Agenda & Abstracts

Advancing Cancer Diagnostics

The International Symposium for Tissue Phenomics™
Agenda
Friday, October 17, 2014

7:00 am  |  Registration (Grand Ballroom Foyer, 3rd floor)
7:00 am  |  Continental Breakfast (Mason Room, 2nd floor)

General Session (Grand Ballroom, 3rd Floor)

9:00 am   |  Keynote Presentation:
          |  Tissue Phenomics – A Novel Big Data Approach in Pathology
          |  Dr. Gerd Binnig, Nobel Laureate and Chief Technology Officer, Definiens,
          |  Munich, Germany

Session I:  Hypothesis or Big Data-Driven Biomarker Discovery

10:00 am  |  Prognostic and Predictive Significance of the Immunological Tumor Environment
          |  Dr. Dirk Jäger, Director Medical Oncology, National Center for Tumor Diseases (NCT)
          |  University Medical Center Heidelberg, Heidelberg, Germany

10:20 am  |  Coffee Break

11:00 am  |  COIN-ing Potential Clinically Translatable Markers in Metastatic Colorectal Cancer Through Tissue Phenomics
          |  Dr. Ryan Hutchinson, Fellow in Molecular Pathology, Department of Pathology, Melbourne and Centre for Translational Pathology, University of Melbourne, Melbourne, Australia

11:20 am  |  Improving Therapy-Relevant Breast Cancer Protein Profiling Using Quantitative Immunofluorescence
          |  Dr. Amy Ryder Peck, Senior Research Scientist, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania, USA

11:40 am  |  Applying Tissue Phenomics to Colorectal Clinical Questions
          |  Peter Caie, Senior Research Fellow, University of St. Andrews, Scotland
Friday, October 17, 2014

12:00 pm    Lunch *(750 Restaurant, 1st floor)*

1:30 pm    Novel Patient Stratification and Predictive Imaging Biomarkers for Development of Oncology Therapies
*Dr. Belma Dogdas*, Associate Principal Scientist, Merck, Rahway, New Jersey, USA

Session II:  Immunotherapy and Tumor Microenvironment

1:50 pm    Use of Computer Tumor Tissue Imaging Algorithms to Assess the Role of Microenvironment in Melanoma Brain Metastases
*Dr. Stergios Moschos*, Clinical Associate Professor of Medicine
The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

2:10 pm    Coffee Break

2:40 pm    Panel Discussion:
Advancing Cancer Diagnostics with Tissue Phenomics in Biomarker Discovery and Immunotherapy

3:45 pm    Closing

4:30 – 6:00 pm    Networking Reception *(Grand Ballroom Foyer, 3rd floor)*
Saturday, October 18, 2014

7:30 am  Continental Breakfast *(Mason Room, 2nd floor)*

General Session *(Columbus Room, 4th floor)*

9:00 am  Tissue Phenomics: From Biomarker Discovery to Clinical Diagnostic Assay Commercialization  
Dr. Thomas Nifong, Executive Vice President, Diagnostic Tests, Definiens, Boston, Massachusetts, USA

Session III:  Assay Optimization, Standardization, and Validation

9:30 am  Tissue Quality and Clinical Data: The Bottleneck for Drug and Biomarker Development  
Dr. Hartmut Juhl, Chief Executive Officer, Indivumed GmbH, Hamburg, Germany

9:50 am  Measurement of Target Engagement and Pharmacodynamic Response to Investigational Anti-Cancer Agents in Phase 1 Clinical Trials  
Dr. Robert J. Kinders, Senior Principal Scientist and Head, Pharmacodynamics, Laboratory of Human Toxicology and Pharmacology, Frederick National Laboratory for Cancer Research, Frederick, Maryland, USA

10:10 am  Coffee Break

10:40 am  Development of an Encapsulated Cell Replacement Therapy for Diabetes  
Dr. Olivia G. Kelly, Director, Cell Biology, ViaCyte, Inc., San Diego, California, USA

11:00 am  Imaging and Analysis of RNA Biomarkers *In Situ* via RNAscope and SpotStudio  
Dr. Xiao-Jun Ma, Chief Scientific Officer, Advanced Cell Diagnostics, Hayward, California, USA
Saturday, October 18, 2014

Session IV: Clinical Trials, Diagnostics, and Companion Diagnostic Tests

11:20 am  Keynote Presentation:
Immunoscore / Immunoprofile: Assessing Anti-Cancer Immunity as a Biomarker to Stratify Patients for Clinical Trials
Dr. Carlo Bifulco, Medical Director, Oncologic Molecular Pathology and Pathology Informatics, Director, Translational Molecular Pathology, Providence Oregon Regional Laboratory & Earle A. Chiles Research Institute, Providence Health & Services, Portland, Oregon, USA

12:05 pm  Lunch (750 Restaurant, 1st floor)

1:30 pm  Immune Landscape and Immunoscore in the Era of Cancer Immunotherapies
Dr. Jérôme Galon, Research Director, INSERM, Head of Integrative Cancer Immunology Laboratory, INSERM, Paris, France

1:50 pm  Implementation of Image Analysis for Biomarker Assessment of Pre-Clinical Tumor Models and Clinical Patient Samples
Dr. Belinda Cancilla, OncoMed Pharmaceuticals, Redwood City, California, USA

2:10 pm  Companion Diagnostics: Aligning Development, Commercialization, and Market Access
Charles Mathews, Vice President, Boston Healthcare, Boston, Massachusetts, USA

2:40 pm  Coffee Break

3:00 pm  Panel Discussion:
Tissue Diagnostics in Clinical Trials

4:00 pm  Closing Remarks
Abstracts & Notes
Tissue Phenomics – A Novel Big Data Approach in Pathology

Data mining techniques and big data approaches have the power to create knowledge that cannot be created otherwise. And while they are well established, only recently have people become aware of the impact these techniques and approaches have on their daily life. The main reason for this is the dramatic growth of data now available in digital form - a first prerequisite for data mining. A second prerequisite is that data need to be meaningful for data mining to be effective. In medicine, however, this condition represents a barrier difficult to overcome. Here the biggest data segment, the pixels in images, represent no value for data mining tools. Advanced image analysis is the way to meaningfully structure images and extract statistical data from relevant objects, regions, and textures for subsequent data mining procedures.

In particular the information contained in tissue slides is medically highly relevant. Tissue Phenomics extracts this data in a much more extensive manner than what can be done manually, enabling increased medical and scientific insight and ultimately improving the treatment of patients. Tissue Phenomics, however, is more than just data collection paired with data mining. It is a comprehensive approach that combines bottom up (systems biology) with top down (statistics and correlation) methods. To manage the combinatorial problem of how to collect data, hypotheses have to be created both ways and be validated statistically. Tissue Phenomics complements genomics, an already established big data approach. Genomics has the limitation that the effect of the gigantic molecular network on diseases is more complex and definitely not only controlled by genes. Tissue Phenomics is closer connected to the state of the patient and therefore has the potential to become within the next years the most relevant science to develop diagnostic and therapeutic methods.

In 1986, Dr. Binnig, along with his colleague Dr. Heinrich Rohrer, was awarded the Nobel Prize in Physics for his work in scanning tunneling microscopy. Dr. Binnig and Dr. Rohrer were recognized for developing the powerful microscopy technique, which can form an image of individual atoms on a metal or semiconductor surface by scanning the tip of a needle over the surface at a height of only a few atomic diameters.

Born in Frankfurt, Germany, Dr. Binnig studied at the J.W. Goethe University in Frankfurt, where he received his bachelor’s degree in 1973 and his doctorate degree in 1978. After earning his doctorate, he joined a physics research group at IBM’s Zurich Research Laboratory prior to being assigned to IBM’s Almaden Research Center in San Jose, Calif. from 1985 to 1986. He was also a visiting professor at Stanford University from 1987 to 1988.

Dr. Binnig developed the Cognition Network Technology®, which uniquely enables Definiens software to analyze large numbers of images automatically, just like the human eye and brain are capable of doing. He started Definiens with the goal to transform the imaging industry by enabling more data to be extracted for better decisions.
Prognostic and Predictive Significance of the Immunological Tumor Environment

The prognostic role of infiltrating T cells has been shown for many tumor entities. We analyzed the predictive significance of T cell infiltrating in patients undergoing chemotherapy. We could show that patients with high density infiltrates at the invasive margin have significant better outcome under chemotherapy, and have significant PFS and OS then the T cell low patients. We currently analyze the mechanisms leading to high immune cell infiltrates in some patients versus low infiltrates in others.

The qualitative analyses of immune cell infiltrates (subsets) in cancer patients combined with cytokine/chemokine analysis give us valuable information regarding the immunological make up of cancer lesions predicting treatment outcome.

Dr. Jäger is head of the Department of Medical Oncology at the University Center Heidelberg and the National Center for Tumor Diseases, Heidelberg. He is also leading a research group “Applied Tumor Immunity” at the German Cancer Research Center (DKFZ)

Dr. Jäger’s research focus is the characterization and better understanding of tumor host immune interactions at the tumor site and the development of novel treatment strategies to therapeutically interact in this immunological interplay. Translational clinical trials evaluating novel immunomodulators, antigen specific immune cells as well as gene modified immune cells are currently ongoing in the center.

Dr. Jäger set up platforms using whole slide tissue analysis (IHC) combined with cytokine/chemokine analysis in microdissected tissue areas to profile the immunological microenvironment in tumors.
COIN-ing Potential Clinically Translatable Markers in Metastatic Colorectal Cancer Through Tissue Phenomics

The MRC COIN trial is the largest metastatic colorectal cancer clinical trial carried out to date with the recruitment of 2,445 patients, which investigated whether the addition of cetuximab to standard chemotherapy could benefit patients by increasing lifespan. Conventional EGFR IHC scoring did not indicate any predictive value for treatment with cetuximab and chemotherapy in first line therapy. In routine clinical practice EGFR IHC is not performed to determine cetuximab eligibility.

The original clinical trial tissue microarrays were scanned on an Aperio ScanScope scanner at 40x magnification. The rule-set was designed to automatically detect tumour and stromal regions, nucleus and membrane detection was carried out within the tumour regions only. EGFR expression thresholds were determined using the image object information table. A novel rule-set was developed in Image miner in collaboration with Dr. Bjöern Reiß to interrogate the vast clinical, genomic and image analysis related data.

The automated EGFR rule-set was found to be highly concordant with the original pathological interpretation. Automated quantification of membranous expression of EGFR was predictive of benefit of adding cetuximab to standard chemotherapy in the KRAS WT cohort, however, excluding NRAS mutations statistical significance was lost. Using Image Miner, a novel feature of interest was found to have prognostic utility in both the KRAS wildtype patient population as well as the KRAS mutant cohort.

We show the benefit of the utility of adopting a tissue phenomics approach in a large scale clinical trial to identify features which have prognostic value across patient cohorts in metastatic colorectal cancer that has yet to be reported. Furthermore this tissue phenotype-genotype correlation provides an insight as the therapeutic combinations. The true potential of big data in the field of oncology has yet to be fully elucidated, using an integrative approach in which phenomic data was combined with clinicopathological data emphasizes the added clinical benefit of a tissue phenomics approach for the evaluation of tissue.

Dr. Hutchinson is in the process of completing his PhD within the Centre for Cancer Research and Cell Biology under the supervision of Professor Peter Hamilton, and has now undertaken a fellowship within the Department of Pathology, University of Melbourne under the supervision of Professor Paul Waring. His research interests are the molecular pathology of colorectal cancer, metastatic colorectal cancer and the clinical applications of image analysis based approaches for patient stratification. His training in pathology started in 2005 under the supervision of Northern Ireland State Pathologist Professor Jack Crane CBE. Dr. Hutchinson is an intern member of Royal Academy of Medicine in Ireland, and was one of 30 residents to be selected for training in clinical genomics by ASCP in 2013.
Improving Therapy-Relevant Breast Cancer Protein Profiling Using Quantitative Immunofluorescence

Improving clinical management of breast cancer will depend on better molecular characterization of diverse breast cancer subtypes in the context of subtype-responsiveness to therapy. Quantitative immunofluorescent detection of druggable protein targets may facilitate identification of novel therapy-relevant breast cancer subtypes that cannot be discerned by standard pathology methods. High-throughput and automated methods will increase the feasibility and accuracy of subtype classifications at the clinical level. Through a multi-disciplinary team of collaborators, we are working to map an initial panel of 250 protein targets in tumors from a cohort of 3000 breast cancer patients using automated and high-throughput quantitative in situ immunofluorescence-based methods. Our initial goal is to establish quantitative immunofluorescence as a more reliable substitute or supplement to standard DAB-based manual scoring methods. Importantly, for some biomarkers, such as phosphorylated Stat5, quantitative immunofluorescence identifies therapy-relevant subpopulations of tumors that are not identifiable by standard pathologist scoring of DAB chromogen stained tumors. We will provide novel evidence that quantitative immunofluorescence protein profiling facilitates characterization of breast cancer and identification of subtypes with increased likelihood of responding to existing or emerging therapeutic agents or their combinations.

Dr. Amy Ryder Peck is a Senior Research Scientist in the laboratory of Dr. Hallgeir Rui at Thomas Jefferson University, Philadelphia, PA, USA. Dr. Ryder Peck’s research identified that loss of active Stat5 is an independent marker of poor prognosis and a predictor of resistance to antiestrogen therapy in breast cancer. Dr. Peck leads the analytical aspects of a major consortium effort by a multidisciplinary team of investigators to identify therapy-relevant biomarkers using quantitative protein profiling of breast tumors.
Applying Tissue Phenomics to Colorectal Clinical Questions

Surgical resection is considered curative for Dukes B colorectal cancer patients, however 20-30% of patients experience disease recurrence and disease specific death. We aim to stratify Dukes B patients into high and low risk subgroups through novel image based analysis algorithms. Firstly we developed an image analysis algorithm to quantify and assess the prognostic value of three histopathological features; lymphatic vessel density and invasion as well as tumour budding. Image analysis provides the ability to standardize quantification across institutes and negates observer variability. Secondly we investigated if novel histopathological features can be identified through a Tissue Phenomics approach.

Colorectal tissue sections were labelled for epithelial cells (pan cytokeratin), lymphatic vessels (D240) and nuclei (DAPI) through immunofluorescence. The labelled tissue was used to segment and quantify tumour buds, lymphatic vessel density and lymphatic vessel invasion through automated image analysis using Definiens software. We next performed quasi-unbiased multi-parametric image analysis on the labelled tissue to quantify the complexity of the cancer microenvironment. The resultant phenome based multi-parametric signature, coupled with data mining statistics, is used to discover novel prognostic features in a Tissue Phenomics approach.

All three histopathological features were able to stratify high and low risk Dukes B patients (p< or = to 0.0001) and were found to be predictors of colorectal cancer specific death in univariate analysis; Tumour buds (HR =5.7; 95% CI, 2.38-13.8), lymphatic vessel density (HR =5.1; 95% CI, 2.04-12.99) and lymphatic vessel invasion (HR =9.9; 95% CI, 3.57-27.98). Lymphatic vessel invasion was an independent predictor of poor prognosis (HR =6.08; 95% CI, 1.17-31.41). A novel infiltrative pattern based histopathological feature was identified through Tissue Phenomics which has the ability to stratify high risk colorectal patients independent of Dukes staging.

Mr. Caie has just accepted the position of Senior Research Fellow in Digital and Systems Pathology at the University of St Andrews. He submitted his PhD in Digital and Quantitative Pathology at the University of Edinburgh, under the supervision of Prof. David Harrison, while building a Scottish wide Quantitative and Systems Pathology collaborative group within the clinical and academic settings. His PhD was focused on implementing quantitative pathology and Tissue Phenomics-based automated image analysis of morphometric and biomarker parameters coupled to molecular pathology in colorectal cancer. Previously, Mr. Caie attained an MRes in Medical biochemistry both from the University of Glasgow prior to working for AstraZeneca for nine years where he developed high content biology assays for in vitro drug discovery.
Novel Patient Stratification and Predictive Imaging Biomarkers for Development of Oncology Therapies

In oncology studies, biomarkers have been effectively used to measure tumor progression and to evaluate efficacy of therapies. Increasingly they have been utilized to stratify different patient groups and develop personalized therapeutic strategies. Towards this goal, immunohistochemistry (IHC) has been playing an increasing role in developing imaging biomarkers to help understand the mechanism of action by measuring molecular targets for particular cancer types. Through quantitative analysis of IHC images, effective biomarkers will facilitate in identifying which patients will respond to certain cancer treatments. At Merck, we have developed IHC imaging assays and analysis strategies for identifying and measuring presence of molecular targets for various cancer types using Definiens platform. Resulting imaging biomarkers are used to identify responder's from patient biopsy samples with high accuracy. Development of such patient selection biomarkers is expected to accelerate enrollment and increase response rates, leading to more effective clinical trial designs.

Dr. Belma Dogdas is an Associate Principal Scientist in Informatics & Early Development Discovery Sciences IT Department at Merck. She leads the Imaging Biomarkers program and her research involves development of mathematical and computational algorithms for multi-dimensional analysis of relevant biological imaging applications. She has supported projects for developing imaging biomarkers in various disease areas such as oncology, Alzheimer’s, cardiovascular, diabetes, infectious disease, bone biology as well as safety and manufacturing studies.

Dr. Dogdas received MS and PhD from University of California, Electrical Engineering Department in 2002 and 2007. She is a member of the IEEE Engineering in Medicine & Biology society.
Use of Computer Tumor Tissue Imaging Algorithms to Assess the Role of Microenvironment in Melanoma Brain Metastases

**Background:** High intratumoral hemorrhage and low degree of immune infiltrates in craniotomy specimens from melanoma brain metastases (MBM) are associated with shorter overall survival (OS). We hypothesize that immune checkpoint proteins (ICP), angiogenic factors, hypoxia, and the density of mature vs. immature blood vessels (MBV, IBV) are important prognostic factors.

**Methods:** Clinicopathologically annotated craniotomy tumor specimens were stained for angiogenic/hypoxic factors (bFGF, VEGF, Ang2, HIF1α), blood vessel (>50 μm²) density (BVD, CD31), ligands for ICP (PD-L1, Galectin-9), and IBV (CD31+SMA—) or MBV (CD31+SMA+) by immunohistochemistry or immunofluorescence. Images from scanned slides were ‘segmented’ into different tissue compartments [melanoma, reactive glia (RG), normal brain (NB), mononuclear cell clusters (MCC)]. The Aperio imaging system and Definiens Tissue Studio were used to analyze expression of each marker within different compartments.

**Results:** Up to 44 cases were analyzed for each stain. When survival information was used to define the optimal cut-point for each unadjusted variable between those with long versus short OS, high melanoma compartment expression of PD-L1 and bFGF, and low melanoma compartment expression of HIF1α were associated with worse OS (p<0.05; hazard ratios 1.92, 2.2, 2.1 respectively). PD-L1 expression was significantly higher within melanoma compartment compared to NB, but insignificantly different between melanoma and RG/MCC. Melanoma compartment expression of bFGF was significantly higher than RG/NB. CD31 BVD within melanoma/RG compartment was significantly higher than that of NB. There was a significant inverse correlation between melanoma PD-L1 expression and MCC density.

**Conclusions:** Sophisticated computer tissue imaging algorithms can differentiate expression of molecules/structures within different tumor compartments, which may play a different role in tumor progression. Tumor response to hypoxia by upregulating HIF1α may actually be a favorable prognostic factor. High expression of PD-L1 in MBM is an adverse prognostic factor, and could be derived from its immunosuppressive effects in the brain microenvironment.

Dr. Moschos received his medical degree in 1997 at the University of Athens, Greece. He completed his Internal Medicine residency training in 2002 at Newton-Wellesley Hospital, Boston, MA and his clinical fellowship training in Hematology/Oncology in 2005 at the University of Pittsburgh Medical Center (UPMC). He remained as a faculty at the UPMC Melanoma Program for 6 ½ years before he moved to the University of North Carolina at Chapel. Dr. Moschos is a melanoma physician-scientist who has been involved in numerous clinical, translational, and basic research studies investigating the prognostic and therapeutic significance of various molecules identified in tumor tissues.
Tissue Phenomics: From Biomarker Discovery to Clinical Diagnostic Assay Commercialization

Definiens’ Cognition Network Technology® (CNT) provides detailed tissue biomarker readouts from digital pathology slides, and enables the correlation of this information with clinical outcome and genomic data, an approach known as Tissue Phenomics. The manual approach to analyzing tissue provides mostly limited, qualitative information. However using CNT, thousands of quantitative, reproducible tissue biomarker features such as histologic scores, intensity measurements, morphologic parameters, and object enumeration have been extracted from all types of tissue stains, including H&E, IHC, ISH, and FISH digital images. Those features have been successfully mined for biomarker signatures for patient stratification, prognostication, and improved molecular diagnostic measurements. With the recent focus on immunotherapy, immunoprofiling of the tumor microenvironment has become a particularly important application of Definiens’ CNT, with clinical relevance demonstrated in both prognostic and predictive settings. The goal at Definiens is to see these phenomics-based assays implemented in the clinical setting to aid in individualized patient care. Although the technical aspects of performing image analysis-based diagnostics with an efficient workflow in the clinical setting have been well documented, diagnostics companies must still navigate a complex regulatory and reimbursement environment in order to commercialize an assay. The regulatory oversight of laboratory developed tests (LDTs) and companion diagnostics will likely increase in both the U.S. and Europe in the near future, and we have looked at ways to meet these requirements through laboratory-based PMAs and partnered IVDs. We have also explored some of the reimbursement and commercialization strategies that can be used along with our CNT to deploy Tissue Phenomics in personalized medicine in the U.S. and globally.

Dr. Nifong has more than 15 years of experience in clinical diagnostics development and operations, laboratory medicine, and project engineering. Prior to joining Definiens, Dr. Nifong was Senior Vice President of Clinical Operations for Metamark Genetics, where he successfully led efforts to translate quantitative multiplex immunofluorescence research protocols into robust, reproducible laboratory developed tests (LDTs) for clinical use. He spent 10 years as a faculty member at the Penn State Hershey Medical Center where he gained extensive experience in molecular pathology, clinical laboratory medicine, and laboratory validation and management.

Dr. Nifong holds a BS in Chemical Engineering from Purdue University and an MD from the University of Rochester. He completed his residency training in Clinical Pathology and is a Diplomat of the American Board of Pathology and a Fellow of the American Society for Clinical Pathology and the College of American Pathologists.
Tissue Quality and Clinical Data: The Bottleneck for Drug and Biomarker Development

Identification of drug targets as stratification and predictive biomarkers in patient populations by analyzing surgically resected tissues has become an important field in drug development. However, considerable research data demonstrate that RNA and protein expression level, including activation or inhibition of signaling pathways and their receptors can change significantly within minutes following surgical resection (“cold ischemia time”). Many of those genes and proteins are possibly involved in growth regulation and might serve as targets or stratification markers for new drugs. In addition, other pre-collection factors can impact tissue-derived data such as drug treatment and anesthesia of patients before surgical tissue removal, intrasurgical ischemia by ligation of main arteries, the location of a biopsy within a given tumor, processing and fixation of the tissue. And, last-but-not least, availability and integration of comprehensive clinical data of the individual patient (medical history, outcome of treatment etc.) significantly support understanding of tissue data in drug and biomarker development programs.

Therefore, controlled and rapid tissue processing and collection of clinical data in a standardized format is a prerequisite for understanding biological differences of or within patient tumors, and when developing targeted molecular therapies. Nowadays, most hospitals and pathology departments use their own, mostly incomparable and often poor standard for biospecimen and clinical data collection – a main reason for the high rate of irreproducible research data.

SOP-guided, ISO-certified processing of tissue in surgical suites and clinical data collection in hospitals and oncology practices have been established by Indivumed in a growing global network of hospitals in Germany and the US. Indivumed’s system generates biospecimens that represent the molecular patterns as they were in the human body. The clinical data base indivuNET allows highly meaningful, reproducible comparative studies. Overall, such labor intensive infrastructures are essential to accelerate drug and biomarker development in personalizing cancer therapy programs.

Prof. Dr. Hartmut Juhl received his MD from Hamburg Medical School in 1986. He worked as a surgical oncologist at the Department of Surgery of the University Hospital in Hamburg and Kiel from 1987 until 1998. In 1998, he was appointed Research Associate Professor at Lombardi Cancer Center at Georgetown University in Washington, DC. Prof. Dr. Juhl became extremely familiar with all aspects, practical problems and scientific needs of biobanking. He founded Indivumed GmbH in Hamburg, Germany in 2002.

Prof. Dr. Juhl is CEO of Indivumed GmbH. He has appointments as Adjunct Professor at Georgetown University in Washington, DC and the University of Hamburg in Germany.
Measurement of Target Engagement and Pharmacodynamic Response to Investigational Anti-Cancer Agents in Phase 1 Clinical Trials

Phase 1 Clinical trials of candidate anti-cancer agents are intended to establish the Maximum Tolerated Dose of the agent. In current practice, evidence of activity of the agent on the intended target is also a requirement in NCI-funded trials. The nature of these early clinical trial designs significantly impacts the ability to generate usable data that addresses target engagement, because of limited numbers of patients enrolled and the inclusion of patients with multiple cancer types. Key factors in success of target engagement measurements are dynamic range of the biomarker signal measured vs background variability in patient specimens, the quality of the tissue obtained for analysis, stability of the biomarker during the course of tissue collection, and timing of the collection after administration of the drug candidate. Analysis of biopsy specimens from patients enrolled in a single agent trial of AZD 1775, and from a combination trial of Irinotecan + Veliparib will illustrate some of the analytical issues and workarounds we have employed. A conclusion of our work in these trials is that biomarker assay multiplexing is critical to increasing the utility of biopsy testing in early stage drug trials. With careful application of assay development and analytical principals this approach has the potential to not only increase our understanding of drug effects on target in patient specimens, but even more importantly can be used to reduce the number of false negatives and increase the number of reportable results within patient trials. Funded by NCI Contract No. HHSN261200800001E.

Dr. Kinders is head of the Pharmacodynamics assay development (PADIS) laboratory at Frederick National Laboratory. The PADIS laboratory is charged with development and validation of assays to measure the effects, at the molecular level, of new, targeted anticancer agents in first-in-man clinical trials at the NCI Clinical Center. He received his Ph.D. in Biology at Kansas State University. His research has focused on cancer diagnostics and biomarkers. Dr. Kinders has worked extensively in the U.S. Diagnostics Devices industry, including stints at Abbott Laboratories and C.R. Bard; and in pharmaceutical development at Abbott Laboratories and CuraGen.
Development of an Encapsulated Cell Replacement Therapy for Diabetes

ViaCyte Inc. is a clinical stage company developing a stem cell-based islet cell replacement therapy for treatment of patients with diabetes. The therapy is a combination product comprised of pancreatic progenitors, PEC-01™ cells, encapsulated within a retrievable delivery device, ENCAPTRA® Drug Delivery System. After implantation, encapsulated progenitor cells mature into endocrine cells that secrete insulin in a regulated manner to control blood glucose levels. The renewable starting material for cell product manufacturing is human embryonic stem cells that are directed to differentiate to PEC-01™ cells in vitro using scalable processes. The bio-stable delivery device is designed to contain cells and to protect cells from immune attack. Use of Definiens’ image analysis platform for product testing and characterization will be presented. The goal is to develop a product that will achieve insulin independence, reduce diabetes-related complications, and eliminate the need for immunosuppressant drugs.

Funding in part from California Institute for Regenerative Medicine; SP1-06513, DR1-01423, TR1-01215 and JDRF.

Olivia G. Kelly, Ph.D. is responsible for various aspects of ViaCyte’s research and development program including assay development, preclinical safety studies, and stem cell differentiation. She is a co-author of high profile publications in renowned scientific journals and a co-inventor on multiple ViaCyte’s patents. Prior to joining ViaCyte, Dr. Kelly performed her post-doctoral and graduate research on the molecular mechanisms of early embryonic patterning and organogenesis using stem cell technologies. Dr. Kelly earned her B.S. in Molecular Biology from the University of Texas at Austin and her Ph.D. in Biochemistry and Molecular Biology from Harvard University.
Imaging and Analysis of RNA Biomarkers In Situ via RNAscope and SpotStudio

RNA in situ hybridization (ISH) is a powerful tool for establishing spatial and cell type-specific gene expression in tissue. Traditional RNA ISH methods are technically challenging to perform and lack sufficient sensitivity and specificity for most clinically relevant biomarkers. RNAscope is a novel RNA ISH technology capable of detecting RNA in situ with single molecule sensitivity and single cell resolution and is compatible with routine formalin-fixed paraffin-embedded clinical specimen. The single-molecule detection capability of RNAscope in particular enables straightforward quantification of RNA in single cells by counting signal dots. We have developed a dedicated image analysis software application (RNAscope SpotStudio) using Definiens’ Cognition Network Technology for quantitative analysis of RNAscope-stained slides. I will discuss how RNAscope and image analysis can be used together in cancer biomarker studies.

Dr. Ma has been involved in cancer biomarker discovery and molecular diagnostics for the last 14 years. Before joining Advanced Cell Diagnostics, he was VP of R&D/Biostatistics at bioTheranostics, where he led the development of several gene signature assays for stratification of breast and prostate cancer patients and for multi-cancer classification for cancer of unknown primary. Prior to bioTheranostics, Dr. Ma worked on drug target discovery at Johnson & Johnson and Monsanto/Searle (now Pfizer). Dr. Ma received his Ph.D. in biochemistry from the University of Iowa.
Immuno score / Immunoprofile: Assessing Anti-Cancer Immunity as a Biomarker to Stratify Patients for Clinical Trials

There is a significant need for innovative prognostic and predictive biomarkers of recurrence, response to therapy, and survival for patients with cancer. Increasing evidence suggests that the number, type and location of tumor-infiltrating lymphocytes in primary tumors has prognostic value, and this has led to the development of an "immuno score". Image analysis technology, such as Definiens, is particularly well suited to objectively and qualitatively characterize the tumor microenvironment and is being used by the Society for Immunotherapy of Cancer (SITC) led global immuno score initiative that seeks to validate the immuno score as a prognostic biomarker for patients with colon cancer. The immuno score concept also has the potential to help predict response to treatment, thereby improving decision making with regard to choice of therapy. However, unlike molecular targeted therapies, where biomarkers are often provided by the tumor's driver mutation profile, strong predictive biomarkers for novel immunotherapies have not yet been clinically validated. This predictive aspect of the tumor microenvironment forms the basis for the concept of immunoprofiling, which can be described as using an individual's immune system signature (or profile) to predict that patient's response to standard therapies as well as novel immunotherapies and to stratify patients enrolled on clinical trials. The characterization of the tumor immune microenvironment and of the tumor-host interaction via immunoprofiling may enable the individual tailoring of immunotherapies such as immune checkpoint blockade. Furthermore, immunoprofiling may also enable and guide the manipulation of the tumor microenvironment, via for example epigenetic modulating drugs, in order to overcome mechanisms of resistance.

Dr. Bifulco is a surgical pathologist, with subspecialty training in Molecular Genetic Pathology and Hematopathology, currently serving as the Director of Molecular Pathology and Pathology Informatics at the Providence Regional Laboratory and as Director of Translational Molecular Pathology at the Earle A. Chiles Research Institute in Portland, OR. Prior to joining Providence Pathology, Dr. Bifulco served on the Pathology faculty at Yale University and completed fellowships in Oncologic Surgical Pathology and Molecular Genetic Pathology at Memorial Sloan Kettering Cancer Center. His translational research work is focused on supporting the characterization of the tumor's microenvironment through immunohistochemical and image analysis techniques.
To date the anatomic extent of tumor (TNM classifications) has been by far the most important factors to predict the prognosis of cancer patients. However, this classification provides limited prognostic information in estimating the outcome in cancer and does not predict response to therapy.

Using large-scale technologies, quantitative measurements, and integrative biology approaches we evaluated the importance of the host-immune response within human tumors.

We showed that tumors from human colorectal cancer with a high density of infiltrating memory and effector memory T-cells (TEM) are less likely to disseminate to lymphovascular and perineural structures and to regional lymph-nodes. We showed that the combination of immune parameters associating the nature, the density, the functional orientation and the location of immune cells within the tumor was essential to accurately define the impact of the local host immune reaction on patients' prognosis. We defined these parameters as the “immune contexture”, and factors modulating it will be discussed. We recently characterize the immune landscape within human tumors, and showed the importance of adaptive immune cells including, cytotoxic T cells, Th1 cells, B cells and T-follicular-helper (Tfh) cells. Analysis of chromosomal instability revealed mechanisms associated with intratumoral lymphocyte proliferation. Based on the immune contexture, a standardized, simple and powerful immune stratification system, termed “Immunoscore”, was delineated that may bear a prognostic power superior to that of the currently used cancer staging system. Tumor invasion parameters were statistically dependent on the host-immune reaction. A worldwide Immunoscore consortium is testing the prognostic value of Immunoscore, using a standardized assay to routinely measure the immune status of a cancer patient.

The functional orientation of the immune contexture is characterized by immune signatures qualitatively similar to those predicting response to immunotherapy, Thus, the continuum of immune response existing, spanning a balance between tumor cell growth and elimination, will be discussed.

Dr Jérôme Galon is Research Director at INSERM and head of the Integrative Cancer Immunology laboratory (Paris, France). Trained at the Pasteur Institute and at the Curie Institute (Paris, France), he worked at the NIH (Bethesda, USA). Work from his laboratory demonstrated that the intratumoral adaptive immune reaction was a better predictor of survival than traditional cancer staging (NEJM, Science, JCO). Dr Galon was awarded by, the Medical Research Foundation (Lamarca Award 2008), the Coley Award for Distinguished Research in Basic and Tumor Immunology (CRI, USA 2010), the National Academy of Science (2011), and the National Academy of Medicine (2011).
Implementation of Image Analysis for Biomarker Assessment of Pre-Clinical Tumor Models and Clinical Patient Samples

Patient derived xenograft (PDX) models have complex histology, preserving the histopathology, gene expression, genetic mutations, and therapeutic response of the original patient tumor, including maintenance of cancer stem cell populations. This complexity provides a basis for biomarker discovery focused on tumor type and targeted therapy. Our implementation of immunohistochemistry (IHC) staining, digital scanning, and image analysis, with use of Definiens Tissue Studio suite for robust analysis of xenograft and human tissues will be discussed. We have developed IHC biomarker antibodies using existing commercial antibodies as well as internal monoclonal antibodies for various stem cell proteins. Their use as biomarkers for retrospective analysis of patients, as well as prospective selection of patients based on IHC protein expression levels will be discussed.

Dr. Belinda Cancilla manages OncoMed's minimally passaged patient derived human tumor bank, and leads histology based translational biomarker discovery and implementation efforts. She has over 18 years of combined experience in cellular and developmental biology, histology, immunohistochemistry (IHC), digital imaging, advanced image analysis and targeted cancer drug discovery and development.
Charles Mathews  
Vice President  
Boston Healthcare

Companion Diagnostics: Aligning Development, Commercialization, and Market Access

Ideally pharmaceutical innovators identify a companion diagnostic need early in the development process and identify an in vitro diagnostic partner that is able to not only support the execution of testing during the clinical trial but also launch the test globally when the drug is approved. However, this is often not the case. Variables in immediate testing needs, difficult commercialization pathways, and misaligned incentives lead to disconnects between the promise of CDx and the realities. This presentation will provide an overview of companion diagnostic development and launch/distribution options and market access pathways. It will review the needs and capabilities of various stakeholders (drug, IVD, lab, CRO, etc.) and not only point out where some of the disconnects lie but also provide suggestions for incorporating stage appropriate activities into drug development and CDx commercialization/market access planning and partnering/deal-terms.

Mr. Mathews has worked on a variety of reimbursement projects identifying public and private payer coverage channels, coding options, and pricing and payment possibilities for drugs, diagnostics, and medical devices. His specific area of expertise is in diagnostics, with a focus on value-based pricing for laboratory tests and molecular diagnostics, as well as with global companion diagnostic launch strategies. His experience further includes work with test and platform developers and laboratories, novel medical/surgical devices, drug/diagnostic combinations, and pharmaceutical product launches.

Mr. Mathews’ prior experience includes working on health policy as a legislative aide on Capitol Hill. He has also worked for the government affairs office of Genentech and has participated in a National Institutes of Health sponsored clinical trial which focused on genetic testing for Alzheimer’s disease.

Mr. Mathews has a master’s degree in public policy from Duke University and completed his undergraduate work at Colgate University.