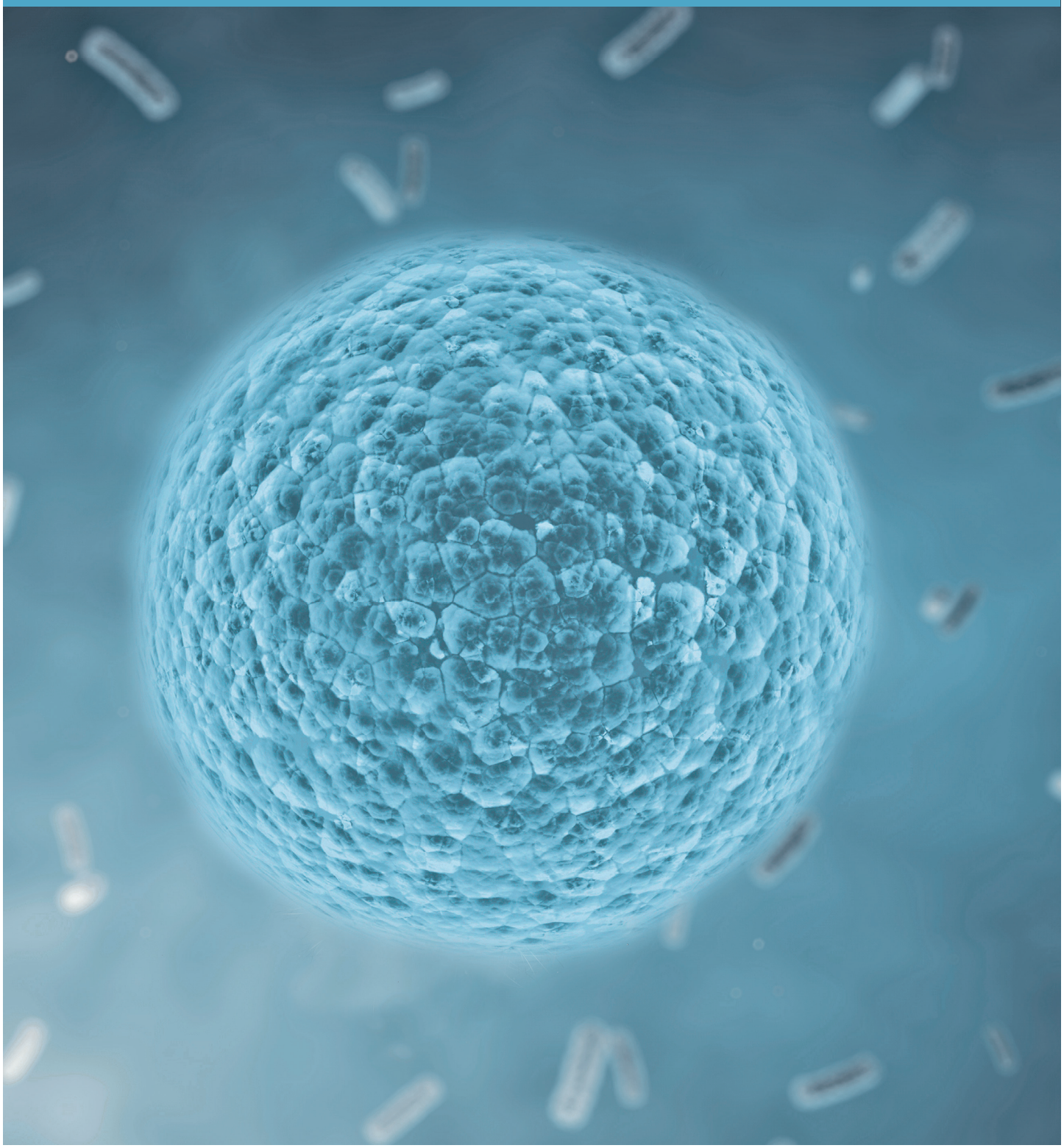
Biofluid Biopsies & High-Value Diagnostics 2015
Conference Abstracts

In collaboration with Journal of Circulating Biomarkers



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This Book of Abstracts covers the titles, authors and abstracts of the presentations given at Selectbio's "Biofluid Biopsies & High-Value Diagnostics 2015" Conference.

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Table of Contents

Conference Plenary Session

Liquid Biopsies in the Management of Cancer

Ellen Beasley
Senior Vice President, Product & Services R&D, Genomic Health, United States of America

Abstract:
Solid tumors that originate in internal organs such as the breast, lung, or bladder often release DNA and tumor cells into the bloodstream or urine. When the presence of tumor-derived DNA in blood is high and persists or increases over time, the cancer is likely growing and a new course of treatment may be appropriate. The ability to reliably measure tumor burden in blood and the evolution of tumor biology over time it will have a transformational impact on the management of cancer. Patients could forego more invasive procedures like tissue biopsies and would not have to wait months to do screenings like PET scans. In real-time management of cancer patients, similar to cholesterol monitoring, we will have the opportunity to guide patients to the right treatment with the right dose more quickly, improving patient outcomes and potentially avoiding unnecessary side effects and cost.

Liquid Biopsies and the Potential to Make Tissue Less of an Issue

Walter Koch
Vice President, Roche Molecular Systems, United States of America

Abstract:
Blood-based testing can reduce test turnaround time and potentially serve as a surrogate for tissue for biopsy ineligible or fragile patients, for whom a tissue sample is not available. Potential uses being explored in clinical studies and trials include 1) identification of genetic determinants that direct targeted therapies (eg EGFR activating mutations in NSCLC for several tyrosine kinase inhibitors), 2) evaluation of early treatment responses, 3) monitoring for minimal residual disease and 4) assessment of disease progression and resistance development. Several technologies are in development for blood based analytes including circulating tumor cells, contents of exosomes released by cancer cells, and cell free circulating tumor DNA (ctDNA). This presentation will exemplify the potential power of Liquid Biopsy approaches using ctDNA analyses with data from several clinical studies involving NSCLC patients undergoing targeted therapy treatment.

Saliva-Based Detection of Actionable EGFR Mutation in Lung Cancer Patients

David Wong
Associate Editor
Journal of Circulating Biomarkers
Director, University of California Los Angeles, United States of America

Abstract:
Constitutive activation of epithelial growth factor receptor (EGFR) is prevalent in epithelial cancers, particularly in non-small-cell lung carcinoma (NSCLC). EGFR mutation predicts sensitivity to EGFR-targeted therapy and mutation detection is mainly based on tissue biopsy, which is invasive, expensive and time-consuming. Non-invasive, real-time, point-of-care, inexpensive detection and monitoring of EGFR mutations in NSCLC patients is highly desirable. We developed a novel core technology, Electric Field-Induced Release and Measurement (EFIRM), relying on a multiplexible electrochemical sensor that can detect EGFR mutations directly from body fluids. EFIRM for EGFR mutation detection was established in vitro, and correlated with tumor size from xenografted mice. In clinical application, we demonstrated that EFIRM can detect EGFR mutations from saliva and serum of 22 NSCLC patients. And finally, a blinded test was performed on saliva from 40 NSCLC patient saliva samples. The receiver operating characteristic analysis indicated that EFIRM detected the exon 19 deletion with an area under the curve (AUC) of 0.94 and the L858R mutation with an AUC of 0.96. Our data indicate that EFIRM is effective, accurate, rapid, user-friendly, and cost effective for the detection of EGFR mutations in saliva of NSCLC patients.

Plasma Extracellular Vesicle Content for Diagnosis and Prognosis of Cardiovascular Disease

Dominique de Kleijn
Professor of Surgery, National University of Singapore, Singapore

Abstract:
Cardiovascular Disease (CVD) is with the cardiovascular events of Ischemic Heart Disease and Stroke, the number 1 and 2 cause of death in the world and expect to increase especially in Asia. Atherosclerosis is the underlying syndrome for CVD and atherosclerotic plaques are detectable already in teenagers. Therefore for cardiovascular disease, we have to determine who is at high risk for a cardiovascular event in a high-risk background of the atherosclerotic syndrome without the possibility of taking a plaque biopsy. The risk for Cardiovascular events is region-dependent so tailored plasma-based biomarkers are essential to guide prevention and treatment of billions. Collecting plasma extracellular vesicles is like taking a liquid biopsy from the pathological tissue that can be used for diagnosis and prognosis of the disease. We show that plasma extracellular vesicle protein content especially when collected from different plasma subfractions can be used as an accurate source for diagnosis and prognosis of cardiovascular disease.

The Evil Little Things About Cancer: EVs as Infiltrators and Informants

Xandra Breakefield
Professor, Mass General Hospital (MGH)/Harvard Medical School

Abstract:
Tumor cells release an abundance of extracellular vesicles which contain protein, RNA and DNA cargo. Studies are actively ongoing to determine the different types of tumor-derived vesicles and the function of their cargo, which appears to be designed to change the phenotype of many types of normal cells in favor of tumor progression. These evil-intentioned vesicles have two possible Achilles' heels. In the first case, they can be isolated from biofluids of the cancer patient and used to inform on the mutational stratagem of the tumors and hence provide clues as to effective treatment strategies. In the second case, vesicles may be transformed into therapeutic vehicles to "silently" attack the tumors.

The Utility of Blood Exosomes from Cancer Patients as Biomarkers for Early Detection

Raghu Kalluri
Professor & Chairman, Department of Cancer Biology; Olla S. Stribling Distinguished Chair for Cancer Research, MD Anderson Cancer Center, United States of America

Abstract:
n/a

Biofluid Biopsies Track

Application of Circulating Tumor DNA Biomarkers to Cancer Drug Development

Brian Dougherty
Translational Genomics Lead, AstraZeneca R&D, United States of America

Abstract:
n/a

Efficacy of Rociletinib in NSCLC Patients Selected with an EGFR Plasma Test

Chris Karlovich
Associate Director, Molecular Diagnostics, Clovis Oncology, Inc., United States of America

Abstract:
We are exploring blood-based molecular testing in clinical trials of rociletinib, a novel third-generation TKI that selectively inhibits the EGFR activating and T790M resistance mutations in NSCLC patients. The utility of plasma-based EGFR mutational analysis for diagnosis, prediction of response to rociletinib, as a PD biomarker, and for ongoing monitoring of response will be described.

Ultrasensitive Detection of Circulating Tumor DNA by Deep Sequencing

Maximilian Diehn
Assistant Professor, Stanford University School of Medicine, United States of America

Abstract:
I will describe recent technical and clinical results from analyses of circulating tumor DNA using Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq).

Circulating Tumor DNA as a Biomarker in Esophageal Adenocarcinoma

Tony Godfrey
Associate Chair – Research, Department of Surgery, Boston University Medical Center, United States of America

Abstract:
n/a

Enabling Urine Biomarker Discovery

Shannon Pendergrast
Chief Scientific Officer, Ymir Genomics, United States of America

Abstract:
The discovery of urinary extra-cellular biomarkers has been impeded by the lack of efficient methods for the isolation of extra-cellular vesicles (exosomes and microvesicles), protein, and nucleic acid. Ultracentrifugation, considered the gold standard for vesicle isolation from many biofluids, is efficacious but laborious, and like most commercially available methods, is unable to consistently isolate enough quality material from small volumes of urine for protein or RNA-based biomarker discovery. Ymir Genomics has invented a novel precipitation reagent that rapidly and efficiently isolates extra-cellular biomolecules and vesicles from urine samples yielding a quantity suitable for biomarker discovery. Ymirite is more efficient than either ultracentrifugation or commercial precipitation methods for isolating extra-cellular nucleic acids and vesicles from a variety of urine samples as evaluated by immunoblot, Nanosight, Electron Microscopy, miRNA microarray, and qRT-PCR. Moreover, unlike all other methods, our reagent was able to isolate vesicles from very dilute samples. Ymir Genomic's Precipitation Reagent offers a method that, in less than 30 minutes, isolates intact extra-cellular vesicles, protein and RNA suitable for biomarker discovery from urine samples.

Technology Spotlight: Multiplexed ICE COLD-PCR (MX-ICP) for Detection of Low-Level Mutations in Liquid Biopsies"

Katherine Richardson
Vice President, Research & Development, Transgenomic, Inc.

Abstract:
The use of "liquid biopsies", where limited or no tumor tissue is available, is increasingly important for molecular demographics, diagnostics and pharmacodynamic monitoring of patients during therapy. The amounts of DNA needed for this low level detection may not be feasible with the limited quantities of blood/plasma/serum obtained from patients on clinical trials. The ability of MX-ICP to enrich alterations can provides a means to evaluate mutations in these samples using lower amount of sample DNA. The benefits of MX-ICP are (1) MX-ICP is a key technology for sensitive detection and monitoring of ALL genetic alterations in multiple targets using a single DNA sample; (2) Less DNA is needed for mutation detection; (3) Mutation quantification is feasible using MX-ICP; and (4) Coupling MX-ICP with platforms like NGS and ddPCR enables high sensitivity detection of mutations at approximately 0.01% in patient samples with limited amounts of DNA. This presentation will describe the technology and give examples of detection and quantification of low-level mutations, including EGFR Exon 19 deletions, the EGFR Exon 21 L858R and both the T790M and C797S mutations in EGFR Exon 20.

Technology Spotlight: Droplet Digital PCR: A Precision Tool for Liquid Biopsy

Svilen Tzonev
Director of Business Development, Science and Technology, Bio-Rad Laboratories

Abstract:
Bio-Rad's QX200 Droplet Digital PCRTM (ddPCRTM) system has become widely accepted as the tool of choice in detecting and quantifying known and actionable cell-free DNA biomarkers. Main advantages include direct quantification, high sensitivity and specificity, digital precision. ddPCR also offers fast turnaround time (less than a day) with an economical and flexible workflow tolerant to PCR inhibitors. A large number of validated assays for detection of individual or multiplexed rare mutations and copy number alteration somatic variants are widely used in cancer research and translational context. This talk will cover latest technological advances and present examples of translational research in lung, colorectal, melanoma, and breast cancer in assessing tumor response and evolution, emergence of resistance markers and monitoring of residual disease via liquid biopsy in blood.

Technology Spotlight: From Sample to Insight: Integrated Solutions from Sample Stabilization, Nucleic Acid Purification and Analysis

Marco Polidori
Global Product Manager, QIAGEN

Abstract:
Liquid Biopsy has the potential to transform healthcare and biomedical research. The presentation will give an overview on QIAGEN's approach to provide integrated solutions for isolation of circulating biomarkers and their analysis in research and diagnostic applications. The presentation will cover new and existing QIAGEN solutions ranging from sample stabilization, nucleic acid purification, and NGS based analysis.

Long Noncoding RNAs as Putative Biomarkers for Prostate Cancer Detection: Urine as a Biofluid for Liquid Biopsies

Ranjan Perera
Associate Professor, Scientific Director Genomics and Bioinformatics, Sanford Burnham Medical Research Institute, USA

Abstract:
Prostate cancer is one of the leading causes of mortality among US males. There is an urgent unmet need to develop sensitive and specific biomarkers for the early detection of prostate cancer to reduce overtreatment and accompanying morbidity. We identified a group of differentially expressed long noncoding RNAs in prostate cancer cell lines and patient samples and further characterized six long noncoding RNAs in prostatic adenocarcinoma tissue samples (Gleason score >6.0) and compared them with matched normal (healthy) tissues. Interestingly, these markers were also successfully detected in patient urine samples and were found to be up-regulated when compared with normal (healthy) urine. Chromogenic in situ hybridization assay was developed to detect long noncoding RNAs in primary prostatic adenocarcinoma tissue samples, paving the way for clinical diagnostics. We believe that these results will set the stage for more extensive studies to develop novel long noncoding RNA-based diagnostic assays for early prostate cancer detection and will help to distinguish benign prostate cancer from precancerous lesions.

Detection of Circulating Tumor DNA by Surface Plasmon Resonance

Sehyun Shin
Professor & Director, Liquid Biopsy Research Center, Korea University, South Korea

Abstract:
Circulating tumor DNA (ctDNA) has been demonstrated as the most promising biomarker for non-invasive assessment of cancer as well as the most accurate predictor of cancer treatment responses. However, detection of ctDNA in blood has been faced with conventional technical limits and called for innovative technologies and methods. Here, we present a stable and selective assay for detecting of epidermal growth factor receptor (EGFR) mutation in plasma (or liquid biopsy) using DNA-DNA hybridization and Au nanoparticle probe with a lab-made surface plasmon resonance (SPR) sensometry. We prepared wild type EGFR, mutant EGFR including point mutation and deletion and control (non-complimentary). Linker DNAs coated on a sensor surface of SPR capture different quantities of three different DNA types. Due to characteristics of SPR, the whole process was monitored in real-time and completed within an hour. This study as a proof of concept can be further expanded into multiplexing detection of major and known ctDNAs, which could provide a solution for clinical unmet needs in cancer treatment and early detection.

NanoVelcro-Embedded Microchips for Detection and Characterization of Circulating Tumor Cells

Hsian-Rong Tseng
Professor, University of California-Los Angeles, United States of America

Abstract:
Inspired by the nanoscale interactions observed in the tissue microenvironment, our research team at UCLA pioneered a unique concept of “NanoVelcro” cell-affinity substrates, in which CTC capture agent-coated nanostructured substrates were utilized to immobilize CTCs with high efficiency. The working mechanism of NanoVelcro cell-affinity substrates mimics that of VelcroTM – when the two fabric strips of a Velcro fastener are pressed together, tangling between the hairy surfaces on two strips leads to strong binding. Through continuous evolution, 3 generations of NanoVelcro CTC Chips have been established to achieve different clinical utilities. My presentation will summarize the continuous evolution of NanoVelcro CTC Assays from the emerge of the original idea all the way to their applications in cancer research and diagnosis. We envision that NanoVelcro CTC Assays will lead the way for powerful and cost-efficient diagnostic platforms for researchers to better understand underlying disease mechanisms and for physicians to monitor real-time disease progression.

Circulating Tumor Cells: From Enumeration to Molecular Characterization

Nicholas Dracopoli
Vice President/Head, Johnson & Johnson, United States of America

Abstract:
n/a

Profiling CTC Heterogeneity in Patients Using Microfluidics

Shana Kelley
Professor, University of Toronto, Canada

Abstract:
The analysis of circulating tumor cells (CTCs) is an important capability that may lead to new approaches for cancer management. CTC capture devices developed to date isolate a bulk population of CTCs and do not differentiate subpopulations that may have varying phenotypes with different levels of clinical relevance. Here, we present a new device for CTC spatial sorting and profiling that sequesters blood-borne tumor cells with different phenotypes into discrete spatial bins. Antibody-functionalized magnetic nanoparticles facilitate CTC sorting, and permit deconvolution of phenotypic subpopulations. Working with patient blood samples, we obtain profiles that elucidate the heterogeneity of CTC populations present in cancer patients. Samples from patients undergoing treatment for prostate, breast, and renal cancers have been analyzed using this approach, and samples collected from xenograft cancer models have also been used for validation of the approach.

Circulating Tumor Cells: The Promise and Reality of the Liquid Biopsy

Minetta Liu
Associate Professor and Chair, Oncology Research, Mayo Clinic, United States of America

Abstract:
The presentation objectives are as follows: To discuss the potential impact of CTC molecular characterization in clinical practice. To review CTC isolation platforms in the context of demonstrating clinical utility as a liquid biopsy.

Monitoring Drug Response Biomarkers in CTCs

Robert Kinders
Sr. Principal Scientist and Head, Laboratory of Human Toxicology and Pharmacology, Frederick National Laboratory for Cancer Research, United States of America

Abstract:
Ongoing Clinical Trials in the NCI network are employing a set of nuclear biomarkers to monitor drug activity on target. This presentation will review specific trials, biomarkers and CTC phenotypes analyzed on the ApoStream or CellSearch 5 channel platform.

Oral and Oropharyngeal Cancer Detection is Spitting Distance Away

Chamindie Punyadeera
Associate Professor, Institute of Health and Biomedical Innovation, Queensland University of Technology, Australia

Abstract:
Oral (OC) and oropharyngeal cancers (OPC) are significantly fatal; only approximately 50% of patients survive to 5 years. Surgery and chemoradiotherapy can leave survivors with pain, disfigurement and disability that further add to the burden of the disease. Unfortunately, more than half of these cancers are only diagnosed at an advanced stage, at which stage their 5-year survival rate plummets. Therefore, there is an urgent need for a reliable early diagnostic method. Current diagnosis is usually made after clinical presentation based on symptoms from the primary cancer and/or when metastatic cervical lymph nodes become obvious. Diagnosis involves biopsy identification of Human Papilloma Virus (HPV) status and histological classification. These diagnostic methods are subjective and become problematic when tumours are too small to be visualised or they are cryptic. These issues commonly result in misdiagnosis. The direct contact between saliva and OC and OPC lesions make the measurement of tumour markers in saliva an attractive alternative to serum and tumour tissue testing.

OncomiRs as Functional Cancer Biomarkers

Frank Slack
Director, Institute for RNA Medicine, Beth Israel Deaconess Medical Center Cancer Center/Harvard Medical School, United States of America

Abstract:
n/a

Optical Imaging of Genitourinary Cancer: A Biofluid Assay

Mathew Thakur
Director, Laboratories of Radiopharmaceutical Research and Molecular Imaging, Thomas Jefferson University, United States of America

Abstract:
Non-invasive diagnosis of genitourinary cancer continues to be challenging. In recent years detecting circulating tumor cells (CTC) in peripheral blood has emerged as one of the most sought-after fields. CTC determination can assess the presence of primary disease, determine effectiveness of therapy and predict disease recurrence. Current approaches for determination of CTC for genitourinary cancer ,however, are time consuming, complex and are not yet widely used in clinical practice. Genitourinary cancer cells express on their surface a genomic biomarker VPAC1, in high density, prior to elevation of PSA and alternations in cell morphology. These cells are shed (STC) in urine. We hypothesize that targeting VPAC1 receptors using a receptor specific biomolecule TP4303, developed in our laboratory, will permit us to detect STC accurately and non-invasively.

An Optimized Procedure for Exosome Isolation and Analysis Using Serum Samples: Application to Cancer Biomarker Discovery

Alexander Sasha Vlassov
Associate Editor
Journal of Circulating Biomarkers
Senior Manager R&D, Thermo Fisher Scientific, United States of America

Abstract:
Exosomes are RNA and protein-containing nanovesicles secreted by all cell types and found in abundance in body fluids, including blood, urine and cerebrospinal fluid. These vesicles seem to be a perfect source of biomarkers, as their cargo largely reflects the content of parental cells, and exosomes originating from all organs can be obtained from circulation through minimally invasive or non-invasive means. Here we describe an optimized procedure for exosome isolation and analysis using clinical samples, starting from quick and robust extraction of exosomes with Total exosome isolation reagent, then isolation of RNA followed by qRT-PCR. Effectiveness of this workflow is exemplified by analysis of the miRNA content of exosomes derived from serum samples – obtained from the patients with metastatic prostate cancer, treated prostate cancer patients who have undergone prostatectomy, and control patients without prostate cancer. Three promising exosomal microRNA biomarkers were identified, discriminating these groups: hsamiR375, hsa-miR21, hsa-miR574.

Liquid Biopsies in NSCLC

Oscar Puig
Biomarker Leader, Hoffmann La Roche, United States of America

Abstract:
ALK inhibitors have shown efficacy in Non-Small Cell Lung cancer (NSCLC) patients whose tumors have activating rearrangements comprising ALK kinase. ALK rearrangements are routinely assessed in formalin-fixed samples by FISH, IHC or NGS. Unfortunately, patients progress after therapy with ALK inhibitors because of the appearance of secondary activating mutations in ALK and other escape pathways, which leads to acquired resistance. Quantitative assessment of dynamic changes of ALK rearrangement in plasma could be used as an indicator of initial treatment response, and appearance of secondary mutations could indicate development of acquired resistance. We will present data from plasma samples of patients enrolled in phase 2 studies treated with the ALK inhibitor alectinib. These results suggest that cell free DNA in plasma is a useful method to assess response and resistance in NSCLC.

microRNA Analysis in Liquid Biopsies

Anton Wellstein
Professor, Georgetown University Medical Center, United States of America

Abstract:
Will discuss the background and technical approaches to monitor microRNAs in the circulation. Applications as a potential tool in prognosis of early stage cancers and in treatment responses will be discussed.

High Value Diagnostics

Technology Spotlight: Circulating Cell-Free DNA (cfDNA) Analysis in Liquid Biopsies

Theresa Zhang
Vice President, Research Services, Personal Genome Diagnostics

Abstract:
n/a

Extracellular RNA in Central Nervous System Injury and Disease

Kendall Van Keuren-Jensen
Associate Professor, Translational Genomics Research Institute, United States of America

Abstract:
Diseases and injuries of the central nervous system are difficult to evaluate or monitor frequently over time. We have been assessing the types of RNAs released from the central nervous system, and its availability for detection in different biofluids. We describe RNA profiles of different biofluids and their correlation with injury and disease.

Extracellular RNAs in the Maternal Circulation: A Window into Placental Function

Louise Laurent
Associate Professor, University of California-San Diego, United States of America

Abstract:
Critical to the success of the pregnancy is the rapid development of a new organ, the placenta, which is of fetal origin, but which must gain access to the maternal bloodstream. This direct contact between placental trophoblast cells and the maternal circulation facilitates the functions of these cells, both as mediators of the exchange of materials between the fetus and the mother, and as sources of signaling molecules that induce changes in maternal physiology necessary for the establishment and maintenance of the pregnancy, such as progesterone, human chorionic gonadotropin and human placental lactogen. It is reasonable, then, to hypothesize that both the physiological changes occurring over the course of normal pregnancy and the pathological changes taking place with complications of pregnancy, are reflected in the ExRNA profiles of maternal biofluids, and may even be partially mediated by ExRNA signals. Here, we will discuss evidence that ExRNAs in the maternal circulation indeed reflect changes in placental function during pregnancy.

Extracellular Vesicles in Cancer: Exosomes, Microvesicles and the Emerging Role of Large Oncosomes

Dolores Di Vizio
Editorial Board
Journal of Circulating Biomarkers
Professor, Cedars Sinai Medical Center, United States of America

Abstract:
n/a

Monitoring Cancer Through the Blood

Cloud Paweletz
Head of the Translational Research Laboratory, Dana Farber Cancer Institute, United States of America

Abstract:
Genotype-directed targeted therapies are revolutionizing cancer care. Genomic alterations in genes such as EGFR, ALK, KRAS, and BRAF have been validated as powerful predictive biomarkers in the management of non-small cell lung cancer (NSCLC), colorectal cancer, and melanoma; testing for these mutations is currently standard to personalize treatment decisions. The challenges associated with routine use of NGS include availability of adequate tumor specimens, slow turnaround time, and evolving tumor biology in response to treatment that may necessitate a repeat biopsy to guide subsequent therapy. Here we describe the use of blood based, non-invasive technologies to overcome tissue based genomics.

Deep Sequencing Analysis of Exosomal and Non-Exosomal Non-Coding RNA in Human Ovarian Follicular Fluid

Shlomit Kenigsberg
Senior Researcher, CReATe Fertility Centre, Canada

Abstract:
n/a

Liquid Biopsies – Cancer Disease Profiling with Less Burden on Patients

Klaus Lücke
CEO & Founder, GILUPI GmbH, Germany

Abstract:
"Liquid biopsy techniques use markers found in blood such as circulating tumor cells (CTCs) for improving the diagnosis and treatment of cancer. Its' significant advantage over highly invasive tumor biopsies is the minimal invasiveness and that it can be performed repetitively, thus being well suited for regular monitoring of disease progression and development of disease resistance following treatment.
The GILUPI CellCollector® liquid biopsy device is uniquely capable of gathering patient-specific disease information critical to the achievement of improved clinical outcomes in major therapeutic areas including oncology and cardiovascular disease. GILUPI's novel in vivo technology has proven highly effective in disease diagnosis, treatment monitoring and drug development via provision of real time biomarker analysis and patient disease data in a minimally invasive manner."

Circulating Tumor Cells via a Novel 4 color FISH Probe for Accurate Diagnosis of Lung Cancer

Ruth Katz
Professor of Pathology, Chief Image Analysis Laboratory, Chief Research Cytopathology, MD Anderson Cancer Center, United States of America

Abstract:
With the advent of early lung cancer diagnosis by spiral CT for small lung nodules, an adjunctive biomarker test that is accurate for diagnosis of malignancy is essential, in order to stratify patients for resection versus observation. We present an overview and results of our antigen independent FISH test, specifically engineered for lung cancer diagnosis, performed in over 100 cases and controls with proven diagnosis of predominantly early lung cancer. We show that this is a highly sensitive and specific test for the diagnosis of all histologic subtypes of lung cancer. Several illustrative cases will be provided.

A Universal Extracellular Vesicle Cancer Screening Test Based on the Warburg Effect

Winston Patrick Kuo
Editor-in-Chief
Journal of Circulating Biomarkers
SAB, Exosomics Siena; Editor-in-Chief, Journal of Circulating Biomarkers, United States of America

Abstract:
Cancer cells adopt a non-oxidative breakdown of glucose to produce energy, in contrast to healthy cells which mainly generate energy from oxidative breakdown of pyruvate. The underlying driver of tumorigenesis is an impaired cellular respiration caused by a dysfunction of mitochondria. It is has been demonstrated that cancer cells, and many cells grown in-vitro, exhibit glucose fermentation even when enough oxygen is present to properly respire, hence the phenomenon, the Warburg Effect. The presentation discusses the novelty and impact of a membrane protein targeting tumour metabolism along with cancer-specific biomarkers in both circulating and specific sub-populations of extracellular vesicles in most solid tumours (colon, gastric, breast, lung, prostate, ovarian and melanoma) for purposes of screening, diagnostics and monitoring therapeutic treatments.

Tissue Print Technologies: An Innovative and Practical Approach to Obtaining High-quality Research Samples from Biopsies and Other Challenging Biospecimens

Sandra Gaston
Director, Molecular Biomarkers Research Laboratory; Scientific Director, Tufts Medical Center Biorepository, Tufts Medical Center, USA

Abstract:
Development of liquid biopsy technologies for use in the clinical management of cancer patients often requires comparisons with tumor tissues. Most human tissue samples obtained from clinical biopsy and surgical resections are only secondarily considered as research specimens, and any plan to collect tissue for research must put first priority on patient care. Remnant tissues obtained from fresh surgical specimens are the mainstay for biorepositories that provide high quality snap-frozen tissues for research, but many critical areas of a surgical resection and most diagnostic biopsies cannot be easily “divvied up” before the tissue is submitted for processing as a formalin-fixed paraffin embedded (FFPE) specimen. Moreover, access to FFPE biospecimens for research is becoming more restricted as clinical molecular testing places increasing demands on those tissue blocks. Our research group has developed a set of tissue print technologies that offer an innovative and practical approach to obtaining high quality RNA and DNA from biopsies and other “high value” specimens without compromising pathology diagnosis. Application of these technologies for cancer biomarker discovery and potential clinical diagnostic applications will be discussed.

Exosomes: Next Generation Diagnostics

Johan Skog
Associate Editor
Journal of Circulating Biomarkers
Chief Scientific Officer, Exosome Diagnostics, Inc., United States of America

Abstract:
n/a

Exosomes in the Pathogenesis and Diagnosis of Alzheimer's and Neurodegenerative Diseases

Lawrence Rajendran
Velux Stiftung Professor for Systems and Cell Biology of Neurodegeneration, University of Zurich, Switzerland

Abstract:
While they play crucial roles in physiological processes, EVs/exosomes also contribute to the development of disease states. Neurodegenerative diseases including Alzheimer's (AD), Parkinson's disease (PD) and Prion diseases, are characterized by protein aggregation and deposition in specific brain regions. While the exact pathological significance of these aggregates remains to be conclusively resolved, the biology behind their formation is unclear. Recent findings, on the release and spread of several amyloid-forming proteins, suggest a model where these proteins can be released from affected cells in the form of amyloid seeds, and then re-enter other cells and aid in the spread of the disease. How are these aggregates released from the cells? Once released, how do they form plaques and propagate in the aqueous extracellular space to gain access to their host counterparts? We propose that exosomes, endocytically derived nanovesicles, are a major way to shuttle amyloids out of the cell and aid in the plaque formation. We provide evidence that Aß is generated in early endosomes and is released in association with exosomes. Immunoelectron microscopy on exosomes showed that Aß peptides not only co-fractionated with exosomes but also are physically associated with them. To investigate whether these findings have any bearing on Alzheimer pathology, we performed immunohistochemistry analysis with antibodies against exosomal proteins on brain sections from AD patients, Parkinson patients and age matched control subjects. Enrichment of Alix, a marker for exosomes, was seen around the small neuritic plaques and a moderate signal in large diffuse plaques in brain sections from all AD patients tested. Alix staining was largely absent in brain sections of control subjects. Similar to Aß, both Tau and a-synuclein are also found in EVs and together this represents a novel mode of amyloid transmissibility presenting opportunity for exosome-based diagnostics.

Carboxypeptidase E: A Prognostic Cancer Biomarker in Tumors and Circulating Exosomes

Y. Peng Loh
Chief and Senior Investigator, Section on Cellular Neurobiology, Eunice Kennedy Shriver National Institute of Child health and Human Development, National Institutes of Health (NIH), United States of America

Abstract:
Carboxypeptidase E (CPE) and/or a splice variant of CPE (CPE-?N) have been reported to be a good prognostic biomarker for hepatocellular carcinoma (HCC), colorectal cancer and glioblastoma. Microarray data from the Gene Expression Omnibus (GEO) profile database have also indicated significant overexpression of CPE mRNA in many different human cancer types compared to normal tissue. These included metastatic non-endocrine cervical cancer, renal (clear cell) carcinoma, Ewing sarcoma, glioblastoma and various types of astrocytomas and oligodendrogliomas. High expression of CPE mRNA was also found in neuroendocrine tumors, such as pheochromocytoma/paraganglioma, (PHEO/PGL), pulmonary neuroendocrine tumors and insulinomas. Circulating exosomes mirror the parent cell. Exosomes derived from cancer cells contain mRNA and protein that reflects the parent tumor. We have detected CPE mRNA in exosomes in cell culture media from cancer cell lines including liver, breast, prostate and colorectal cancer cell lines. We have also isolated exosomes from serum of PHEO/PGL patients and showed that they contain CPE/CPE-?N mRNA. In a pilot study the levels of CPE/CPE-?N mRNA was determined by qRT-PCR in circulating exosomes of cancer patients. There was a significantly elevated level of CPE/CPE-?N mRNA levels compared to normal controls. Since this biomarker is elevated in many types of cancers, a serum assay using circulating exsomes could be very useful for: 1. routine screening of population > 60 y of age for early diagnosis of cancer. 2. screening high risk patients for early detection of many cancers including patients with liver cirrhosis, smokers, men with positive PSA test, women with solid ovarian cysts, or carry the gene for breast cancer, PHEO/PGL patients with inherited gene mutations for the disease; 3. post-surgery screening of Stage I & II patients for early detection of recurrence; 4. in clinical trials to test the efficacy of the cancer treatment.

Isolating Circulating Cell Free DNA from Plasma and Serum Using MagMAX Cell Free DNA isolation kit

Susan Magdaleno
Sr. Manager, R&D, Thermo Fisher Scientific, United States of America

Abstract:
Cell-free DNA (cfDNA) circulating in blood and other body fluids is a sample type of broad interest in applications for non-invasive detection and monitoring of human cancer and other diseases. cfDNA in body fluids is typically rare in abundance making sample preparation very challenging. Current commercially available methods of preparation do not allow for enrichment of the smaller cfDNA fraction away from the contaminating genomic DNA which may lower sensitivity of detection of the cfDNA. And current workflows for preparing cfDNA from body fluids can be lengthy and cumbersome, especially when processing multiple samples simultaneously. We have developed an easier method to isolate cell free DNA from liquid biopsies. The MagMAX™ cell-free DNA isolation kit is a new magnetic bead-based sample preparation kit designed to enrich for cfDNA using a quick and simple workflow that can be performed manually or using automated platform. The enriched cfDNA is compatible with downstream assays like digital PCR, qPCR and next generation sequencing technologies. The MagMAX™ cell-free DNA workflow and analysis of the isolated cfDNA using various analytical methods will be presented.

Technology Spotlight: Maximizing Next Generation Sequencing Capabilities of Circulating, Cell-Free DNA

Tim Harkins
President and CEO, Swift Biosciences

Abstract:
Swift Biosciences presents NGS methods that are cost effective, sensitive, and specific to assess cfDNA. Methods discussed for whole genome sequencing from PCR-free libraries, point mutation detection with hyb/capture and multiplex amplicons, and methylation patterns all from single liquid biopsy samples.

The Landscape of Cancer Biomarkers and Challenges and Opportunities for the Translation of Research

Lynn Sorbara
Program Director, National Cancer Institute, United States of America

Abstract:
The talk will be a review the challenges, gaps, and potential clinical utility of liquid biomarkers.

Technology Spotlight: An Effective NGS Workflow for microRNA Profiling from Liquid Biopsies, Extracellular Vesicles and Small Sample Input - Sample Preservation, RNA Extraction, Amplification and Profiling

Bernard Lam[1] and Moemen Abdalla[2]
[1]*Senior Research Scientist, Norgen Biotek Corporation, Canada*
[2]*Senior Research Scientist, Norgen Biotek Corporation, Canada*

Abstract:
Here, we present an effective workflow for studying miRNAs from various bodily fluids. The workflow involves four important modules - (1) Sample Preservation, (2) RNA Extraction, (3) miRNA Amplification, (4) Detection. Using urine RNA as an example, we demonstrated the importance of preservation as a significant amount of miRNA transcripts could be lost during standard freezing storage. For RNA purification, we demonstrated that the use of silicon carbide-resin technology worked more effectively than traditional phenol:chloroform/silica column-based methods in recovering miRNAs from bodily fluids such as plasma and urine. In particular, the silicon carbide technology does not require the use of carrier RNA and it does not have any bias in GC contents of the miRNAs. Even with effective preservation and RNA extraction, many bodily fluids samples may yield very low amount of RNA (sometimes at the nanogram or picogram range). We will also discuss the methods of quantification of RNA extracted from these liquid biopsies and the expected outcome. Finally, we will present data of a novel procedure that allows amplification of miRNAs from sub-nanogram amount for applications such as RT-qPCR array, microarray and small RNA sequencing.

The Role Of The Pathologist In The Measurement Of Circulating Tumor Cells (CTCs): A Pathologist's Perspective

Malini Harigopal
Associate Professor, Dept of Pathology, Breast and Cytopathology, Yale University, United States of America

Abstract:
The use of Circulating Tumor Cells (CTCs) is a rapidly growing new diagnostic test to help manage oncology patients. The test is being done at different institutions, even though it requires morphologic skills highly similar to those of the cytopathologist. The dramatic reduction in Pap smear specimens due to molecular technologies leaves potential capacity in the cytopathology lab. Thus, CTC analysis is an ideal new test to introduce into the cytopathology practice. After many years of basic research for assessment of circulating tumor cells (CTCs), there is now an FDA approved method available for use in clinical labs. The CellSearch System (Veridex) requires morphology skills highly similar to those of the cytopathologist to enumerate CTCs. The cytopathology lab is a natural location for this technology in the healthcare delivery system. The cytotechnologist under the supervision of the pathologist is involved in the final interpretation and enumeration of CTC. Molecular characterization of CTC provides additional prognostic information.

Technology Spotlight: The First Cytomic Signature of Vascular Health

Roy Overton
VP, Laboratory Operations, CytoVas, LLC

Abstract:
The CytoVas Vascular Health Profile™ (VHP) is the first-in-class diagnostic and prognostic test, integrating cytomic biomarkers and computational biology to provide a highly precise, cell-based signature of the state of the vascular endothelium and, by extension, vascular health.

Extracellular Vesicles in Human Reproduction

Clifford Librach
Medical and Scientific Director, CReATe Fertility Centre, Canada

Abstract:
Investigation of the functional role of extracellular vesicles and their RNA content in 4 fluids associated with human reproduction including seminal fluid, follicular fluid, embryo conditioned culture media and pregnancy serum.

Use of ctDNA and CTCs in the Biomarker Identification and Monitoring of Patients with Cancer

Lyle Arnold
Chief Scientific Officer, Biocept, United States of America

Abstract:
Liquid biopsies offer the opportunity to interrogate a number of different target sample types, including ctDNA and CTCs. At Biocept both ctDNA and CTCs are used for identifying medically actionable biomarkers to assist in the optimal treatment of patients. A highly sensitive Target-SelectorTM assay is used for analysis of ctDNA which can detect better than 1:10,000 (mutant:wild-type). At the same time, a patented microchannel is used for isolating and interrogating CTCs at the single cell level. CTCs are enriched up to 50,000 fold and clinically actionable biomarkers are analyzed directly in the microchannel. The combination of these technologies has enabled the clinical validation of an array of biomarkers from single blood samples. These include mutational analysis for EGFR, KRAS, and BRAF, as well as FISH and protein analysis for HER2, FGFR1, MET, ALK, ROS1, ER, and PDL-1 across a range of cancer types.

Novel Technologies for Quantitative Enrichment of Mutations Prior to Targeted Re-Sequencing of Circulating DNA

Mike Makrigiorgos
Professor, Dana-Farber Cancer Institute/Harvard Medical School, United States of America

Abstract:
n/a

Upcoming Events: