First Look

The Next Wave of Cancer Breakthroughs

Monday, 25th | 8AM – 12PM

TABLE OF CONTENTS

Modeling of Cancer Therapeutic Response In Vitro: Identification of Personalized Therapies and Potential of Functional Diagnostics to Complement Molecular Profiling of Tumors

CYRIL BENES, PHD

Director, Center for Molecular Therapeutics, MGH Cancer Center, Assistant Professor of Medicine, Harvard Medical School

Email: **CBENES@mgh.harvard.edu**

The research in the Benes laboratory aims at discovering novel therapeutics strategies for the treatment of cancers and to find better ways to match individual cancer patients with particular targeted drug therapies in order to maximize the likelihood of a clinical response.

Over the past decade there has been a paradigm shift in the approach to treatment of cancer patients due to the development of a new generation of targeted cancer therapies. These include several FDA-approved agents such as Herceptin, Imatinib, Vemurafenib and Crizotinib, which are associated with reduced toxicity and significantly improved patient responses in molecularly defined subsets of patients. The success of targeted therapies is based on an increased understanding of how the molecular features of cancer can influence patient response to the anti-cancer therapies. Cancers vary enormously in their genetic and epigenetic constitution, and this variation is a key determinant of drug response. For example, while most patients whose melanoma harbors a BRAF mutation demonstrate a dramatic (but short lived) response to the B-RAF inhibitor Vemurafinib, BRAF-mutant colon cancers may require simultaneous suppression of BRAF and EGFR pathway to achieve a response. Yet other drug-gene interactions are unpredictable from currently understood pathways.

Genetic based stratification of patients has had a profound impact on the care of a subset of cancer patients. However, the mechanistic understanding of cancer cells' drug response is still very limited such that most of the genetic information acquired is not readily leveraged. Moreover, even for patients with a genotype matched therapy depth and duration of therapeutic response are not predictable. To tackle these issues, we aim to establish a platform for the *in vitro* assessment of tumor response to clinically actionable drugs using cells derived from tumor biopsies. Through a close collaboration with a network of clinicians at the MGH Cancer Center we are developing new ways to inform the care of cancer patients. This functional diagnostic platform will complement currently implemented genomic profiling. Our previous studies have used similar tumor derived material to discover candidate strategies to counter acquired resistance. These efforts aimed at informing the development of combinatorial therapeutics for cohorts of patients similar to those from whom biopsies had been obtained. Encouraged by the results of these studies published in Science in December 2014, we are now aiming to test biopsy derived cells (tumor and stromal cells) within 4-5 weeks to directly inform the care of the biopsied patients. We are currently developing a process to rapidly obtain viable cells from patients' biopsies and assays to test the effect of drugs on these cells using high content imaging. Further development will include miniaturization, development of different assay technologies with the longer-term goal of producing a robust and easy to implement clinical test allowing chemotyping in clinics across the country. We believe that this chemotyping approach has the potential to improve the care of patients in the next few years.

Shifting Clinical Paradigms in Primary and Metastatic Brain Tumors: Unleashing the Power of Genomics to Clinical Care

PRISCILLA BRASTIANOS, MD

Assistant Physician in Medicine, Hematology and Oncology, Massachusetts General Hospital, Instructor of Medicine, Harvard Medical School

Email: **PBRASTIANOS@mgh.harvard.edu**

Incomplete knowledge of the molecular mechanisms that drive many types of brain tumors has hampered the development of novel therapeutic approaches for these tumors. Genomics will undoubtedly shift clinical paradigms in a number of brain tumors, including meningiomas, craniopharyngiomas and brain metastases.

Meningiomas are the most common primary nervous system tumor, with no effective systemic therapy after failure of radiation and surgery. We comprehensively characterized the genomics of meningiomas (Brastianos et al. Nature Genetics 2013) and demonstrated that a subset of meningiomas harbor recurrent clinically actionable mutations in the genes *AKT1, SMO* and *NF2*. Notably, *AKT1* and *SMO* mutations are present in therapeutically challenging tumors of the skull base. The results of this study begin to define the spectrum of genetic alterations in meningiomas and identify potential targets that can be translated to the clinical setting. We have now initiated a prospective national, multicenter NCTN Phase 2 study of an AKT1, SMO or FAK inhibitor in patients with meningiomas harboring *AKT1*, *SMO*, or *NF2* mutations, respectively. ***This study, sponsored by the National Cancer Institute, represents the first national multicenter trial of personalized medicine in brain tumors and a novel therapeutic approach in meningioma, a disease with a critical need for effective systemic therapy.***

Craniopharyngiomas are rare epithelial tumors that can cause profound clinical sequelae both through mass effect at presentation and through morbidity of treatment. No effective treatment besides surgery and radiation is known for craniopharyngiomas. We comprehensively characterized craniopharyngiomas (Brastianos et al. Nature Genetics 2014) and identified mutations in *CTNNB1* in nearly all adamantinomatous craniopharyngiomas and recurrent mutations in *BRAF* in nearly all papillary craniopharyngiomas. This was the first study to demonstrate that adamantinomatous and papillary craniopharyngiomas harbor mutations that are mutually exclusive and clonal. These findings have important implications for the diagnosis and treatment of these neoplasms. We recently treated a patient with a multiply recurrent papillary craniopharyngioma at MGH using a combination of BRAF and MEK inhibitors and achieved an exceptional therapeutic response (Brastianos et al. JNCI 2015). ***This is the first time that systemic therapy has demonstrated such a remarkable response in craniopharyngioma.*** Given this promising and unique tumor response, and the near ubiquitous occurrence of the BRAF V600E mutation in nearly all papillary craniopharyngiomas, we are initiating a national, multicenter Phase II study evaluating the combination of BRAF and MEK inhibition in patients with papillary craniopharyngiomas.

Brain metastases are a common and devastating complication of cancer. Our understanding of how brain metastases genetically evolve from their primary tumors is limited. In the largest genomic study in brain metastases to date, we subjected more than 88 matched brain metastases, primary tumors and normal tissues to whole exome sequencing and used novel computational techniques to map their evolutionary relationships (Brastianos, Cancer et al. Cancer Discovery 2015*)*. In all cancer samples, we observed branched evolution, in that all metastatic and primary sites shared a common ancestor yet continued to evolve independently. In 53% of cases, we found clinically actionable alterations in the brain metastases that were not detectable in the clinically sampled primary tumor. In contrast, spatially and temporally separated brain metastasis sites were more genetically homogenous. Extracranial sites were highly divergent from brain metastases. These observations suggest that when clinically feasible, genomic characterization of brain metastasis tissue should be considered when selecting therapeutic agents for patients with brain metastases. ***Based on these discoveries, we are now initiating a genomically driven trial in patients with brain metastases, the first of its kind in the US.***

Tumor Antigens in Cancer and How to Manipulate Antigens to Stimulate Immunity

MARK COBBOLD, MD, PHD

Associate Professor of Medicine, Massachusetts General Hospital

E-mail: **MCOBBOLD@MGH.HARVARD.EDU**

The role of anti-tumor immunity in the protection and control of arising tumors has been an intense focus of research for many decades. While it is clear from strong correlative clinical data combined with definitive experimental evidence from mouse cancer models that T-cells mediate this protection; the nature of the antigens targeted remains poorly characterized. Over the past decade, the role of altered-self antigens, termed neoantigens, has become clear. Tumor-specific neoantigens are known to act as targets of spontaneously arising adaptive immunity to cancer and thereby determine the ultimate fate of developing tumors. Nonsynonymous mutations in coding regions of expressed proteins are termed mutational neoantigens and, perhaps critically, are not subject to central tolerance. In cancers with high mutational loads such as non-small cell lung cancer and melanoma CD8+ T-cells can be identified within the tumor against MHC class-I restricted neoantigens in patients responding to immunotherapy. Despite these advance questions remain in the field, for example tumor-resident immunity against mutational neoantigens is typically at very low frequencies and it is surprising that this magnitude of immunity can be responsible for dramatic reductions in tumor volume. Additionally, some of the tumors with the best clinical responses to immunotherapy have some of the lowest mutational loads for example renal cell carcinoma and leukemia. Hematological malignancies in particular are known to be amongst the most immunogenic cancers. Therefore, it is likely that the antigens in these malignancies derive from other classes of antigens.

Since dysregulation of cell signaling pathways plays a prominent role in cancer, leukemia-specific antigens may derive from the posttranslational modifications (PTMs) associated with aberrant signaling. Indeed, we have previously shown that a number of phosphorylated peptides as potent cancer antigens. Interestingly, immunity to these antigens was seen in healthy donors, but lost in a subset of leukemia patients with poor clinical outcome and restored after SCT, suggesting a role for these neoantigens in the graft-vs-leukemia response. O-GlcNAcylation is another process involved in the dysregulation of cell signaling pathways in cancer. It functions as a nutrient sensor and regulates numerous cell signaling pathways by blocking and unblocking phosphorylation sites.

Overcoming Tumor Heterogeneity Associated with Drug Resistance

RYAN CORCORAN, MD, PHD

Assistant Professor of Hematology and Oncology, Massachusetts General Hospital

E-mail: **rbcorcoran@partners.org**

Personalized cancer medicine approaches, inhibiting kinases in tumors driven by defined genomic alterations, have demonstrated striking efficacy in many cancer types. However, the development of drug resistance is a major limitation to the efficacy of targeted therapies in oncology. Identifying and understanding the molecular mechanisms driving resistance may foster opportunities to develop therapeutic strategies to overcome resistance. For example, in the ~10% of colorectal cancer patients whose tumors harbor BRAF V600 mutations, we have found that feedback reactivation of MAPK signaling, often mediated by EGFR, underlies the relative insensitivity of these cancers to RAF inhibitors. The development of targeted therapy combination strategies to block feedback reactivation of MAPK signaling has led to marked improvements in response rates for these patients from ~5% to >30% over the past few years. However, in patients who respond to these targeted combinations, the rapid development of acquired resistance still limits clinical benefit. Acquired resistance in BRAF mutant colorectal cancer and in other molecularly-defined tumor types can be marked by the development of extensive molecular heterogeneity due to the selection of sub-clonal tumor cell populations, capable of growing under drug pressures, which poses significant diagnostic and therapeutic challenges. We will present data demonstrating how a single-lesion biopsy at disease progression to diagnose the mechanism of acquired resistance can vastly underrepresent the molecular heterogeneity of resistant tumor clones in an individual patient, and may fail to detect the existence of distinct but important resistance mechanisms that can drive mixed or lesion-specific responses and treatment failure to subsequent targeted therapy. By contrast, new “liquid biopsy” approaches analyzing circulating tumor DNA have the potential to detect the presence of simultaneous resistance mechanisms residing in separate metastases in a single patient and to monitor the effects of subsequent therapies on specific sub-clonal tumor cell populations. These findings highlight the critical role of tumor heterogeneity in driving therapeutic resistance and how convergent targeted therapy strategies blocking common signaling nodes capable of overcoming multiple resistance mechanisms may be needed.

Tumor Immunity Against Early Stages of Cancer Development

SHAWN DEMEHRI, MD, PHD

Assistant Professor of Medicine, MGH Cancer Center, Harvard Medical School

E-mail: **SDEMEHRI1@mgh.harvard.edu**

Our laboratory is focused on understanding the role of the immune system in regulating the early stages of cancer development in order to harness its anti-tumor potential for cancer therapy. Despite the recent success of immunotherapies in treating patients with late-stage metastatic cancers, the role of the immune system in preventing the early development of cancer remains uncertain. To determine the efficacy of the immune system in cancer prevention, our laboratory aims to identify the immune mechanisms that drive an immune activation sufficient to prevent cancer formation from pre-cancerous lesions. This approach raises a great opportunity to discover novel immune pathways that can be leveraged in cancer therapy and prevention.

One of the directions we have pursued is founded on mechanisms of T cell activation against early cancer. Our laboratory has studied the mechanism of thymic stromal lymphopoietin (TSLP) in evoking resistance to cancer development. TSLP is an epithelial-derived cytokine that plays a central role in stimulating $CD4^+$ T helper 2 (Th2)-mediated allergic diseases like atopic dermatitis and asthma. We have shown that high TSLP levels establish a dominant anti-tumorigenic immune environment preventing cancer promotion. Currently, our team investigates the detailed mechanism of TSLP anti-tumor function against solid cancers and examines its application for the treatment of pre-cancerous skin lesions in patients.

Opportunities and Challenges for a New Era in Translational Cancer Research

DANIELA DINULESCU, PHD

Principal Investigator, Division of Women's and Perinatal Pathology Eugene Braunwald Research Center

E-mail: **ddinulescu@rics.bwh.harvard.edu**

Background: Recent advances in cancer research reveal that distinct aberrant epigenetic events, including abnormal histone modification and DNA methylation, are important cancer hallmarks. However, our understanding of the precise role of these epigenetic modifications and how their dysregulation contribute to the development and progression of solid tumors, including ovarian cancer, is still largely unknown. Furthermore, the importance of systemic DNA hypomethylation for predicting clinical prognosis and response to platinum therapy in ovarian cancer is rarely explored. The recent discovery of the TET family of 5-methylcytosine (5-mC) hydroxylases, which convert 5-mC to hydroxymethylcytosine (5-hmC), has added an additional layer of complexity to the epigenetic regulation of DNA methylation. Our genome-wide mapping studies further indicate a global loss of 5-hmC levels in the epigenome of aggressive tumors.

Innovation and Clinical Impact: A great challenge in platinum cancer therapy is the management of chemoresistant tumor cells, including cancer stem cells, which have a largely unknown molecular pathogenesis at the level of epigenetic regulation. Our understanding of chemotherapy resistance is vastly enriched by the ever-expanding knowledge originating from epigenetic studies. We now understand that no single genetic mutation is responsible for the malignant transformation/progression or the development of a chemotherapy resistant phenotype. Our work focuses on the development and validation of sensitive and reliable methods to detect global patterns of epigenetic 5-hmC loss in newly diagnosed aggressive cancers and/or chemoresistant relapsed tumors. These assays can be easily translated and implemented into routine clinical practice. We are currently conducting a whole genome-wide DNA methylation mapping of 5-hmC levels in ovarian tumors and compare key differences within the 5-hmC landscape between platinum sensitive and resistant tumors. In addition, our comprehensive study investigates the DNA methylation patterns and 5-hmC landscape of chemoresistant ovarian cancer stem cells and the findings will allow us to develop novel epigenetic strategies targeting these cells. Thus, a huge impact of our current studies is to open new avenues to reverse the platinum chemoresistance of ovarian tumors and cancer stem cells within each tumor. This will be especially important for relapsed ovarian cancer patients for which current therapeutic options are extremely limited. The findings will help us learn how best to translate novel epigenetic discoveries into routine clinical care with the aim of improving current therapies for chemoresistant patients and develop reliable chemosensitivity markers and assays, which could accurately predict clinical outcome and response to platinum therapy. Our long-term goal is to develop combinatorial therapies that merge platinum compounds with epigenetic adjuvants for effective therapeutic intervention.

Defining Functional Protein Networks with High-Throughput Proteomics

WILHELM HAAS, PHD

Assistant Professor of Medicine, Massachusetts General Hospital, Harvard Medical School

E-mail: **whaas@mgh.harvard.edu**

Function, activity, localization, and other properties of a protein are often regulated through protein-protein interactions (PPIs). Therefore, it is believed that a comprehensive map of the functional and physical interactions of all proteins will be crucial information to fully understand genotype-phenotype relationships and many fundamental questions in biology. Major efforts are currently made to generate global PPI maps, either by characterizing physical interactions using the yeast-two hybrid (Y2H) assay and protein affinity-purification/mass spectrometry (AP-MS) or functionally by using genetic interaction screens. These methods require enormous experimental effort to generate a static interaction catalogue of a comprehensive PPI networks and it is notorious that the PPI data generated with these technologies show very little overlap.

We have developed a technology that allows global mapping of PPIs and their dynamics at an unprecedented high-throughput and at high accuracy. The technology is based on quantitative mass spectrometry-based proteomics and it allows the global characterization of functional and physical interactions of a proteome in hours. Beyond the identification associated proteins we are able to measure if this association is deregulated in analyzed proteome. The technology allows us to do this for thousands of protein-protein interactions in almost any sample upon data acquisition of less than 10 hours.

High Resolution Optical Imaging for Early Detection and Diagnosis of Cancer

LIDA HARIRI, MD, PHD
Assistant Pathologist, Massachusetts General Hospital, Instructor of Pathology, Harvard Medical School

E-mail: **LHARIRI@mgh.harvard.edu**

Early lung cancer diagnosis is often impeded by low diagnostic yields on biopsy. Early, accurate diagnosis of lung cancer is essential for patient survival. CT is highly sensitive for detecting lung nodules, but cannot diagnose malignancy. Diagnosis must be made at the microscopic level. Unfortunately, low-risk biopsy techniques can have poor diagnostic yields due to sampling error, especially when lesions are < 2 cm. Many patients undergo followup CT imaging, rather than biopsy, to assess nodule growth as an indicator of malignancy. Yet, nodule growth often represents tumor advancement, and thus defeats the goal of early detection.

Our research focuses on developing an optical imaging tool for: 1) intraprocedural guidance of biopsy site selection in real time to increase diagnostic yield and 2) virtual "optical biopsy" for in vivo diagnosis. Optical coherence tomography (OCT) conducts rapid, 3D optical imaging of tissue volumes orders of magnitude larger than biopsy, with microscopic resolution (< 10 μm) and penetration depths of 2-3 mm. We have developed OCT catheters compatible with clinical bronchoscopy procedures for endobronchial and needle-based transbronchial imaging.[1]

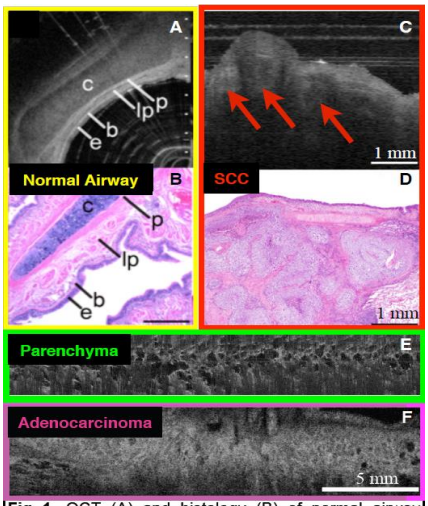

Fig 1. OCT (A) and histology (B) of normal airway depicting epithelium (e), basement membrane (b), lamina propria (lp), perichondrium (p) and cartilage (c). (C, D) OCT and histology of SCC with signal-intense tumor nests (arrows). (E) OCT of lung parenchyma and (F) adenocarcinoma.

OCT for microscopic biopsy guidance to targeted lung nodules. To determine whether OCT can confirm biopsy location within a targeted nodule, we conducted a blinded study where readers were asked to differentiate normal or atelectatic lung from lung nodules (Fig 1E-F). Six readers (pathologists, bronchoscopists, and OCT experts) were trained with 2 OCT images, and asked to interpret a test set of 109 OCT datasets. Interpretation accuracy was > 95% for all readers, and average time for interpretation was < 20 seconds.[2]

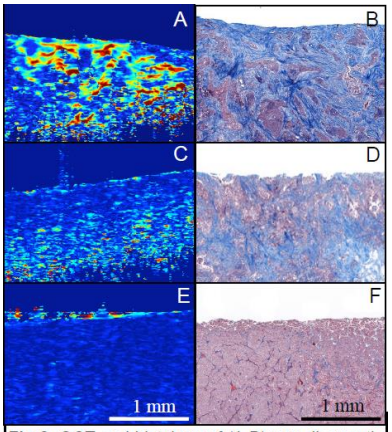

Fig 2. OCT and histology of (A,B) non-diagnostic tumor fibrosis evident by strong birefringence, (C,D) diagnostic adenocarcinoma with early fibrosis with moderate birefringence, and (E,F) diagnostic carcinoma with no birefringence.

OCT for intratumoral discrimination of non-diagnostic fibrosis and necrosis from diagnostic tumor. Biopsies are often contaminated by nondiagnostic tissues, such as bronchial wall, tumor fibrosis and necrosis. We have shown OCT can identify bronchial wall (Fig 1A-B) and necrosis. OCT can measure birefringence from organized tissues, such as collagen, and differentiate non-diagnostic fibrosis from tumor as shown in Fig 2.[3]

Large volume OCT "optical biopsy" for in vivo lung cancer diagnosis. To investigate whether OCT can serve as a form of in vivo microscopy to diagnose lung cancer as a complement to biopsy, we have developed and validated OCT diagnostic criteria for squamous cell carcinoma (SCC, Fig 1C-D), adenocarcinoma (Fig 1F), and poorly differentiated carcinoma, and demonstrated high sensitivity and specificity for each diagnosis (ranging 80-97%) in a blinded ex vivo validation assessment.[4,5] We conducted a pilot in vivo OCT clinical study in patients undergoing bronchoscopy to evaluate suspected lung cancer. A blinded reader applied OCT diagnostic criteria[4] to 19 patients (2 adenocarcinoma, 5 SCC, 1 poorly differentiated carcinoma, and 11 normal), and 89.5% were diagnosed correctly with 100% sensitivity and 94% specificity for adenocarcinoma, and 80% sensitivity and 100% specificity for SCC.

REFERENCES
1. Tan KM … Suter MJ. Flexible transbronchial optical frequency domain imaging smart needle for biopsy guidance. Biomed Opt Express 2012;3:1947-54.
2. Hariri LP … Suter MJ. Toward the guidance of transbronchial biopsy: identifying pulmonary nodules with optical coherence tomography. CHEST 2013;144:1261-8.
3. Hariri LP … Suter MJ. Seeing beyond the bronchoscope to increase the diagnostic yield of bronchoscopic biopsy. Am J Respir Crit Care Med 2013;187:125-9.
4. Hariri LP … Suter MJ. Diagnosing lung carcinomas with optical coherence tomography. Ann Am Thorac Soc 2015;12:193-201.
5. Hariri LP … Suter MJ. Volumetric optical frequency domain imaging of pulmonary pathology with precise correlation to histopathology. CHEST 2013;143:64-74.

Precision Prevention of Invasive Breast Cancer

ADITI HAZRA, PHD

Associate Epidemiologist, Brigham and Women's Hospital, Assistant Professor of Medicine, Harvard Medical School

E-mail: **aditi.hazra@channing.harvard.edu**

Precision-Enabled Image-Guided Surgery in the Advanced Multimodality Image-Guided Operating (AMIGO) Suite

JAYENDER JAGADEESAN, PHD

Research Associate, Brigham and Women's Hospital, Assistant Professor of Radiology, Harvard Medical School

E-mail: **jayender@bwh.harvard.edu**

Traditional cancer interventions and surgeries rely heavily on the eye-hand coordination skills of the physician and are limited by the inadequacies of human visualization and dexterity. As visible light cannot penetrate the skin or exposed surfaces, without the guidance of advanced imaging technologies, the treating physician – be it a surgeon or an interventional radiologist – cannot physically visualize a 3D operating volume and is forced to access the targeted tissues layer by layer. Further, the human eye cannot distinguish most of the malignant, infiltrating tumors such as found in breast from the surrounding normal tissue. Imaging can play a new and important role in compensating for these deficits to achieve the optimal target definition that is the prerequisite of all successful interventions or surgeries. Prior to all such procedures, the patient undergoes a set of diagnostic imaging tests. These images are then processed in various ways, converted into 3D images and models representing the patient's disease and anatomy. This information is subsequently used for preoperative surgical planning and/or intraoperative surgical decision-making.

I will present work done in the Advanced Multimodality Image Guided Operating (AMIGO) suite, which is a state-of-the-art surgical research environment that houses a complete array of advanced imaging equipment and interventional surgical systems at BWH. Multidisciplinary teams of specialists use this equipment array and the unique design of the suite to efficiently and precisely guide treatment — before, during, and after surgery — without the patient or medical team ever leaving the operating room. Three specific examples will be described - image-guided breast conserving surgery, image-guided video-assisted thoracic surgery (iVATS) and image-guided Parathyroidectomy – where we use intraoperative imaging to accurately localize the lesion and ensure complete tumor resection. Further, I will also describe some of the recent work on the development of mixed reality devices for diagnostic and surgical applications.

New Diagnostic Platforms with Wide-Reaching Applications in Medicine and Life Sciences

HAKHO LEE, PHD

Director, Biomedical Engineering Program, Center for Systems Biology, Massachusetts General Hospital, Associate Professor of Radiology, Harvard Medical School

E-mail: **HLEE@MGH.HARVARD.EDU**

Recent progresses in digital sensors and computational approaches create new opportunities for point-of-care (POC) cancer diagnostics and care delivery. By integrating ideas and techniques embodied in microelectronics, and nanotechnology, my research focuses on advancing sensitive, fast, and cost-effective diagnostic platforms.

Digital diffraction diagnostics (D3)

Smartphones and wearable electronics have advanced tremendously over the last several years but fall short of allowing their use for molecular diagnostics. We have developed a generic approach to enable molecular diagnostics on smartphones. Termed D3 for digital diffraction diagnostics, the method utilizes molecular-specific microbeads to generate unique diffraction patterns which can be recorded and deconvoluted by digital processing (Fig. 1). We applied the D3 to resolve individual pre-cancerous and cancerous cells as well as to detect cancer-associated DNA targets. Because the system is compact, easy to operate and readily integrated with the standard, portable smartphone, this approach could enable medical diagnostics in geographically and/or socioeconomically limited settings with pathology bottlenecks.

Point-of-care exosome screening.

Exosomes are an emerging new biomarker for cancer management. Exosomes are nanoscale vesicles (50 – 200 nm in diameter) actively secreted by cancer cells. These extracellular vesicles carry molecular constituents of their originating cells, and can thus serve as cellular surrogates that can be repeatedly and conveniently obtained with minimal complications. We have been developing new biosensors to streamline clinical exosome analyses. Developed systems include acoustic-wave based microfluidics for exosome isolation and nanotechnology-inspired sensors exosome molecular screening (Fig. 2). The ensuing clinical studies with patient samples (glioblastoma multiforme and ovarian cancer) have demonstrated the clinical utility of exosomes as a novel biomarker for cancer detection, treatment monitoring, and resistance prediction.

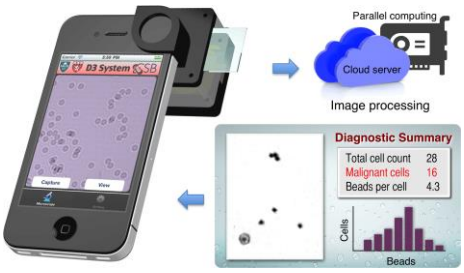

Fig. 1. Digital diffraction diagnostics (D3). A smartphone is used to record the diffraction images of the specimen. The recorded images are transferred to a server via the cloud service for real-time image reconstruction and analyses.

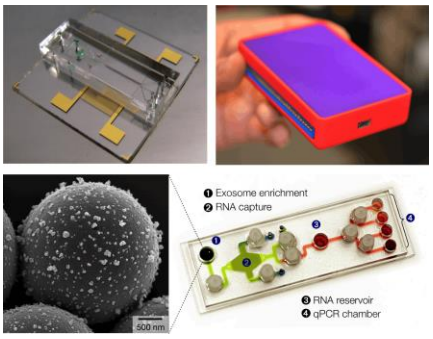

Fig. 2. Exosome diagnostic systems. New miniaturized system have been developed to facilitate exosome diagnostics. (Top left) a microfluidic chip for exosome separation in blood. (Top right) a portable sensor for exosome protein screening. (Bottom) a cartridge for on-chip exosome RNA profiling.

Making the Virtual Biopsy a Reality: Advances in MR Spectroscopy of Cancer

ALEXANDER LIN, PHD

Director, Center for Clinical Spectroscopy, Brigham and Women's Hospital, Assistant Professor of Radiology, Harvard Medical School

E-mail: **aplin@bwh.harvard.edu**

Gliomas represent the most common primary malignant brain tumor in adults, with nearly 20,000 cases per year in the US[1]. MRI is used to monitor treatment response however it is limited by providing only anatomical and not functional information of tumor viability where progression could occur well before increases in tumor size are detectable, and has problems distinguishing radiation effects from tumor progression, the so-called 'pseudo-progression' in which early post-treatment brain MRIs show increased contrast-enhancement or FLAIR edema which falsely suggests tumor progression but is actually radiation-induced increased vascular permeability[2]. The development of a noninvasive molecular tool to monitor tumor progression or response to treatment is a vital. Recent reports[3,4] indicate that 80% of grade II gliomas, 50% of grade III gliomas, as well as 10% of glioblastomas, harbor a unique somatic mutation of the IDH1 gene which produces the oncometabolite 2-hydroxyglutarate (2HG)[5] which can be detected using magnetic resonance spectroscopy (MRS) or what we call the virtual biopsy, enabling a non-invasive, quantitative and accurate biomarker for tumors[6-9]. In addition, MRS can measure other metabolites related to cancer such as 1) n-acetyl aspartate, a putative neuronal marker that depletes as tumors destroy healthy brain cells; 2) choline, a constituent of membrane phosphatidylcholine that increases in concentration as the tumor breaks down cell membranes, or glutamate, an excitatory neurotransmitter, that is affected by the IDH mutation, and finally, glutathione, an important brain oxidant tied directly to neuro inflammation. Together combined with the 2HG oncometabolite, a powerful diagnostic and prognostic tool that is both non-invasive and quantitative, lending itself to treatment mirroring as well.

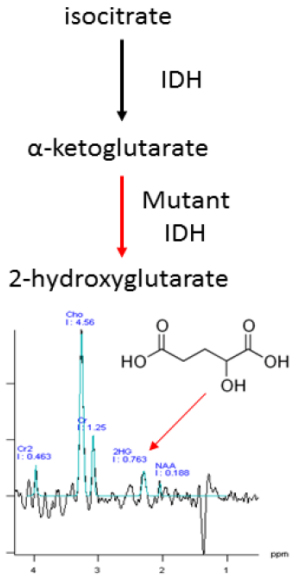

Figure 1. IDH mutations result in the production of 2HG which can be measured using MRS

Despite thousands of publications demonstrating the accuracy and diagnostic value of MRS[10], this promising technique has not been fully utilized in clinical practice due to both provider and system barriers. MRS is only performed at a small number of centers as the current processing software is either too basic to provide useful results or so opaque that it requires expert users and considerable processing time. Our solution addresses this important unmet need by lowering the barriers of entry to utilizing MRS technology by incorporating over 15 years of clinical experience into an automated web-based software platform that enables efficient, accurate, and non-invasive diagnosis to improve that patient care. We therefore present BrainSpec, a web-based software platform to provide diagnosis of brain cancer within minutes without any surgical intervention and 100% specificity. BrainSpec utilizes a linear combination model based post-processing backend that utilizes the current state of the art method for measuring 2HG and other brain chemicals as demonstrated in numerous publications, combined with a novel, highly intuitive and user-friendly front-end web-based user interface that was designed by industrial engineers and clinicians, together. Special emphasis was placed on data visualization by providing intuitive color maps as well as important data as shown on the screenshot of the prototype in Figure 1. A prototype of BrainSpec was supported by the BWH Innovations Hub Radiology accelerator program as a finalist in two rounds of funding. Integration of these methods into the clinical workflow will both significantly accelerate research progress by providing a tool that can be used in clinical studies but also has high potential for commercialization. The long-term goal will be to reduce healthcare costs and improve quality of life for patients.

*References available upon request

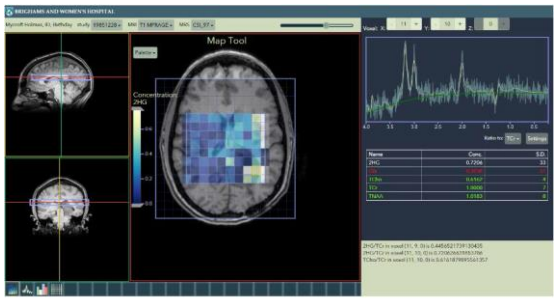

Figure 2. Screenshot of the BrainSpec prototype

The Systemic Nature of Breast Cancer: New Diagnostic and Therapeutic Opportunities

SANDRA MCALLISTER, PHD

Associate Scientist, Hematology Division, Brigham and Women's Hospital, Assistant Professor of Medicine, Harvard Medical School

E-mail: **smcallister1@partners.org**

Nearly 600,000 cancer patients are expected to die in the United States this year. The vast majority of cancer-related deaths are due to metastatic disease, but by the time metastases are detected, patients are not treated with curative intent. In these patients, tumor cells had clearly disseminated from the primary tumor prior to surgery but remained indolent for varying periods of time. Complicating our approaches to effective treatment is the fact that it is currently impossible to accurately predict which patients will experience disease relapse. Our lab thus created new animal models that mimic the early phases of metastatic disease when patients harbor indolent tumor cells in the periphery at the time of their primary diagnosis, with the idea that a deeper understanding of disease at an early point in time would offer suggestions for more effective diagnostic, prognostic, and therapeutic modalities.

Our novel preclinical models enable us to study cancer cells, patient blood samples, and surgical tumor tissue specimens in order to test our new strategies in mice before trying them in patients. Using these models, we reported that certain breast cancers (triple-negative breast cancer) systemically support the outgrowth of disseminated, otherwise indolent tumor cells by secreting cytokines that activate bone marrow hematopoietic cells to become pro-tumorigenic (McAllister, et al., *Cell*, 2008; Elkabets, et al., *JCI*, 2011; Castaño, et al, *Ca Disc*, 2013). Using models of luminal breast cancer, we discovered that certain tumors promote metastatic outgrowth by supporting angiogenesis via platelet activation (Kuznetsov, et al., *Ca Disc*, 2012). We also identified drugs that interdict these specific tumor-promoting systemic events, thus halting disease progression. Our findings highlight the systemic environment as an important component of disease progression that can be exploited to more accurately identify patients who would benefit from the appropriate therapies before they relapse.

We are now using a new, highly innovative and sensitive technology that enables us to study rare events related to metastatic outgrowth in vivo, which has previously been impossible to do. Our studies are designed to provide us with the first precise identity of life threatening human cancer cells before they convert to a malignant state. We also use unique co-culture assays, developed in our laboratory, to identify mechanisms by which indolent cells convert to malignancy and to identify drugs that can prevent their conversion. We have brought together a team of researchers, clinical oncologists, breast pathologists, patient/research advocates, and computational biologists in order to leverage opportunities for immediate clinical translation of our research findings.

The preclinical studies conducted in our laboratory are beginning to suggest completely new diagnostic and treatment strategies not previously proposed for cancer therapy. Our ultimate goal is to develop new non-invasive tests that would allow oncologists to more accurately identify breast cancer patients who are likely to suffer from disease relapse and to identify new treatment therapies that can be given to those patients *before* their disease recurs.

Manipulating Cancer-Immune Cell Interactions

MIKAEL PITTET, PHD

Samana Cay MGH Research Scholar, Associate Professor, Harvard Medical School, Center for Systems Biology, Massachusetts General Hospital

E-mail: **MPITTET@MGH.HARVARD.EDU**

Immunotherapy is revolutionizing cancer treatment because it can durably control disease in patients who otherwise resist other treatment options. For example, drugs targeting T cell inhibitory checkpoint signaling pathways provide durable responses at unprecedented rates against various common cancer types. Yet, these treatments benefit a minority of patients only.

Considering that immune checkpoint therapy may preferentially benefit patients whose tumors are pre-infiltrated by CD8 T cells (and consequently fail against tumors that lack CD8 T cells), my laboratory is interested in answering the following questions: Can we identify strategies for instigating CD8 T cell infiltration into tumors? Can such transformation be used to sensitize tumors to checkpoint therapy to durably control cancer progression?

To answer the above questions we explore and manipulate genetically engineered lung adenocarcinoma mouse models that are inadequately infiltrated by CD8 T cells and resist all current therapeutic options. Lung cancer is the leading cause of cancer mortality worldwide. Also, we use various modalities, including in vivo imaging, to interrogate the unfolding of tumor-associated immune responses and therapeutic effects directly in tissue microenvironments.

In evaluating drugs for their ability to induce immunogenic phenotypes in tumor cells in vitro and testing the most promising candidate treatments in mice, we found that the drugs currently given to lung cancer patients typically fail to induce immunogenic phenotypes in tumor cells. However, we discovered other therapeutics that not only trigger these immunogenic phenotypes efficiently but also provoke CD8 T cell infiltration into tumor areas.

Furthermore, we identified that drug-induced CD8 T cell recruitment into lung tumors can successfully sensitize these tumors to checkpoint inhibition. This approach also sensitizes mice to checkpoint blockade inhibitors in models of fibrosarcoma and colon carcinoma, indicating that the findings are applicable to diverse tumor types.

Thus, drugs selected for their ability to induce tumor immunogenicity can be used to transform 'cold' tumor tissues into immunologically 'hot' T cell-rich environments, which then become responsive to immune checkpoint therapy. These findings have exciting implications for current and future clinical efforts because they indicate that the proportion of cancers responding to immune checkpoint therapy can be feasibly and substantially expanded by combining checkpoint blockade with rationally selected immunogenic drugs.

Selected work from Pittet lab

Weissleder R, Pittet MJ. *Nature*. 2008; 452:580
Swirski FK et al.. *Science*. 2009; 325:612
Pittet MJ, Weissleder R. *Cell*, 2011; 147:983.
Cortez-Retamozo V et al. *PNAS*. 2012; 109:2491
Cortez-Retamozo V et al. *Immunity*. 2013; 38:296
Pfirschke C et al. *Immunity*. 2016; 44:343
Pucci F, et al. *Science*. 2016, in press

Long-Acting, Biocompatible, Biodegradable, Flexible Polymer Films Eluting Chemotherapy Reduce Local Recurrence Rates in Sarcoma

CHANDRAJIT RAUT, MD

Director, Dana-Farber/Partners Cancer Care Surgical Oncology Fellowship, Brigham and Women's Hospital, Associate Professor, Harvard Medical School

E-mail: **CRAUT@BWH.HARVARD.EDU**

Discussion Topic

Long-Acting, Biocompatible, Biodegradable, Flexible Polymer Films Eluting Chemotherapy Reduce Local Recurrence Rates in Sarcoma

Synopsis

Sarcomas arising in the retroperitoneum/pelvis have a high risk of local recurrence due to their large size at diagnosis (> 20 cm) and complex anatomic relationships. Patients succumb to progression of locally recurrent disease rather than distant metastases. Therefore, improving local control should improve survival.

Intravenous chemotherapy is not effective as an adjuvant therapy. Heated intraperitoneal chemotherapy has no benefit for sarcomas and carries significant toxicity. Preoperative radiation therapy is currently under investigation in a transatlantic phase III trial.

Local delivery of chemotherapy without systemic absorption is an attractive alternative. Existing options, such as Gliadel wafers used for gliomas, can be effective, but they are too small to adequately cover the involved surfaces for these sarcomas and release drug too quickly to impact the cell cycle of the different histologic subtypes arising in the retroperitoneum.

We have developed a biocompatible, biodegradable polymer film that can be loaded with a chemotherapeutic agent effective in sarcoma and contoured to the large, complex 3-dimensional surface of the retroperitoneum. Drug can be released locally over a prolonged period of time (> 60 days) without systemic absorption. These films have reduced local recurrence rates and improved overall survival rates in mouse models of sarcoma.

In-Situ RNA Diagnostics

MIGUEL RIVERA, MD

Assistant Molecular Pathologist, Massachusetts General Hospital, Assistant Professor of Pathology, Harvard Medical School

E-mail: **MNRIVERA@mgh.harvard.edu**

Immunohistochemistry (IHC) is the most commonly used technique for the detection of biomarkers in tissue samples but it is limited by the availability of suitable antibodies. RNA in-situ hybridization (RNA-ISH) is an alternative often used in basic research to rapidly test for gene expression patterns. This approach has not been widely utilized in clinical assays due to its complexity and low throughput. The incorporation of oligonucleotide based detection and branched DNA amplification has now greatly improved the performance of RNA-ISH protocols, paving the way for the development of novel in-situ RNA biomarkers in a large scale. This includes genes for which antibodies are not available and also targets such as non-coding RNAs and splicing variants where RNA testing is the only means of detection.

In order to facilitate translational projects using in-situ RNA diagnostics, we have established an RNA Biomarker Discovery Laboratory at the MGH Department of Pathology and MGH Cancer Center in collaboration with Affymetrix. We find that branched DNA amplification provides robust RNA-ISH signals for a variety of targets in paraffin embedded clinical tissue samples and that it can be readily automated using standard clinical laboratory equipment. Our initial studies successfully validated a set of markers that are now entering clinical use and we are currently setting up additional projects that capitalize on the ability to reliably detect RNA in-situ.

Tissue Molecular Imaging – Diagnosis, Surgical Guidance and Drug Development

SANDRO SANTAGATA, MD, PHD

Neuropathologist, Brigham and Women's Hospital, Assistant Professor of Pathology, Harvard Medical School

E-mail: **ssantagata@BICS.BWH.Harvard.edu**

Our goal is to develop and implement tools that will provide pathologists and surgeons an unprecedented ability to visualize the molecules within tissues. In pathology, we currently lack advanced tools that permit us to quickly determine the molecular characteristics of surgical biopsy and resection specimens during a tumor resection. Our central tool for analyzing tissue in the intraoperative setting – the microscopic review of H&E stained frozen sections – was developed over a century ago. The value of this approach and the diagnostic expertise provided daily by pathologists is unquestionable. Yet molecular information is left behind in the tissue, untapped until later in the molecular diagnostic evaluation of specimens – either with immunohistochemistry, genetic analyses or other molecular techniques.

Our multidisciplinary team of pathologists, surgeons and chemists is advancing tools for rapid mass spectrometry-based approaches that permit the characterization of lipids, metabolites, proteins and drugs, within the operating room. This data can be acquired on a single strip of tissue (line scan) or we can generate two-dimensional molecular images from tissue sectioned on a slide. The spatial resolved data can be overlaid on top of a histology image, thereby allowing correlation of histology with signatures (multiple peaks) or specific single peaks (from one molecule). The line scan is rapid and useful during a surgical procedure. 2D molecular imaging is an important research tool that allows us to validate mass spectrometry data against the gold standard of histopathology.

Such molecular guided surgery based upon tissue molecular imaging will become increasingly common in clinical trials and will provide a novel set of biomarkers to improve patient diagnosis, surgical resection and the development and implementation of advanced therapeutics. This work is done in our Advanced Multimodality Image Guided Operating (AMIGO) Room at Brigham and Women's Hospital which is the home of the National Center for Image-Guided Therapy (NCIGT), a National Institutes of Health's (NIH) central resource for research into image-guided procedures.

Supramolecular Therapeutics: Preferentially Modulating the Tumor Immune Contexture

SHILADITYA SENGUPTA, PHD

Associate Bioengineer, Brigham and Women's Hospital, Assistant Professor of Medicine, Harvard Medical School

E-mail: **Shiladit@mit.edu**

Cancer immunotherapy has emerged as one of the most promising approaches in the treatment of cancer. However, it is increasingly being realized that current immunotherapy treatments have limitations, and durable responses are seen in only a subset of patients. Additionally, systemic immune-related adverse effects can be serious. Thus there is an urgent need to develop technologies that can overcome the above challenges by amplifying the immune response but at the same time focus it to the tumor immune contexture and thereby avoid the systemic immune-related adverse effects.

Our laboratory has been developing 'first in class' supramolecular therapeutics to address the above unmet needs. Supramolecular therapeutics are LEGO-like super-structures in the nanoscale that are formed via the weak interactions of molecular building blocks. We design the building blocks using a proprietary Quantum Mechanical- All Atomistic Simulation (QMAS) platform.

We show that the supramolecular therapeutics preferentially home into the tumor. In a recent *PNAS* report1, we demonstrated that a supramolecule can be used to elicit an effective cytotoxic T cell response in the tumor, and additionally monitor this response in real time. Here we will show that supramolecular therapeutics can be designed to modulate macrophage-based innate immunity. We show that BWH-750, a novel CSF1R-targeted supramolecular therapeutic enables a M2 to M1 macrophage switch in the tumor, leading to increased efficacy in comparison to biologics and small molecule inhibitors targeting CSF1R.

The ability of the supramolecules to exert a sustained inhibitory effect on the target combined with the preferential accumulation in tumor indicates that supramolecular therapeutics could emerge as a new platform for effective tumor immunotherapy. Furthermore, the LEGO-like fabrication means that a supramolecule could be used as a 'plug and play' platform, where targeting antibodies and drugs or immunotherapies and molecularly targeted therapies could be assembled together, thus truly enabling an integrative approach in the treatment of cancer.

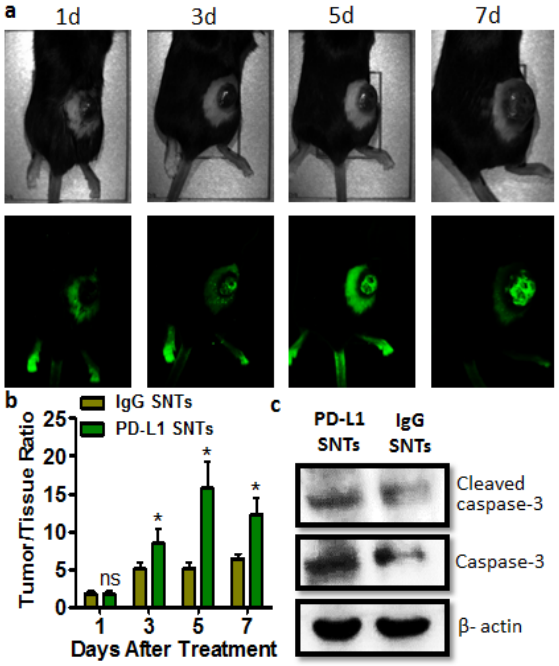

Fig. 8. PD-L1 SNTs enable early immunotherapy response monitoring in melanoma model (a) Representative pictures of B16F10 tumor bearing mice from PD-L1 SNTs treated groups at different time points; **(b)** Graph shows the quantification of tumor response to immunotherapy treatment as measured in terms of NIR intensity ratio between tumor and normal tissues at different time intervals; **(c)**Western-blot showing expression of cleaved caspase-3 in tumor samples treated with either PD-L1 SNTs or control IgG SNTs.

REFERENCES

1. Kulkarni A, et al. Reporter nanoparticle that monitors its anticancer efficacy in real time. Proc Natl Acad Sci U S A. 2016. pii: 201603455.

Costs of Managing Bleeding Risks Before and After Image-Guided Biopsies: An Inexpensive Solution?

PAUL SHYN, MD

Associate Radiologist, Brigham and Women's Hospital, Assistant Professor of Radiology, Harvard Medical School

E-mail: **PSHYN@PARTNERS.ORG**

Percutaneous image-guided needle biopsies are common, effective and relatively safe procedures used to obtain tissue samples for diagnosis of focal and diffuse disease processes affecting solid organs (liver, kidney, spleen, etc.) of patients. The total number of biopsies performed in the U.S. grew from 428,144 per year to 677,811 per year between 1997 and 2008 in Medicare patients alone, and have reported accuracies of approximately 93-95%. Percutaneous solid organ biopsies are critical to disease diagnosis and therapeutic management; an example is selecting a molecular targeted therapy for cancer based on receptor analysis of biopsy specimens. However, there are significant risks including major bleeding that may occur in any patient, but more commonly in patients who have bleeding disorders or who must take blood thinners. The existing methods of reducing bleeding events involve the use of fibrin sealants and gelfoam, but these have been inconsistent in their results and cumbersome in their preparation and administration; these methods lack regulatory approval in the US and carry risks of their own. Alternative procedures, such as transjugular liver parenchymal biopsies, are performed in some high-risk patients, despite higher costs, lower technical success rates, inability to target focal lesions, and questionable ability to mitigate bleeding risks.

An important additional problem with needle biopsies is seeding of cancer cells along the needle biopsy track due to microdeposits of malignant cells that may result in new tumor growths. The problem of tumor seeding of the biopsy track has been reported to occur in up to 12.5% of liver mass biopsies performed for hepatocellular carcinoma. This has dramatically impacted the policies surrounding liver transplant allocation, which currently discourage liver biopsies for diagnosis of hepatocellular carcinoma (a diagnosis which increases patient listing priority) and instead emphasizes the use of imaging tests to predict the presence of cancer.

Our proposed device is expected to decrease the need for and costs of pre-procedural coagulation testing and blood product transfusions used to correct bleeding disorders before biopsy procedures, and to prevent bleeding complications during biopsy procedures. The device may therefore reduce the costs and adverse events associated with blood transfusions, as well as the costs of extended hospital stays and additional procedures used to manage bleeding complications. No viable device addressing these needs is commercially available in the medical market.

Key design features of our initial prototype include 1) an 18 gauge outer diameter enabling the device to fit through any commercially available 17 gauge thin-walled introducer needle, 2) a simple disposable design making the device cost-effective for routine use, 3) low power requirements enabling use of common electrocautery generators already owned by most hospitals, and 5) a sliding stop that preselects the length of the device for coaxial insertion through biopsy introducer needles. The proposed novel radiofrequency technology device has the potential to reduce overall healthcare costs while reducing the incidence of bleeding complications and tumor seeding events associated with image-guided biopsies.

Multi-Isotope Imaging Mass Spectrometry: Leveraging Metabolic Interrogation of Tumors at Subcellular Resolution for Precision Medicine

MATTHEW STEINHAUSER, MD

Principal Investigator, Division of Genetics, Brigham and Women's Hospital, Assistant Professor of Medicine, Harvard Medical School

E-mail: **msteinhauser@partners.org**

For over a century, tumors have been recognized to display cellular morphologic heterogeneity. Genetic sequencing and lineage-tracing studies now support the respective heterogeneity attributable to evolving genetic subclones and to the diverse developmental states that support tumor growth. Although less well studied from the standpoint of heterogeneity, the metabolic adaptations engaged by cancer cells were first proposed by Warburg in the 1920s, when he hypothesized the predilection of cancer cells for non-oxidative glycolysis. It is now recognized that cancer cells undergo complex, albeit incompletely elucidated, metabolic reprogramming of not only glucose metabolism, but also amino acid and fatty acid metabolic pathways to support anabolism and proliferation. Moreover, metabolic imaging studies at gross tissue level resolution suggest that these metabolic adaptations may also be manifested heterogeneously. Despite the generally accepted existence of tumor heterogeneity, a recently convened expert working group on the topic acknowledged fundamental knowledge gaps, including the critical question of what aspects of tumor heterogeneity affect prognosis and treatment responsiveness? The group also raised the equally important question of what technologies can be used to obtain clinically useful information about tumor heterogeneity? We hypothesize that **the metabolic adaption of cancer cells to promote survival and growth – representing the integrated output of genetic, epigenetic, and microenvironmental regulatory networks – is a critical indicator of cancer cell function and therefore directly relevant to disease progression, prognosis, and therapeutic response. Furthermore, we propose that a new imaging technology, multi-isotope imaging mass spectrometry (MIMS), is an answer to this unmet need.**

MIMS is a state-of-the-art quantitative metabolic imaging technology with subcellular resolution, pioneered at the Brigham and Women's Hospital over the past decade. MIMS couples high-resolution ion microscopy with quantitative stable isotope tracer methodology to functionally illuminate subcellular domains much smaller than a cubic micron. We pioneered the biological application of MIMS with functional measurements at the single-cell level, demonstrating nitrogen fixation in symbiotic bacteria, inner ear protein metabolism in frogs and mice, chromosome segregation by mitotic intestinal stem cells, cardiac regeneration in mice, and the hyperplastic potential of adipose tissue. Furthermore, we have now also successfully translated MIMS to humans with a proof-of-concept study of adipocyte turnover in subcutaneous adipose tissue.

In this presentation, we present our preliminary experience in several murine tumor models, demonstrating marked intra-tumor cell-to-cell heterogeneity of glucose and glutamine metabolism. We also present proof-of-concept data in murine malignant peripheral nerve sheath tumors that targeted therapy selects for survival of glutamine avid tumor cells, therefore supporting the concept that MIMS measurements are functionally significant. In leveraging our translational experience with MIMS together with data demonstrating the power of MIMS to reveal intra-tumor metabolic heterogeneity, we propose a vision in which MIMS can be used **to dramatically refine the pathologic diagnosis and grading of human tumor specimens in a way that augments the goal of precision cancer therapy.**

Microfluidic Technology for Isolation of Exosomes from Cancer Patients

SHANNON STOTT, PHD

Assistant in Genetics, Massachusetts General Hospital, Assistant Professor of Medicine, Harvard Medical School

E-mail: **SSTOTT@mgh.harvard.edu**

Malignant tumors will aggressively invade surrounding tissue due to rapidly dividing cancer cells that are nourished by an ample blood supply. As these cancer cells are multiplying, they release thousands of tiny particles into the blood stream, referred to as exosomes, which contain genetic information about the tumor. These tiny (70-200nm) particles are shed by most cells in the body, with some cells producing thousands of exosomes per day. Exosomes were originally thought to be a means for cells to dispose of unwanted waste, but it is now thought that they shuttle genetic material, cytokines, and proteins between cells, fulfilling an important role in cellular communication. Most cancers have been shown to release exosomes that contain genetic information about the primary tumor and their release is thought to occur early in tumor development. Consequently, their isolation may be an ideal way to detect and monitor cancer progression through a simple blood draw. In this presentation, a new microfluidic technology will be presented, $^{HB\text{-}Exo}$Chip, to capture and analyze these extracellular vesicles from glioblastoma patients treated at the Massachusetts General Hospital. Clinically, there is a dire need to diagnose and monitor brain tumor recurrence and to detect mutations in real time to guide patient treatment. A blood-based 'liquid biopsy' that captures and analyzes exosomes would be ideal approach better predict tumor response in glioblastoma without the need for highly invasive brain surgery. Through these blood-on-a-chip assays, we aim to gain a better understanding of when these important tumor derived extracellular vesicles are released and how we can exploit their molecular content to better guide patient treatment.

My laboratory has optimized the $^{HB\text{-}Exo}$Chip such that tumor specific exosomes are isolated with a 100 fold enrichment in comparison to conventional ultracentrifugation approaches. Working in partnership with Drs. Xandra Breakefield and Brian Nahed at MGH, my laboratory has used this technology to isolate exosomes from the serum and plasma of patients with advanced glioblastoma multiforme. Following isolation, we have interrogated their molecular content to identify the EGFRviii mutation using droplet based PCR and screened their expression profile using next generation RNA sequencing. Using this approach, we have successfully identified tumor specific transcripts from our patient co-hort and plan to expand our analysis to more patients, tracking their exosomes during the course of their treatment.

The Stott laboratory is comprised of bioengineers and chemists focused on translating technological advances to relevant applications in clinical medicine. Specifically, we are interested in using microfluidics, biomaterials and imaging technologies to create tools that increase our understanding of cancer biology and of the metastatic process. In partnership with others at the Mass General Cancer Center, we have developed a microfluidic device that can isolate extraordinarily rare circulating tumor cells (CTCs) from the blood of cancer patients. We continue to develop new microfluidic technologies to isolate other blood-based biomarkers such as microvesicles and oncosomes. Additionally, we are employing new imaging modalities and biomaterials to probe and characterize cancer cells in novel ways. Ultimately, we hope that by working in close partnership with the molecular and cell biologists at the Mass General Cancer Center, we can create new tools that directly impact patient care.

Redefining the Cellular Architecture of Human Gliomas Through Large-Scale Single-Cell Analyses

MARIO SUVA, MD, PHD

Assistant Molecular Pathologist, Massachusetts General Hospital, Assistant Professor of Pathology, Harvard Medical School

E-mail: **Suva.Mario@mgh.harvard.edu**

While human tumors are shaped by the genetic evolution of cancer cells, evidence also suggests that they display superimposed hierarchies reminiscent of normal development, where cancer stem cells (CSC) can drive tumor growth and give rise to differentiated progeny. Yet, unbiased evidence for CSC in solid human malignancies remains elusive. Here, using human IDH1-mutant gliomas as a model, we profiled ~10,000 single cells from 10 fresh tumors by RNA-seq and reconstructed the genetic and epigenetic cellular architecture of tumors in patients. We found that most cancer cells are differentiated along two specialized glial programs, while a rare subpopulation of cells is undifferentiated and associated with a neural stem cell expression program. Surprisingly, cellular proliferation was restricted to this rare population, consistent with a stem cell compartment that is solely responsible for fueling growth of IDH1-mutant gliomas in humans. By identifying distinct genetic clones, we show that this cellular architecture evolves during the progression of the tumor, suggesting that developmental programs and genetic influences coordinately determine tumor architecture. These results provide unprecedented insight into the cellular composition of brain tumors at single-cell resolution and may help harmonize the cancer stem cell and the genetic models of cancer, with critical implications for disease management.

Disruption of Repetitive Elements as Novel Cancer Therapeutics

DAVID TING, MD

Assistant Physician, Hematology and Oncology, Massachusetts General Hospital, Assistant Professor, Harvard Medical School

E-mail: **DTING1@mgh.harvard.edu**

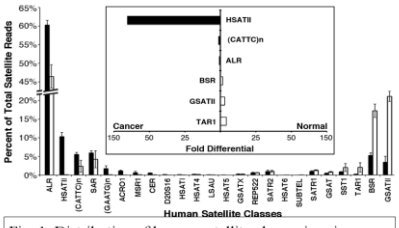

Fig. 1: Distribution of human satellite classes in primary pancreatic tumors and normal tissues. Percent of satellite reads shown from highest differential in cancers (Black bars, right) to highest in normal tissues (White bars, left). Inset showing differential by fold change.

Next generation sequencing technology has recently provided unprecedented resolution of the cancer transcriptome and has revealed significant expression of non-coding sequences (ncRNAs) in normal and cancer tissues. These ncRNAs provide a previously unexplored source of novel cancer biomarkers and therapeutic targets (1, 2). We performed RNA sequencing of primary tumors and a variety of normal tissues and identified significant transcription emanating from pericentromeric heterochromatic regions of the genome previously thought to be inactive due to heavy epigenetic silencing. Our analysis of all human satellites identified the ncRNA HSATII satellite as being exquisitely specific for a wide variety of cancers including pancreatic, prostate, lung, ovarian, and renal cell carcinomas compared to normal tissues (Fig. 1) (3). Recently, we have discovered that these satellite sequences are actively reverse transcribed (RT) in cancers and lead to progressive expansion of satellite sequences in tumor genomes (Fig. 2) (4). Notably, HSATII expression and RT was completely absent in cancer cell lines grown in standard adherent 2D conditions, while it was massively induced in the setting of tumorigenesis as a xenograft or 3D tumorspheres *in vitro*. Using the nucleoside reverse transcriptase inhibitors (NRTI) ddC and d4T, as well as HSATII specific locked nucleic acids, we have found significant reduction of tumor growth in 3D tumorspheres and xenografts, but not in standard 2D growth conditions (Fig. 3). Together, this indicates that ***HSATII RT inhibition is a novel anti-cancer therapeutic target that is seen in vivo, but would have otherwise been missed in standard 2D cell line drug screens***.

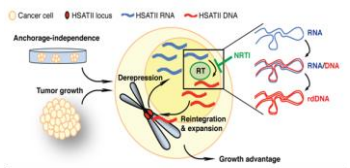

Fig. 2: Model of HSATII expression and RT in cancers

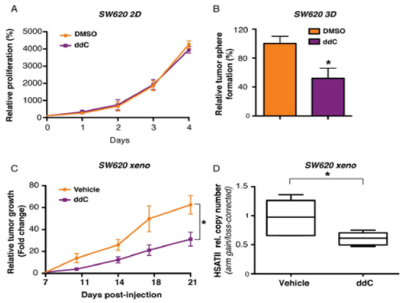

Fig. 3: NRTI ddC differential effect in SW620 cell line grown in (A) 2D vs (B) 3D and NRTI ddC with reduction in SW620 xenograft (C) proliferation and (D) HSATII CN gain

SPECIFIC AIMS

Aim 1) Elucidation of the HSATII reverse transcriptase

Although we have described that HSATII sequences are reverse transcribed, we are uncertain what is the precise reverse transcriptase that is active on these repeats. Using the 2D vs 3D differences in HSATII RNA expression, we will be able to identify proteins that are specifically interacting with HSATII. Together, this approach may not only reveal the critical RT, but also client proteins that may provide a better target for HSATII RT inhibition.

Aim 2) Identification of optimal HSATII RT inhibitor

We are evaluating a panel of NRTIs and testing them in 2D vs 3D culture across a panel of colon, breast, and pancreatic cancer cell lines to identify the best NRTI in blocking HSATII RT. This will provide mechanistic insight into HSATII RT and identify tool compounds for therapeutic development.

Aim 3) Characterization of anti-cancer effects of HSATII RT disruption in multiple cancer cell lines

We are testing cell lines that have been already characterized for RNA expression patterns and genomic alterations (Sanger COSMIC, Broad CCLE) (5, 6). We will use this existing genomic and transcriptional data to identify candidate genetic aberrations and RNA expression profiles that predict cancer cell line sensitivity to HSATII RTi. Together, this work will be important in stratifying patients for HSATII RT inhibition as a paired diagnostic test.

REFERENCES

1. Berretta J, Morillon A. Pervasive transcription constitutes a new level of eukaryotic genome regulation. EMBO Rep. 2009;10:973-82.
2. Jacquier A. The complex eukaryotic transcriptome: unexpected pervasive transcription and novel small RNAs. Nat Rev Genet. 2009;10:833-44.
3. Ting DT, Lipson D, Paul S, Brannigan BW, Akhavanfard S, Coffman EJ, et al. Aberrant overexpression of satellite repeats in pancreatic and other epithelial cancers. Science. 2011;331:593-6.
4. Bersani F, Lee E, Kharchenko PV, Xu AW, Liu M, Xega K, et al. Pericentromeric satellite repeat expansions through RNA-derived DNA intermediates in cancer. Proceedings of the National Academy of Sciences. 2015.
5. Garnett MJ, Edelman EJ, Heidorn SJ, Greenman CD, Dastur A, Lau KW, et al. Systematic identification of genomic markers of drug sensitivity in cancer cells. Nature. 2012;483:570-5.
6. Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature. 2012;483:603-7.