Cancer Steering Committee Annual Meeting Executive Summary

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Executive Summary

The Foundation for the National Institutes of Health (FNIH) was established by Congress in 1990 to support the mission of the National Institutes of Health (NIH) through developing public-private partnerships. The Biomarkers Consortium (BC) was founded in 2007 to foster pre-competitive collaboration of stakeholders to develop biomarkers for specific applications to accelerate drug development and improve clinical practice. The focus of the BC is on advancing biomarkers from mid-stage exploration and development to regulatory acceptance. All data generated by BC-supported projects are made publicly available.

The BC Cancer Steering Committee (CSC) held its annual meeting on October 15 and 16, 2015. Attendees included representatives from the NIH, FNIH, academic, non-profit and industry partners, and invited subject matter experts. The meeting objectives were to discuss the science of biomarker development and resources supporting biomarker research with a focus on precision medicine, to identify the best ideas for consideration for CSC project development, and to consider next steps. As appropriate, each session of the meeting included presentations on current and planned CSC projects and/or external initiatives to which new CSC projects could contribute, followed by group discussion. The agenda also featured three keynote speakers who highlighted applications of biomarkers in precision medicine. A recurring theme was the need for standards to support biomarker development and applications—*e.g.*, in protocols for biospecimen and data collection, reference materials for comparing results across multiple assays, clinical protocols, and data analysis.

Keynote: Cancer Biomarkers: Opportunities and Obstacles

Rich Simon, NCI

Dr. Simon discussed strategies for overcoming challenges to biomarker development. He described the types of biomarkers that can inform clinical research and patient care, including prognostic, predictive, early detection, and clinical endpoint biomarkers and noted that a predefined intended use is key to the successful development of any type of biomarker. That is, the most common errors in biomarker development are the failure to specify the intended use at an early stage and the failure to realize the kinds of studies needed to establish clinical utility, *i.e.*, benefit to patient of using the biomarker relative to not using it. He emphasized that biomarker development should focus on specific intended uses with potential for major clinical utility and described several designs for identifying and evaluating biomarkers in clinical studies for different intended uses.

Precision Medicine Studies: Lung-MAP, NCI-MATCH, ALCHEMIST, MPACT, New Protocols and Resources

The changing paradigm of oncology research from pre-2000 to the anticipated post-2020 approach with increasing focus on precision medicine requires a parallel change in the paradigm for drug development incorporating biomarker strategies. This new paradigm was discussed from the perspective of the drug developer, who will need to explore new resources (*e.g.*, genomics profiles, advanced technologies for evaluating cancer biology) and nonlinear, iterative routes for incorporating biomarker data from these resources into evidence for effective cancer treatment regimens (*e.g.*, expanded cohort early clinical trials). The changing paradigm also was discussed from FDA's perspective on incorporation of precision medicine into regulatory thinking. This perspective includes consideration of 1) segmentation of patient populations based on specific 'omics profiles; 2) reliance on multi-drug, multi-histology, multi-biomarker "Master Protocols" for drug and companion diagnostics (CDx) development and approvals; 3) use of multiplex assays rather than single molecule tests to define CDx; and 4) use of patient reported outcomes in evaluating new treatment regimens.

The design of, progress on, and challenges to four of NCI's precision medicine trials were described as examples of drug and biomarker development in precision medicine. Each study has a screening component to identify relevant actionable genetic abnormalities (potentially predictive biomarkers) followed by a treatment component that matches patients to drugs targeting their biomarkers.

The Lung Cancer Master Protocol Trial (Lung-MAP) in advanced, previously treated squamous non-small cell lung cancer (NSCLC) was launched in June 2014 in the National Clinical Trials Network (NCTN). The study uses next-generation DNA sequencing (NGS) to profile patients' tumors and to define the biomarkers of interest, supplemented as needed with immunohistochemical (IHC) assays in a setting where the individual biomarkers occur with low frequencies. Sub-studies of therapies matched to these biomarkers, as well as non-match therapies for patients with no targeted mutation, are included. The new, faster pace of drug approvals can be challenging for trials such as Lung-MAP. In its original design, the sub-studies included randomization between the targeted drug and docetaxel, a current standard of care chemotherapy. The 2015 approval of the more effective immunotherapy nivolumab for second line treatment of advanced squamous NSCLC made randomization to docetaxel infeasible, and unethical, and so the sub-studies have been re-designed to accommodate this change and to incorporate nivolumab. The lesson learned is the need to be strategic and nimble in reacting to the evolving treatment landscape.

The Molecular Profiling-based Assignment of Cancer Therapy (MPACT) and the NCI-Molecular Analysis for Therapy Choice (NCI-MATCH) trials also use NGS-based tumor profiling to determine treatment assignment, but with the goal of identifying patients likely to show an exceptional response to the study drugs without regard to tumor histology. MPACT is a pilot randomized controlled trial (RCT) enrolling patients with advanced, treatment refractory solid tumors. NCI-MATCH is a non-randomized trial that seeks to understand the relative efficacy of the same therapy applied to patient subgroups harboring specific molecular abnormalities in solid tumors and lymphomas. A particular challenge in the use of NGS assays for patient tumor profiling is complexity and variability that add new sources of measurement error, especially when conducted at multiple sites. This concern was addressed in the NGS assay development and validation plan for MPACT and NCI-MATCH. The plan includes definition of intended use of the assays, expected performance criteria, standard operating procedures (SOPs), and an assay validation protocol. In addition, the assay system was discussed with regulatory bodies during its development.

The Adjuvant Lung Cancer Enrichment Marker Identification and Sequencing Trials (ALCHEMIST) is a simply designed NCTN project consisting of three integrated trials: one screening trial and two Phase III treatment trials for patients with early stage lung cancer. ALCHEMIST objectives are 1) to perform central genotyping of lung cancers for *EGFR* mutations and *ALK* rearrangements to facilitate accrual to the treatment trials and 2) to obtain clinically annotated tumor tissue and DNA from blood for genomic analyses at the NCI Center for Cancer Genomics, as well as to obtain epidemiological and follow-up data for additional analyses. Tissue is obtained at registration for screening and on progression in the treatment trials. In the spirit of the Precision Medicine Initiative, the collected data will become a public resource for further research.

It was noted that sophisticated analytic tools could improve the evaluation of targeted therapies in these precision medicine settings. For example, in light of emerging designs such as NCI-MATCH, the use of novel Bayesian strategies to provide robust statistical evaluation of effects of the same targeted therapy across multiple tumor types or subtypes was discussed. The strategies involve specific algorithms for combining the results of this testing to make the analysis more robust (*e.g.*, adaptation of the James-Stein Estimator).

Precision medicine clearly requires the use of robust diagnostics to identify relevant targets in patients. In this regard, the roles of CDx and complementary diagnostics in precision medicine settings were discussed. CDx are well defined scientifically and by regulators. They provide information that is essential for the safe and effective use of a corresponding therapeutic product, and the label for the therapeutic product requires that patients treated with the therapeutic express the CDx appropriately. Complementary diagnostics were defined more recently. A complementary diagnostic provides information that helps a provider understand a patient's likely response to a therapeutic product, but is not mandatory for use of the drug. For example, PD-L1 IHC 28-8 pharmDx, the first FDA approved complementary diagnostic, identifies PD-L1 expression levels on the surface of NSCLC cells and provides information on the survival benefit of treatment with nivolumab. However, the benefits of nivolumab are not limited to patients who overexpress PD-L1 and other tests for PD-L1 expression may be useful in characterizing response to nivolumab or other immune checkpoint inhibitors. In fact, a second PD-L1 assay with a different antibody, PD-L1 IHC 22C3 pharmDx assay, is an approved CDx for the use of the PD-1 inhibitor pembrolizumab in treatment of PD-L1 positive NSCLC. Pharma and diagnostic companies are collaborating to set standards for the use and evaluation of these assays in lung cancer (see also under BIOMARKER STUDIES FOR IMMUNOTHERAPY). Many complementary diagnostics are laboratory-developed tests (LDTs) and do not go through the FDA regulatory clearance process. However, FDA recognizes the potential utility of complementary diagnostics and is working on guidance for their use in drug development.

Conclusions. The overall sense of the meeting participants was that these precision medicine approaches are valuable and may improve patient outcomes. As noted by both presenters and other meeting participants, obstacles to be addressed include appropriate, and potentially changing, trial designs for rare subpopulations, quality of biospecimens used to screen patients, validity of genomics screening assays, development of regulatory guidelines around the use of complementary diagnostics, the cost of such tests, and reimbursement of test costs by payers. The meeting participants agreed on the utility of the flexible, common screening platform, multi-site, multi-drug, multi-target Master Protocol clinical trial designs.

Minimal Residual Disease in Blood Based Cancers—Acute Lymphoblastic Leukemia and Multiple Myeloma

Acute Lymphoblastic Leukemia (ALL)

The MRD in ALL Project Team presented the status of their efforts to demonstrate that MRD may provide a response biomarker and surrogate endpoint that will shorten the time required for drug development in hematologic malignancies. The ALL project started in 2014 with four aims: 1) meta-analysis of existing data to determine the utility of MRD for predicting survival in pediatric and adult ALL, 2) standardization of MRD testing using flow cytometry; 3) exportation of the new standards to the community, and 4) comparison of NGS sequencing to current state-of-the-art flow cytometry techniques for measurement of MRD.

Aim 1. The MRD Project Team performed a Bayesian meta-analysis that shows a robust separation in EFS and overall survival (OS) probability curves based on MRD status for both pediatric and adult ALL populations. This literature-based, study-level meta-analysis is a first step in demonstrating the utility of MRD as an endpoint. The next step is collaboration with the FDA on a meta-analysis of individual patient-level data.

*Aims 2 & 3.*The goals of this effort are to standardize and optimize the selection of antibodies, clones, and fluorochromes; standardize sample processing procedures and instrument settings; create a common analysis template; educate analysts using list mode data files of normal, regenerating, and

abnormal samples; and reach concordance at multiple sites, as monitored by continuing quality assessment. Eight laboratories participated in several rounds of quality control that involved analysis of shared list mode data. Laboratories also are completing analysis of spiked blood samples for two wet sample challenges. Deficiencies have been few, minor, and easy to fix. Development of an 8-color assay is under consideration to allow better evaluation of newer antibody drugs. The NCI will decentralize MRD testing of ALL clinical trial participants within the Children's Oncology Group (COG), where much of the clinical trial activity in ALL is seated, by June 16, 2016, and the standards developed in this project will be distributed and used to qualify local laboratories within the Group.

Aim 4: The Project Team will use archival samples from COG to compare flow cytometry with NGS for MRD testing. Advantages of flow cytometry over NGS include fast turnaround, applicability to most patients, and no pretreatment required for immunophenotype. However, NGS is highly sensitive, more objectively interpretable, and more easily standardized, and will likely be the method of choice for future evaluation of MRD.

Conclusions. The Project Team and other meeting participants discussed additional work remaining to qualify MRD as a drug development tool for ALL, such as determining the optimal time point for measuring MRD and the critical burden of MRD. FDA is invested in establishing the utility of MRD in ALL and as a precedent for extension to evaluation of MRD in chronic lymphocytic leukemia and acute myeloid leukemia. The group endorsed close collaboration with FDA on the patient level meta-analysis and other activities.

Multiple Myeloma (MM)

Over the past year, leaders from NCI, the Multiple Myeloma Research Foundation, academia, pharma, FDA, the diagnostics industry, and FNIH have met frequently to discuss ways of improving drug development and patient care for MM, including use of MRD. At the CSC meeting, presentations were made on the complex characteristics of the clinical disease, strategies for incorporating MRD to evaluate therapeutic effects at various stages of MM development, and strategies to qualify MRD as a drug development tool (DDT) in MM.

MM likely begins with a precursor state called monoclonal gammopathy of undetermined significance (MGUS) then advances to an intermediate form called smoldering myeloma (SMM) where M-protein and plasma cells (PC) are elevated but the patient is still asymptomatic. Progression through these stages takes place at about 1%/year for MGUS and at a higher rate for SMM. Drug intervention at the MGUS/SMM stage may contravene progression. The disease often takes the form of a relapsing remitting disorder making it difficult to monitor the success of treatment intervention. However, new therapies have increased the 5-year OS rate of MM to greater than 73%. Discovery of cohorts with high-risk cytogenetic translocations vs. hyperdiploid forms of the disease has allowed for risk stratification and application of more aggressive treatments and drug combinations for those who have a poorer prognosis. NGS analysis and gene expression profiling have shown that there is no single mutational route to advanced MM. In addition, the role of the bone marrow microenvironment in influencing evolution and selection of malignant PC clones is now recognized.

A meta-analysis of the prognostic utility of MRD in MM is in progress and designs for a prospective trial using MRD by flow cytometry (using the CD138 marker for PC) or by NGS analysis. This trial may answer questions regarding how to use existing drugs better by examining MRD in maintenance therapy, or if treatment intensification/de-escalation studies, or continuous *vs*. intermittent treatment protocols could be of value. Similarly, use of MRD to monitor the performance of new drugs in inducing longer or faster remissions could be used as the basis of regulatory approval.

The role of the macrophage, osteoblast/osteoclast complex, the mesenchymal stroma, and adhesion molecules in mediating the initial bone and marrow insult that precipitates MM could be evaluated with MRD. MRD also could be used to assess the clonal diversity of PCs, given the differing responses of the dominant clone and successor clones to treatment and the development of resistance.

FDA acknowledged on-going efforts in the research community to use MRD as a response biomarker in clinical studies and re-iterated its support for qualifying MRD as a DDT via either the formal Biomarker Qualification Program or the less formal qualification processes of interaction with a specific FDA drug development program and collaborative group interactions with the FDA.

Conclusions. The meeting participants agreed on the potential utility of MRD as a surrogate endpoint in MM research, comparing it to viral load as a surrogate endpoint in clinical trials of HIV drugs and to pathologic complete response in early breast cancer. The participants agreed that no matter what approach is taken to biomarker qualification, an integrated, pre-competitive effort is needed that could be effected by the BC. They also agreed on the need to begin routinely including MRD measurement of treatment effect in MM RCTs.

Proving the Liquid Biopsy—Circulating Tumor DNA (ctDNA)

More than 25 companies and laboratories have developed or are developing assays for circulating ctDNA. The importance of developing standards for clinical use was discussed, including the need to avoid the missteps and omissions in the development of the forerunner technology, circulating tumor cell (CTC) assays. Particularly, these missteps included lack of standard protocols for acquiring and processing blood specimens, lack of clinical validation, and lack of reference material for use in comparing the results among the multiple assays in development.

Currently, the main technologies for detecting ctDNA use polymerase chain reaction (PCR) and NGS. PCR works well for individual point mutations, can be used for crude copy number analysis, and multiplexing is possible. However, it is necessary to know the mutations of interest ahead of time. PCR is fast (1 to 2 hours), highly reproducible, inexpensive, and requires minimal bioinformatics. With NGS, it is not necessary to pre-define mutations of interest, but NGS has a high false discovery rate that requires error suppression. As with PCR, sensitivity depends on the mutations assayed. NGS is more expensive than PCR, and the turnaround time is longer (at least 1 to 2 days).

Two recently developed methods for assessing ctDNA in plasma from NSCLC patients were presented as examples of these technologies: a digital drop PCR technique and an NGS actionable gene mutation panel. It was suggested that findings from ctDNA approaches, at least in some cases, might provide more reliable results than biopsy findings, for example, in detection of *EGFR T790M* mutations in patients with NSCLC.

FDA recognizes that ctDNA assays still require extensive analytical validation and reference materials would be useful in this regard. Further, clinical validation and evidence of clinical utility are needed before ctDNA is acceptable for use as a DDT or in patient care. For example, the assay result should be clearly associated with a cancer and be medically important. If the assay is used as a predictive biomarker or CDx, the genetic abnormality measured should also be found in the tumor. If used as a response biomarker, the result should correlated to an objective clinical response.

An opinion on how insurance plans view biomarker innovation was presented, stating that the evidence supporting a new product must meet criteria that allow coverage by insurance carriers. These decisions are not based on cost, but on evidence that the product benefits patients. It is critical that developers of innovative diagnostic products clearly communicate performance standards and intended uses. In a session later in the day, it was noted that policies of the Center for Medicare & Medicaid Service (CMS) provide some help to assay developers. CMS policies cover certain interventions for which clinical utility

has not been demonstrated while further evidence is collected, consider approval for routine costs incurred in clinical trials, and cover selected Investigational Device Exemption studies.

The National Institute of Standards and Technology (NIST) is already engaged in efforts to standardize DNA sequencing technologies. Through the Genome in a Bottle Consortium, NIST is providing infrastructure to assess confidence in human genome variant calls as measured by sequencing technologies. NIST also participates in the Global Alliance for Genomics and Health Benchmarking Task Team, which has developed standardized definitions for sequencing performance metrics and is developing sophisticated benchmarking tools. In an effort directly applicable to any future BC project on creation of ctDNA reference materials, NIST has also begun working with the NCI Early Detection Research Network to improve measurements of ctDNA.

Conclusions. The meeting participants agreed on the need for a "must have" ctDNA application to spur development and regulatory acceptance of the technology, suggesting that ctDNA determination of *EGFR T790M* mutations in lung cancer patients to guide treatment choice at the time of diagnosis or during treatment monitoring may represent such an application.

Using Big Data for Biomarker Development and Analysis

Examples of current projects in Precision Medicine using "Big Data" concepts were presented. These included efforts at NCI, FDA, the Veterans Administration, American Society of Clinical Oncology (ASCO), and the CEO Roundtable's Project Datasphere. The presentations emphasized the need for standards for data collection and analysis, interoperability of diverse data sets, and enhanced capacity for managing large data resources.

Informatics goals of NCI supporting the Precision Medicine Initiative were presented. The first goal is to develop a Cancer Knowledge System by establishing a national database that integrates genomic information with clinical response and outcomes. A second goal is to develop molecular, imaging, pathology, and clinical signatures that predict therapeutic response, outcomes, and tumor resistance. A third goal is to build multi-scale, predictive computational biology models for understanding cancer biology and informing therapy.

In pursuing these goals, NCI has established the Cancer Genomic Data Commons (GDC) to standardize and simplify submission of genomic data to NCI. GDC is part of the NIH Big Data to Knowledge (BD2K) initiative and is expected to go live in spring 2016. It will be a single repository for all NCI cancer genomics data, including primary sequence data, which is currently stored in many different repositories and is confusing to users, inefficient, and a barrier to research. The amount of data will exceed all available resources and will not be able to be stored locally, and so NCI has initiated three separate cloud-computing pilots to consider how to provide access to genomic data without requiring the user to download the data.

CancerLinQ is ASCO's planned rapid learning health system for oncology that will use real-world patient care data to inform research. The data will be used to measure quality of care, continually improve the performance of providers and patient outcomes, provide physicians with clinical decision support tools, and provide insights for exploration and hypothesis generation. Challenges include overcoming the reluctance of sites to provide protected health information, building electronic health records (EHR) interoperability, mapping diverse data sources to common data model, data blocking by commercial entities, and patient education and advocacy. A second ASCO initiative, the Targeted Agent and Profiling Utilization Registry (TAPUR), was designed to describe the anti-tumor activity and toxicity of commercially available targeted anti-cancer drugs in patients with advanced solid tumors, B cell non-Hodgkin's lymphoma, or MM with molecular targets of interest. TAPUR also seeks to facilitate patient access to commercially available targeted anti-cancer drugs of potential efficacy for the treatment of

these cancers. Secondary objectives are to record treatment-related adverse events, create prospective registries of patient outcomes following treatment and of commercially available tumor genome profiling tests used in clinical care settings, and to determine concordance of treatment plans proposed by treating oncologists with those recommended by molecular tumor board in applicable situations.

The FDA's Information Exchange and Data Transformation (INFORMED) initiative seeks to build capabilities and infrastructure to optimize data analysis, enable new data exploration, and serve as a platform for dialogue and stakeholder engagement to advance regulatory science and the FDA mission of protecting the public health. INFORMED outputs will include enhanced post-market surveillance and better predictive models for the development of new therapies and for providing actionable guidance to patients and providers to optimize use of new therapies. There are three components: transformation of FDA's existing clinical trial datasets into a common standard, development of a big data environment for storage and mining of transformed datasets, and incorporation of diverse pipelines of data (*e.g.*, EHRs, wearable technologies, biometric monitoring devices, unstructured content (*e.g.*, social media), and omics) into the big data environment. A particular interest is capturing DNA sequencing data. The growth of these data has greater velocity than anticipated, doubling every seven months. In addition, there is individual diversity with over 88 million germline variants, and so far, this diversity has been ignored in precision medicine. The priority is to determine a core set of standardized data modules to allow collection and analysis of these data.

The Massachusetts Veterans Epidemiology Research and Information Center's new program in precision medicine was described. The Center has core facilities in epidemiology, biobanking, clinical trials, and informatics and production programs including Million Veteran Program, Point-of Care Program, and NODES (network of distributed enrollment sites).The New England Precision Oncology Program (POP) is a start-up program at the Center targeting individuals served by the Veteran's Integrated Service Network. The POP proposes to match patients to clinical trials based on molecular variants identified by targeted sequencing. The POP model also includes a biomarker research resource that will be shared with VA and non-VA investigators.

Project Data Sphere, LLC , an independent, not-for-profit initiative of the CEO Roundtable on Cancer's Life Sciences Consortium, operates the Project Data Sphere platform, a free digital library-laboratory that provides a place where the research community can broadly share, integrate and analyze historical, patient-level, comparator-arm data from academic and industry Phase III cancer clinical trials. The Prostate Cancer DREAM Challenge (PCDC) of Project Data Sphere was a recent crowd-sourced research challenge pilot in prostate cancer. The Challenge was initiated by Project Data Sphere and Sage Bionetworks in collaboration with 15 institutions and is guided by the PCDC. The process for one of two sub-challenges was described. Researchers participating in the Challenge were asked to use provided data to develop a prognostic model to predict OS of metastatic castrate-resistant prostate cancer patients based on the patients' baseline clinical variables. The Challenge fostered collaboration to advance prostate cancer research; models from 11 teams performed better than a previously published prognostic model, with one clear overall winner.

Conclusions. Meeting participants recognized the high potential value to precision medicine studies as well as the immense challenges in developing and using big data. The group recommended that the presenters meet again to flesh out the design of a few projects that could be conducted via the CSC.

Keynote: Human Papillomavirus (HPV) Vaccination FDA Approval Was Just the Beginning

Doug Lowy, NCI

Dr. Lowy discussed the research and development of the Cervarix (GlaxoSmithKline) bivalent HPV vaccine and Gardasil (Merck) quadrivalent HPV vaccines. The two goals of HPV vaccination are to directly reduce the individual risk of infection and disease and indirectly reduce population risks by reducing the prevalence of HPV in the general population through community immunity, also known as "herd" immunity.

The high efficacy of the HPV vaccines is related to several factors, including the intrinsically immunogenic nature of the virus-like particle (VLP) immunogen, the presence of tissue-associated neutralizing antibodies at potential sites of infection, and high antibody levels at these sites. Furthermore, HPV is highly susceptible to neutralizing antibodies with data suggesting that one or two doses of HPV vaccine, rather than the original three dose regimen, may be sufficient to confer protection.

Merck has developed a 9-valent VLP vaccine, adding five oncogenic HPV types to their quadrivalent vaccine. This second-generation vaccine, which gained FDA approval in December 2014, demonstrated 96 percent protection against infection with added HPV types. Such a vaccine with activity against a broad range of HPV types may achieve the greatest reduction in HPV-associated disease.

The example of the precision medicine HPV VLP for cervical cancer prevention may inform other potential uses of VLP technology. Because VLP vaccines can abrogate B cell tolerance and induce autoantibodies against self-antigens, VLP vaccine technology might replace some of the monoclonal antibody-based vaccines with potential application in low resource settings. Potential genetic immunization applications include vaccination against HPV pseudoviruses in the female genital tract. VLP treatment applications might include near infrared phototherapy with dye-coupled VLPs for treatment of epithelial tumors.

Imaging—Update, Promise, and Opportunities for Biomarker Development

Progress on and further plans for the CSC Volumetric Computed Tomography Metrics for Precision Analysis of Clinical Trials (CTvol-PACT) project were presented. The project has three specific aims: 1) to assess feasibility of collection and analysis of images from completed Phase III trials to simulate Phase II trial results, and to develop quantitative metrics for improved prediction of Phase III trial results; 2) to identify quantitative metrics that most accurately and reliably predict Phase III results across different trials; 3) to quantify the added value of volumetric tumor measurement as compared with conventional measurement. The need for improved response/progression metrics for immunotherapeutic drugs given the atypical response kinetics seen in patients treated with immune checkpoint inhibitors and the possibility that these metrics could be addressed by CTvol-PACT were also discussed.

More than 20 completed Phase III trials sponsored by pharmaceutical companies have been identified as being of high value to this study because of large size (≥150 subjects per arm) and availability of centrally collected imaging data. The first step is to collect existing trial data of Digital Imaging and Communications in Medicine (DICOM) images and clinical metadata, focusing on trials of measureable carcinomas, *i.e.,* NSCLC, renal cell carcinoma, colorectal cancer, and melanoma. Thus far, the project has secured Data Sharing Agreements with four companies for seven trials containing data of more than 5,000 patients. The Project Team is currently in discussion on lung cancer trials of targeted therapy and melanoma trials of immunotherapy with three other companies.

In connection with its FDG-PET in Lung and Lymphoma Projects, the CSC has initiated efforts to work with the FDA in its formal Biomarker Qualification process. A briefing document supporting the use of FDG-PET as a response biomarker in oncology has been prepared and revised, and several meetings have been held with the FDA Biomarker Qualification Review Team to discuss qualification claims and evidence requirements. The design and conduct of a prospective imaging sub-study embedded within the Alliance/Cancer and Leukemia Group B 50303 RCT have been key to this effort. The parent trial tests R-CHOP immunochemotherapy versus dose-adjusted EPOCH-R in untreated *de novo* diffuse large B-cell lymphoma patients. The sub-study seeks to identify FDG-PET/CT imaging biomarkers of response to chemotherapy that are predictive of remission and survival outcomes. The RCT and the sub-study are nearing completion with publication of initial results and methods are expected in 2016.

Two resources supporting imaging-based biomarker development, the Quantitative Imaging Biomarkers Alliance (QIBA) and The Cancer Imaging Archive (TCIA) were described. QIBA, a public-private partnership started by the Radiological Society of North America in 2007, seeks to improve the value and practicality of quantitative imaging biomarkers by reducing variability across devices, patients, and time. The variation in imaging technologies related to image acquisition, measurement method, and interpretation led to the need for QIBA. QIBA generates profile documents that describe a specific performance claim for quantitative imaging and how it can be achieved.

NCI's TCIA seeks to advance imaging biomarker research with its collections of freely accessible DICOM images and supplementary data. The collections are intended to foster data re-use and reproducibility within the cancer imaging research community. TCIA enables discovery in the areas of computer aided diagnosis, medical image processing, image protocol development, grand challenges, radiomics, and imaging genomics.

Conclusions. Meeting participants discussed the CTvol-PACT project as a promising technology for drug development that will permit the conduct of smaller, more efficient clinical trials. They agreed that better metrics are needed to assess the clinical benefit of immunotherapies; volumetric imaging could help define these metrics. The pre-competitive collaborations to develop these technologies will take energy, commitment, and data sharing but should be well worthwhile.

Advancing Novel Technologies/Methods for Translating Research and Clinical Biomarker Assays

The BC CSC High Content Data Integration Working Group was formed in early 2013 to promote translation of novel methods for integration of high content data into translational research and clinical biomarker assays. The goal is to employ the heterogeneity definable from analysis of single and bulk cell populations for development of tailored signatures that can assist in patient stratification and identification of patient responses to treatment.

Two projects developed by this Working Group have been approved by the EC and will be launched in 2016. The BC CSC high definition single-cell analysis (HD-SCA) project seeks to determine whether liquid biopsies (CTCs) correlate with solid tumor biopsies in metastatic colorectal cancer. Expected benefits of the project include confirmation that results of liquid biopsies correlate with those of solid biopsies and can be used for characterizing disease states. High content bio-signatures will be generated out of the liquid biopsies that should support characterization of metastatic states, therapeutic mechanisms of action and resistance, and may influence how compounds will be developed in the future.

The BC CSC Community Assay Repository (CAR) multiple reaction monitoring (MRM) project aims to enable the use of high resolution, accurate mass spectrometry-based protein measurements across the research and drug development community. The project calls for building a community assay repository to standardize proteomic measurements using MRM; foster the manufacture and distribution of

standardized assay kits and reagents; encourage implementation of standardized assays by contract research organizations and core facilities; and reach a "tipping point" at which there is a demonstrable market for MRM-based kits.

The background and methodology for a new project concept on single-cell mass accumulation was presented. This concept is grounded in the need for a biomarker to quickly predict response to cancer treatment in real time. The project concept introduces two predictive biomarkers for assessing patient response to therapy: single-cell mass (Mass) and single-cell mass accumulation rate (MAR). A measurement system for Mass and MAR using a suspended microchannel resonator has been developed.

Practical considerations in developing assays based on new technologies for use in community clinical settings were discussed. The example described was an assay to detect Philadelphia chromosome-like (Ph-like) gene signatures. Studies in children with ALL show that the Ph-like signature is associated with a spectrum of kinase alterations and poor prognosis. An inexpensive, fast, reliable, reproducible, and easy-to-use clinical laboratory platform was needed to get uptake of assays for the Ph-like gene signatures in the community setting. These features are being addressed by using low density array cards for detecting the signature. Use of this platform identified kinase fusions in Ph-like ALL that are amenable to therapy with commercially available tyrosine kinase inhibitors.

Conclusions. The meeting participants were very interested in the projects presented and discussed some of the challenges faced by the investigators, for example, the complex analysis need to develop bio-signatures required for the HD-SCA project. The need for the investigators to continue collaboration with the FDA and with expert assay developers was discussed. It is expected that the Working Group will continue to be a venue in which this collaboration can take place.

Keynote: Mutation Driven Immune Responses in Cancer

Luis Diaz, Johns Hopkins School of Medicine

Dr. Diaz described his and his colleagues' research that is based on the observation that while the average tumor has dozens of somatic mutations that potentially can be recognized by the immune system, mismatch repair (MMR) deficient tumors harbor thousands. Therefore, immune augmentation with PD-1 blockade may be highly effective in MMR deficient tumors.

Dr. Diaz and colleagues conducted a small trial to test the hypothesis that PD-1 blockage therapy with pembrolizumab would be effective in cancer patients with MMR deficient tumors. The data show that MMR deficient tumors were highly responsive to checkpoint blockade with pembrolizumab. The trial ended early when it met pre-determined criteria for efficacy. Clinical benefit was noted across tumors with MMR deficiency including cancers of the colon, uterus, stomach, pancreas, prostate, duodenum, and bile ducts. Furthermore, the biochemical response to treatment correlated with radiographic response as well as with PFS and OS. Analyses also showed that MMR deficient tumors are rich in CD8+ T cells and PD-L1 expression at the tumors' invasive front. The single arm KEYNOTE-164 study in patients with MMR deficient colorectal tumors will attempt to validate these promising results.

Biomarker Studies for Immunotherapy

A new CSC Working Group has been formed on the timely topic of biomarker studies for immunotherapy. The objective of this session was to discuss potential CSC projects that could contribute to the understanding of the effects of immunotherapy on tumors and to the development of immunotherapies.

The first topic considered was how biomarkers might be developed that provide information on immunotherapies effect on cellular biology. In this regard, the use of single-cell genomic analysis, such

as by single-cell RNAseq was discussed. RNAseq can be used to dissect the complexity of the tumor cell ecosystem to help determine which cells are malignant and provide information about their molecular states, interactions with other cells, and functional effects. The technique works for samples obtained by resection, biopsy, or pleural effusion. In single-cell RNAseq, the sample is disaggregated and the cells are recovered and separated in single-cell lysates. This takes less than two hours to perform but relies on having fresh, not frozen, samples. The identification of biomarkers is one useful application of RNAseq.

The use of PD-L1 expression as a predictive biomarker and companion diagnostic in the development of pembrolizumab, a PD-1 checkpoint inhibitor, was the second topic discussed. Pembrolizumab is FDA-approved for treatment of ipilimumab-refractory melanoma and notably, ORR, PFS and OS were correlated with PD-L1 expression in the registration trial. More recently, the drug was approved for treatment of advanced PD-L1 positive NSCLC, where PD-L1 expression is measured by the PD-L1 IHC 22C3 pharmDx assay. Another potential use of PD-L1 expression is selection of patients who are most likely to respond to pembrolizumab in other tumor types. Several clinical trials have been exploring this possibility, including KEYNOTE 012 and KEYNOTE 013).

The problems inherent with multiple tests using potentially different characteristics to measure the same biomarker were discussed under PRECISION MEDICINE STUDIES. PD-L1 assays have been of concern in this regard. The Treatment and Diagnostic Industry PD-L1 Blueprint Proposal that emerged from a March 2015 Public Workshop sponsored by FDA, the American Association for Cancer Research, and ASCO was presented as a means to address this issue. The Workshop focused on the need for pre-competitive collaboration to address concerns that multiple PD-L1 assays are being developed in a "one assay, one drug" paradigm. At the Workshop, four pharma and two diagnostic companies agreed on a pre-competitive Blueprint to achieve some level of standardization for these assays in lung cancer. A possible project for the CSC would be to evaluate the use of PD-1 assays in other target organs.

A third topic was exploration of technologies beyond IHC that could provide more detailed understanding of the tumor microenvironment. For example, the NanoString platform for gene expression profiling (nCounter PanCancer Immune Profiling Panel) may prove useful for patient selection for immunotherapy trials. A custom platform with 800 immune-focused genes was used to look in FFPE tissue for gene signatures associated with response to pembrolizumab. A 10 gene IFN-γ signature, indicative of a T cell inflamed microenvironment, as well as an expanded immune signature of 28 genes correlated with response. This was also a pharmacodynamic biomarker—a postdose change in the 10 gene IFN-γ signature in blood also predicted response (CR + PR and PFS), suggesting an inflamed tumor microenvironment is a good predictor of response to checkpoint blockade. Comparing PFS and the IFN-γ signature score, non-T cell inflamed tumors do not respond, while there were both responders and non-responders in subjects with inflamed tumors. The key will be to understand the differences between the various groups.

Potential roles for the BC were presented that include efforts to:

- Perform meta-analyses on topics such as predictors for rapid progression or long-term survival. Such an endeavor would require sharing of patient-level data from immunotherapy RCTs sponsored by various companies.
- Standardize the definition of mutational load in the context of immunotherapy.
- Develop continuous variable (versus dichotomous) biomarkers as surrogate endpoints.
- Validate biomarkers in development, especially for rarer cancers. For example, validate *PD-L1* as a biomarker for prediction of PD-1 blockade treatment response in multiple cancers.
- Assemble publicly available data to explore immunology questions needed to inform the design of master protocol immunotherapy trials testing several therapies and utilizing several

predictive biomarkers. Compare such data to prospective data collected using newer single-cell technologies.

- Review the existing initiatives for biomarker development to determine if there are components that can be leveraged for research on biomarkers for use in immunotherapy.

Conclusions. The meeting participants agreed that projects in immunotherapy should be pursued. The Immunotherapy Working Group will meet to plan specific projects over the next few months.