



DCP Division of
Cancer Prevention

**NCI-FDA-NIST Workshop on Standards in Molecular
Diagnostics for the Discovery and Validation of
Clinically Useful Cancer Biomarkers:**

Recommendations from the
National Cancer Institute
U.S. Food and Drug Administration
National Institute of Standards and Technology

Workshop: December 7, 2012
REPORT

Division of Cancer Prevention, National Cancer Institute
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Workshop on Standards in Molecular Diagnostics for the Discovery and Validation of Clinically Useful Cancer Biomarkers: Recommendations from the NCI-FDA-NIST

Executive Summary

Recent discoveries in cancer biology have greatly increased our understanding of cancer at the molecular and cellular level, but translating this knowledge into safe and effective therapies for cancer patients has proved to be challenging. More efficient transfer of new molecular tests into patient care requires that greater standardization of laboratory practices, measurement methods, and data management be put into place to ensure that all stakeholders involved are conducting studies and interpreting study results consistently.

The one-day **NCI-FDA-NIST Workshop on Standards in Molecular Diagnostic** was sponsored by the National Cancer Institute (NCI) in collaboration with the U.S. Food and Drug Administration (FDA) and the National Institute on Standards and Technology (NIST) to better understand disparate stakeholders' motivations, obstacles and challenges to moving promising biomarkers beyond the discovery laboratories into validation laboratories and ultimately into clinically accessible assays for the early detection and treatment of cancer.

The workshop focused on four areas: 1) Standardization of pre-analytical variables during specimen collection, stabilization, and processing; 2) Validation of the clinical and analytical performance of newly discovered biomarkers; 3) Evaluation of the analytical performance of the final diagnostic assay; and 4) Meeting regulatory requirements.

The workshop featured presentations and panel discussions by 20 experts from NCI, FDA and NIST and academia and industry and was attended by more than 60 participants involved in biomarker research. The outcome of the workshop was the development of a set of recommendations for standardizing many of the processes, materials and techniques involved in biomarker discovery.

The absence of common terminology, laboratory practices, measurement methods and other elements used by researchers and clinicians can bias outcomes and add time and cost to the process of validating useful cancer biomarkers.

Adhering to standards would ensure that a greater number of valid cancer biomarkers are translated into patient care as efficiently and smoothly as possible.

State of the Science and Current Challenges

Workshop speakers described the current environment in the United States in which more than 200 validated cancer biomarkers are in use in clinical practice with patients today but where myriad obstacles cause as many as 90 percent of candidate biomarkers to

fail to make the transition to common clinical and regulatory practice.¹

Workshop speakers described development of clinically useful, FDA-approved cancer biomarkers as a long process. Candidate biomarkers must endure a rigorous validation process, especially those that are intended to guide treatment decisions. Many problems associated with this process – from initial discovery through potential FDA approval – exist, for investigators and regulators. Through the course of the workshop, speakers and participants described the issues that set back progress, including: Laboratories that do not follow Standard Operating Procedures (SOPs) and guidelines for operating labs set by the College of American Pathologists (CAP); inadequate training of laboratory personnel in biostatistics and data analysis; investigators' failure to define the biomarker's intended use or clinical need before initiating a clinical trial and inadequately designed trials that lack power or invite results bias; lack of quality specimens from appropriate patient populations; lack of technology measurement reproducibility and transferability across labs caused by a shortage of materials for analytical validation and inadequate supply or use of reference data sets for investigators; poor communication between labs in inter-laboratory studies and between labs and the regulatory agencies.

In addition to the challenges faced by investigators, the Federal agencies (NCI, FDA and NIST) face demands from investigators for a streamlined consultation and review process for biomarker approval submissions to the FDA and a clear set of guidelines for submitting applications. The agencies struggle to keep pace with increasing demand for genomic testing, educating staffs on analytic test terminology and procedures, and a general dearth of uniform biomarker databases to store and share common data emerging from labs. The broader biomarker community lacks global biomarker standards, especially among the U.S., Europe and Japan, and operates on a daily basis in a decentralized environment for biomarker research, driven by how tests are funded and controlled and the varying technology capabilities of laboratories across the country.

RECOMMENDATIONS

The challenges elicited in the workshop point to the need for assays, materials and procedures to be standardized and for the quality of biomarker assay results to be continually monitored by proficiency testing and quality control measures. The challenges call for establishing a working environment within each laboratory in which standards are practiced and collaboration is encouraged. Workshop presenters emphasized that creating and adopting standards will require ongoing, active participation by virtually everyone involved in biomarker discovery and validation. It was generally thought that addressing these challenges successfully would help to accelerate biomarkers validation.

While many of the workshop presentations focused on procedures that take place in CLIA/CAP-certified reference labs, speakers encouraged discovery labs to incorporate guidelines early on in biomarker discovery work to enhance data confidence. In addition, investigators cannot pursue validation and approval of every protein or RNA they discover, but should educate themselves on

¹ Source: 2010 Stanford University meta-analysis

when an assay may be worthy of further testing, e.g., after verification in a small blinded set, and on ways to improve the initial discovery process, e.g., through use of miRNA standard reference material. Among all laboratory investigators, education on the importance of standards and how to adopt them is essential.

Workshop presenters and participants presented the following set of recommendations on what stakeholders can do to assure success in their areas of expertise.

Workshop Recommendations for Enhancing, Establishing and Enforcing Standards in Molecular Diagnostics

I. Biomarker Discovery and Development – What Labs Should Do

1. Enhance analytic accuracy and precision of biomarkers

While all CLIA/CAP-certified clinical diagnostic laboratories must strictly adhere to CLIA and CAP guidelines, discovery/research laboratories should incorporate some or all of the following recommendations in the early stages of biomarker discovery work to enable researchers to have confidence in their pre-analytical and analytical data:

- Document procedures to ensure proper specimen handling and storage; include written instructions for acceptable specimen criteria and a troubleshooting guide.
- Formulate written checklists for calibration and tolerance thresholds on equipment and instruments; include maintenance schedules.
- Formulate written SOPs for aliquoting of specimens; preparation of samples for nucleic acids or other products; and evaluation of quality and quantity of those products.
- Written SOPs should specify quantitative and qualitative controls, tolerance limits, corrective actions, statistics, and verification of review of all results.
- If possible, formulate written guidelines for analytic assay interpretations and potential clinical performance.
- Train and periodically test personnel to ensure that SOPs are adhered to.

2. Enhance the rigor of laboratories to ensure consistent results

- Improve the initial discovery process by developing miRNA standard reference material.

- Develop and follow SOPs and ensure that partnering labs are operating under the same SOPs to ensure consistent results and avoid bias.
- Create a culture in which lab directors encourage investigators to identify and air potential problems.
- Adopt formal, informal and ongoing investigator training on biostatistics, data interpretation and other topics.
- Understand the business of developing biomarkers and tests, to look for obstacles in the process that slow progress.

3. Improve trial design elements needed to test potential biomarkers

- Develop parameters to validate that a biomarker can be accurately measured, ensuring that it is associated with the clinical outcome of concern, and confirming that it is appropriate for the proposed use.
- Pay greater attention to intended clinical use of biomarkers to make efficient use of time and budget. Ensure the research assay is credible and research is believable in terms of intended clinical use before initiating testing.
- Institute more proficiency and parallel testing between two or more labs involved in trials; require proficiency testing conducted in a research lab as part of the process.
- Require biomarkers to demonstrate clinical and statistical significance as an independent variable in a multivariable analysis including stage, grade, etc.
- Instigate greater communication between research labs and clinical labs before commencing testing.
- Identify early the platform or procedure to use and discuss preparation for technology transfer with the CLIA/CAP lab to avoid delays.

II. Biomarker Evaluation and Approval – What NCI, FDA and NIST Should Do

1. Develop or strengthen guidelines for biomarker development

- Clearly define the steps from discovery to validation.

- Stage allocation of materials to enable testing in appropriate fashion.
- FDA should issue more detailed guidelines on approval submission requirements and make existing guidelines more accessible.
- NIST should develop more reference materials to be used for many of the molecular assays being developed.
- Consider development of additional translational labs.
- Increase oversight of biorepositories to ensure standards are being applied.
- Encourage stakeholders to look for already available FDA guidance on required data for FDA approval on FDA's website.

2. Streamline the biomarker approval process

- Break down the approval process into steps to indicate when biomarkers can qualify to move to the next step, to make the transfer process more efficient and cost effective.
- To speed the approval process, FDA should abolish the consultation step in the biomarker approval process.
- Give proper funding consideration to validation studies.

3. Require or encourage greater communication and interaction among the agencies

- Enhance training of IRB personnel on the biomarker development process.
- FDA should simplify language in its guidance to investigators on submitting applications.
- NCI and FDA should work together as early as possible in the development process to identify potential obstacles to biomarker validation and approval.

4. Develop uniform global lab standards

- Consider working with Europe, Japan and other international communities on developing standardized lab and testing requirements and procedures.

Conclusion

Workshop participants acknowledged several areas of progress in biomarker standardization over the past years, however were in unanimous agreement that greater progress must be made to speed delivery of FDA-approved cancer biomarkers for use with patients. Presenters expressed a sense of urgency that changes must be made quickly and broadly to keep pace with the rapidly growing field of molecular diagnostic research. Participants stated that all laboratories need to adhere to the standards that already exist; operate under rigorous SOPs; communicate more effectively with partnering labs and the Federal agencies (NCI, FDA, NIST); educate lab personnel on the discovery, development and validation process, and constantly look for ways to avoid the problems in study design and execution that often set back progress and stifle innovation. The agencies must continually seek input and consensus from the research community on where the process is hindered by too much or too little regulation; seek clearly defined requirements for biomarker approval; make tools and resources available for all stakeholder to promote consistency; and work together to continue to make improvements to speed delivery of biomarkers into clinical use.

Summary of Workshop Recommendations

I. Biomarker Discovery and Development – What Labs Should Do

- 1. Enhance analytic accuracy and precision of biomarkers by following CLIA/CAP guidelines**
- 2. Enhance the culture of laboratories to improve consistency**
- 3. Improve trial design elements needed to test potential biomarkers**
- 4. Clearly define the steps from discovery to validation**

II. Biomarker Evaluation and Approval – What the Agencies Should Do

- 1. Develop strong guidelines for biomarker development**
- 2. Streamline the biomarker approval process**
- 3. Require or encourage greater communication among the Federal agencies**
- 4. Develop uniform global laboratory standards**

Overview of the NCI-FDA-NIST Workshop on Standards in Molecular Diagnostics

NCI works closely with FDA and NIST to establish principles and guidelines for the development of validated biomarkers to accelerate the translation of cancer therapeutics from the laboratory into the clinic. Biomarkers hold great promise for the detection, diagnosis, and management of some types of cancer on an individualized level. Hundreds of potential biomarkers are tested for potential clinical use each year. Yet despite the heightened interest in personalized medicine, and considerable time and funds spent on biomarker research, only a small percentage of biomarkers have been successfully translated into validated diagnostic tools for use in patients. The deluge of molecular data available today presents challenges to researchers to find reliable ways to stratify the many kinds of cancer and degrees of cancer risk, and scientists at every stage in biomarker translation—from the initial idea to acceptance in clinical practice—are confronted with the problems inherent to an enterprise lacking in universal standards.

A newly discovered biomarker assay must move through clearly defined stages of assay confirmation to make the successful transition from a research setting to the clinical diagnostic laboratory. The validation laboratory's first task is to evaluate research assay technology, performance, and specifications (*analytical validation*), with the ultimate goal of validating the test to identify early stage cancer (*clinical validation*). After that, assays progress toward a standardized, reproducible, high-throughput format for clinical diagnostic implementation. With laboratory performance rigorously established, the clinical variables can subsequently be analyzed to define limitations, applications, and clinical utility.²

Scientists at every stage in biomarker translation—from the initial idea to acceptance in clinical practice—are confronted with the problems inherent to an enterprise lacking in universal standards.

Establishing standards in molecular diagnostics is an urgent issue in biomarker research. The absence of common terminology, laboratory practices, measurement methods and other elements used by researchers and clinicians research can bias outcomes and add time and cost to the process of validating useful cancer biomarkers. The field of biomarker research involves extensive interactions between academic researchers, biomedical developers, clinicians, and regulators, all with varying goals and ambitions. Stronger industry standards, adherence to laboratory SOPs, and clear guidance from the Federal regulatory

agencies would strengthen virtually all dimensions of biomarker development, validation and approval.

Purpose of Workshop

The one-day workshop sponsored by NCI in collaboration with FDA and NIST focused on several areas of biomarker development in need of standardization, including laboratory practices, measurement methods, and data standards. Over the past decade, the agencies have co-hosted

² Source: NIST

several workshops on this theme to move closer to understanding stakeholders' challenges to biomarker discovery to move the process beyond the discovery labs, into validation labs and ultimately into clinically accessible assays as efficiently as possible.

The workshop focused on four areas: 1) Standardization of pre-analytical variables during specimen collection, stabilization, and processing; 2) Validation of the clinical and analytical performance of the newly discovered biomarkers; 3) Evaluation of the analytical performance of the final diagnostic assay; and 4) Meeting regulatory requirements.

The workshop assembled more than 20 experts from the three agencies as presenters, and 60 attendees from industry, academia, and cancer centers. The intended outcome of the workshop was to educate participants on the state of the science in standardization of molecular diagnostics and develop a set of recommendations for improving the process of validating biomarkers for the early detection and treatment of cancer. The resulting report makes a series of recommendations for implementation by both investigators and regulators. Summaries of the presentations, discussions, and recommendations are contained in this report.

State of the Science and Current Challenges

Moderator Nadarajen Vydelingum, Ph.D., NCI, opened the workshop, stating that standardization has been a critical issue for several years but is more urgent now as new diagnostics become available and microarrays become routinely used for selection, assessment, and quality control. Dr. Vydelingum cited a 2003 report *Molecular Diagnostics: An FDA Perspective* that gave early impetus to the drive for standardization in biomarker research. The report called for the development of a co-operative framework between regulators, product sponsors and technology experts in order to realize the revolutionary promise biomarkers could have on the evolution of drug development, regulatory science, the practice of medicine, and public health.³ The 2003 FDA report was followed by a 2007 Institute of Medicine (IOM) report that called for a more organized, comprehensive approach to discovering biomarkers among the Federal agencies.⁴ The IOM report challenged stakeholders to develop improved methods, tools and resources for biomarker discovery and development, as well as better guidelines, standards and oversight, concluding that success will

The workshop brought together the three Federal agencies with primary oversight of the biomarker development enterprise: NCI (the research body), FDA (the regulatory body), and NIST (the standards body). Each agency plays a unique role in the process of developing and validating cancer biomarkers.

³ Expert Rev Mol Diag, 2003 Mar; 3(2):129-40 Molecular diagnostics: an FDA perspective. Ardekani,AM, Petricoin, EF 3rd, Hackett JL.

⁴ Cancer Biomarkers: The Promises and Challenges of Improving Detection and Treatment, IOM 2007 <http://iom.edu/~media/Files/Report%20Files/2007/Cancer-Biomarkers-The-Promises-and-Challenges-of-Improving-Detection-and-Treatment/biomarkers.pdf>

have a broad impact on biomarker development and help reduce the burden of cancer and other diseases.

The workshop included didactic presentation of several areas of biomarker development, including measurement, submission of applications to the FDA, and bioinformatics. The workshop included case studies from scientists involved in developing and validating some of the most commonly used biomarkers today, including CA 125 for ovarian cancer and PSA for prostate cancer. Two panel discussions focused on what has worked, what needs improvement and how to best to fill gaps in the process. Throughout the workshop, presenters described an ambitious field of investigators, regulators, industry and clinicians working together – and sometimes at odds – to develop the next fully validated cancer biomarker. Challenges will involve the entire scientific community because the vast majority of breakthroughs that show promise on the bench fail to meet the requirements of clinicians and regulatory scientists or make the transition to clinical and regulatory practice.

Challenges

Issues faced by laboratories

- Lack of laboratory Standard Operating Procedures (SOPs) in place;
- Failure to follow guidelines for operating labs set by the College of American Pathologists (CAP);
- Inadequate training of laboratory personnel in biostatistics and data analysis;
- Inadequately designed clinical trials that lack power or invite results bias;
- Failure to define the biomarker's intended use or clinical need before initiating trials;
- Difficulty in validating both the clinical and analytical performance of the diagnostic;
- Failure to meet high demand for quality assurance, scientific validity, maintaining consistent methodology, and quality control;
- Lack of quality specimens from appropriate patient populations;
- Lack of technology measurement reproducibility and transferability across labs;
- Shortage of materials for analytical validation and inadequate supply or use of reference data sets for cancer researchers;
- Lack of funding for validation studies;
- Poor communication between labs and among agencies;
- Poor understanding of regulatory requirements by investigators.

Issues faced by regulators and agencies

- Lengthy consultation and review process for biomarker submissions to the FDA;
- Lack of global biomarker standards, especially among the U.S., Europe and Japan;
- Lack of clear guidance from FDA on what makes a successful approval submission;
- Increasing demand for genomics testing;
- Lack of uniform databanks;

- Poor understanding of analytic test terminology by FDA and Institutional Review Board (IRB) personnel;
- Decentralized environment for biomarker research, driven by how tests are funded, control of studies, and technology construction across labs.

Response to Challenges – Emerging Themes of Workshop

Workshop presenters from NCI, FDA and NIST expressed their eagerness to take on the issues of biomarker standards but acknowledged it will require ongoing, active participation by many stakeholders to accelerate the pace of biomarker discovery and validation. Presenters stated that the challenges presented by their colleagues are critical issues for clinical research and that patient care, unfortunately, has not gotten proper attention until now.

It was generally thought that addressing these challenges successfully would help to accelerate biomarkers from discovery to clinical use. Workshop presenters offered advice on what stakeholders can do to assure success in their areas of expertise:

Follow CLIA and CAP guidelines to get reliable, reproducible results. Speakers described the importance of the Clinical Laboratory Improvement Amendments (CLIA) that certify laboratories, and the College of American Pathology (CAP) Laboratory Accreditation Program that inspects and accredits laboratories every two years. They stressed the imperative that research and clinical

Standardization has been a critical issue for several years but is more urgent now as new diagnostics become available and microarrays become routinely used for selection, assessment, and quality control.

laboratories strive to adhere to CAP guidelines “to the letter” as the only way to ensure consistency from lab to lab and to avoid biasing results. Following CLIA and CAP guidelines will become increasingly important as more developmental labs make the transition to becoming clinical labs. Following CAP guidelines is not just important for those with CAP/CLIA labs but also for scientists doing the groundwork in discovery labs to incorporate the guidelines at the beginning of assay development.

Use shared and common tools to expedite validation. NCI’s Sudhir Srivastava, Ph.D., MPH, described quality assurance as a major challenge to biomarker validation and urged labs to utilize standard reference sets developed by NCI’s Early Detection Research Network (EDRN) to overcome logistical and design issues in biomarker validation. EDRN’s creation of shared and common sets of specimens from well characterized and matched cases and controls from specific disease spectra enables provides investigators a common and transparent set of criteria used to evaluate applications.

Lack of comparability sets back progress. NIST’s Marc Salit, Ph.D., stated that comparability over space and time is an essential piece often missing in biomarker studies. To enable scientists to build confidence around a measurement, NIST and NCI’s EDRN are working to establish a systematic approach composed of traceability, measurement uncertainty, and validation. NIST is also working in the next generation of sequencing RNA to establish approaches for gene expression; developing

mixed tissue ratiometric controls for gene expression and for conducting complex sample development; quality system approaches, including proficiency testing; and study design standards.

Learn from experts and past examples. Among the case studies presented, Sanford A. Stass, M.D., described lessons learned from a study in which biomarkers being tested at his laboratory at the University of Maryland did not turn out to be as relevant as hoped, and provided suggestions for how the study could have been improved – through better defined SOPs, more rigorous criteria for assay parameters and data interpretation, continual proficiency testing, and greater quality assurance oversight of the two labs conducting the testing.

New challenges from whole genome assays. The FDA's Zivana Tezak, Ph.D., said that FDA efforts to regulate highly multiplexed genomic tests and policies are keeping pace in this rapidly changing field, including regulations for genetic tests and implications of moving whole genome sequencing into wider clinical use. FDA is working on new policies and approaches for valid instrumentation, quality tests, understanding test performance, and fostering databases by working with other Federal agencies.

Learn what is required for best practices in a laboratory setting. Laboratory personnel, including directors, often lack knowledge of what is required in conducting tests. Workshops such as this provide opportunity to learn from colleagues who have been successful in developing biomarkers to avoid mistakes and make good use of time and funds. To be successful, laboratory personnel need a plan; a commitment from their institution for financial resources, personnel, funds, space, and standardized equipment; a working environment and culture that makes it acceptable to bring up problems; and evidence-based practice, validation, consistency in testing and policy, and sustained quality control throughout the testing process.

Good communications and collaboration is critical. At least three sets of people must interact in the biomarker development process: 1) the scientist with the idea; 2) clinicians who must have the potential to apply the assay to patients; and 3) scientists in a CLIA/CAP lab who will perform, certify, validate and interpret the test and ultimately interact with the clinicians to provide that information. Clinicians who conduct clinical trials must collaborate with assay developers early in the process to understand what developers need to yield the best use of assays in trials.

A biomarker must be useful to physicians or there is no use in developing it. A major criterion for testing biomarker effectiveness is whether or not the biomarker is able to detect disease in a clinical setting. Researchers need to ask: Will the biomarker provide clinical benefit to the patient? Does the information improve diagnostic accuracy of cancer testing? Does the biomarker add new or more information? Will the biomarker help the clinician manage patients? If the answer is no to these questions, then the biomarker will have no meaning and will be of little use in detecting or treating cancer.

Knowledge of FDA regulations is important. The FDA's Lakshman Ramamurthy, Ph.D., and other speakers encouraged communication among investigators and FDA to resolve issues with applications for biomarker approval to avoid slowing down the process. FDA encourages interaction between lab directors and the FDA, especially when the lab director questions any decision made by FDA or IRBs. NCI is working with FDA to engage research lab personnel earlier in the process to better understand practices in research labs.

Clinical studies design needs improvement. The FDA has witnessed issues with the design of increasing numbers of clinical studies to test biomarker validity, especially in the areas of selection or verification bias. Studies often fail due to lack of defining clinical intended use. In 2011, FDA CTRR published guidance for clinical trial design to assist scientists in trial design.

Resources are available. Speakers emphasized the host of resources available to the research community to support assay testing, including the EDRN Resource Network Exchange that allows specimen databases to be viewed across the world through the EDRN portal, and FDA's set of guidelines for submitting successful applications.

Conclusion

In summary, workshop speakers presented a spectrum of challenges facing the biomarker research community and were in unanimous agreement that greater progress must be made to speed delivery of FDA-approved cancer biomarkers for use with patients. Presenters expressed a sense of urgency that changes must be made quickly and broadly to keep pace with the rapidly growing field of molecular diagnostic research. Participants stated that all laboratories need to adhere to the standards that already exist; operate under rigorous SOPs; communicate more effectively with partnering labs and the Federal agencies (NCI, FDA, NIST); educate lab personnel on the discovery, development and validation process, and constantly look for ways to avoid the problems in study design and execution that often set back progress and stifle innovation. The agencies must continually seek input and consensus from the research community on where the process is hindered by too much or too little regulation; seek clearly defined requirements for biomarker approval; make tools and resources available for all stakeholder to promote consistency; and work together to continue to make improvements to speed delivery of biomarkers into clinical use.

SUMMARY of Workshop Presentations

Workshop Introduction and Overview

*Nadarajen Vydelingum, Ph.D., FACB, NCI Cancer Biomarkers Research Group,
and Barry Kramer, M.D., M.P.H., NCI Division of Cancer Prevention*

As workshop moderator, Dr. Vydelingum welcomed participants and provided the workshop goals and format. Dr. Kramer followed up with comments on the workshop goals.

Dr. Vydelingum stated that it is difficult to imagine a scientific enterprise in the 21st century that does not have sophisticated standards and universally acceptable forms of standardization. In the medical sciences, well executed standardization programs greatly improve the quality of laboratory measures used to detect signs of illnesses, guide interventions to prevent or treat diseases, and assure production of credible and comparable data across laboratories, which is especially critical when extracting data from multiple sources and across disciplines.

The 2003 publication, *Molecular Diagnostics: An FDA Perspective*, describes several challenges facing the medical science community in the next decade, concluding that the development of a cooperative framework between regulators, product sponsors and technology experts will be essential for realizing the revolutionary promise these platforms could have on the evolution of drug development, regulatory science, the practice of medicine and public health.⁵

The goal of the workshop is to stimulate discussion across several areas of cancer biomarker research with an emphasis on challenges from the discovery phase to ultimate routine clinical use.

Dr. Vydelingum said that in 2012, “we are at that point now,” as new diagnostics become available and microarrays become routinely used for selection, assessment, and quality control. Realizing potential is a challenge to the entire scientific community because breakthroughs that show promise on the bench often fail to meet the requirements of clinicians and regulatory scientists and to make the transition to common clinical and regulatory practice.

Many of these challenges outlined in the 2003 report still exist. Within EDNRN, scientists have made significant progress in biomarker research, yet challenges in standardization of assay methods remain, including the need to: 1) Validate both the clinical and analytical performance of the diagnostic; 2) Standardize pre-analytical variables during specimen collection, stabilization, and processing; 3) Pay rigorous attention to the analytical performance and validation of the assay; and 4) Meet regulatory requirements.

⁵ Expert Rev Mol Diag, 2003 Mar;3(2):129-40 Molecular diagnostics: an FDA perspective. Ardekani,AM, Petricoin, EF 3rd, Hackett JL.

Dr. Kramer followed up on Dr. Vydellingum's remarks. The workshop goal, he said, is *to stimulate discussion across several areas of cancer biomarker research with an emphasis on challenges from the discovery phase to ultimate routine clinical use*. Dr. Kramer stated that investigators have spent years looking at better ways to diagnose cancer at sufficiently early stages to reduce mortality, however the challenges are many. While advances in molecular biology and genomics provide important tools for the development of early detection, prognosis and predictive markers of cancer, standardization and clinical validation of biomarkers is an arduous process.

Dr. Kramer said that much of the impetus behind this and other workshops came from the 2007 consensus report by the Institute of Medicine (IOM) that stated that improved methods, tools and resources are necessary for the discovery and development of biomarkers, as well as better guidelines, standards and oversight. The IOM report concluded that success will have a broad impact on the development of biomarkers and will help to reduce the burden of cancer and other chronic diseases, and called for a more organized, comprehensive approach to discovering biomarkers among the Federal agencies.

To address the IOM's challenge goals, Dr. Kramer said that NCI organized regular workshops over the past several years. EDRN has made considerable progress in developing platforms for developing and validating molecular diagnostics and creating Standard Operating Procedures (SOPs) to improve the validity of test results and quality assurance. However, the task is incomplete. Dr. Kramer stated the workshop's goals: provide an overview of the state of the science in standardization of molecular diagnosis; provide a survey on what has been successful; identify remaining challenges; describe submissions to the FDA; and discuss future priorities in research and practice.

The Role of Biomarkers in Early Cancer Detection

Cancer biomarkers are molecules that indicate the presence of cancer in the body, mostly based on abnormal mutations in genes, RNA, proteins and metabolites. Since the molecular changes that occur during tumor development can take place over a number of years, biomarkers potentially can be used to detect cancers early, determine prognosis and monitor disease progression and therapeutic response. Candidate biomarkers, however, frequently are found only in relatively low concentrations amid many other biomolecules, so both biomarker research and possible diagnostic tests depend critically on the ability to make highly sensitive and accurate biochemical measurements.

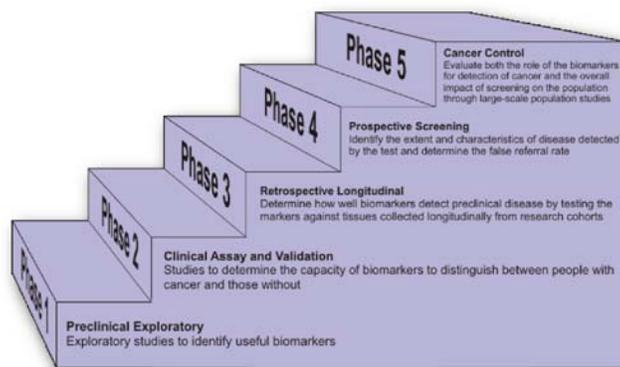
Biomarkers are made by normal cells as well as by cancer cells; however, they are produced at much higher levels in cancerous conditions. These substances can be found in the blood, urine, stool, tumor tissue, or other tissues or bodily fluids of some patients with cancer. Most tumor markers are proteins. However, more recently, patterns of gene expression and changes to DNA have also begun to be used as tumor markers. Markers of the latter type are assessed in tumor tissue specifically. Thus far, more than 20 different tumor markers have been characterized and are in clinical use. The value of biomarkers is great: As molecularly-informed research moves us closer to personalized cancer interventions with less toxicity, targeted testing using validated biomarkers to screen individuals at differing risk levels many one day take the place of general cancer screening guidelines. Source: NCI

Topic 1

Using Standard Reference Sets for Expediting Clinical Validation of Biomarkers

Sudhir Srivastava, Ph.D., M.P.H., NCI Cancer Biomarkers Research Group

Dr. Srivastava provided an overview of the challenges facing the biomarker research community, and areas of opportunity, including the use of standard reference sets. A major challenge to biomarker validation is quality assurance, he said. Lab work performed on convenience samples from cases and controls may have been collected in a variety of ways, making comparisons difficult. With the creation of shared and common sets of specimens from well characterized and matched cases and controls from specific disease spectra, EDRN has overcome many of the logistical and design issues in biomarker validation. Creation of standard reference sets from EDRN provides investigators a common and transparent set of criteria used to evaluate applications.



The five phases of biomarker validation.

Dr. Srivastava stated that many biomarkers fail to make it into clinical use because they have failed to prove their scientific validity, lack a sound methodology, have poor quality control, and lack specificity. He addressed these shortcomings and made recommendations for overcoming them, including through the use of standard reference sets.

To prove *scientific validity*, a test used to detect biomarkers must be associated with the occurrence of a disease and have a positive predictive value appropriate to the intended clinical use. To establish the clinical validity of biomarkers, data must be collected under an investigative protocol to demonstrate the benefit and risk that come from both positive and negative results. In terms of *methodology* – the way in which assays are designed and performed – scientists must have a rationale for selection of the target sequence, probe, and other elements, and a method for looking at single vs. multiple targets. To drive biomarker testing in a specific specimen type, a diagnostic lab must specify the kind of specimens it is using, what they are intended to do, and the clinical goal the lab is trying to achieve.

All laboratories must have rigorous *quality control* standards to avoid biasing test results. Laboratories engaging in high-throughput assays, as in genome sequencing, must have stringent internal controls, especially for contamination and quantitation standards, and well- characterized panels of reference reagents.

Dr. Srivastava noted that EDRN validates biomarkers through a five-phase process. Each phase has associated statistical conditions that must be met before moving up to the next step. As it moves up, evidence for the biomarker increases and the scientific validation of the biomarker increases. EDRN works mainly on the first three phases and works with cooperative groups and others on phases four and five.

National Cancer Institute

The majority of cancer biomarker research takes place in NCI's Early Detection Research Network (EDRN). Since it was established in 2000, EDRN laboratories have researched hundreds of biomarkers and through a process of well-defined methods, has identified a large number of candidate molecular markers in different cancers that represent both potential markers for novel diagnostics and targets for a new generation of therapeutic cancer agents. EDRN has developed a set of guidelines for study design in the discovery, evaluation, and initial clinical development of biomarkers, and maintains a list of currently available biomarkers and associated results on its website.

The assay must be validated in a Phase Two test in which EDRN verifies the performance characteristics of the test. The goal at this phase is for the assay to be analytically validated for each biomarker that it is intended to measure. The test must be easy, cost effective and not time consuming for it to pass to the next phase. The lab must validate its test using the same internal controls as those used by other clinical labs. At multicenter studies and multicenter crosschecks for pooled specimens, inter- and intra-laboratory interfering factors often exist. Dr. Srivastava noted that this stage is often where an assay fails.

EDRN has established reference sets, which are high quality, clinically annotated specimens (urine, plasma, serum, etc.) that are labeled as an EDRN reference. If a lab has a biomarker to prove they can use, it can

apply to have the biomarkers included as a reference set.

Dr. Srivastava provided an example of a test EDRN conducted on the process of validating SELDI for prostate cancer. After reviewing the study design, EDRN confirmed that SELDI is not an effective biomarker for detecting prostate cancer. Since the three-year study was issued, many clinicians stopped using SELDI as it was determined not to be a diagnostic platform. EDRN often conducts this type of study not just to validate an important quality finding but the finding that will lead to better improvement of future research. Dr. Srivastava recommended that there be a credited program for labs to promote standardization and that a formal training program be established in testing laboratories on how to interpret the data.

On the topic of quality control, Dr. Srivastava provided an example of the process of validating CA 125 for ovarian cancer. Under the five-step criteria, EDRN was able to narrow down the thousands of potential biomarkers for ovarian cancer to 120 markers and finally to 10 candidate samples. EDRN was challenged in working with scientists who agreed to test their biomarkers but who had different mindsets and interests. Many criteria led to the point in which EDRN had 10 promising biomarkers, whose platforms had the lowest CV (coefficient of variation), scored highest under ROC (receiver-operating characteristics), and had high specificity and sensitivity. In the end, EDRN found the top

performing biomarkers to be HE4, Decoy receptor 3, and CA 125. EDRN conducted final testing on this panel using preclinical samples and found that CA 125 outperformed the other markers. A major criterion for testing biomarker effectiveness is whether or not the biomarker is able to detect disease in a clinical setting. FDA ultimately approved CA 125 and HE4 in 2010.

Topic 2

The Role of Standards in Realization of Robust Molecular Biomarkers

Marc Salit, Ph.D., NIST Multiplexed Biomolecular Science Group

Dr. Salit addressed his vision for applying principles used in chemical metrology to biological metrology, or the measurement of results that builds confidence in results around which sound decisions can be made. To enable scientists to build confidence around a measurement, NIST and EDRN are working to establish a systematic approach composed of traceability, measurement uncertainty, and validation. Dr. Salit likened this approach to a 3-legged stool, in which each leg is indispensable to its purpose.

Traceability is the way in which results are tied to one another so two or more labs can share a common reference and results can be compared across space and time. Basic physical metrology, e.g., a pan balance or meter stick, and standards from the International Standard of Units and System Internationale are regarded as reliable reference sets. Biological measurement however has not proven to be particularly effective because most biological experiments have traceability as a control built into the experiment, as opposed to an external reference, presenting a shift from what is commonly practiced in chemical and physical measurement domains.

Measurement uncertainty is a way to estimate a reasonable expectation for the dispersion of measurement results about a truth. Measurement uncertainty of the dispersion ends in a circumstance in which one applies the measurement and elements in the measurement system as a coefficient of variants. For example, CVs are partial estimates of measurement uncertainty; but they do not encompass all the considerations one would have in quantitative uncertainty estimates. It is important to consider all possible sources of variability, including those that arise from calibration, lab-to-lab variability, and bias from different platforms, to be prepared for what could go wrong and undermine reliable measurement results.

Method validation involves developing an evidence base that ensures the measure being made is in fact representative of what is intended to be measured. If measurement interferences occur, they need to be assured that they do not sway results in such a way to lose confidence in making the measurement. A number of ways exist to arrive at method validation although they are not as reliable

Dr. Salit likened metrology to a stool with three equal legs constructed of traceability, measurement uncertainty, and method validation.



or precise a scientific endeavor as they should be. Scientists must be assured they are not reporting artifacts and that they have data and lab-to-lab repeatability. This is distinct from clinical validation and the ability to demonstrate the clinical utility of a biomarker.

[Dr. Salit defined several additional terms contained in the report's Glossary of Terms.]

National Institute of Standards and Technology

NIST is the measurement standards laboratory within the U.S. Department of Commerce responsible for advancing measurement science, standards, and technology in a wide variety of industries, including biosciences and health care. NIST collaborates with other organizations to provide new measurements and standards methods, tools, data, and reference materials for laboratory test methods to advance biosciences research. NIST's Multiplexed Biomolecular Science Group conducts research in measurement science, technology development and standards for measurements; establishes methods for asserting traceability, assesses measurement uncertainty, and validates measurements for multiplexed measurands; and establishes approaches for measurement assurance of multiplexed and multivariate measurands, including standards and methods development.

Dr. Salit noted the definitions of the "tools of the trade," defined by the Joint Committee for Guides in Metrology: *Measurement Uncertainty* is a non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used. *Metrological Traceability* is a property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty. *Validation* is the provision of objective evidence that a given item fulfills specified requirements where the specified requirements are adequate for an intended use.

Dr. Salit stated that his preferred definition of metrological traceability is: "property of the result of a measurement or the value of a standard whereby it can be related to stated references,

usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties." He emphasized *usually* because it might not be important that standards for biomarkers be related to national or international standards. Scientists need a reference and a way of comparing results against that reference in order to establish traceability.

On the issue of comparability of results, results are only useful when they can be compared to other results within a single study or to other results over a long term for a trend against a limit or threshold. Comparability over space and time is an essential piece often missing in biomarker studies. Comparability of results is essential to traceability; WHO standards are commonly used in biomedical measurements and enable comparability of results.

Measurement uncertainty can be challenging to assess quantitatively. It raises several questions: Can two values be compared? Are they the same or not, even if they have the same value but a different name and cannot be distinguished with any statistical confidence? How well is the result trusted? What has been done to get a good sense of the possible dispersion?

An emerging issue for the biomarker research community is how to validate markers in the present especially with new technology approaches of molecular profiling using sequencing. Traceability using molecular diagnostics is different from experiences in physics and chemistry. NIST is working to develop methodology approaches with type standards. Validating a method involves checking the measurement model, testing completeness, and assumptions. Method validation helps to establish an uncertainty budget, identify the parameters that need to be kept under control, and tests the scope of the model.

Dr. Salit posed several questions: Has the biomarker enterprise suffered from a lack of standards? How often are biomarkers frail instead of robust? How often do biomarkers fail to repeat in a new study in a different laboratory? Why doesn't one always get the same biomarkers performance on a different instrument platform? Where are biomarkers failing because of poor analytic validation, poor comparability, or poor study design? Are there opportunities to establish documentary standards for study design and does that fit for validation design? Are we already doing a good enough job at this? Does the enterprise not suffer with this problem? What are the possible roles of EDNRN in establishing best practices?

NIST is developing two kinds of analytical controls for genome scale measurements and analytical performance controls and type controls. Opportunities exist outside of these types of control development or quality systems which some may call the fourth leg of the 3-legged stool of metrology. Proficiency testing can aid in a quality system in laboratory accreditation, and is an essential quality of methods validation.

In conclusion, Dr. Salit said several standards opportunities exist:

1. Analytical performance controls, e.g., mRNAs, miRNAs, proteins and PTM-proteins, are external controls developed at NIST in collaboration with reagent developers working in the next generation of sequencing RNA to establish approaches or a dashboard for gene expression. NIST developed a set of mimics or messenger RNA and some methods for characterization, and is looking at establishing statistical tests to define the linear dynamic range of gene expression to support confidence in signatures for biomarker applications.

2. Type controls are standards that are effective mimics for clinical samples (complex, but very useful for method validation). NIST is developing mixed tissue ratiometric controls for gene expression. These approaches are useful for performing complex sample development that might be reference samples where the composition of the sample components is unknown but the difference between the two samples when the pair is developed is known. NIST is developing approaches for mixture samples that can be used to validate complex, multiplex measurements. NIST is working with Dr. Stass's lab at the University of Maryland to demonstrate the utility of this approach for conducting miRNA measurements and will be conducting inter-laboratory studies with EDNRN with this approach for miRNAs.

Among other NIST priorities are quality system approaches/accreditation, including proficiency testing, and establishing study design standards.

Topic 3

Standardization of Molecular Biomarker Assays from Discovery and Development to the Clinical Laboratory: Lessons Learned

Sanford A. Stass, M.D., University of Maryland School of Medicine, University of Maryland Medical Center

Dr. Stass provided a broad view of standardization related to molecular biomarkers, background on laboratory standards and testing, and lessons learned from managing his developmental and clinical laboratories at the University of Maryland, focusing on transferring molecular testing from a research lab to a clinical lab. At the end of his presentation, Dr. Stass offered several recommendations for improving the molecular biomarker standardization process.

Dr. Stass described the importance of the Clinical Laboratory Improvement Act (CLIA), established by Congress to certify laboratories, and the College of American Pathology (CAP) Laboratory Accreditation Program that inspects and accredits labs every two years.

During a research or clinical laboratory inspection, CAP evaluates laboratory SOPs, quality control procedures, the staff and director qualifications, laboratory equipment and facilities, and overall laboratory management, among other features. Using a checklist, CAP evaluates a laboratory's analytical sensitivity, analytical specificity, calibration, and other criteria against a gold standard. The CAP checklist includes a range of issues that the lab must address to become accredited. CAP also evaluates laboratory-developed tests (LDT) developed in the clinical lab. LDTs are not FDA approved but can be implemented based on appropriate validation. Laboratories that fall short on a CAP

Cancer Biomarkers in Clinical Use

A number of biomarkers are currently in use by clinicians for a wide range of cancer types. Although most of these biomarkers can be tested in laboratories that meet CLIA requirements, some cannot be and may therefore be considered experimental. Some markers in common use are: CA 125 to help monitor patients with ovarian cancer; HER2/neu to determine whether treatment with trastuzumab is appropriate for breast cancer patients; prostate-specific antigen (PSA) to help diagnose, assess response to treatment, and look for recurrence of prostate cancer; and 5-protein signature (OVA 1) to pre-operatively assess pelvic mass for suspected ovarian cancer, among others.

inspection are subject to either a Phase I citation, which covers violations that do not seriously affect the quality of patient care or endanger laboratory work, and require a written response, or a Phase II citation, given when a violation may seriously affect the quality of patient care or health and the safety of hospital or laboratory personnel. A Phase II violation must be corrected with a plan of action and supporting documentation that the plan is being implemented.¹

Dr. Stass was a founding member of the CAP Molecular Pathology Resource Committee in 1991. The committee established the national standards for operation of clinical molecular diagnostic laboratories and criteria for their inspection and accreditation throughout the country. Criteria were included based on knowledge of clinical and research laboratories at the time. CAP regulations change with time. Today, an important issue in laboratory accreditation is neoplastic cell content. CAP requires that when using paraffin embossed tissue, 5 percent of the tissue must be neoplastic content to prevent an extraneous event from lacking appropriate tumor present. Another issue is gene sequencing, whereby CAP requires assay optimization and cross documentation.

Case Study: Assay Qualification Study 1

Dr. Stass provided a case study in which biomarkers being tested did not turn out to be as relevant as hoped, and provided suggestions for how the study could have been improved. In this case study, Dr. Stass's CLIA/CAP-certified laboratory, the University of Maryland Baltimore Biomarker Reference Laboratory (UMB BRL), was involved as a quality assurance reference laboratory conducting a validation assay for microsatellite analysis (MSA) for detection of bladder cancer, along with a CLIA/CAP-certified reference lab in Virginia.² Dr. Stass's laboratory was only to provide quality assurance on 10 percent of the blood and urine samples. All tests were blinded. The assay – a short tandem repeat/polymerase chain reaction (STR/PCR) analysis of 15 previously validated microsatellite markers located within 14 gene loci – had an expected assay sensitivity of 83 to 95 percent and specificity of 100 percent for normal vs. abnormal determination of the sample pair. Dr. Stass said the task seemed simple – two CLIA labs were testing the same assay, both had SOPs, and both were experienced in conducting this type of test. However the process exposed problems.

Prior to validation, Dr. Stass's lab conducted five rounds of qualification testing on previously extracted DNA from blood and urine sample pairs. The samples were split and jointly assayed by the two laboratories and sent to a main data center to assess and assure concordance. The first round of testing revealed just 78 percent concordance, although the two laboratories were working from a previously developed SOP. The laboratories identified a difference in the primer pairs that were used. A second qualifying round revealed 73 percent concordance and the need for a new positive control. The laboratories had established as a variable a clear acceptance criterion for allele peak heights so analyzed additional data results. In the third round, the SOP was refined in terms of interpretation and acceptance criteria, and the test attained 87 percent concordance. In the fourth round, new reagents, a new PCR master mix, and high performance liquid chromatography (HPLC) primers were used, and an analysis of peak heights was again an issue. Prior to the fifth round, the laboratories performed an unblinded study to determine optimal conditions for a number of factors, including electrophoresis conditions, and finally reached 92 percent concordance. The concordance for loci that were evaluable for both laboratories was 96 percent and the overall evaluation of the samples (positive or negative for cancer) was identical (20/20).

Dr. Stass described several lessons learned from this nearly yearlong study:

- Very early on in the testing process, the quality assurance laboratory should be involved in development of the SOP and the data interpretation.
- The laboratories should perform unblinded parallel studies during the assay development phase to assess efficacy and include instrumentation reagent and the general assay performance. (These were two CLIA-certified labs; research laboratories may present other issues.)
- Better criteria for assay parameters and data interpretation are needed; the interpretation guidelines needed to be revised for consistency between the two labs.
- The SOPs needed to be defined better.
- Continued proficiency testing is needed in the research lab and in both CLIA/CAP labs.
- Better quality assurance oversight was needed.

Case Study: Assay Qualification Study 2

Dr. Stass presented another case study: MiRNA Assay Discovery Laboratory to Clinical Laboratory.^{3, 4, 5} Dr. Stass referred to the miRNA assay that Dr. Salit discussed earlier in the workshop. Dr. Stass's laboratory is working on this assay using the reference assays discussed. The reference assays are materials based on cell mixing. miRNAs have different levels of expression and potentially different effects in terms of identification. Although Dr. Stass's laboratory set up a reference sample for these miRNAs (which are of interest because of the development lab) for each miRNA that was developed in other labs, the research lab is required to do its own mixing study with those relevant miRNAs to establish the reference test.

Dr. Stass explained that there are different ways of conducting miRNA sequencing and standardization and conducting quantitative reverse transcription PCR (qRT-PCR). His lab is conducting qRT-PCR because of their experience in it. He stated that this is a basic tenet of moving from a research lab to a clinical lab. He provided an example of an assay typically used in a clinical lab. His lab had an issue in which it used a piece of equipment that is not typically used in a clinical lab, presenting significant delays. Dr. Stass's lab has established a standardized protocol for miRNA. His lab developed guidelines for inter-laboratory result interpretation and is creating a set of standards for these miRNAs that can be used to conduct a clinical trial on detection of stage 1 non-small cell lung cancer. His lab will use this as a reference material when testing clinical samples.

Dr. Stass described another test on the application of a vimentin gene methylation assay as a potential biomarker for colon cancer, along with Sandy Markowitz, M.D., Ph.D., at Case Western University and Dean Brenner, M.D., at the University of Michigan. The labs are looking at aberrant methylation of exon-1 sequences within the non-transcribed vimentin gene - a novel molecular biomarker of colon cancer that can be successfully detected in fecal DNA. It was previously reported sensitivity of 50 percent and specificity of 90 percent. Dr. Stass's laboratory will be the CLIA/CAP-

certified reference lab for vimentin assays using real time PCR SOP being developed for transfer from Dr. Markowitz's research laboratory to UMB-BRL.

During this study, several issues emerged. UMB-BRL has conducted methylation studies on other biomarkers but this is a unique assay developed by Dr. Markowitz at Case Western. UMB-BRL has run parallel samples to establish a consistent level of sensitivity for methylated and unmethylated vimentin appropriate with the assay developed by Dr. Markowitz, and is currently evaluating buffer sensitivity using ABI equipment and a universal buffer. Two types of equipment are involved: Biorad at Case Western and ABI at UMB-BRL. The labs are addressing issues related to buffer type, presence or absence of ROX, use of HPLC purified primers, and parameters for performing standard curve. Dr. Stass said these are interesting issues because his clinical lab has had experience with this type of assay. UMB-BRL and Case Western are discussing the best way to perform the assay in the UMB-BRL CLIA/CAP environment.

Dr. Stass offered his conclusion of lessons learned in this second case study:

- Developmental or research laboratories have been involved in assays long prior to the discovery of biomarkers. Their SOPs often have been modified making it difficult to ask a lab "what's your SOP?" and "why did you do this?" making it difficult for the CLIA lab to track what has been performed.
- The reference lab should identify early a platform or procedure it will use for technology transfer and discussed it with the CLIA lab. Assays used by research labs that are not standardized can present delays because of a piece of equipment that was not typically used may impact results.
- Lack of appropriate quality control in the research lab is an issue. A research laboratory's test costs may be different from a clinical lab because they did not include controls, standard curves and other issues.
- Better and earlier communication between the research lab and the clinical lab is needed. The development lab may use different methods for the same assay than what the clinical lab is using that may be difficult to transfer.
- It can be difficult to know how the research lab calculates its results, and whether the specimens and reagents are not stored appropriately, and if they need to better document labeling and tracking.
- Adequate staff training is needed to avoid inconsistencies. All staff might not follow the SOPs, instead doing things their own way.

Dr. Stass offered the following recommendations:

- Early in the process, have a conversation between the research labs and clinical labs;
- Proficiency testing and parallel testing are needed between the two labs;

- Working with NIST, develop more reference materials to be used for many of the molecular assays being developed;
- Develop a translational research lab that is CLIA-CAP-accredited.

Dr. Stass concluded that in 2010, he was involved in starting a CLIA-accredited translational genomics lab in a research environment at the University of Maryland. He had observed that the university's School of Medicine had a number of research activities that were such low volume that they were not cost effective to be conducted in a clinical lab. The translational lab proved to be a bridge between the clinical research in the School of Medicine and the clinical laboratory in the Medical Center. The translational lab provides genomic tools for translational research for clinical trials and includes data analysis and genome sequencing.

Discussion

A workshop participant pointed to industry studies that reveal that up to 90 percent of biomarkers that start out in basic research studies fail to get validated and asked whether instituting standards would increase that number. Dr. Stass said that his example of MSA was one such test that did not get validated, not necessarily because of the assay but it did not appear to be a biomarker that could be used. That experience differs from the miRNA assays being conducted in his development lab in which early results have been promising. An important element in a marker's failure could be lack of appropriate validation in the reference laboratory – a fundamental issue that needs discussion. If the research community is to invest heavily in a developmental lab to run a large amount of samples and intend to transfer them to a clinical lab, only to find them ineffective, one needs to ask whether the assay does not work because the appropriate test was not done, or the test was not appropriately validated and was not stringent enough, or was it not a good marker? Dr. Stass concluded that EDNRN should plan to embrace these questions. He also noted that, as part of the miRNA process, Dr. Sorbara's solution of proficiency testing, made earlier in the workshop, was wise and that EDNRN could be a model for requiring that proficiency testing done in a research lab as part of the process.

A workshop participant said another reason research assays fail is because they lacked spatial heterogeneity. Could this aspect be addressed during assay development? Dr. Stass reiterated that when clinical labs conduct assays, they must assure a minimum of 5 percent tumor in the specimen being tested. Many assays are conducted using blood or urine but if the test is done on tissue the lab must be sure to have a good specimen and a minimum amount of tumor present in the specimen to be assayed. If the tumor field type changes over time and the lab does not have the original sample to go back to and reanalyze, then it must use a broader panel based on known variations of the tumor. For example, in Leukemia, evolution in certain genetic changes occur in which the assay can be broadened to include changes that might be present in an evolving specimen.

- 1 College of American Pathologists (CAP) Molecular Pathology Checklist, Sept. 25, 2012.
- 2 Helber, S., Ames, J., KuKuruga, D., Lewis, L., Poore, B., Agbo, E., Reynolds, T., Kagan, J., Srivastava, S., Thornquist, M., Schoenberg, M., Stass, S. Demonstration of Performance

Qualification of the Microsatellite Marker Assay for Detection of Bladder Cancer. Presented at the NCI Translational Science Meeting, Washington, DC, November 7-9, 2008.

- 3 Jiang F, Todd NW, Qiu Q, Liu Z, Katz RL, Stass SA. Combined genetic analysis of sputum and computed tomography for noninvasive diagnosis of non-small-cell lung cancer. *Lung Cancer*. 2009 Oct; 66(1):58-63. Epub 2009 Jan. 31.
- 4 Xie Y, Todd NW, Liu Z, Zhan M, Fang H, Peng H, Alattar M, Deepak J, Stass SA, Jiang F. Altered miRNA expression in sputum for diagnosis of non-small cell lung cancer. *Lung Cancer*. 67(2010) 170-176.
- 5 Shen J, Stass SA, Jiang F. MicroRNA as Potential Biomarkers in Human Solid Tumors. *Cancer Letters*, Nov. 27, 2012.

Topic 4

CLIA/CAP Standardization from the Ground Up

Lynn Sorbara, Ph.D., NCI Cancer Biomarkers Research Group

Dr. Sorbara expanded upon Dr. Stass's overview of CLIA and CAP requirements and relayed experiences from starting her own laboratory. She stressed the importance of following the CAP checklist "to the letter" to not only gain laboratory accreditation but to improve laboratory performance in all facets of research.

Dr. Sorbara told a story of baking as a metaphor for a laboratory SOP. Her mother had a recipe for cheesecake that she had made for years. When Dr. Sorbara tried her mother's recipe, the cheesecake did not turn out like her mother's, even though she followed the recipe carefully. She repeated the recipe several times with the same results. Finally, she asked her mother why her results were different. Her responded was: "Yes, the recipe says to use three eggs, but I use five eggs. It says to use one cup of sugar, but I use two cups," and so on. Dr. Sorbara's mother had changed the recipe over time but never wrote the changes down. When her mother rewrote the recipe using the measurements she now uses, the cheesecake Dr. Sorbara made came out well. The lesson is: When you follow an SOP and if the SOP is correct, you have reproducible results. If you change the SOP, your results will vary. In the kitchen or in the laboratory, it is the same situation.

Laboratory Standardization

For measurements in laboratory medicine, it is important that laboratory results will be comparable or standardized and be independent of the laboratory where the testing was performed to allow correct medical interpretation. Routine measurement procedures that are traceable to the same system of reference standards should produce numerical values for clinical samples that are comparable regardless of time, place, or laboratory generating the result. Standardization of laboratory measurements is key to providing accurate and reliable results from investigational studies and for optimal patient care.

With the proliferation of potential cancer biomarkers due to genomic research, the number of laboratories involved in biomarker testing has grown, and more peripheral laboratories have become involved in providing routine testing. Consequently, the need for laboratory standardization is great. A standardized system is one in which each laboratory at the same level uses the same techniques, equipment and SOPs, generating consistent, comparable and reliable test results.

Dr. Sorbara stressed that CAP and CLIA requirements are not just important for those with CAP and CLIA-certified labs but for researchers doing the ground work in discovery labs. Those who know the parameters can start incorporating them at the very beginning of assay development. Dr. Sorbara said that down the road, the analytical validation of the assay will be much easier and there will be a clearer path for having a valid, clinically useful biomarker.

Dr. Sorbara referenced CAP's Molecular Diagnostics Checklist, designed to rigorously determine all aspects of regulation for a lab to be certified. CAP and CLIA are vital to ensuring reliable, reproducible and trustworthy assays. It is important to point out that many of the tests conducted for clinical use are not FDA-approved. For these tests to be clinically useful and reliable, standardization or accreditation is needed. LDTs may or may not be FDA-approved. If they are approved but at some point are modified, e.g., a different collection mechanism is used, or a sample is stored, it is considered an LDT and needs to be reevaluated and accredited by CAP or CLIA.

Dr. Sorbara said that much of what she did in her lab – gene rearrangement studies for malignancies and soft tissue tumors – were LDTs. All labs conducting the same testing are evaluated by CAP the same way, regardless of size or location of lab. A CAP inspector accompanies each team during the evaluation. If there are any phase violations, the inspection teams call a summation conference in front of colleagues to know what to improve upon.

Dr. Sorbara offered advice from managing a basic science lab: The most important activity to remember to do is to document everything. This includes test results, specimen handling, storage and preservation, quality control and other activities. For example, on specimen handling, she said CAP looks for documentation and type of specimens, why a specimen might be rejected, what the lab plans to do if the specimen does not meet its criteria, how to reject a sample and dispose of a sample, how to report results, and the clinical performance of the assay. She stressed the need to have proof of possession, like in forensics. Laboratories need to know from the time a specimen comes into its hands to when the result is reported, a specific and unique identifier must be carried through in all documentation of that specimen.

On the topic of storage and preservation, CAP requires documentation of how long a sample is stored, proof that that sample is still good after the preservation time, that SOPs exist for processing and preparation of samples, and other issues so at the end of the isolation the lab knows the sample is good and in not, has a plan for disposing of it.

Dr. Sorbara said that an issue she found difficult but necessary is having to “quality control” every component of the assay. If a lab uses a lot of buffer and changes the lot, it must go back and perform a quality control check against the older lot to ensure there was proper results, and if not, perform trouble shooting. For the assay itself, labs need qualitative and quantitative controls, know what their tolerance limits are, and have corrective actions in place if they do not meet tolerance limits. Every lab needs a written protocol of how it will record quality record statistics, who reviews statistics and who

signs off on them. She said the CAP checklist “goes on and on” in terms of specific guidelines on assays.

Dr. Sorbara said a major issue transitioning from a basic lab to a CLIA-accredited lab was equipment. One might have a machine in a basic lab that was never calibrated but will be unacceptable in a CLIA-CAP lab, where equipment must have routine calibrations and documentation. Each well must be documented and the lab must have a planned, scheduled trouble-shooting plan if the well does not meet requirements. CAP and CLIA labs must have comparability of instruments and methods so if one part of the lab uses one kind of centrifuge and another part uses another, they must be calibrated together. The same requirements apply to instruments. Labs must take calibrations every day on balances, power supplies, freezers, refrigerators, water baths, incubators, gels, etc.

Dr. Sorbara concluded that these requirements may be daunting, especially if lab personnel is not used to them, but they become easier once you have a routine. “It’s like brushing your teeth,” she said. “In the long term it saves you hundreds of thousands of dollars if you’re barking up the wrong tree or if you’re using the recipe card for the cheesecake that is the wrong card. Even in a basic science lab, incorporate some of these things into your daily routine so that ultimately you will have a much better analytical evaluation of your assay.”

Topic 5

Case Study: Regulatory Aspects When Reviewing a Device – PCA3

Nisar A. Pampori, Ph.D., FDA

Dr. Pampori provided a case study of what FDA learned from the PCA3 assay review process and provided a review of intended use elements and analytical studies performance.

Following biomarker discovery and assay development, the FDA reviews the clinical and analytical studies performance of the assay and sees that there is a reasonable assurance of safety and effectiveness and valid scientific evidence before the device moves into the clinics.

Dr. Pampori noted several challenges exist between the research community and FDA. 1) A general lack of knowledge of FDA requirements in the research community. 2) Differing goals – NCI has research/exploratory goals and must establish relationship of biomarkers to the disease; FDA has regulatory goals of assuring safety and effectiveness of a test, including adequate clinical and analytical performances. 3) Significant growth in new technologies and increased public expectations for rigorous review.

FDA reviews six types of device submissions: Investigational Device Exemptions (IDE); Premarket Approval Applications (PMA); Premarket notifications [510(k)]; Device Classifications Requests

[513(g)]; Humanitarian Device Exemptions (HDE); and Pre-Submissions. FDA also reviews de-novo and CLIA waivers.

Three regulatory classes of devices – investigational devices, approval applications, and notifications and classifications – are based on the level of control necessary to provide assurance of safety and effectiveness. If risk is very low, premarket submission is exempt in most cases. With medium risk, FDA has premarket notifications with special controls in place. If risk is high, FDA requires premarket applications, quality system requirements, and premarket approval, and evaluates the safety and effectiveness of the device.

U.S. Food and Drug Administration

The FDA's Center for Drug Evaluation and Research (CDER) ensures the safety and effectiveness of available drugs, including over-the-counter, prescription and generic drugs, and biological therapeutics. CDER's Biomarker Qualification Program works with laboratories to provide guidance for regulatory submissions of candidate biomarkers.

The FDA's Office of In Vitro Diagnostics and Radiological Health (OIR) in the Center for Device Evaluation and Radiological Health (CDRH) regulates in-home and laboratory diagnostic tests (in vitro diagnostic devices, or IVDs); radiological medical devices; radiation-emitting non-medical products; and implements the Mammography Quality Program. To foster innovation, assure patient access to safe, effective and high quality medical devices and prevent the marketing of unsafe or ineffective devices, OIR combines pre-market and post-market responsibilities into one multi-disciplinary office. OIR is responsible for ensuring safety and effectiveness of in vitro diagnostics devices, which include biomarker-based laboratory assays for clinical use.

Risk is dependent on the intended use of the device, for example, whether it is to be used for screening, diagnosis or prognosis. One test might be used for different purposes, for example, a molecular assay intended for cancer screening in asymptomatic patients is considered a high-risk device but if it is used for prognosis, after patients are diagnosed, it is considered of moderate risk. When reviewing a device submission, FDA looks at the analyte, technology, and result outcome, e.g., quantitative, semi-quantitative or qualitative (with two outcomes, either negative or positive, or with three outcomes, negative, equivalent or positive). FDA also looks at the intended

patient population, clinical use (diagnostic, prognostic, screening, monitoring, predictive, etc.) and intended users (clinical lab, home use, over the counter, etc.).

Dr. Pampori provided a case study of the PROGENSA PCA3 assay, an in vitro nucleic acid amplification test used with patients who have previously had a prostate biopsy. This was the first test reviewed by FDA that measured PCA3 and PSA for prostate cancer. The patent for PCA3 was applied for in 1997 after a long route of discovery.

Dr. Pampori stated that of the approximately 1 million prostate biopsy procedures conducted in the U.S. each year only approximately 25 percent detect the presence of cancer. The theory behind the PCA3 assay is to reduce the number of biopsies conducted through use of the test. The PCA3 assay

measures the concentration of prostate cancer gene 3 (PCA3) and prostate-specific antigen (PSA) RNA molecules, and calculates the ratio of both molecules (PCA3 Score) in post-digital rectal exam (DRE) first catch male urine specimens. The PCA3 assay is intended for use in conjunction with other patient information to aid in the decision to conduct a repeat biopsy in men age 50 and older who have had one or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended by a urologist based on current standard of care, before consideration of PCA3 assay results. A PCA3 score lower than 25 is associated with a decreased likelihood of a positive biopsy. PCA3 is a “rule out test.”

When reviewing the assay, FDA asked: What are the important issues of the clinical validity of the test? Does the test have a relationship with the expected clinical presentation and if so, how reliably? FDA reviewed the assay’s clinical performance, including clinical sensitivity, or how often the test detects patients with disease (the frequency of false negatives). Because a PCA3 score of less than 25 is associated with a decreased likelihood of a positive biopsy at the next biopsy visit, the potential for a false negative result means the patient will not get a biopsy. FDA also looked at clinical specificity, or how well the test detects patients without the disease (the frequency of false positives). In the PCA3 assay, the implication of a false positive result is that the patient will get a biopsy, which is no different from recommendations under standard-of-care. For PCA3, clinical sensitivity should be high enough that the negative predictive value will be clinically acceptable.

FDA reviewed specific performance characteristics of the PCA3/PSA assay. The test is intended to rule out patients who need a repeat biopsy, so the negative predictive value is important. With the PCA3, the higher the negative predictive value (NPV), the better. In this case, NPV was 90 percent. FDA conducted multivariable logistic regression analysis to determine whether the addition of the PCA3 assay information improved diagnostic accuracy over the standard-of-care information that is currently used for repeat biopsy decisions, including age, family history, number of previous negative biopsies and other information. In this analysis, the odds ratio (OR) for PCA3 Score, expressed as a binary categorical variable (positive or negative using a cutoff of 25) was statistically significant. (OR = 4.56 [95 percent CI - 2.65, 7.83])

In the clinical study of 466 men recommended for repeat biopsy, 231 had a PCA3 score of less than 25 and 235 had a score of more than 25. After 6 to 12 months, among 235 patients with negative PCA3 scores, 208 men turned out to have negative biopsy results and 23 had positive biopsy results. This meant that 90 percent (208 out of 231) of the men were saved from repeat biopsy and the decisions were correct. The other 10 percent (23 out of 231) were saved from repeat biopsy but if they would have repeat biopsies their biopsies would be positive, meaning these patients will get a delayed diagnosis. Patients enrolled in the study were already recommended to receive a repeat biopsy so a warning was added to labels: “The study only included men who were recommended by urologists for repeat biopsy. Therefore, the performance of the PCA3 assay has not been established in men for whom a repeat biopsy was not already recommended.”

In a subgroup analysis, certain groups did not perform well, including those men with atypical small acinar proliferation (ASAP) on their most recent biopsy. The NPV in this group was just 66.7 percent. For this purpose, another warning was added to the labeling: “The PCA3 assay should not be used for men with atypical small acinar proliferation (ASAP) on their most recent biopsy. Men with ASAP on their most recent biopsy should be treated in accordance with current medical guidelines.”

FDA also reviewed the analytical performance of the assay. When positive-repeat biopsies and negative-repeat biopsies are not well separated, precision studies near cut-off become important. Additional statistical modeling showed that 94 percent of study patients with a PCA3 score close to the cutoff of 25 had total imprecision of 14 to 18 percent (6 percent of patients had total imprecisions of 18 to 25 percent). (Additional simulation on the score was discussed in detail by Dr. Kondratovich later in the workshop.)

Due to normal assay variability, specimens with PCA3 scores near the cutoff of 25 (18 to 31) could yield a different overall interpretation of positive or negative upon repeat testing. Therefore, FDA added a statement in the labeling that states: “PCA3 scores in the range from 18 to 31 should be interpreted with caution.”

Finally, Dr. Pampori noted that in the PCA3 assay, FDA was challenged in validating specificity of multiplex assays. When reviewing analytical specificity performance of the device it was difficult to ensure whether the test was measuring an analyte specifically and not something else, and to state with certainty that the test specifically identifies and measures the analyte.

Discussion

Discussion centered on how and who makes the cutoff decision. Dr. Pampori said that selection of the cutoff at 25 was up to the laboratory. FDA looks only at whether the lab has determined the cutoff point before starting the clinical study, and if so, whether the clinical study test works with that cutoff level. One participant noted that Dr. Pampori had discussed why a simple cutoff makes little sense to most people – that small vagaries from testing from lab to lab can go from positive to negative. He asked why the “grey zone” is not part of a commercial product and why FDA allows a product to proceed when simple cutoffs do not make sense. Dr. Pampori replied that FDA asks when a sponsor proposes a cutoff, that they have samples in the precision studies around the cutoff zone and that they demonstrate ability to call the positives positive and the negatives negative. A sponsor must demonstrate analytical and clinical validity for the cutoff. It is possible that samples close to the cutoff are inconclusive, and a number of tests with equivocal zone around the cutoff exist. He also noted that that every test has a zone in which the samples close to the cutoff can have different results under repeated testing and it is important to evaluate the span of the zone around the cutoff and the percentage of the intended use population in the zone. The precision study should have samples near the cutoff level. When a relatively high percentage of the intended use population is near the cutoff level, FDA requires the sponsor to demonstrate its acceptable analytical and clinical performance. If the decision is critical, a doctor may choose to watch the patient more carefully.

Another participant stated that the most optimistic performance of a clinical test is often during the pre-market clinical study, however real-life performance of the test can be lower once approved. She asked whether FDA considers post-approval studies to gauge actual test performance. Dr. Pampori replied that in some cases, FDA considers post-approval studies when additional information about test performance is required and post-market surveillance on issues that develop that may prompt recalls.

Dr. Pampori also addressed the issue of off-label uses of biomarkers, including using PCA3/PSA before conducting a first biopsy so that the patient might possibly not have to have a biopsy at all. He acknowledged that physicians may order use of any drug or device for off-label use, however FDA has not evaluated the PCA3/PSA test for this intended use.

Topic 6

The Role of Bioinformatics in Standardization

Kristen Anton, M.S., Dartmouth University

Kristen Anton provided an overview of the knowledge environment in bioinformatics. She described the concept of turning data into information and then into data structures and onto a knowledge circle to illustrate the concept of bringing data that is mapped to standards and integrated into richer information to develop wisdom. The bioinformatics field moves information to knowledge environments or knowledge bases, instead of databases, in a cyclical process: What is learned in the information phase is brought back to the data phase, and so on. When embarking on a project it is important to think how far to drive the information and how much data can be integrated to make it more useful and rich.

Ms. Anton described bioinformatics as the use of information technology to facilitate biology-related scientific tasks. From EDRN's perspective, it is similar to architecture. In biology, challenges are in defining and managing views of bioinformatics models that are important starting points for systems, model tracking of validation, consistency, and enabling real-time access to information that crosses institutional boundaries. EDRN sources are in many places. Biomedical information is decentralized, driven by how research is funded and studies are conducted, and technology use within labs, with some having strong informatics structure and others using spreadsheets.

In theory, Ms. Anton said that science informatics needs to be unobtrusive and science must drive bioinformatics. The goal of bioinformatics is to support, not hinder, science-driven research needs. The data model, which brings standards to the science, is often overlooked. Data ownership, user training, and security requirements are also important issues.

Ms. Anton described how EDRN has worked with NASA to develop a biomarker-centric infrastructure to enable real-time access to information that crosses institutional and international boundaries for the

many institutions working on identifying and validating biomarkers. EDRN strives for coordinated discovery and validation of biomarkers across cancer research centers, facilitation of analysis through data integration and single point access to data sets, and supporting work flows. EDRN has worked to link highly diverse systems together with data types and structures, providing tools and other resources. EDRN's focus is to link data together to enable accountability when a specimen is collected and link the metadata around the activity to when the specimen is used in an experiment and tied to results.

Data models allow for relationships between and among objects. Metadata describe the inception, composition of data, and multiple schemata to make the metadata machine usable so the engine in the EDRN system can be searchable across the knowledge system and has a common language associated with common data elements that have uniformed research identifier (a URL that points to a page that describes standards for each data element).

Ms. Anton described helpful bioinformatics tools for organizations that do not have substantial data support: the EDRN Resource Network Exchange (ERNE) that allows specimen databases to be viewed across the world through the EDRN portal, and the Validation Study Information Management System (VSIMS), a standard system for validation studies that allows common data elements to provide information about participants. Red Cap is an open source database with EDRN common data elements for EDRN investigators to obtain products through the EDRN portal. The EDRM Catalog and Archive System (eCAS) is a catalog of data files. All EDRN tools were developed in collaboration with standards groups to make them comparable. EDRN is currently working with the Clinical Proteomic Technology Assessment for Cancer (CPTAC) to process mass spec data to provide a standardized process, bring files into eCAS and allow investigators conducting proteomic studies to use the validated pipeline.

In summary, Ms. Anton said that EDRN has seen tremendous success from a bioinformatics perspective. The EDRN database holds more than 4,000 curated biomarkers and is growing. In building the database, EDRN took an iterative approach, aimed not at having all software available right away but in starting small and allowing the system to grow. The database was built with open source tools available and architecture that can be laid on any data source. She concluded that bioinformatics is infused with standards and helps promote standards in the research that is supported by these systems.

Topic 7

The Role of the FDA pre-IDE as a Means to Improve Clinical Assays

J. Milburn Jessup, M.D., NCI, DCTD Diagnostic Evaluation Branch

Dr. Jessup's talk focused on the process of moving biomarkers into clinical trials and ultimately into the community for use with patients. He discussed the need for standardization, classes and uses of biomarkers, the FDA review and application process, and the role of the Institutional Review Board

(IRB) in this process. Dr. Jessup concluded this presentation with recommendations for improving the process and improving clinical assays.

Dr. Jessup stated that in cancer treatment, it is important to bring together clinicians who conduct clinical trials and assay developers to understand what the developers need to get the best use of the assays in trials. This is magnified when the assays are moved into clinical use. A small number of approved diagnostics are available today, with a recent increase in companion diagnostics. However, some of these therapies may benefit a marker-positive subset of patients than a marker-negative group, for example in non-small cell lung cancer. The main goal is to assure that trial results can be moved to clinical application. FDA has until now not enforced its regulatory oversight of LDTs but may be reconsidering this, as it places greater emphasis by NCI and other clinical research organizations to adapt more rigorous standards for diagnostics.

Certain protocols require extra consideration because their markers pose significant risk to patients such as protocols that include markers essential for trials because they require extra biopsies or pose a collection risk; or because the presence or absence of a biomarker may predict response to a drug and/or increased toxicity for patients.

Uses of Biomarkers

Integral biomarkers are essential for trial performance and are used for medical decision-making in specimen donors. For example, eligibility criterion, treatment assignment, risk stratification, and dose modification all must be performed in a CLIA-accredited laboratory. Integral markers (three types) are performed on all patients but are not used in decision-making, or they are performed by a predefined subset, for example, on quality of life studies, or to test a hypothesis.

There are five (or more) classes of biomarkers. Pharmacokinetic and pharmacodynamics are research markers that do not affect patient decision-making and do not have to be performed in a clinical lab. Prognostic, predictive, and pharmacogenomics markers, if used in patient decision-making, need to be performed in a CLIA-certified lab and may be subject to FDA's IDE review. An investigational device is used in a clinical study to collect safety and effectiveness data. Investigational use includes clinical evaluation of certain modifications or new intended uses of legally marketed devices. All clinical evaluations of investigational devices, unless exempt, must have an approved IDE before the study is initiated. An investigational device (assay and its marker) that does not pose a significant risk may be approved by an IRB but all IDEs that may have a significant risk need to be reviewed by the FDA. When questions arise about risk, the PI and assay developer should engage in pre-IDE review with the FDA.

Regulations

Several regulations revolve around significant risk. IDE regulations require that significant risk device studies follow all IDE regulations and have an IDE application approved by FDA. Integral markers are high risk, since the patient may be exposed to harm. The IRB and FDA determine significant risk. An

IDE may be bundled with an investigational new drug (IND) application (CDRH and CDER/CBER may do a bundled approval). Even if the marker and its assay are cleared of significant risk, the impact of the marker measurement on the patient must be assessed.

As defined by FDA, good clinical practices (GCPs) include the regulations and requirements that must be complied with while conducting a clinical study, and apply to the manufacturers, sponsors, clinical investigators, IRBs, and the medical device. Primary regulations are included in the Code of Federal Regulations, Title 21 (21 CFR).

Pre-submission guidance on medical devices

FDA recently released draft guidance on pre-submission for devices (assays) containing current recommendations on assay development and how to work with FDA offices. FDA requires an environmental assessment. When a laboratory fills out a pre-IDE review or pre-submission, it sets up an IDE application, which like the IND has a 30-day review period. The pre-IDE has been extended to 75 days. The elements of standardization and analytical performance are part of risk assessment and elements of assay assessment and elements of the trial document. One benefit for trialists is that the application for the pre IDE can be minimally modified to answer questions for the review. NCI's CTEP has an operational review working group. Trialists are under pressure to move quickly from concept development to trial activation. Any integral marker now has to go into pre and IDE review. This must be done in parallel as soon as the concept is approved to integrate it into the workflow with little delay.

FDA recently released guidance on IRB procedures on how to handle these issues because they can be difficult for IRB members to interpret. For example, what is the risk to patients of a false positive or false negative result? Many IRB members may not be versed in analytical performance, so an investigator must present it in a way an IRB can understand. NCI assists trialists and assay developers through its Clinical Assay Development Program, a resource of a network of CLIA-certified labs where they take an assay into readiness for clinical trials, and documents that allow for putting data on the analytical performance on validity into forms for assays, reviewed by CDRH and external panels.

Recommendation

Dr. Jessup concluded that IRBs may want their institution's protocol to include a section that concisely documents whether an IDE or IND is required, although sometimes this is not clear. For protocols that include integral markers, the risk of false positive and false negative assay results and their consequences should be described for patients and it may be necessary for IRB to include clinical assay developers on the IRB for protocols with integral markers.

In summary, he said that the IRB reviews the role of the protocol investigators, assay developers, performers, and sponsors to determine if an IDE or IND is needed. If the trial has an integral marker, then an IDE is likely and the IRB investigator sponsor needs a pre-IDE submission to FDA. He suggested laboratory personnel contact FDA early and often. It can be difficult for IRB and FDA to find

the risk attendant to an integral marker. The investigator working with the assay developers needs to know the accuracy, reproducibility, and reliability of the clinical assay performance to be able to translate that into what it means for toxicity in patients. Both FDA and IRB need to know what the patient is told about this in informed consent.

Topic 8

Introduction to Investigational Device Exemption: Pre-IDEs, IDEs and Related Submissions to FDA

Lakshman Ramamurthy, Ph.D., FDA

Dr. Ramamurthy discussed Investigational Device Exemptions (IDEs) – a regulatory submission that permits clinical investigation of devices and In Vitro Diagnostics (IVDs). An approved IDE permits a device to be shipped lawfully for conducting investigations of it without complying with the legal requirements that apply to commercial devices. Because it is a study to understand how a device works, labs are exempt from other requirements of interstate commerce, including premarket notification or approval application.

All device investigations are either subject to or exempt from IDE regulation. An investigational device is exempt if the test does not require an invasive sampling procedure that presents significant risk to the patient, such as a scan, and is not used as a diagnostic procedure without confirmation by another procedure. For example, an observational trial that includes treatment but is also collecting information that will not impact trial progress and will not be used to predict therapeutic outcome or stratify patients in a clinical trial is IDE exempt. But if the marker will be used to decide which patients get a certain therapy, the test is not IDE exempt. One cannot stratify patients or make a therapy choice based on the biomarker without an IDE.

An example of a significant risk study is a clinical trial in which marker-positive patients receive a new treatment, and marker-negative patients receive standard of care. Dr. Ramamurthy presented a dilemma for FDA in which both marker-positive and marker-negative patients in a study are given the same treatment. In preclinical studies, if a chemotherapy drug is only helpful to patients with a certain biomarker, ethically, it should not be given to patients who do not have the marker because they will not benefit from it. The FDA is often challenged when it receives data only on trial patients who are marker-positive and no data on marker-negative patients and is asked to interrogate whether the marker is valid in doing what it is intended to do. In these cases, FDA requires a pre-IDE or pre-submission discussion with the laboratory to determine how to design both an ethically correct and clinically robust clinical trial.

When conducting a significant risk study, sponsors must submit an IDE application. FDA will approve, approve with conditions, or disapprove the application within 30 days. Before submitting an IDE, FDA

encourages investigators to contact the Office of In Vitro Diagnostics and Radiological Health (OIR) for guidance on FDA expectations for pre-submission packages.

Sponsors must obtain IRB approval before the study begins. IRBs assess whether the proposed study is considered significant risk or non-significant risk. If the IRB or the sponsor needs assistance or requests that FDA make risk determinations, then FDA's determination is final. Dr. Ramamurthy encouraged dialogue between the sponsor and the FDA. He said that IRBs are now centralized or regionalized with high workloads and over time quality may suffer. FDA has faced situations in which a centralized IRB has made a decision that differs from FDA's. The sponsor is ultimately responsible for the study so should take the case to the FDA when there is doubt. Dr. Ramamurthy said FDA is working to make the IDE application process easier for labs and reminded workshop participants that IRBs charge a review fee while FDA does not.

Of importance, Dr. Ramamurthy said while enforcement discretion is applied to LDTs, it is not applied to investigational use of LDTs. Laboratories using an LDT in a trial must submit an IDE or a pre-IDE if there are questions.

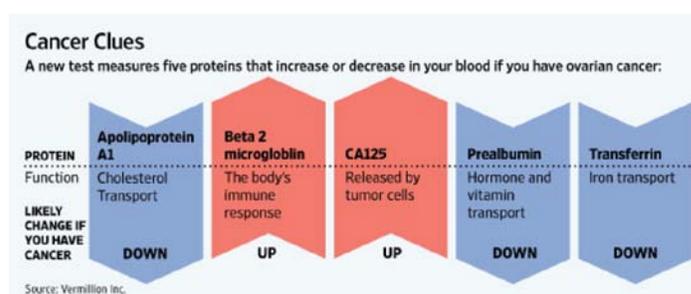
Finally, Dr. Ramamurthy provided a hypothetical situation in which a clinician with an IDE wishes to conduct a clinical trial involving 90 sick patients. The FDA would advise the clinician to first conduct a feasibility study with 10 patients to assess how the patients will fare and then conduct a pivotal trial. Both trials would require an IDE but the requirements would differ.

Topic 9

Case Study – FDA Approval of OVA 1 Blood Test

Daniel Chan, Ph.D., Johns Hopkins University, and Marina Kondratovich Ph.D., FDA

Dr. Chan provided a case study on the FDA approval of the OVA 1 blood test for detecting the presence of ovarian cancer in patients who have ovarian adnexal mass and for whom surgery is planned. OVA 1 is the first proteomics in vitro diagnostic multivariate index assay (IVDMIA) approved by the FDA (in 2009). Vermillion Inc. licensed the invention and conducted the clinical studies. The test measures five proteins that increase or decrease in the blood in the presence of ovarian cancer: CA 125, a protein released by tumor cells; Beta 2 microglobulin, involved in the body's immune response; apolipoprotein, a protein that functions as a cholesterol transport; prealbumin, a hormone and vitamin transport; and



OVA 1 is a qualitative serum test that combines the results of five immunoassays into a single numeric score.

transferrin, a protein involved in iron transport. OVA 1 combines the results of the five immunoassays into a single numerical score.

The intended use of OVA 1 is for women age 18 and older, with an ovarian adnexal mass for which surgery is planned, and who have not yet been referred to a gynecologic oncologist. The OVA 1 test is an aid to further assess the likelihood that malignancy is present when the physician's independent clinical and radiological evaluation does not indicate malignancy. The test is not intended as a screening or stand-alone diagnostic assay.

OVA 1 was developed out of Dr. Chan's lab at Johns Hopkins Hospital Clinical Chemistry, a 250-person CLIA/CAP-certified lab that conducts 14 million tests per year. Johns Hopkins University's (JHU) Center for Biomarker Discovery works in biomarker discovery, validation and translation to select the right technologies and clinical specimens, develop bioinformatics tools for data analysis and multiplexing of biomarkers, design multi-center case control study, discover and identify biomarkers, and translate biomarker into multiplex clinical diagnostics.

Dr. Chan's lab presented at the 2002 Human Proteome Organisation (HUPO) World Congress, on finding a proteomic biomarker for ovarian cancer, and again in 2003, on cancer proteomics. In finding the biomarker, JHU collaborated with other cancer centers to gain serum samples of early stages of ovarian cancer to enable a large study using several sets of biomarkers. The lab used protein chips – the latest technology available. Dr. Chan said that many scientists discounted this approach, but if conducted correctly, it can be successful. Through EDRN, JHU conducted the study with careful standardization to develop the right results. Because technology changes over time, Dr. Chan advised labs to use what is available and not to wait for better technology to become available to develop a useful biomarker.

Dr. Chan presented several lessons learned over the course of the OVA 1 study.

The 4Bs & 4Gs for Biomarker Translation

1. Define clearly a specific clinical "intended use" for unmet needs. Select the right population and conduct validation trial.
2. Generate sufficient evidence in preliminary studies to support the investment for a large-scale validation study.
3. Select/develop assays with analytical performance suitable for clinical use.
4. Conduct a clinical trial to demonstrate clinical utility to obtain regulatory approval and gain acceptance "by the clinical community."

Lesson 1: Clearly define intended use. If the goal is to discover biomarkers for aggressive cancer, for example, specimens should be obtained from patients both with and without aggressive cancer in order to find markers. With OVA 1, the target population was patients with pelvic mass, not the general patient population. The consequence for false negative is significant. Gaining a false positive is less significant because the test is attempting to detect ovarian cancer with a high sensitivity and high negative predictive value.

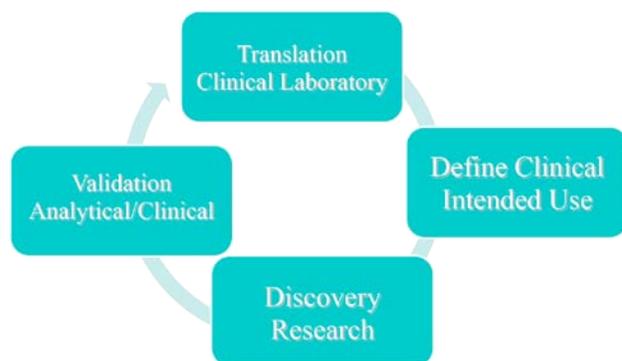
Lesson 2: Early on, determine whether the right approach has been identified for biomarker discovery and validation. Discovery studies usually require a small sample size before conducting a larger validation study, however, this may not always be the best practice. Starting with a large sample early could generate more reliable clinical biomarkers. Dr. Chan's lab initially designed the study to work with several hospitals, using each hospital's specimens for a specific task. If the marker survived in the discovery process, it was sent to another hospital for validation, a process he said worked very well.

In summary, Dr. Chan said his lab developed a concept called *4Bs and 4Gs* – or four bridges and four gates for biomarker translation. He explained that each bridge needs gates. When building a bridge or developing a biomarker important decisions must be made at each phase whether to move forward or not, to save money and time. Dr. Chan's laboratory published a paper on lessons learned from the OVA 1 study based on the 4Bs and 4Gs:⁶ 1) Define intended use; 2) Generate evidence in preliminary studies before conducting a large-scale validation study; 3) Select assays with analytical performance suitable for clinical use; and 4) Conduct a trial to demonstrate clinical utility to obtain regulatory approval by the clinical community.

Discussion

Participants debated whether a stepped approach, described as bridges and gates, might lead to missing a good marker by defining intended use too narrowly. The example was provided of the drug Viagra that was originally developed for cardiac use. One participant said there are so few good biomarkers available that it would be regrettable to have one rejected if it did not make it through the first or second "gate." Dr.

Chan replied that if a lab develops a set of biomarkers that meets the clinical intended use, it makes no difference the ones that may have been missed. However, if the laboratory does not have the right panel, it would need to go back and look for additional markers to achieve what was intended. For example, in 2007, his laboratory invited 14 EDRN investigators to present results from a prostate cancer study. They selected five top markers to move forward to validation. One reference set was used. Three out of the five markers failed, despite strong data. In the end, PCA3 and pro PSA were approved by FDA in 2012. Dr. Chan concluded that some markers may have been missed but it was important to identify the successful biomarkers.



Marina Kondratovich: In Vitro Diagnostic Multivariate Index Assay

⁶ Cancer Epidemiology, Biomarkers & Prevention, 19, 2995-2999, 2010

As a follow up to Dr. Chan's case study, Dr. Kondratovich discussed how scientists used IVDMIA to arrive at a score in the OVA 1 test. The IVDMIA is a device that combines the values of multiple variables using an interpretation function to yield a single, patient-specific result, such as a classification, score, or index, intended for use in disease diagnosis, cure, mitigation, treatment or prevention.

Dr. Kondratovich explained that the usual precision study provides information about precision for particular combinations of the individual analyte amounts, which were present in the precision study samples. Many possible combinations of the individual analyte amounts exist. The combinations can provide the same value of the test score so the samples with the same score but different combinations of the individual analyte amounts can have different precisions. The additional simulation provides information about precision profiles of the test score system for different combinations on individual analyte values. The basic steps are: the precision profile for each individual analyte should be constructed by performing linear interpolation using the known precision data from the precision studies with actual samples. For each possible combination of the values of the individual analytes, estimate the value of the score corresponding to this combination of values of analytes and precision of the score based on the corresponding precision profiles. Because the score is based on separate measures of individual analytes in a sample, random measurement errors of each analyte can be considered as uncorrelated. The precision profile for the score is a relationship between the mean value of the score and the interval of %CV values (not one %CV value), which can be obtained with different combinations of analytes. Clinical study data should be used to understand what combinations of analytes are clinically possible and whether enough information about precision of the score for the clinically possible combinations is available.

In summary, Dr. Kondratovich concluded that:

- 1) Precision profile for the score based on precision profiles of individual analytes and Monte Carlo simulation of error propagation provides additional valuable information.
- 2) Percent subjects in the clinical study with score values close to the cutoff provides information about clinical impact of score random measurement error.
- 3) Further investigation is needed for different IVDMIA scenarios (as multiplex assay) because random measurement errors of analytes in the multiplex assay can be correlated.

Topic 10

Standards of Operation and Best Practices for Future Biomarker Evaluation

Robert Christenson, Ph. D., University of Maryland

Dr. Christenson described criteria for the assessment of novel biomarkers. He noted three reasons why biomarkers are measured based on three key words: Measure, more and manage. 1) Can the

clinician *measure* the biomarker? 2) Does the biomarker add new or *more* information? 3) Will the biomarker help the clinician *manage* patients? If the answer is no to these questions, then the biomarker will have no meaning and will be of little use.

Dr. Christenson briefly reviewed the history of clinical lab regulations that relate to quality, citing CLIA's establishment in 1967. He stressed the important role of the laboratory director, who by law is responsible for the operation and administration of the laboratory, including personnel, record keeping and assuring regulation compliance. Dr. Christenson emphasized that "the buck stops with the lab director," who is responsible for "everything" that goes on in the lab. The lab director must establish a culture in which validation and documentation are paramount.

Dr. Christenson described CAP as a "real asset" and reiterated Dr. Sorbara's advice for investigators to document every activity that the CAP checklist requires. He advised laboratory personnel to develop a culture of validating and documenting everything. "It doesn't count until it's written down," he said.

From his experience of managing a laboratory, Dr. Christenson said the best way to get into the practice of documentation is to divide testing into its three logical phases: pre-analytical (before getting the specimen); analytical (conducting the testing) and post analytical (disseminating the data in a format that can be used by clinicians).

1. The **pre-analytical phase** involves events prior to sample analysis. Investigators must examine the condition of the specimen and requisition upon receipt in the laboratory, and accept it only with proper labeling and identification. If a specimen is unacceptable, the disposal or retention of the specimen must be recorded. In molecular testing, cross contamination must be avoided. It is important to begin a study by determining the type, size and volume of specimen that can be used, including fresh, frozen, or formalin fixed paraffin embedded (FFPE) tissues, for example, all of which must be validated. Dr. Christenson referred to Dr. Sorbara likening this to a chain of custody. He said that with 5,000 or more samples a lab might handle in a day, the specimen cannot be labeled wrong or switched or "catastrophe will happen."

2. The **analytical phase** involves SOPs, extraction and specimen storage, contamination control, laboratory design, test validation, equipment maintenance, personnel competency and other areas. Again, during this phase, everything must be written down and all methods must be specified, from the quality of a lab's air pressure, to dedicated lab coats. Test performance requires validation of the performance of clinical test methods and reagents. If a lab modifies an FDA-approved test in any way, it must repeat the validation process to avoid adulterating it. FDA requires validation of the performance of clinical test methods and reagents in accurately detecting or measuring analytes prior to use in human testing. Proficiency testing refers to external specimens from a reference source supplied to independent laboratories. CAP and other organizations supply specimens for molecular analysis. If proficiency specimens are not commercially available, labs can exchange blinded split

specimens, or test blinded specimens measured or documented by independent means such as chart review.

3. The **post-analytical phase** involves documentation of test results. Test results in the form of electropherograms, gel images, etc. should be of sufficiently high quality that results are unequivocal. Documentation of assay conditions, reagent lot numbers and quality and quantity of the isolated DNA or RNA is required. In situ results are correlated with histological findings (stained sections) of tissue morphology. Raw data are retained with the final report and clinical interpretation of the test results. The results report must convey the method or manufactured kit used, locus, mutation or organism tested, analytical interpretation of the raw data, the clinical interpretation of the analytical result, and the likelihood of false-positive or false-negative results.

Dr. Christenson noted examples when a disclaimer may be included in a test result report. When Class I Analyte Specific Reagents (ASR) are used in an analytical method, the test report must include: "This test was developed and its performance characteristics determined by [lab name]. It has not been cleared or approved by FDA." Some institutions add: "The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 as qualified to perform high complexity clinical laboratory testing." FDA notes that a disclaimer that reads, "The FDA has determined that such clearance or approval is not necessary" is inappropriate. This disclaimer seems to have been borrowed from the ASR rule that relates to a reagent and not the test.

In summary, Dr. Christenson said to achieve a laboratory operating with best practices, "You must be the person with the plan. You need a commitment from your institution for enough financial resources, personnel, funds, and the right space and standardized equipment. Staff is your most valuable resources, and you need a culture of being able to bring up a problem." He concluded that evidence-based practice is important, as is validation, consistency in testing and policy, all sustained quality through ongoing quality control meetings.

Discussion

Participants reiterated that adhering to CAP and CLIA requirements can work to a lab's advantage by making it more efficient. One participant stressed that modifying an LDT test is not necessarily adulterating, but it does require a full test validation, not just verification. One participant asked whether it is realistic for discovery labs to operate under a quality system of accreditation. Dr. Christensen said that if research labs read the CAP guidelines correctly, they should not have problems running an efficient lab. The systematic approach championed today is appropriate even for discovery labs. He reiterated that a lab director must develop a work environment in which documentation is routine. Dr. Christenson said it may seem like a laborious process to remain a CLIA-licensed lab but it is a worthy process to help labs keep up with improvements and advances in technology.

Dr. Christenson said he was glad to hear in earlier presentations that “perfect is not the enemy of good” and that all stakeholders want biomarkers that will work, not necessarily “the ideal.” On this note, one participant said that “perfect” may be the enemy of innovation and creativeness but the biomarker community must be incentivized to conduct studies with quality samples, not convenient samples. Dr. Christenson responded that balance is required. “We don’t want to stifle innovation and creativity. But you have to design the right trials. Once in the lab, you are compelled to go to the perfectionist end of spectrum, for repeatability,” he said.

Topic 11

Whole Genome, High Density Platforms, Standards, and New Approaches to Evaluation of Molecular Assays

Zivana Tezak, Ph.D., FDA

Dr. Tezak provided background on FDA efforts to regulate highly multiplexed genomic tests and policies in place to keep pace in this rapidly changing field, including regulations for genetic tests and implications of moving whole genome sequencing into wider clinical use. Her presentation looked at the whole genome and policy and regulatory implications. She also provided an overview of regulatory requirements and FDA evaluation of genomic tests, performance information, current developments and future challenges.

Dr. Tezak said that many discoveries involving whole genome sequencing have been transferred to the clinic just in the last year. While many predicted genome sequencing would change clinical practice, FDA may not be certain about this but is focusing now on how to regulate it. There are currently more than 200 genomic tests that have FDA clearance.

For any traditional genomic test (not high-density platforms), FDA looks for intended use; device description (platform, software); pre-analytical, analytical and clinical performance; instrumentation and software validation (if applicable); labeling, and other factors. For analytical performance, FDA reviews genomic tests similarly as for in vitro diagnostics’ clinical and analytical performance of the test. FDA asks if the test is measuring what it is intended to measure. FDA needs real samples for many performance studies.

Dr. Tezak illustrated differences in accuracy of whole tests vs. individual reported analytes. Any number of unexpected variations may occur with whole genome sequencing, so investigators cannot ask for specifics of each but must look at different approaches. When determining the clinical performance of genomic tests, an abundance of genotype information and professional guidelines may be available so FDA may not require clinical studies. When there is not enough information, e.g., on a new or different marker, laboratories may have to conduct a trial.

For high-density platforms, one can have any number of results so cannot often predict expected variants, nor evaluate every expected result. If an unexpected result arises, the question becomes how to interpret clinical significance and use standards to interpret it. The challenges to high-density platforms include unlimited results in which clinical significance is open to interpretation; adequate demonstration of analytical reliability of all possible outputs (cannot expect clinical samples that span all possible variation in the genome for studies); evaluation of accuracy; difficulty in capturing all sources of analytical variation; defining expected accuracy and precision; and lack of controls and standards.

Dr. Tezak stated that in July 2011, FDA held a public workshop with experts translating genome sequencing. FDA asked specific questions on analytical validation, starting with the technical performance of the platform, and how to assess the analytical validity and data analysis methods. In response, FDA received more questions and almost no answers. Since the last meeting, however, questions are getting answered as technology and use is progressing.

In April 2012, the American College of Medical Geneticists issued a statement on when it makes sense to use whole genome sequencing. At the time, several research labs were performing whole genome sequencing with a CLIA-certified lab confirming positives that were found. Dr. Tezak said that today this may be changing.

Any clinical test involves many instruments, reagents, and software and elements, but these elements are amplified in next generation sequencing-based genome testing. Dr. Tezak described a “conundrum” in the field when all of these elements are specialized and produced by different manufacturers. Since the 2011 workshop, FDA is looking at possibly regulating separately the performance of the next generation sequencing platform to assess if it is reliable and accurate. Dr. Tezak said this path may be more accommodating if changes need to be made and is something that FDA is considering.

Validation strategies cannot validate every expected outcome so labs may choose a subset of clinically important and analytically challenging markers. The question of the size of the subset and percentage of the genome being analyzed is important. In validating the subset laboratories confirm whether the whole platform works, what must decide what to do with the rest of it. Clinical labs may have confirmed the positive results but must consider whether they can develop standard samples that can be used as part of validation and add that to the examples mentioned before. There are different sample types for cancer.

Dr. Tezak said that in past years, much sequencing has been done in academic institutions, and a great deal of accumulated knowledge exists on ways to evaluate performance. However, questions arise, including: Should the whole sequence be evaluated? Some regions are impossible to sequence so should those regions be expected to have the same performance? Work being done on clinical interpretation is fragmented. Labs are getting unexpected results and often must interpret it manually.

Dr. Tezak asked, “How good is this information? We don’t have great clinical databases as a lot of it is research data only, and do not know how good it is or how good the interpretation is.” In addition, a study author may not publish negative results of the study. Many databases are being developed, so FDA is looking at connecting databases together so clinicians can have access to common interpretation.

Dr. Tezak projected where the research community must go from here. FDA is working with NIST and other groups on new approaches for valid instrumentation, quality tests, understanding test performance, and fostering databases. FDA tries not to hinder innovation but must ensure tests are accurate. FDA has in place a system to regulate genetic tests and has approved many tests but does not have a completely formulated policy for high-throughput sequencing- based tests. Each new submission raises different regulatory and scientific issues.

Topic 12

From Validation to Qualification of Biomarkers and Alternative Paths

Federico Goodsaid, Ph.D. Vertex Pharmaceuticals

Dr. Goodsaid centered his presentation on three questions: 1) What impact are regulatory qualification processes having on collaborative efforts to develop and qualify new biomarkers? 2) What is the most effective path for regulatory acceptance of biomarkers? 3) Should additional biomarker qualification acceptance paths be developed?

Dr. Goodsaid said a major goal for labs is to ensure that biomarker information in regulatory submissions is acceptable to regulatory agencies. The concept of qualification in this case is circumscribed to the requirements of regulatory review. Not all biomarkers need to be qualified, and not all biomarkers may be qualified through a biomarker qualification regulatory process.

Over the years, several attempts have been made to instill a process for qualifying biomarkers, including the Pilot Biomarker Qualification Process (2005); the Formal Biomarker Qualification Process (2009); the FDA’s Draft Guidance (2010); and the IOM Report (2010). In the end, these efforts concluded that evidence can be gathered to make a decision, but often the missing piece is that the biomarker is not a good marker. “It is not the silver bullet,” Dr. Goodsaid said. A biomarker must be developed for the purpose of expediting development of successful market applications. The regulator’s perspective of use does not go beyond application of the biomarker. This approach has been used for 50 years. Dr.



Many biomarker approval submissions to FDA get caught in a “continuous loop” in the consultation and advice stage, delaying or often halting the process.

Goodsaid said that, “People accept them when they are tired of arguing their usefulness, which has little to do with science. This is an example of perfect being the enemy of the good.”

Dr. Goodsaid defined *context of use* as a statement of how the biomarker will be used and its potential value. Data from additional studies obtained over time may be submitted to expand the qualified context of use and may include the range of clinical disorders, drug classes, species, procedures and criteria for how samples are obtained, and interpretation of results. Within the stated context of use, the results of an assessment can be relied upon to have a specific interpretation and application in drug development and regulatory decision-making. If a biomarker is qualified, analytically valid measurements of it can be relied upon to have a specific use and interpretable meaning in drug development, and the qualification process is expected to expedite development of successful marketing applications. If a biomarker is qualified for a specific context of use, industry can use the biomarker for that purpose during drug development. FDA reviewers can be confident in applying the drug development tools (DDT) for the qualified use without the need to reconfirm the DDT’s utility.

Dr. Goodsaid described FDA’s three-step biomarker qualification process: 1) initiation; 2) consultation and advice; 3) review. In step 2, FDA reviews the submission to determine whether there is adequate data and may ask for additional clarification or more data. Often during this step, FDA and the submitter engage in an “infinite loop” in which the back-and-forth exchange of consultation and information sharing “never ends.” He said many biomarker submissions stay in this stage for months or years. While the infinite consultation loop continues, the submitting organization may run out of time, money and will to continue the process. He advocated for “abolishing” the consultation and advice step of the FDA qualification process.

Dr. Goodsaid described a successful submission, including cover letter and completed forms. He also described the Biomarker Qualification Process and the Predictive Safety Testing Consortium, involving FDA and its European and Japanese counterparts. Harmony exists between the three agencies but conflict may exist in a biomarker meaning something different to each agency. He advocated for a universal agency, instead of three independent groups.

Discussion

Discussion centered around the concept of whether to hold out to get the perfect biomarker, and perhaps spend months or years proving its value, to provide modest use. One participant said, “We cannot wait 50 years to lower cholesterol until you get perfect silver bullet. Just provide modest use.”

Panel Discussion 1

Lessons learned on assay development and application from research/developmental laboratories to clinical use

Panel: Frederick Barr, M.D., Ph.D., NCI (Chair); Sanford Stass, M.D., University of Maryland; Yun, Fu Hu, Ph.D., FDA; Jim Vaught, Ph.D., NCI; Lynn Sorbara, Ph.D., NCI

Dr. Barr introduced the workshop's first panel of experts who would discuss how research assays are transferred from the basic research laboratory to the molecular pathology laboratory, and the decisions and challenges in the process. Dr. Barr acknowledged three groups that must interact in the process: 1) the research scientist who comes up with the basic idea; 2) clinicians who must have the potential to apply the assay to patients; and 3) scientists in a CLIA/CAP-certified molecular pathology laboratory who will perform, certify, validate and interpret the test and ultimately interact with the clinicians to provide that information. He stressed that if the test will not impact clinicians' decision making, then any good assay is meaningless and the development process will end there.

An important decision is whether the initial idea is intended for transfer directly to the molecular pathology lab as an LDT that the lab will have to validate for use, or if it will become part of a larger idea that will be transported into an FDA-approved test? Much thought must be put into this if a lab is to seek FDA approval or an LDT.

Dr. Barr told a story involving a lab he ran 10 years ago that was working on discovery of an infusion in sarcomas that was transferred into an LDT but that did not have enough power to seek FDA approval. He was contacted by an attorney representing a clinician being sued for "failure to treat in a timely fashion." The attorney had a molecular pathology lab perform a test to show that the patient's tumor should have been detected by a molecular pathology test earlier. Dr. Barr determined that the test was a research test and not a clinical test. The lab had never written SOPs and was involved in a transitional period in which molecular pathology labs often did not have clear guidelines and were conducting research tests but calling them clinical tests. Labs needed to transition from research testing to clinical testing by establishing guidelines to begin conducting clinical testing – an important linchpin the community needed to understand.

Dr. Vaught provided a brief synopsis of activities within the NCI Biorepositories and Biospecimen Research Branch (BBRB). The group's first job was to draft the *NCI Best Practices for Biospecimen Resources*, highlighting the technical aspects of management and oversight of biorepositories, the importance of biospecimen research and the ethics around it. The best practices were published in 2007, and updated in 2011.⁷ In addition, Dr. Vaught's office formed the Biospecimen Research Network (BRN) to study questions of effects of preanalytical variables on blood samples, and time, temperature effects on formalin fixation, and other biospecimen research issues. BBRB has been asking questions in an effort to develop evidence-based SOPs that ultimately can have clinical applications.

BBRB's goal is to create evidence-based practices that can be incorporated into NCI best practices and ultimately into clinical practices. Dr. Vaught said that merging BBRB's new program with NCI's clinical assay development program has been helpful in applying what has been learned in NCI's

⁷ Available on <http://biospecimens.cancer.gov/default.asp>

biospecimen research program into the new program. BBRB's approach is different but complementary to EDRN's program. BBRB and NIST have been in discussion about creating standard reference materials and approaching problems to developing tests that can predict the quality of the samples that have been stored in freezers or in paraffin blocks. BBRB held a joint workshop with NIST and FDA on the topic in 2010 and talks continue.

BBRB's impetus for creating standard reference materials came from experience in procuring samples for The Cancer Genome Atlas (TCGA) from various biorepositories around the country in 2009. During analysis of the initial samples, 70 percent failed either molecular or pathology quality control or both. Dr. Vaught said they were not poor samples, but were collected in different labs and stored in different ways so they may have been fit for the purpose for which they were collected, but they did not meet TCGA's strict criteria. For multi-center trials trying to develop consistencies across samples, SOPs and quality standards must be applied to ensure samples are consistent across different geographical and programmatic areas.

BBRB is interested in CAP's Biorepository Accreditation Program. NCI does not have regulatory authority so an agency is needed to inspect biorepositories to ensure standards are being applied. Dr. Vaught said BBRB encourages programs to require strict quality standards, however the consistent application of biospecimen best practices has a long way to go.

Dr. Hu provided background on the FDA biomarker submission review process. FDA is often challenged by a lack of reference materials provided by labs for most tests. He explained that some gene mutations or variants are so rare that it is difficult to find sufficient and appropriate materials to evaluate or validate the test. Dr. Hu said that investigators often misunderstood FDA terms. For example, test validation – proof that a test performs similarly in the lab compared to what the device manufacturer demonstrated – differs from test verification, which requires a different sample size than what is required for test validation. A test system differs from a test component. FDA views a test as a system, including reagents, instruments, software and instructions for use. An antibody from an FDA-approved kit does not mean it is FDA-approved if it is used with any other system or protocol. Exploratory studies differ from registrational studies. A device may be modified before, during or after an exploratory study, but the device should be prespecified and analytically validated prior to use in registrational studies. FDA may require IDE or stringent test requirements for registrational studies, however, it recommends use of a prespecified and validated test in all studies, considering an early-stage study could eventually become a registrational study.

Dr. Hu stressed the differences of risk determination by the IRB and biomarker use in studies. If the IRB says a lab does not need an IDE, but FDA considers the study a significant risk study requiring an IDE, the lab or its IRB may consult FDA. In many cases the lab will not need an IDE; but if test results are used in patient management (e.g., selection of patients for a drug), an IDE is required. A predictive marker differs from a selective marker. The same test can be used in selection of patients for treatment in a drug trial. If only the marker-positive group or marker-negative group is enrolled in

the drug trial, a lab can only make a selection for the device because the efficacy of the drug is not tested in the other group (e.g., marker-negative group in a trial with marker-positive only group). If both marker-positive and marker-negative patients are enrolled and the lab demonstrates that the drug worked in one group and not in the other, investigators can make a predictive claim for the test.

Dr. Hu also addressed temporal and tissue heterogeneity issues. An analyte of interest may be present at different levels during disease progression or at different tissue locations. In Dr. Pampori's presentation, the test was specifically intended for use with the first catch of urine from a man 50 years and older, not the second catch or in younger patients. If a physician chooses to use the test with the second catch, it is considered off-label use.

Occasionally, test performance may be better in the study population used for test validation than in the general population after FDA approval. FDA requires the study to be performed in a setting as close to how it will be used in clinics as possible, i.e., a lab cannot validate a test on one set of patients and use it in another with different characteristics. For test validation, data collection and analysis should be defined prior to trial initiation. If they are modified based on trial results, the trial is considered exploratory and another study is required for validation.

Discussion

Following the panel discussion, workshop participants made several recommendations to make the assay validation process more efficient.

One participant recommended the biomarker industry develop clearly delineated steps, in which each step indicates when biomarkers can qualify to move to the next step, to make the transfer process more efficient and cost effective for laboratories to perform. EDRN has made a good start with its five-step process. However, at each stage of development, a team of experts needs to ensure the research assay is credible and research is believable in terms of eventual clinical use. As a first step, NCI needs the funding to get reliable outcome data. As a second step, the lab needs to transfer the assay to the validation step to see if the assay should be evolving to the diagnostic assay where there is clinical utility. Another participant suggested biomarkers be required to demonstrate significance as an independent variant in a multivariate analysis that includes stage, grade, and other components.

Other suggestions included instating staged allocation of materials to clinicians with biomarkers to prevent labs from using materials too quickly and running out of supply; require analytical performance of the assay, in addition to clinical performance; adopt new attitudes toward funding validation studies (not being innovative does not mean not worth funding).

Panel Discussion II

Standards in Molecular Diagnostics: What has been done well? What are the remaining gaps? What needs improving?

Panel: Henry Rodriguez, Ph.D., NCI (Chair); Daniel Chan, Ph.D.; Johns Hopkins University; Marina V. Kondratovich Ph.D., FDA; Federico Goodsaid, Ph.D., Vertex Pharmaceuticals; Robert Christenson, Ph.D., University of Maryland

Panel Chair Dr. Rodriguez stated that the workshop's wrap-up session would allow each panel member to address two key questions: What has been done well on the landscape of standards in molecular diagnostics? What are the remaining gaps that need improving? He appealed to workshop attendees to participate in the discussion, as they will ultimately play a major role in the workshop's outcome.

Dr. Chan opened discussion naming three areas that need addressing to improve biomarker performance: 1) standardization; 2) process or SOPs; and 3) the material used to test whether the performance is correct or not.

Dr. Chan said that in 1997 he was working with a CAP-certified laboratory to test PSA for detecting the presence of prostate cancer. His lab was using survey materials sent to multiple labs that ranged in value from three to 10. Years later, the labs were able to narrow the range from nine to 10 after World Health Organizations (WHO) standards for PSA became available. Most assays at the time measured pre PSA differently. He said the laboratory learned that each method tested reacted differently and there had been problems with the survey material, concluding that standards must be in place for calculating the equation, method, and substance used in testing. With immunoassays, laboratories must ensure the standard material measured is the same as what is measured in patient samples in order to get accurate results and performance. He concluded that the industry still has a long way to go in these areas.

Dr. Kondratovich stated that from the regulatory side, FDA appreciates NCI's attempts to bring FDA in on the validation process earlier so that regulators understand the process of biomarker testing being conducted in research labs, including the technologies and work flows. For diagnostic areas it is important to know clinical performance. FDA often encounters issues with clinical studies design, especially in selection or verification bias. FDA made progress in understanding clinical trials design for diagnostic devices when CDRH published guidance for trial design in 2011. This was the first time FDA provided basic, plain language advice for what it was looking for in clinical trials, including types of devices. FDA is now using a Clinical and Laboratory Standards Institute (CLSI) document for nearly all analytical parameters and is focusing on multiplex and measuring a single analyte. Dr. Kondratovich concluded that FDA faces a heavy workload. The agency is continually challenged to keep abreast of the increasing complexities of study design and ways in which the agency can aid analytical performance. She said FDA often encounters studies that are underpowered in their statistical capabilities or designed improperly, and that sample size and other issues are important but not as critical as the issue of bias. She stressed that every element in clinical trials must be unbiased.

The third speaker, **Dr. Christenson**, spoke from the perspective of running a laboratory and discussed issues surrounding LDTs, which are conducted in every lab. He described a hypothetical situation in which a patient presents with fluid on his side after a renal transplant. The doctor needs to know if the fluid is urine or serum and if it is urine, the patient must receive urgent care. The laboratory needs to be able to tell the doctor the creatine level in the sample. There is no FDA-approved test on body fluid creatine and the need is critical for a validated test for it. He concluded that every lab has to do that or patient care suffers.

Among the three Federal agencies, Dr. Christenson acknowledged that FDA has the difficult job of being risk adverse because of its mission to provide patient safety. However, he said it would be very helpful if FDA provided a clear guidance document on what is required of labs to gain FDA approval of biomarkers. He acknowledged that a document would not be “one size fits all” but any document would be helpful. At NIST, there is a shift toward complicated proteins with many measurands for antibodies for harmonization. Dr. Christenson said that NIST is on the right track with its PT/INR (prothrombin time/international normalized ratio) survey. Finally, he emphasized that NCI values innovation. He asked how a laboratory can be both innovative and “take a giant step” toward practical utilization and ensure the patient being tested has a better outcome than the patient not being tested. Dr. Christenson concluded that the industry needs to “keep its eye on the clinical ball.” Clinical trials should be set up to ask: Will patients be better off with this biomarker?

Dr. Goodsaid offered his perspective from his experience in industry and with FDA. He stressed the importance of developing standards regulating operation of labs and noted no single uniform standard exists for what makes a quality operation worldwide. He recommended making global standards more uniform but asked whether setting a universal baseline among the U.S., Europe and Japan would call for more stringent criteria. Europe, for example, does not have the equivalent of a FDA CDRH so it is difficult to maintain a minimum standard for analytic test performance. Clinical utility of a test is important but a fundamental issue for most biomarkers is whether they work or not. Dr. Goodsaid concluded, “If we can’t address this, the second part about clinical utility is irrelevant.”

Discussion

A participant commented that Dr. Goodsaid’s recommendation for global biomarkers standards was interesting but questioned the role of societal and political considerations in biomarker validation. As an example, he said biomarker GP73 was approved in China but not in the U.S. and asked “Is the science that China accepted bad? Or is there something that U.S. society would not accept?” In response, participants questioned whether GP73 was ever submitted to U.S. FDA, “because it can’t be approved if it was never submitted.” An FDA presenter said that FDA does not take into consideration whether a test has been approved in other country. Another participant suggested that perhaps China had more clinical data to approve the test, or the test approved in China may not be as relevant to the U.S. population.

Another participant urged investigators to “know the business of biomarker development.” More than 20,000 biomarker tests are conducted in the U.S. each year. When all are conducted using identical methodology, the model that has regulatory approval works very well. But as that changes, the model is no longer applicable because new obstacles arise. A biomarker may not be submitted for regulatory approval because of the thought that doing so will limit chances to compete with the next wave coming up. The participant referenced Dr. Christenson’s remarks when he said labs must consider whether they want to remain a lab or become a manufacturer.

Other recommendations from participants included requiring analytical assay review (in addition to requirements for statistical review) during grant interview of papers for journals; and a new minimal usefulness checklist for biomarkers. A presenter from FDA reminded participants that FDA does issue guidance for certain tests: “We do not have guidance of specific creatine sample type (referenced in the panel discussion) but we have summaries of what we ask.” She said guidance summaries are on the FDA website, prompting one participant to suggest revising the website to make it easier to find useful documents.

End of workshop

Division of Cancer Prevention
National Cancer Institute - National Institutes of Health
U.S. Department of Health and Human Services

NCI-FDA-NIST Workshop on Standards in Molecular Diagnostics

Friday December 7, 2012
NIH Neuroscience Building, Room C

AGENDA

8:00 a.m. – 8:10 a.m.	Welcome & Overview Nadarajen A. Vydelingum, Ph.D., FACB, NCI
8:10 a.m. – 8:25 a.m.	Welcome & Introductory Remarks Barry Kramer, M.D., MPH, NCI
8:25 a.m. – 8:45 a.m.	Standard Reference Sets for Expediting Clinical Validation of Biomarkers Sudhir Srivastava, Ph.D., MPH, NCI
8:45 a.m. – 8:50 a.m.	Q/A
SESSION I:	State of the Science
Moderators:	Nadarajen A. Vydelingum, Ph.D., NCI Lakshman Ramamurthy, Ph.D., FDA
8:50 a.m. – 9:10 a.m.	The Role of Standards in Realization of Robust Molecular Biomarkers Marc Salit, Ph.D., NIST
9:10 a.m. – 9:20 a.m.	Q/A
9:20 a.m. – 9:40 a.m.	Standardization of Molecular Biomarker Assays from Discovery and Development to the Clinical Laboratory: Lessons Learned Sanford Stass, M.D., University of Maryland
9:40 a.m. – 9:50 a.m.	Q/A
9:50 a.m. – 10:05 a.m.	CLIA/CAP Standardization from the Ground Up Lynn Sorbara, Ph.D., NCI
10:05 a.m. – 10:10 a.m.	Q/A
10:10 a.m. – 10:30 a.m.	Break

10:30 a.m. – 10:50 a.m.	Regulatory Aspects: When Reviewing a Device – PCA3 Nisar A. Pampori, Ph.D., FDA
10:50 a.m. – 11:00 a.m.	Q/A
11:00 a.m. – 11:20 a.m.	The Role of Bioinformatics in Standardization Kristen Anton, M.S., Dartmouth University
11:20 a.m. – 11:30 a.m.	Q/A
11:30 a.m. – 11:50 a.m.	The Role of the FDA pre-IDE as a Means to Improve Clinical Assays J. Milburn Jessup, M.D., NCI
11:50 a.m. – 12:00 p.m.	Q/A
12:00 p.m. – 12:30 p.m.	Panel Discussion I <ul style="list-style-type: none"> ➤ Transfer of research assays to assays validated for clinical use in a CLIA/CAP environment (requirements for SOPs, etc.) ➤ Lessons learned related to assay development and application from research/developmental laboratories to clinical use <p>Panel Members: Frederick Barr, M.D., Ph.D., NCI (Chair); Sanford Stass, M.D., University of Maryland, Yun-Fu Hu, Ph.D., FDA, Jim Vaught, Ph.D., NCI, Lynn Sorbara, Ph.D., NCI</p>
12:30 p.m. – 1:30 p.m.	Lunch
SESSION II:	What has worked? What has not? Where are the Gaps?
Moderator:	Lynn Sorbara, Ph.D., NCI
1:30 p.m. – 1:50 p.m.	Pre-IDEs, IDEs and Related Submissions to FDA Lakshman Ramamurthy, Ph.D., FDA
1:50 p.m. – 2:00 p.m.	Q/A
2:00 p.m. – 2:30 p.m.	Case Study – FDA Approval of OVA 1 Blood Test Daniel Chan, Ph.D., JHU/Marina Kondratovich Ph.D., FDA
2:30 p.m. – 2:40 p.m.	Q/A
2:40 p.m. – 3:00 p.m.	Break
3:00 p.m. – 3:20 p.m.	Standards of Operation and Best Practices for Future Biomarker Evaluation Robert Christenson, Ph.D., University of Maryland

3:20 p.m. – 3:30 p.m.	Q/A
3:30 p.m. – 3:50 p.m.	Whole Genome, High Density Platforms, Standards, and New Approaches to Evaluation of Molecular Assays Zivana Tezak, Ph.D., FDA
3:50 p.m. – 4:00 p.m.	Q/A
4:00 p.m. – 4:20 p.m.	From Validation to Qualification of Biomarkers and Alternative Paths Federico Goodsaid, Ph.D. Vertex Pharmaceuticals
4:20 p.m. – 4:30 p.m.	Q/A
4:30 p.m. – 4:50 p.m.	Panel Discussion II <ul style="list-style-type: none"> ➤ What has been done well on the landscape of standards in molecular diagnostics? ➤ What are the remaining gaps and what need improving? <p>Panel Members: Henry Rodriguez, Ph.D., NCI (Chair); Federico Goodsaid, Ph.D., Vertex Pharmaceuticals; Robert Christenson, Ph.D., University of Maryland; Daniel Chan, Ph.D.; John Hopkins University; Marina V. Kondratovich Ph.D., FDA</p>
4:50 p.m. – 5:00 p.m.	Closing Remarks & Adjournment Nadarajen A. Vydelingum, Ph.D., NCI

Speaker Roster

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Workshop Speaker Biographies

Kristen Anton, M.S., is director of bio-informatics at the Geisel School of Medicine at Dartmouth, and the University of North Carolina, Division of Gastroenterology and Hepatology in Chapel Hill. Kristen and her team of 22 computer programming, systems design, data management and technical support professionals at both institutions are dedicated to making high-quality information management systems available to collaborating researchers. The team has developed and maintained data capture, management, integration and sharing systems for clinical research registries, clinical trials and prevention studies, as well as genomics and proteomics projects associated with epidemiological studies, for more than 20 years. In 2011, Kristen and the EDRN Informatics Center Team were awarded the prestigious NASA Group Achievement Award for “the innovative and pioneering use of NASA data system technologies to construct a remarkably successful national bioinformatics network of cancer biomarker research.” Kristen co-teaches computational molecular biology (specifically database theory and biological databases) at Dartmouth College. Before coming to Dartmouth in 1997, Kristen worked in commercial bioinformatics, as an associate scientist and team leader for Genetics Institute in Cambridge, Mass. Kristen holds an M.S. degree in Biomedical Engineering from Boston University and an A.B. degree in Human Biology from Stanford University.

Frederic Barr, M.D., Ph.D., is head of the Cancer Molecular Pathology Section and deputy laboratory chief at NCI. He received his undergraduate education at Williams College and attended Washington University School of Medicine, where he obtained his M.D. and Ph.D. Subsequently, he received residency training in anatomic pathology at the Hospital of the University of Pennsylvania and performed postdoctoral research in the Division of Human Genetics and Molecular Biology at the Children's Hospital of Philadelphia. Before coming to the NIH, Dr. Barr was a faculty member in the Department of Pathology and Laboratory Medicine at the University of Pennsylvania School of Medicine. In addition to his research activities, Dr. Barr serves on the steering committee of the Soft Tissue Sarcoma Committee of the Children's Oncology Group, and is director of the Advanced Molecular Pathology Course of the Council of the United States and Canadian Academy of Pathology.

Daniel W. Chan, Ph.D., DABCC, FACB, is professor of Pathology, Oncology, Radiology and Urology and the director of the Center for Biomarker Discovery and Translation at Johns Hopkins University and director of Clinical Chemistry Division and co-director of Pathology Core Laboratory at Johns Hopkins Hospital. Dr. Chan is a diplomat of the American Board of Clinical Chemistry and a fellow of the National Academy of Clinical Biochemistry (NACB). He is an internationally recognized expert in clinical proteomics, cancer biomarkers and molecular diagnostics. He has written five books, 40 book chapters and over 250 scientific articles. He is the principal investigator of several NIH research grants including the Cancer Proteomic Tumor Analysis Consortium and NCI Early Detection Research Network. Dr. Chan served as chair of the Proteomics Division (a founder) of the American Association for Clinical Chemistry; board of directors of NACB and president of the National Registry in Clinical Chemistry. Currently, he is president of the International Society of Enzymology and on the board of the U.S. Human Proteome Organization. He is a senior editor of cancer screening and detection of *Cancer Epidemiology Biomarkers & Prevention* and editor-in-chief of *Clinical Proteomics*.

Robert Christenson, Ph.D., is director of Clinical Chemistry Laboratories & Rapid Response, Division of Pathology and Medical and Research Technology, Greenebaum Cancer Center, Surgical Oncology, University of Maryland. He is president elect of the American Association of Clinical Chemistry (2013). Dr. Christenson obtained his medical degree at the Florida State

University followed by a fellowship in clinical chemistry at the University of North Carolina, Chapel Hill. His research interests include: Clinical Chemistry, Red Blood Cell Substitutes, Laboratory-Assisted Diagnosis of Neurologic Diseases, Improved Strategies for Utilization of Laboratory Tests, and Characterization of Biochemical Markers for Assessing Cell Injury.

Federico Goodsaid, Ph.D., is vice president for strategic regulatory intelligence at Vertex Pharmaceuticals where he focuses on early and effective interaction and collaborations on exploratory and product biomarkers with regulatory agencies. He was previously associate director for operations in genomics and biomarker qualification coordinator at the Office of Clinical Pharmacology, Office of Translational Sciences, Center for Drug Evaluation and Research at the U.S. FDA, working on the regulatory application and development of genomics and biomarkers. He holds a B.A. degree in biochemistry and biophysics from the University of California at Berkeley and his Ph.D. from Yale University in molecular biophysics and biochemistry. He was a postdoctoral fellow at Cornell University and Washington University in St. Louis. Before joining FDA, he was senior staff scientist at Applied Biosystems and lead for the molecular toxicology group at the Schering-Plough Research Institute.

Yun-Fu Hu, Ph.D., RAC, completed his studies in animal sciences and veterinary medicine at Central China Agricultural University. He holds an M.S. degree in reproductive endocrinology and Ph.D. in cancer biology from Ohio State University. He spent five years, eventually as a staff fellow, at Fox Chase Cancer Center in Philadelphia. His research interest centers on molecular mechanisms of carcinogenesis. He joined Becton Dickinson in Baltimore as a project scientist leading development of a molecular diagnostic test for melanoma for two years before being recruited to GlaxoSmithKline as an investigator where his group was responsible for discovery of biomarkers and development of biomarker tests. After GSK, he joined a biotech company's diagnostics program as director of diagnostics development where he oversaw discovery of metabolomic biomarkers and development of in vitro diagnostics. He joined FDA in 2009 as a scientific reviewer in CDRH's Office of In Vitro Diagnostic Device Evaluation and Safety and was promoted to associate director of the Immunology branch of the division responsible for review and clearance or approval of immunology and molecular diagnostic devices such as cancer diagnostics and genetic tests, and tests for autoimmune diseases, e.g., Celiac disease, Crohn's, and allergies. Upon re-organization of the FDA Office of In Vitro Diagnostic Device Evaluation and Safety (currently known as Office of In Vitro Diagnostics and Radiological Health), he is currently chief of the Molecular Pathology and Cytology Branch.

J. Milburn Jessup, M.D., is a surgical oncologist and scientist and chief of the Diagnostics Evaluation Branch of the Cancer Diagnosis Program in DCTD and an adjunct investigator in the Laboratory of Experimental Carcinogenesis in CCR. In 25 years of academic practice he focused on the multidisciplinary treatment of gastrointestinal and breast cancer, melanoma and soft tissue/skeletal sarcomas in several different academic settings. He also led a research effort to study the mechanisms by which human colorectal carcinomas form hepatic metastasis and identified roles for the marker Carcinoembryonic Antigen in modulating inflammatory responses and promoting metastasis. He is currently Chair of the AJCC Hindgut Task Force that formulates the TNM staging classification in the U.S. Dr. Jessup also aids investigators in facilitating the development of biomarkers for clinical use in patients with cancer.

Marina V. Kondratovich, Ph.D., is an associate director for clinical studies, personalized medicine, in the FDA Office of In Vitro Diagnostic and Radiological Health. She has been FDA for 13 years and has served as spokesperson at multiple FDA Advisory Panel meetings. Some of her interests are personalized medicine tests, analytical evaluation of tests, design of clinical studies for diagnostic devices, missing data (as verification bias, multiple imputations). She

participates in CLSI standards development and in the ISO. Dr. Kondratovich received her Ph.D. in Mathematical Statistics from the Department of Statistical Modeling at St. Petersburg State University in Russia.

Barry Kramer, M.D., is director of the NCI Division of Cancer Prevention. He was editor-in-chief of the *Journal of the National Cancer Institute* from 1994 to 2012. He serves as chairman of the Physician Data Query (PDQ) editorial board on Screening and Prevention and is a member of the PDQ Treatment editorial board. Dr. Kramer has served on the cancer prevention committee of the American Society of Clinical Oncology and was the committee chairperson from 2006 to 2007. Dr. Kramer received his M.D. from the University of Maryland Medical School, and completed his internship and residency in internal medicine at Barnes Hospital in St. Louis. He completed a medical oncology fellowship at NCI. He is board-certified in internal medicine and medical oncology, and received a master's degree in public health from Johns Hopkins University Bloomberg School of Public Health. Dr. Kramer has extensive experience in primary cancer prevention studies, as well as clinical screening trials of lung, ovarian, breast and prostate cancers. He is an investigator and on the steering committee for two large NCI cancer screening trials: the Prostate, Lung, Colorectal, Ovarian (PLCO) Trial; and the National Lung Screening Trial (NLST). He has a strong interest in weighing and reporting the strength of medical evidence and created an annual Medicine in the Media Workshop to help working journalists develop methods of reporting medical evidence.

Nisar A Pampori, Ph.D., is a biologist and in-charge branch chief of the Immunology and Flow Cytometry Branch at the FDA Center for Devices and Radiological Health, Division of Immunology and Hematology Devices, where he reviews diagnostic devices' pre-market and post-market submissions. Prior to joining FDA, Dr. Pampori worked in the Biotechnology Industry Research and Development for 10 years and developed immunoassays, proteomic assays and high throughput screening assays in the singleplex and multiplex formats. His work in academia used a genetic engineering model to create novel ligand mimetic Fabs as strategies for clinical diagnostics and therapies. Dr. Pampori has a number of publications in peer-reviewed journals and proceedings. He contributes to FDA guidance development and participates in CLSI standards development.

Lakshman Ramamurthy, Ph.D., is a senior reviewer in the Division of Immunology and Hematology Devices in OIR, CDRH, FDA, where his focus is in cancer diagnostics, screening tests and companion diagnostics devices utilizing genetic and genomic biomarkers. He was variously acting associate director, OIVD, policy advisor at the Office of Center Director, CDRH and was a Congressional affairs specialist in the Office of the FDA Commissioner. Prior to joining FDA in 2007, Dr. Ramamurthy spent more than a decade in the biopharmaceutical industry serving as an R&D scientist at GlaxoSmithKline and as manager in a genomics services company. Dr. Ramamurthy received a Ph.D. in molecular biology from the University of North Carolina at Chapel Hill. He has over a dozen publications including book chapters and review articles. He is also member of the CLSI Committee on Nucleic Acid Sequencing Methods and an organizer of FDA-NCI-NIST workshop on Standards in Molecular Diagnostics.

Henry Rodriguez, Ph.D., is an internationally recognized leader in cancer research, FDA regulatory affairs, government policy, and business finance. His research focuses on development of molecular-based high throughput technologies, to enhance the understanding of cancer biology and improve cancer diagnosis, treatment and prevention. He has authored more than 90 papers in peer-reviewed journals, including co-editing a bestselling book on oxidative stress and aging. Dr. Rodriguez serves as director of the NCI Office of Cancer Clinical Proteomics Research where he oversees research decisions, development, and oncology

programs in proteomics. Prior to NCI he worked at NIST, where he developed and directed the Cell and Tissue Measurements Group, and was program analyst in the Office of the Director, director of the Tissue Engineering program, and principal scientist in the DNA Damage and Repair program. His fellowship was at The Scripps Research Institute and the City of Hope Cancer Center. Dr. Rodriguez has received numerous awards, including the Sigma Xi Young Scientific Investigator award, FDA Leveraging Award, MARC/MBRS programs of NIGMS Distinguished Alumni Award, and the Florida International University Distinguished Alumni Award. He holds a B.S. degree in biology/chemistry and a M.S. degree in toxicology from Florida International University, a Ph.D. in cell and molecular biology from Boston University, and a M.B.A. in finance and management from Johns Hopkins University School of Business.

Marc Salit, Ph.D., is leading a NIST group dedicated to technology development and measurement infrastructure (standards, reference data, predictive models) for massively multiplexed genome-scale measurement methods. The Multiplexed Biomolecular Science group is a multidisciplinary team growing out of work to address microarray measurement science issues, and a long-running effort in technology and measurement science in microfluidics. Dr. Salit has worked extensively in measurement science in chemistry and physics, with emphasis on precision measurements, lab automation, algorithm development, measurement uncertainty, traceability, and standards development. His research is now focused on bringing experience from the chemical metrology community to the emerging biometrology community.

Lynn Sorbara, Ph.D., is a program director in the NCI Division of Cancer Prevention, Cancer Biomarkers Research Group since 2007. She earned her doctoral degree studying the mechanisms of action of the drug Taxol at the Albert Einstein College of Medicine. Her post-doctoral training in insulin and signal transduction was done at the Rockefeller University. After a few years as junior faculty at Mt. Sinai School of Medicine, Dr. Sorbara came to NIH in 1992, first in NIDDK, and then as the technical supervisor of the CLIA/CAP-approved molecular diagnostics unit of the NCI Laboratory of Pathology.

Sudhir Srivastava, Ph.D., M.P.H., is chief of the Cancer Biomarkers Research Group in the NCI Division of Cancer Prevention. Dr. Srivastava joined NCI in 1988. Since 1990, he has served as program director in the DCP focused on developing and managing programs in molecular diagnostics with primary emphasis on cancer screening, early detection, risk assessment and informatics. In 2000, Dr. Srivastava developed and implemented a novel approach to collaborative clinical research on cancer biomarkers through establishment of NCI's flagship EDRN. In collaboration with NASA's Jet Propulsion Laboratory he has played a key role in EDRN informatics infrastructure, a model collaboration being followed elsewhere.

Sanford A. Stass, M.D., is a board certified pathologist in anatomic, clinical and hematologic pathology with expertise in molecular pathology. He is chair of the Department of Pathology and Department of Medical and Research Technology at the University of Maryland School of Medicine, and director of the Laboratories of Pathology at the University of Maryland Medical Center. Dr. Stass has more than 25 years of administrative and research experience in anatomic/clinical and hematologic pathology at the University of North Carolina, Chapel Hill, St. Jude Children's Research Hospital, and the University of Texas M.D. Anderson Cancer Center and other institutions. He is recognized internationally for his research in the molecular biology of hematopoietic neoplasia; has published over 200 peer-reviewed articles; and is recognized for contributions in the molecular biology and immunologic characterizations of leukemias and lymphomas, including identification and clinical application of novel biomarkers for diagnosis, prognosis, and therapeutic monitoring. Dr. Stass was a founding member and chair of the CAP molecular pathology resource committee that established the national standards for operation of

clinical molecular diagnostic laboratories and criteria for their inspection and U.S. accreditation. As a CAP fellow, he was chair of the molecular pathology resource committee, and a founding member of the Ad Hoc Committee for stored tissue/genetic privacy, which dealt with genetic testing and patient privacy, and a member of the Clinical Laboratory Improvement Advisory Committee Subcommittee on Genetics Testing. As PI of the University of Maryland Baltimore Biomarker Reference Laboratory, Dr. Stass is developing biomarkers for lung cancer and collaborating with several laboratories within the EDRN to standardize methodologies to evaluate and validate cancer biomarker assay performance between research-oriented discovery laboratories and CLIA/CAP accredited diagnostic laboratories.

Živana Težak, Ph.D., is an associate director for science and technology in the Office of In Vitro Diagnostic Device Evaluation and Safety, Personalized Medicine Staff, at the Center for Devices and Radiological Health, FDA. Prior to joining the FDA in 2004, as a scientific reviewer in microbiology, genomics and molecular biology, Dr. Težak worked in biotechnology industry, holding research and development scientist positions in a bioinformatics and array developer company. Dr. Težak received a Ph.D. in biochemistry/molecular biology from Florida State University in 1997. From 1998 to 2001, she was a research fellow at the University of Pittsburgh Medical Center and Children's National Medical Center, Research Center for Genetic Medicine, working on neuromuscular disorders, human genetics, gene therapy, and high-throughput screening technologies. Her work resulted in a number of publications in peer-reviewed journals, book chapters and proceedings.

Jim Vaught, Ph.D., is chief of the NCI Biorepositories and Biospecimen Research Branch (BBRB). After working as a laboratory scientist in the mechanisms of chemical carcinogenesis, Dr. Vaught has worked in biorepository and biospecimen science for more than 15 years. In 1999, he was a founding member of the International Society for Biological and Environmental Repositories, and was its second president. He participated in the development of the first edition of ISBER's Best Practices for Repositories. In 2005, Dr. Vaught joined the OBBR and has participated in the development of NCI's Best Practices for Biospecimen Resources and the office's other strategic initiatives.

Nadarajen A. Vydelingum, Ph.D., FACB, has several years experience in the medical field as a basic and clinical investigator, educator, and administrator. He joined the NCI Division of Cancer Prevention, Cancer Biomarkers Research Group in 2012 where he participates in programs for the development of novel molecular strategies in biomarker research. In 2007 he spent a year in the DCP Office of Preventive Oncology, when he served as associate director and co-led the Cancer Prevention Fellowship Program. In 2008, he worked in the Basic Prevention Science Research Group where he participated in the development of systems/integrated biology approach to cancer prevention research and worked on the NIH-wide Genes, Environment and Health Initiative. In 2002, he was appointed deputy director of NCI's Center to Reduce Cancer Health Disparities. In 1991, Vydelingum joined the Center for Scientific Review where he headed a scientific review group on peer review in bioengineering and physiology. Vydelingum earned a Ph.D. in clinical biochemistry from the University of London, U.K. in 1977, and joined the Medical College of Wisconsin in the departments of medicine and pharmacology and later as the director of the lipid laboratory in the General Clinical Research Center. His early research interest in insulin action and fat metabolism as related to type II diabetes and obesity attracted him to Memorial Sloan-Kettering Cancer Center where he focused on the cellular mechanisms of cancer cachexia.

Resources Referenced in Workshop Presentations

College of American Pathologists (CAP) Accreditation Checklists

http://www.cap.org/apps/cap.portal?_nfpb=true&cntvwrPtl_t_actionOverride=%2Fportlets%2FcontentViewer%2Fshow&_windowLabel=cntvwrPtl_t&cntvwrPtl_t%7BactionForm.contentReference%7D=laboratory_accréditation%2Fchecklists%2Fchecklist.html&_state=maximized&_pageLabel=cntvwr

FDA Bioinformatics - Data Format, Storage, Data Analysis

<http://www.fda.gov/MedicalDevices/NewsEvents/WorkshopsConferences/ucm255327.htm>

FDA Biomarker Qualification Program

<http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/ucm284076.htm>

FDA Center for Devices and Radiological Health

<http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDRH/default.htm>

FDA CDRH Learn – Sponsor/Investigator/IRB Responsibilities, Bioresearch Monitoring Program

<http://www.fda.gov/Training/CDRHLearn/default.htm>

FDA Device Advice

<http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm>

FDA IDE and Pre-IDE Approval Process

<http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/InvestigationalDeviceExemptionIDE/ucm046164.htm>

FDA Information Sheet Guidance For IRBs, Clinical Investigators and Sponsors

<http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM328855.pdf>

FDA Medical Device User Fee Amendments 2012 (MDUFA III)

<http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Overview/MDUFAlII/default.htm>

FDA Office of In Vitro Diagnostic Device Evaluation and Safety (OVID) Guidance Documents

<http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/>

FDA PCA3 Assay: Summary of Safety and Effectiveness Data

<http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfTopic/pma/pma.cfm?num=P100033>

FDA Pre-submission Draft Guidance

<http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm310375.htm>

FDA Recognized Consensus Standards

<http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm>

FDA Performance of FDA Approved Genomic Tests/510(k) Decision Summaries

<http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmnm.cfm>

FDA Premarket Approvals (PMAs)

<http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMA/pma.cfm>

NCI Best Practices for Biospecimen Resources

<http://biospecimens.cancer.gov/bestpractices/>

NCI Cancer Biomarkers Research Group

<http://prevention.cancer.gov/programs-resources/groups/cb/>

NCI Cancer Diagnosis Program - Templates for IHC, FISH/CISH, or Somatic Mutations

<http://cdp.cancer.gov/diagnostics/templates.htm>

NCI Clinical Assay Development Program

<http://cadp.cancer.gov/>

NCI EDNRN: Research Tools and Informatics Resources

<http://edrn.nci.nih.gov/resources>

NIST Materials Measurement Science

<http://www.nist.gov/mml/bmd/index.cfm>

Glossary of Terms Used in Workshop

Biomarker: A characteristic objectively measured and evaluated as an indicator of normal biologic or pathogenic processes, or responses to a therapeutic intervention; typically measured using a diagnostic test or instrument (e.g. an *in vitro* laboratory diagnostic test, imaging diagnostic) or other objective measurement method (e.g. blood pressure); may refer to any biological measurement obtained using an objective instrument or test, without subjective judgment (patient, clinician, or observer assessment).⁸

- **Robust biomarker:** Compared to other biomarkers, a robust biomarker is strongly built, can be trusted, and therapeutic decisions can be based on it.
- **Validated biomarker:** A biomarker measured in a test with well-established performance characteristics and for which there is an established scientific framework or body of evidence that elucidates the physiologic, toxicologic, pharmacologic, or clinical significance of the test results.⁹

Confidence: Demonstration that results can be compared to one another; that they will likely take on a range of values that can be discussed and used in confidence bands. Calculations can be based on these results and predictions and good decisions can be made.¹⁰

Documentary standards: Sourced from regulatory body consensus standards to bring a measurement system into control, enabling traceability, measurement uncertainty and results comparison.¹¹

Qualification: The evidentiary process of linking a biomarker (using data obtained by a biomarker assay) with meaningful biological or clinical outcomes.¹²

Scientific validity: The scientific basis of biomarkers in terms of intended clinical use; a test to measure or detect biomarkers must be scientifically valid for it to be associated with the occurrence of a disease.¹³

Type materials: Complex materials representative of the sample being studied, often used in inter-laboratory studies to check the process and control bias; key tools in conducting method validation to enable sharing a sample with other labs to confirm both labs are getting the same results.¹⁴

Validation: The process of assessing the assay and its performance characteristics, and determining the optimal conditions that will generate a reliable, reproducible, and accurate biomarker assay for the intended application.¹⁵ Confirmation, through the provision of objective evidence that the requirements for a specific intended use or application have been fulfilled.¹⁶

⁸ Biomarkers Definitions Working Group (2001). *Clinical Pharmacology and Therapeutics*, 69, p. 89 – 95.

⁹ U.S. FDA Regulatory Information Guidances.pdf.

¹⁰ Salit, workshop presentation

¹¹ Salit

¹² Chau CH et al.

¹³ Salit

¹⁴ Salit

¹⁵ Chau CH, Rixe O, McLeod H, Figg WD. Validation of analytic methods for biomarkers used in drug development. *Clin Cancer Res* 2008;14:5967–76

¹⁶ [ISO 9000:2000, 3.8.5]