

12th IMAT PI Meeting Report
January 6, 2012

**Innovative Molecular Analysis Technologies Program
12th Principal Investigators Meeting
Summary Report**

November 14-15, 2011

Bethesda, MD

Office of Biorepositories and Biospecimen Research
Center for Strategic Scientific Initiatives
National Institutes of Health
U.S. Department of Health and Human Services

INTRODUCTION

In 1998, the National Cancer Institute (NCI) established the groundwork for a highly successful program focused on innovative technology development to meet the specific needs of cancer researchers and clinicians by stimulating the next wave of technologies capable of being applied toward the field of cancer research. Unlike other initiatives of the time, the Innovative Molecular Analysis Technologies (IMAT) program solicited only the most cutting-edge ideas, thus restricting its application pool to those projects that had the potential to be truly transformative. By doing so, the program filled a void that no other program at NCI or the larger National Institutes of Health (NIH) filled.

Taking risks on early stage potentially transformative technologies, IMAT has contributed to many block-buster technologies that are now on the market and in almost ubiquitous use across the cancer research and clinical care continuum. Successfully developed and commercialized products such as RNALater, Affymetrix gene chips, Illumina bead platforms, quantum dot labeling, and ICAT technology were all considered high-risk ideas at the time of their initial funding through the IMAT program. Yet, their widespread use and applicability to multiple clinical and basic sciences research settings are a testament to the high pay-off and impact that such transformative technologies have provided to the field of cancer research. By soliciting and supporting these otherwise risky technologies during their earliest stages of development, NCI (through the IMAT program) has supported not only the development of these new transformative technologies in and of themselves but also supported them in a manner consistent with providing researchers and the individual investigator community rapid access to such platforms through appropriate commercialization and dissemination. NCI has thus taken risks to substantiate the ultimate value and utility of such technologies in cases where even venture capital (VC) firms have been reluctant to do so due to the inherent risks associated with innovative technology development.

Currently, there are new challenges facing cancer researchers and clinicians and, as such, the need for a sustained technology development pipeline encompassing inception and initiation (i.e. the 'bright idea' stage) through dissemination and commercialization has never been greater. Challenges represented by the need to rapidly assess all of the epigenetic changes in single cells, directly measuring microenvironment impact on cancer metastasis, collecting rare cells from the blood of patients with recurrent disease require *creative thinking* and *risk-taking* to enable research in a manner similar to the way that gene expression profiling is currently enabled. IMAT seeks to fill this void by:

- 1) empowering individual investigators across the whole of the research community and especially those researchers not traditionally engaged in cancer research to think creatively,
- 2) stimulating technologists and engineers from multiple fields to partner with biologists and clinicians who face similar or common technical challenges, and
- 3) taking the risks needed to overcome or break through the most common and pressing technical barriers that currently impede progress, effective research, and clinical decision making

By accomplishing these goals, the IMAT program seeks to stimulate progress in the field of cancer research at a pace that is *revolutionary* rather than evolutionary and to ensure the adequate, fair, and equal dissemination of knowledge that stems from such an approach.

The intent of the annual IMAT Principal Investigators (PI) meeting – this being the 12th PI meeting – is to provide the opportunity for participants in this program to share their ideas and progress

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in an open forum with investigators representing a broad range of scientific expertise. In addition, NCI program staff used the meeting to share information about complementary NCI programs and NCI research resources as well as to solicit ideas on how NCI can accelerate progress in developing new molecular analysis technologies for cancer research. In accordance with guidelines in the grant, all PIs were expected to personally participate in the meeting and to submit an abstract for a poster presentation.

MONDAY NOVEMBER 14, 2011

Welcome and Program Update

Carolyn Compton, M.D., Ph.D.

Acting Director, IMAT

Director, Office of Biorepositories and Biospecimen Research, NCI/NIH

After welcoming everyone to NCI's 12th Annual IMAT Principal Investigator Meeting, Dr. Compton began her talking by informing the audience that the IMAT program had been renewed following a presentation to the NCI Board of Scientific Advisors by new program coordinator Tony Dickherber, and that the Board had recommended a significant budget increase for the program. She noted that IMAT hopes to announce receipt dates for new applications and resubmissions in early 2012, and that Dr. Dickherber will be playing a more active role in helping IMAT-funded investigators focus on developing applications that will meet NCI's evolving agenda. She reminded the audience that IMAT's purpose is to create new solutions for problems in translational research and ultimately in the clinic.

Dr. Compton also said that NCI's new Provocative Questions (PQ) Program, started by the Institute's Director, is focusing much of NCI's research effort on filling in the gaps in fundamental knowledge that could produce significant breakthroughs in our understanding of cancer and how we diagnose, treat, and prevent cancer*. Many of these gaps in knowledge are related to a lack of tools to explore various aspects of cancer, and so the IMAT program has the potential to make a major contribution to the PQ Program. She also remarked that NCI recognizes the enormous contributions that IMAT has already made to the field and firmly believes that the program's efforts will play a critical role in meeting NCI's goals.

To illustrate IMAT's role in the PQ Questions Program, Dr. Compton reviewed a few of these questions and showed how they actually solicit technology development. For example, PQ 9 (out of 24) asks, "Can we identify mutations most critical to maintenance of oncogenic phenotype?" To answer this question, the research community needs new tools to elucidate the relationship between individual low-frequency mutations and pathway-related mutations and tumor development as well as tools to track the order of mutation development and corresponding metastatic potential in models. PQ 11 asks, "How do changes in RNA processing contribute to tumor development?" Tools to measure RNA regulation *en masse* and *in situ* and to monitor activity of protein products of targeted RNA transcripts are needed to answer this question.

Continuing on, Dr. Compton discussed PQ 14, which asks, "Are there properties of benign lesions that predict invasive or metastatic disease?" Opportunities in this area include the development of tools that allow for the more effective identification of key differences between cell types such as

* The complete list of Provocative Questions is available at <http://provocativequestions.nci.nih.gov/rfa>

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non-malignant and malignant cells in the same tissue, and for technologies that can characterize metastatic potential based on a multi-parameter screening assessment across larger volumes of tissue. She also described PQ 20, which asks, “Can biomarkers or signatures be identified as predictors or surrogates of therapeutic efficacy for immunotherapy?” Answering this question requires new tools to facilitate the search for surrogate markers that predict or track immunotherapy efficacy.

Dr. Compton then listed four other technology development targets arising from the PQs:

- PQ 3. Are there ways to objectively ascertain exposure to cancer risk using modern measurement technologies?
- PQ17. Since current methods to assess potential cancer treatments are cumbersome, expensive, and often inaccurate, can we develop other methods to rapidly test interventions for cancer treatment or prevention?
- PQ18. Are there new technologies to inhibit traditionally "undruggable" target molecules, such as transcription factors, that are required for the oncogenic phenotype?
- PQ24. Given the difficulty of studying metastasis, can we develop new approaches, such as engineered tissue grafts, to investigate the biology of tumor spread?

She also noted other areas of scientific priority identified by NCI leadership for which technology development has yet to occur and for which IMAT investigators could perhaps apply their technologies. For example, technologies are needed to study the mechanisms of metastasis and cell migration in more detail and for probing the physics and mechanics of the cancer cell. Ultimately, all of the technologies developed by IMAT will be applied to the study or characterization of real biospecimens from real individuals with cancer, and so the need for technologies to assure that biospecimens are of sufficient quality for a given purpose. This is biospecimen science and it is the focus of the Office of Biorepositories and Biospecimen Science (OBBR) in which the IMAT program resides.

Dr. Compton also stressed the need for IMAT investigators to seek out opportunities for collaboration with other NCI programs beyond their formal IMAT grants. As a prime example, she discussed NCI’s Cancer Human Biobank (caHUB) Program, which is focused entirely on biospecimen science. The goal of caHUB, she explained, is to investigate the impact of the procedural and environmental variables on the quality of human biospecimens and data prior to downstream molecular analysis on various platforms in order to develop Standard Operating Procedures (SOPs) that will minimize or at least standardize preanalytical variables. The value proposition for IMAT investigators is that NCI will be able to collect fit-for-purpose biospecimens and data of established quality according to strict SOPs to support technology development and compile specimen sets with systematically embedded pre-analytical variation for assessment by various molecular analysis platforms to determine required biospecimen quality.

After describing the need to systematically study the effect of preanalytical variables on biospecimen quality and the approach that OBBR and caHUB has developed to conduct this systematic study, Dr. Compton noted three particular areas of biospecimen science that can benefit from technology development efforts. Tools are needed, she said, to identify what happens to known analytes as preanalytical factors are varied. New technologies that can identify molecular signatures associated with particular sample handling variables will certainly benefit biospecimen science, as will methods that can provide a quick readout of biospecimen quality, perhaps through the automated identification of specific molecular signatures that correlate with biospecimen quality. In summary, Dr. Compton said that caHUB needs tools that the IMAT community can develop. In closing, she quoted Arnold Beckman, the

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founder of Beckman Instruments, who said, “I have done more for science in general by making instruments available for thousands to use than what I could do in my own laboratory by myself.” She said that this can be a guiding principle for the IMAT community given its proven history and its focus on developing technologies that the broader cancer research community will be able to use in its efforts to better understand cancer and translate that understanding into the clinic for the benefit of patients.

TOPIC 1: SYSTEM ANALYSIS TOOLS

Moderator: J. Randy Knowlton, Ph.D.

Program Director, Structural Biology and Molecular Applications Branch
Division of Cancer Biology, NCI/NIH

Nonlinear Optical Imaging of Cellular Processes in the In Vivo Breast Cancer Environment

Patricia J. Keely, Ph.D.

University of Wisconsin at Madison

Dr. Keely started the scientific presentations by noting that the environment in which a tumor develops is a three-dimensional one and that this environment is surrounded by connective tissue in ways that are intimate and mechanical. This is relevant to patient outcome because dense breast tissue is linked to a four- to six-fold increased risk of developing breast carcinoma. A significant part of this density is related to the collagen surround the fat, ducts, and lobules in the breast. She noted, too, that dense breast tissue makes it difficult to see small tumors on radiographic images.

The goal of her study was to image deep into tissue *in vivo* to understand the cancer microenvironment with the aim of characterizing endogenous fluorophores, collagen and several metabolic markers in particular, and to image Rho signaling in a three-dimensional context. The approach that her team took was to use two nonlinear optical imaging techniques – multi-photon microscopy (MPM) and second harmonic generation microscopy (SGHM) – to conduct *in vitro* and *in vivo* cellular studies. Using this technique the researchers identified three different types of collagen structures that correlate with tumor development, and they showed that tumor cells invade out of the primary tumor along the radially aligned fibers that show up in MPM images during tumor development. They explored this phenomenon further using aligned collagen matrices that they developed and showed that tumor cells greatly prefer to migrate along collagen fibers arranged in parallel and will not cross fibers arranged perpendicularly. This suggested that the changes in collagen organization that the researchers were observing had real functional significance.

Working with 200 human archived biopsy specimens with 20 years of associated outcome data, the investigators were able to identify these collagen signatures in the human samples and show that this aligned collagen signature predicted the potential of a patient to relapse or die from breast cancer with a risk factor of about five-fold. This predictive signature, Dr. Keely noted, was independent of other risk factors commonly used as prognostic indicators. She and her colleagues plan to expand their study to see if they can identify patient subsets based on responses to specific types of therapies.

Dr. Keely then discussed her team’s use of these imaging techniques in combination with intravital microscopy to develop a more dynamic view of tumor development as opposed to the static view she had just described. She showed images of tumors in live mice in which collagen fibers were clearly visible and in which tumor cells were migrating along collagen fibers. The next step will be to record these images over time to study how these collagen signatures develop in three-dimensions.

Next, she discussed her team’s work on characterizing endogenous metabolic markers using the inherent autofluorescence of the metabolites FAD and NADH. Because these molecules are intimately

linked to metabolism, which itself is intimately linked to cancer, they may provide an endogenous marker relevant to the processes going on in the development of cancer. For example, their studies have shown that the optical signatures of these endogenous markers in mitochondria differ between normal and transformed cells. They found, too, that they could observe these same signatures in paraffin-embedded tissues and that they could easily distinguish tumor from normal epithelium in their unstained images even in the earliest stages of cancer. They also showed that they could see these signatures in a live animal using intravital microscopy.

The second aim of her project was to investigate the activation of Rho pathway as tumor cells engage the extracellular matrix, realign it, and migrate along it to move out of a tumor. These studies showed that regions of high Rho activity corresponded to regions where collagen was being actively remodeled rather than where collagen concentrations were the highest. Moreover, in live animals using intravital microscopy, the investigators were able to image areas of high Rho activity where cells were protruding from the tumor and beginning to migrate out along aligned collagen fibers.

Global Profiling of Tyrosine Kinases Deregulated in Cancer Using Peptide Microarrays
Benjamin E. Turk, Ph.D.

Yale University

Protein tyrosine kinases (PTKs) have emerged as major drug targets in cancer therapy, Dr. Turk noted in starting the morning's second presentation, with over a dozen protein kinase inhibitors approved for clinical use and another 150 in clinical trials. Among the 518 human protein kinases that are coded in the human genome, there are likely to be many more drugable targets, but he said that actual drug development efforts targeting kinases are focused on a relatively small number of well-studied kinases. To help develop a better understanding of the "untargeted cancer kinome," his group has been working on a technology using peptide microarrays to identify consensus phosphorylation sequences for the entire set of 85 human PTKs.

This approach uses positional scanning libraries that have a fixed phosphorylation sequence surrounded by random sequences that are scanned five residues upstream and four residues downstream of the phosphorylation site, which in this case is a tyrosine residue. Dr. Turk's team has developed an automated microarray approach to then analyze phosphorylation site specificity and determine those residues that are preferred or disfavored by a given kinase at each position and to do this analysis on a kinome scale. He described the method his team developed to analyze multiple kinases simultaneously, and showed how this method was used to study the 32 human non-receptor PTKs. The data from this study is now being analyzed using computational methods to see if the sequence data can be matched to data of known kinase targets.

In the meantime, his group is now using the data to create probes for PTK activity in cell lines or tumor samples as a means of identifying potential targets for drug development efforts. The challenge is that kinases are not constitutively active but rather they toggle back and forth between active and inactive states. As a result, while approaches such as expression profiling can identify which kinases are present in a cell, they do not identify which ones are active in the cell. Dr. Turk's group created kinase activity-based probes that only bind to the active conformation of a kinase. These probes contain photoaffinity labels that will covalently bind in the active site upon exposure to light and that can then be isolated for analysis via a biotin label. His group then was able to create specific peptide-based affinity labels that can target specific kinases in an activity-dependent manner. These activity-based probes, he said, can serve as tools for assessing the activity state of specific kinases *in vivo* and identify

those kinases that are most active in key processes involved in cellular transformation, tumor maintenance, and metastasis.

FRET-Based Biosensors to Monitor Redox in Cell Cycle Regulation

Vladimir Kolossov, Ph.D.

University of Illinois at Urbana-Champaign

Dr. Kolossov discussed his group's work developing intracellular FRET-based redox sensors capable of measuring physiologically relevant changes in the glutathione redox couple. Disruption of redox homeostasis, he noted, is a common feature of many diseases, including cancer. In particular, the glutathione redox couple is particularly germane to the regulation of cell transformation and the response of tumor cells to cytotoxic drugs and ionizing radiation. A more reduced intracellular environment, he added, is thought to facilitate proliferation and protect from apoptosis.

To better characterize the functioning of the glutathione redox couple in cells, his team created a FRET sensor that generates an intense signal when oxidized and that is quenched in a reversible manner as the sensor is reduced. Using this probe, his team demonstrated that a reduced intracellular redox environment is restored within minutes after removal of an acute oxidative insult, and that this recovery is controlled to a large degree by the glutathione redox couple. He and his collaborators also demonstrated that redox recovery is delayed significantly by inhibition of glutathione synthesis and by inactivation of glutathione recycling. Redox recovery is also delayed by a factor of two in transformed cells relative to isogenic non-tumorigenic cells when glutathione homeostasis is altered. These observations, he noted, suggest that this method can be used to examine specific pathways by which glutathione contributes to cellular homeostasis and that it can be used to monitor responses in live cells in real time.

Dr. Kolossov's team has also developed a FRET-based biosensor that can monitor glutathione potentials in the relatively high oxidative environments of the endoplasmic reticulum, Golgi, and in lysosomes. Using this probe, his team was able to monitor in real time the response of the endoplasmic reticulum to elevated levels of glutathione and to sequential treatment with exogenous oxidants or reductants. In closing, he noted that these sensors should provide new opportunities to study the mode of action of anticancer agents and to suggest novel therapeutic approaches to cancer based on altering internal redox conditions.

Functional Metabolomics and Metabolic Flux Analysis in Cancer

Adam Richardson, Ph.D.

Sanford-Burnham Medical Research Institute

Dr. Richardson ended this session with a recap of the work his group has done developing stable isotope approaches to quantifying metabolic flux measurements in mammalian cells and tumors. He noted that it is now widely accepted that cancer cell metabolism is an important area of study. He also commented that understanding about the expression of metabolic enzymes is not equal to understanding the metabolic fluxes going through an enzyme or through a particular pathway. He also noted that recent work has shown that metabolites can actually drive transformation and that metabolic enzymes are inherently drugable targets. From biological standpoint, it is also clear that metabolism is close to a cellular phenotype, and that the metabolome is therefore an important source of targets for treating cancer.

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His group has been working on the problem of cancer metabolism at three scales. The first scale is discovery metabolomics, which analyzes the full cellular metabolome to identify known and unknown metabolite abundances, as well as in normal and cancer cells. Metabolic characterization maps the core metabolic characteristics of a specific cell type, while hypothesis testing quantifies the absolute or relative activity of specific metabolic pathways in response to a cellular perturbation, such as with a small molecule drug or using RNA interference.

Using stable isotope methods, Dr. Richardson's group is mapping cellular metabolic activity in terms of metabolic fluxes, metabolite pool sizes, and transport and exchange rates. He reviewed the basics of stable isotope methods and how isotope ratios are used to track pathways, flux, and pool size, and then outlined the experimental workflow that he and his collaborators have developed, which relies on gas chromatography/mass spectrometry (GC/MS) as the analytical tool. He noted that a typical experiment requires about one million cells and that his group is working to reduce that number by a factor of 10. He showed an example of how this approach can quantify the forward and reverse activities of the TCA metabolic cycle using ¹³C-labeled glucose and glutamine. Experiments with melanoma cells and normal melanocytes showed how changing conditions from normal to anoxic conditions caused the TCA cycle to switch from the forward to the reverse direction in order to maintain the pool size of specific metabolites that was characteristic of either a normal or malignant cell.

Dr. Richardson then discussed his group's work studying serine biosynthesis, which recently published work from two other groups suggests provides an upstream signal involved in the development of breast cancer. Coincidentally, his group had been looking at the same pathway in a colon cancer cell line, and what this work showed was that glutamine is actively metabolized in glycolysis as well as the TCA cycle and that glutamine actually undergoes what appears to be reverse glycolysis. To map the metabolic pathways to serine, Dr. Richardson's team used both ¹³C- and ¹⁵N-labeled probes. He listed the various tracers that his group has developed and the pathways that they can be used to study, and he noted that his group is now working to develop methods for conducting these studies in both tumor xenografts *in vivo* and resected or cultured cells. His team is also developing a new *in silico* model of human cellular metabolism, one constrained by expression and metabolite data and that will therefore do a better job of predicting metabolic fluxes and active pathways than is possible using the available computational models of metabolism.

TOPIC 2: GENETIC SCREENING

Moderator: Lynn Sorbara, Ph.D.

Program Director, Cancer Biomarkers Research Group
Division of Cancer Prevention, NCI/NIH

Long-Range Massively Parallel Mate-Pair Sequencing of Breast Cancer Cell Lines

Aleksandar Milosavljevic, Ph.D.

Baylor College of Medicine

In this session's first talk, Dr. Milosavljevic discussed his team's work developing a method to improve cancer rearrangement detection using two massively parallel sequencing strategies. He reviewed the use of mate-pair sequencing to map structural aberrations but noted that the application of existing technologies to mapping structural aberrations in tumors is hard because they lack the combination of sensitivity and specificity needed to deal with the unique features of tumors. In particular, mapping chromosomal aberrations is hard because of the association of breakpoints with non-unique portions of the genome, that is, those with high copy repeats and low copy repeats.

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The solution, said Dr. Milosavljevic, is to jump over those repeats using long-range fosmid diTags of 36 kilobase pairs (Kbp). He reviewed the details of how fosmid diTags are used to map breakpoints and described the bioinformatic methods that his group developed to analyze the data and incorporate copy number into the analysis. He also noted that his team has conducted over 30 mapping experiments, some used to characterize new species, and is now collaborating with two other groups that have developed similar technologies to expand their mapping work.

For cancer-related studies, his team has worked with the MCF-7 and HCC1954 breast tumor cell lines. These studies involved pair-end sequencing of 1.5 million fosmid diTags and 5 million Illumina 5 Kbp pair-ends, both representing about 15-fold coverage of the genome, for each cell line, and then mapping the aberrant joins using the bioinformatics tools his group developed. His team then used a semi-automated process to design PCR primers across the predicted aberrant joins and then performed PCR amplification across the predicted joins in both cell lines and controls in order to discriminate somatic from germline variants. He showed the results of these experiments and noted that this approach allowed for the identification of much larger numbers of copy variation compared to standard array-based techniques. These studies detected and validated 91 somatic rearrangements in MCF7 cells and 25 in HCC1954 cells.

Other patterns emerged from these studies, said Dr. Milosavljevic. Clustered breakpoints – those that occurred within one to five megabase pairs of each other – coincided with high copy-number amplifications, and non-clustered breakpoints were enriched in low copy-number repeats. The latter are known markers of genomic instability in germline cells, and these data showed that the same holds true for malignant cells. Additional analysis showed that over 50 percent of known chimeric transcripts in the two cell lines could be explained by genomic DNA fusions detected by these paired-end methods.

The investigators also observed a large number of fusions in tumor suppressor genes rather than oncogenes, which is contrary to the current thinking that holds the opposite to be true. One such fusion involved the *SULF2* gene, which codes for a human sulfatase that modulates the activity of many growth factors and the interactions of cells with the extracellular matrix. Indeed, Dr. Milosavljevic's group showed that knocking down *SULF2* promotes the development of cancer phenotypes in three different cell lines. Other experiments found that fusions and truncations affected members of the BRCA1 complex. He noted in summary that these studies suggest that gene fusions and aberrations point to the role of DNA repair genes in the genetic predisposition to breast cancer. In addition, the discovery of gene fusions and truncations with dominant negative effects points to the possibility of developing therapeutic strategies based on synthetic lethality.

Enhanced Genetic Screening

Stephen J. Elledge, Ph.D.

Harvard Medical School

In his talk, Dr. Elledge described his group's work developing methods to increase the utility of genome-wide retroviral short hairpin (shRNA) libraries in loss-of-function genetic screening strategies. He reviewed how RNA interference (RNAi) has become a revolutionary biomedical research tool and the use of microRNA-based shRNAs developed in his laboratory as potent triggers of RNAi both *in vitro* and *in vivo*. However, creating the necessary high-potency single-copy shRNAs has proven to be challenging. Most research groups have used sophisticated prediction algorithms followed by trial-and-error experiments of multiple shRNAs, an approach that is both expensive and time-consuming.

To develop a more efficient approach, Dr. Elledge and his collaborators have developed a functional high-throughput strategy to assess the potency of every possible shRNA for a mammalian

gene transcript. The basic idea is to create a quantitative a RNAi reporter that produces an optical signal which varies depending on whether a given shRNA has no activity or if it is an intermediate or strong trigger of siRNA. These reporters are then synthesized in massively parallel fashion using array-based technologies and screened in a high-throughput manner. Dr. Elledge showed the results of several experiments that validated this approach, which he calls the Sensor Ping-Pong strategy – for identifying single shRNAs with potent siRNA triggering activity starting with pools of as many as 55,000 constructs. These experiments have demonstrated that the selected shRNAs work at the single copy level and that the Sensor Ping-Pong strategy identifies many potent shRNAs that are not predicted using computational methods. In fact, over 70 percent of the Sensor-identified shRNAs are not predicted, and conversely, most of the predicted shRNAs do not score in the Sensor assay. Dr. Elledge's group explored the influence of target secondary structure on potency and found that there was a good correlation between the two.

In a final set of experiments, Dr. Elledge's group demonstrated that highly efficient shRNAs predict highly efficient siRNAs. These experiments show the potential of using this approach to enhance the therapeutic uses of siRNA.

Development of Recombomice for Revealing the Roles of Exposure and Proliferation on Recombination In Vivo

Bevin Engelward, Sc.D.

Massachusetts Institute of Technology

Dr. Engelward's group is interested in revealing the genetic and environmental factors that promote homologous recombination in mammalian cells and to do that her team has developed an *in vivo* recombination reporter system in which cells that have undergone a homologous recombination event will fluoresce yellow. She reviewed the processes that cells use to repair double-stranded breaks DNA, including homologous recombination. However, too much homologous recombination, because of the large number of repeats in the human genome, can lead to deletions, insertions, and loss of heterozygosity. She also noted that heritable mutations in homologous recombination genes can increase the risk of cancer and that a number of environmental carcinogens increase the demand for homologous recombination and that in turn can result in deleterious sequence rearrangements.

Dr. Engelward then described the Recombomice that her team engineered to express a yellow fluorescent reporter when homologous recombination occurs. She discussed the use of ear tissue taken from these mice to demonstrate that this system works and then showed that they could detect rare recombination events in the pancreas of these mice.

Using these mice to study aging, Dr. Engelward and her collaborators showed that homologous recombination events increase in frequency as these mice age. She noted that this increase will raise the odds that over time the same cell will experience multiple mutations of the sort that raises the risk of a cell becoming malignant. To further explore how these multiple mutations arise, her team, working with collaborator Peter So, developed an *in situ* three-dimensional imaging system that can pinpoint the cells over the entire pancreas in which these homologous recombination events are occurring. In a set of experiments using this microscopy technique, the researchers were able to show that clonal expansion accounts for over 90 percent of the burden of recombinant cells.

In another set of experiments, Dr. Engelward's group studied alkylation-induced homologous recombination and found that exposure to this type of agent does, as expected, increase homologous recombination. However, these experiments revealed a flaw in their system which is that most of the cells in the pancreas are in the G1 phase of the cell cycle, but homologous recombination occurs in the S

phase. To increase the number of pancreatic cells in S phase, they fed their mice a diet rich in thyroid hormone triiodothyronine (T3), which stimulates cell growth and division. Using these mice, Dr. Engelward's team asked the question if cells undergoing cell division are more susceptible to the effects of environmental mutagens, and the answer is yes. From a biological perspective, these results point to the importance of not just looking at compounds in isolation as far as determining mutagenic potential, but also in combination with growth-inducing hormones. It also points to the need to consider that the time when humans experience the highest levels of cell division are in utero and in childhood.

Inflammation can also lead to cell proliferation, and Dr. Engelward's team used their Recombomice to study the effect of repeated bouts of inflammation in homologous recombination. These studies showed that there was in fact an increase in homologous recombination following three episodes of inflammation. These experiments, she noted, are among the first to demonstrate *in vivo* effects of inflammation on homologous recombination. Dr. Engelward finished her talk with a brief description of a second generation mouse that provides strong expression of homologous recombination in a greater range of tissues including liver, heart, kidney, brain, spleen, colon, intestine, and lungs. She noted that this system is fast and easy, producing results in under seven minutes per mouse. The assay is short term, identifying evidence for homologous recombination within a month of first exposing the mice to a suspected agent and therefore it should be useful to screen for genotoxic side effects of potential drugs or for identifying risk factors associated with environmental exposures. She added that the three-dimensional imaging system is now being commercialized by a startup company.

Quantum Dots and Microfluidic Single Molecule Detection for Screening

Tza-Huei (Jeff) Wang, Ph.D.

Johns Hopkins University

Circulating DNA, said Dr. Wang in the morning's final presentation, is an ideal source of non-invasive biomarkers, but detecting these molecules is incredibly challenging because of their low concentration and the high levels of obscuring background circulating DNAs. To address this problem, his team has developed quantum dot-based FRET nanosensors capable of detecting very low levels of specific DNA molecules. The use of quantum dots minimizes the background fluorescence associated with FRET because of their uniquely narrow emission bands and large Stokes shift. As a result, it is possible to detect single quantum dot probes with high signal-to-background ratios.

Dr. Wang's group has developed quantum dot-FRET nanosensors for point mutation detection and has used one of these nanosensors to detect *K-ras* point mutations in clinical samples from patients with ovarian serous borderline tumors. His group has also used these probes to detect methylated DNA in the presence of a 10,000-fold excess of unmethylated alleles at a level of 15 picograms of DNA, the equivalent of five methylation events. Dr. Wang presented data showing that these probes could detect DNA methylation in clinical sputum samples.

To create a system that would be amenable to large-scale clinical use, Dr. Wang's team also developed a method for using confocal fluorescence microscopy in combination with microfluidics to detect changes in circulating DNA. It has been hypothesized, Dr. Wang explained, that tumor necrosis causes the release of DNA of random sizes, while normal cell apoptosis releases DNA of uniform size around 200 base pairs, and recent published reports support this idea and suggest that circulating DNA size distribution measurements could serve as a cancer biomarker and as a means of monitoring therapeutic efficacy. His group has used its microfluidic system to measure size distribution of circulating DNA and shown that it is far easier to use than standard nested PCR technique and is not subject to sampling error associated with this other technology. In addition, the microfluidic technique

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does not require DNA isolation or separation, which can shear DNA and introduce artifacts, and requires no amplification. In one set of experiments on a small number of samples, Dr. Wang's group showed that the number of large DNA fragments present in clinical samples from lung cancer patients correlated strongly with disease stage.

Keynote Address

Carol A. Dahl, Ph.D.

Executive Director, The Lemelson Foundation
Founder of the IMAT Program

In the day's Keynote Address, Dr. Dahl reflected on the genesis of IMAT, made some comments on whether the program is fulfilling its intended purpose, and talked about some of the challenges in taking technology and applying it meet the health needs of the poorest populations in the world. She started her talk by harkening back to 1996-1997 when the program got launched conceptually and in actuality with its first request for proposals. At that time, it was understood that the genetic basis of cancer was critically important and that new technologies were revealing the complexity of the genetic changes that were happening in solid tumors. There was the realization at NCI then that there needed to be something transformational that would happen that move the field beyond its approach of the individual investigators studying one gene or one pathway, and that transformation was going to be enabled by the Human Genome Project. The real impact of the Human Genome Project was that it changed the way in which molecular and genetic information would be interpreted by enabling us to look at the function of any one gene or gene product in the context of the entire genome.

That was a transformational event for cancer research, and it was with that as a backdrop that NCI launched IMAT. At the time, the experiences gained from the Human Genome Project made it clear that new technologies were going to drive biomedical research in the post-genomic era and that there was in fact a need for a wide range of new technologies based on the principles of automation, parallelism, and miniaturization that could be used to create the toolkit to enable molecular discovery and speed cancer research. It was also apparent that academia could not be the sole source of this innovation but that it would have to be a joint venture involving academia, foundations, government, and industry with the goal of not just serving research but actually producing commercial instrumentation and services. The other key recognition was that informatics was going to play a huge role in the successful development and implementation of these technologies.

When NCI created IMAT, Dr. Dahl explained, it had the ambitious goal of being able to analyze anything at any scale over any timeframe. To enable technology development, NCI had to create a new funding and review mechanism that was more friendly to technology development and that involved releasing funds as milestones were met. The result was the Phased Innovation Award with two flavors of grants – the R21 phase for technology development and the R33 phase for application of the technology – with parallel mechanisms in the Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) programs. IMAT made a point of searching for new contributors, particularly in the chemical sciences and engineering fields, and it stressed multidisciplinary approaches and encouraged collaborations, which was the genesis of the annual IMAT PI meeting. IMAT also stressed the development of technologies that met the specific needs of NCI, and included milestone-based performance review. IMAT engaged reviewers from industry, a new concept, and demanded that successful proposals included a credible technology development plan, which was also a new concept at NCI.

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Turning to the subject of measuring IMAT's impact, Dr. Dahl said that from an administrative view, the program has been tremendously successful. IMAT brought in many new and first-time investigators and contributed most of the new technologies being brought into NCI. In addition, the program was succeeding at translating discoveries into applications that were being patented and turned into commercial products. She highlighted just a few examples of IMAT's successes, including Isotope Coded Affinity Tags (ICAT), RNA Later and its derivatives, and Microfluidic Genetic Analysis (MGA) Technology. She added that while she was not qualified to judge the impact that IMAT has had on progress in the battle against cancer, she felt that the brainpower engaged in IMAT-funded research and the number of IMAT-funded technologies that are coming to market has to bode well for the future.

Dr. Dahl then spoke about the global health crisis and the growing attention that it is receiving. While much of the focus has rightly been on addressing the most serious issues, particularly infectious diseases and maternal and child health, there is an increasing recognition that cancer is a growing problem in poorer nations as they begin to solve these other problems. She noted that the leadership of both NCI and NIH have expressed their support for efforts that can benefit the world's poorer populations. She challenged the IMAT community to think about how the technologies being developed can translate into applications for these populations.

To set the context, Dr. Dahl presented the chilling statistics on global poverty, which is that 1.4 billion of the 6.8 billion people in the world live on less than \$1.25 per day and therefore cannot afford most modern medical treatments. Some 2.5 billion people lack sanitation and nearly a billion lack access to clean water. About 25 percent of children in developing countries are undernourished, and malnutrition contributes over one third of child deaths in those countries.

Providing health solutions for the poorest faces a number of obstacles. On the basic science front, the poorest populations often have many things going on that complicate their health, and so the lack of understanding about the relationship between malnutrition, concurrent infections, and immune status and their impact on the body's ability to respond to therapy is a real obstacle to progress. It is also important to develop an understanding of the role that the microbiome plays in nutritional and immune status and resulting response to intervention. Developing solutions require integrating across complex systems and diseases states – silos, Dr. Dahl said, will not solve these problems.

Appropriate technologies are also missing in these countries. Large numbers of people do not have effective access to the health system infrastructure, nor do they have access to clean water, reliable electricity, and well-trained healthcare providers. Social and cultural issues, she said, can limit options for solutions, and neglected ethical considerations can bring research to a halt. Greater attention needs to be paid to defining the target product profiles needed for the world's most poor settings, and technologies cannot be driven solely by the developed world's perspectives

Another obstacle is that industry has not fully engaged in the problems of the poorer nations because market opportunities are not apparent, nor do they match the developed world's expectation in terms of return on investment even when these opportunities may support viable local businesses. Target product profiles are unclear – there is little or no market research conducted in poorer populations. Even when partners with experience relevant to the targeted settings want to attack a particular problem, they must deal with daunting regulatory ambiguities and complexities, as well as a public health community that is often resistant to industry involvement. It is critical to reach out local regulators and public health leaders early in the development process and to map out a critical path by which new technology can be approved and disseminated throughout the target population.

Finally, sustainable solutions need local ownership. Parachuting developed world solutions into these poorer populations almost never works over the long haul. Ethical, social, and cultural issues influence the likelihood of uptake and therefore impact. Investigators from the developed world have

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had a poor history in engaging equal partnerships and appropriately valuing the scientific contributions of local scientific partners. It is local leaders, after all, that shape regulatory and policy change and not fully involving them is missing an important opportunity.

Several years ago, when Dr. Dahl was at the Gates Foundation, she led an effort to look at the diagnostic needs of the global health community. This examination recommended that there be a research initiative created to develop new diagnostic platforms that would detect multiple diseases on a common platform that was accessible to these poorer populations. One of the major considerations is that there is very little advanced or moderate infrastructure in these poorer countries. Healthcare is provided in settings with no reliable electricity or clean water, with no laboratories, and with minimal expertise in laboratory skills. Given these constraints, diagnostic technologies have to be portable, they have to run off a car battery or even no power, produce no waste, and do not require clean water. They have to be capable of handling multiple specimen types and of multiplexed testing with nearly fully automated operation by operators with minimal training. On top of these requirements, the technologies have to be affordable to these poorer countries.

While restrictive, these challenges create plenty of opportunities for innovation that should appeal to the IMAT community. But also, Dr. Dahl said, there is a great deal of thought in the global health community that many of the innovations that would serve the poorer nations should be turned around and used in the developed world to lower costs and increase access to care across the globe. She encouraged the IMAT community to engage in this effort and to focus on the defining the context of their work in the broader context of the global health community. She also encouraged IMAT investigators to understand the value proposition better, to consider and embrace social, ethical, and cultural issues, and above all, to partner with local experts who may lack formal training but who understand and have expertise in dealing with the health concerns of these poorer populations.

She closed her talk by noting that there is a tremendous opportunity to make an enormous impact on a large percentage of the world's people. These populations are very receptive to new technologies that benefit them. For example, there is an amazing level of compliance with treatment regimens among AIDS patients in these communities. These people are smart, they are motivated, and not resistant to change, and they want the opportunity to be partners on the development of technologies that can help their communities. But being successful will mean thinking about how technology is developed and used in a different context, without prejudice about what is and is not possible, and it will also require thinking ahead 10 years and considering whether a technology is sustainable without foundation support but as a technology that involves local businesses. She encouraged the IMAT community to reach out to the global health community and to colleagues who work and teach in these countries, to get their perspectives and expertise and then use those inputs to guide development efforts in innovative ways to leapfrog these barriers.

In response to a question about industrial involvement and industry's need to a return on investment, Dr. Dahl said she was thinking more about entrepreneurial companies that have the ability to develop a new business model that relies more on lower margins and higher volumes. She cited some examples in the vaccine world where companies in India and Brazil are really taking the market by storm by looking at how to reduce the cost of making vaccine on a system-wide basis. Dr. Dahl said that taking a broader approach to rethinking how to develop a technology to be affordable and accessible is more likely to produce the needed breakthroughs rather than taking an incremental approach of wringing costs out of a technology after it has been developed.

Focused Innovation—Clinical Applications for Technology: Observations From a Long-Time Reviewer

Marc F. Hansen, Ph.D.

University of Connecticut Health Center

Dr. Hansen has been a long-time reviewer of IMAT proposals and from that context he offered some ideas of where there may be opportunities for applying technology in new areas. Going forward, he said, it is clear that IMAT is ideally situated to develop technologies to address the Provocative Questions. This is a wonderful opportunity for the program to make a major contribution in this area. One place where IMAT does need to make inroads, however, is in the area of clinical adoption. Clinicians are a conservative bunch who do not always see a need to move to replace an effective technology with a one that is clearly better.

The other key issue for the IMAT program is the need to join technology development with unaddressed clinical needs. There are many clinical problems in cancer diagnosis and therapy that are not benefitting from new technologies, said Dr. Hansen, and the reason is not because of neglect but because the technology development community is not aware of those needs. Communication between clinicians and technology developers is not happening to the degree needed.

Dr. Hansen also talked about two problems that he sees in IMAT proposals, issues that he said are related to what he called the “lamp post” effect and the “limitations of the hammer” effect. The lamp post effect has to do with the fact that it is very easy to search for new things in places where you already have the tools to search for them. There are large parts of cancer biology for which we have no good search mechanisms so they tend to be ignored even though they represent important questions that need addressing. In colloquial terms, he explained the hammer effect as follows: if you only have a hammer, all problems look like nails. Sometimes, no matter how much you improve the hammer, what a problem really needs is a saw.

There is also a tendency to propose technologies to address the biggest cancers. The advantage of the IMAT program is that it is driven by the technology, not the biological problem, and so this program will fund technologies that address important biological questions even when those problems at the moment are not associated with one of the five major cancers. As an example, he cited an area that he works in – jaw osteosarcoma, a cancer that affects perhaps 100 people in the United States annually. However, this is an ideal cancer to study surgical margins and in which it is critical to define margins accurately. Any imaging technology that could accurately define tumor margins would be a boon to surgeons and be well-received.

As a final point, he asked the IMAT community to please highlight milestones in proposals and directly address whether those milestones were met. That would make the review process go much more smoothly.

Beyond Inspiration and Invention to Innovation: Translational Science at NIH

Rosemarie Hunziker, Ph.D.

National Institute of Biomedical Imaging and Bioengineering, NIH

In her presentation, Dr. Hunziker highlighted the importance of translational science and the need to move groundbreaking science out of the laboratory and into the clinic. She focused immediately on a term that she feels is overused, and possibly misused; the Valley of Death. Significantly, she noted that there are in fact two valleys to be aware of. The first Valley of Death – the gap in funding between basic research or invention and applied research or technology validation – has received a great deal of attention. The bigger threat to new technologies, though, lies in the Darwinian Sea of the marketplace

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that ultimately determines the fate of a new technology where only the most creative, agile, and persistent succeed. She also said that NIH, contrary to popular notion, does have funding available to help promising technologies cross the first Valley of Death. The problems that researchers experience in accessing that funding arises more from the complexity of NIH's funding machine and the confusion within the research community about which of the many funding mechanisms to access for technology validation and translational science.

Two important funding mechanisms are the SBIR and STTR programs, each of which has three phases that run from feasibility to full research and development and on to commercialization. The main issues with these programs have to do with budgets that are often inadequate to meet expectations and the fact that there can be a gap between the first two phases of almost two years in some cases. NIH is addressing these problems with a phase I/phase II fast-track program in which applications for both phases are reviewed together. Such applications must include convincing preliminary data and contain clear, measureable, and achievable milestones, particularly for the transition to phase II. These applications must also include a well-conceived and compelling commercialization plan and letters of phase III support or interest. NIH has also created a phase II-plus award to support the development of complex instrumentation, clinical research tools, and behavioral interventions.

Dr. Hunziker then discussed the Clinical and Translational Science Awards program that aims to create definable academic homes for clinical and translational research and transform the local, regional, and national environment for clinical research and training. This program integrates diverse teams from across academia, university hospitals, and industry and currently involves 60 institutions in 30 states and the District of Columbia. More information about this program is available at <http://ctsaweb.org>. She also noted that the NIH Common Fund (<http://commonfund.nih.gov>) supports a wide range of cross-cutting, trans-NIH programs. The Common Fund supports high-risk research to explore ideas that have strong potential to improve health through three grant mechanisms. The Transformative RO1 award emphasizes creative ideas and projects with the potential to overturn paradigm. Pioneer Awards support individual scientists of exceptional creativity who propose pioneering approaches to major challenges in biomedical and behavior research. The New Innovator award stimulates highly innovative research being conducted by promising new investigators. The NIH is also involved in comparative effectiveness research, particularly research aimed at the creation of early standards that can accelerate effective translation efforts.

High risk/high reward research stands a better chance of getting funded by NIH, Dr. Hunziker explained, if there is the clear potential for high impact by taking a novel approach, not necessarily a new idea. Successful proposals demonstrate that team members have deep expertise in the area and a compelling research plan that anticipates obstacles and proposes alternatives.

Dr. Hunziker then briefly discussed NIH's Public-Private Partnership Program (<http://ppp.od.nih.gov/pppinfo/description.asp>) and the Center for Translational Therapeutics (<http://nctt.nih.gov/ScientificPrograms>), which both aim to accelerate translation of research advances into the clinic. She also mentioned that funding opportunities were available through the Bridging Interventional Development Gaps (BrIDGs) program, formerly known as the RAID program; the Therapeutics for Rare and Neglected Diseases (TRND) program; and the Production Assistance for Cellular Therapies (PACT) program administered by the National Heart Lung and Blood Program (<http://www.pactgroup.net>).

She noted, too, that the NIH-FDA Regulatory Science Initiative is a Common Fund program that supports translational science. Some common themes of NIH's translation research efforts are that they are outcome- and milestone-driven and that they leverage diverse resources. Projects involve multi- and

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interdisciplinary research teams and have active involvement of the FDA. They emphasize training and are trans-NIH in scope. They tend to be early stage and pilot programs that evaluate new technologies.

As a last example, Dr. Hunziker discussed the National Center for Advancing Translational Science (NCATS) that is now being established as a new NIH institute. The proposed mission for NCATS is to catalyze the generation of innovative methods and technologies that will enhance the development, testing, and implementation of diagnostics and therapeutics across a wide range of human diseases and conditions. NCATS will complement, not compete with, the private sector by studying the steps in diagnostics and therapeutics development, testing, and implementation into patient care, identifying bottlenecks amenable to re-engineering, and experimenting with innovative methods to streamline the process.

SBIR Development Center: IMAT Update

Amir Rahbar, Ph.D., M.B.A.

National Cancer Institute, NIH

Dr. Rahbar discussed funding opportunities available through the SBIR and STTR programs, set-asides that together generate about \$110 million annually in funding from NCI and \$650 million from the NIH. These grants not only provide seed funding for innovative technology development, but they also provide recognition, verification, and visibility that investigators can use to attract additional funding and support from the private sector. SBIR and STTR grants are just that – they are not loans and they are non-dilutive as far as ownership is concerned.

As Dr. Hunziker mentioned in her talk, the SBIR/STTR phase I grant totals can be up to \$150,000 and run six months to one year. Phase II grants can be up to \$1 million, run two years, and require a commercialization plan. Phase III is the commercialization stage of development, requiring non-SBIR/STTR funding. He detailed the requirements for SBIR and STTR applicants and noted that NCI recently changed the way SBIR and STTR grants are managed. A team of nine program managers and directors and one center director, entirely funded by NCI, focuses exclusively on NCI's SBIR and STTR portfolio. All of the directors have industry experience and professional networks to mentor awardees in commercialization strategies and processes. The NCI Development Center staff interacts with NCI program staff to identify areas of focus and is using that information to develop new activities to help small businesses. He noted that having a team that understands what it takes to commercialize a product is enabling the Center to do a much better job in guiding NCI's awardees toward commercialization.

Recently, the NCI Development Center initiated a phase II bridge award that is designed to help early-stage companies cross the Valley of Death by facilitating partnerships with third-party investors and strategic partners. In this new program, NCI is effectively sharing in the investment risk with other investors. The incentive structure it uses gives competitive preferences and funding priority to applicants that can raise third-party funds in a one-to-one match. Grantees can receive up to \$1 million in funding per year for three years and is available to current phase II awardees and to those whose phase II grant ended within the last two years. Matching funds can come from another company, venture capital firm, angel investor, foundation, state or local government, or any combination of the above.

Dr. Rahbar also described the NCI SBIR Investor Forum, which provides the opportunity for 14 NCI-funded companies to showcase their technologies to a large group of investors and potential strategic partners. More information on the Forum is available at <http://sbir.cancer.gov/investorforum/>. He then discussed SBIR/IMAT collaborations that aim to catalyze targeted technology development and commercial validation in the area of molecular analysis technologies through a dedicated set-aside. This

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effort provides a cohesive program that is aligned with the goals of the IMAT program with an emphasis on commercialization. He noted that IMAT R33 awards will flow smoothly into SBIR R43/44 awards. More information on this and NCI's other SBIR opportunities is available at <http://sbir.cancer.gov/funding/>.

POSTER HIGHLIGHTS

Moderator: Tony Dickherber, Ph.D.

Program Manager, IMAT Program
Office of Biorepositories and Biospecimen Research, NCI

This year, IMAT investigators presented results of their projects in 53 posters. Program managers decided that seven of these merited special attention and asked the investigators to summarize their posters in a series of 10-minute presentations to the general assembly. These presentations are described here.

CITP-Based Proteome Enrichment

Cheng Lee, Ph.D.

University of Maryland

Dr. Lee's project aims to evaluate the performance of a capillary isotachopheresis (CITP)-based proteomics coupled with tissue microdissection to enable targeted biomarker discovery. He and his collaborators evaluated this system using fresh-frozen and formalin-fixed microdissected tissues and assessed reproducibility of protein identification in replicate analyses of the same proteomic sample and among samples from two different lesions of the same tumor specimen. They were able to identify more than 5,000 high-confidence proteins in amounts as small as 10 attomoles. Reproducibility was 85 percent within triplicate replicates of the same proteomic sample and 80 percent among different cancer lesions of the same tumor specimen. The 5,000-plus high-confidence proteins provided comprehensive protein subclass coverage across major functional categories relevant to cancer and afforded superior pathway coverage across cytoplasmic and nuclear proteins.

Using this system, Dr. Lee's group compared protein expression profiles in astrocytomas. They identified nearly 4,000 proteins in common from 10,000 cells in 12 cases. They also identified 225 proteins that were unique to glioblastomas, 31 that were unique to grade II astrocytomas, and 30 that were unique to grade III astrocytomas. They were also able to show that increased expression of one specific protein, IQGAP1, correlated with astrocytoma progression. These results were validated using immunohistochemical staining in a tissue microarray system.

Bead Array Flow Cytometer with Mass Spectrometry Detection

Victor Baranov, Ph.D.

University of Toronto

In this poster, Dr. Baranov and his colleagues described the bead array flow cytometer/mass spectrometer system that they are developing as a means of performing biomarker discovery and detection using metal-labeled affinity probes. The investigators demonstrated the resolving power of mass cytometry and showed that it can be used to discriminate polystyrene beads tagged with five

different concentrations of four different lanthanide ions in a manner that makes it suitable for use in a high-throughput assay system. This poster also detailed the team's synthesis of a library of functionalized metal-encoded beads suitable for conjugation to affinity probes. The group has now amassed a library of 150 different beads and proved that they can identify the individual members of this library with 95 percent accuracy. In addition, the team has demonstrated that they can functionalize the surface of these beads with oligonucleotides and antibodies to serve as affinity probes.

Mutation Analysis of Formalin-Fixed Paraffin-Embedded Tissues

Hanlee P. Ji, M.D.

Stanford University

Dr. Ji and his collaborators have developed a method for capturing targeted DNA fragments for resequencing from archived tissue samples, and in this poster they described the technique and presented data demonstrating its performance characteristics. Their method provides high DNA sequencing coverage in a single reaction volume, can be completed in a two-day workflow, and integrates with fast clinical sequencers. It relies on an inverted bridge probe that allows for amplification and resequencing of multiplexed targets. When tested on formalin-fixed, paraffin-embedded (FFPE) archived tissue samples, this method achieved capture efficiency exceeding 90 percent at over 100-fold coverage in a single sequencing run. In a second of experiments, Dr. Ji and his colleagues compared results from matched low-quality FFPE samples and high-quality flash frozen samples and demonstrated that this technique could capture the same single-nucleotide variants from both types of samples. Based on the results of these comparisons, Dr. Ji concluded that the method could have potential in both clinical diagnosis from contemporary biospecimens and for biomarker discovery using the vast numbers of archival tumor samples that are available.

A+PSA Assay for Prostate Cancer Diagnosis and Beyond

Gang Zeng, Ph.D.

University of California, Los Angeles

In this poster, Dr. Zeng and his collaborators describe a method for quantifying auto-antibodies against a panel of clinically relevant tumor-associated antigens and using this technology to create a potential new diagnostic for prostate cancer. Dr. Zeng's team first identified B cell epitopes from a six previously identified prostate cancer-associated antigens and then conjugated these peptides to colored seroMAP microspheres that could be used in a Luminex multiplexed instrument to both quantify and identify prostate cancer-associated auto-antibodies in human serum samples. They confirmed that the auto-antibodies that reacted with these peptides also bound to the full-length proteins and that the peptide-bead conjugates did not interfere with simultaneous prostate-specific antigen (PSA) determination on the same instrument.

After validating the combined auto-antibody and PSA assay, which they named A+PSA, Dr. Zeng and his collaborators conducted a pilot study using pre-surgery sera from 131 biopsy-confirmed cancer patients and 121 benign prostatic hyperplasia and/or prostatitis patients. Compared to PSA measurements alone, a logistic-regression-based A+PSA index increased sensitivity from 52 percent to 79 percent, specificity from 79 percent to 84 percent, and reduced false positives from 21 percent to 16 percent. Overall accuracy of the A+PSA index was 81 percent compared to 65 percent for PSA alone.

Handheld Biomarker Monitor

Guodong Liu, Ph.D.

North Dakota State University

Dr. Liu and his colleagues reported in their poster on the progress they have made developing an aptamer-based electrochemical bioassay to measure the tumor marker carcinoembryonic antigen (CEA). The assay involves four steps. First, a biotinylated probe that recognizes CEA is added to serum. Streptavidin-coated magnetic beads are then used to capture the CEA-aptamer complex. Next, a streptavidin-horseradish peroxidase polymer is added and finally, electrochemical detection reaction occurs after adding hydroquinone and hydrogen peroxide to the mixture. The poster presentation describes the work done to optimize the system and compared its performance to that of the standard antibody-based assay. The aptamer-electrochemical system had a detection limit down to 0.54 nanograms of CEA per milliliter, which meets the requirement for CEA analysis in biological samples. The investigators are now developing an automated microfluidic test device for this assay.

Proteomic Profiling of Redox Signaling

Cristina M. Furdui, Ph.D.

Wake Forest University, School of Medicine

The goal of the work described in this poster is to identify and characterize the function of the cysteine sites in proteins that are oxidized by reactive oxygen species. The approach that Dr. Furdui, Dr. Poole and their colleagues have taken is to chemically trap the cysteine sulfenic acid residues on oxidized proteins with a set of reagents that do not react with free sulfhydryl groups or possible confounding species. Dr. Furdui and her team used one of the reagents (DCP-Bio1) to show that PDGR-dependent oxidation inhibits the Akt2 kinase, but not Akt1 or Akt3, and that this occurs through oxidation of a specific cysteine residue located in the regulatory linker region of this kinase. In another set of experiments, the investigators used a different reagent (DCP-FL1) to show that VEGF signaling increases protein oxidation at the leading edge of migrating endothelial cells during angiogenesis. The poster also reported a set of experiments in which a third reagent (DCP-Rho1) was used to label sites of protein oxidation *in situ* in prostate cancer and ovarian cancer cells. These experiments showed that protein oxidation occurs at specific regions in the cell membrane that surround endocytosed receptor complexes following treatment with lysophosphatidic acid, an endogenously produced growth factor.

2-Dimensional PCR Mapping of Gene Changes in Tumor Sections

Disha Pant, Ph.D.

University of Maryland

In her poster, Dr. Pant described the technology that she and her colleagues are developing to spatially map changes in gene methylation across human tissue sections with high spatial resolution. The researchers start by placing a tissue section on a piece of packing tape and then affixing it to a multi-well plate preloaded with lysis buffer. The lysate is then moved to a 96-well PCR plate for methylation analysis using qPCR. Because the wells map to the original tissue, the resulting data provides a methylation map that is dependent on cell type and proximity to the tumor area. Initial studies of prostate cancer biopsy specimens identified higher levels of methylation in specific genes in tumor cells versus normal prostate tissue.

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NCI's Continuing Need for Technology Innovation and Opportunity Going Forward

Douglas R. Lowy, M.D.

Deputy Director, National Cancer Institute, NIH

In his address to the IMAT community, Dr. Lowy said that IMAT exemplifies the high risk/high payoff type of research that NIH in general and NCI in particular wants to support. There is the tendency in times of challenging budgets to become more conservative, yet what sets IMAT apart is that it has set-aside money that is not micromanaged in terms of what the community proposes and what NCI funds. He noted, too, that NCI understands that there is going to be a high rate of failure with this approach, yet the program is structured so that it has sufficient size and scope to produce great successes as well. To illustrate the enthusiasm that NCI has for IMAT, Dr. Lowy recounted that at the recent Board of Scientific Advisors meeting the Board did not ask its usual question of why is NCI setting aside money for a particular program but instead asked why NCI wasn't setting aside more money for IMAT.

One aspect of the program that Dr. Lowy said he appreciates is its flexibility. He said, too, that this program is unique across NIH in that its R21 application provides three years of support rather than the usual two years. He noted that NCI is going to be announcing a new omnibus R21 proposal that will allow investigators to propose innovative ideas on any aspect of cancer research, rather than restricting them to specific categories of cancer research. He said that he hopes that this new program will be just one example of how even in this challenging environment NCI is creating new funding opportunities for the extramural community to put forward its ideas, even risky ones where there is a limited amount of preliminary data. In closing, Dr. Lowy said that it is very gratifying to him that the IMAT program has tremendous support among the program officers of the different divisions of NCI and from its oversight Board of Scientific Advisors.

In response to a question about whether the new omnibus R21 applications will be reviewed by the usual study sections, Dr. Lowy said that the plan is that there will be a special study section devoted to R21 applications. NCI is hoping that the review will be conducted by the Division of Extramural Activities.

TOPIC 3: NANOTECHNOLOGIES

Moderator: Paul Wagner, Ph.D.

Program Director, Cancer Biomarkers Research Group
Division of Cancer Prevention, NCI

Epigenetic Profiling in Nanochannels

Robert Riehn, Ph.D.

North Carolina State University

In the first presentation of this session, Dr. Riehn described the progress his group has made in using nanochannels to stretch DNA and then read cytosine methylation and histone modification profiles with gene-relevant positional precision. He showed images of stretched DNA within 100 nanometer-diameter channels and remarked that the device his group built can stretch DNA reliably and uniformly. He also showed images demonstrating the ability to image cassettes of transcription factors bound to DNA and to perform restriction mapping at high resolution using the nanochannels. He explained that the ability of DNA in the nanochannels to fluctuate leads to the superior performance of methods that

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use nanochannel stretching of individual DNA molecules. The optimal readout length is about 200 kilobase pairs, a scale long enough to map across multiple genes or repeat sequences.

Dr. Riehn then noted that it is important to be able to analyze epigenetic plasticity in single cells, as opposed to an ensemble of cells, because cancers arise from a single cell origin, not from wholesale changes in multiple cells in the same organ. The goal is to examine every single chromosome in every single cell in a tissue sample with a resolution of 1 kilobase pairs (kbp). So far, his group used their nanochannel techniques to study the cytosine methylation state in DNA pieces of up to a few hundred kilobases (kb) in length with a resolution of better than 10 kbp. The current method is based on the binding of a fluorescently labeled protein fragment that binds specifically to the CpG pattern found in the promoter regions of many higher organisms. False positives with this detection system occur at a rate of under one percent and false negatives at a rate of under 0.6 percent.

His group is also using its technology to look at histone modifications, which he believes will require getting down to a resolution of 5 kbp. The challenge in working with chromatin is that it is positively charged while the walls of the nanochannel are negatively charged, raising the possibility that chromatin will stick inside the nanochannels rather than move through them. To overcome this problem, Dr. Riehn's team uses an electric field to drive chromatin into the nanochannels. Using fluorescent antibodies directed to specific histones, they could then visualize histone modifications on the stretched chromatin at the single gene level. The one problem that they have not been able to solve is that they have not been able to create a benchmark sample of histone modification to use to demonstrate specificity.

The final part of this project involves creating a label-free system that uses Raman spectroscopy to detect chemical modifications on DNA or chromatin. To boost the Raman signal, his group built a device that contains a nanoantenna to concentrate the light hitting the molecules in the nanochannels and that excites the molecules close to a resonant absorption line. Tests with benzene as a model system showed that these two additions enhanced the Raman signal by about 10^8 , a pleasant surprise. Recently, Dr. Riehn's group has shown that they can detect cytosine methylation on DNA. His group is now working to build a device that adds on-chip DNA extraction from single cells that would feed long pieces of DNA or chromatin into the Raman imaging nanochannel portion of the device.

Nanoelectrode and Nanofluidic-Based Assay of Mitochondria Membrane Potential and Apoptosis
Peter J. Burke, Ph.D.

University of California, Irvine

This project, which Dr. Burke explained is a collaboration with Douglas Wallace's group at the University of Pennsylvania, aims to develop microfluidic chambers that will enable the evaluation of mitochondrial metabolic and biophysical parameters on just a few or even one mitochondria. He reviewed the overall workings of the mitochondria and noted that mitochondria are tied to cancer through the link between the mitochondrial membrane potential and apoptosis. The mitochondria, in fact, acts as the key decision maker on the apoptosis pathways, yet because of our inability to study small numbers of mitochondria, little is actually known about the exact steps that occur in the mitochondria that lead to the irreversible decision for the cell to enter apoptosis. The need to tease out these mechanisms is what drives the effort to develop technologies capable of measuring the membrane potential in single mitochondria with high spatial resolution and at high-throughput under variable conditions.

It is almost impossible to study mitochondria inside the cell, said Dr. Burke, but it is possible to gently lyse the cell, isolate mitochondria, and keep them functioning for about an hour in a respiration

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buffer that mimics the cytosol. He described the standard electrochemical methods used to measure mitochondrial membrane potential and described the microfluidic chip his team built to make these measurements on small numbers of mitochondria. The first generation of this device, built by hand on a glass slide using hand-cut PDMS chambers, had the capability of making membrane potential measurements while introducing agents that blocked specific ion channels and pumps in the mitochondrial membrane. His group has since designed a second-generation chip that is produced on a wafer scale using semiconductor processing technologies and that has much faster response times and can make measurements on mitochondria from as few as 1000 cells with a 10 millivolt resolution over 10 milliseconds; in contrast, the best current method for assessing mitochondrial membrane potential requires mitochondria isolated from about 10 million cells. With this device, the researchers have measured response of various substrates for and inhibitors of mitochondrial function. They are still working to reach the single mitochondria level and one micron spatial resolution. Nonetheless, Dr. Burke said that there is commercial interest in this device as it stands now for use in detecting various mitochondrial diseases.

He then discussed one possible application of this device, which is to study the unique state of mitochondria in human embryonic stem cells. It turns out, he said, that the mitochondria of human embryonic stem cells have a different morphology than those in regular cells. Their inner membranes are not convoluted and they produce less ATP and reactive oxygen species, and in fact, their pluripotency is related to their metabolic activity. Using their device, Dr. Burke and his collaborators demonstrated that the mitochondrial membrane potential is about half that of human somatic or cancer cells, which is consistent to a respiratory rate that is also about 50 percent less. Furthermore, this reduced mitochondrial function appears to be a feature of the stem cell mitochondria themselves. His team demonstrated this by transferring human stem cell mitochondria into somatic cells and observing that the recipient cells then acquired several stem cell features, including morphology and expression of the key pluripotency protein OCT4. This research is still in its early stages, and it is only possible, Dr. Burke noted, because of the technology development that IMAT funded.

Dr. Burke then discussed the next step in technology development which is to build a device capable of assaying single mitochondria using nanochannels and carbon nanotube sensors. The first part of this effort is focused on characterizing the electrical behavior of carbon nanotubes coated with lipid bilayers into which are embedded biological ion channels. The idea is that with the right electrical properties, such constructs could serve as incredibly sensitive electrodes for measuring membrane potentials in individual mitochondria at high spatial resolution. Data collected so far suggest that these carbon nanotube electrodes are in fact measuring ion flow through individual ion channels supported in the lipid bilayer coating.

The second part of this effort involves learning how to drive individual mitochondria into carbon nanotubes and hold them within the nanotube channel in order to monitor fluctuations in membrane potential as it is manipulated chemically. Dr. Burke showed fluorescence microscopy images demonstrating the movement of individual mitochondria into nanochannels, and he noted that the probability of having more than one mitochondrion in contact with a given nanoelectrode in the nanofluidic channel is less than one percent. The goal now is to merge these two technologies into a single device and to demonstrate its utility in studying not only mitochondria but other subcellular organelles, too.

Development of a Nanoscale Calorimeter

Gregory Kowalski, Ph.D.

Northeastern University

This project, which represents the joint efforts of Dr. Kowalski's group and Dale Larson's group at the Draper Laboratory, is developing a nanoscale calorimeter that can be placed in a grid and coupled to a microfluidics system. Such a device would be valuable for use in drug development and optimization efforts. Dr. Kowalski reminded the audience about the value of calorimetry and drug development. The best in-class drugs, he said, have the largest negative enthalpy of reaction values, which are reflections of the binding of a drug to its target. Being able to make calorimetry measurements on a multiplexed scale early in the drug development process would enable researchers to optimize this important property at the initial stages of lead compound development. The problem with calorimetry as practiced today, though, is that it requires large amounts of drug compound and these compounds are expensive.

After explaining the basic physical principles of calorimetry measurements, Dr. Kowalski described the use of nanohole sensors to measure concentration and temperature changes at high spatial resolution in a chemical reaction chamber. These sensors use a property called extraordinary optical transmission (EOT) as a temperature and concentration sensor that requires no wires. He detailed the construction of the device and the optics and processing technologies used to collect and analyze the data. He noted that the total reaction volume in the device is 25 nanoliters, and he presented data confirming that the device can indeed measure enthalpies of reaction on a very small scale. One experiment, for example, used 0.167 nanograms of material and measured an energy change of -353.6 picoJoules. Today's state of the art calorimeters require milligrams of material, so the cost savings that this device can achieve are substantial, Dr. Kowalski concluded. He added that these nanohole sensors are easily multiplexed.

TOPIC 4: PLATFORMS/ASSAYS

Moderator: Mukesh Verma, Ph.D.

Branch Chief, Methods and Technologies Branch
Division of Cancer Control and Population Sciences, NCI

Method for Detection of Secreted Proteins in Single Cell Assays

Henryk Szmecinski, Ph.D.

University of Maryland

Dr. Szmecinski and his team have been developing an assay platform for measuring single cell secretion of cytokines using metal-enhanced fluorescence from plasmonic substrates. He first noted that the cytokine and chemokine signaling networks used to coordinate the activity of the immune system is not only incredibly complex, but it takes relies on signals that are present at levels that are below the sensitivity of most measuring technologies. His team has successfully developed plasmonic nanostructures that are compatible with standard immunoassay formats and that can detect cytokines with picogram per milliliter sensitivity. The use of plasmonic nanostructures is critical because they overcome the many limits of classical fluorescence detection technologies, increasing sensitivity.

After describing the physics and design of multi-layered plasmonic structures created using metal-enhanced fluorescence, Dr. Szmecinski showed that the use of these structures increased the intensity of the fluorescence signal from every dye tested. In one example that he discussed, this system increased the fluorescent signal from Cy5-labeled DNA by about 20 fold and increased photostability by

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a factor of 30 while decreasing the fluorescence lifetime by a factor of 6. Taken together, these improvements create a very sensitive detection system that is easily manufactured in a format that can use standard fluorescence microscopes present in laboratories today. He showed time-lapse images of TNF- α and IFN- γ secretion from individual macrophage cells that had been measured using the MEFspot system in conjunction with fluorescence lifetime imaging microscopy (FILM) both before and after immune stimulation. He also showed that it is possible using software developed by the microscope manufacturer to analyze the data and directly quantify the amount of cytokine secretion at concentrations in the picogram per milliliter range. His group is now working to demonstrate that they can measure real-time secretion and demonstrate the detection of 50 to 100 secreting cells per millions of cells.

Developing a Single Cell Growth Assay Platform for Monitoring Response to Cancer Therapies
Scott R. Manalis, Ph.D.

Massachusetts Institute of Technology

This project, explained Dr. Manalis, aims to develop a device that can measure mass changes in individual cells as a means of quantifying changes in cell growth kinetics. Such a device, he said, would be useful for studying the effect of anticancer agents, for example, on both healthy and malignant cells, or for studying the effect of targeted anticancer agents on tumor cells from a specific individual. The technology that Dr. Manalis and his colleagues developed to measure growth in individual cells is the microchannel resonator. They have now shown that they can use this to track the growth kinetics of single cells in a manner that enables the cells to grow over multiple generations as if they were in bulk culture, at least in terms of doubling times. The precision of the device is such that it can detect changes as small as 0.01 percent of the cell mass, permitting the observation of subtle changes in cell growth. Dr. Manalis noted that it proved to be a significant challenge to create a device that would enable measurement of cell growth while adding drugs to the growth media in a way that was compatible with the microchannel resonator and that did not subject the cells to shear stress.

Using mouse lymphoblasts, Dr. Manalis's team used its device to study the response to three different anticancer agents, and was able to show that each drug affected growth kinetics in unique ways. They also showed that a change in instantaneous growth rate correlated with response time to the drug. His team has since begun studies on patient-derived glioblastoma cell lines and shown that the device can measure their growth in real time. His team has since run cells dispersed from biopsy samples within two hours of their removal from the brain and shown that they can detect and trap glioblastoma cells out of the large mass of other types of cells present in the biopsy sample. In this initial study, Dr. Manalis's team found that less than a third of the cells within glioblastoma tumors are proliferating. Given that low level, a higher throughput device is needed if this device is to be clinically useful, and his team is now working to develop an array-based device capable that can meet this need.

Microfluidic 3D Scaffold Assay for Cancer Cell Migration and Intravasation
Joseph Charest, Ph.D.

Draper Laboratory

The goal of Dr. Charest and his collaborators is to develop a microfluidic three-dimensional scaffold system for studying cancer cell migration and extravasation in an environment that accurately models physiologic conditions. After detailing the construction of the microfluidic system, which can include a layer of endothelial cells to mimic the vasculature if desired, he discussed the experiments his

group has conducted to better understand how interstitial flow affects the metastatic potential of tumor cells. Pressure gradients within the extracellular space produce slow, creeping flows in the tenths to several micrometers per second range. This fluid drains into vasculature or lymph. Under normal conditions surrounding healthy vasculature, the pressure within the vasculature is greater than the interstitial pressure. However, in tumors the pressure is equalized and interstitial fluid can drain into the vasculature.

Initial studies in their microfluidic device have demonstrated that interstitial flow distributes a cell-secreted morphogen, creating a gradient that a tumor cell migrates along using the CCR-7 receptor. Dr. Charest described the experiments his group conducted using MDA231 breast cancer cells and explained that they measure interstitial migration using two metrics, streamline and directional migration. Streamline migration looks at whether the migration is parallel or anti-parallel to the flow while directional migration measures whether migration is going with or against the direction of the flow. The streamline flow metric shows that tumor cells migrate parallel or inline to the flow and that cells have aligned along the flow within 36 hours. From a directional migration viewpoint, the tumor cells migrate downstream with the flow, but upstream when the CCR-7 receptor is blocked. Cell density also affects directional migration – at low cell density the cells migrate downstream parallel to the flow, but as cell density increases migration becomes directionally neutral and eventually turns to the upstream direction. Dr. Charest believes this latter behavior is the result of local cells secreting enough of the morphogen to saturate the system and eliminate the chemotactic gradient.

His group is now working to move beyond building prototypes to fabricating devices that can be used outside of his laboratory. They have developed a hot-embossing process using a thermoplastics such as polystyrene with highly consistent surface properties that can then be modified further using an oxygen plasma treatment process. The resulting surfaces allow for more stable gel formation and promotes cell survival in the device without altering the material's optical properties. This manufacturing process is easily scaled for commercial production.

Next-Generation Sequencing and Clonal Analyses of Flow Sorted FFPE Clinical Samples

Michael Barrett, Ph.D.

The Translational Genomics Research Institute

The first aim of Dr. Barrett's project is to develop flow cytometry-based assays for the identification and purification of clonal cell populations from pancreatic carcinoma samples to then use in genomic studies. He described the specific instrument and experimental method that his group uses to take dilute samples from biopsy tissue and get efficient DNA-based sorting based on whether cells are diploid or aneuploid. He then demonstrated how this approach generates clonal level resolution of pancreatic adenocarcinoma genomes from tissue samples that are only eight percent tumor cells.

His team is now testing this technology in the context of a phase II clinical trial to determine if molecular profiling can help select specific therapies for patients with previously treated metastatic cancer. The goal of this aspect of the project is to use this selection to increase the one-year survival of patients with pancreatic cancer. The technical challenge is that the biopsy samples are small and must be divided into three for analysis by different approaches, but nonetheless, Dr. Barrett's team was able to identify aneuploid cells and identify genomic markers for resistance to previously administered drugs and the presence of molecular targets suitable for second line therapy. This technique was also able to resolve two different aneuploid populations of cells had nearly identical genomic profiles but they were still able to detect genomic differences relevant to therapeutic options. Dr. Barrett's group has also used this approach to analyze tissue samples taken from different metastatic locations in the same patient. The

genomic analysis showed that the different metastases had many important similarities, reflecting their common origin, but that there were also tissue-specific genomic differences.

The second aim of Dr. Barrett's project is to validate DNA content and multiparameter flow sorting for detecting and purifying clonal populations from FFPE pancreatic samples. His team procured matched fresh frozen and FFPE samples from various laboratories and developed a metric for similarities between the matched samples. This analysis found a very high level of concordance between the two sample types, and they have done this in samples from rare tumors obtained from a variety of sources. They have also done multiparameter sorting that enabled them to maximize recovery of cell populations representing very small fractions of the original FFPE tissue sample. Dr. Barrett's team is now optimizing the use of flow-sorted FFPE clinical samples for high-resolution genomic profiling. This work has shown that they need about 50,000 nuclei for optimal results, but given that it is rather laborious to obtain this many nuclei, his team wants to maximize the number of analyses it can conduct on this sample size. They worked with a reagent manufacturer to maximize the isolation process and were able to use the resulting samples to perform next generation sequencing and identify from FFPE samples all of the mutations identified in matching fresh frozen samples. They have now also shown that they can separate six separate cell populations from FFPE samples.

TOPIC 5: CIRCULATING TUMOR CELL PLATFORMS

Moderator: Avi Rasooly, Ph.D.

Chief, Disparities Research Branch

Center to Reduce Cancer Health Disparities, NCI

Isolation of Rare Circulating Tumor Cells From Blood Using Microbubbles

Dmitri Simberg, Ph.D.

University of California, San Diego

In this project, Dr. Simberg and his collaborators are developing a method that will use perfluorocarbon (PFC) microbubbles to quickly and easily isolate rare circulating tumor cells (CTCs) from whole blood. Buoyant PFC microbubbles avidly bind cells, and theoretical calculations by Dr. Simberg's group have suggested that if one microbubble coated with CTC-specific antibodies were to bind to a CTC, the combination would have a buoyancy that would enable it to be separated from all other cells in whole blood with very gentle centrifugation. To test this hypothesis, Dr. Simberg's group prepared PFC microbubbles coated with both anti-EpCAM and anti-CEA antibodies and used them to isolate lung carcinoma or pancreatic adenocarcinoma cells spiked into whole blood. Under optimized conditions, dual-targeted PFC microbubbles recovered about 80 percent of the cells spiked into blood, results comparable to those obtained using immunomagnetic beads. Dr. Simberg noted that microbubble harvesting results in small sample volumes compatible with PCR and immunostaining and that there is only a small amount of contamination by leukocytes.

Isolating Viable Circulating Tumor Cells for Functional and Molecular Analysis

Hsian-Rong Tseng, Ph.D.

University of California, Los Angeles

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In his presentation, Dr. Tseng reported on the progress he and his collaborators have made in developing a microfluidic device for capturing CTCs from whole blood. The key feature of this device is its nanostructured surface that harnesses Velcro-like interactions to achieve unprecedented efficiency at capturing CTCs. Dr. Tseng described his team's fabrication of silicon nanowire substrates using state-of-the-art nanofabrication techniques developed for the semiconductor industry. He then discussed how his team functionalizes the nanowires using anti-EpCAM antibodies to confer specificity for CTCs and how they incorporate this substrate in a microfluidic device that can be imaged with a standard fluorescent microscope. The latter capability enables the researchers to take advantage of a unique near-infrared dye that exhibits preferential uptake and retention in living tumor cells. Validation assays using cancer-cell spiked human blood demonstrated capture efficiencies of greater than 80 percent.

Dr. Tseng's group is now collaborating on several clinical applications of this technology. In one project run in collaboration with Matthew Rittig at UCLA, the investigators are monitoring dynamic CTC numbers in castration-resistant prostate cancer patients who received treatment with the androgen synthesis inhibitor abiraterone acetate. Measurements of CTC numbers in patient blood samples showed a strong correlation between CTC number and tumor size. In one patient, treatment with Provenge resulted in CTC numbers plummeting. When CTC numbers started rising again, the patient was treated with abiraterone acetate, which caused CTC numbers to fall nearly zero. Dr. Tseng also briefly discussed results from trials in metastatic breast cancer, gastric cancer, melanoma, and adrenal cancer.

Real-Time Observations of High-Efficiency Capture of Cancer Cells in a Three-Dimensional Magnetic Prep Device

Shan Wang, Ph.D.
Stanford University

In the meeting's final scientific presentation, Dr. Wang discussed his group's efforts to develop a three-dimensional magnetic sifter for enriching circulating tumor cells. This device achieves high capture efficiency and throughput through the use of parallel flows through a densely porous, magnetically soft membrane to which a high magnetic gradient is applied. The procedure that Dr. Wang's group developed takes whole blood, adds a magnetic and fluorescent capture probe, and then after a two-hour incubation runs the sample through the sifter, which can be imaged in real-time using standard fluorescence microscopy. Images that Dr. Wang presented clearly showed cells becoming trapped on the sifter when a magnetic field was applied and then being released when the magnetic field was turned off. He also reported that this device achieves capture efficiencies that exceed 90 percent at flow rates of 10 milliliters per hour, a flow rate that is 10-fold higher on a device 30 times smaller than other cell capture microdevices.

Gap Analysis Reports

During lunch on Day 2, meeting attendees were asked to participate in one of four discussion groups whose task was to identify gaps that innovative molecular analysis technologies could address. After the last scientific presentation of the day, one member of each group reported on these discussions. Below is a summary of the gap analysis conducted by each of the five groups.

Proteomics Gap Analysis

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- Better quality control samples for analysis with standards development for comparison across laboratories and across platforms at each stage of analysis
- Gaps in sample preparation techniques, chromatography and bioinformatics in mass spectrometry based proteomics.
 - Better and more variety in proteases through engineering
 - Chemical probes and methods for selective enrichment of specific classes of proteins or posttranslational modifications
 - Better and faster chromatographic separation techniques - current methodologies are time-consuming and expensive
 - Bioinformatics: databases are missing information from The Cancer Genome Atlas Project (TCGA);
 - Bioinformatics: National Center for Biotechnology Information (NCBI) needs working groups to address the bioinformatics needs, perhaps with input from NCI's Clinical Proteomic Tumor Analysis Consortium (CPTAC)

Pathways Gap Analysis

- Methods are need to quantify signaling pathways
 - Relative quantitation of stoichiometry and activation
 - Measuring flux through pathways
 - Simultaneous tracking of molecular populations and associations with cellular phenotype
 - Pathway dynamics
- Tracking single molecules through a pathway
 - Assess cross-talk across cellular pathways
 - Collect from multiple signaling pathways at once and track the activity across multiple cells at once to understand degree and significance of tumor heterogeneity
 - Multiple signaling nodes being tracked simultaneously
 - Track pathways in initial cell progeny over multiple generations
 - Put in context of evolution of tumor to deal with that kind of information
 - Where/when to collect data and how this impacts conclusion
- Areas to be addressed
 - Data collection
 - Missing is interfacing different kinds of data
 - How to compare measurements from boundary of cells
 - Need to be able to make kinetic measurements, e.g. as enzymes are operating
 - Ultimately need computational analytical capabilities
 - How to collect and analyze enormous data sets and represent them in a way that is clear to the research community. How to represent the data is key
 - How to standardize (and maybe centralize) data so that it remains useful to the broad scientific community years after it has been collected
 - How to develop better models based on these quantitative data and how to model very large networks
 - Modelers need good data and good context in order to create useful models. Need to figure out how to make sure of all the numbers we are generating.
 - Need to have good ways to validate these models (this is a challenge for the biologists)

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Genomics Gap Analysis

- General impression: Great technologies out there already filling the gaps, but there is plenty of room for optimization and more effective dissemination to have more impact
 - Better selectivity and specificity and affordability still needed
 - Usability is still limiting
- Ideal is to have genetic, epigenetic and spatial position information collected on the individual cell level at the least
 - Technologies would have to be embedded in small devices and much faster to be usable in a clinical setting. Ease of use is important of course
- Things useful in the lab do not exhibit the right properties for applicability in the clinic. Incremental changes in this field would have significant impact on impact for patients.
 - Usability threshold still exists for empowering clinicians with genomic information
 - Real emphasis on potential for pushing quantitative for making qualitative impact
- Possible to develop a rapid assessment platform that could test susceptibility to a variety of therapies?
 - If you use a small number of cells, could you make a determination on likely clinical outcome
- For population-based studies, hi-throughput studies are certainly valuable, but improvement on cost and throughput still required.
 - Getting genomic information on thousands of patients for making statistical arguments on patient prognosis
 - Ideally, technologies should be able to work past limitations inherent in long-term stored materials (e.g. FFPE tissue or long frozen blood)
- There's a lot of information from certain regions of genome, but areas outside of coding regions still not well understood.
 - Need mapping tools that allow us to better guide us for these regions.
 - 3D maps of structural impact on functional relationships across genome
- No unbiased technologies for identification of miRNA and their regulatory function.

Biomarker Gap Analysis

- Not enough teamwork or interdisciplinary efforts.
 - Need a Web-based network to centralize information for new collaborations.
- New workshop to identify critical needs from clinical end-users
 - Follow-up with technology development experts to identify places where there already are solutions (requiring better awareness or dissemination), opportunities (ripe for technology development) and where there is poor feasibility at this time (i.e. kick the can to later).
- Better multiplexing tools and better modeling

Biospecimen Science Gap Analysis

- Useful to have a mechanism and standard for longer term storage of blood with sufficient annotation.
- Better live sample collection and preservation for single-cell analyses, especially for drug efficacy/safety testing (with the microenvironment, if possible)

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- Minimize and document time for obtaining samples. Too much variability in this across the country.
- Need surrogate indicators to better understand whether or not the sample is good.
- Coordinate metastatic lesions with primary tumor, perhaps using CTCs
- Consent and collection challenges generally need to be addressed/standardized
- Underserved populations with varying biology not sufficiently sampled.
- Increase supply to human samples for technology development and validation in order to avoid developing technologies that work well on animal samples but not human samples.