Outcome Evaluation of FY2011 IMAT Awards

Background

The National Cancer Institute's (NCI) Innovative Molecular Analysis Technologies (IMAT) program was launched in 1998 on the notion that innovative technologies may radically accelerate progress in any field for which they are developed. The first solicitation was a broad call for the development of highly innovative cancer-relevant technologies. Since then, NCI Program Officers have periodically modified the structure of the IMAT program to meet the changing priorities of the NCI and the landscape of technology development, but primary motivations and goals for the program remain largely intact. In order to properly monitor the effectiveness of the IMAT program and maximize its utility for the continuum of cancer researchers, clinicians and ultimately patients, it is important to engage in an on-going evaluation of the IMAT portfolio and assess progress on the intended mission and goals of the program.

The outcome evaluation described in this document assesses the extent to which the IMAT program has been successful in making progress on these goals by focusing on a limited selection of supported projects from FY2011. Anticipating the need to periodically request approval for reissuance of IMAT program solicitations in the future, an evaluation strategy has been devised for assessing outcomes from the program for only the most recently completed awards to support each request without duplicating findings from prior evaluations. To support the current reissuance request, this document provides the assessment of outcomes from FY2011 IMAT grants for which applications were submitted in FY2010. This strategy for assessing the most recently completed awards was first pursued during the summer of 2013, leading to approval from both the NCI Scientific Program Leaders (SPL) committee as well as the NCI Board of Scientific Advisors (BSA) for reissuance of IMAT program solicitations. BSA members strongly suggested that future evaluations focus more directly on the scientific contributions from the program, rather than some of the indirect measures provided the previous year. The outcome evaluation described in this document represents a shift in strategy commensurate with this request.

In addition, a parallel effort to pursue a comprehensive evaluation of the full history of the program by a professional evaluation firm is also underway. The trans-divisional IMAT program team has been awarded NIH evaluation set-aside funds to pursue this comprehensive process and outcome evaluation, with anticipated completion in FY2016.

Program Goals

Mission: *To support the development, maturation, and dissemination of novel and potentially transformative next-generation technologies in support of basic, clinical, and epidemiological cancer research.*

The intended purpose of the IMAT program is to empower basic and translational research through targeted (and potentially disruptive) technology innovation. While the structure of the program has evolved, the goals remain largely unchanged:

- To catalyze innovative technology development for cancer research;
- To focus efforts from the technology-development community on cancer-related issues; and
- To accelerate the maturation of meritorious technologies from feasibility through development and into the hands of researchers and clinicians.

Program Details

The IMAT program currently utilizes an atypical R21 award mechanism and a standard R33 award mechanism to support highly innovation technology platforms and approaches. The IMAT program was most recently authorized to grant up to $10.5M (total costs) for new awards per year (resulting in roughly 30-40 awards, annually), where both the R21 and R33 awards may support up to 3 years of technology development research. To date, the program has issued more than 500 R21 and R33 awards.

The program is organized into two thematic areas supporting technology development.

1. *Innovative and emerging molecular and cellular analysis technologies*. These awards are designed to support highly-innovative molecular and/or cellular analysis technologies with significant potential for having a transformative impact in cancer research and/or clinic application.
2. *Innovative and emerging cancer-relevant biospecimen science technologies*. These awards focus on the development and application of novel and potentially transformative technologies to improve the quality and utility of biospecimens used in cancer research. Applications should offer novel capabilities to procure, process, and/or preserve human biospecimens and derivatives, or offer means to assess the biological integrity or quality of analytes for cancer research.

Awards for either theme support establishment of feasibility (R21) through validation (R33) of the technology for application in a basic, clinical, and/or epidemiological research settings. The program is managed by a trans-divisional team of program officers from across the extramural divisions of the NCI, and centrally coordinated by a program director from the NCI Office of the Director in the Center for Strategic Scientific Initiatives (CSSI). A list of participating program officers is included in Appendix A of this report.

Program Evaluation

Evaluation Strategy

The NCI has obtained funding from the NIH Evaluation Set-Aside program to pursue a comprehensive process and outcome evaluation of the IMAT program, with anticipated completion in FY2016. While program officers have consistently been able to identify examples

of success for the program, the full program has never been comprehensively evaluated to determine its unique contributions to the NCI's mission. The NCI has issued a contract to a professional evaluation organization to pursue this comprehensive process and outcome evaluation. The evaluation is based on a strategy developed specifically for the IMAT program through a 2007 Feasibility Study by Macro International (now ICF Macro) to assess the *unique* contributions, if any, from the IMAT program towards the successful development of technologies that have advanced cancer research or clinical care. While this comprehensive evaluation is underway, an evaluation of program outcomes of the most recently completed round of awards (applications submitted in FY2010 for awards issued in FY2011) was pursued to support the current reissuance request. The evaluation criteria used were those approved by both SPL and BSA in the last approval for reissuance of the IMAT solicitations in 2013 and include:

- number of publications that cite a specific IMAT award number;
- number of patent applications submitted to the US Patent & Trademark Office (USPTO);
- number of patent applications granted or approved by the USPTO based on patent applications that cite a specific IMAT award number in one of four government interest fields;
- number of IMAT-funded technologies now used in other NCI and NIH strategic initiatives; and
- follow-up case studies on previously funded technology development projects and platforms, including their current use by and utility to the extramural scientific and clinical communities.

The NCI IMAT program team collected some of the data necessary for the evaluation, with bibliometric and patent analysis produced by the Thomson Reuters – Research Analytics group.

Evaluation Scope

As indicated above, the evaluation of program outcomes supporting this reissuance request was limited to the most recently completed round of awards, which includes applications submitted in FY2010 for awards issued in FY2011. The IMAT program issued five individual Request For Applications (RFA) solicitations during FY2010, with three separate receipt dates, and two rounds of awards made in FY2011[1]. Consistent with the current request, solicitations were organized into the following two areas:

1. *Innovative and Applied Emerging Technologies for Cancer Biospecimen Sciences* focuses on the development and application of novel and potentially transformative technologies to improve the quality and utility of biospecimens used in cancer research. Applications should offer novel capabilities to procure, process, and/or preserve human biospecimens and derivatives, or offer means to assess the biological integrity or quality of analytes for cancer research. Also within scope is adapting downstream molecular analysis formats to the challenges presented by the quality and complexity of clinical samples.

[1] Due to administrative complications, the first two rounds of applications received were presented as a single funding plan to SPL.

- *BSP R21* [RFA-CA-10-001] – Innovative and Applied Emerging Technologies for Biospecimen Science
- *BSP R33* [RFA-CA-10-002] – Innovative and Applied Emerging Technologies for Biospecimen Science

2. *Innovative and Emerging Molecular Analysis Technologies for Cancer Research* emphasizes research projects on the inception, fabrication, and preliminary development of very early-stage, highly innovative and potentially transformative molecular and cellular analysis technologies for cancer. Awards support initial development through validation of the technology for application in a basic, clinical, and/or epidemiological research setting.
    - *EMT R21* [RFA-CA-10-003] – Early Stage Application of Transformative Emerging Technologies for Cancer Research
    - *EMT R33* [RFA-CA-10-004] – Advanced Stage Application and Validation of Transformative Emerging Technologies for Cancer Research
    - *IMT R21* [RFA-CA-10-005] – Innovative Technology Development for Cancer Research

It is important to note that R21 awards from RFA-CA-10-005 were permitted up to 3-years of support and up to $500k in direct costs, as opposed to use of the standard NIH R21 award for RFA-CA-10-001 and RFA-CA-10-003. This atypical award structure was developed in response to a recommendation made by the BSA during reauthorization of the IMAT program in June 2007. Applications and awards are summarized in Table 1 below for each cycle of all five RFAs. Appendix B provides a list of all 41 awards.

Receipt Date	IMT R21 RFA-CA10-005		EMT R21 RFA-CA10-003		EMT R33 RFA-CA10-004		BSP R21 RFA-CA10-001		BSP R33 RFA-CA10-002	
	Apps	**Awards**	**Apps**	**Awards**	**Apps**	**Awards**	**Apps**	**Awards**	**Apps**	**Awards**
Feb 2010	86	8	34	3	22	3	12	1	1	0
May 2010	55	5	25	1	9	2	4	1	7	1
Sept 2010	82	3	46	7	20	4	14	1	2	1
Total	**223**	**16**	**105**	**11**	**51**	**9**	**30**	**3**	**10**	**2**

Table 1. Summary of applications and awards associated with IMAT RFAs with FY2010 receipt dates

Evaluation Findings

Publications and Associated Bibliometrics

Tracking publications generated by funded research projects, and various associated bibliometrics, are a standard means of assessing outcomes for any research program. Provided in Table 2 below is a summary of the bibliometric analysis for the 41 projects supported through FY2010 IMAT RFAs. Publications were obtained through SPIRES and confirmed by principle investigators (PIs) associated with the grant award, with associated bibliometrics assembled by the Thomson Reuters evaluation team. Many research projects yield additional publications beyond the award closeout, however, and an additional six months (at a minimum) should be allowed for the accumulation of citation activity to assess the potential impact of any publications. Therefore, the findings summarized below should be considered as preliminary, rather than as final, bibliometric finding for these projects.

	2-yr R21 (16 projects)	3-yr R21 (14 projects)	R33 (11 projects)	Total (41 projects)
Total Publications Reported	44	30	75	149
Total Publications Indexed[2]	33	21	51	105
Average Publications (Maximum)	2.8 (8)	2.1 (7)	6.8 (10)	3.6 (10)
Average Total Citations (Maximum)	9.1 (24)	7.5 (29)	56 (118)	21.1 (118)
Median Impact Factor Quartile[3] (Minimum)	1 (1)	1 (1)	1 (1)	1 (1)

Table 2. Record of publications and associated bibliometrics for projects supported by IMAT awards initiated in FY2011.

Follow-up Research Support

The NIH IMPAC II database was screened using the Query View Report (QVR) search tool to find evidence of IMAT-funded technologies now used in other NCI and NIH strategic initiatives. Specifically, QVR was used to search for new NIH applications submitted by PIs associated with the 41 awards targeted in this evaluation to identify new project applications that included some significant use of the technology supported by IMAT. 53 new applications were identified that met these criteria, based on 19 (of 30) IMAT R21 projects and 9 (of 11) IMAT R33 projects. Of these 53 applications, 15 have been funded and 17 are still pending. 37% of these applications were for further development of the technology (20 of 53), with nearly half of those directed to

[2] These publications are indexed in Web of Science with citation data available.

[3] All journals in a Journal Subject Category (262 categories) are ranked and assigned a quartile according to their Journal Impact Factor within each journal subject category. Quartile 1 is the quartile with the higher journal impact factors.

one of the IMAT R33 solicitations (9 total) for follow-up support on successful R21-supported projects. The remaining applications were submitted to a broad variety of NIH FOAs, including 27 new applications for R01 support. Regardless of the solicitation focus and the review panel, summary statements (SS) from reviewed applications included some expression of enthusiasm specifically for the IMAT-supported technology platform in a significant majority of all applications (38 of 41 available summary statements). The tables provided below provide further breakdown of this analysis. A list of successful awards is included as Appendix C.

Table 3.Status of all new applications

Pending	17
Funded	15
Not funded	21
Total	53

Table 4. Follow-up applications to IMAT program

Pending R33	1
Funded R33	2
Not funded R33	6
Total	9

Table 5. Based on IMAT R21

Pending	12
Funded	9
Not funded	10
Total	31

Table 6. New R01 applications

Pending	12
Funded	5
Not funded	10
Total	27

Table 7. Based on IMAT R33

Pending	5
Funded	6
Not funded	11
Total	22

Table 8. New R21 applications

Pending	0
Funded	3
Not funded	1
Total	4

Table 9. IRG[4] expressed enthusiasm for IMAT-supported technology in review summary statement (SS)

Focus of FOA	Technology focused	Non-Tech focused
# Submitted Applications	20	33
Mentioned in SS*	20	18

*No mention of technology from review in SS for 3 applications, with remaining applications pending IRG

Table 10. Primary IC referral for application

NCI	31
Other ICs	22

NOTE: Many applications with referral to other institutes of NIH are still focused on cancer

Biomedical research funding support may obviously come from a number of sources beyond the NIH, and dialogue with the investigators associated the 41 FY2011 IMAT awards revealed a follow-up support from non-NIH funding sources specifically enabled by the technology

[4] Initial Review Group

development research supported by IMAT. These include awards from private industry (including pharmaceutical and biotechnology companies), private non-profit organizations, the Congressionally Directed Medical Research Program (CDMRP) and other Department of Defense related research programs.

Patents and Commercialization Efforts

Another method of assessing the appropriateness of the IMAT program for supporting innovative technology development is to monitor evidence that patent protection is being sought for the supported technology. Investing the time and resources necessary to seek patent protection suggests an unmet market need has been identified, and successful commercialization often (but not always) involves securing intellectual property rights through patents. The Thomson Reuters evaluation team queried the USPTO database for evidence of patent applications and awards stemming from the same group of FY2011 awardees. This information is provided in Table 11, along with evidence derived from technical progress reports evaluated by NCI program officers.

Supported Project Type	Patent Application	Patent Award	Licensure
R21	19	7	7
R33	15	2	5
Distinct Total	**34**	**9**	**12**

Table 11. Patent activity associated with FY2011 IMAT-awarded projects.

Discussion of Successfully Developed Technologies

In order to provide some context for the numbers and tables provided above, the following section provides a brief overview of particularly noteworthy technology development projects supported by the 41 awards that are the subject of this evaluation. The discussion is subdivided into the thematic areas of support described in the Background section. It should be noted that in addition to the noteworthy scientific achievements described throughout this document, IMAT awards contributed substantially to the interdisciplinary training of many postdoctoral and graduate researchers involved in all of the awards.

Innovative Molecular Analysis Technologies

As indicated in Table 1 above, 223 R21 applications were submitted to this solicitation over three rounds of receipt, with 16 selected for awards. Of these 16 awards, nine projects mostly or completely met their self-imposed milestones indicating that the project was successful. Of the seven that didn't meet their milestones, at least two led to incidental findings that have yielded yet more promising directions for the PI to pursue potentially significant new technologies that have received funding from NIH. Most of the PIs associated with the remaining five are still hopeful that they can overcome remaining hurdles, but likely will need to obtain additional funding to pursue these efforts. Three of these *innovative technology* projects were considered particularly noteworthy by the IMAT program team and are described below.

PI: **Jianghong Rao**
Institution: **Stanford University**
Project #: **R21CA138353**
Title: **Nanotechnology for multiplex detection of enzymes**

The goal of this project was to develop novel sensors for the multiplexed detection of protease activity, such as matrix metalloproteinase (MMP) and urokinase plasminogen activator (uPA) in biological samples of cancer patients. The sensor design was based on competitive quantum dot bioluminescence resonance energy transfer (cQD-BRET) and involved a novel nano-detector design in which the intensity of the fluorescence from the quantum dots increased in proportion to the activity of the protease. The strength of the approach was to offer a simpler, faster, and more sensitive assay for determining both concentrations and activity level of proteases.

Developmental complications led to the discovery by the PI of polymer nanoparticles which were more robust and had broader application potential than the original sensor design. cQD-BRET was replaced with a combination of chemiluminescent resonance energy transfer (CRET) and fluorescence energy transfer (FRET) based semiconducting polymer nanoparticles (CF-SPN) which were more sensitive, cheaper to make, and could be applied to a broader diversity of targets, including reactive oxidative species (ROS), reactive nitrogen species (RNS) and also for monitoring and enhancing drug activity. The PI has been using CF-SPN sensors for detection of cancer cells as well. He has submitted two new R01 applications for NIH support to use these as ROS sensors in tracking tumor response to radiation therapy and as one of a suite of imaging-based sensors to monitor the activity of specific oncogenes within a tumor and make predictions about oncogene addiction. The PI has also launched new collaborations beyond those for tracking the efficacy of novel therapeutics. He is working with the NCI Nanotechnology Characterization Laboratory to further develop these particles for testing therapeutic efficacy and liver toxicity testing (as described in a recent Nature Biotechnology article[5]).

The progress on this project has resulted in six accepted journal publications with the PI reporting another six having been submitted this year and pending review or acceptance. The PI has submitted three unique patent applications to secure intellectual property associated with the CF-SPN sensor design, with a report that they have been approached by several large companies to commercialize the platform. The technology facilitated data collections on a standing NCI-supported R01 project, and was central to a new R01 award from NIDDK[6] (R01DK099800). The PI further reports that they have received a great deal of interest from industry and academic scientists alike for the ROS sensing capabilities of CF-SPN.

PI: **Philip Santangelo**
Institution: **Georgia Institute of Technology**
Project #: **R21CA147922**
Title: **Characterizing gene regulation with single molecule sensitive probes**

The goal of this project was to adapt an innovative technology for imaging low copy number native mRNAs in living cells towards characterization of mRNA and RNA-binding protein interactions in cells with single interaction sensitivity. The project involved combining the PI's

[5] Shuhendler et al, Nature Biotechnology, Apr 2014, PMID 24658645
[6] National Institute of Diabetes and Digestive and Kidney Diseases

recently developed multiply-labeled tetravalent RNA imaging probes (MTRIPs) with antibody-based proximity ligation assays (PLAs), rolling circle amplification (RCA) and multicolor fluorescence microscopy to examine mRNA-protein interactions during the development and progression of prostate cancer, an approach collectively termed FMTRIP-PLA.

The PI was successful in developing the approach as proposed and using this method, detected and quantified the localization and frequency of interactions of the human respiratory syncytial virus (hRSV) nucleocapsid protein with viral genomic RNA, and with single-interaction sensitivity. Other demonstrations of the technology have also been published, including mRNA-cytoskeleton interactions and in characterizing changes in the translational potential of specific mRNAs. One particular advantage of using FMTRIP-PLA over traditional immunoprecipitation-based approaches is the relative ease with which competitive binding versus cooperative binding *in situ* can be accomplished.

The PI has reported two accepted journal publications plus another currently under review. A full patent application has been submitted regarding FMTRIP-PLA, and the PI has an R01 application currently pending review to track macromolecular complexes critical to the hRSV life cycle. The PI intends to submit a follow-up IMAT application for R33 support to further develop this technology this year.

PI: **Jay Shendure**
Institution: **University of Washington**
Project #: **R21CA160080**
Title: **Ultrasensitive identification and precise quantitation of low frequency somatic mutations by molecular counting**

The goal of this project was to develop a method for detection of rare, low-frequency somatic mutations through RCA of bar-coded primer-initiated templates in order to overcome detection limitations in sequencing PCR-amplified products. The approach involved a novel variation of RCA that utilizes gapped molecular inversion probes with unique sequences ("bar codes") to identify each molecule from which resulting amplicons are generated. Massively parallel sequencing of all products yields the sequence of each target and its associated bar code, where the bar codes allow for filtering of all deviant sequences. The approach thereby yields an extremely sensitive determination of different mutations in the target regions.

The PI was successful in developing the approach and demonstrated that somatic mutations present at a frequency of 1 mutant copy in a background of 100,000 normal copies can be detected while incurring at most 1 false positive detection event per ~1 kilobase of aggregate target, for each target nucleotide. The PI was able to demonstrate 100% reproducibility in replicate specimens. The approach is termed single-molecule Molecular Inversion Probes (smMIP), and beyond having extremely high sensitivity, it is fast, simple and parallelizable, and can be used with formalin fixed paraffin embedded samples. The PI has extended smMIP to tag circulating cells and circulating cell-free DNA, and also for applications beyond cancer-relevance; most notably in targeted sequencing studies for neuropsychiatric disorders.

This project yielded seven accepted publications with an assertion from the PI that many more are in preparation. The PI has been awarded a patent on elements of this approach with another

patent application for smMIP still under review, and has reported ongoing negotiations with at least three different companies for non-exclusive licensing of the software associated smMIP. This innovation contributed to the PI's successful application for a 2013 NIH Director's Pioneer Award (DP1). An application for follow-up R33 support from the IMAT program has been submitted and will be reviewed in November 2014.

Emerging Molecular Analysis Technologies

As indicated in Table 1 above, 156 applications were submitted to this solicitation over three rounds of receipt (105 R21s and 51 R33s), with 20 selected for awards (11 R21s and 9 R33s). 10 of the 11 R21 awards either mostly or completely met their self-imposed milestones indicating that the project was successful. All R33 projects were successfully completed, with broad variance in evidence of dissemination of the new capabilities. Eight of these *emerging technology* projects (three R21 and five R33) were considered particularly noteworthy by the IMAT program team and are described below.

PI: **Sarah Blair**
Institution: **University of California – San Diego**
Project #: **R21CA151140**
Title: **Non-circulating microparticles for improved localization and resection cancer**

The goal of this project was to develop a technique for the synthesis of silica-shell fluorocarbon particles and to validate in a rabbit model the use of encapsulated microbubbles for ultrasound contrast enhancement identifying surgical margins for small breast tumors identified during mammography. The approach takes advantage of the recent spread of ultrasound in surgical suites and could replace the stubbornly persistent and suboptimal practice of marking such pre-palpable tumors with either a simple barbed wire or with radioactive seeds. The PI proposed testing two stationary microparticle formulations, and associated toxicity studies.

The project team successfully met their proposed milestones, with the best results coming from a formulation that involved 500 nm Fe-doped silica shells. These nanoshells were demonstrated to retain their position and contrast capability for up to 10 days, with no adverse effects identified from systemic toxicity studies of these particles. The PI has succeeded in securing follow-up support with an IMAT R33 award (R33CA177449), and is currently engaged in early stage clinical trials with the nanoshells.

This progress has been documented in four accepted journal publications with two additional manuscripts currently under review. The PI has submitted a patent application to secure intellectual property rights, and has begun the preparation of an investigational new drug (IND) application for submission to the Food and Drug Administration.

PI: **Claudia Fischbach**
Institution: **Cornell University**
Project #: **R21CA157383**
Title: **Mineralized 3-D tumor models to study breast cancer bone metastasis**

The goal of this project was to develop and validate a mineralized 3-D tumor model to be used to study molecular mechanisms that promote breast cancer bone metastasis. The original project was cut to only a single year of support to pursue Aim 1 and parts of Aim 2, encompassing the development of the 3-D model itself with control of interactions between tumor cells and the scaffolding materials mimicking bone. The remaining aims were considered beyond the scope of the IMAT program as they involved use of the tool to explore hypotheses about breast cancer metastasis to bone.

The PI was successful in developing the model, which was documented in two accepted publications with a report from the PI that five more are in preparation. The PI received a new R01 (R01CA173083, Score - 10) based on the development of this model to study breast micro-calcifications and their role in breast cancer bone metastasis. She was also awarded a Humboldt fellowship based on this work. The PI reports that these studies have further led to several new collaborations with collaborators such as Dr. Cliff Hudis (MSKCC, Chief of Breast Medicine) and Dr. Peter Fratzl (Max Planck Institute for Colloids) for further development and application of the model.

PI: **Laurie L. Parker**
Institution: **Purdue University**
Project #: **R21CA160129**
Title: **Biosensor technology to monitor leukemia-related kinase activity in patient cells**

The goal of this project was to develop methods using novel nano-scale sensors for detecting kinase activity from intact cells with potential application as a diagnostic tool for clinical use. The key novelty of the proposed approach was the use of phosphorylated nanosensors that could be taken up by active cells to measure kinase activity within the context of a live cell. Sensors are interrogated by multiple-reaction monitoring (MRM)-based mass spectrometry which offers a high degree of sensitivity and dynamic range.

The PI met her proposed milestones and has secured follow-up support through an IMAT R33 (R33CA183671, Score – 10) to continue development and validation of this method. Proof-of-concept studies supported by the R21 award demonstrated the ability to detect Bcr-Abl activity and the percentage of phosphorylation (at femtomolar sensitivity) from cell lysate equivalent to the contents of ~15,000 cells with coefficients of variation within limits acceptable for clinical assays (16-40%). The PI has also optimized assay conditions for use in multi-well plates for high-throughput screening applications. The follow-up R33 award involves the use of additional sensors that have been functionalized for detection of tyrosine kinases other than Bcr-Abl leveraging work from another IMAT R21 (R21CA147993, PI: Benjamin Turk).

These successes have been documented in two accepted publications and two applications for patent protection. Beyond the follow-up R33 award, the PI has also been awarded a new R01 grant (R01CA182543, Score - 20) to develop a kinase-inhibitor screening assay, as well as a 2013 AB SCIEX Young Investigator Award based on this work. A former graduate student for the PI is pursuing a commercial venture based on this technology as well.

PI: **Jingfang Ju**

Institution: **State University of New York at Stonybrook**
Project #: **R33CA147966**
Title: **Identification of post-transcriptionally regulated targets by TrIP-Chip/Seq**

The goal of this project was to further develop and validate a method to measure actively translating mRNA levels affected by miR215 in colon cancer cell using a novel technology called translational immunoprecipitation-microarray analysis (TrIP-chip), initially developed under support from a prior IMAT R21 award. Specifically, the goal of the R33 award was to increase both the coverage and sensitivity to detecting rare transcripts potentially at the single cell level, for discovery of post-transcriptionally regulated RNA targets.

The PI has successfully completed the validation studies and has received an R01 award (R01CA155019) to use TrIP-chip to study the role of miR140 in colon cancer, with the reported intention of continuing to seek further R01 support for studying miRNA using TrIP-chip. While several other applications for R01 have been unsuccessful, the reviews nearly all contain expressions of enthusiasm for the TrIP-chip technology.

The progress in developing and demonstrating the capabilities of TrIP-chip has been documented in 24 accepted publications (12 under the prior R21 award and 12 under the R33 award). The PI reports a large number of new collaborations that were catalyzed by the early demonstration of TrIP-chip (largely captured in the referenced publications), with an assertion from the PI that he will be seeking patent protection and licensing to make this technology commercially available.

PI: **David Muddiman**
Institution: **North Carolina State University at Raleigh**
Project #: **R33CA147988**
Title: **Development and Application of Novel Glycan-Specific Reagents to Facilitate Early Detection of Epithelial Ovarian Cancer**

The goal of this project was the advanced development of novel tags to facilitate quantitative mass spectrometric analysis of N-linked glycans with improved limits-of-detection. The tags and methods were originally developed under support from a prior IMAT R21 award (R21CA134250) and would be applied to the experimental chicken model of spontaneous epithelial ovarian cancer for biomarker discovery related to the early detection of human epithelial ovarian cancer. Enthusiasm for this project was grounded in the persistent lack of adequate labeling approaches to screen glycans beyond selected systems.

The PI was successful in validating this technology which has generated a significant number of technological innovations for the analysis of *N*-glycans, allowing for advances in fundamental measurement science and ovarian cancer applications of *N*-glycan profiling. A library of hydrazide reagents was synthesized with the intention of increasing the electrospray ionization (ESI) efficiency of tagged *N*-linked glycans, thereby increasing ion abundance in a mass spectrometer and enhancing the limit of detection (LoD) for low abundance species. The technology is currently called Individuality Normalization when Labeling with Isotopic Glycan Hydrazide Tags (INLIGHT^{TM}). Reagents from the synthesized library were able to demonstrate a 2-10 fold (glycan and reagent dependent) enhancement in glycan signal with a >97% tagging efficiency and short (<4 hr) preparation time. During validation studies with collaborators at the Mayo Clinic, application of the INLIGHT-based approach with human plasma revealed

statistically significant variations in glycosylation patters, a small number of N-glycans appearing relevant as a diagnostic tool for ovarian cancer.[7]

Progress in developing and validating this technology has been documented in eight accepted publications (one in association with the R21 award and seven with the R33 award). The PI has also engaged a partnership with Cambridge Isotope Laboratories to disseminate a commercial INLIGHT™ kit and associated methods, with additional improvements continuing to accrue and become available through Cambridge Isotope Laboratories. The PI is collaborating on a new R01 supported project (R01GM112662, Score – 13) to develop a method for an improved front end interface for mass spectrometry-based proteomic and glycomic analyses, generally. The INLIGHT technology will be used for evaluating resulting glycans in this project.

PI: **Levi Garraway**
Institution: **Dana-Farber Cancer Institute**
Project #: **R33CA155554**
Title: **High-Throughput Tumor Genomic Profiling by Massively Parallel Sequencing**

The goal of this project was to develop a high-throughput sequencing pipeline for profiling of hundreds of known mutations across cancer genes in a large number of clinical specimens. Specifically, the PI wished to transform an MS-based screening assay (OncoMap) developed by the PI under a prior IMAT R21/R33 award) to an Illumina-based massively parallel sequencing microarray assay (MPS-OncoMap). The goals involved optimizing the methodology for sample barcoding technology, solution-phase exon capture, and single-molecule sequencing to enable robust mutation profiling (base mutations, amplifications, and deletions) across ~150 cancer genes in at least 12 tumor samples simultaneously.

The PI was successful in developing and validating the MPS-OncoMap approach and obtaining CLIA certification[8] for the assay, which is currently being used to profile patients at both Dana-Farber Cancer Institute and Brigham and Women's Hospital in Boston, MA. The PI anticipates as many as 7,000-8,000 patients will be profiled over the next two years with what is now called OncoPanel. The PI reports that OncoPanel is offered routinely to all eligible patients at both institutions with a reported 80-90% consent rate. The current panel includes 250 genes with 250X coverage, with the intention of incorporating an additional 24 genes during the summer of 2014. Limitations in either the breadth or depth of coverage is currently limited only by cost considerations and having a sufficient amount of sample, and is currently being run in a trial against whole exome sequencing to assess strengths and weaknesses by comparison.

Successful development has been documented in 11 accepted publications. The MPS-OncoMap technology is a significant component of several other projects supported by NCI, including a P50 award (project 3 of P50CA090381) as well as a current U01 (U01CA162148, Score – 11) to systematically characterize genetic traits of African Americans with prostate cancer.

PI: **Hsian-Rong Tseng**

[7] Manuscript under review with Mol. Cell. Proteomics
[8] Clinical Laboratory Improvements Amendments, a necessary certification for use of a laboratory developed test for diagnostic, treatment, health assessment or prevention purposes. http://www.cms.gov/clia/

Institution: **University of California - Los Angeles**
Project #: **R33CA157396**
Title: **Advanced Development of An Integrated CTC Enrichment Technology**

The goal of this project was to further develop a technology originally supported by a prior IMAT R21 (R21CA151159) called NanoVelcro, for the enrichment and isolation of circulating tumor cells (CTC). Briefly, the approach involves silicon nanopillars functionalized with aptamers for affinity to CTCs and built into a chamber that causes fluid mixing to maximize exposure of constituent cells to the fibers. High capture rates (99%) and viability of captured CTCs (84-91%) using NanoVelcro suggested the platform could be a superior tool to yield clinical material for subsequent molecular analyses that can be used to direct appropriate therapies for individual patients.

The PI successfully completed development and validation of the platform for enumerating CTCs with samples from more than 400 prostate cancer patients, with an incidental discovery made during the course of these studies of a neuroendocrine small cell phenotype among prostate cancer CTCs.[9] The current version of the NanoVelcro platforms utilizes very high resolution imaging for the very purpose of having better information for classifying cell types, so were able to make such an observation. Of great potential interest is preliminary work from the PI suggesting this particular small CTC phenotype may be better correlated with a more aggressive cancer condition. The NanoVelcro platform has served to launch a number of new collaborations, including with Cedars Sinai Medical Center Uro-Oncology program (P01CA098912-Project 1, Score – 15), the UCLA Pancreatic Cancer Program, and with the Beijing Genome Institute, where the platform demonstrated the highest sensitivity of those compared in a 400 patients screen. The PI suggests that, when paired with an Illumina HiSeq 2000 platform, one can obtain genome sequences of CTCs from patient samples within 24 hours. Based on this pipeline, the PI is pursuing establishment of an independent CLIA-certified laboratory. The PI is currently developing an assay panel for diagnosing non-small cell lung cancer patients from collected CTCs.

This progress has been documented in 18 accepted publications (eight from the R21 and 10 from the R33), with an assertion from the PI that three more manuscripts are pending publication and many more under preparation. The PI has submitted four applications for patent protection, the latest of which having to do with a diagnostic assay for the small cell phenotype CTC. A company was formed in 2010 called CytoLumina Technologies Corp. to commercialize NanoVelcro, which was awarded a SBIR phase 1 (R43CA180482) and currently seeking phase 2 (R44) support. The PI reports also having received grant support from the Department of Defense and the Prostate Cancer Foundation for applying the NanoVelcro platform towards new research aims.

PI: **David Beebe**
Institution: **University of Wisconsin - Madison**
Project #: **R33CA160344**
Title: **Integrated Micro Scale transcriptional profiling of cell communication networks**

[9] Manuscript under review

The goal of this project was to employ their Phase-Gate technology in creating a seamless nucleic acid purification and amplification capability directly in line with a co-culture platform to examine intercellular interactions in heterogeneous patient specimens. The approach involved streamlining extraction of target transcripts from the culture chamber, reverse transcription and amplification on a single microscale device, which could potentially be used with patient primary cells in a highly multiplexed manner.

The PI was successful in developing the platform and has called it Exclusion-based Sample Prep (ESP) technology. Conventional approaches targeting multiple analyte types require separate aliquots for each type, but ESP allows for targeting of multiple analytes from a single aliquot. The PI has used ESP to successfully identify resistance markers to first line therapies for multiple myeloma patients in which patient cancer cells were co-cultured with bone-marrow derived stroma. The PI reports substantial interest from the community for using ESP to screen weak interacting constituents, where this is particularly difficult to study with alternative approaches.

The successful development of this technology has been documented in four accepted publications and in four submitted patent applications. The PI has pursued commercialization of the platform through a small business venture called Salis Discovery, with term sheets for two partnerships now signed and a manufacturer engaged to make ESP products available before the end of 2014. The PI reports ongoing negotiations with cancer-focused diagnostic companies, with a suggestion that the platform is most competitive in a variety of companion diagnostic formulations. The PI is pursuing R01 support to continue developing and demonstrating the capabilities of ESP, as well as through other mechanisms of support.

Biospecimen Science Technologies

As indicated in Table 1 above, 40 applications were submitted to this solicitation over three rounds of receipt (30 R21s and 10 R33s), with 5 selected for awards (3 R21s and 2 R33s). All awarded projects successfully accomplished the proposed aims, with broad variance in evidence of dissemination of the new capabilities. Two of these *biospecimen science technology* projects (one R21 and one R33) were considered particularly noteworthy by the IMAT program team and are described below.

PI: **Curt Hagedorn**
Institution: **University of Arkansas for Medical Sciences**
Project #: **R21CA148068**
Title: **Sentinel Pol II RNAs for Measuring RNA Integrity in Biospecimens**

The goal of this project was to develop a method for measuring the integrity of mRNA in tissue samples by identifying “sentinel” mRNAs which are susceptible to degradation during normal handling of clinical and research samples. Enthusiasm for the proposal focused on the need for directly measuring mRNA, rather than the Agilent Bioanalyzer-based approach which measures ribosomal RNA as a surrogate. Specifically, the approach involved using an RNA isolation procedure based on the 5’ m7G rather than the poly A+ tail, then RNA-Seq analysis to identify and quantitate both protein coding and non-coding regulatory RNAs in biospecimens and

determine the entire length of each RNA, define 5'-3' pattern of degradation, then develop a qRT-PCR based assay of their 3' and 5' regions to measure their level of intactness.

The PI was successful in developing a system to identify several sentinel RNA, based on degradation from the 3' end, with a panel for liver completed and panels for breast and colon epithelium underway. While review concerns that the entire approach was not likely to become a widely disseminated tool for standard quality assessment of sample RNA continues to be justified, this work was important for providing awareness of these degradation patterns and the biases introduced when analyzing poly-A selected RNA. The PI's methods findings and methods were discussed at a recent meeting for The Cancer Genome Atlas initiative, for example. A more immediate impact is participation from the PI in a new 12-15 year cooperative study supported by the US Dept of Veterans Affairs involving polyp sample collection and analysis from 50,000 veterans patients, where the quality assessment principles developed under the IMAT R21 award were reported to be an important element for involving Dr. Hagedorn.

This progress has been documented in six accepted publications, and a patent application has been submitted. Data generated from these studies have been deposited in NCBI GEO. The PI has also received a new R21 award (R21CA176130, Score – 12) applying the methods developed under the IMAT R21.

PI: **Lance Liotta**
Institution: **George Mason University**
Project #: **R33CA157403**
Title: **Implementation of phosphoprotein preservation technology for cancer biospecimens**

The goal of this project was to develop and validate a novel tissue fixative called Biomarker and Histology Preservative (BHP) developed by the PI as a replacement for formalin fixation, especially for the ability to preserve phosphoproteins. A particular focus of the proposed approach was to introduce a product that could be seamlessly incorporated into standard pathology workflows and deliver a fixative that maintained or improved analysis of morphology and histology available through traditional formalin fixation. The goal of the project was one-step paraffin block stabilization of all classes of cellular phosphoproteins, diagnostic histomorphology, and diagnostic immunohistochemistry antigens, while at the same time maintaining full diagnostic morphology equivalent or superior to standard formalin fixation. The project team would collect fresh surgical tissue covering a broad variety of organs and cancer histology to develop an archive of 150 cases of matched paraffin and frozen specimens.

The PI successfully met the aims of the project and completed development and validation of a novel non-formalin one-step phosphoprotein preservation chemistry which was characterized with a wide variety of human and animal tissues, and independently validated by pathologists. BHP has been demonstrated to stabilize phosphoproteins, immunohistochemical antigens, glycoproteins, and nucleic acids and decalcifies bone. BHP was also shown to render diagnostic cellular histomorphology at least equivalent to formalin. The PI has responded to interest from the College of American Pathologists to begin developing policies and appropriate guidelines for using BHP. Several clinical trials have incorporated use of BHP, including two breast cancer

trials (NSABP and GSK)[10] and a multiple myeloma trial (Walker Foundation and Virginia Oncology Services).

Progress from this project has been documented in 10 publications, with an additional three pending publication. One patent has been awarded, with an additional four applications submitted. The technology has been licensed to Theranostics Health, Inc and Grace BioLabs has been sublicensed to manufacture and distribute the fixative. The PI has received funds from the CDMRP Breast Cancer Research Program, the Komen Foundation, and the Walker Foundation involving the use of BHP. The PI intends to use BHP as a component of future research applications seeking NIH support.

[10] National Surgical Adjuvant Breast and Bowel Project and Glaxo-Smith Kline

Appendix A. NCI IMAT Program Team

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NOTE: Significant efforts on behalf of the NCI Division of Extramural Activities, are also critical for success of this program. Efforts from this branch have been particularly significant from Drs. Thomas Vollberg, Jeffrey DeClue, and Donald Coppock.

Appendix B. FY2011 Awards associated with IMAT RFAs receiving applications during FY2010

IMT R21 Awards

Project #	PI Name(s)	Project Title	Institution
R21CA128692	CLARY, BRYAN M	*In vivo* selection of tumor-specific RNA binding motifs	DUKE UNIVERSITY
R21CA138333	MO, YIN-YUAN	Methods of systematic microRNA target validation and identification	SOUTHERN ILLINOIS UNIV
R21CA138353	RAO, JIANGHONG	Nanotechnology for multiplex detection of enzymes	STANFORD UNIVERSITY
R21CA140036	DECAPRIO, ANTHONY BERMUDEZ, HARRY ROTELLO, VINCENT	Platform for high-throughput analysis of protein adducts for carcinogen exposure	FLORIDA INTERNATIONAL UNIVERSITY
R21CA140080	STRAUSS, STEVEN H. BUCHANAN, JANICE	Nanocaged metal tags in massively multiplexed leukemia bioassay and beyond	COLORADO STATE UNIVERSITY
R21CA143362	MESSMER, BRADLEY	Molecular evolution of multifunctional DNA nanoparticles	UC SAN DIEGO
R21CA143408	HUANG, SONGPING	Prussian Blue nanoparticles as cellular T1 MRI contrast agents	KENT STATE UNIVERSITY
R21CA147922	SANTANGELO, PHILIP	Characterizing gene regulation with single molecule sensitive probes	GEORGIA INSTITUTE OF TECHNOLOGY
R21CA155424	LAVIE, ARNON KAY, BRIAN KENNETH	Enzyme-delivery scaffold technology for targeted cancer killing.	UNIVERSITY OF ILLINOIS AT CHICAGO
R21CA155472	LAI, JONATHAN	Methods to identify high-affinity antibodies that target tumor-associated glycans	ALBERT EINSTEIN COL OF MEDICINE
R21CA155479	MCDONALD, JOHN F	Use of nanogels to target delivery of siRNA to cancer cells in mice	GEORGIA INSTITUTE OF TECHNOLOGY
R21CA157366	LEVY, MATTHEW	Targeting cancer cells with functionalized nanoparticle libraries	ALBERT EINSTEIN COL OF MEDICINE
R21CA157395	PARKER, LAURIE L.	Label-free, real-time detection of kinase activity *in vitro* and in single cells using surface-enhanced Raman spectroscopy	PURDUE UNIVERSITY
R21CA157417	ZEICHNER, STEVEN L	Development of an *in vivo* screening technology for cancer vaccine immunogens	CHILDREN'S RESEARCH INSTITUTE
R21CA160052	LAWRENCE, DAVID	Holistic diagnostics of host during development of cancer	WADSWORTH CENTER
R21CA160080	SHENDURE, JAY ASHOK	Ultrasensitive identification and precise quantitation of low frequency somatic mutations by molecular counting	UNIVERSITY OF WASHINGTON

EMT R21 Awards

Project #	PI Name(s)	Project Title	Institution
R21CA151140	BLAIR, SARAH L	Non-circulating microparticles for improved localization and resection cancer	UC SAN DIEGO
R21CA151164	MAKRIGIORGOS, G. M	High-throughput technology that enables sequencing depth for colorectal CA	DANA-FARBER CANCER INST

R21CA154958	ALEXANDRAKIS, GEORGIOS	Scanning correlation microscopy methods for quantifying DNA repair kinetics	UNIVERSITY OF TEXAS ARLINGTON
R21CA155535	EVANS, CONOR LEE	Hyperspectral and structural microscopy platform for therapy of resistant cancer	MASSACHUSETTS GENERAL HOSPITAL
R21CA155536	YAO, XUDONG	Ultra-throughput multiple reaction monitoring mass spectrometry for large-scale cancer biomarker validation	UNIVERSITY OF CONNECTICUT
R21CA155568	BIEBERICH, CHARLES	Application of an innovative technology to develop low toxicity kinase inhibitors	UNIVERSITY OF MARYLAND
R21CA155572	LI, DEYU CHEN, JIN WEBB, DONNA J	VEC3-valve enabled cell co-culture platforms for cancer biology study	VANDERBILT UNIVERSITY
R21CA155615	HARISMENDY, OLIVIER	Identification of somatic mutations in rare subclones of solid tumors	UC SAN DIEGO
R21CA157383	FISCHBACH, CLAUDIA ESTROFF, LARA A	Mineralized 3-D tumor models to study breast cancer bone metastasis	CORNELL UNIVERSITY
R21CA160129	PARKER, LAURIE L.	Biosensor technology to monitor leukemia-related kinase activity in patient cells	PURDUE UNIVERSITY
R21CA160157	KOPELMAN, RAOUL	Magnetorotation: a rapid Assay for single cell drug sensitivity of cancer cells	UNIVERSITY OF MICHIGAN

EMT R33 Awards

Project #	PI Name(s)	Project Title	Institution
R33CA147966	JU, JINGFANG	Identification of post-transcriptionally regulated targets by TrIP-Chip/Seq	SUNY STONY BROOK
R33CA147988	MUDDIMAN, DAVID C. COMINS, DANIEL L HAWKRIDGE, ADAM PETITTE, JAMES N	Development and application of novel glycan-specific reagents to facilitate early detection of epithelial ovarian cancer	NORTH CAROLINA STATE UNIVERSITY
R33CA151210	GREIS, KENNETH DONALD	Validation of MALDI-MS-based inhibitor screening technologies for cancer targets	UNIVERSITY OF CINCINNATI
R33CA155252	TANG, KEQI	Mass spectrometry based assays for high throughput and quantitative biomarker validation	BATTELLE PACIFIC NORTHWEST LABORATORIES
R33CA155554	GARRAWAY, LEVI MACCONAILL, LAURA	High-throughput tumor genomic profiling by massively parallel sequencing	DANA-FARBER CANCER INST
R33CA155586	PORTER, MARC MULVIHILL, SEAN J	Advanced development of a multiplexed SERS-based biomarker detection platform: A multiplexed panel approach to early stage cancer diagnosis	UNIVERSITY OF UTAH
R33CA155618	SUPERFINE, RICHARD	Array microscope assay for cancer cell mechanics	UNC - CHAPEL HILL
R33CA157396	TSENG, HSIAN-RONG	Advanced development of an integrated CTC enrichment technology	UC LOS ANGELES
R33CA160344	BEEBE, DAVID ALARID, ELAINE T	Integrated micro-scale transcriptional profiling of cell communication networks	UNIVERSITY OF WISCONSIN-MADISON

BSP R21 Awards

Project #	PI Name(s)	Project Title	Institution
R21CA148068	HAGEDORN, CURT H.	Sentinel Pol-II RNAs for measuring RNA integrity in biospecimens	UNIVERSITY OF UTAH
R21CA155478	HRUDKA, BRIAN	Development of a system (devices and protocols) to improve biospecimen preservation and shipping	BIOSPECIMEN PROCUREMENT SOLUTIONS, INC.
R21CA155543	GULLEY, MARGARET L	Enhanced formalin fixation to improve tests on solid tissues	UNC - CHAPEL HILL

BSP R33 Awards

Project #	PI Name(s)	Project Title	Institution
R33CA157403	LIOTTA, LANCE ALLEN	Implementation of phosphoprotein preservation technology for cancer biospecimens	GEORGE MASON UNIVERSITY
R33CA160138	THOMAS, NANCY E DORSEY, KATHLEEN	High-throughput DNA-methylation profiling from fixed melanocytic tissues	UNC - CHAPEL HILL

Appendix C. Successful follow-up awards from NIH

Further Technology Development

Project #	PI Name(s)	Project Title	Score
R01DK099800	RAO, JIANGHONG	Nanoprobes for imaging RONS and drug-induced hepatotoxicity	25
R21CA182330	LEVY, MATTHEW	Lectimers: Glycan-anchored scaffold libraries for targeting carbohydrate-binding	20
R21EB016925	PORTER, MARC D	Real-time internal calibration for multiplexed microarray analysis	20
R33CA177449	BLAIR, SARAH L	Non-circulating microparticles for improved localization and resection of cancer	27
R41CA180389	MAKRIGIORGOS, G. M.	Temperature-Tolerant COLD-PCR enables mutation-enriched targeted re-sequencing	25
R33CA183671	PARKER, LAURIE L.	Multiplexed kinase biosensor technology to detect leukemia signaling with mass spectrometry	10

Application of Technology

Project #	PI Name(s)	Project Title	Score
DP1HG007811	SHENDURE, JAY ASHOK	Interpreting genetic variants of uncertain significance	12
R01CA155019	JU, JINGFANG	Molecular mechanism of miR-140 in colon cancer	24
P50CA090381	GARRAWAY, LEVI ALEXANDER	Genomic determinants of resistance to primary androgen deprivation therapy and aggressive disease	13
U01CA162148	GARRAWAY, LEVI ALEXANDER	Systematic genetic characterization of African American prostate cancer	24
R01AI111495	PORTER, MARC D	Surface-enhanced Raman spectroscopy immunoassay for detection of category A pathogens	29
P01CA168585	TSENG, HSIAN-RONG	Microfluidic diagnostics for monitoring of BRAF inhibitor resistance in melanoma	17
P01CA098912	CHUNG, LELAND	Prostate cancer bone metastasis biology and targeting	15
R01CA173083	FISCHBACH, CLAUDIA	Breast micro-calcifications and their role in breast cancer bone metastasis	10
R01CA182543	PARKER, LAURIE L.	Biosensor assay to screen for signaling pathway inhibition in cancer	20
R21CA176130	HAGEDORN, CURT H.	Molecular phenotype of polyps in serrated polyposis syndrome	12