WORKSHOP ON BIOSPECIMEN REFERENCE SETS AND DRUG-DIAGNOSTIC CODEVELOPMENT

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Office of Biorepositories and Biospecimen Research

National Cancer Institute

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EXECUTIVE SUMMARY

The Workshop on Biospecimen Reference Sets and Drug-Diagnostic Co-Development was convened to inform the NCI Office of Biorepositories and Biospecimen Research (OBBR) about the biospecimen needs and challenges in the co-development of a drug and diagnostic assay. OBBR recognizes the importance of high-quality, well annotated biospecimens for the advancement of personalized medicine, and a better understanding of the issues associated with biospecimen availability for co-development will allow OBBR to assist in overcoming the biospecimen challenges that limit development of diagnostics.

Over the course of the day, experts in pathology, pharmaceutical development, regulatory oversight, clinical-trials research, and assay development described the importance of high-quality, appropriately consented, well-annotated biospecimens for the development, registration, and commercialization of candidate drugs and diagnostics. The meeting discussions highlighted a number of common themes, including the need for access to matched specimens from a variety of tumor types, better annotation, better control of collection, processing and storage variables, and ethical standards that allow for broader use of tissue.

To operate optimally, co-development researchers would benefit from access to matched frozen and formalin-fixed, paraffin-embedded biospecimens from multiple tumor types—common and rare; archival and prospective; pre-malignant, primary, and metastatic, with collection performed in a manner that emulates clinical practice. Biospecimen collections for the development of companion diagnostics have more requirements than development of any other types of assay, including samples that are positive and negative for the biomarker of interest and samples from treated and non-treated patients, with significant annotation that includes sample handling and patient treatment and outcome data. Ideally such biospecimens will be obtained from the beginning of an adjunct oncology clinical trial, but it is a significant logistical and financial challenge to build proper biospecimen procurement and annotation, as well as appropriate informed consent, into a clinical trial.

Numerous biospecimen challenges limit the optimal development of diagnostic assays. Assay developers frequently have difficulty in obtaining sufficient numbers of appropriate biospecimens for each step in the development pipeline. When available, variations in how biospecimens are collected and annotated often lead to questionable or unreplicable assay results, which can put an immediate end to the development of the diagnostic. Preanalytical variables that influence assay results must be managed to ensure standardized biospecimen collection. It is essential for diagnostic developers to have access to standardized high-quality biospecimens for preliminary steps in assay development and other molecular pathology

applications. Greater recognition of the difficulties associated with biospecimen procurement, by those developing assays and conducting clinical trials, and the regulatory agencies pertinent to co-developed drugs and diagnostics, would also be an important step towards ameliorating biospecimen challenges.

A national biobank would be ideally suited to collect high-quality biospecimen sets that include blood samples from multiple time points before, during, and after treatment, as well as matched tissue and fluid specimens, accompanied by treatment and outcome data and broadly applicable consent. Another opportunity of which a national biorepository might avail itself is in the development of publicly available protocols for high-quality biospecimen collection, and education of all stakeholders in the biobanking process.

For a diagnostic submission, a number of requirements must be met concerning the device's intended use and clinical validation. Often several disparate entities are involved in the steps that lead up to drug/diagnostic submission. Improved harmonization of protocols and informatics systems among participating organizations, and of guidance issued by regulatory agencies, would benefit the field immensely.

The workshop succeeded in initiating a dialogue with content experts around supporting drug and diagnostic-assay co-development and validation. OBBR welcomes additional input concerning the facilitation of this co-development and the feasibility of the proposed approaches.

SUMMARY & FINDINGS

A. BIOSPECIMENS AND CO-DEVELOPMENT: ASSESSING THE LANDSCAPE

Diagnostic assays are critical components of personalized medicine for cancer. Modern cancer therapeutics tailored around molecular "lesions" require both drugs that target specific biomolecules and companion diagnostics that can accurately detect those same targets. Such assays can be used in a variety of ways, including for assessment of safety, efficacy, pharmacodynamics, and pharmacokinetics. However, one of the most common uses is for the stratification of patients in the clinic, to determine whether individuals should be included or excluded from a given therapy. An assay with this use is considered a companion diagnostic, and is defined in recently released FDA draft guidance as "…in vitro device that provides information that is essential for the safe and effective use of a corresponding therapeutic product."[1]

The development of companion diagnostics relies on the availability of biospecimens of adequate and consistent quality, with appropriate annotation and consent. Unfortunately, such biospecimens are rare commodities, due to the limited dissemination of good biobanking practices, the lack of sufficient evidence guiding the collection of biospecimens, and a series of logistical and institutional hurdles that prevent consistent and efficient policies and procedures around banking. To address these barriers, the Office of Biorepositories and Biospecimen Research (OBBR)[2] of the National Cancer Institute convened a one-day workshop to address biospecimen needs in the field of drug-diagnostic codevelopment, with participants from the NIH, the private sector, and the FDA (Appendix 1). The purpose of this workshop was to identify the major scientific, economic, and regulatory barriers related to biospecimens, and to more fully understand the needs of diagnostic developers in biotechnology and pharmaceutical companies. The outcomes of this workshop are important for OBBR as it plans further initiatives in the field of biospecimen research and quality standards and as it establishes a national biobanking resource (the Cancer Human Biobank [caHUB]).[3] While this workshop focused on codevelopment, as companion diagnostics have the most stringent needs, many of the issues and recommendations discussed are broadly applicable to assay developers in general.

[1] http://www.fda.gov/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm262292.htm
[2] http://biospecimens.cancer.gov/default.asp
[3] http://cahub.cancer.gov/

B. NEEDS AND BARRIERS RELATED TO BIOSPECIMEN USAGE IN DRUG-DIAGNOSTIC CODEVELOPMENT

B.1. The assay development pipeline for co-development: process and biospecimen requirements

Biospecimens are required at every stage of the co-development pipeline, from basic R&D to assay validation and qualification (Figure 1). Initial efforts to identify biomarkers and to develop assays to detect those biomarkers, while often initially involving animal models or cell lines, must eventually be translated to human specimens. Specimens must be available to assess the expression profiles of the biomarkers of interest, the feasibility of detection, and assay performance in the context of critical parameters including reproducibility, sensitivity, and specificity, as well as the basic expression profiles of the biomarkers of interest.

The initial development phase of an assay is followed by Phase 1-3 trials to meet the requirements for regulatory approval. For codevelopment, while an Investigational Device Exemption (IDE) must be obtained for a diagnostic test used for inclusion/exclusion of patients during a trial, the implication of the current definition of a companion diagnostic is that a New Drug Application (NDA) for a novel drug cannot be approved without a Premarket Approved (PMA) diagnostic test to go with it (although approval for the diagnostic can be obtained at the same time as the drug). Such approval necessitates that a diagnostic with a specified intended use has successfully undergone both analytical and clinical validation. Part of the intended use must address specimen usage--it must define the target population, the type of specimen to be collected, the matrix, the analyte to be detected, and the platform being used for detection.

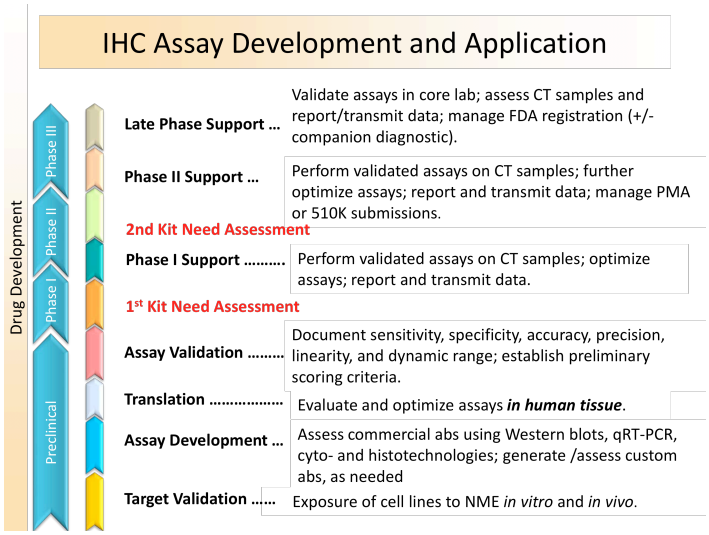

Figure 1. IHC Assay Development (courtesy of John Bloom).

Current FDA draft guidance lays out an ideal process whereby companion diagnostics are truly "co-developed" with a therapeutic, and envisions that developers have planned this outcome in advance. However, it is usually the case that an approved diagnostic is unavailable at the beginning of a pivotal trial. This may occur for several reasons. Small populations that experience adverse effects, or in which efficacy or resistance is observed—and for which a predictive marker is subsequently identified—may not be characterized prior to a large phase III or post-marketing study. In the most extreme case, no assay may exist at the time of a drug trial, a specific biomarker may not have been identified, and/or a diagnostic strategy for patient selection or stratification is only identified after its completion. Alternatively, existing FDA-approved assays may not be available for the correct indication, intended use, or sample type. For example, an assay may have been developed using tissue collected in a different format: basic research is often performed on fresh-frozen tissue, whereas assays in the clinic are typically performed on FFPE tissue. An assay may not have been shown to function in the appropriate matrix, which will involve demonstrating uniformity, analyte stability, and the effects of the matrix on the diagnostic measurement. Finally, when multiple versions of an assay exist, the assay(s) used in the pivotal trial may be different and therefore "bridging" to the assay(s) used become very complex and ill-defined.

In general, when the study design involves stratification using a known biomarker but there is not an approved assay, a laboratory-developed test(s) (LDT) may be used instead at the outset of the trial. This represents a bottleneck in co-development, as the assay can only be approved for clinical use in special circumstances. In the scenarios described above, bridging studies to the assay(s) (LDT) are required if the tests used in pivotal studies will not be approved by the FDA. Such bridging studies will require the use of retrospective collections from clinical trials, and such collections (as well as those used in the ideal case described above) must be adequately representative and compliant with the quality and annotations standards discussed below. Sufficient numbers of specimens, in the correct matrix, must be available. It is therefore unsurprising that there exist significant barriers that must be overcome for successful development of a diagnostic. Many were identified by participants, and can be classified into three major categories: specimen quality, quantity and type.

Addressing specimen needs are critical for regulatory approval. The FDA requires sample uniformity, which requires uniformity of the entire process (from sample collection to diagnosis). Sample type, sample handling protocol, and hardware and software must also be uniform. This requires a sufficient number of specimens—in the form of independently

collected sample sets--with adequate quality and annotation. For this reason, many of the barriers to regulatory approval are intimately linked to those described above, both to cover drug trials and clinical studies. A major limitation is the insufficient number of specimens that meet the necessary requirement to easily move an assay through the regulatory process for co-development.

B.2. Lack of standardized practices results in variability that can prevent the development of clinically valid tests.

Quality

Participants repeatedly pointed to specimen quality as a crucial issue facing assay developers. From the standpoint of the assay developer, quality typically refers to analyte stability. Many of the diagnostic development problems related to poor specimen handling result from the differential stability of analytes. Poor quality tissue will result in the alteration or degradation of analytes, preventing meaningful assay readings and making development or validation impossible. Workshop participants noted that the lability of some classes of analytes—particularly phosphoproteins—can pose an insurmountable barrier to assay development, and some companies will not purse diagnostics around these targets.

Specimen quality relies upon a number of factors that must be controlled or at least recorded during collection, processing, shipping, and storage. Molecular assays are sensitive to a number of these factors—such as time in fixative--with different assays having different requirements. When biobanking is not performed properly, quality drops off, yielding specimens that are not fit-for-purpose.

The failure to control preanalytical factors also means that it is difficult to accrue a sufficient number of biospecimens that have been handled in the same way to achieve valid assay results. This is unsurprising, as samples obtained from patients with the same diagnosis, but processed in different ways may end up yielding different results on a particular platform—even if those two tumors had identical analyte profiles *in situ*. Tests and standards for assessing specimen quality are lacking, and where they exist are inadequate for most uses, so there is no way of knowing *a priori* if a particular specimen is adequate for an assay.

The resulting variation in specimen quality can make it difficult to demonstrate analytical and clinical validity and clinical utility for a single product. Variable specimen quality effectively reduces the number of biospecimens that are useful for the development of molecular assays, as only a fraction of any collection will have specimens of sufficient quality for use in a

particular assay. These barriers are even higher when developing diagnostics for low-prevalence diseases, as even fewer specimens are available at the outset.

For these reasons, participants agreed that one of the most important needs is to control preanalytical factors through standardization. Standardization will allow for the collection of a set of specimens with uniform quality. As specimen quality is fit-for-purpose, with different assays requiring different levels of specimen integrity, collection guidelines should also be fit-for-purpose. The development and use of such guidelines require tools to preserve and evaluate biospecimen quality, and participants specifically noted needs around reference materials, standards, best practices, and SOPs. The provision of such tools demands a better understanding of biospecimen science and the factors that affect analyte stability and presentation. Particularly for retrospective samples, understanding the effects of storage conditions on specimen quality is critical.

Despite all of this, participants noted that even if preanalytical factors are understood by key personnel and specimen collection is standardized, some of the barriers related to specimens are inherent to biology. No specimen will exactly mimic the internal milieu in which it developed. By the time they are obtained, specimens may have been exposed to numerous drugs that can have large biological effects, and both surgical and autopsy specimens necessarily experience anoxic conditions for often lengthy periods of time. Such conditions can significantly alter gene expression within a specimen, meaning that biomarker discovery within a specimen runs the risk of identifying targets that do not reflect biology within the patient, but rather reactions to the conditions to which the specimen has been exposed. Understanding the effect that preanalytical factors have on specific analytes will help to address this issue.

Annotation

Annotation is an important component of biobanking. Providing information on how samples are collected, processed, and stored allows for construction of high-quality specimen sets, for instance, by selecting specimens that have experienced similar and acceptable exposures to uncontrolled variables (e.g., warm ischemic time, defined as the amount of time elapsing from arterial clamp to removal of the specimen from the patient). Therefore, proper annotation will allow assay developers to identify potential uses for banked specimens. The absence of such data results in specimen collections with samples of variable quality, which in turn will yield inconsistent or invalid results. Patient data, including treatment information, is also critical to interpreting data. Annotation is particularly crucial for the use of retrospective samples—particularly with respect to archived paraffin blocks--where knowledge of storage conditions is particularly important but usually not well-tracked. Further downstream in the process, outcome data are critical to establish whether a biomarker is diagnostic or prognostic.

Education

It was the consensus of workshop participants that even when a scientific basis exists for specific biobanking procedures, there is a general lack of understanding of the importance of preanalytical variables to specimen quality and analyte stability. Pathologists don't always understand the importance of the specimen to basic researchers or trial sponsors, and in any case are not economically incentivized to prioritize standardized collection and annotation. Best practices, standard operating procedures, and collection kits are helpful in attempting to standardize practice, but are nonetheless insufficient to ensure proper specimen handling, as they do not guarantee proper usage at the site of collection. For this reason, participants stressed the importance of education of biobanking personnel and of sponsors—who need to understand the value proposition and consequently prioritize procedures involving specimen collection and handling. The use of global central labs or contract research organizations capable of routine tissue handling and processing for molecular pathology applications also was recommended as a way to promote standardization. Such organizations must follow instructions and protocols, again pointing to the need for education.

B.3. Specific tissue types and formats are often limiting factors during co-development.

Biospecimens are required in every phase of diagnostics development, with given diagnostics requiring specific tissue types and formats. However, these are often either unavailable or available only in limited quantities. Generally stated, the specimens used for assay development must match what is found in the clinical populations. This includes the stage and subtype of the disease for which the assay is being developed. Additionally, specimen collection, whether it involves a fine-needle aspirate, needle biopsy, resection, or another method, also must match what is being done in the clinic. When assay development is performed on specimens that do not match the clinical population, additional studies are required, adding cost and complexity. Providing appropriate tissue will therefore allow tests to be developed that have more easily demonstrated clinical validity and utility.

Participants noted a number of diverse needs related to specimen availability. Matched samples of normal and diseased tissue from the same individual are very desirable, particularly given inter-individual variability in biomarker expression. Furthermore, having multiple well-characterized sample sets is important for the development of a single diagnostic assay, as characterization and verification must be repeated with independent samples. The number of these independent sample sets must be sufficient to allow for multiple studies.

Tissue sets from rapid autopsies were seen to be of particular use in understanding the basic science of a disease, target discovery, genomic analysis, and assay development. Moreover, this would allow for the collection of metastases, as often autopsy cases have undiagnosed cancer. Participants also noted other needs, including solid tissue, blood prior to death and rare tissue (including specific cancer types).

The availability of metastatic tissue was identified as a critical issue. Metastasis is a key event in cancer progression, and represents a significant problem in the development of molecular-targeted therapeutics. For example, the biomarker readout from a primary tumor may not reflect that of metastatic tumors, as metastases often have accumulated additional mutations when compared to the primary tumor. Therefore, targeted drugs may not be effective as the disease progresses. However, most clinical trials are not aimed at metastatic tumors, and collection of these would require additional biopsies, which is not standard of care. As a result, most available samples are from biopsies of tumors. Even retrospectively, archived paraffin blocks represent the tumor at the time of initial diagnosis. This discordance means that developed assays may target biomarkers that do not accurately reflect disease progression. Alternatively, diagnostics may be developed around a primary tumor, while treatment itself may target later stages of disease, meaning that the test may not be a good method of selecting therapies for patients. For these reasons, participants indicated the need for untreated and treated metastatic tumors for core research and analytical product development (particularly when dealing with cancer of unknown primary site), as well as primary tumors and metastases from the same patient. This need is equally valid for both common and rare tumors.

Participants also elaborated on special logistical hurdles presented when trying to obtain tissue of limited quantity. Technicians often over-trim paraffin blocks, limiting the use of those blocks for multiple purposes. Moreover, collection sites are reluctant to part with tissue blocks, and will instead just provide a small collection of unstained slides, which may be of limited utility. Again, better protocols and more effective education were seen as strategies to address this issue.

B.4. Biospecimen usage related to clinical trials involving diagnostic assays pose unique logistical and ethical barriers.

Drug-diagnostic co-development requires the collection of patient samples from clinical trials. While prospective studies, in which specimen collection associated with a trial is planned in advance, requires more logistical complexity up front, it does allow for better control of preanalytical variables than does a retrospective study.

Attrition is also a major issue related to bridging studies that must be performed to obtain approval of the companion diagnostic once the clinical trial has ended, and to IVD devices that want to add an indication to their product labeling. Such studies require an unbiased set of specimens that have been collected in accordance with the intended use of the assay. These bridging studies require a plan for biospecimen use that includes a description of how samples will be acquired, processed, and stored, and the inclusion of both screen-positive and screen negative patients. An inability to accrue specimens can lead to bias, and consequently a failure to validate. Therefore, successful bridging from the assay(s) used to enroll to a test to be approved requires a high ascertainment of specimens for testing[4], as well as both positive and negative samples by the enrollment tests, and a high concordance rate between the tests. This means that attrition can present a significant barrier to assay developers, with the number of viable biospecimens usable for analysis representing a small fraction of the number of patients initially enrolled in a trial. Attrition occurs first because of consent (consent issues are discussed in more detail below), then because not all consenting patients actually provide samples, and finally because not all samples that are provided are of sufficient quality for analysis[5]. For example, archived paraffin blocks typically are not collected or stored under standardized conditions, and they are not well-annotated; all of these factors add additional variability to studies. Attrition is even more pronounced when multiple biomarkers must be analyzed, as not all biospecimens are of sufficient quality to allow for assessment of all necessary molecules.

Attrition can also result from logistical hurdles. As clinical trials involving biomarkers usually involve multiple collection sites and analytical labs, problems with specimen collection, shipment, storage, and processing often result. Increasing the number of sites that must handle tissue increases cost and variability, and also involves multiple Institutional Review Boards (IRBs) with different standards for protocol approval.

B.5. Restrictive and non-standardized consent requirements may limit the accrual of specimens for assay development.

Participants identified strict and differing consent requirements as a major barrier to unbiased specimen accrual, and outlined several ways in which such requirements may curtail diagnostic

[4] It is often stated that the FDA desires that the diagnostic be tested on 90% of the patients from the clinical trial.

[5] Hirsch FR, Varella-Garcia M, Bunn PA Jr, et al. Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non-small-cell lung cancer. J Clin Oncol. 2006; 24(31):5034-42; Tsao MS, Sakurada A, Cutz JC, et al. Erlotinib in lung cancer –molecular and clinical predictors of outcome. N Engl J Med. 2005;353(2):133-44.; Mok TS, Wu YL, Thongprasert S. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009;361(10):947-57.

development. First, the common requirement by IRBs that informed consent (IC) documents detail the research to be performed using donated specimens does not easily accommodate bridging or other retrospective studies. This can preclude the use of specimens from clinical trials in the development of companion diagnostics, as at the time consent is given for participation in a clinical trail, needs related to diagnostics may not be known. Second, varying IRB requirements prevent specimen accrual. Clinical trials face unique Health Insurance Portability and Accountability Act (HIPAA) and IC procedures instituted by IRBs at each participating site. The lack of standardized rules means that different sites will face different requirements related to specimen collection and use[6]. Furthermore, many IRBs may view applications involving industry partners with more suspicion, and this may result in more stringent HIPAA and IC requirements than would otherwise be necessary. Resulting limitations in patient or specimen accrual can lead to biased collections and as a result may present a barrier to the development of effective diagnostic assays. Third, it is difficult to access specimens from patients screened out of clinical trials. While there is a need to obtain these specimens, sites may not allow collection from patients that are not enrolled in a trial; when collected, such specimens may not be fully tracked. This results in the need for yet another prospective specimen collection protocol by the device or diagnostic manufacturer to obtain specimens from the excluded population.

As a result of these barriers, participants felt strongly that broader and standardized consent appropriate for the use of retrospective samples is a critical need. Breadth of consent, particular consent for unspecified purposes, would allow the use of banked samples as required for specific assays. Standardized consent, perhaps guided by evidence-based best practices, would address the logistical difficulties faced by assay developers in dealing with multiple sites, and would more easily allow for collection from marker-negative patients. Standardized consent would also help industry more effectively approach IRBs.

[6] While the inclusion and exclusion criteria associated with a study do not vary between sites, the varying patient information and consent requirements may differ, adding time and leading to patients withholding consent.

C. RECOMMENDATIONS and OPPORTUNITIES for OBBR/caHUB

C.1. caHUB: a national biobank supporting biospecimen science

Using funds provided by the American Recovery and Reinvestment Act of 2009, the National Cancer Institute (NCI) established caHUB as a national center for biospecimen science and standards. The mission of caHUB is to provide high-quality, well-annotated biospecimens via a centralized biobanking infrastructure to develop standards, best practices, standard operating procedures, and other public products that will improve the use of human samples for basic and clinical cancer research. To achieve this goal during its pilot phase, caHUB will form a series of research collaborations with outside entities that have defined biospecimen science questions to address. As an example, a developer of immunohistochemical assays may need to determine acceptable fixation parameters for pancreatic tumors when detecting specified protein targets. caHUB will be able to provide that developer with pancreatic tumor sets, collected through a series of tissue source sites and distributed through a centralized infrastructure, that represent a range of treatment conditions such as fixation times, perhaps in the form of a tissue microarray, which would then be assessed using the relevant platform. The outcome of such an analysis will be used to develop the types of public products mentioned above.

C.2. caHUB can address the lack of well-annotated human specimens of standard quality through its public products

Given its mission and organizational structure, OBBR/caHUB can take a longer view than companies with regards to a number of identified needs, including best practices, evidence, education, and standards development. This will give caHUB the opportunity to provide a number of products and services that can ameliorate many of the technical and logistical challenges faced during co-development.

In its pilot phase, caHUB's focus on biospecimen science will directly address issues of quality. Primarily, caHUB will be able to provide aliquots of samples, including solid tissue, with controlled variation of preanalytical factors. These samples will be collected in a clinical setting and provided as tissue microarrays or in other formats. Data biomarker expression in these sample sets will provide guidance to diagnostic developers on fit-for-purpose biospecimen protocols. In particular, SOPs will be developed that will reduce variability and document tissue status. Tissue quality metric and assays will also be provided, and these can form a fit-for-purpose grading system. In this context, OBBR's recent initiative with the National Institute of Standards and Technology (NIST)[7] will result in the development of specific standards, certified

[7] http://biospecimens.cancer.gov/resources/publications/workshop/psr.asp

reference materials, and assays around tissue quality assessment. All such outputs will also allow caHUB to provide input to the FDA on quality standards around biospecimens.

At the same time, caHUB can provide hard-to-obtain specimens. Aliquots prepared as described above can be provided as matched sample sets, including pre- and post-treatment matched specimens, and sample sets pre-characterized for markers of known interest. All of these specimens will be annotated with relevant preanalytical factors, including time from patient to fixation (cold ischemia time), time of fixation, fixative type, and the age of cut specimens at the time of analysis.

Tissue sets and unique samples (e.g., metastatic tumors) from rapid autopsy programs are a particular niche that can be filled by caHUB. caHUB has the potential to provide access to pre-cancerous lesions, primary tumors, and/or metastases and blood along with clinical data from elderly patients who are foregoing treatment. Particularly in this last case, tissue can be used for evaluation of preanalytical factors, and to compare different sampling methods (biopsy, core sample, touch prep, etc.). In the post-pilot phase, it is envisioned that expanding the collection capabilities of caHUB will fulfill further needs, including the provision of reference sets, paired frozen and FFPE tissue, low-prevalence tissue, longitudinal samples (from multiple timepoints during disease progression or treatment course), and independent samples for assay validation.

Guard banding around particular variables for FDA submissions was cited as a specific need, as sample aliquots with controlled variation, matched with clinical specimens, are necessary to define assay parameters. Such sample sets, presented in a format such as a tissue microarray, could be provided by caHUB. During the pilot phase, such sample sets would allow caHUB to address specific questions in biospecimen science by further defining important prenalytical factors for specific analytes, tissue types, and platforms, while at the same time helping assay developers meet the standards necessary for regulatory approval of in vitro diagnostics (IVDs).

During the post-pilot phase, caHUB can also fulfill needs by acting as a global centralized lab for routine tissue handling and processing for molecular pathology applications. By banking biospecimens for unspecified purposes during the post-pilot phase, caHUB can help developers avoid delays associated with prospective collection and clinical follow-up, allowing needs to be rapidly met. caHUB could also provide banking services for particular projects. Such banking could support analytical validation as well as clinical trials involving IVDs. With regard to the latter, participants felt that caHUB can add value by providing improved methodologies, increased number of donors for diverse clinical trials, and better treatment and longitudinal data that can interface with electronic medical records.

C.3. Evidence-based standards for informed consent

Participants also envisioned a role for caHUB in addressing barriers related to informed consent. As it recruits patients for specific collections aimed at addressing biospecimen science, caHUB can simultaneously work with NIH's Office of Human Research Protection (OHRP) to understand how these patients react to requests for broader consent. This will help develop better evidence-based practices around patient consent. Dissemination of that research can help institutional IRBs remove unnecessary requirements for the provision of experimental detail in consent forms. To disseminate new evidence-based recommendations, participants recommended that caHUB publish an open letter to IRBs in conjunction with partners such as FDA and IOM.

C.4. Education and Outreach

Participants expressed the concern that, even when evidence is available to support specific biobanking practices, such practices may not be implemented due to ignorance on the part of key individuals. For this reason, participants felt that caHUB should take an active role in educating stakeholders on both technical and logistical aspects of biospecimen handling, and help them understand the difference between the optimal and pragmatic handling of a specimen. Education at procurement sites (provided to pathologists and technicians) will improve biobanking logistics, and should include clear protocols, collection kits, onsite tumor qualification, and instructions to histotechnicians for limited tissue quantities. Similarly, evidence collected by caHUB can be communicated to FDA to improve regulatory requirements. Finally, participants expressed the opinion that caHUB can play an important role in improving patient awareness of sample donation.

D. CONCERNS and BARRIERS

Despite the areas of opportunity for caHUB, participants identified a number of potential barriers to its success. While caHUB has a primary focus on supporting biospecimen science to enable the collection and use of specimens of standardized and sufficient quality, there may exist limitations its ability to completely address needs for co-development. For example, caHUB will not be able to control many of the known preanalytical factors—such as warm ischemic time—and specimen sets will therefore still contain significant variability. For this reason, assays under development will need to be sufficiently robust to be independent of uncontrollable variables.

The nature of available tissue may also represent a limitation. Sets of autopsy samples—while appropriate for some purposes--cannot be used for validating companion diagnostics. Moreover, the dynamic nature of tumors, involving complex genotypes, phenotypes, and signaling pathways may present technical challenges that caHUB will not be able to overcome. Participants also felt that, despite the best efforts of caHUB, many specimens will still have limited availability.

Participants expressed concern that the need for data transparency may come into conflict with intellectual property considerations. Users of caHUB specimens will be expected to share data collected on those specimens. However, companies will not be willing to share data publicly until all relevant patents have been filed[8]. Moreover, limitations imposed by informed consent may prevent data sharing even if data were anonymized.

Participants also felt that financial barriers may pose limitations to the use of caHUB by outside parties. caHUB's biobanking procedures, as well as more inclusive consent and extensive annotation, will lead to higher costs for specimens. Participants worried that the cost of obtaining specimens from caHUB will lead assay developers to turn to other sources for specimens, even if that means using less standardized and lower quality samples sets.

Finally, it was felt that even in the face of evidence, there will be resistance among various communities to changing traditional procedures and protocols, and this will provide a barrier to the adoption of best practices.

[8] In preparing this report, one participant pointed out later that this will be particularly true given the revisions to the patent system enacted in September 2011 by the America Invents Act.

APPENDIX 1. WORKSHOP DISCUSSION

I. Participants

Dr. John C. Bloom	Bloom Consulting and Food and Drug Administration (FDA)
Dr. Carolyn Compton	Office of Biorepositories and Biospecimen Research (OBBR)
Dr. Jeff Cossman	United States Diagnostics Standards (USDS)
Dr. Maryellen de Mars	USDS
Dr. Anthony Dickherber	OBBR
Mr. Noel Doheny	OpGen, Inc.
Dr. Myla Lai-Goldman	Personalized Science, LLC
Dr. Yun-Fu Hu	FDA
Dr. David Jackson	QIAGEN Manchester
Dr. Diane Leong	Genentech
Dr. David Litwack	OBBR
Dr. Yuan Liu	Oncology, GlaxoSmithKline
Ms. Karen Long	Abbott Molecular
Dr. Scott Patterson	Amgen Inc.
Dr. Reena Philip	FDA
Mr. William Pignato	Novartis
Dr. Rajesh Ranganathan	NIH
Dr. James Robb	SAIC-Frederick, Inc.
Dr. Sherilyn Sawyer	OBBR
Dr. Stephen Vincent	Athena Diagnostics
Dr. Eric Walk	Ventana Medical Systems/Roche Tissue Diagnostics

II. Introduction (Attachment 1)

Carolyn Compton, MD, PhD, Office of Biorepositories and Biospecimen Research

In her opening remarks, Dr. Carolyn Compton, Director of OBBR and the Executive Director of the Cancer Human Biobank (caHUB), explained that this meeting of experts was convened so that OBBR and caHUB representatives could obtain a better understanding of the biospecimen limitations in the area of co-development of a drug and a diagnostic assay. Such assays are imperative to the advancement of personalized medicine, as are biospecimens. OBBR's interest in biospecimens stems from its mission to advance personalized medicine. A major hurdle to the development of diagnostic tests is the scarcity of an adequate supply of appropriate biospecimens from patients in clinical trials of the associated drugs, and OBBR is interested in understanding and helping to address the biospecimen challenges that limit development of diagnostics.

In the United States, biospecimen collection for clinical trials and research tends to be done by individuals according to their own protocols, with varying annotation and informed consent procedures, resulting in widely varying quality of biospecimens and data available for diagnostic decisions and for research such as biomarker discovery and assay development. OBBR was formed to address this variation, which is generally recognized to be the major factor limiting progress in translational research. It has been promoting standards through its release of the NCI Best Practices for Biospecimen Resources; collaboration with the National Institute of Standards and Technology; support for research in biospecimen science through the Biospecimen Research Network and new technologies through the Innovative Molecular Analysis Technologies Program; and formation of the caHUB. The objectives of the caHUB include unifying policies and procedures for NCI-supported biospecimen resources for cancer research, and providing a baseline for operating standards on which to build as the state of the science evolves.

One of the activities of the caHUB might be to support diagnostic-assay development and validation. Such support would include offering special collections of biospecimens to address specified research questions; providing biospecimens for standards development, specifically in support of advanced genomics platforms; and building biospecimen infrastructure support for clinical trials. OBBR is interested in biospecimen requirements for developing diagnostic assays, including understanding where the current supply of biospecimens is lacking, and how to help ensure that sufficient, appropriate biospecimens are available.

III. Perspectives from an Industry Pathologist (Attachment 2)

Myla Lai-Goldman, MD, Personalized Science, LLC ®

As an anatomic and clinical pathologist, Dr. Goldman noted that even highly standardized commercial pathology laboratories, ones that rigorously control conditions between biospecimen receipt and data analysis, must consider significant issues of preanalytical variation introduced at the point of collection. Institutions such as LabCorp receive hundreds of thousands of biospecimens daily, and are rarely given information pertaining to such critical factors in sample quality as cold-ischemia time or blood collection protocol. This makes it difficult to determine their impact on the results of assays run in the laboratory.

Over the past two decades, Dr. Goldman has participated in the development of hundreds of clinical assays, some destined for clearance by the Food and Drug Administration (FDA) and others developed for in-house use. Access to adequate numbers of appropriate, high-quality, biospecimens collected in a consistent manner was often a determining factor in the

development of an assay. For example, a great deal of the research in drug-diagnostic co-development is performed on frozen tissue samples because these are most readily available. However, frozen tissue is not practical for clinical assays, which must be adapted to formalin-fixed, paraffin embedded (FFPE) tissues. An extremely useful product for a biobank to offer would be paired blocks of FFPE and fresh or frozen tissue. Another barrier to successful assay development is access to biospecimens from low-prevalence diseases; these must be collected over time, and sets of equally handled biospecimens in sufficient quantities are difficult to access.

In her more recent experience as a diagnostics consultant, Dr. Goldman has advised innovators working to develop assays or analysis platforms. These researchers often need to rely on the data integrity of licensed technology. Demonstrating analytical and clinical validity and clinical utility of a single product requires multiple, well-characterized biospecimen sets. It is not uncommon for biospecimen variability to render tests unrepeatable, quashing development of a product.

The biospecimen requirements for drug-diagnostic co-development will depend on the stage of the project. Single or multiple tumor types might be required. For example, if a research group has identified a genomic signature that might be associated with cancer, the group will need to investigate a number of tumor types to identify which have the highest percentage of lesions containing that signature. Ideally, the group would like to assess archival tumors—common as well as rare—and metastases from the same patients to evaluate any expression changes.

Academic researchers have some unique challenges in terms of biospecimen requirements. It can be challenging to obtain sufficient funding to collect high-quality specimens and associated data in clinical trials, for example. Typically more funding is available for treatment trials than for correlative laboratory studies, including assay development and validation. This problem might be addressed by a funding mechanism whereby applications are reviewed by pathologists and other experienced laboratory scientists who are intimately familiar with the challenges of drug-diagnostic co-development.

The process of discovering, translating, and validating new assays requires multiple validations, which need to be repeated on independent sample sets. Access to such sample sets would be significantly improved by widespread standardization in biospecimen collection, preservation, and storage; to translate most efficiently into benefits to the patient, every biospecimen tested has to have been collected in the same manner.

IV. Roche Sample Needs in Oncology Research (Attachment 3)

Diane Leong, PhD, Roche Sample Repository

Dr. Leong described the biospecimen challenges and opportunities she has experienced at Genentech and Roche banking fluid biospecimens primarily from clinical trials for exploratory research. She often works closely with tissue biobankers who frequently handle samples from the same patients, as well as commercial vendors, academic collaborators, and members of the diagnostics and regulatory community.

The biospecimen needs at Roche are driven by the drug-development pipeline; Roche currently has several potential oncology drugs in Phase I, II, and III trials. The new molecular entities (NMEs) in the pipeline address a variety of biological pathways, and in some cases have overlapping indications—some for more common cancers such as breast or colon, others more specialized—selected for the highest probability of success in developing a therapeutic and having efficacy.

Therapeutics developed at Genentech and Roche, such as Herceptin®, have become standard of care. Although this has been of ample benefit to patients, it presents a challenge to researchers, particularly early development researchers, who need to anticipate the next advance in the therapeutics pipeline and to identify non-responders and patients likely to relapse. The more that is known about differences between patients for whom a treatment is fully effective and those for whom it is not, the better the chance of identifying the pathways involved in disease and how the drugs influence disease progression. For example, a recent request for biospecimens involved fresh blood and plasma samples from colorectal cancer patients at multiple time points before and during Avastin® treatment, in addition to tumor tissue samples if possible, to help researchers identify biomarkers correlated with drug response or lack thereof. Another recent request included fresh frozen tumor and normal-adjacent tissue, plasma, serum, sputum, and fresh blood from lung cancer patients at the time of surgery, and plasma, serum, sputum, and fresh blood at 1 to 3 months post-surgery and again at 3 to 5 months post-surgery. Markers in tissue specimens may be followed in fluid samples for development of companion diagnostics using more easily accessible samples.

Biospecimens collected in conjunction with clinical trials fulfill some, but not all, needs of researchers. In the past, researchers acquired biospecimens from vendors in an ad-hoc manner, but quality was sometimes compromised because of time constraints, pricing was not always negotiated, and competition for scarce samples was generated among different parts of the organization. These challenges to acquisition have been addressed by creating the centralized sample procurement team, which supports activities of the Genentech Research and Early

Development (gRED) group. This service receives in-house requests for biospecimens relevant to a variety of indications and phases, maps the requests to variety of commercial vendors, and manages those vendors for the company.

A recent analysis of the trends in biospecimen requests across the company noted a significant increase in demand over the past two years, reflecting increased activity in the direction of personalized medicine to support companion diagnostic development. Specifically, requests have increased for samples from multiple time points before and during treatment or longitudinally over several years, for prospective collection driven by patient criteria and project requirements, and for sample sets of tissue plus matched fluid from the same patient. Accompanying patient-treatment data and information on outcomes, such as metastases, are now considered mandatory.

The company has established criteria for selection of external biospecimen vendors. Currently, gRED has 25 vendors with which it works; only 5 of these can provide highly detailed annotation for FFPE tissues, and only 4 offer a variety of approaches to prospective collection. Designated, prospective collection of high-quality biospecimens with detailed treatment information is crucial for biomarker discovery and research, but comes at a high cost. Lower-cost residual or remnant tissue from dissections that would otherwise be discarded, or residual blood samples from chemistry laboratories, may be used for testing assays or instrumentation, but are insufficient for biomarker development.

In its active management of commercial vendors, the sample procurement team uses a number of selection criteria. Several of these are mandatory, including that the vendor has direct access to oncology or immunology clinics and surgical centers; has direct access to hospital pathology archives and patient treatment information; is knowledgeable about the disease of interest; has the ability to initiate or modify procurement activity quickly; can provide high-quality dedicated biospecimens, not residual samples; and can deliver fresh blood samples within 24 hours of the draw. It is not mandatory that institutional review board (IRB) approval be already in place, but this is preferred. Other criteria are based on specific requests and pertain to cost and availability of banked or prospectively collected biospecimens. The sample procurement team also performs site visits and monitors the performance of biospecimen vendors, for example, for responsiveness to any arising issues and on adherence to guidelines on shipping notification, manifest format, and barcoding. Researchers assess the quality of the samples according to the assays they run, supplying that information to the sample procurement team, which then provides feedback to the vendor.

Dr. Leong related some observations she has made on vendor site visits that might indicate areas of opportunity. Some of the smaller clinics do not always appreciate the value of the biospecimens to biomarker research and translational science. Vendors tend to have high enrollment in procurement projects and a high rate of obtaining informed consent from participants.

A centralized, national biorepository presents the opportunity to plan for future needs more than the current system typically does. Growing needs in the drug-diagnostic co-development community involve more treatment information, clinical data, and longitudinal information; a centralized biobank might have the ability to leverage electronic medical records to deliver clinical data along with biospecimens. Additional needs include increasing involvement of donors from clinical sites; a national biorepository might be ideally situated to improve public awareness of the utility of biospecimen donation. Finally, matched sample sets are increasingly in demand; a centralized biobank might have the resources and foresight to procure these routinely.

V. The Importance of High-Quality, Appropriately Annotated Specimens in the Development, Registration, and Commercialization of Candidate Drugs and Diagnostics: Observations from the Perspective of a Pharmaceutical Sponsor and Government Consultant (Attachment 4)

John C. Bloom, VMD, PhD, Bloom Consulting Services, LLC, and Food and Drug Administration

A number of developments in the business of translational medicine have presented increasing challenges to stakeholders relating to the access to and use of biospecimens. These include shifting portfolios due to mergers, acquisitions, and partnerships; transformation of research-and-development (R&D) efforts as pharmaceutical companies downsize; and aggressive outsourcing efforts. In addition, translational science and enabling technologies are rapidly evolving and continue to identify and characterize major signaling pathways and key mutations. With the increasing emphasis on personalized medicine, there has been significant growth in the number of specialized life-sciences and drug-development service providers as biomarker and diagnostic development strategies become more integrated.

Stakeholders in biospecimen research include drug and diagnostics developers, regulators, contract-, academic-, and cooperative-research organizations, biospecimen providers and bankers, sample analytical service providers, medication prescribers, payers, and patients. Among these are rapidly evolving and interdependent value propositions with strategic, technical, and operational implications.

Biospecimen access is an enabling resource in pharmaceutical and diagnostics R&D. Appropriately collected, stored, and annotated samples provide for molecular characterization of the patient or clinical-trial subject and that person's disease, which in turn enables drug discovery, translational medicine, clinical development, diagnostic development, product differentiation, and personalized medicine. Optimal performance of these activities requires access to a range of biospecimens that includes blood components, urine, cerebrospinal fluid, tissue, and extracted biomolecules, among others. These may be collected fresh or, more commonly, stored in biorepositories. They may be obtained commercially or collected from clinical-trial subjects during development of a drug or diagnostic. The requirements concerning the collection process, handling and storage conditions, and level of annotation generally are defined by the application to ensure that they are "fit-for-purpose."

Commercially obtained biospecimens are in great demand for use in the drug development pipeline, frequently in conjunction with cell lines, xenografts, or related in vivo models. They may be used for target identification, validation, and characterization; for biomarker discovery; to validate animal models for efficacy and preclinical safety in humans; for patient claims; or for regulatory submissions. Additionally, they may be used to inform clinical development strategies for screening or stratifying patients and to provide references or standards for tumor molecular profiling in terms of disease stage, therapy effects, or relapse. Finally, they may be used for development of diagnostics either prospectively or retrospectively in conjunction with biospecimens from clinical trials. To obtain biospecimens for these purposes typically requires access to specialized biorepositories through pharmaceutical service providers or strategic partnerships and collaborations.

The identification of biomarkers is foundational to diagnostics development. Access to tissue is the biggest challenge to finding biomarkers that enable cancer drug development and provide candidate diagnostics; in fact, NCI identified access to quality tissue as the largest impediment to translational oncology research. There is limited availability of high-quality, fully annotated tissue that has been handled with appropriate quality control in order to minimize artifacts due to collection, storage, and fixation. There also is minimal quality control of representative samples; for example, The Cancer Genome Atlas procurement team found that greater than 98 percent of archival FFPE biospecimens failed to meet set tissue standards (such as percent tumor nuclei and necrosis), and nearly 30 percent of those that passed failed molecular quality standards (such as RNA integrity number). Assuring that appropriate informed consent has been obtained from patient donors is another obstacle to accessing biospecimens, as is cost, particularly for rare-disease biospecimens. Many biospecimens have been preserved as FFPE blocks, which have significant limitations in terms of research utility.

Tissue acquisition in clinical trials presents particular challenges. It can be burdensome to find subjects and caregivers who will agree to biopsies pre- and post-therapy. If procurement begins after therapy has been started, the success rate in retrieving pre-therapy diagnostic tissue that is appropriately annotated, quality controlled, and consented can be as low as 30 percent. There is reluctance to have the collection site perform the measures necessary to ensure biopsy quality.

Another challenge arises from the reliance on solid-tissue markers for assay development. For example, to develop and validate an immunohistochemistry (IHC) assay involves a series of steps from exposure of cell lines to the NME, developing antibody assays for the NME, evaluating and optimizing the antibody assays in human tissue, validating the assay, performing and optimizing the validated assay on Phase-I clinical-trial samples, assessing the needs for developing an assay kit, performing and optimizing the validated assay on Phase-II clinical-trial samples, performing and optimizing the validated assay on late-phase clinical-trial samples in a core laboratory, and managing FDA registration with or without a companion diagnostic. The stakeholders in each step of the process tend to be distinct, making it impossible for all of this to occur with one vendor or partner.

A spectrum of partnerships characterizes the development of a diagnostic between biomarker discovery and commercialization. Academic, government, and research institutions are primarily focused on biomarker discovery and assay development. Biomarker discovery companies focus on these aspects as well as assay validation. Small or niche diagnostic companies have a similar focus to biomarker discovery companies, but also might participate in the development, validation, and approval of an in vitro diagnostic. Medium or large diagnostic companies might participate on assay development and validation, but are more focused on in vitro diagnostic development, validation, approval, and commercialization. Understanding this difference in the strategic foci of various partners is critical to meeting the sponsor's needs, including fit-for-purpose use of biospecimens.

At the American Association of Cancer Research 2010 Annual Meeting, Dr. Bert Vogelstein noted that of 130,072 mutations in 3,142 genes in 353 cancer subtypes, only approximately 320 "driver genes"—286 tumor suppressor and 33 oncogenes—have been identified; almost all of these fall into 12 core signaling pathways. This has profound implications for diagnosis and treatment: this vast tumor heterogeneity means that tumors with identical presentations might be due to mutations at distinct locations, therefore molecular profiling of each patient's tumor will be needed to determine appropriate treatment. Biomarker-Integrated Approaches of Targeted Therapies for Lung Cancer Elimination (BATTLE) is the first completed biopsy-

mandated study in stage-IV non-small-cell lung cancer patients. The biopsies were evaluated for markers from four molecular pathways implicated in non-small-cell lung cancer, and the patients were adaptively randomized for one of four therapies based on eligibility criteria and biomarker analysis; this personalized placement resulted in disease control in 46 percent of patients, whereas typical trials in this patient population would expect only about a 30 percent response rate. This confirmed the value and feasibility of routine biopsy and profiling for non-small-cell lung cancer patients.

Although the results of the BATTLE study were groundbreaking in terms of personalized medicine, the number of full-time pathologists required for just the 255 patients in this study makes such an approach impractical for routine care at this time. To integrate molecular markers into clinical trials and clinical practice will involve standardization of protocols and testing, biomarker validation, commitment to tissue collection and feasible implementation of tissue collection, access to high-quality yet practical technical platforms and services, and collaboration of the sponsor, clinical research organization, and analytical service provider. Access to well-annotated biospecimens will be crucial throughout the process.

In addition to biospecimen access and reliance on solid tissue biomarkers, another challenge to biomarker discovery is the dynamic nature of the genotype, phenotype, and signaling pathways involved in cancer. Access to pre- and post-treatment biospecimens and matched controls is essential to distinguishing the effects of tumor progression from those of the intervention, meaning that phase-specific biospecimens that are appropriately collected and profiled is necessary. FDA desires assessment of at least 90 percent of subjects to support claims based on changes in tumor molecular profile, which is a high bar to reach.

A number of principles guide diagnostics development in an R&D setting. The overarching priority of a pharmaceutical company is to optimize sales of therapeutics; diagnostics are a means to optimize the value of the sponsor's drugs in the marketplace. The range of assay-driven technologies and commercialization needs requires multiple, and often novel, partnerships. The indication or opportunity for a value-added diagnostic can occur at any point in the drug-development/commercialization chain.

Because the need for a diagnostic might arise at any point in the drug-development process, it is not always possible to follow the optimal sequence of events in diagnostic development, in which proof-of-concept for an investigational-use-only assay triggers production of the in vitro diagnostic early so that the in vitro diagnostic may be used in the drug's clinical trials and the data therefrom used for regulatory registration. Frequently, investigational-use-only or "home-brew" assays are adapted to in vitro diagnostics later, requiring bridging studies to demonstrate

that the home-brew or investigational-use-only assays are effective in clinical-trial samples and/or the equivalency between home-brew or investigational-use-only assays and the in vitro diagnostics. Having ready access to clinical trial samples is the key to enabling registration of the in vitro diagnostic in a timely manner in these late-start situations so that the diagnostic is available when the therapeutic is ready for launch. Commercially derived biospecimens are unacceptable—the biospecimens used for assessing in vitro diagnostic must be those collected from patients in the drug trials.

Biospecimen banking during clinical trials is essential. Properly annotated and collected biospecimens are a critical resource for follow-up candidate discovery, optimization, and development; essential for candidate- and clinical-trial, subject-specific biomarker development; and necessary for post-registration challenges and opportunities. Some regulatory agencies and IRBs proscribe biospecimen collection without a specific hypothesis prior to the study; this would preclude a wide swath of drug-diagnostic co-development.

Dr. Bloom concluded with his thoughts on opportunities and challenges for OBBR. The field would benefit greatly from caHUB methodologies being applied to clinical-trial biospecimens. The FDA and the caHUB might collaborate to encourage and facilitate biospecimen collection in clinical trials. OBBR might educate stakeholders concerning biospecimen collection in clinical trials as to the value proposition, research expectations, fit-for-purpose practices, and logistics. Finally, the caHUB might expand protocol development beyond traditional tissues and fluids to such biospecimens as circulating tumor cells and DNA, exosomes, and microRNA.

VI. Discussion

Dr. David Litwack framed the discussion by stating that reference sets of normal tissue might be useful to diagnostics developers for biomarker discovery and assay development. Assistance with biospecimen collection in clinical trials might also be welcome. When asked about most pressing needs, Dr. Scott Patterson replied that well-annotated and -handled biospecimens are needed for biomarker discovery work, and outcome data are critical to establish whether a biomarker is predictive or prognostic. Analytical validation must be done on biospecimens collected in a manner identical to that in a clinical setting. Dr. Leong added that while oncologists might be the physicians running a clinical trial, pathologists are the physicians banking the biospecimens. It is not always clear to the banking pathologists that the biospecimen is important to the pharmaceutical sponsor of the trial, nor to the pharmaceutical sponsor that the biospecimens are valuable to the diagnostics developer when the two

companies are distinct. In addition, pathologists rarely are reimbursed for their involvement.[9] Incentivizing pathologists might result in clinical-trial biospecimens being available. Another participant added that sponsors should indicate their biospecimen needs in the contract with the biospecimen-procurement organization, which is likely to comply rather than risk losing its sponsor. OBBR might help alleviate this issue by promoting collection of sufficient quantities of high-quality biospecimens for the purpose of companion-diagnostics development from early stages of clinical trials.

Another participant raised the issue of informed consent. As mentioned by Dr. Bloom, frequently the in vitro diagnostics used in Phase III trials are not fully analytically validated, and bridging studies with high ascertainment rates are required. However, informed consent documents often do not accommodate bridging studies; voluntary participation in biospecimen donation with clinical trial participation precludes a 90 percent ascertainment rate. This contributes to the difficulty in accessing sufficient biospecimens for diagnostics development. In addition, bridging studies can deplete sample stores, particularly if multiple bridging studies need to be done to achieve concordance. Dr. Yun-Fu Hu added that biospecimens from the clinical-trial patient pool also are needed for regulatory submissions, and rarely are sufficient quantities of these collected.

This reflects a larger issue noted by several participants, which is that even pharmaceutical companies fail to appreciate what is necessary to develop a companion diagnostic. This failure extends to the FDA and IRBs, as evidenced by the requirement for companion diagnostics to receive FDA approval and IRB requirements that informed-consent forms specify exactly the experiments to be performed on the collected biospecimens, which may preclude the biospecimens' use in diagnostics development. Dr. Compton commented that the Office of Human Research Protections (OHRP) does not impose the requirement of IRBs that informed consent forms must clearly detail the research to be performed. OBBR is hoping to work with OHRP to support research concerning patient's reactions to broader consent, and from that OHRP might be able to assure IRBs that this restriction is unnecessary. The group agreed that this would be extremely helpful.

Dr. Compton commented that biospecimens from commercial vendors are rarely accompanied by sufficient data on preanalytical variables—such as clamp time or cold-ischemia time—to assess the biospecimen's quality. Several participants agreed that it would be very useful to

[9] Billing codes currently do not exist that pathologists may use to specifically biobank remnant tissue biospeciens for research purposes only. Coverage of remnant tissue collection for biobanking is possible if that activity is requested by the submitting surgeon. Furthermore, codes that allow for molecular analysis only cover those activities when relevant for patient care, not for research biobanking.

drug-diagnostics developers to have access to an assay that would enable evaluation of the quality of the biospecimen. Ventana Medical Systems, for example, has chosen not to develop companion diagnostics for phosphorylation markers because these markers are notoriously unstable, and clinical biospecimens are highly unlikely to have been handled carefully enough to ensure a meaningful assay reading. A standard or metric that would inform researchers of the quality of the biospecimen would help to ameliorate this problem. Dr. Liu added that education of the personnel at the procurement organization is critical to ensuring biospecimen quality; without detailed understanding of the requirement of controlling preanalytical variables, technicians are unlikely to practice optimal handling techniques.

Dr. Compton noted that little about biospecimen handling is evidence-based; even cryopreservation, which is held up as the gold standard for biomolecule preservation, has little objective evidence supporting its vaunted position. Dr. Leong added that pharmaceutical companies developing companion diagnostics have to be pragmatic. Understanding that variables such as clamp or cold-ischemia times often cannot be controlled, developers know that the assay being developed must be robust. Dr. Compton added that biospecimen research will quantify measurable quality differences between optimal handling and pragmatic handling once optimal handling has been established. To date, little work has been done to establish that.

Dr. Litwack mentioned that the term “high-quality biospecimens” has come up several times in the discussion and asked what criteria define high quality in a biospecimen among diagnostics developers. Dr. Liu replied that high quality will involve careful attention to the human portion of sample management, including accommodation of local and national laws and obtaining proper informed-consent; another determining factor in sample quality is the accompanying data. This can be problematic when dealing with commercial biospecimen vendors because requests for more inclusive consent or more annotation result in higher costs. Although specific quality requirements will vary depending on the tissue type and biomolecule of interest, frequently organizations that are well run and have provided samples that performed well in the past provide high-quality samples. This also can be determined when assays performed on multiple platforms yield comparable results. Dr. Litwack commented that OBBR is collaborating with the National Institute of Standards and Technology to establish biospecimen quality standards for DNA, RNA, serum proteins, and FFPE tissue; this is important because no common definition is widely in use. Dr. Hu added that standards for antibody quality, in terms of their performance and cross-reactivity, would also be of value. It would be helpful in promoting biobanking best practices if higher quality standards were to be reimbursed at a higher rate, because it is certainly the case that collecting high-quality biospecimens and annotation is more expensive than the alternative.

Dr. Compton described an experiment that the caHUB might perform or sponsor: aliquots of rigorously collected biospecimens may be subjected to known preanalytical variations and made into microarrays for evaluating an assay's feasible boundaries of such factors as fixation time or cold-ischemia time. Such microarrays may be provided to assay development companies for guard banding experiments. There was agreement among attendees that this is something diagnostics developers might be interested in purchasing; such work is currently done on human tissue xenografts, and there would be great value in being able to perform such studies on tumor tissue of the diagnostics' target subtype and grade that had been collected in the same manner as in a clinical setting. Dr. Bloom noted that results from experimentation such as this performed by the caHUB might be useful in providing guidance to developers of like assays or on like targets, to supply a set of parameters to being their guard banding experiments.

Dr. Leong summarized that the opportunities for OBBR and the caHUB are those that take a longer view than pharmaceutical companies tend to take, in promoting best practices in biospecimen collection, educating stakeholders about the requirement for companion diagnostics development, providing an evidence base for biospecimen handling, contributing to the development of standards, and offering biospecimen arrays for guard banding studies. It also would be beneficial for the caHUB to bank biospecimens for unspecified purposes so that they are available when needed, thus avoiding the delays inherent in prospective collection and clinical follow-up. Dr. Bloom added that strategic partners could be brought into the process to assist with the financing, because all of these functions would provide ongoing benefits to those partners. Dr. Compton affirmed that this was the initial intent of the caHUB, to begin operations via government funding and then transition into a public-private-partnership business model. Dr. Bloom likened that model to the Alzheimer's Disease Neuroimaging Initiative, which is a consortium of academic, government, and pharmaceutical partners that share data and resources to accelerate development of effective Alzheimer's disease treatments. To do something similar in the biospecimen arena would require standardization of biospecimens, analyses, and information technology.

Development of companion diagnostics also would be streamlined with buy-in from regulatory agencies in the adoption or promotion of standard informed consent or material transfer agreements. Dr. David Jackson added that regulatory agencies also should consider adjusting the evaluation parameters to accommodate changes to assays over time. For example, a current test evaluates seven Kirsten ras oncogene (KRAS) mutations; as additional mutations are discovered the test is likely to be expanded. It would be useful if the expanded test could be validated on biospecimens that were not collected in the clinical trial with which the test was

developed. Dr. Reena Philip agreed that a mechanism is required so that such assays can be brought to market. It is not always possible or feasible to run another clinical trial—pharmaceutical companies are unlikely to sponsor an additional trial to support diagnostics development—yet validation would need to be performed on clinical samples, which would be useful for the caHUB to offer. Dr. Jackson commented that it is a challenge to identify markers of negative predictive value, that is, markers that indicate a treatment is unlikely to be beneficial to a patient.

Dr. James Robb asked when is a reasonable time post-resection to perform a blood collection, if only one may be performed. Dr. Liu responded that this will depend on the drug cycle and the willingness of patients, but typical timing is 4 to 8 weeks after the end of treatment. It also would be useful to obtain progression samples at a number of time points.

Dr. Compton asked whether assays on urine, as a non-invasive and renewable biospecimen, have proven useful. Dr. Bloom replied that urine typically is not banked because of the resources required to freeze and store it. Dr. Hu added that no good cancer biomarkers have been found in urine.

VII. Biospecimens and Companion Diagnostics: FDA Perspective (Attachment 5)

Reena Philip, PhD, FDA

Dr. Philip explained that the FDA received approximately three times the number of submissions of companion diagnostics device consults (tandem submission of the companion diagnostic device and the drug with which it is associated) in 2010 than 2009. Companion diagnostics devices are in vitro diagnostics that are necessary for the safe and effective use of the corresponding therapeutic products, are included in the instructions for use in the labeling of those products, and are themselves labeled to guide use of a particular therapeutic or its generic equivalent, or a class of therapeutics. Co-development occurs when the test is intended to identify patients for whom the drug is expected to be effective or is expected to have minimal or no effect, who would likely have serious adverse events, or who would likely receive greater benefit or have lower probability for adverse events on one drug than another. Co-development also occurs when the test is intended to be used to monitor response to drug therapy or select doses of the drug most likely to be effective and/or safe for the patient.

Intended use of the device is a key factor in the evaluation of a pre-market submission. Specific claims made as to the intended use must be supported by data on appropriate performance characteristics. The “Intended Use” portion of the submission must document the target

condition; purpose of the test, i.e., whether it is for a diagnostic, predictive, or other purpose; analyte to be measured, such as DNA, RNA, protein, or metabolite; target population; specimen type, such as primary tumor, fine-needle or bone-marrow aspirate, or biopsy; matrix, i.e., whole blood, FFPE tissue, serum, etc.; the result type, i.e., whether it is a quantitative or qualitative test; and setting in which the diagnostic will be used.

The test should be clinically validated in Phase III of the drug trial, so that the clinical performance is supported by the Phase-III clinical-trial data. The training set should be distinct from validation sample set, and the test should be analytically validated before the clinical validation. However, this optimal situation is not always possible. For example, occasionally the test used in drug trial is not the version intended for marketing. In this case, sponsors should be prepared with a bridging study, and need to plan for sample acquisition, storage, and access for retest analysis by saving biospecimens from both screen-negative and screen-positive patients. Concordance is critical between the two tests at the negative/positive cut-off. Another challenge arises when samples are missing. In this situation, the sponsor needs to demonstrate that bias due to lost samples has been controlled by retaining inclusion of biospecimens from both screen-negative and screen-positive patients and by presenting well-annotated records, e.g., demographics, previous treatments, and evaluating other factors that affect the test such as tumor size, percent tumor content, and sample quality.

Sometimes prospective samples are unavailable because biospecimen collection was not part of the clinical trial until late in the process, and retrospective specimens are needed. Whether data from retrospective biospecimens will be considered is conditional on several key factors including whether the storage conditions of the specimens affect the assay; the specimens, as indicated by well-annotated records, are representative of the intended use of the device in terms of such factors as demographics, diagnosis, treatment history, age, and disease stage; the specimens are consecutive cases meeting a set of inclusion/exclusion criteria; and the performance assessed is comparable to that expected with prospective biospecimens. It must also be shown that the retrospective biospecimens are accompanied by appropriate informed consent, and are unbiased in terms of such factors as collection setting and specimen age.

Occasionally a bridging study is needed to demonstrate that a device works as well in one matrix as another. Factors that the FDA considers include the uniformity of the matrix, the length of time the analyte is stable in the matrix, the storage conditions required, the purification or concentration requirements, and any interference with assay measurement.

For any device submission, the FDA considers whether the test has been developed and validated in a manner that supports clinical diagnostic use in the intended population. The

studies to validate the test must have been controlled both analytically and for patient safety. The test instructions for use must be feasible in real-world settings.

High-quality biospecimens, when used in diagnostics development, will lead to better discovery, better tests, and good science. All of these will ultimately benefit patients.

VIII. Discussion

Dr. Litwack asked whether a variety of matched samples from the same patient would be useful in addressing the validation of tests in multiple matrices. Dr. Philip explained that access to such samples would be helpful; they would not be needed from the entire clinical-trial population, but the samples tested would need to cover the measurement range of the device.

Dr. Sherilyn Sawyer asked how the FDA judged biospecimen quality. Dr. Philip replied that it depends on the analyte; percent tumor content, for example, is one measure of tumor quality and standards such as the 260:280 nm ratio are used for stored DNA. Dr. Sawyer noted that NCI has received complaints when percent tumor or molecular quality are used as measures of biospecimen quality because these also can be measures of tumor quality, over which the collection site has no control. Dr. Philip requested any input OBBR might be able to offer as to quality standards. Dr. Jackson noted that he has experienced situations in which the validation method is less sensitive than the assay being validated. Whether sequencing will be required is negotiated on an ad hoc basis, and he recommended that the FDA develop a firm policy on this.

In response to a query from Dr. Jackson, Dr. Philip explained that the FDA Office of In vitro Devices has close working relationships with the Center for Drug Evaluation and Research and the Center for Biologics Evaluation and Research to ensure accuracy in drug labels.

Dr. Patterson asked how the cut-point for a drug's predictive biomarker should be established when therapies are becoming more targeted and as such, individuals in Phase III trials may be pre-screened to enroll only biomarker-positive subjects. Dr. Philip responded that this is why it is necessary to retain data about screen-negative patients in Phase III.

IX. Tissue Collection Challenges in Co-Development Clinical Trials (Attachment 6)

Eric Walk, MD, FCAP, Ventana Medical Systems/Roche Tissue Diagnostics

Dr. Walk has experience with biospecimen issues from the perspective of a surgeon and that of a pharmaceutical researcher. The types of biospecimens and associated annotation needed will depend on the intended use of the diagnostic device, although the preanalytical variables should be standardized and the quality fit-for-purpose. For a routine diagnostics assay for cytokeratin or transformation-related protein 63, for example, biospecimen might be obtained from public or private tissue providers and would need to be accompanied by anatomic pathology diagnostics information and, ideally, characterization data with results from comparative assays or technologies. Biospecimens for a prognostic assay, such as Oncotype DX®, might typically be obtained from an NCI Clinical Trials Cooperative Group and would have to be accompanied by patient outcome data. A companion diagnostic assay, for example to measure human epidermal growth factor receptor (EGFR) 2 or an EGFR gene mutation, has the highest biospecimen requirements, including some samples that are biomarker-positive and others biomarker-negative, and some should have been drug treated and others non-drug treated. The annotation needs to include response and outcome data. The typical source for such biospecimens is pharmaceutical clinical trials.

Many challenges arise in companion diagnostic co-development, including logistics such as biospecimen collection; technical challenges such as controlling preanalytical variables, selecting primary antibodies, and biospecimen limitations; and conceptual or scientific, such as whether the target is a primary or metastatic tumor or whether single or multiple biomarkers are in play. The experience of the Iressa Survival Evaluation in Lung Cancer (ISEL) Trial exemplifies the difficulty of biospecimen availability. This was a Phase III trial that compared gefitinib with placebo in 1,692 patients with refractory advanced non-small-cell lung cancer. Four biomarkers were evaluated at the DNA, RNA, and protein level, and were found in between 118 and 382 participants. However, tumor samples were only available in 460 of the 1,692 participants, and only 91 patients were available for all biomarkers.[10] Similarly, in the National Cancer Institute of Canada Clinical Trials Group Study BR.21, only 17 to 44 percent of patients' biospecimens were useable and successfully analyzed for IHC, in situ hybridization (ISH), and molecular sequencing.[11] The Iressa Pan-Asia Study had only 36 percent of samples available for EGFR mutation analysis, 33 percent for EGFR copy-number evaluation, and 30 percent for EGFR expression.[12]

[10] Hirsch FR, Varella-Garcia M, Bunn PA Jr, et al. Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non-small-cell lung cancer. J Clin Oncol. 2006; 24(31):5034-42.

[11] Tsao MS, Sakurada A, Cutz JC, et al. Erlotinib in lung cancer –molecular and clinical predictors of outcome. N Engl J Med. 2005;353(2):133-44.

[12] Mok TS, Wu YL, Thongprasert S. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009;361(10):947-57.

Clearly, tissue collection remains challenging in oncology clinical trials. Inclusion of biospecimen collection in a clinical-trial design increases logistic complexity, adds potential IRB issues, has the potential to slow enrollment, and increases cost. Mandatory tissue collection is becoming more common but is not yet standard. Enrollment of and biospecimen collection from biomarker-negative patients is crucial for diagnostic regulatory approval. Although prospective biopsies offer the greatest control over preanalytical variables, they add the most logistical complexity and cost to the trial and result in limited tissue. Archival paraffin blocks are more easily obtained, but offer no control over preanalytical variables and typically represent primary tumor at the time of initial diagnosis.

The logistical challenges in sample management and disposition relate to the involvement of multiple sites. For example, tissue samples from patients in a trial are likely to be stored at the site of the resection; portions of these need to be shipped to the laboratories performing the IHC, ISH, and sequencing—which are typically not all at the same location—and the shipping conditions vary depending on the method of preservation. Patient consent forms and compliance with the Health Insurance Portability and Accountability Act need to be considered throughout. The logistical complexity increases for Phases II and III trials.

Dr. Walk noted that simply including instructions in a laboratory manual is frequently insufficient to ensure appropriate biospecimen handling. For example, in one trial the collection site was instructed to secure biopsies in cassettes and label the cassettes with the subject number, patient initials, date of collection, time point of the biopsy in relation to the treatment, and protocol number. Some cassettes arrived with three layers of writing; others were labeled with just the company name. Some biospecimens were sent to the wrong site, i.e., the blood samples went to the IHC laboratory; some were shipped under the wrong conditions, others included insufficient tumor content.

To ensure that such logistics are handled appropriately, a great deal of detailed education is needed, preferably onsite, including a clear written protocol. Collection kits and onsite tumor qualification also can help minimize these issues. In cases of limited tissue quantities, instructions to histotechnicians should be to minimize trimming of the sample; employing a centralized laboratory should minimize the number of times a block is sliced; and assays should be multiplexed when possible. Sites tend to be reluctant to part with the entire tissue block, frequently offering 10 to 20 unstained slides instead.

A number of preanalytical variables should be annotated and controlled, particularly time to fixation, time of fixation, type of fixative, and the age of cut sections at the time of analysis. Other factors to consider include the use of phosphatase inhibitors, tissue processing protocol,

embedding paraffin temperature, type of glass slides, use of a tape transfer system, and thoroughness of deparaffinization. These variables are rarely annotated. Other errors may be introduced in these steps; for example, Dr. Walk displayed a photograph of uterine fibroids in formalin that did not fully cover the tissue.

Ventana Medical Systems conducted a study in xenografts tumors of the impact of six fixatives for six durations (between 1 and 48 hours) on the performance of Ventana HER2 dual ISH compared to Vysis HER2 fluorescent ISH. Hematoxylin and eosin (H&E) staining and both assays were optimal with fixation in neutral buffered formalin for 6 to 24 hours; longer or shorter fixation times were less ideal. Davidson's alcohol-formalin-acetic acid fixative gave the best results at 6 hours fixation for both assays, although the results were not optimal at any time point and the fluorescence assay had significant difficulties with morphology and background staining. The other fixatives produced even more disappointing results.

A poster presented by Indivumed at a 2008 symposium presented results from an experiment evaluating delay time between tissue removal and fixation. Phosphorylation markers increased between 5 and 90 minutes delay. A literature search revealed the opposite result or no change in phosphorylation markers, depending on the tissue and marker in question.

A study concerning the stability of immunohistochemical markers in cut sections on slides indicated significantly reduced staining for estrogen receptor by day 6 and virtually no staining by day 30. Antigenicity mostly could be preserved by coating slides in paraffin and storing them in nitrogen; addition of an oxidation inhibitor increased the rate of signal degradation.[13]

Another challenge for biomarker studies is the question of whether the biomarker readout from the primary tumor accurately reflects metastatic disease. Samples from primary tumors are fairly readily accessible, but most clinical trials are aimed at treatment of metastatic tumors, samples of which are rarely available without an additional biopsy, which is not standard of care. Increasing numbers of studies are evaluating the biomarker concordance between primary and metastatic tumors and are finding that it cannot be assumed.[14] The concern is that

[13] Atkins D, Reiffen KA, Tegtmeier CL, et al. Immunohistochemical detection of EGFR in paraffin-embedded tumor tissues: variation in staining intensity due to choice of fixative and storage time of tissue sections. J Histochem Cytochem. 2004;52(7):893-901.

[14] Scartozzi M, Bearzi I, Berardi R, et al. Epidermal growth factor receptor (EGFR) status in primary colorectal tumors does not correlate with EGFR expression in related metastatic sites: implications for treatment with EGFR-targeted monoclonal antibodies. J Clin Oncol. 2004;22(23):4772-8; Italiano A, Vandenbos FB, Otto J, et al. Comparison of the epidermal growth factor receptor gene and protein in primary non-small-cell-lung cancer and metastatic sites: implications for treatment with EGFR-inhibitors. Ann Oncol. 2006;17(6):981-5; Xiao C, Gong Y, Han EY, et al. Stability of HER2-positive status in breast carcinoma: a comparison between primary and paired metastatic tumors with regard to the possible impact of intervening trastuzumab treatment. Ann Oncol. 2011 Jan 14. [Epub ahead of

patients are being stratified for treatment based on the biomarker status of the primary tumor, but the drug is judged by the effectiveness in the metastasis. It would be ideal to have samples of primary and metastatic tumor from the same cases for biomarker analyses in drug studies.

Dr. Walk concluded with a biospecimen wish-list for diagnostic co-development. The first item was standardized sample-collection procedures that reduce or document preanalytical variability, accompanied by tissue quality metrics or assays with a fit-for-purpose grading system: low grade for morphology only, medium grade for routine ISH or IHC, and high grade for sequencing and phosphoprotein analysis. The second item was widespread availability of materials to enable IHC assay development, including clinical sample sets pre-characterized for known markers of interest and characterized cell lines and xenografts for use as assay controls. The third item was global central laboratories or contract research organizations capable of routinely handling and processing tissue samples for molecular pathology applications.

X. Discussion

Dr. Robb commented that several groups, including the National Community Cancer Center Program, American Society of Clinical Oncology (ASCO), College of American Pathologists (CAP), and Clinical Laboratory Standards Institute (CLSI) have issued guidelines for preanalytical variables such as cold-ischemia and formalin-fixation times. He added that Dr. Stephen Hewitt (NIH) has shown that degradation in paraffin-embedded sections is due to residual water (hydrolysis rather than oxidation). Finally, he contended that if the Automated Quantitative Analysis (AQUA®) method of quantitative IHC becomes the norm, the quality of fixation and annotation should improve. Dr. Goldman expressed concern that if a diagnostic's technology platform is only in use at limited sites, adoption and access to the test will be likewise limited.

Dr. Liu commented that the discordance between the primary and metastatic tumors can be a product of the biology of the tumor; metastases that develop early will be more similar to the primary tumor. The treatment regimen also can influence development of discordant metastases, as can the site of the metastasis. Dr. Goldman noted that some groups are beginning to request access to match primary and metastatic tumor for the reasons Dr. Walk discussed. Dr. Jackson added that challenges in sample availability will only be exacerbated if primary and metastatic tissues are needed.

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print]; Gong Y, Han EY, Guo M, et al. Stability of estrogen receptor status in breast carcinoma: a comparison between primary and metastatic tumors with regard to disease course and intervening systemic therapy. Cancer. 2011;117(4):705-13.

Dr. Compton added that these issues are further complicated for tumors in which surgical removal is not the standard of care, i.e., for which biopsies are used for diagnosis and then a non-surgical treatment pursued. This makes collection of tumors from esophageal or rectal cancer, for example, difficult to obtain prior to exposure to neoadjuvant therapy. Dr. Patterson replied that metastases might be obtained from rapid autopsy and evaluated with previously banked primary tumor. Dr. Compton explained that the caHUB has recently partnered with a rapid autopsy program. This arrangement might enable access to pre-cancerous lesions, primary tumors, and/or metastases and blood from elderly patients who chose not to receive treatment, along with pertinent clinical data. Dr. Jackson remarked that such tissue sets could be very valuable. Dr. Litwack asked what the group would recommend that such sample sets include. Dr. Philip replied that cancer of unknown primary site is in great demand. Several attendees stated that sets of autopsy samples would be quite useful for understanding the basic science of the disease and might be useful for assay development, genomic analysis, or target discovery, but being distinct from clinical samples and obtained differently, could not be used for development of companion diagnostics. Autopsy samples could be used for evaluation of preanalytical variables, such as type of fixative, on a variety of tissue types and antigens, particularly if samples were obtained in a variety of ways, such as biopsies, core samples, and touch-preps. Ms. Karen Long suggested that participants take the question of the utility of autopsy specimens to the core researchers of their pharmaceutical and diagnostics companies. Dr. Compton pointed out that although autopsy specimens have the drawbacks of lack of circulation and oxygen prior to excision, no biospecimen will exactly mimic the internal milieu—surgical biospecimens, for example, have been exposed to dozens of drugs with huge biological effects in the course of surgery.

Dr. Jackson asked how the caHUB will handle the informatics requirements of a large biobank. Dr. Compton explained that de-identified data from collection, processing, and storage will be publicly available for caHUB biospecimens. In addition, raw data from experiments using caHUB biospecimens will be deposited in a publicly accessible database. The Cancer Biomedical Informatics Grid (caBIG®) will underlie caHUB informatics for maximum interoperability. Reposited data will not be quality-controlled by the caHUB, but any validation or verification studies performed on aliquots also would be publicly available. Repositing data would increase the value of remaining aliquots and help prevent replication of work.

XI. Real-World Drug-Diagnostic Co-Development Learnings (Attachment 7)

David Jackson, PhD, QIAGEN

Dr. Jackson is part of QIAGEN's Companion Diagnostic Partnerships group, which develops molecular in vitro devices in support of pharmaceutical partners' newly developed drugs. This work primarily covers oncology, in which mutation or expression changes are identified at early stage of the clinical trial and it needs to be determined whether these correlate with clinical outcome.

The goal in co-development, largely driven by FDA requirements, is to have contemporaneous approval (within two weeks) of diagnostic and drug. In the absence of the accompanying in vitro device the drug will not be approved. The diagnostic, in the form of a standardized test, should be available for trial participants, and if it is to be used for determining trial inclusion and exclusion, an investigational device exemption must be obtained, otherwise later concordance studies are needed to demonstrate that the assay used is equivalent to the test submitted for approval.

To develop the test using the appropriate biospecimens, the diagnostic company depends on access to clinical trial specimens. It is consistently difficult to get access to samples from the pharmaceutical companies or the collecting institutions in the quantities needed. The FDA tends to evaluate the entire development process as a single system, yet multiple players are involved, leading to issues with sample uniformity in terms of preanalytical variables, uniformity of analytical platforms, and interoperability of informatics systems.

Once developed, a diagnostic device has to be validated on several levels. The biomarker being tested has to be demonstrated to correlate with the disease or dysfunction; the analytical capability has to be validated to show that the test detects what it claims to detect; and correlation with clinical outcome must be demonstrated.

Issues pertaining to tissue include acquisition, preservation, annotation, and consent. Two hypothetical companies might approach biospecimens quite differently: company A might have the samples centrally archived, handled according to protocol, and fully consented; nucleic acids have been recovered and assessed in a central laboratory using a validated laboratory-developed test, and most tissue blocks remain available. Company B might have samples that have been poorly archived and handled and that have not all been fully consented; nucleic acids have been recovered and evaluated using multiple laboratory-developed tests, and few tissue blocks remain available. Company B has a significantly higher risk of failing to demonstrate that the marker is predictive, yet this is by no means guaranteed for Company A.

Dr. Jackson concluded that uniformity of technologies and protocols is important to ensure parity among samples. Harmonization is a challenge; although the Center for Devices and

Radiological Health regulates in vitro devices well, there is discordance between CAP and CLIA regulations; in addition, CAP and CLIA regulate laboratories, not tests. Greater regulation of laboratory-developed tests would help reduce noise in the system.

XII. Discussion

In response to an observation by Dr. Jackson that some validation tests are less sensitive than the assays they are validating, Dr. Hu commented that perhaps some of the testing that is done in the development laboratory prior to submission might be used as a validation panel.

Dr. Goldman contended that laboratory-developed tests are used only when approved in vitro devices are unavailable. Dr. Jackson agreed, noting that patient care should, of course, be the utmost consideration. However, more approved companion diagnostics would be available if the limitations discussed throughout the workshop were alleviated. Lack of sufficient biospecimens nearly precludes development of a predictive device after the clinical trial has ended, meaning that the only option for predictive assays on patients is a laboratory-developed test, even though the laboratory-developed test might not have been subject to the same stringent development standards as an approved in vitro device.

Dr. Goldman expects the FDA to regulate laboratory-developed tests, depending on the risk category. Dr. Philip added that the FDA is planning to release a guidance regarding this issue. Laboratory-developed tests that are being used as companion diagnostics are likely to be at the top of the list. Dr. Compton asked how a laboratory-developed test being used as the standard of care could pass FDA approval; it is extremely unlikely that an additional clinical trial will be run from which to obtain biospecimens. Dr. Philip replied that such issues will be addressed on a case-by-case basis while considering clinicians' demands; another name might be given to laboratory-developed tests that have received FDA clearance.

Dr. Sawyer commented that the workshop has been very useful for the caHUB to understand the needs of companion diagnostic developers. She asked what might make developers hesitant to approach a national biobank for biospecimens. Dr. Jackson said that economics would influence this; if a membership fee were required and yet biospecimen access was limited because other customers have priority access, then the membership would be terminated. Dr. Compton explained that the caHUB biospecimen access policy would not favor academic researchers over commercial users; science and the public benefit are the interests the caHUB hopes to promote. Dr. Liu added that proprietary information would need to be kept private for long periods of time, and data transparency requirements might cause developers to be reluctant to use caHUB biospecimens. According to Dr. Compton, the caHUB is interested in

working with commercial partners to determine where the line should be drawn with respect to intellectual property. Dr. Robb commented that a great deal of the information submitted to the FDA for product approval is not proprietary—such as the effects of fixation and cold-ischemia times as presented by Dr. Walk—and would be useful for others to be able to access, perhaps through the Biospecimen Research Database (BRD). Dr. Compton explained that the BRD is an effort that OBBR has undertaken to survey and curate research data that define the precise relationships between biospecimen handling and the quality and reproducibility of scientific results. To include in the BRD the valuable, unpublished data that pharmaceutical and diagnostics companies generate, OBBR would need to work with others, such as workshop attendees, to determine the level of data qualification needed. In Ms. Long's view, companies are unlikely to be willing to share data publicly until after any applicable patents have been issued. It is not clear whether data obtained in the development of drugs that are already patented are still available, and whether the pertinent informed-consent forms would allow the data to be made public, even if the data were anonymized.

Drs. Compton and Sawyer explained that the caHUB is open to forming intellectual partnerships, such as the one recently signed with the European Union's Standardisation and improvement of generic pre-analytical tools and procedures for in-vitro diagnostics (SPIDIA) project. It is difficult for the caHUB to assess the needs of industry stakeholders. Some opportunities for collaboration include studying the economics of biospecimens in drug-diagnostic co-development, development of assays, and improvement of informed-consent documents. An attendee noted that in return for assistance in assessing the needs of the business community, OBBR could assist the business community in communicating with the Centers for Medicare and Medicaid Services concerning payment issues, such as offering reimbursement to pathologists for biospecimen processing.

Dr. Litwack asked whether workshop participants thought it would be of value to prepare a mock FDA submission of a companion diagnostic, akin to the one recently submitted on multiplex proteomics, to obtain FDA feedback concerning requirements. Dr. Philip commented that the proteomics mock submission was done to receive feedback concerning how to present the new platform for FDA approval. Any mock submission concerning companion drug and diagnostic would have to be geared toward an intended use. It would be difficult to address all aspects of a submission at once; therefore, it might be prudent to focus on preanalytical variables. Dr. Sawyer added that the mock submission might pertain to a set of reference samples exposed to a variety of preanalytical variables. Dr. Jackson noted that the value of the samples is as a portion of the companion diagnostic submission; typically discussions with FDA representatives prior to a submission help to clarify the FDA's expectations. Dr. Philip saw value

in such a submission, and once such sample sets with controlled variations in preanalytical variables have been established, they might be a good way for sponsors to evaluate assays.

Dr. Hu asked for opinions concerning blood versus bone marrow biospecimens, and how biomarkers might differ between the two. Drs. Compton and Robb commented that such comparisons might be performed with matched samples from rapid autopsies or amputations.

Dr. Compton concluded the meeting by noting that the next steps will be for the caHUB to develop ideas around supporting drug-diagnostic co-development, and then to consult with the experts present as to the feasibility of that approach in a continuing dialogue.