate inventions that may be patented or may become part of a future collaboration.

KEY ACCOMPLISHMENTS

Responsive Mission Support

- Supported the IDP, which aims to accelerate the development timeline of NCI intramural inventions by advancing the technologies through critical, early stages of validation. The PDO provides project management support for IDP and coordinates the preclinical validation work to be performed using FNL resources, including the Nanotechnology Characterization Laboratory, Cancer Research Technology Program, and Laboratory Animal Sciences Program, and external resources when necessary. In FY2020, IDP funded three new projects and had six ongoing projects.
- Managed the LDER Program, which provides funding for investigator-driven projects that are outside the scope of current work under the Federally Funded Research and Development Center contract. Thirteen new proposals were submitted for FY2021 funding, and five were awarded funding after a competitive review process. In addition, three FY2020 LDER projects were renewed for funding for the upcoming year.
- Completed task order negotiations where PDO would support operations and management of FNL's Visiting Scholar Program starting in FY2021.)

Stewardship of the National Laboratory

- Executed six cCRADAs at the time of writing, totaling more than $4,345,000 in partner contributions. An additional five cCRADAs are close to execution. The cCRADAs represent a variety of high-impact research, such as a Bill and Melissa Gates Foundation-funded collaboration between the AIDS and Cancer Virus Program and Beth Israel Deaconess Medical Center to identify potential viral reservoir biomarkers in simian immunodeficiency virus-infected macaques.
- Executed five material cCRADAs.
- Executed 11 amendments to expand, increase funding, or otherwise modify existing cCRADAs and material CRADAs. An additional six are in development and nearing execution.
- Executed three Research Collaboration Agreements.
- Executed one Beta-Testing Agreement, 320 Material Transfer Agreements, and 60 Confidentiality Agreements.
- License activity increased as a direct result of COVID-19 due to increased licensing of the FLU-PRO Questionnaire. Thirty licenses were executed with various academic and contract research organizations during the period.
- Executed 12 new TSAs, 8 TSA amendments, and 34 technical service orders—generating over $1,027,000 in cost recovery funds and enabling a large body of research that would otherwise not be possible. Additional technical service requests are expected before the end of August.
- Added one new technical service to the Technical Services Program to make it available to the external research community: *Polymerase chain reaction testing for* Mycobacterium tuberculosis *DNA.*
- Handled 12 new employee invention reports that were reported to the NCI during the period.
- Provided critical business development support to the Accelerating Therapeutics for Opportunities in Medicine (ATOM) consortium, a public–private partnership with the mission of transforming drug discovery by accelerating the development of more effective therapies for patients. The FNL helped found ATOM in 2017.
- Participated in more than 50 scientific, community, and business development networking events, including running a high-traffic booth at the 2019 TEDCO Entrepreneur Expo; participating in the Foundation for the National Institutes of Health's Biomarkers Symposium and 2019 FLC Mid-Atlantic Meeting; and speaking at SMART PROC GovCON 2019 and Defense Techconnect.

- Networked with over 80 companies and organizations via one-on-one partnering meetings at the digital BIO International Convention in June 2020.
- Organized the 2020 Technology Showcase (scheduled as a virtual event on September 9, 2020) in collaboration with NCI colleagues and partners in the community.
- Won the 2020 NCI Director's Award in collaboration with NCI colleagues, under the "Making an Impact" category for establishing the Annual Technology Showcase to foster collaboration and licensing of NCI technologies.
- Organized the virtual Federal Laboratory Consortium Mid-Atlantic Meeting (scheduled as a virtual event on November 10–12, 2020).
- Organized the quarterly Biotech Connector seminar series and moved it to a virtual format to continue it while adhering to COVID-19 safety considerations.
- Published four articles on partnership-related work on the public-facing FNL website.

Mission-Aligned and Engaged Employees

- Hired two new PDO staff members (Partnership Alliance Manager and Business Development Manager) to support expanded collaborative work.
- Hired a new IPSA attorney to provide support to collaborative transactions and contracts.
- Launched a new quarterly partnership email newsletter for all FNL staff to keep everyone apprised of collaborative opportunities.
- Hosted four internal partnership workshops to improve understanding of collaborative capabilities and processes across the FNL.
- Published 11 articles on the FNL's partnership-related activities on Insite.

Tailored Organizational Governance

- Developed new Standard Operating Procedures for the entire cCRADA process.

Public Affairs and Communications Office

The Public Affairs and Communications Office (PACO) supports the public service mission of the FNL through external communications and community relations, employee engagement, and creative multimedia services. We inform local, regional, and national constituencies about FNL's scientific accomplishments, opportunities for collaboration and partnership, shared national resources, business and career opportunities, and community impact. We protect and enhance FNL's identity and reputation. We stimulate internal collaborations across organizational boundaries to improve efficiency.

Stewardship of the National Laboratory

The FNL staff in PACO has improved the quality and expanded the reach of the FNL website and FNL's relatively new social media channels. This makes it easier for external constituents to find FNL online; learn about its science; access its shared technologies; and discover opportunities for collaboration, partnership, transacting business, and employment.

With existing resources, PACO published 37 science and technology articles in FY2020, an 80 percent increase over the previous year. FNL's social media channels continued to grow: Twitter increased 38 percent to 1,012 followers, LinkedIn increased 171 percent to 1,719 followers, and Facebook increased 24 percent to 845 followers. PACO hired a new digital media specialist who implemented social media management and scheduling tools and is using creative approaches, such as turning out short animations to make posts stand out.

FNL's pandemic response, which has drawn national attention, and the National Cryo-Electron Microscopy Facility are among major growth areas on the website. PACO collaborated with the National Cancer Institute (NCI) on national publicity for FNL's SARS-CoV-2 serology testing for the Food and Drug Administration and for the new multi-institutional Serology Sciences Network to be headquartered at FNL.

PACO expanded its participation on social media before, during, and after major scientific meetings to engage with participants and increase visibility of FNL science. For instance, PACO staff highlighted FNL abstracts on Twitter during this spring's virtual American Society of Clinical Oncology and American Association for Cancer Research meetings. PACO also collaborated with the National Cryo-Electron Microscopy Facility team on a Twitter strategy to highlight their attendance at the Biophysical Society Annual Meeting in February. Beginning January 28, PACO posted tweets promoting FNL's booth at the 2020 Annual Meeting of the Biophysical Society using the hashtag #BPS20; from Feburary 16–18, staff posted tweets and photos from the team on the ground in San Diego, getting more than 20 retweets and 50 likes. Ten cryo-electron microscopy researchers started following FNL on Twitter during the session. FNL is now directly connected to 1,000 Twitter followers that include a large share of high-quality, well-connected organizations and individuals. PACO continues to expand this network.

Cancer Survivors Day and other designated "disease" days or weeks provide an opportunity to join others in featuring FNL's scientific staff. For example, FNL's Twitter channel showcased Drs. Natalia de Val and Marina Dobrovolskia for Women in Science Day.

PACO supported Human Resources with its social media recruitment campaign through an enhanced LinkedIn profile and Life page, and a dedicated @FredNatLabJobs Twitter ID.

PACO staff represented FNL in the Frederick Cancer Coalition's Breast Cancer Awareness Month kickoff with County Executive Jan Gardner. We hosted Lakshman

Bindu, a scientist with the RAS Initiative, who spoke about the role of basic cancer biology research in the development of better treatments for cancer, and the passion she and her colleagues have for their work.

FNL scientists received media coverage for their research accomplishments and expertise. The national laboratory was also cited in multiple outlets with news of the HPV Serology Laboratory's central role in validation of SARS-CoV-2 serology assays.

PACO provided the Partnership Development Office communication support for the annual NCI FNL Technology Showcase, which won a 2020 NCI Director's Award. PACO, the Partnership Development Office, and the Frederick County Chamber of Commerce also cohosted a series of local biotech briefings, the Biotech Connector.

Mission-Aligned and Engaged Employees

With the onset of the pandemic, FNL's Power of Community launched the Caring for One Another campaign to help the community persevere. The two-part outreach program included employee contributions and an employer match, capped at $1,000 per organization, from FNL contractor Leidos Biomedical Research's corporate funds. The first phase of giving focused on businesses and families adversely affected by the economic impact of the pandemic. It also included monetary donations to help Frederick Health. The second phase benefited Frederick County food banks and the Maryland Food Bank. During the initial months of the pandemic, the Power of Community supported seven organizations and generated more than $15,000 in charitable giving.

As employees adjusted to telework changes, PACO partnered with Environment, Health, and Safety (EHS) to create a user-friendly COVID-19 information hub on the NCI at Frederick website. PACO collaborated with staff in Scientific Publications, Graphics and Media to structure the content and ensure a professional look and feel. The site was designed with graphic elements to tie in with LISTSERV and Communications Center messaging about returning to the workplace. PACO collaborated with EHS and the FNL President's Office on messaging designed to provide clear and complete direction for employees to safely return to their work spaces. Messages were delivered as joint dispatches with the NCI Office of Scientific Operations to all government and contractor staff. Contractor-specific messages went to just FNL staff. Messages were archived for reference on Insite, alongside links to the Centers for Disease Control and Prevention website and state and local websites. The site continues to highlight workplace updates and guidelines, as well as a link to EHS's return to the workplace website.

As FNL made scientific contributions to responding to the COVID-19 pandemic, PACO showcased articles about these success stories, as appropriate, on Insite and the FNL website. The PACO team also helped EHS develop resources for employees returning to work after the stay-at-home order and helped with communications to bring these resources to employees' attention.

The new teleworking environment brought a growing need for employees to connect with one another. Insite featured "A Break from the Outbreak," a place to share pictures, discover coping strategies, and stay connected during a time of uncertainty and isolation. During non-work hours, employees shared photos of pets, family members, and gardens. They also shared a retirement Zoom party, suggested board games, and recommended carry-out food. A Break from the Outbreak received 3,419 views and 151 comments and generated 85 photos with 879 corresponding likes.

Each year, FNL's annual Achievement Awards Program recognizes staff for outstanding contributions to FNL's mission and science. Employees look forward to nominating their colleagues and honoring them. Because of the pandemic, PACO used creative approaches to convert the 22nd Annual Achievement Awards into a virtual event and a month-long celebration. A dedicated page on Insite offered congratulations and welcomed comments and employee engagement. The page featured recognition "tiles" for all 99 Achievement Award winners, along with video clips and award spotlights. The virtual event achieved full impact through the following tactics:

- FNL Laboratory Director Dr. Ethan Dmitrovksy kicked off the month-long celebration with a video message featured on Insite and distributed through an all-employee email.
- Achievement Award spotlights were posted on Insite and sent through all-employee email, profiling recipients and sharing why the award was important to them.
- Award recipients received a virtual "swag bag" which included gift choices and an option to donate to charities.
- A concluding video featured quotes and candid pictures from award winners.

The technical setup made it difficult to track engagement but gave the event maximum visibility on the site, augmented by LISTSERV messaging and supervisor engagement. The site is rich with comments and congratulatory notes from across the organization and has enduring visibility.

Financial Operations Directorate

The Financial Operations Directorate (FOD) oversees all finance-related activities for the FNL, including the following functions: general accounting, payroll, accounts payable, billing, accounts receivable, financial planning and analysis, and core business information systems. The directorate's mission is to administer an enterprise-level, integrated financial management program to support FNL's full mission; establish fiscal policies that ensure accountability for, and control of, government funds; provide timely, relevant, and clear financial analysis and reporting to assist in managing fiscal resources; and deliver accurate, timely, and complete financial information to FNL stakeholders.

Responsive Mission Support

The **General Accounting department** performed a broad range of activities under each of the following categories: maintained daily activity of the Special Bank Account (SBA), processed corporate-funded accounts payable, processed billings and receivables, prepared National Cancer Institute (NCI) cost-incurred invoices, ensured compliance with accounts payable legal requirements, and forecasted and monitored fringe and general and administrative costs.

The Accounts Payable team processed invoices for vendors and subcontractors and addressed vendor inquiries related to payment. The group interacts with Purchasing, Receiving, Contracts, and programs daily. All vendor invoices are received at a processing center and are transmitted and approved electronically, thus eliminating the need for manual entry and filing. In FY2020, the group processed more than 50,000 invoices and check requests.

In addition to the ongoing general accounting operations, the Controller's Office certified Sarbanes–Oxley controls each quarter, effectively administered the SBA, and effectively monitored the fringe benefit indirect rate throughout the fiscal year. Significant effort was expended on the development of the 2019 Incurred Cost submission.

The Accounting department spent significant effort transitioning to the new Federally Funded Research and Development Center (FFRDC) contract. The new contract resulted in new projects, organizations, account structures, and SBA requirements. The group also continues to focus efforts on close-out of open orders and vouchers. In addition, as NCI moved to a decentralized invoice review and approval procedure, FNL developed Cognos training material and trained more than 80 government personnel who would be responsible for reviewing and approving FNL invoices going in the FFRDC contract environment. Beyond the Cognos training, FNL established a new e-mail box specifically to receive the NCI reviewers' invoice questions so that FNL can help them finalize payment approval. Over the course of the contract year, FNL successfully responded to hundreds of invoice questions from the government. Although there were some corrections made, no invoice rejections occurred as a result of the inquiries.

FNL successfully underwent an audit, with no findings identified, that was conducted in response to the revised disclosure statement that FNL submitted in August 2019.

FNL also submitted a request for approval to make adjustments to its provisional billing rates for FY2016 through FY2019, which the Division of Financial Advisory Services ultimately approved. This resulted in reductions in billing amounts for the prior periods, which was appropriate and beneficial to the government.

During FY2020, the FOD team continued to use the FocusPoint platform to enhance its capabilities to time-phase and track project costs, develop and maintain estimates at completion, and provide improved reporting. In addition, redesign work on the Costpoint 2.0 ledger system proceeded to enhance FOD's accounting capabilities to support the new FFRDC contract requirements.

FOD continued to sponsor the Finance Working Group forum between FNL and key government stakeholders (Management Operations Support Branch [MOSB] and the Office of Scientific Operations [OSO]). These meetings were held consistently on a biweekly basis and provided an effective forum to facilitate communication and interaction on finance-related priorities and initiatives to align both legacy and emerging contract requirements to FNL stakeholders.

FOD provided timely submission of all finance-related contract data requirement list requirements for the past fiscal year.

The Financial Planning and Analysis (FP&A) group supports cost management, budget development, and coordination of fiscal processes for the NCI at Frederick community. For FY2020, the group has monitored approximately 23,095 individual projects, including annual tracking of operating costs and associated funding levels for the FFRDC and indefinite delivery/indefinite quantity (IDIQ) contracts of approximately $1.9 billion. FP&A directly supported the proposal pricing, award, and implementation of 23 new task orders (TOs) awarded under the FFRDC contract, with an awarded value of approximately $855 million. FP&A project controls is actively supporting the tracking of costs, ceilings, and associated program funding for all awarded TOs.

During FY2020, FP&A has been involved in the following major activities:

- Continued to develop and refine the use of FocusPoint, a financial planning and reporting program, for FNL's IDIQ task orders, enhancing FNL's ability to time-phase and track project costs, develop and maintain estimates at completion, and provide improved reporting
    - Used FocusPoint to provide two estimate-at-completion analyses for operational TOs, with program input to be submitted to the government as the target for FY2020 full-year funding; to track additional effort added to contract via Yellow Task/FFRDC Contract Administration System in the operational contract baseline; and to provide Impact Assessment Report information for changes to TOs. FP&A also extensively tested systems; provided requirements for reporting, validated reporting, and validated systems; and provided training to end users.
- Evaluated the model for SBA requirements to determine whether advance payment percentages for TOs would continue to provide sufficient working capital
- Provided an annual update to the interim rate submission and the contractor's Cost Accounting Standards Board disclosure statement, which included updates to the indirect cost pools/bases. An additional update was provided to update the interim rate proposal for FY2021.

- Directly supported the ongoing financial reporting and analysis, Impact Assessment Reports, and ceiling monitoring of 101 TOs awarded under the IDIQ and FFRDC contracts; current TOs have an awarded value of approximately $1.4 billion.
- Provided support and analysis for the FY2015 expiring projects to help NCI understand rate adjustments and funding requirements through the end of projects' period of performance, thereby allowing for funding decommitted if necessary.
- Continued to evaluate and report on Operations and Technical Support contract funding status, providing additional information, as requested, on unbilled details and open-commitment details for required funding by the various divisions, offices, or centers; institutes; and agencies.
- Completed changes to systems and reporting as a result of the new bridge contract.
- Participated on the team that developed and implemented the Laboratory Directed Exploratory Research Program at FNL
- Prepared limitation of funds/limitation of costs analysis on awarded TOs as required.
- Provided multiple funding analyses near the end of the period of performance on the operational TOs to evaluate funding status and open commitments. FP&A followed up with OSO and MOSB to ensure that adequate funding was added to the contract to continue to deliver service.
- In conjunction with Partnership Development Office and program areas, implemented seven new contractor Cooperative Research and Development Agreement (cCRADA) efforts, with a value of $1.8 million and additional in-kind effort, and provided support for 21 additional ongoing cCRADAs, with a value of $14.3 million and additional in-kind effort.
- Coordinated the review of the Office of the Director (OD) at Frederick credit status, quarterly labor savings, and the capital equipment pool. In conjunction with the program areas, FP&A offered recommendations for use of labor savings and capital equipment. This practice has provided funding for purchases outside of the proposed operating budgets. With OD-Frederick budget reductions eliminating many of the open positions that fund this pool, FP&A has closely monitored any potential savings to ensure it stays within the overall OD-Frederick budget.
- Provided monthly and quarterly cost management/ status reports for various high-profile projects/ divisions (i.e., Vaccine Research Center; Biopharmaceutical Development Program; Office of Cancer Genomics; Division of Cancer Epidemiology and Genetics; agencies; NCI Center for Biomedical Informatics and Information Technology; Office of the Director, Center for Strategic Scientific Initiatives; Office of the Director, Immediate Office of the Director; NCI Experimental Therapeutics Program), which the customer uses to make financial decisions on the future operations of each of these programs.

Contract Deliverables

- Provided pricing recommendation for various shared services
- Provided out-of-cycle plan requests for OSO
- Provided the quarterly AIDS cost and budget report
- Provided the quarterly Division of Cancer Epidemiology and Genetics progress report
- Provided quarterly Cost, Schedule, Performance reporting for TOs
- Provided limitation of funds and limitation of costs reports as required

Project Management Operations Office

The Project Management Operations Office (PMOO) standardizes, guides, oversees, and leads the development, implementation, improvement, and extension of project management standards and processes across the FNL. PMOO has developed a functional project management framework with refined and robust processes, procedures, and principles adapted to FNL's mission. These principles are reinforced with resources and project management tools, training, expertise, surge staff, and templates. Trust, communication, and transparency between directorates, members of the senior management team, and the government are founded in this discipline.

Responsive Mission Support

Last year, we introduced the PMOO concierge concept, which was well received by the technical directorates. As part of the concierge concept, the PMOO provides a dedicated partner to develop the response to task order (TO) Requests for Proposals, coordinate the extended project teams, and author project management best practice documents for the monitoring of awarded TOs. Under the Federally Funded Research and Development Center (FFRDC) contract, many activities are now executed under a TO as well. The change from the Operations and Technical Support contract required the extension of project management processes to areas and organizations not familiar with the TO environment. The concierge simplified the transition to the new TOs for all teams, regardless of their experience.

The PMOO uses multiple methods to promote a professional project management culture in the FNL. PMOO's suite of processes and procedures facilitates documenting, monitoring, and reporting of Cost, Schedule, and Performance; changes; impacts; risks; issues; and resolutions. It also supports established accountability, transparent data-sharing, and open communication among stakeholders.

To monitor the performance of certain TOs, we primarily used the In-Process Review (IPR) site, which provides stakeholders continuous access to the status of the work scope, cost, and schedule. The IPR site is accessible to government stakeholders and serves as the foundation for the development of the quarterly Cost, Schedule, and Performance report that is submitted as a deliverable. In addition to serving as a historical record, the site provides executive leadership visibility into the collection of more than 100 projects across our diverse customer base via regularly scheduled presentations. The IPR is a central tool in project performance monitoring. It provides a venue for project managers to describe and pursue targeted solutions to challenges and risks; focuses senior management on areas requiring escalated engagement; highlights significant achievements; and monitors progress against project milestones.

The President's Acclamation and the President's Watch Lists are a synthesis of current monitoring systems and IPR inputs. These monthly reports highlight topics of significance to FNL's performance for commendation or correction. The PMOO is responsible for compiling significant project-performance-related data and issues from the IPR sites, written government feedback, and other project management documentation for inclusion in the reports. Directorates and group leads review and verify project performance data and issues, make additions/deletions, describe corrective actions and progress to resolution, and populate the Acclamation List. Documents are curated by the PMOO and briefed to the president of Leidos Biomed the second week of the month.

Stewardship of the National Laboratory

The PMOO is committed to collaborate with the government in shaping and developing effective and prompt responses to the scientific challenges facing the nation. The PMOO serves a key role as a liaison between government stakeholders and Leidos Biomed technical programs. In this function, the PMOO reviews, curates, and coordinates responses to new work requests from the government. In addition, the PMOO participates in government-organized pre-award kickoffs, ensuring the common understanding of project requirements. These efforts have led to significant improvements in TO proposal development, which, in turn, has facilitated project execution.

Mission-Aligned and Engaged Employees

The PMOO has developed guidelines and established a process for a new human capital management program called "The Bench." The purpose of The Bench is to ameliorate the impact of TO completion, scheduled breaks, and realized risks on the retention of seasoned scientific staff. Additionally, The Bench acts as an adjunct for cross-training, career development, and succession planning. This past year, The Bench was successfully deployed ensuring the retention of key staff within the Drug Discovery and Development Program.

In addition, the PMOO continues to support the community of project managers by sponsoring two tables at the Project Management Institute, Baltimore Chapter Dinner Meetings and serving as key subject matter experts at a monthly project management lunch-and-learn seminar series.

Tailored Organizational Governance

The Bridge Contract, awarded in August of 2019, presented Leidos Biomed with seven new TOs requesting support for the ongoing activities and projects funded by the NCI and NIAID, Moonshoot and Leases. After contract award, the PMOO led the effort to reconcile the government-accepted technical response documents with the question-and-answer documents that were generated during the negotiations process. These technical response documents served as the operational record and the starting point for the next proposal. In addition to the modifications required from the proposal negotiation process, PMOO led the effort to ensure all change requests approved after the cutoff for the proposal period and during performance of the new TOs were integrated to the technical volumes prior to releasing copies for use as templates for the next TO proposals, thus ensuring the most up-to-date understanding of the work. Updates were made to the technical volume structure for the Fiscal Year 2020 through 2024 proposal based on lessons learned from the previous proposal. The PMOO was instrumental in communicating changes to the directorates as the proposal process evolved and in tracking and reporting progress back to executive leadership. The PMOO led the Fiscal Year 2020 through 2024 proposal response effort, serving as the communication and coordination center for all operational and technical groups. The PMOO established a response schedule and tracking system, developed templates and procedures, and served as the central communication hub between directorates, government customers, and administrative and operations groups. The PMOO also coordinated the development of technical responses, advising the directorates on structure and required content during development, reviewing them to ensure accuracy and responsiveness to the statements of work, and compiling responses from all technical areas into each comprehensive TO response.

The PMOO led the effort to repurpose the Yellow Task system from an outside-facing request system to an internal change management tracking system for the new TO environment. In its new role, the system tracks both government (FFRDC Contract Administration System requests) and Leidos Biomed-initiated changes (Impact Assessment Reports, contracting officer approvals, and contracting officer's representative concurrences that change the operational baseline and/or level of effort). In the current reporting period, Leidos Biomed has processed:

- 112 approved requests from 23 different divisions, offices, or centers;

- 14 COR Concurrences from 10 different divisions, offices, or centers; and
- 23 task requests that are pending approval.

The PMOO also oversees NCI at Frederick Accessioning System request submission and approval through a systems administrator, processing over 1,200 requests from 15 institutes and agencies. This past year, the PMOO continued to collaborate with the technical program areas, requesters, and service area managers to develop new features and continuously improve the services provided by the system. The new features introduced this year allow:

- Interagency access to the NCI at Frederick Accessioning System;
- Service area managers to submit new requests on behalf of the PI; and
- Linking of requests across contract years.

The PMOO also coordinated the onboarding of three new labs (two from the Basic Science Program and one from the Laboratory Animal Sciences Program) and facilitated the transition of billing for one lab from its old legacy system, ensuring greater transparency, efficiency, and control over project costs and budgets.

The PMOO is an active participant in the team assigned to define requirements for the acquisition and implementation of a contracts life cycle management system. As part of this process, PMOO is closely collaborating with the Enterprise Information Technology Directorate to define requirements for acquiring and subsequently implementing an enterprise program management system that will seamlessly integrate with contracts and financial management tools.

Contracts

The Contracts team is responsible for administering the two NCI-awarded IDIQ contracts and the associated TOs awarded under each, totaling more than 100 TOs.

Responsive Mission Support

The Contract team's responsibilities include proposal development and negotiation of all proposal submissions with the government. Proposal development and negotiations are conducted in a professional and collaborative manner with the NCI and their government customers and the scientific community responsible for performing the work.

There is close coordination with the Business Operations Group, such as the Program Management Operations Office, Financial Operations Directorate, and Human Resources Directorate, and across all areas of the Contracts & Acquisitions organization.

After a TO is awarded, Contracts proactively interfaces with scientific program managers during performance, review, and submission of all required contract deliverables; participates in the change management process when statements of work are being revised; and ensures resolution of contractual issues if they arise during work performance.

Research Subcontracts

As a crucial part of C&A, the Research Subcontracts (RS) team works with the internal scientific teams and internal operational support teams to ensure that subcontracts are awarded in support of both NCI and FNL requirements. Some examples of our support are detailed below.

Responsive Mission Support

- **Rapid design and deployment of the COVID-19 therapy guidelines for treatment website**: We engaged an FNL vendor to rapidly design and deploy a website as part of the effort to disseminate current information about COVID-19 therapy and guidelines to a wide audience of consumers, ranging from clinicians to the general public. In close coordination and collaboration with a highly focused and dedicated technical team, C&A personnel rapidly engaged and secured subcontractor services under a temporary authorization to proceed. Within two weeks of receiving a draft statement of work, subcontractors negotiated and awarded an IDIQ TO subcontract. Before awarding and executing the subcontract, the proposal was reviewed and received approval from NIAID and the White House COVID-19 emergency response team.
- **Maximo re-implementation**: RS personnel identified and engaged an FNL vendor to re-implement the Maximo tracking system, a sophisticated equipment tagging and tracking system used by NCI warehouse property, technical program, and government personnel. Two prior attempts to re-implement the system were unsuccessful.
    - Several RS team members were recognized at the 22nd Annual Achievement Awards and received the Outstanding Team Achievement Award in the Administrative or Infrastructure Category.
        - The Outstanding Team Achievement Award was presented with the following citation: "The dedication, knowledge, and support of the Maximo project team was paramount in overcoming the challenges and obstacles associated with the implementation of the new version of Maximo while preserving the core functionality. The execution was a success and great feedback was received from the users and the customer."
- **Cancer data aggregator competitive acquisition:** To facilitate this acquisition, RS interconnected the Genomic Data Commons (GDC), Proteomic Data Commons, and Imaging Data Commons to enable all participants across the cancer research and care

continuum to contribute to, access, combine, and analyze diverse data that will enable new discoveries and lower the burden of cancer.

  - This large-scale (approximately $4.5 million) project required significant collaboration and facilitation skills and effort on the part of the senior subcontract administrator and the scientific technical evaluation team in order to navigate through and mitigate identified potential risks.

- **Genomic Data Commons:** RS continues support for the GDC with the primary university subcontractor and lower-tier subcontractor institute for cancer research whose mission it is to provide the cancer research community with a unified data repository that enables data sharing across cancer genomic studies in support of precision medicine. The GDC, currently now in Phase VII, harmonizes raw sequence data, applies state-of-the-art methods for generating high-level data, such as mutation cells and structural variants, and provides scalable downloads and web-based analysis tools. A Phase VIII is anticipated.
- **Cancer Moonshot**: Last year's subcontractor support for an automated patient portal, centralized institutional review board, and clinical data management system continues through subcontracts with FNL vendors. C&A personnel have added additional subcontracted support to serve as the Biospecimen Core Repository and the Biospecimen Source Site Coordinator. Now that these key components have been put in place, the Cancer Moonshot protocol is anticipated to launch in Fall 2020.
- **Biopharmaceutical Development Program:** RS continued to support and assist the Biopharmaceutical Development Program's mission to develop leading-edge analytical technologies for antibodies, recombinant proteins, peptide and DNA vaccines, virus vaccines and oncolytic viruses, gene therapy products, and other biological and immunomodulating agents in addition to their subcontract partnerships for the development or manufacture of a canine antibody, ATI-1013 antibody, hAnnA-1 antibody, and AAV9 gene vectors. Multiple additional initiatives are anticipated in the future.
- **Molecular Profiling to Predict Response to Treatment:** In an effort to support retrospective characterization and analysis of biospecimens collected from NCI-sponsored trials of the National Clinical Trials Network and NCI Community Oncology Research Program through the Molecular Profiling to Predict Response to Treatment Program, RS personnel initiated subcontracts with a number of highly qualified entities for various study support areas, such as operations, statistics, biobanking, genomic characterization, and genomic data analysis support. FNL anticipates additional awards to support Phase 2 of the program.
- **Clinical Proteomic Tumor Analysis Consortium:** RS continues to support the Clinical Proteomic Tumor Analysis Consortium's efforts in antibody generation through subcontracts with several FNL vendors, nonprofit organizations, and universities. These collaborations are expected to continue into the next fiscal year and beyond.
- **SARS-CoV-2 serology:** RS received an urgent request to award a subcontract to Quest Diagnostics Incorporated due to the urgent need to respond to the COVID-19 pandemic. The objective is to determine seroprevalence of SARS-CoV-2 as well as to determine the percentage of adults in the United States who may have been exposed to SARS-CoV-2. This objective will be accomplished by screening 350,000 samples using a validated SARS-CoV-2 serological assay with evidence of high specificity and sensitivity.
- **Serological Sciences Network Capacity Building Centers:** RS issued a solicitation for Capacity Building Centers (CBCs) to support the Serological Sciences Network (SSN). The objective is to mobilize collaborative efforts to develop serological assays of high specificity and high sensitivity for deployment to test for SARS-CoV-2 induced immune responses and to rapidly expand national serological testing capacity. The SSN for SARS-CoV-2 (SeroNet) will contain several components: (i) CBCs; (ii) U01 grants; and (iii) U54 grants. We anticipate the solicitation will result in several awards.
- **Support to NIAID DCR Ebola Response Program:** In November 2019, RS issued competitive solicitation, known as the Liberia-U.S. Partnership for Research on Vaccines and Infectious Diseases in Liberia (PREVAIL), formerly the Liberia-U.S. Partnership for Research on Ebola Virus in Liberia. This effort was a recompete of existing work. The solicitation consisted of an IDIQ agreement specific to the Liberia-U.S. Partnership for Research on Vaccines and Infectious Diseases and seven TOs to be awarded to one subcontractor for a base and four option years. This was a complex and high-profile procurement that required extensive collaboration between RS and the government. Four subcontractors formally submitted proposals, including the incumbent. A final award was made in May 2020.
- **Vaccine Research Center (Zika virus):** RS awarded subcontracts for live attenuated Zika virus vaccine candidates as well as a subcontract to provide clinical research services for a Phase I challenge study and protocol development and planning for future Zika virus vaccine candidate studies to support the work previously considered with a different entity.
- **NIAID Division of Clinical Research expert emergency clinical research response:** In February 2020, FNL was tasked with an emergency response

to the COVID-19 pandemic. RS was tasked with working with the program to rapidly put subcontracts in place.

- RS utilized the emergency response (ER) IDIQ subcontracts previously put in place in 2018 to multiple subcontractors capable of rapidly responding to this type of health emergency. Under the ER IDIQ holders in support of COVID-19, RS awarded multiple separate TOs as of June 2020 through a limited competition among the ER-qualified IDIQ holders. Outside of the ER IDIQs, RS worked rapidly to implement subcontract agreements to two other qualified entities. Additionally, RS implemented modifications quickly to existing subcontracts in multiple countries to provide COVID-19 support.

Construction and Facilities Services Procurement/Subcontracts

Responsive Mission Support

Construction and Facilities Services (CFS) procurement is responsible supporting acquisitions for all architecture and engineering (A/E), construction, refurbishment, and other facilities service requirements, including the lease and utilities of off-site locations supporting NCI at Frederick operations. CFS's primary focus this year was supporting the ongoing major refurbishment projects throughout the FNL campus. Examples are detailed below.

- **IDIQ contracts for general construction services and A/E services**
  - CFS maximized use of IDIQ contracts for general construction services and A/E services.
  - In response to the NCI's request to improve the timelines associated with awarding subcontracts, the four IDIQ subcontracts for construction services were restructured to allow for awards up to $1 million (previously a $150,000 maximum) until the end of FY2020. Additionally, a new IDIQ solicitation was issued for contract awards between $150,000 and $2 million, which will be awarded by FY2021. Beginning in September 2020, CFS will have additional IDIQ contractors to cover awards up to $2 million, reducing the cycle time for the solicitation/award phase.
  - IDIQ agreements for A/E services were reissued to four large engineering firms. The previous A/E IDIQ agreement was awarded to three A/E companies (two large and one small firm). The awards to the four large A/E firms provide greater access to A/E firms specializing in designing biological laboratories and vivariums.
  - CFS is also currently managing 16 requirements contracts issued to single-trade and specialty contractors, providing the ability to address requirements previously performed in house and requiring immediate attention.
- **Lease facilities and utilities support**
  - In the Fall 2019, CFS managed the execution of a new lease in support of the Center for Biomedical Informatics and Information Technology Technical Operations Support team. The new lease space was in shell condition, requiring a complete buildout by the landlord. The space was to be built out in two phases: Phase 1, consisting of 17,000 square feet, would be occupied immediately upon completion and Phase 2, consisting of an additional 8,040 square feet, would be built out later.

Small Business Office Outreach

Responsive Mission Support

The FNL Small Business Office is always looking for ways to expand the FNL outreach program supporting FNL's use of small businesses, including Alaskan Native Corporations and Native American-owned, women-owned, veteran-owned, service-disabled veteran-owned, and HUBZone businesses.

During the past year, the Small Business Office has very proactively participated in both FNL activities and NCI and NIH events. Recently, in support of the NIH Small Business Programs Office (SBPO) Historically Black Colleges & Universities (HBCU) Initiative per Presidential Executive Order 13779, the FNL small business liaison officer implemented an NIH-HBCU integration strategy to create ties between the FNL and the HBCU community. By partnering with HBCUs in their local HUBZone communities to build small business capacity and capabilities, the SBLO seeks to better achieve the federal government statutory three percent HUBZone subcontracting goal.

The strategy includes HBCU student/faculty exchanges, formal collaborative agreements, and complimentary cradle-to-grave subcontracts training. In July 2020, FNL issued an HBCU request for information to assess current HBCU capabilities and to better identify areas for FNL collaboration. The request for information was distributed to the NIH SBPO Path to Excellence and Innovation participants via the SBPO PEI listserv and the White House Initiative on HBCU listserv.

Purchasing

Mission-Aligned and Engaged Employees

Purchasing is responsible for acquiring commercial goods and services, including equipment maintenance and fleet services, as well as overseeing, managing, and training program areas on using the blanket order and credit card programs.

In order to meet ever-increasing demand, Purchasing management and their team focuses on many areas, including:

- **Strategic sourcing and commodity management program:** Purchasing continues to focus on our commodity management program by evaluating spend and volume on a quarterly basis for the top commodities, and working on opportunities for strategic sourcing, improved cycle times, supplier service levels, and cost savings. The top commodities represent more than 90 percent of total spend for the first three quarters of FY2020. The top commodities (by spend) include animals and animal supplies, capital equipment, industrial supplies, laboratory supplies, biologicals, commercial research services, IT hardware and software, office supplies, and service maintenance agreements.

  The strategic sourcing blanket purchase agreements (BPAs), an integral part of the program, are the facility's preferred sources for purchasing applicable products and services. Benefits of using the strategic sourcing BPAs are as follows: (i) lower prices (significant cost savings) and more consistent pricing and service/delivery levels; (ii) lower administrative costs due to the reduction/elimination of duplicate contracts for similar items; and (iii) customized, user-friendly websites for efficient online purchasing.
- **Increased competition:** One of goals this year within this program was to increase competition where possible. In the first three quarters of FY2020, an average of 61 percent of the spend on commodities was competitive. The range for competitive procurements ranged from 12 percent for animals up to 96 percent for service maintenance agreements. Animals are usually sole-sourced due to research requirements.
- **Service-level performance:** Service-level performance is a key metric for the Purchasing department. The following service-level performance data is from September 2019 through July 2020:
  - The service-level agreement for materials and supplies is to award at least 85 percent of the requests within 10 days from receipt of the request. 90 percent were awarded within the 10 days.
  - The service-level agreement for radioisotopes is to award at least 90 percent in one day. 95 percent were awarded within one day.
  - The service-level agreement for capital orders up to $150,000 is to award at least 85 percent within 25 days. 76 percent were awarded within 25 days; however, from September 2019 through May 2020, 91 percent were awarded within 25 days. We are not able to keep up with the volume in June, July, and August.
  - The service-level agreement for capital orders over $150,000 is to award at least 85 percent within 50 days. 83 percent were awarded within 50 days; however, from September 2019 through April 2020, 90 percent were awarded within 50 days. The volume of capital orders between May and July 2020 was very large, making it more difficult to attain the 85-percent service level due to volume.

Human Resources Directorate

Mission-Aligned and Engaged Employees

The Human Resources Directorate (HR) commits to staff quality and excellence by acquiring the right talent and enabling employees to engage, learn, and develop, while continually adapting to the emerging needs of the mission. HR fosters a positive work environment by providing leadership and guidance in the development, implementation, and equitable administration of policies and procedures.

Recruiting, retaining, and developing the FNL's talent remains HR's primary focus. This year, in recognition of our efforts to establish a strong organizational culture were employees feel valued and can grow and develop with the company, FNL was named a Top 50 Workplace in Frederick County. Awardees were selected based on powerful employee testimonials and each workplace's unique strategies to attract and retain top talent. This is the third consecutive year the FNL has been recognized by the Frederick County Office of Economic Development: in 2018, FNL was named a Top 50 Business; in 2019, Dr. Ethan Dmitrovsky was named a Top 50 CEO; and, in 2020, FNL was named a Top 50 Workplace.

Over the past year, we have worked diligently on enhancing our recruitment capabilities to attract world-class talent and develop tools that align processes and programs that further enhance our ability to develop FNL staff. With competition for talent on the rise, organizations cannot be content to manage their business as they have in the past. Continuous improvement must be at the forefront of every organization's strategy to attract, develop, and retain their talent. To ensure we continuously improve on our capabilities, FNL partnered with Shaker Recruitment Marketing (Shaker) to develop a branding and marketing campaign. This campaign was designed to enhance the visibility of the FNL, not only in the markets in which we recruit, but also on social media sites to educate the public about who we are and what we do, and to draw more candidates to our open positions. By partnering with Shaker, we were able to enhance our diversity recruiting and reduce costs by changing some of our previous vendors and recruitment platforms. We also developed recruitment campaigns on sites such as LinkedIn, Facebook, Twitter, and Google to enhance our ability to attract talent. Lastly, we implemented a program called Shaker Programmatic. This initiative allowed FNL to reach more job boards more efficiently. By establishing a metrics dashboard, we will measure the effectiveness of these changes in 2020 as well as develop future strategies based on the data we collect. Additionally, FNL was able to recruit and fill 272 positions this year, of which 74 percent were exempt and 26 percent were nonexempt. Talent acquisition staff

continue to provide specialized recruitment strategies in response to the unique hiring needs of each program, including our recent support in response to crucial clinical and research needs of the COVID-19 pandemic.

Through our hard work, creativity, and innovation, we have made significant strides toward enhancing our ability to retain talent in addition to our recruitment efforts. We have used branding for HR are the programs and initiatives to build awareness of these efforts. For example, we branded our new learning management system, EDGE (Empowering Development, Growth, and Excellence), and our newly redesigned 401(k) savings plan, The Capital Accumulation Plan (The Cap). By branding our tools and services, we enable employees to relate and engage with their benefits. The naming conventions have become part of the employee lexicon.

The Cap, formerly called the Leidos Biomedical Research, Inc. 401(k) Plan, reflects our commitment to our employees. We've improved upon the standard 401(k) plan. It's more than just a new name; we've enhanced the plan to make it easier for employees to save and invest, reduced administrative burden, and provided a broader portfolio of investment options. With these changes, employees can now manage their account and access useful resources to help them obtain the most value from their retirement savings. Now, employees have access to everything they need to create a personal retirement plan, a program that can grow with them as they work toward retirement and meet their retirement needs.

To further support our commitment to developing and growing FNL's talent, in partnership with the Enterprise Information Technology Directorate and the Environmental Health and Safety Directorate, we implemented EDGE. This tool will allow the FNL to support and meet the training compliance requirements for the organization in a more efficient and integrated way. Additionally, EDGE will build a learning culture for enhancing and developing FNL staff skills and knowledge and provide a career path for professional growth and development. This is evident in our recent review of the company's annual voluntary turnover, which was seven percent for this period and has been steady since 2019. This turnover level continues to compare favorably with the marketplace and validates that employees do not leave the FNL at the same rate as they do at other organizations. Annual involuntary turnover related to performance was less than one percent for this period. At the contract year-end, FNL's employee population was 2,335; of these employees, 56 percent were female and 32 percent were from minority groups.

The FNL has also developed and implemented a new career framework model. This framework will provide the organization with a means to link performance with employee development and ensure that the FNL remains competitive in talent acquisition. With the implementation of this framework, we will firmly align our compensation programs with current business strategy and industry best practices. Our mission is to attract and retain the best employees, and this structure will provide the tools to meet this goal. The concept is simple; we reclassified positions from the previous job structure to a new job structure that will provide many benefits to the FNL as an employer as well as to our employees. From an employer perspective, the new framework allows us to easily define every job, compare jobs with the market, and identify trends or issues in the market. From an employee perspective, job families in the framework have been enhanced and enlarged to provide many levels for career growth and development and a more defined path for succession planning and career growth.

As the FNL continues to grow and evolve, HR is prepared to foster FNL's core values of accountability, collaboration, compassion, dedication, integrity, and versatility, which will allow FNL to continue to address the growing public health needs.

APPENDIX A:
COMPANY OVERVIEW

Frederick National Laboratory
for Cancer Research

sponsored by the National Cancer Institute

Leidos Biomedical Research, Inc.

2019-2020 Annual Report

Appendix A

Company Overview

OFFICE OF THE PRESIDENT

Ethan Dmitrovsky, M.D., President

SCIENCE AND TECHNOLOGY GROUP

Leonard Freedman, Ph.D., Chief Science Officer

Cancer Research Technology Program Directorate

Dwight V. Nissley, Ph.D., Director

The Cancer Research Technology Program (CRTP) leads scientific initiatives and provides technical solutions to the National Cancer Institute (NCI) and National Institutes of Health to meet the challenges of and carry out mission-driven biomedical research. A major research area of CRTP is the NCI RAS Initiative, which was charged with the mission of developing therapeutic strategies against oncogenic RAS in RAS-driven cancers.

The RAS Initiative is using emerging technologies to solve RAS protein structure, define RAS interactions with the plasma membrane, characterize and disrupt RAS complexes with effector and regulatory protein partners, validate KRAS as a therapeutic target, and identify compounds that would inhibit RAS-driven tumors. To accomplish this goal, the RAS Initiative implemented a hub-and-spoke model to develop and foster collaborations that will increase knowledge and resource sharing. These efforts include collaborations between researchers at the Frederick National Laboratory for Cancer Research hub, extramural NCI-supported labs, pharma, and intramural labs. These collaborations were initiated via partnerships facilitated through NCI and contractor mechanisms, including material transfer agreements, technical services agreements, contractor collaboration agreements, and cooperative research and development agreements. Significant advances have been made through collaborations with the University of California, San Francisco; Theras, Inc; the Weizmann Institute; and Sanofi.

In addition, CRTP continues to lead and support NCI efforts to enable the extramural research community through the Antibody Characterization Laboratory, Nanotechnology Characterization Laboratory, and Optical Microscopy and Analysis Laboratory. CRTP also oversees dedicated laboratories to support ongoing research efforts in collaboration with the Center for Cancer Research (CCR) and the Division of Cancer Epidemiology and Genetics. The work of the National Cryo-Electron Microscopy Facility, in conjunction with the extramural cancer research community and a newly added cryo-EM R&D component, has resulted in publications in high-impact journals. The Single Cell Analysis Facility has expanded and is providing support to CCR investigators in performing analyses at single-cell resolution.

CRTP is composed of the following:

NCI RAS Initiative

Extramural-Enabling Programs:

- Antibody Characterization Laboratory
- Nanotechnology Characterization Laboratory
- National Cryo-Electron Microscopy Facility

NCI-Dedicated Programs:

- Center for Cancer Research Dedicated Core Laboratories
- Division of Cancer Epidemiology and Genetics Core Laboratory

AIDS and Cancer Virus Program Directorate

Jeffrey D. Lifson, M.D., Director

The AIDS and Cancer Virus Program (ACVP) consists of both investigator-initiated research sections and research support core laboratories that support work by both the ACVP and collaborating investigators from outside the ACVP. During the review period, the research portion of the ACVP comprised the laboratories of five Principal Investigator (PI)–headed research sections pursuing independent yet related multidisciplinary research programs in basic or applied molecular virology, viral immunology, retroviral pathogenesis, and viral oncology. The scientific staff of the ACVP encompasses expertise in a broad range of scientific disciplines, and there is a strong tradition of collaboration between the PIs. The studies pursued by the laboratories have, as a unifying feature, their direct or potential relevance to the overall goal of developing an effective vaccine or other approaches for the prevention or treatment of HIV infection and AIDS, as well as relevance to the study of viruses involved in cancer. The program helps fulfill the mission of NCI by contributing to the advancement of our understanding of HIV and AIDS, which are major causes of morbidity and mortality in the United States and around the world and are predisposing factors for AIDS-associated malignancies. Through research on vaccines and other approaches for the prevention and treatment of HIV infection and AIDS, including treatments that go beyond daily combination antiretroviral therapy, the ACVP also seeks to have a practical impact on this global problem. Finally, the ACVP seeks to add to the legacy of important contributions to AIDS research and viral oncology made by NCI scientists. The ACVP has been very productive over the last year, contributing to 51 articles submitted or published in peer-reviewed journals, including multiple high-impact publications. The ACVP also provided unique cutting-edge, state-of-the-art services in virus production and purification, viral characterization and imaging, as well as viral sequencing and quantitation, in collaborative support of numerous researchers in the AIDS and cancer research community. This year, the program is also making important contributions to the SARS-CoV-2/COVID-19 research effort. For example, our program is participating in a nationwide clinical study examining the effects of SARS-CoV-2 infection on HIV-1 viral loads in dually infected patients; has developed RNAScope, DNAScope, immunofluorescent and immunohistochemistry approaches for detection of SARS-CoV-2 in tissues from infected individuals; has provided technical consultation and precision flow cytometry and cell sorting instrumentation for COVID-19–related research; and has collaborated with the Uganda Virus Research Institute on a large SARS-CoV-2 population-based monitoring study investigating the effect of SARS-CoV-2 infection on herpesvirus reactivation.

Basic Science Program Directorate

Mary N. Carrington, Ph.D., Director

The Basic Science Program (BSP) consists of both investigator-initiated research laboratories and personnel who work in support of the National Cancer Institute (NCI) Center for Cancer Research (CCR) laboratories. Six principal investigators (PIs) run laboratory sections that pursue independent, multidisciplinary research. The PI laboratories include: the Human Leukocyte Antigens Immunogenetics Section, headed by Dr. Mary Carrington; the Molecular Immunology Section, headed by Dr. Stephen Anderson; the Hematopoiesis and Stem Cell Biology Section, headed by Dr. Jonathan Keller; the Molecular Genetic Epidemiology Section, headed by Dr. Cheryl Winkler; the Computational Structural Biology Section, headed by Dr. Ruth Nussinov; and the Epigenetics Section, headed by Dr. Kathrin Muegge. Researchers who are embedded in CCR laboratories are organized into four scientific sections: The Chemistry and Nanotechnology Section, the Cancer and Immunology Section, the Basic Research Section, and the Genetics Section.

BSP also provides services and products to support CCR's research efforts. The Cancer and Inflammation Program (CIP) Genetics and Microbiome Core performs statistical and bioinformatics analysis in support of CIP investigators; the Fluorescence-Activated Cell Sorting Core conducts flow cytometry and analysis for CIP and CCR scientists; the Media Laboratory produces media and reagents for more than 45 CCR laboratories; and the BSP program office provides logistical and administrative support to CCR laboratories.

The scientific staff has expertise in a broad range of the basic science disciplines, and a strong collaborative tradition exists between the research staff and their CCR colleagues. The unifying feature of the studies pursued by the investigators is their direct or potential relevance to the overall goal of gaining knowledge and developing cutting-edge tools that can be applied to human diseases. PIs' progress and scientific efforts are monitored through CCR site visits. BSP's contributions focus on cancer and retrovirology, and its researchers seek to understand basic biology, the cellular mechanisms that contribute to carcinogenesis, and the genetic factors that influence disease susceptibility and progression.

Laboratory Animal Sciences Program Directorate

Stephen N. Jones, Ph.D., Director

The Laboratory Animal Sciences Program (LASP) is a comprehensive resource for National Cancer Institute (NCI) scientists performing animal-based research at the Frederick and Bethesda campuses. The program provides the highest possible quality of animal care and assists NCI investigators in the use of healthy animals appropriate for their research objectives. In addition, LASP ensures that all animals are housed, handled, and cared for in a humane manner in accordance with regulatory guidelines and provides robust scientific support for investigators performing animal-based research.

To support the diverse research requirements of the scientific community, LASP operates the Animal Research Facilities, including Laboratory Animal Medicine (LAM), the Animal Health Diagnostic Laboratory, and Receiving and Quarantine. Through these facilities, LASP guards against the accidental introduction of pathogens in experiments and provides accurate clinical diagnoses and preoperative care. LASP also offers quality animal holding facilities and related services, including the quarantine of imported animals, rederivation of pathogen-carrying strains, and monitoring of the health status of animal research colonies.

LASP also provides Scientific Support Programs (cores) to NCI investigators:

- Mouse Modeling & Cryopreservation Core: Facilitates the production of genetically engineered mouse models (GEMM) via pronuclear microinjection and gene targeting in embryonic stem cells. Mouse Modeling & Cryopreservation also provides a Cryopreservation Laboratory to freeze germplasm of unique and valuable GEMM strains, thereby guarding against their loss due to disease outbreak or other genetic or environmental factors.
- Molecular Histopathology Laboratory: A comprehensive veterinary pathology and molecular histopathology service that focuses on the phenotypic characterization of animal disease models. The Molecular Histopathology Laboratory capabilities include immunohistochemistry, in situ hybridization, laser-capture microdissection, blood chemistry analysis, and digital whole-slide image capture and analysis.
- Small Animal Imaging Program: Offers state-of-the-art animal imaging facilities (MRI and magnetic resonance spectroscopy imaging, multi-modality positron emission tomography with X-ray computed tomography and MRI, ultrasound, and optical imaging) for real-time in vivo monitoring of tumor cells and metastases, and assessment of the effects of pharmacological interventions.
- Animal Diagnostics Laboratory: Employs molecular-based technologies for the detection of animal pathogens, the assessment of the genetic purity of animal-related reagents, and the genotyping of complex genetically engineered mouse strains. The Animal Diagnostics Laboratory also runs the Animal Health Diagnostic Lab component of LASP and the High-Throughput Animal Genotyping Laboratory, which provides a platform for large-scale genetic monitoring and management of complex, GEMM colonies.
- Animal Research Technology Support: Assists investigators with complex procedures and experiments in rodents by providing expertise in the development and implementation of specialized animal research protocols. The Animal Research Technology Support group also operates the Gnotobiotics Facility, a facility that generates and characterizes mice maintained in axenic (germ-free), gnotophoric (single-agent), or gnotobiotic (known multiple agents) conditions to better assess the role of the environment or infections on GEMM phenotypes or in treatment modalities in mouse models of cancer.
- Genome Modification Core: This new Center for Cancer Research (CCR)-funded core resource offers advanced expertise in nuclease-mediated mutagenesis and provides CCR researchers with validated tools to effectively apply and use nuclease methodologies to model and investigate human cancer in cells and animals.
- NCI Mouse Repository: A central Division of Cancer Biology resource that maintains and propagates mouse cancer models and distributes strains throughout the scientific community (academic, nonprofit, and commercial).

LASP also provides management oversight and technical expertise to the Center for Advanced Preclinical Research, a program initiative funded by the CCR that focuses on the generation of novel animal models of human cancer and the use of established GEMMs in the development and preclinical testing of cancer diagnostics and therapies. In addition, LASP has instituted several programs to train the next generation of animal research-based scientists, including several postdoctoral positions in core biotechnology, a one-year veterinary internship in laboratory animal medicine, and a one-year internship in veterinary pathology.

Biomedical Informatics and Data Science Directorate

Eric Stahlberg, Ph.D., Director

The Biomedical Informatics and Data Science (BIDS) Directorate works collaboratively and helps to fulfill the Frederick National Laboratory's mission in the areas of biomedical informatics and data science in the following ways to enable, advance, and accelerate disease research:

- Developing and applying world-leading data science and computing technologies to basic and applied biomedical research challenges
- Developing and delivering national data resources
- Employing leading-edge software, computational, and data science

ADVANCED BIOMEDICAL COMPUTATIONAL SCIENCE

Jack Collins, Ph.D., Director

The Advanced Biomedical Computational Science (ABCS) group supports scientific research at the Frederick National Laboratory, National Cancer Institute (NCI) at Frederick, NCI at Bethesda, National Institutes of Health (NIH), and other federal agencies. ABCS provides expertise, consultation, and collaborative research and project support in a broad range of scientific domains to NCI and NIH scientists and staff. Typical collaborative and support tasks and projects include:

- Bioinformatics
- Next-generation sequence analysis
- Clinical data analysis
- Data and computational science
- Advanced scientific computing
- Mathematical and statistical analyses
- Systems biology
- Data integration
- Database development and application
- Image analysis and machine learning
- Structural biology
- Biophysics
- Molecular modeling
- Computational chemistry
- Chemoinformatics
- Scientific programming and application support

This support is provided through dedicated cores, embedded technical and scientific staff, and a pool of scientists with a wide variety of domain expertise.

CBIIT TECHNICAL OPERATIONS SUPPORT GROUP

Braulio Cabral, Ph.D., Director

The Center for Biomedical Informatics and Information Technology (CBIIT) Technical Operations Support Group is involved in the operation and execution of the projects sponsored by NCI CBIIT, including the following areas:

- Acquisition and subcontracting
- Project management and oversight
- Deliverable review
- Intellectual property and licensing negotiation
- Financial management
- Coordination with other CBIIT and NCI programs

Primary activities include:

- Support for the definition of scope, time, and budget for information technology activities
- Development of biomedical informatics software products
- Development of other information technology products, including vocabularies/ontologies, common data elements, data standards, and data or other biomedical capabilities associated with information technology systems
- Acquisition of commercial information technology products
- Review and assessment of information technology resources from all subcontract activities and from such noncontract activities as designated by the contracting officer's technical representative

Coordination and participation in the National Cancer Informatics Program

BIOMEDICAL APPLICATIONS DEVELOPMENT CENTER

Brent Coffey, Director

The BIDS Biomedical Applications Development Center (BADC) is responsible for the development of enterprise-level artificially intelligent study-participant-assignment systems for use in precision medicine clinical trials. BADC provides software development, bioinformatics support, biomarker advisement, cloud-based development, artificial intelligence, and data analysis for NCI scientists and staff, with specific expertise in the following areas:

- Software engineering
- Cloud solutions
- Bioinformatics
- Artificial intelligence
- Expert systems
- Precision medicine study participant assignment
- Data science
- Data warehouse/data mart
- Biomarker selection
- Manuscript support
- Statistical support

STRATEGIC AND DATA SCIENCE INITIATIVES PROGRAM

Eric Stahlberg, Ph.D., Director

The Strategic and Data Science Initiatives Program aims to establish and extend collaborations in computing and data science to address cancer challenges and accelerate cancer research. The group's efforts include the following areas:

- Collaboratively developing and deploying capabilities to accelerate cancer research by applying increasing levels of high-performance computing
- Establishing, deploying, and supporting approaches to transform the use of large-scale data in cancer research—ranging from effective data management, to education, to cutting-edge data-driven artificial intelligence applications and technologies
- Establishing and developing a sustainable direction and path forward for broader NCI, academic, government, international, and private industry collaborations to advance the frontier for research and clinical application of predictive oncology

PROGRAM ADMINISTRATION AND OPERATIONS OFFICE

Bobbie Jo McCord, Director

The Program Administration and Operations Office provides support to BIDS to ensure sound business operations by managing budgets, contractual compliance, procurement, and other operational functions. The office serves as an operational hub for collaboration both within BIDS and across the Frederick National Laboratory. The office also facilitates and executes technical project management to support the complex portfolio of projects within BIDS.

CLINICAL GROUP

Barry L. Gause, M.D., Director

Clinical Research Directorate

Barry L. Gause, M.D., Director

The Clinical Research Directorate (CRD) brings programs at the clinical end of the translational spectrum under an umbrella that fosters interactions in areas of overlap and provides clinical supervision of such activities. The quality assurance programs provide the required autonomy and transparency of Good Manufacturing Practice quality assurance operations. CRD includes the Biospecimen Research Group, which provides scientific and technical program support for a number of NCI-sponsored basic and translational research projects, such as the Molecular Profiling to Predict Response to Treatment Program, the Clinical Proteomic Tumor Atlas Consortium, the Genomic Data Commons, and the Human Cancer Models Initiative. The Cancer Genomics Research Laboratory investigates how germline and somatic genetic variations contribute to cancer susceptibility. The Molecular Characterization Laboratory is a CLIA-certified laboratory that that provides histology, pathology, molecular, and genomic assay support for various projects and clinical studies. CRD's Drug Discovery and Development Program provides support to Therapeutics for Rare and Neglected Diseases /National Center for Advancing Translational Sciences and other directorates within the Frederick National Laboratory Clinical Group.

The objectives of the directorate are to (i) provide clinical research support for clinical trials, (ii) provide quality assurance for the production of vaccines and biological agents at the National Institutes of Health (NIH), (iii) develop standardized approaches to the acquisition of tumor samples, (iv) manage the collection and analysis of tumor and normal tissue on a molecular level, (v) advance the field of precision medicine by using next-generation sequencing to direct targeted agents in the treatment of malignancy, and (vi) support the development of agents for the treatment of rare and neglected diseases through the management of various projects. CRD provides operational support for clinical research and supports clinical trials management, regulation, pharmacovigilance, and protocol development and navigation. The directorate also provides comprehensive, dedicated clinical research professionals who provide patient care services and support to major clinical programs within NIH.

In addition, the directorate establishes quality systems at the Vaccine Clinical Materials Program (VCMP) Pilot Plant and the Biopharmaceutical Development Program (BDP) before the initiation of manufacturing, and it follows through on all regulatory aspects of production, including providing support for Investigational New Drugs. Detailed descriptions of quality assurance activities are presented under the sections for VCMP and BDP.

Clinical Monitoring Research Program Directorate

Beth Baseler, Director

The primary mission of the Clinical Monitoring Research Program Directorate (CMRPD) is to provide comprehensive, dedicated clinical research support to major programs within the National Cancer Institute (NCI); the National Institute of Allergy and Infectious Diseases (NIAID); the National Heart, Lung, and Blood Institute; the National Institute for Arthritis and Musculoskeletal and Skin Diseases; the National Institute of Mental Health; the National Institute of Neurological Disorders and Stroke; the National Human Genome Research Institute; and the National Institutes of Health (NIH) Clinical Center. To support the clinical research community's diverse research requirements, CMRPD provides an integrated range of quality services that are functionally organized within the Clinical Group. CMRPD represents a comprehensive resource for many of the intramural clinical research programs at NIH.

CMRPD provides high-quality clinical research support services to meet the expanding and emerging challenges faced by NIH researchers. CMRPD recognizes the numerous barriers to conducting clinical research both domestically and internationally. Successful completion of CMRPD's mission directly benefits the mission of NCI, NIAID, and other institutes, and it has contributed to improving the overall standards of public health globally. The repertoire of support services provided to clinical researchers throughout the world has expanded dramatically over the years, helping researchers provide the highest-quality clinical research that is compliant with applicable regulations and guidelines and maintaining data integrity, with the overall goal of protecting individuals participating in clinical research. CMRPD continues to support the goal of increasing international sites' capability to participate and partner in clinical research, and it has assisted in the critical development of clinical trial networks across the world.

CMRPD continues to provide regulatory, clinical trials management, pharmacovigilance, protocol development and navigation, and project/program management services to support domestic and international studies related to cancer; HIV; avian flu/severe human influenza; heart, lung, and blood diseases and conditions; parasitic diseases; rheumatic and inflammatory diseases; musculoskeletal and skin diseases; neurological diseases; and emerging and re-emerging infectious diseases such as Ebola and COVID-19.

Applied and Developmental Research Directorate

Michael W. Baseler, Ph.D., Director

The Applied and Developmental Research Directorate (ADRD) consists of two main program areas: the Clinical Services Program (CSP) and support to the National Cancer Institute (NCI) Division of Cancer Treatment and Diagnosis (DCTD). In FY2020, CSP laboratories supported over 150 NCI and National Institute of Allergy and Infectious Diseases (NIAID) clinical trials, as well as trials from additional institutes. Clinical trial support included processing and cryopreserving clinical materials; database tracking of clinical samples received; performing sequential studies of immune function in patients with cancer, AIDS, other infectious diseases, chronic granulomatous disease, and other diseases associated with immune deficiency or autoimmunity; testing viral burden; identifying viral quasi-species; and determining viral mutations associated with drug resistance. These efforts include the evaluation of new technologies and the development of new assays that can be used to monitor patients during therapy. Seven program laboratories performed high-complexity testing under the auspices of the Clinical Laboratory Improvement Amendments (CLIA), with test results used to aid in patient diagnosis or treatment decisions.

Laboratories within CSP provide dedicated support to the clinical trials programs at NCI, including the Division of Cancer Epidemiology and Genetics, the Center for Cancer Research, and the Division of Cancer Prevention, as well as NIAID. Support also extends to preclinical and translational research. Several program laboratories provide shared services that can be accessed by other institutes within the National Institutes of Health through the NCI at Frederick Accessioning System. CSP laboratories have also provided support to other government agencies through the Economy Act. ADRD also directs the NCI at Frederick Repository and provides oversight to the American Type Culture Collection subcontractor operating the Repository.

Biopharmaceutical Development Program Directorate

George Mitra, Ph.D., Technical Director/Program Director

KEY ACCOMPLISHMENTS

- The key accomplishment in this period was establishing cell therapy expertise.
- Within 12 months, the technology transfer of the Department of Transfusion Medicine's Prodigy production platform was successfully completed and an Investigational New Drug application for CD33 CAR T-cell product was submitted and approved.

The Biopharmaceutical Development Program (BDP), formerly the Monoclonal Antibody Recombinant Production Program, was established in 1993 to provide dedicated services to the Biological Resources Branch (BRB); Developmental Therapeutics Program (DTP); and the National Cancer Institute (NCI)'s Division of Cancer Treatment and Diagnosis (DCTD), as well as to provide support to intramural and extramural National Institutes of Health investigators, government agencies, and independent parties through interagency agreements or cooperative research and development agreements. BDP continues to take on new challenges in support of the BRB, DTP, and DCTD.

BDP provides leading-edge development of monoclonal antibodies, recombinant proteins, peptide and DNA vaccines, virus vaccines and oncolytic viruses, gene therapy products, and other biological and immunomodulating agents. BDP maintains biopharmaceutical production and testing facilities that are compliant with relevant current Good Manufacturing Practices. BDP provides complete support, from manufacturing feasibility through process development and clinical manufacturing, with all required regulatory documentation. With a staff of 54 highly trained and experienced personnel, BDP's facilities are designed to be flexible, which enables staff to work on multiple projects for a variety of different therapies. BDP concentrates on products that are in early development, beginning with the demonstration of product feasibility on the bench through the production and biomolecular characterization of Phase I/II clinical supplies.

During this period, BDP participated in the development and clinical production of the following class of biological entities: CAR T cells (GD33, GD2); monoclonal antibodies (Canine, Antinicotine); recombinant proteins (TTHX1114); gene therapy for rare and neglected diseases (Propionyl-CoA Carboxylase A); vaccine as virus-like particles (Epstein-Barr Virus-2); and gene vectors (lentivirus, retrovirus).

Vaccine Clinical Materials Program Directorate

David Lindsay, Ph.D., Director

The Vaccine Clinical Materials Program (VCMP) supports National Institute of Allergy and Infectious Diseases (NIAID)'s Vaccine Research Center (VRC) in advancing preclinical and clinical research, development, production, and supply of investigational phase biologics and/or vaccine candidates to address infectious diseases of global significance. The VCMP is responsible for the operation of a vaccine pilot-plant facility in Frederick, MD.

We handled procurement services for research and development; managed subcontracting of preclinical and Phase I–enabling studies with universities/academia and industry; oversaw the technology transfer of cell-culture/fermentation manufacturing processes and analytical methods from VRC's Vaccine Production Program to the Frederick, MD-based pilot plant, and qualification/validation of test methods; manufactured multiproduct drug substance and formulated, vialed drug product under Good Manufacturing Practice; performed all analytical release and stability testing; provided regulatory support content input (chemistry, manufacturing, and controls) for Investigational New Drug applications sponsored by the VRC; controlled temperature unit storage of starting materials, intermediates, and final product; and supplied a wide range of Phase I/II clinical materials (including placebos and adjuvants) to sites in the United States and worldwide on demand for planned and/or ongoing clinical trials.

The VCMP manufactures and supplies NIAID/VRC with kilogram quantities of anti-HIV broadly neutralizing monoclonal antibodies for evaluation in clinical trials conducted globally. Additionally, the directorate manufactures alum- and non-alum-based adjuvants. The VCMP also manufactures/supplies several investigational products using different platform technologies (e.g., recombinant glycoproteins, monoclonal antibodies, nanocage-based particles, peptide-conjugate vaccines, virus-like particles, and plasmid DNA) to tackle current or emerging/re-emerging diseases caused by Ebola virus, Equine Encephalitis virus, influenza, Malaria, respiratory syncytial virus, and Zika virus. The VCMP employs 149 full-time staff members.

OPERATIONS GROUP

Kathy Terlesky, Ph.D., Chief Operating Officer

Business Services Directorate

Richard Pendleton, Director

The Business Services Directorate provides operational, logistical, and administrative support across the contract in the areas of purchasing, travel, seminar speaker and event planning, and the provision of clean, sanitized laboratory glass from Central Glassware Services.

Contracts and Acquisitions Directorate

Beverly Hayes, Director

The Contracts and Acquisitions Directorate (C&A) is responsible for all contract administrative functions associated with proposals, prime contracts, and task orders awarded to the Frederick National Laboratory for Cancer Research (FNL); purchasing commercial goods and services; soliciting, negotiating, and awarding third-party research and construction subcontracts; identifying and protecting intellectual property and establishing strategic agreements; tracking all accountable government-furnished property; and logistics support necessary to support the FNL mission.

Enterprise Information Technology Directorate

Brett Smith, Director

The Enterprise Information Technology Directorate (EIT) provides supporting IT services to the Frederick National Laboratory for Cancer Research and the National Cancer Institute at Frederick scientific community. This support includes core infrastructure as well as conferencing audio/video, information security, web hosting, software solutions, end user support, and business systems. EIT data centers hold the backbone of networking, compute, and storage across the enterprise, and they provide the scientific community with high-performance computing and tiered-storage capabilities. EIT software solutions, custom and commercial off-the-shelf, provide the scientific community with web and logistical automation solutions, as well as the back-office backbone, the enterprise resource planning solutions. End user services include personal computing machines, desktop support, and help desk activities for handling service requests and resolving issues.

Environment, Health, and Safety Directorate

Terri S. Bray, Director

The Environment, Health, and Safety Directorate (EHS) is dedicated to ensuring a safe, healthful, secure, and environmentally friendly workplace for all employees of, and visitors to, the National Cancer Institute (NCI) at Frederick.

It is the policy of NCI at Frederick to create and maintain a secure, healthy, and safe workplace and to promote a healthy workforce as its most valuable and enduring resource. EHS provides occupational health services to NCI at Frederick employees, as well as treatment for occupational injury or illness sustained by NCI at Frederick employees.

EHS's goal is to maintain and develop programs that establish expectations and requirements to ensure full compliance with all applicable federal, state, and local occupational safety and environmental laws and regulations.

Facilities Maintenance and Engineering Directorate

Dante Tedaldi, Ph.D., P.E., Director

The Facilities Maintenance and Engineering Directorate (FME) provides in-house design, construction management, maintenance, and facilities management functions. An emphasis on interactive working relationships with scientists, directorate staff, and administrative management results in cost-effective solutions in response to the researchers' changing needs. Whether serving routine daily operations and maintenance duties or addressing emergency maintenance/repair services, FME's team of professional staff with diversified credentials remains committed to serving the evolving needs at the Frederick National Laboratory.

Financial Operations Directorate

Sue Bruce, Director

The Financial Operations Directorate (FOD) oversees all finance-related activities for the Frederick National Laboratory, including general accounting, payroll, accounts payable, billing, accounts receivable, financial planning and analysis, and core business information systems. The directorate's mission is to administer an enterprise-level, integrated financial management program to support the full mission of the Frederick National Laboratory; establish fiscal policies that ensure accountability for, and control of, government funds; provide timely, relevant, and clear financial analysis and reporting to assist in managing fiscal resources; and deliver accurate, timely, and complete financial information to Frederick National Laboratory stakeholders.

Human Resources Directorate

Christopher March, Director

The Human Resources Directorate (HR) works in partnership with managers and their teams to support the mission of staff as they work on behalf of the Frederick National Laboratory for Cancer Research.

HR fosters a positive work environment through leadership and guidance in the development, implementation, and equitable administration of policies and procedures. HR's core services and competencies include talent acquisition, organizational and employee development, compensation and benefits, HR information management, employee relations, and counseling and regulatory compliance.

Project Management Operations Office

Bernard Courtney, Ph.D., Director

The Project Management Operations Office (PMOO) integrates, standardizes, guides, oversees, and leads the development and implementation of project management practices across the FNL. The functional project management framework has evolved under continuous improvement to provide refined and beneficial processes and procedures. The PMOO has established sound project management principles adapted to FNL's mission, strengthened by its concierge service model and using project management tools, training, expertise, surge staff, and templates. The enhanced trust, communication, and transparency between directorates, members of the senior management team, and the government is a result of this discipline.

The In-Process Review (IPR), a key tool for project oversight, provides executive leadership visibility into more than 80 projects across our diverse customer base via regularly scheduled presentations. The IPR provides project managers a venue to describe and pursue targeted solutions to challenges and risks encountered, and brings senior management's attention to areas requiring escalated engagement. Though less than ideal for this purpose, the tool facilitates documentation, communication, and monitoring of Cost, Schedule, and Performance critical to the publishing of a quarterly contract deliverable. Additionally, a monthly President's Acclamation and Watch List synthesizes the IPR data, sponsor assessments, change management, impacts, risks, and issues and resolutions for Dr. Ethan Dmitrovsky's awareness.

Promoting a professional and nimble project management culture is demonstrated through Insite content, new employee orientation briefs, training, recognition, peer mentoring, and engagement opportunities. Certified Project Management Professionals are supported with regular presentations sponsored by Human Resources and endorsed by the PMOO and PMOO-sponsored Project Management Institute Chapter Dinners.

Public Affairs and Communications Office

Frank Blanchard, Director

The Public Affairs and Communications Office (PACO) supports the Frederick National Laboratory's public service mission through external communications and community relations, internal business communications, and creative multimedia services. The office informs key local, regional, and national audiences about the national laboratory's scientific accomplishments, opportunities for collaboration and partnership, shared national resources, and local community impact. The office also protects and enhances the national laboratory's identity and reputation.

APPENDIX B:
PUBLICATIONS

Frederick National Laboratory
for Cancer Research

sponsored by the National Cancer Institute

Leidos Biomedical Research, Inc.

2019-2020 Annual Report

Appendix B

Publications

OFFICE OF THE PRESIDENT

SCIENCE AND TECHNOLOGY GROUP

Cancer Research Technology Program

JOURNAL ARTICLES

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