that microRNA-145 (miR-145) expression decreases with the progression of PACa, allowing aberrant expression of MUC13. Restitution of lost levels of miR-145 may prevent PACa progression by down-regulating MUC13 expression.
Design: A pilot study using six-week-old athymic Nu/nu mice was carried out to investigate the effect of miR-145 restitution. Mice were divided into three treatment groups,control (six mice), non-targeting control mimics (NC, six mice) and miR-145 (six mice). Tumors in mice were implanted by injecting PACa HPAF-II cells, having high MUC13 expression, into the right flank subcutaneously. After the tumors developed, (avg. volume 80 mm^3) miR-145 mice group was injected intratumorally with miR145 mimics 9 times. Tumor growth was followed for 21 days until the tumors reach 700 mm^3. The mice were then euthanized followed by the analysis of tumor growth, volume and metastasis. Tumor volumes were examined as a function of time (discreet), group (control, NC, miR-145) and interaction between them.
Results: We demonstrate thatthe tumor growth and volume regressed drastically with miR-145 replenishment in mice compared to control and NC treated mice. On investigation of mice tumors, we found that the tumors from miR-145 treated mice had drastic reduction in the expression of MUC13 and its target HER2 using *in-situ* hybridization and immunohistochemistry techniques. This suggested an important role of miR-145 in the inhibition of pancreatic tumor growth by directly targeting MUC13.
Conclusions: The current study provides important insights into the tumor suppressor role of miR-145 in a well-known tumor-promoting network that includes MUC13. The study delineates the association of alterations in miR-145 levels with MUC13 expression that may have a potential role in initiation and progression of pancreatic ductal carcinoma. miR-145 induced down-regulation of MUC13 is associated with tumor growth reduction in pancreatic xenograft mice model, highlighting miR-145 as an attractive modulator of MUC-13 in stemming the initiation and growth of PACa.

1809 Morphology and Molecular Evidences of Malignant Potential of Entrapped Ducts in Pancreatic Neuronendocrine Tumors

Wendi Zhou, Jayalakshmi Panicker Balakrishna, Wei Zhang, Abdelsalam Sharabi, Violette Ghali. Mount Sinai St. Luke's – Roosevelt Hospital and Beth Israel Medical Center, New York, NY.
Background: Pancreatic endocrine tumors are well documented for containing entrapped small, cytologically bland ductules. However, pre-neoplastic changes of those entrapped glands on both morphology and molecular levels are not sufficiently investigated.
Design: Total of 25 cases of pancreatic well-differentiated neuronendocrine tumor between 2006 and 2013 were retrospectively analyzed. Pre-neoplastic changes (pancreatic intraepithelial lesions, PanIN) were detected in 9 cases. To investigate whether these PanIN like changes in entrapped glands are preneoplastic or reactive, immunohistochemistry for DPC4, p53, P16, EGFR were performed in all cases. In two cases with PanIN-2 lesions, EGFR gene amplification was analyzed by FISH, followed with mutational analyses for EGFR and KRAS genes using DNA extracted from FFPE tissue after microdissection. Epithelial mucin overexpression was evaluated by staining for MUC1, MUC2, MUC5.
Results: Entrapped ductules were proven to be present in 18 cases by immunohistochemistry staining for CK19, CEA, and CA19.9. Neuronendocrine components were confirmed by staining for synaptophysin and chromogranin. All 9 cases with PanIN changes in entrpped ducts are significantly larger in size (average 4.5 cm, range 3.0-6.6 cm) compared to PanIn negative tumors (average 2.0 cm, range 1.5-3.5 cm). In two cases, entrapped ducts showed extensive proliferation and acquired at least PanIN-2 like changes with profound papillary structures and moderate nuclear atypia. Positive EGFR immunostaining was detected in 6 of 9 PanIN lesions (including two PanIN2 lesions), among which 4 exhibited diffuse 2+ or 3+ immunoreactivity. However, EGFR gene amplification was only detected in one PanIN2 lesion. Mutations in K-RAS were detected in both PanIN-2 lesions. No mutations were found in the matched nonlesional ductal areas, suggesting that mutations were acquired during transformation. Immunohistochemically, two PanIN2 lesions expressed MUC1 and MUC5, one PanIN2 lesion had weaker staining of P16. No lesions showed aberrant P53 expression, or loss of DPC4 staining.
Conclusions: Entrapped ducts in pancreatic neuronendocrine tumor can undergo extensive proliferation and acquire PanIN-2 like morphology, apposing challenges in distinguishing from true mixed ductal-endocrine neoplasms. Molecular and immunohistochemical evidences support these lesions to be pre-malignant rather than reactive, and are found in larger size tumors. Further investigation are needed to understand the significance of this pre-malignant changes.

Pathobiology (including Pan-genomic/ Pan-proteomic approaches to cancer)

1810 Molecular Profiling of Breast Ductal Carcinoma In Situ (DCIS) – Gene Expression Signature of HER2 Positive DCIS

Emmanuel Agosto-Arroyo, Tatyana Isayeva, Shi Wei, Jonas Almeida, Shuko Harada. University of Alabama, Birmingham, AL.
Background: Although significant advance has been made in understanding the molecular changes in invasive breast carcinoma (IBC), the exact molecular behavior of DCIS is not well understood. We have shown HER2 overexpression in DCIS is associated with developing IBC. Our hypothesis is that differentially expressed genes in HER2+ DCIS are associated with high risk of developing IBC. The aim of this study is to investigate the differences in gene expression profile between HER2 positive and HER2 negative DCIS.
Design: Resection specimens received in 2012-13 with the diagnosis of DCIS without IBC were screened. Based on ER, PR, and HER2 status, a total of 18 cases with high nuclear grade DCIS [6 ER/PR+, HER2–(ER/PR+), 6 ER/PR-, HER2+(HER2), and 6 ER/PR/HER2–(triple negative; TN)] were selected. RNA was extracted from DCIS areas of the formalin fixed paraffin embedded blocks with 6 non-neoplastic breast tissue controls. Expression array was performed with Affymetrix Human Gene 1.0 ST array at Heflin Center for Genomic Science Core Laboratories. The data was analyzed using the MATLAB software.
Results: A total of 1,682 transcripts were clustered by the specific subtype and the difference of their signal intensity. Thirty genes were identified to be under or overexpressed in the HER2 subtype as compared to the two other groups (Figure 1). Among those, the PROM1 gene was consistently found to be overexpressed in HER2 subtype, as compared to other two subtypes. Expression of this gene is associated with several types of cancer. PEG10 gene was overexpressed in the HER2 group compared to TN and the control groups.

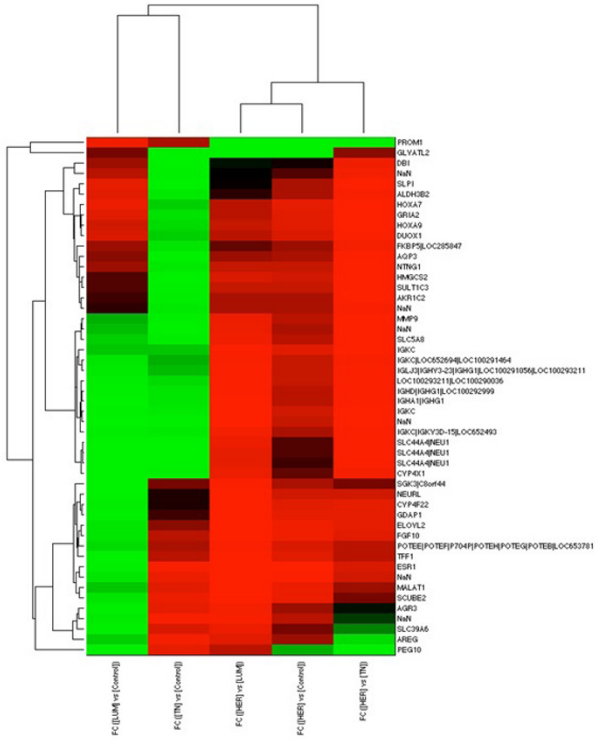

Figure 1. Hierarchical analysis of genes differentially expressed (green-over, red-under) in HER2 subtype compared to control, ER/PR+ (desiganated as LUM) or TN groups.
Conclusions: We identified unique gene signatures expressed differently in HER2+ DCIS, which may be associated with development of IBC. The result may enhance our understanding of the mechanisms of breast cancer progression and become the basis for developing new predictive biomarkers and/or therapeutic targets.

1811 Comprehensive Evaluation of DOG1 in Squamous and Urothelial Carcinomas Reveals Frequent Expression Across Anatomic Sites

Deema Alkapalan, Emily McMullen, Andrew Bellizzi. University of Iowa, Iowa City, IA.
Background: Discovered on GIST1 (DOG1), a calcium-activated chloride channel, is most familiar as a gastrointestinal stromal tumor marker. DOG1 was independently discovered as part of a chromosomal region (11q13) frequently amplified in head and neck squamous cell carcinoma (HNSCC). DOG1 overexpression is prognostically significant in HNSCC, and overexpression correlates with gene amplification. We performed DOG1 immunohistochemistry (IHC) on a cohort of SCCs and urothelial carcinomas (UCs) to determine whether expression was HNSCC-specific or represented a more generalized phenomenon.
Design: DOG1 IHC was performed on tissue microarrays constructed from 243 SCCs (anus 22, uterine cervix 25, esophagus 16, larynx 21, lung 28, penis 21, skin 21, ventral tongue 21, tongue base 11, tonsil 16, vagina 19, vulva 22) and 45 UCs (bladder), using polyclonal (Spring) and monoclonal (DOG1.1, Zeta) antibodies. Tumors were arrayed in triplicate. Extent (%) and intensity (0, 1+, 2+, 3+) of staining were assessed and an H-score (extent x intensity) calculated. H-scores >0 were considered positive.
Results: DOG1-positivity was noted in 48% (polyclonal) and 26% (monoclonal) of 288 squamotransitional carcinomas (STCs). 2 or 3+ positivity was seen in 22% (polyclonal) and 13% (monoclonal). Detailed expression data are presented in the Table.

DOG1 Expression in by Anatomic Site						
	Polyclonal			Monoclonal		
	% Positive (any staining)	% Positive (2 or 3+)	Average H-score (if positive)	% Positive (any staining)	% Positive (2 or 3+)	Average H-score (if positive)
Anus	36	5	41	9	0	7
Bladder (UC)	38	22	95	22	13	102
Cervix	20	0	30	4	0	5
Esophagus	75	63	126	75	44	69
Larynx	67	29	35	38	24	30
Lung	42	21	51	21	14	60
Penis	43	19	31	14	5	40
Skin	76	14	22	48	10	7
Tongue (Ventral)	67	38	64	33	19	51
Tongue Base	45	45	86	45	18	33
Tonsil	38	25	57	25	13	40
Vagina	32	5	17	0	0	0
Vulva	73	23	31	36	18	23

Conclusions: DOG1 expression was detected across anatomic sites. The highest rate of positivity, including high-level expression, was seen in the esophagus. Rates of staining with a polyclonal antibody were twice those with a monoclonal antibody, possibly reflecting cross reactivity with other anoctamins. DOG1 could represent a potential therapeutic target and pathologists should be aware of frequent DOG1-positivity in STCs to avoid a potential misdiagnosis of GIST.

1812 Discriminating Uterine Serous Carcinoma From Uterine High Grade Endometrioid Carcinoma By Whole Genome Expression Analysis

Baraa Alosh, Sumi Thomas, Michele Cote, Sudeshna Bandyopadhyay, Haleema Saeed, Gregory Dyson, Aliccia Bollig-Fisher, Robert Morris, Adnan Munkarah, Rouba Ali-Fehmi. Wayne State University and Karmanos Cancer Institute, Detroit, MI.

Background: The morphologic and clinical features of uterine serous carcinoma (USC), high grade endometrioid carcinoma (HEC) are well known. The aim of our study was to analyze and highlight the differences at the molecular level.

Design: We retrieved 24 cases of primary high grade endometrial carcinomas: 12 USC and 12 HEC, matched by age and stage, from our pathology database. Whole genome expression analysis was performed on mRNA from formalin-fixed paraffin (FFPE) tissue using Illumina HT12 DASL (cDNA-mediated annealing, selection, extension, and ligation) microarray assays. A t-test determined significant differential gene expression.

Results: 338 gene probes demonstrated a statistically significant difference at the 0.05 level and a fold-change difference of at least 2. The majority of these showed increased expression in HEC relative to USC and system-level analysis of the probe set revealed significant over-representation of genes linked to important disease functional categories, including the top ranked: (1) Endocrine System Disorders, (2) Immunological Disease and (3) Inflammatory Disease. Genes in the analyzed data set associated with these functions include well-studied cancer genes: cytokines/interleukins, NFKB, P38 MAPK, PI3K and AKT.

Conclusions: Understanding molecular distinctions across these diseases can help in clarifying observed clinical differences and establish rationale for further molecular work to pursue prognostic biomarkers and new targeted treatment strategies toward an objective to improve therapeutic outcomes.

1813 FOXO3 Inhibits Vascular Tumor Growth

Sriram Ayyaswamy, Divya Thomas Mani, Andrea Haws, Wa Du, Thuy Phung. Texas Children's Hospital, Houston, TX; University of Texas, Houston, TX.

Background: Vascular tumors are endothelial cell neoplasms whose molecular pathogenesis is poorly understood and current therapies have limited efficacy. Forkhead box protein O (FOXO) family of transcription factors are dysregulated in human cancer and loss of FOXO genes leads to vascular tumor formation in mice. We have examined the role of FOXO3, a member of the FOXO gene family, in the pathogenesis of human vascular tumors.

Design: FOXO3 expression was examined in infantile hemangioma, hemangioendothelioma and angiosarcoma tissues by immunohistochemical stains. To delete FOXO3 gene expression, we used short-hairpin RNA to knock down FOXO3 mRNA. To overexpress FOXO3, tumor cells were transfected with FOXO3 cDNA. Cell migration, proliferation and apoptosis were assessed by scratch area closure, cellular DNA content, and levels of the apoptosis marker Annexin V, respectively.

Results: Vascular tumors expressed lower levels of FOXO3 as compared with normal blood vessels. Loss of FOXO3 increased vascular tumor cell migration and proliferation and reduced apoptosis in vitro. Conversely, over-expression of FOXO3 suppressed tumor cell migration and proliferation and increased apoptosis. Loss of FOXO3 increased tumor growth in animal xenograft models, demonstrating the biological relevance of FOXO3 in vivo. Mechanistically, knockdown of FOXO3 inhibited the expression of the pro-apoptotic genes BIM and TRAIL and the cell cycle arrest genes p21WAF1 and p27KIP1 in tumor cells, suggesting that FOXO3 induces apoptosis and inhibits cell proliferation by regulating the expression of these genes.

Conclusions: These findings reveal the important regulatory role of FOXO3 in vascular tumor growth. Targeting FOXO3 expression or activity is a potential novel therapeutic strategy in these tumors.

1814 PBX-1 Is a Key Regulator of Hemangioendothelioma and Angiosarcoma Growth

Sriram Ayyaswamy, Wa Du, Divya Thomas Mani, Haider Mejbel, Thuy Phung. Texas Children's Hospital, Houston, TX.

Background: Hemangioendothelioma and angiosarcoma are malignant vascular tumors whose growth regulatory pathways are poorly understood, and current therapies have limited efficacy. Pre-B-cell leukemia transcription factor 1 (PBX-1) has been shown to be dysregulated in human cancer and is a potential important regulator of vascular tumor development in experimental animal models. We have investigated the biological role of PBX-1 in human vascular tumors and explored molecular pathways regulated by PBX-1.

Design: PBX-1 expression was examined in hemangioendothelioma and angiosarcoma by immunohistochemical stains and western blots. To delete PBX-1 gene expression, we used short-hairpin RNA to knock down PBX-1 mRNA. Cell migration, proliferation and apoptosis were assessed by scratch area closure, cellular DNA content, and levels of the apoptosis marker Annexin V, respectively.

Results: Hemangioendothelioma and angiosarcoma cells expressed higher levels of PBX-1 than normal endothelial cells. Genetic deletion of PBX-1 by knocking down PBX-1 mRNA with short hairpin RNA decreased vascular tumor cell migration and proliferation, and increased apoptosis in vitro. Loss of PBX-1 significantly reduced tumor growth in tumor xenograft models in mice. To assess the molecular pathway(s) by which PBX-1 regulates tumor growth, transcriptional gene expression profiling of cells with loss of PBX-1 was performed. Through pathway analysis using bioinformatics tools, we have identified selective groups of PBX-1-regulated genes with shared functional pathways that are involved in angiogenesis, cell cycle regulation and cytoskeleton modification. We will further investigate the functional and biological significance of these candidate genes in vascular tumors.

Conclusions: These findings demonstrate the important biological role of PBX-1 in vascular tumorigenesis, and uncover novel molecular pathways regulated by PBX-1 that may have essential functions in hemangioendothelioma and angiosarcoma development.

1815 Immunohistochemical Staining Patterns of p53 in Barrett Esophagus With Molecular Pathobiologic Implications

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Background: Mutations within p53 are one of the most commonly detected genetic abnormalities in neoplasia. However, the status of p53 can be expressed in a variety of staining patterns by immunohistochemistry (IHC) in both distribution and intensity. When p53 harbors a truncation or insertion/deletion type mutation, the antigenic site for IHC is essentially unavailable and there is no staining. With an amino acid substitution or point mutation, the protein becomes stabilized and expression is more diffuse with stronger intensity. Weak staining can be seen in wild type, unmutated p53 with hyperexpression due to physiological response to other genetic changes induced by stress. The purpose of this study was to correlate p53 IHC patterns with mutational load analysis in patients with Barrett esophagus (BE) in progressors (P), defined by patients who initially presented with intestinal metaplasia (IM), low grade dysplasia (LGD) or indefinite for dysplasia (IND) and subsequently developing high grade dysplasia (HGD) or esophageal adenocarcinoma (EAC) and non-progressors (NP), characterized by patients with stable IM, LGD, or IND.

Design: Slides were prepared and stained with IHC for p53 in 50 previous H & E cases of BE with known mutational load analysis, (P n=20, NP n=30). P53 staining was scored by percentage of nuclear staining (0: 0-5%; 1: 5-25%; 2: 25-50%; 3: 50-75%; 4: 75-100%) and intensity of staining (1+: mild; 2+: moderate; 3+: strong). Strong staining was defined as nuclear staining of 3-4 and intensity of 3+. Weak staining was defined as nuclear staining of 0-2 and intensity of 0-2+. The IHC for p53 was blindly read and subsequently correlated to previous mutational load analysis results.

Results: In the P group, 80% (17/20) exhibited strong p53 staining. One highly mutated case exhibited complete lack of staining with p53, which most likely represents a truncated mutation. In the NP group, 30% (9/30) exhibited complete lack of staining, which correlated with a mutational load of 0. In the remainder of the NP group, 70% (21/30) showed weak staining, 42.8% (9/21) of which showed low mutational loads.

Conclusions: 1. IHC staining patterns can give insight into the pathobiologic molecular status of p53.

2. Strong, diffuse p53 correlates with P having increased mutational load.

3. Weak p53 staining in low or no mutational load cases of BE in NP, most likely demonstrates an unmutated, wild type p53 with hyperexpression.

1816 Expression of ORAI3: A Novel Marker of Poor Prognosis of Lung Adenocarcinoma

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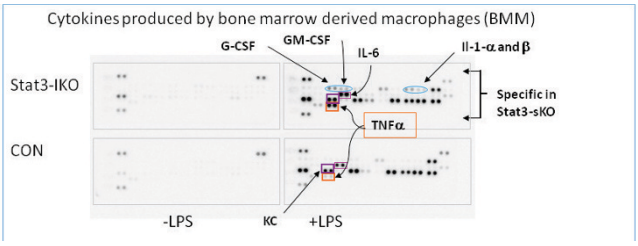
Background: The 5-year survival rate of lung cancer patients are only 15%. Adenocarcinomas (AC) represent 40 to 76% of Non-Small Cell Lung Cancer Cells. Tobacco use is known as the most significant risk factors leading to lung cancer. However, the prognosis of the patients with the same stage of lung cancer is variable. Therefore, it is of great importance to identify novel biomarkers to predict the prognosis of lung cancer patients. Furthermore, tissue homeostasis is based on a balance between cell proliferation and apoptosis. Calcium is known to play an important role in regulation of cell proliferation and apoptosis. ORAI1,2 and 3 channels play a major role in

providing calcium influx. ORAI3 is involved in the proliferation of lung cancer cells. In breast cancer, it has been reported that ORAI3 is an estrogen receptor α (ERα) and that EGF-regulates its activity. Moreover, both ERα and EGFR play a significant role in the growth of lung carcinomas.
Design: Immunohistochemistry was performed on 200 samples of primitive lung AC (ORAI3, 1:100, Sigma ; ERα, SP1, Ventana ; EGFR, 3C6, Ventana). Staining levels were determined by subjective visual scoring by two operators independently.The aim was to investigate the ORAI3 expression in primitive lung AC (n=200) in relation to ERa and EGFR and its potential relevance to clinicopathological variables and prognosis.
Results: ORAI3 staining was present in 66.5% (N=133/200) of AC. Statistical analysis showed that overexpression of ORAI3 was significantly associated with tobacco use, necrosis tumor, visceral pleura invasion, ERα expression and EGFR status. The Kaplan-Meier survival analysis showed that the expression of ORAI3 was related to the poor overall survival (OS) (expression, N=133/200, 40.71 months vs no expression, N=67/200, 95.6 months; p<0.001) and metastatic progression-free survival (MP-FS) (expression, N=133/200, 27.21 months vs no expression, N=67/200, 69.52 months; p<0.001) of patients with lung AC. Multivariate Cox analysis showed that ORAI3 was an independent prognostic factor for both OS and MP-FS.
Conclusions: We highlight the major role of ORAI3 regulation by ERα and EGFR in lung AC. We show, for the first time, that the overexpression of ORAI3 was associated with tobacco use, histological variables and poor prognosis in lung AC and thus may serve as a new molecular marker to predict the prognosis of lung AC patients.

1817 Insight Into the Pathogenesis of Inflammatory Bowel Disease-Related Osteoporosis: Specific Bone Marrow Macrophage Cytokine Production in Mice

William Bivin, Elizabeth Richards, Andrew Nguyen, Elaine Lin, Tamara Avezbadalov, Terry Dowd, Katherine Sun, Qiang Liu. Montefiore Medical Center, Bronx, NY.
Background: Patients with inflammatory bowel disease (IBD) are at an increased risk for osteoporosis (OP). Bone loss in IBD appears to be multifactorial, with IBD activity, malabsorption, and corticosteroid therapy proposed to play important roles. Understanding the pathogenesis of OP in IBD is necessary for effective treatment and development of targeted therapy. Proinflammatory cytokines produced by immune cells have been shown to regulate osteoclast differentiation and activity. Diagnostic and management guidelines for OP in IBD have been issued; however, the pathogenetic mechanisms have not yet been elucidated.
Design: A mouse colitis model was made by targeted deletion of STAT3 in macrophages (STAT3-IKO). To analyze bone mineralization, longitudinal nondecalcified femur sections from STAT3-IKO mice (n=6) and control mice (n=5) were embedded in PMMA, cut at 2 µm, and mounted on infrared windows. Spectral images were acquired via a Fourier transform infrared microscope. Mineral-to-matrix ratio and collagen cross-linking were calculated. To analyze bone marrow macrophage (BMM) cytokine production, bone marrow was flushed and cultured with CSF-1 for 7 days. BMMs were induced with lipopolysaccharide (LPS) for 24 hours. Supernatants were collected for cytokine analysis using a Proteome Profiler Array.
Results: Severe colorectal inflammation was observed in STAT3-IKO mice. Following colitis onset, scoliosis developed. Femur sections from STAT3-IKO mice had a lower bone mineral-to-matrix ratio than control mice (6.4 vs 8.5) and less collagen cross-linking (2.0 vs 2.4). STAT3-IKO BMMs produced a significantly higher level of specific cytokines: TNFα, KC, IL-6, G-CSF, GM-CSF, IL-1α and IL-1β.



Conclusions: STAT3 knockout in macrophages induces severe colitis and subsequent bone defects in mice. The lower bone mineral-to-matrix ratio and decreased collagen cross-linking suggests less collagen maturation occurs due to increased bone resorption. STAT3-IKO BMMs produce a significantly higher level of specific cytokines, which are known to regulate osteoclastogenesis and are highly expressed in IBD. These results imply that specific cytokines may be useful predictive biomarkers for OP in IBD and potentially serve as therapeutic targets.

1818 Mutational Landscape of the Mediator Complex across Human Cancers

Diana Bohm, Zaki Shaikhibrahim, Anne Offermann, Mario Deng, Sven Perner. Institute of Pathology, Bonn, NRW, Germany.
Background: The Mediator complex serves as an integrative hub for signaling pathways. It consists of 33 subunits, comprised of head, middle, tail and kinase modules. As a component associated with the transcription machinery, Mediator is a key regulator of the transcription of all protein-coding genes and is implicated in the regulation of several cancer-related genes. Several key pathways, which control critical functions in cancer cells, such as differentiation, growth, survival and apoptosis, have been shown to depend critically upon specific Mediator subunits.
Based on this integrative role, it is conceivable that alterations in Mediator subunits have major pathophysiological consequences in cancers.
Design: The Cancer Genome Atlas (TCGA), which catalogues mutations found in cancer genomes was used to analyze somatic mutations. We utilized deposited exome data of 6,014 patients and analyzed the mutation status of all 33 Mediator subunits in 22 different human cancer types. Non-synonymous mutations were considered for analysis. SNPnexus web server (http://www.snp-nexus.org) was used to retrieve pre-computed SIFT and Poly-Phen2 predictions of the probability of an amino acid substitution affecting protein function.
Results: We found a unique mutational landscape, identifying the kinase and tail as hot spots across all cancers. Furthermore, a recurrent mutation in *MED12L* was found across six cancer types whereas two recurrent mutations in *MED15 were* detected in five cancer types. Multiple mutations across different subunits within a tumor specimen were observed. Mutations in MED12, MED12L, MED13 and MED13L show the highest numbers of connectivity to mutations in other subunits of the Mediator complex. Also mutations in the kinase module of the Mediator subunit result mainly in a damaging protein function.
Conclusions: Our systematic and comprehensive analysis reveals, for the first time, the mutational landscape of the whole Mediator complex in cancer, and suggests that mutations are frequently found in the kinase module of the Mediator but also are widespread among different tumor entities.

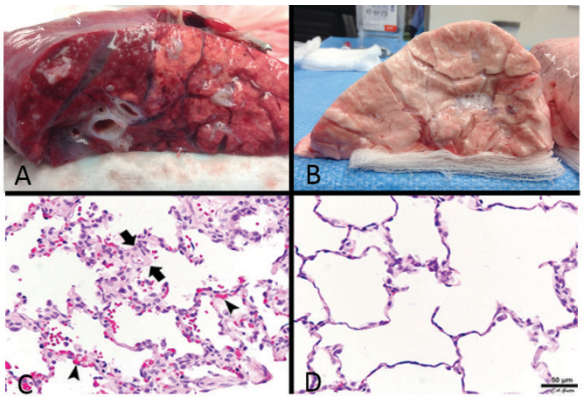
1819 Two-Staged Approach in Identifying Therapeutic Targets for Patients With Advanced Cancers

Russell Broaddus, Beate Litzenburger, Kenna Shaw, J Jack Lee, Walter Kinyua, Blessy Sajan, Mark Routbort, Keyur Patel, Rajyalakshmi Luthra, Rajesh Singh, Scott Kopetz. University of Texas MD Anderson Cancer Center, Houston, TX.
Background: The efficacy of small next generation sequencing (NGS) panels for the initial therapeutic management of patients with solid tumor malignancies is established, especially for melanoma, colorectal cancer, and lung cancer. The utility of such panels for identifying therapeutic targets after front-line therapies have failed is less clear. We initiated a prospective study to assess the utility of systematic identification of potential therapeutic targets by NGS in patients with advanced solid tumor malignancies. A two-staged molecular diagnostics approach was used in which the patients were initially evaluated with a 50 gene next generation sequencing (NGS) panel (CMS50) that analyzes for hot spot mutations. If no therapeutic trials were identified based on the CMS50 testing, or if the patient suffered recurrence following an initial targeted therapy trial, a next generation sequencing panel of 409 full exomes (CMS400) was employed.
Design: To be eligible for the expanded CMS400 panel, patients had to have completed standard front-line therapy, have a performance status of 0 or 1, and be agreeable to participation in a clinical trial if a possible therapeutic target was identified. All NGS was performed on formalin-fixed, paraffin-embedded tumors. An interim analysis was performed after the first 117 patients to determine the initial clinical utility. Clinically actionable was defined as a somatic mutation for which a matched genotype selected therapeutic clinical trial exists. Actionable mutations had associated functional data demonstrating that they could be targeted by a specific agent.
Results: For 24% of patients (28/117), at least one mutation in a targetable gene was identified in the initial CMS50 screen. Overall, CMS400 identified 96 mutations in actionable genes. 44% of patients (51/117) had at least one mutation in an actionable gene identified by the more expanded testing that was not identified in the initial screen. 29% of these patients (15/51) had mutations that were known to have functional impact. The functional significance of the remaining somatic mutations is currently unknown.
Conclusions: Expanded molecular testing does provide benefit by identifying more patients who are potentially eligible for targeted therapy trials. Most of the specific mutations identified, while in actionable genes, have unknown functional impact. At this point, only the patients with variants known to have functional impact are enrolled in clinical trials. Once the relevance of these other variants is better defined, it is likely that more of these patients can be enrolled in trials.

1820 Molecular Mechanisms of Prevention of Septic Acute Respiratory Distress Syndrome By Airway Pressure Release Ventilation

Tiffany Caza, Kerry Whiting, Michaela Kollisch-Singule, Sumeet Jain, Joshua Satalin, Kathleen Snyder, Louis Gatto, Frank Middleton, Penny Andrews, Gary Nieman, Nader Habashi, Steve Landas. SUNY Upstate Medical University, Syracuse, NY; SUNY Cortland, Cortland, NY; University of Maryland School of Medicine, Baltimore, MD.
Background: Acute respiratory distress syndrome (ARDS) is refractory to treatment once established and the only therapy is supportive mechanical ventilation. Preemptive application of airway pressure release ventilation (APRV) prevents ARDS in animal models and in a human trial by preserving surfactant, reducing pulmonary edema, and minimizing alveolar microstrain. Molecular mechanisms underlying protection in APRV-protected lung are unknown. The purpose of this study is to elucidate mediators of lung protection through a genome-wide gene expression analysis.
Design: Pigs were anesthetized and instrumented. Fecal peritonitis was established by SMA clamping then release for 30 min to induce an ischemia/reperfusion injury. Pigs were randomized to early ARDSnet (n=3) with TV 6 mL/kg, PEEP 5cmH_2O, F_iO_2 21% and titrated according to the low PEEP or early APRV (n=3) with P_{High} 16cmH_2O, P_{Low} 0cmH_2O, T_{High} 4.5 s, T_{Low} set to terminate the peak expiratory flow at 75% and F_iO_2 21%. After 48h, necropsy was performed. RNA was extracted from lung, cDNA synthesized, and hybridized on Affymetrix porcine gene 1.0 ST arrays. Microarrays were analyzed by Ingenuity Pathway Analysis. Gene expression at the <5 or >95 percentile with a p value of <0.05 were considered significant.
Results: APRV prevented ARDS. APRV activates TGFb1 and targets that negatively regulate inflammation ($p=1.3 \times 10^{-7}$). APRV-treated lung tissue had reduced expression of transcripts promoting leukocyte, NK, and CTL function and downregulation of downstream cytokines IFNγ and IL-15 (cluster $p=6.6 \times 10^{-6}$). APRV reduced expression of transcripts that reduce apoptosis and promote survival in endothelial cells, regulated

by AGT, C5, CSF2, IL-1, SMAD3, and VEGF, and endothelin 1 ($p=3.1 \times 10^{-6}$ of cluster). APRV enhances expression of genes encoding surfactant by 52-382 percent. Matrix metalloproteinase (MMP) transcripts are reduced by ≥50%.



Preemptive use of APRV during sepsis reduces lung pathology and prevents ARDS. A. Gross lung – ARDSnet ventilation; B. Gross lung – APRV; C – Microscopic – ARDSnet ventilation. Note thickening of alveolar septae (thick arrows) and early hyaline membrane formation (arrowheads). D. Microscopic – APRV.

Conclusions: APRV prevents development of ARDS secondary to ischemia-reperfusion injury and sepsis by reducing inflammation, endothelial cell apoptosis, and MMP-mediated breakdown of the ECM.

1821 Scavenger Receptor Class B1 (SR-B1) Is Expressed in Diverse Human Cancers Including Most Triple Negative Breast Adenocarcinomas

Dianne Chadwick, Nicole Baryla, Brian Barber, Frank Gleeson, Michael Roehrl. University Health Network, Toronto, ON, Canada; Ontario Institute for Cancer Research, Toronto, ON, Canada.

Background: Scavenger Receptor Class B1 (SR-B1), the high affinity cell surface receptor for high density lipoprotein (HDL), facilitates the transport of cholesterol into liver cells as part of cholesterol regulation. HDL-inspired nanoparticles (designated HPPS) have been shown to deliver functionally viable siRNA into cancer cell lines that express SR-B1. The purpose of this study was to perform SR-B1 expression analysis on a comprehensive series of patient tumors and normal tissues towards identifying potential targets for HPPS-mediated siRNA therapeutics.

Design: IHC staining of SR-B1 was optimized on fixed sections of normal human liver. Tissue microarrays (TMAs) were constructed with cores from a wide variety of banked fixed solid tumors and matched normal tissues. Pathology diagnoses and other clinical information were collected.

Results: Anti-SR-B1 antibody optimization was achieved using a rabbit monoclonal antibody from Epitomics (Burlington, CA). IHC analysis confirmed that normal human hepatocytes and adrenal cortical cells express SR-B1 on the cell membrane, whereas normal breast, skin, colon, rectum, lung, prostate, ovary and adrenal medulla are SR-B1 negative. There was some cytoplasmic (non-specific) staining of normal pancreatic and kidney cells. Analysis of patient tumor TMAs revealed membranous staining on all cases of hepatocellular carcinoma (8/8), most cases of renal cell carcinoma (14/15) and melanoma (7/9), and some cases of breast carcinoma (6/13), mesothelioma (3/7), and adrenal cortical carcinoma (3/7). There was some focal, non-specific staining of colorectal and pancreatic carcinoma cases. All other tumors tested (prostate, ovary and lung adenocarcinoma and squamous cell carcinoma) stained negatively. SR-B1 expression was validated on TMAs containing additional cases of breast adenocarcinoma. 28/46 (61%) of triple negative breast cancers were positive for SR-B1 versus 8/57 (14%) of non-triple negative cases (two-tailed $p<0.0001$, Fisher's exact test).

Conclusions: The results of this survey indicate that diverse human tumors, especially triple-negative breast cancers, express SR-B1 and therefore represent potential targets for HPPS-mediated siRNA therapeutics. SR-B1-specific IHC methodology may provide a companion diagnostic strategy for identifying cancer patients who would benefit from this type of siRNA delivery.

1822 Comprehensive Analysis of Karyotypically Normal Adult ALL (Acute Lymphoblastic Leukemia) Cases: Utility of SNP (Single Nucleotide Polymorphism) Microarrays in Identifying New Regions of Oncogenic Potential

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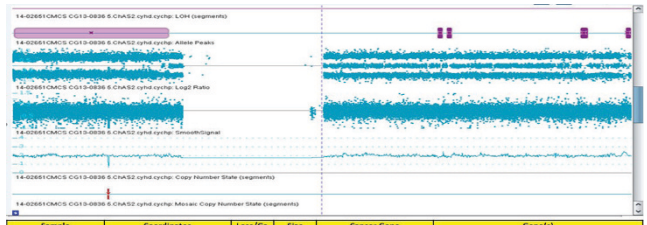
Background: Conventional cytogenetics (CC), including karyotyping & FISH, has long been the gold standard tool in the management of ALL. ALL is complex on multiple levels, & laboratory efforts over the past 3 decades have focused on better understanding of it's molecular pathogenisis. One of the biggest strengths of SNP arrays is in its ability to identify copy neutral LOH (a limitation of CC) which have a significant prognostic implications in ALL. Aim of the current study is to investigate the clinical utility of SNP technology in facilitating in the diagnosis, prognosis and management of karyotypically normal (kn) ALL.

Design: We identified 18 cases of ALL which had normal karyotype and FISH in our system & showed >20% blasts in marrow. Archival reports & slides were retrieved & reviewed. Subsequently, high resolution SNP microarray using CytoScan HD Microarray (Affymetrix Inc,) was performed on DNA isolated from methanol-acetic acid fixed marrow pellets of 3 cases following the American College of Medical Genetics (ACMG) guidelines for neoplastic disorders.

Results: In our study all the kn-ALL's showed multiple genomic abnormalities. Clinically important findings revealed (B-ALL) a 1.2 Mb mosaic deletion of chromosome 22q13.1q13.2 which includes the PDGFB & ATF4 cancer genes. Interesting other cases (T-ALL) revealed a 2.7 Mb mosaic loss of chromosome 1q31.3q32.1 which includes the ASPM cancer gene & a ~35 Mb LOH on chromosome 9p24.3p13.3. Also, we identified a 150 kb deletion within this LOH block including CDKN2A gene.



Sample	Coordinates	Loss/Gain	Size	Cancer Gene	Gene(s)
14-02601CMCS_13-150	chr22:39,272,060-40,538,716	Loss	1.266 Mb	*PDGFB, ATF4*	NPTXR, CBX6, APOBEC3A, APOBEC3B, APOBEC3C, APOBEC3D, APOBEC3F, APOBEC3G, LOC101927202, APOBEC3H, COX5BP7, CBX7, LOC100127979, PDGFB, RPL3, SNORD83B, SNORD83A, RNU86, SNORD43, SYNGR1, TAB1, LOC100506472, MGAT3, MIEF1, ATF4, RPS19BP1, CACNA1I, ENTHD1, LOC100132621, UQCRFS1P1, GRAP2, FAM83F, LOC100130899, TNRC6B, RPL7P52
14-02615CMCS_13-290	chr1:195,887,472-198,660,143	Loss	2.772 Mb	*ASPM*	KCNT2, MIR4735, CFH,CFHR3, CFHR1, LOC100996886, CFHR4, CFHR2, CFHR5, F13B, ASPM, ZBTB41, CRB1, LOC127011, MRPS21P3, DENND1B, EEF1A1P32, RPL24P5, FAM204BP, C1orf53, LHX9, NEK7, PRR13P1, ATP6V1G3, PTPRC, PEBP1P3
14-02651CMCS_13_836	chr9: 454,022 - 35,719,940	LOH	35 Mb	*IAK2, RLN2, KDM4C,PTPRD, NFIB, PSIP1, SH3GL2, MLLT3, CDKN2A, CDKN2B, TEK, TOPORS, BAG1, FANCG, STOML2*	362 genes
14-02651CMCS_13_836	chr9: 21,846,426 - 21,996,603	Loss	150 Kb	*MTAP, CDKN2A*	MTAP, ERVFRD-3, C9orf53, CDKN2A, CDKN2B-AS1

Conclusions: While we recognize that this is a small cohort, but this work is intriguing for the new information it provides on genomic abnormalities in kn-ALL. Our data highlights important role of SNP microarray technology in understanding the fundamental aspect of leukemogenesis especially in those cases which are clinically labeled with a normal karyotype. We are in the process to expanding the study by including additional kn cases of ALL.

1823 Recurrent Abnormalities of chr3q12-13 Contribute To Pediatric Acute Lymphoid Leukemogenesis

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Background: By using a combination of array-based comparativegenomic hybridization and genomic sequencing, a number of recurrent mutations have been identified in pediatric acute lymphoid leukemia (ALL). The most commonly targeted genes were PAX5, EBF1, and IKZF. A number of other rarer lesions were also identified, including focal deletions confined to the genomic loci chr3q12-13 containing *CD200* and *BTLA* in about 7% of ALLs.

Design: By mapping the breakpoint of these deletions in primary ALL blasts, the deletions resulted in the loss of both CD200 and BTLA in all but one case. Moreover, analyzing the sequence at the breakpoints suggested that deletion was the result of aberrant RAG-mediated recombination. Importantly, no point mutations in CD200 or BTLA were detected in any of the analyzed pediatric ALL cases, suggesting that haploinsufficiency of these two genes was likely to contribute to leukemogenesis. To explore whether loss of both genes contributes to leukemogenesis, we crossed mice heterozygous for a Cd200-null allele (Cd200 +/-) with mice heterozygous for a Btla-null allele (Btla +/-) to generate mice that were heterozygous for both genes (Cd200+/-: Btla+/-).

Results: Our analysis revealed no significant alteration in B-cell development in mice heterozygous for either Cd200 or Btla alone. In contrast, the Cd200+/-: Btla+/- mice had a subtle decrease in mature B cells and a modest increase in pre-B cells, suggesting that loss of both genes cooperated to dysregulate normal B-cell development. To determine if this defect could cooperate with other genetic lesions in leukemogenesis, bone marrow cells from wild-type, Cd200 +/-, Btla +/-, and Cd200+/-: Btla+/- mice were transduced with a retrovirus expressing BCR-ABL1 and GFP and then transplanted into lethally irradiated syngeneic mice. No significant difference was noted in the penetrance, latency, or disease phenotype of bone marrow cells obtained from mice heterozygous for Cd200 or Btla. In contrast, expression of BCR-ABL1 in marrow obtained from Cd200+/-: Btla+/- mice resulted in a significant decrease in latency and increase in penetrance of ALL without affecting the immunophenotype of the resultant leukemia.

Conclusions: These data suggests that the co-deletion of CD200 and BTLA induces a subtle block in normal B-cell development and can cooperate with the expression of BCR-ABL1 in leukemogenesis.

1824 Endoscopic Mucosal Resection Specimens as the Source in Identifying Markers for The Early Neoplastic Process

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Background: Accurate diagnosis of an early neoplastic lesion has a major implication in patient management. Diagnosing borderline, low or high grade dysplasia, or early carcinoma has been a major challenge. Substantial interobserver diagnostic variation emphasizes the need for routinely applicable marker(s) as an adjunct to histological diagnosis. Endoscopic mucosal resection (EMR) specimen is the best source to learn about the process. We tested EMR specimens with a panel of markers.

Design: Seven gastric adenocarcinoma(CA) and 5 esophageal squamous cell CA(SCA) obtained by EMR specimens were tested for the expression of p53, Ki-67, minichromosome maintenance proteins (Mcm2), β-catenin, cyclin D1 and p16 by immunohistochemistry, together with 3 colon adenoCA, 19 adenomas (7tubulovillous, 6 tubular, and 6 serrated) and 6 hyperplastic polyps of colon. Tubulo-villous adenomas were used as positive controls for the immunoreaction of dysplastic cells. Study cases were interpreted by comparison with controls. Only strongly stained columnar cells in continuity (not scattered) were considered positive. Only cytoplasmic p16, nuclear cyclin D1,Mcm2 and P53, and cytoplasmic and /or nuclear β-catenin were considered positive.

Results: P53, Mcm2 and cyclin D1 stains in EMR colocalized with the dysplastic area and showed clear delineation from low (LGD) and high grade(HGD) dysplasia to CA and uniformly strong positive staining in CA, distinct from the adjacent atypical or normal areas. TVA showed strong and uniform staining, while tubular adenomas showed heterogenous reaction admixed strong and weak staining. Serrated adenoma showed positive staining in the basal crypts, labeling extended from the lower crypts to the surface, easily distinguishable from normal and hyperplastic polyps. p53 stains were negative in 2 of 7 (28.6%) gastric adenoCA and 1 SCA (20%). Ki-67 stains was less-specific than that of Mcm2. β-catenin staining was mostly cytoplasmic in LGD and focally lost or cytoplasmic with occasional nuclear staining in the area of HGD/CA. p16 cytoplasmic expression was present in 60% of HGD/CA but weak and spotty in LGD.

Conclusions: p53, cyclin D1 and Mcm2 stains were consistently and significantly associated with the degree of dysplasia and carcinoma. P16 and β-catenin stains are of diagnostic adjunct in HGD/CA. The panel of marker(s) would serve to confirm a suspected diagnosis of early neoplastic process for the risk stratification. It is noteworthy that p53 can be negative in adenocarcinoma or dysplasia.

1825 Co-Inhibition of MYC and BCL2 Signaling Is a Potentially Effective Strategy for Treatment of Double Hit and Triple Hit B-Cell Lymphomas

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Background: Overexpression of MYC and BCL2 or/and BCL6 due to genomic rearrangements is the key molecular feature of double hit lymphoma (DHL) or triple hit lymphoma (THL). Patients with DHL and THL show very aggressive disease course and poor survival because there is no effective treatment modality. The goal of this study is to assess the impact of potent MYC inhibitors, 10058-F4 and JQ-1, and a BH3 mimetic selective BCL2 inhibitor, ABT-199, on patient-derived THL and established DHL cell growth with or without combination of chemotherapeutic agent, vincristine or doxorubicin.

Design: Primary THL cell line as named CS-THL1 was established from an 85 year-old lymphoma patient. Immunohistochemistry (IHC) was conducted to determine the levels of MYC, BCL2, BCL6, or Ki-67 expression in formalin-fixed, paraffin-embedded tissues. MTS assay were employed to evaluate the anti-growth efficacy of 10058-F4, JQ-1, ABT-199 in CS-THL1 and DoGKiT cell alone or together and with or without vincristine or doxorubicin exposures. Flow cytometric analysis of propidium iodide and Annexin V and/or 7AAD stained cells or cytochrome c release was performed to assess apoptosis. Intracellular expression of BCL2, BCL-xL, BIM, and MCL-1 protein was analyzed by flow cytometry.

Results: As shown by IHC analysis, expression of MYC, BCL2, BCL6 and proliferation factor Ki67 was increased in THL. Analysis of cell viability demonstrated that inhibition of MYC by 10058-F4 or JQ-1 or BCL2 by ABT-199 as a single agent significantly inhibited the growth of CS-THL1 and DoGKiT cells in a dose and time-dependent manner. In addition, combination of 10058-F4 or JQ-1 and ABT-199 or together with vincristine or doxorubicin synergistically attenuated the growth of CS-THL1 and DoGKiT cells compared with single agent treatment. Moreover, using multiple approaches, we demonstrated that apoptosis induced by the inhibition of anti-apoptotic BCL2 with ABT-199 and the inhibition of strong growth promoting factor MYC with 10058-F4 or JQ-1 was the underlying cause of the observed growth retardation.

Conclusions: These observations suggest that concurrent inhibition of MYC and BCL2 pathway signaling is a promising treatment strategy for patients with aggressive DH and TH B-cell lymphomas.

1826 Platelet Cloaking of Cancer Cells Inhibits Immune Surveillance

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Background: We have previously described platelet cloaking of cancer cells as part of the metastatic cascade. The platelet cloak confers on cancer cells a pro-growth survival signal through inhibition of apoptosis, autophagy and promotion of pro-cell cycling, angiogenesis and EMT. The aim of this study was to investigate whether platelet cloaking of cancer cells inhibits NK cell function as part of natural immune surveillance in cancer.

Design: Cell line models including SKMES-1, K562, SKOV3, 59M and SK-Mel-28 were used and interacted with peripheral blood mononuclear cells including NK cells (with and without platelet cloaking and platelet releasate). The NK cancer cell interaction receptor ligand systems: NKG2D, MICA/MICB and CD115, CD96 and CD226 were examined in dynamic interaction assays monitoring CD107a (NK cell degranulation) and IFNγ secretion.

Results: Platelet cloaking induced significant down-regulation of NKG2D on NK cells and its ligands MICA/B on cancer cells. In addition, the cloak promotes the release of soluble MICA/B from the cancer cell (an immune decoy mechanism). The platelet cloak significantly deregulated CD155 on cancer cells and CD96 on NK cells. Both of these mechanisms inhibit NK cell function and immune surveillance.

Conclusions: Platelet cloaking of cancer cells directly inhibits NK cancer cell killing function, directly inhibiting innate immune surveillance.

*Joint Senior Authors.

1827 Analysis of the Urinary Proteome and Peptidome To Identify Prognostic Biomarkers for Small Renal Masses

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Background: The incidence of renal cell carcinoma (RCC) has doubled due to increased detection of asymptomatic small renal masses (SRMs). There are no clinical or radiographic features able to predict the behaviour of SRMs. Non-invasive prognostic biomarkers able to predict aggressive behaviour are urgently required.

Design: We performed label-free quantitative liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) urine peptidomics analysis on 6 patients with progressive and 6 with non-progressive with clear cell RCC-SRM as well as 6 matched normal urines. Bioinformatic analysis was performed to identify significantly altered pathways.

Results: We identified 1,963 unique endogenous urine peptides arising from 392 proteins. Initial comparative analysis identified 227 unique peptides in non-progressive disease and 289 unique peptides in progressive disease. Endogenous peptides from monocyte differentiation antigen CD14 (CD14), serotransferrin (TF), fibronectin 1 (FN1), filamin A (FLNA), and aminoacylase 1 (ACY1) were only identified in urine from patients with progressive clear cell RCC-SRMs. Pathway analysis identified CD14 to be a positive regulator of tumor necrosis factor production ($p=0.0016$) and associated with NF-kB signalling ($p<0.01$). In addition, pathway analysis identified TF as a positive regulator of the platelet-derived growth factor receptor signalling pathway ($p<0.0001$), endothelial cell proliferation ($p<0.01$), protein kinase B signalling ($p<0.01$), angiogenesis ($p<0.01$), and epithelial cell proliferation ($p=0.01$). FN1 was shown to be involved in cell-matrix adhesion ($p<0.01$) as well as migration processes such as angiogenesis ($p=0.01$) and wound healing ($p=0.027$). FLNA has a role in epithelial to mesenchymal transition (EMT) ($p<0.01$) as well as the G-protein coupled receptor signalling pathway ($p<0.01$) and NF-kB signalling pathway ($p<0.01$). Furthermore, pathway analysis found ACY1 to have a role in cellular amino acid metabolism ($p=0.018$).

Conclusions: Taken together, CD14, TF, FN1, FLNA, and ACY1 represent potential prognostic biomarkers able to distinguish between patients with progressive and non-progressive RCC-SRMs.

1828 Functional Mutation Signatures of 5 Cancer Differentiation Subtypes and Epithelial Tumor Grading: Utility of Exome Sequence Data and Random Forest Analysis

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Background: Cancer is morphologically and genetically heterogeneous and the Pan-Cancer Analysis Project aimed to identify the genomic changes present in 12 cancer types from the Cancer Genome Atlas (TCGA) set. We aimed to identify predictors of the 5 main differentiation subtypes in the Pan-Cancer Analysis and a general molecular grading for epithelial malignancies.

Design: Whole exome sequencing was performed on tumor and normal tissue samples from 3129 patients enabling the identification of cancer related mutations in each patient. Clinical data were also collected for all patients, including gender and age. We used a Random Forest machine learning approach to compare the 5 differentiation subtypes in a pairwise fashion and a low (grade 1-2) vs. high (grade 3-4) grade.

Functional somatic mutations unique to tumors were identified and represented as a samples x genes mutation matrix (mutated=1, non-mutated=0). ► Pairwise Random Forest models were built for the 5 cancer differentiation subtypes (Adenocarcinoma, Squamous, Urothelial, Brain, Hematological) ► Recursive feature selection was used in a 5x10 fold cross validation design. Random Forest models were based on the training set using the caret package in R ► Predictive accuracy of each model was measured in an independent test set.

Results: We were able to discriminate between Bladder Urothelial Cancer and Acute Myeloid Leukemia in unseen samples with 87.8% accuracy (95% CI : (78.71, 93.99), Specificity 0.77 Sensitivity 0.94 AUC 0.9677) 5 binary predictors of tumor class membership of Urothelial or Hematological subtypes and epithelial grading (low vs. high). Average accuracy in the other comparisons is 85.0% ► Using a 5 variable random forest ensemble classification model (MLL3, TTN, ARID1A, MLL2, FRG1B), we achieved near 90% accuracy in class assignment task (after excluding any statistically significant gender and age bias between the high grade and low grade tumors) and identifying high grade carcinomas (glandular, squamous and urothelial). Grade-predictive genes included ARID1A, CTCF, CTNNB1, PIK3CA, PIK3R1, PTEN, and TP53.

Conclusions: Exome sequence provide reliable data to classify common malignancies and may be clinically useful for the subtyping of malignancies and grading of carcinomas in case of limited tissue (i.e. biopsies). We now aim to produce a 5-class subtyping and 2-tier grading prediction models to assign test samples prospectively.

1829 Microscopic Colitis Associated With Inflammatory Bowel Diseases Is Characterized By Increased Th1 and TNFα-Producing Cells in Colonic Mucosal Lamina Propria

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Background: An association between microscopic colitis (MC), i.e. lymphocytic colitis (LC) and collagenous colitis (CC), and inflammatory bowel diseases (IBD) has been noted. A subset of MC cases evolve into IBD, either ulcerative colitis (UC) or Crohn's disease (CD). IBD in remission may present a histologic pattern of MC. MC and IBD can coexist in different regions of bowel. All of these suggest a link between MC and IBD in their pathogenesis. Abnormal mucosal immunity is likely the key.

Design: We reviewed totally 2324 MC cases in Calgary region over 14 years and identified a small number of IBD-related cases in 2 subgroups: 1)MC evolved into IBD, 13 cases (5 LC-CD, 3 LC-UC, 1 LC-IBDU, 2 CC-UC, 2 CC-IBDU), 2) mixed MC and IBD, 5 cases (2 LC/CD, 1 LC/UC, 1 CC/UC, 1 LC/IBDU). They were further investigated for their colonic mucosal lamina propria mononuclear cells, as opposed to 20 cases (LC 10, CC 10) that were quickly resolved after treatment. The first colonic biopsies of these cases were retrieved and immunohistochemistry was used to detect various T cell subsets characterized by key cytokines and master transcription factors (IFNγ and T-bet for Th1, IL-13 and Gata3 for Th2, IL-17 and RoRgt for Th17, FoxP3 for Treg) as well as TNFα^+ cells (partly representing Th1). The lymphocytes positive for each marker were counted (average number per HPF of 5 to 10 HPFs).

Results: Overall, as compared to the normalized cases, the IBD-related cases showed a significantly higher population of Th1-type cells including IFN-γ^+ (20.07 ± 11.80 vs 13.92 ± 8.12) and T-bet$^+$ cells (158.66 ± 52.36 vs 122.35 ± 48.31), and TNF-α^+ cells (19.97 ± 18.35 vs 11.18 ± 10.85). ($p < 0.01$ for all). This difference was particularly seen in cases that evolved into IBD (subgroup 1). In addition, this subgroup had more Gata-3$^+$ (Th2) cells (283.12 ± 75.98 vs 219.87 ± 45.32, $p < 0.01$). No difference existed between the two groups for Th17 (ROR-gt$^+$ and IL-17$^+$), Treg (FoxP3$^+$), and IL-13$^+$ cells. Within the normalized cases, more TNF-α^+ and ROR-gt$^+$ cells, but no difference for other subsets, were seen in LC than that in CC cases.

Conclusions: Th1 and TNFα-producing cells, and likely a subset of Th2 (Gata-3$^+$) cells as well, may be involved in the MC cases that are associated with IBD transformation.

1830 Additional KRAS, NRAS and BRAF Mutations Revealed By Next Generation Sequencing Cancer Gene Panel: Necessity of Comprehensive Mutation Detection for Scrutinizing Colorectal Cancer Candidates for Epidermal Growth Factor Receptor (EGFR) Targeted Therapy

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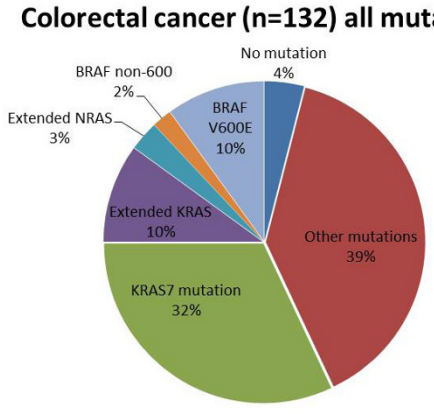
Background: EGFR targeted therapies have been combined with standard chemotherapy regimens for treatment of metastatic colorectal cancer (CRC) in patient's whose tumors lack KRAS mutations. In 2012, the FDA approved a theranostic mutation test covering 7 mutations in codons 12 and 13 of KRAS only. However, more recent data suggests that expanded "RAS" testing to include alternate mutant codons in KRAS, BRAF, and NRAS may better predict whether patients will respond to EGFR targeted therapies. The goal of this study was to assess the clinical utility of running a 50 gene cancer panel (including the expanded RAS coverage) versus the FDA-approved KRAS test in a clinically tested cohort of patients with CRC.

Design: Mutation data was retrospectively collected from 132 consecutive CRC patients undergoing routine clinical testing with a 50 gene next generation sequencing (NGS) cancer panel, which included expanded testing of KRAS, NRAS and BRAF genes. All variants detected with the primary panel were verified using an orthogonal NGS test.

Results: Pathogenic mutations were detected in 96% of cases, and 57% had mutations in KRAS, BRAF and/or NRAS genes (Figure 1). Overall, 25% of tested patients had a KRAS, BRAF or NRAS activating mutation that would not have been detected by the FDA approved KRAS assay, including alternate KRAS mutations (10%), NRAS mutations (3%), BRAF V600E mutation (10%) and BRAF non-600 mutations (2%). In addition, 39% of specimens had mutations in genes other than RAS/RAF genes.



Conclusions: The FDA approved KRAS single gene assay does not detect a significant portion of RAS/RAF mutations known to be associated with non-response to EGFR inhibitors. As a result, using only the FDA approved test will result in non-efficacious, expensive, and potentially harmful anti-EGFR therapy treatments for a significant fraction of CRC patients. These results suggest that all tumors from patients being considered for treatment with EGFR inhibitors should, at minimum, be tested for mutations using expanded RAS/RAF coverage.

1831 Synergistic Effect of EZH2 Knockdown and Irradiation Against Pancreatic Cancer Cells

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Background: Pancreatic ductal carcinoma is the fourth leading cause of cancer-related death, with a 5-year survival rate below 5%; mostly due to advanced-stage diagnosis, relative scarcity of treatment options, and resistance to chemotherapy. Therefore, development of novel treatment approaches, including targeted treatment as an adjuvant modality or as a radiosensitizer, are urgently needed. EZH2 is a catalytic subunit of a histone methyltransferase and is involved in transcriptional repression of genes involved in cell proliferation. EZH2 has been implicated in several aggressive cancers including pancreatic carcinoma. The use of small interfering RNA (siRNA) to downregulate its expression has been explored as a therapeutic option in multiple tumors. Here we explore the antitumor effect of siRNA knockdown of EZH2 on pancreatic cancer cells when applied in combination with irradiation.

Design: EZH2 expression was evaluated in human pancreatic adenocarcinoma tissue sections (n=10) and in human pancreatic carcinoma cells (PANC1) by immunohistochemistry. PANC1 cells were seeded and allowed to adhere for 24 hrs before transfection. At 72 hrs post-transfection, the cells were γ-irradiated (4 Gy) and left undisturbed for 6 hrs before plating or harvesting for various assays. Cell cycle analysis of propidium iodide-stained cells was performed by flow cytometry at 6 hrs after irradiation. Western blot analysis for EZH2 expression was performed on 3, 5 and 7 days post-transfection. Cell proliferation assays were performed at 24, 48, 72 and 96 hrs following irradiation.

Results: All of the human pancreatic adenocarcinomas and PANC1 cells were strongly positive for EZH2. siRNA-EZH2 downregulated EZH2 expression by 90%. Western blot showed the knockdown of EZH2 levels in transfected cells at 72 hrs post-transfection and the day of irradiation. EZH2 knockdown arrested the cells in the G2/M phase as compared to control siRNA-treated cells. The combination of siRNA-EZH2 and irradiation led to cell cycle arrest at G2/M and increased apoptosis. An increase in the number of floating cells was noted in the combination treatment of EZH2 knockdown and irradiation.

Conclusions: These results suggest that siRNA mediated knockdown of EZH2 together with radiation induces a therapeutic effect in PANC1 cells as determined by cell proliferation, cell cycle progression and apoptosis. Combination therapies using radiotherapy and gene silencing may prove useful in treating pancreatic carcinoma.

1832 Histologic Characterization of Tumors With "No Reportable Alteration" By Next-Generation Sequencing

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Background: Next-generation sequencing (NGS) is a powerful technique to acquire high-throughput data on genetic alterations in various malignancies. The FoundationOne pan-cancer NGS panel interrogates the entire coding sequence of 236 cancer-related genes plus 47 select introns from 19 genes involved in fusion. These genes are demonstrated to be somatically altered in many human solid cancers. However, reportable alterations are not identified in every malignancy.

Design: Surgical pathology formalin-fixed paraffin embedded tumor specimens from 179 patients seen at our institution since November 2012 were sequenced by NGS using the FoundationOne platform at a depth of 500-1,000X. Histopathological features of all specimens with "No Reportable Alteration" were analyzed.

Results: 11 out of 179 cases (6.1% of total 186 specimens) returned results of "no reportable alteration". One case had three specimens tested. All paraffin blocks had adequate viable tumor tissue based on the FoundationOne standard. These 11 cases included 4 female and 7 male patients with a median age of 34.9 years (range: 3-56 years). Specimen interval from collection to NGS ranged from 0.5 to 41 months (median 1.5 months). Specimen sites included soft tissue (4), lymph node (3), lung (2), colon (1) and brain (1). Specimen types were known in 10 cases, including resection (5), excisional biopsy (3), core biopsy (1) and fine needle aspiration cell block (1). These 11 cases included 4 soft tissue sarcomas (synovial sarcomas, desmoplastic small round cell tumor, rhabdomyosarcoma), 2 neuroblastomas, 1 angioimmunoblastic T-cell lymphoma, 1 metastatic poorly differentiated squamous cell carcinoma of unknown primary, 1 metastatic medullary thyroid carcinoma, and 1 well-differentiated neuroendocrine carcinoma. In comparison with cases with reportable alterations, this group of patients were younger and had tumors predominately originating from mesenchymal, neuroectodermal, or neuroendocrine origins.

Conclusions: Certain tumor types, such as those arising from mesenchymal, neuroectodermal, or neuroendocrine origin may have alterations currently unknown or not covered by current pan-cancer NGS panels but potentially reportable. Increasing the breadth of sequencing (whole exome sequencing) or applying more focused approaches on select cases (using sarcoma or hematopoietic panels) may uncover more useful and clinically-oriented data in these types of cases.

1833 Comprehensive Assessment of RAS/RAF Mutation Status and Identification of Alternative Targetable Driver Alterations in RAS/RAF Wild Type Colorectal Carcinoma Using a Hybrid Capture-Based Next-Generation Sequencing Assay

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Background: Clinical molecular testing of advanced colorectal carcinoma (CRC) is currently limited to detecting activating mutations in *KRAS*, *NRAS*, and *BRAF* to guide treatment decisions regarding anti-EGFR therapy. Here, we report our clinical experience with a next-generation sequencing (NGS) assay that enables a more comprehensive molecular assessment of CRC.

Design: We use a hybrid capture-based NGS assay encompassing all exons of 341 cancer genes (MSK-IMPACT) to sequence tumor against matched normal to detect potentially actionable somatic alterations including point and indel mutations, copy number alterations, and select structural rearrangements.

Results: Eighty-six CRC were sequenced over 9 months. The most commonly mutated genes, regardless of *RAS*/ *RAF* status, were *APC* and *TP53*, respectively mutated in 75% and 74% of cases. RAS/RAF mutations were found in 53 (62%) CRC; 42 (49%) cases harbored mutations in *KRAS* codons 12 (n=27), 13 (n=10), 61 (n=1), 117 (n=1), and 146 (n=3), 10 (12%) cases had *BRAF* mutations in codons 600 (n=7), 466 (n=1), 469 (n=1) and 601 (n=1), while 1 case had an *NRAS* mutation in codon 61. Thirty-three (38%) were *RAS*/ *RAF* WT CRC. Several oncogene alterations were unique to this WT group, including mutations in the ras homolog family member *RHOA* (Y34F, G62E), a MAP2K1 (MEK1) mutation (K57E), and activating in-frame fusions of tyrosine kinase receptors (*ERBB2-GRB7*, *RET-NCOA4*) previously reported in other cancers. While these alterations were limited to the *RAS*/ *RAF* WT CRC, potentially actionable alterations common to both *RAS*/*RAF* mutant and WT CRC were also observed, including amplifications of *MET* (n=5), *ERBB2* (n=4), and *FGFR1* (n=1); and hotspot mutations in *PIK3CA* (n=14).

Conclusions: More comprehensive NGS-based testing of CRC in routine clinical practice identified *RAS*/ *RAF* mutations, including rare mutations in *NRAS*, *KRAS* exons 3 and 4, and *BRAF* exon 11 implicated in primary resistance to anti-EGFR therapy, in 62% of cases. Alternative potential targetable driver oncogene alterations that may predict resistance or response to targeted therapies were also detected.

1834 Value of High Throughput Screening for Predictive Biomarkers in Lung Oncology Using a Large Collection of Tissue Microarrays

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Background: Immunohistochemistry can provide a valuable screening tool for "druggable" genomic alterations in lung cancer by taking advantage of a growing list of commercialized mutation-specific antibodies. However, some of these alterations can be rare leading to test hundreds of samples to get positive cases. We assessed the value of a large collection of NSCLC tissue microarrays to screen by IHC the expression of targets of interest such as *EGFR* mutations (p.L858R and p.E746_A750del exon 19 deletion), *BRAF* p.V600E mutation, *ROS1* and *ALK* gene rearrangements, c-MET overexpression, and *NRAS* p.Q61R mutation.

Design: Tissue microarrays (TMAs) constructed from 2650 NSCL (1850 non epidermoid and 800 epidermoid carcinoma) were screened with mutated *EGFR, BRAF*, *ALK*, *c-MET* (Ventana) *ROS1* (Cell Signaling Technology) and *NRAS* (Spring Bioscience) antibodies. IHC assessment was made blindly by 4 pathologists or by using the Calopix (Tribvn, France) software. Positive IHC results for *EGFR*, *BRAF*, *ROS1*, *ALK* and *NRAS* was correlated with molecular biology approaches (pyrosequencing or FISH).

Results: 11%, 3%, 1.9%, 2.3%, 0.5%, and 0.1% of NSCLC were positively stained with *EGFR*, *BRAF*, *ROS1*, *ALK*, *c-MET*, *NRAS* antibodies, respectively. The concordance rate with the molecular biology approaches range between 91% and 99% according to the selected genomic alteration.

Conclusions: IHC on TMAs can be useful to screen rare genomic alterations in lung carcinoma, particularly in order to set up clinicopathological and epidemiological studies on large cohort of patients. TMA is emerging as an essential tool in the discovery and validation of tissue biomarkers for use in personalized medicine.

1835 Histopathologic Distribution of Cancers in a Nigerian Tertiary Hospital

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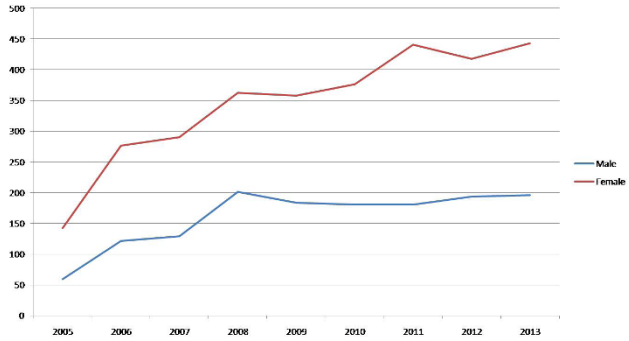
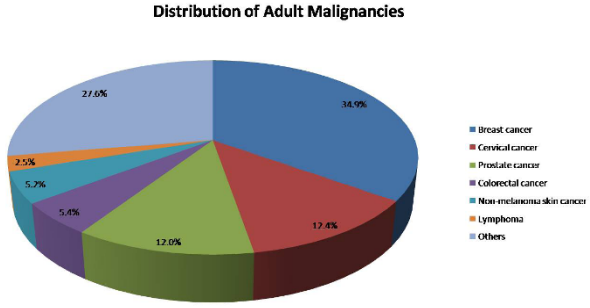
Background: There has been a significant increase in the incidence of cancer in sub-Saharan Africa with an estimated 100, 000 cases occurring yearly in Nigeria. However there are considerable difficulties in sustaining cancer registration in developing countries and many cancer registries in Nigeria have suffered setbacks over various years.

Design: This is a retrospective study carried out to review and categorize all malignancies received in our department from January 2005 to December 2013.

Results: A total of 4548 malignancies were analyzed during the study period. There was a general increase in cancer incidence over the 9 years of study (Figure1). The male to female ratio was 1:2.1 for all malignancies. Carcinomas accounted for 84.3% of cases, sarcomas 7.1%, lymphomas 4%, blastomas 1.7%, melanomas 0.7% and other hisologic subtypes 2.2%. The peak age of occurrence was the 60s for men, 40s for women and under 5 for children. The distribution of malignancies in adults is shown in figure 2. Chilldhood cancers accounted for 3.6% of all malignancies and had no significant sex predilection. The commonest childhood cancers were retinoblastoma (21.3%), nephroblastoma (15.2%), lymphoma (9.8%) and rhabdomyosarcoma (9.8%).





Conclusions: Cancer incidence in Lagos, Nigeria is on the rise. The establishment of comprehensive cancer control programmes for breast, cervical, prostate and colorectal cancers is imperative.

1836 Multicenter Evaluation of the Immunohistochemical Approach for the Detection of the NRASQ61R Mutation in Patients With Metastatic Melanoma

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Background: Somatic mutations in *NRAS* gene have been detected in 13–25% of all malignant melanomas. Among these mutations, the Q61R *NRAS* mutation occurring in exon 2 is the most frequent. It is of strong interest to routinely assess the *NRAS* status in patients with metastatic melanoma since *NRAS*-activating mutations confer resistance to RAF inhibitors. Moreover, *NRAS* mutated patients appear to be sensitive to MEK inhibitors in phase I and phase II trials. A mutation-specific antibody targeted against mutated $NRAS^{Q61R}$ has been recently developed. The aim of this study was to assess the diagnostic accuracy of the $NRAS^{Q61R}$ antibody on FFPE tissue samples from patients with metastatic melanoma.

Design: This retrospective multicenter study compared the performance of IHC with DNA sequencing in identifying the $NRAS^{Q61R}$ mutational status in 172 FFPE specimens from metastatic melanoma, including primary and metastatic tumors. Automated IHC assay was performed by using the SP174 clone.

Results: DNA sequencing detected either a Q61R mutation in 24% (42/172) or a non-Q61R mutation in 14% (24/172). Evaluation of a test cohort (10 cases) with knowledge of mutation status established a specific IHC pattern for the mutation: cytoplasmic, sometimes with membrane reinforcement, with moderate 2+ or strong 3+ intensity in ≥50% tumor cells. Using this pattern, pathologists at 3 levels of training independently performed blinded evaluation of the remaining cases. The IHC SP174 assay accurately identified all $NRAS^{Q61R}$ mutated tumors, and demonstrated 100% sensitivity and specificity.

Conclusions: $NRAS^{Q61R}$ IHC is reliable and specific for the evaluation of mutational status in metastatic melanoma and may be an alternative to molecular biology in metastatic melanoma.

1837 DNA Methylation in Endocrine Therapy Resistant Breast Cancer

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Background: Therapy resistant tumors may develop through several routes in women who receive endocrine therapy for estrogen receptor (ER) positive breast cancer. DNA promoter methylation leading to gene silencing is one mechanism implicated in acquired endocrine-resistance, but remains poorly understood. Accordingly, we compared DNA methylation in paired primary and recurrent breast tumors to elucidate markers and mechanisms that may be associated with therapy failures and potential targets for targeted therapies.

Design: A total of 70 breast tumor tissue blocks were obtained including 25 matched primary and recurrent pairs. DNA was extracted from 3 macro-dissected FFPE tissue

sections per tumor and methylation was assessed using the Illumina HM450 BeadChip. Methylation of primary and recurrent tumors, sorted by ER status, were compared using Mean β values (degree of methylation).
Results: Among all primary tumors, ER+ tumors had higher mean β values than ER- tumors. Furthermore, ER+ primary tumors from women with an ER+ recurrence(n = 34 tumors) showed slightly greater mean methylation than the ER+ primary tumors from women with an ER-recurrence(n = 13 tumors). However, in this group(ER+ primary to ER- recurrent), methylation was significantly increased in the ER- recurrent tumors. Specifically, 97% of the top deferentially methylated CpGs(dmCpGs) in the set showed greater methylation in the ER- recurrent tumor as compared to the ER+ primary tumor. In contrast, when both primary and recurrent were ER+, only 57% of the top dmCpGs showed increased methylation in the recurrent tumor. Likewise, when both primary and recurrent were ER-, only 29% of top dmCpGs were more methylated in the recurrent. Among the top CpGs in ER- recurrent tumors from women who had ER+ primary tumors, only two CpGs had decreased methylation in the recurrent tumors: Both were in AFF3, a gene shown to be upregulated in breast cancer. Heat map analysis of AFF3 reveals an overall methylation increase in the ER- recurrent tumors, with the greatest increase occurring in promoter and 5'UTR regions.
Conclusions: To our knowledge, this is the first report showing that DNA methylation is significantly increased in ER- recurrent tumors occurring in women whose primary breast tumor was ER+. However, our results confirm earlier reports of greater methylation associated with ER+ as compared to ER- breast tumors. These observations may affect selection of patients for treatments targeting gene methylation.

1838 Comparison of Protein Biomarker Expression in Bronchoalveolar Lavage (BAL) Between Primary Lung Adenocarcinoma and Squamous Cell Carcinoma To Improve Detection of NSCLC

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Background: Following the findings of the National Lung Cancer Screening Trial (NLST), the detection of lung cancers has been substantially improved with the use of highly sensitive low-dose computed tomography (LDCT). However, NLST also found high repeat screening rates and high false-positive rates, causing unnecessary second-line invasive procedures and/or surgery. New strategies are still needed to improve the specificity of lung cancer screenings. Bronchoalveolar lavage (BAL) is commonly used for the cytological evaluation of lung parenchymal cells in patients with a suspicion for lung cancer. Our preliminary study has shown that proteins in the BAL fluid can be analyzed to identify potential protein biomarkers. In this study, we have further evaluated the protein signature between lung adenocarcinoma (ADC) and squamous cell carcinoma (SqCC), and the potential utility of these signatures as biomarkers for the diagnosis of lung cancers.
Design: BAL samples from patients with benign lung disease, lung adenocarcinoma, and lung squamous cell carcinoma, were collected and analyzed by using SPEG and LC-MS/MS techniques. After initial identification, we further tested sensitivity and specificity of a subset of proteins using independently-collected BAL specimens by ELISA assays.
Results: A total of 462 glycoproteins were identified and quantified from BAL fluids of both ADC and SqCC patients . Among them, 290 proteins were identified in the BAL of lung ADC, 376 in lung SqCC, and 318 in benign BALs. In addition to proteins found in all groups, we identified 123 unique proteins that were differentially expresssed exclusively in either benign disease, ADC or SqCC. The levels of several proteins in cancer and benign BALs were further validated by ELISA assays, using an independently collected subset of BAL specimens. Most notably among these proteins, periostin was significantly elevated in cancer BAL specimens compared to levels seen in benign lung diseases.
Conclusions: Proteins in the airway fluid (i.e. BAL fluid) from lung cancer patients can be detected by a highly sensitive proteomic approach. They can be used as protein biomarkers to enhance the detection of lung cancers, therefore improving the specificity of lung screening tests.

1839 Cellular and Epiallelic Composition of Oral Fluid

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Background: Expectorated human oral fluid (a.k.a. saliva, spit) is a popular, facile, non-invasive specimen for constitutional genome sequencing, epigenomic analyses, epidemiological studies, and nucleic-acid-based detection of pathologic conditions. Despite the prevalence of oral fluid molecular profiling, there are few published reference values for cellular composition in routine expectorated oral fluid DNA extracts; in addition, CpG methylation reference levels based on a large population of individuals are not well established for detecting constitutional or malignancy-associated epimutations as may be present in oral fluid.
Design: Oral fluid DNA methylation profiles from >300 specimens were derived from bisulfite microarray analysis of >450K CpG targets, for cellular composition and proportions, and for methylation levels of several genes subject to epimutation in the human population.
Results: Principal component analysis was consistent with a two-lineage mixture of leukocytes and buccal mucosal epithelial cells (BECs), and further showed that leukocytes outnumber desquamated epithelial cells several fold. Additional comparisons incorporating purified lymphoid and myeloid white blood cell subsets revealed that the oral fluid leukocyte component is largely derived from neutrophils (PMN), while lymphocyte-specific epialleles were essentially undetectable by DNA methylation microarray in whole oral fluid DNA extracts.
Conclusions: Thus, oral fluid molecular profiling captures the same predominant cell component as blood—the neutrophil-- while epithelial cells comprise ~20%. Differentially methylated targets contributing to oral fluid composition are significantly enriched for enhancers of genes that function in leukocyte and epithelial cell biology. Pediatric versus adult age, but not sex, was found to have a significant correlation with the epithelial/leukocyte ratio in oral fluid. Overall, our results demonstrate that expectorated oral fluid is a biological resource with a definable cell composition and copious DNA yields, and provide reference values for leukocyte and epithelial fractions for molecular profiling studies. Additionally, oral fluid DNA methylation reference databases are useful assets for estimating population frequency of constitutional epialleles/epimutations.

1840 Spectrum of Notch Gene Mutations in Diverse Human Cancers

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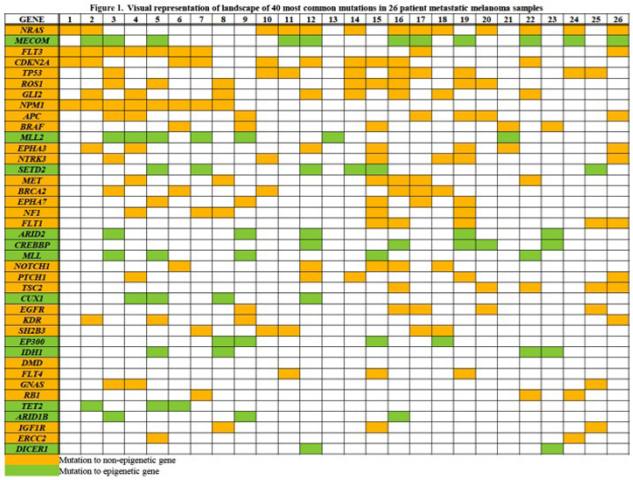
Background: Oncogenic Notch signaling is implicated in diverse cancers and is an attractive therapeutic target, but context specific tumor suppressive roles for Notch have also been recognized. Patient selection for clinical trials of Notch inhibitors requires a more complete understanding of the spectrum of oncogenic and tumor suppressive Notch driver mutations in cancer.
Design: DNA was prepared from FFPE tumor specimens from adult patients at the Dana Farber Cancer Institute. Exons 1-34 of Notch1 and Notch2 were captured and subjected to next generation sequencing on an Illumina Hi-Seq 2000 instrument. Single nucleotide variants and InDels were identified by comparing tumor sequences with reference DNA after filtering germline SNPs (NCBI, dbSNP). Non-SNP sequence variants were called gain-of-function, loss-of-function, or unknown significance based on prior structure-function analyses.
Results: Sequencing was carried out on 4,092 tumors, including diverse carcinomas, sarcomas, lymphomas, and leukemias. 617 sequence variants were identified, 376 involving Notch1 and 241 involving Notch2. Gain-of-function mutations were identified in ~0.5% of tumors, including T-ALL (7), adenoid cystic carcinoma (5), B cell lymphomas and leukemias (3), breast adenocarcinoma (2), melanoma (1), lung adenocarcinoma (1), and carcinoma of unknown primary (1). One adenoid cystic carcinoma had dual gain-of-function mutations, a pattern previously described only in T-ALL, and all 5 Notch-mutated adenoid cystic carcinomas had high-grade features. In evaluable cases, gain-of-function mutations were associated with high levels of activated Notch in tumor cells, judged by IHC staining. By contrast, loss-of-function mutations were seen in 1.8% of tumors (71 tumors total), of which 46 (65%) were squamous cell carcinomas arising from skin, oropharynx, lung, anus, and salivary gland. The large majority of sequence variants (85%) were in-frame SNVs of uncertain significance that mapped throughout Notch1 and Notch2.
Conclusions: These findings are consistent with the emerging view that Notch can function as either an oncogene or a tumor suppressor in a context-specific fashion. Gain-of-function mutations are enriched in T-ALL, peripheral B cell tumors, and adenoid cystic carcinoma, whereas loss-of-function mutations are prevalent in squamous cell carcinoma. Most sequence variants are of uncertain functional significance and may be passenger mutations acquired stochastically during tumor evolution and progression.

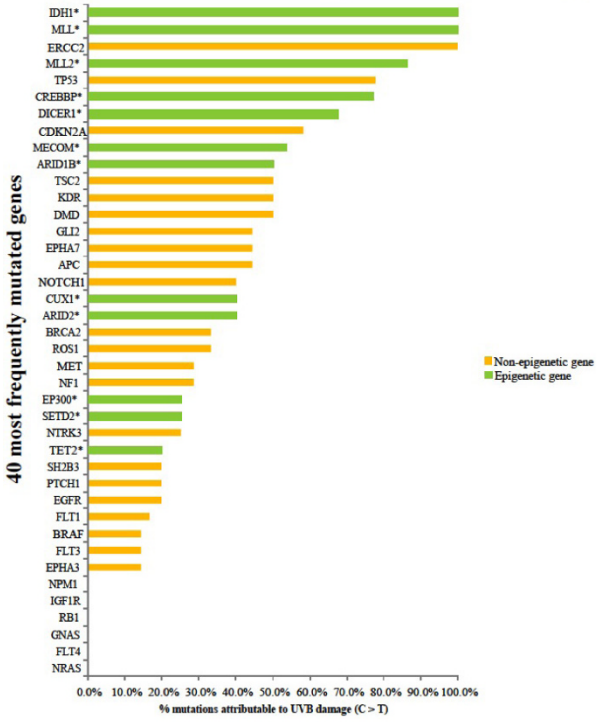
1841 Targeted Next Generation Sequencing of Treatment-Naïve Metastatic Melanoma Specimens Reveals High Frequency of Epigenetic Alterations

Jonathan Lee, Neal Lindeman, Gary Chin, Jason Luke, Patrick Ott, F Stephen Hodi, Scott Granter, Alvaro Laga, Charles Yoon, Harley Haynes, Andrew Werchniak, Jennifer Lin, Robert Liu, Nancy Bailey, George Murphy, Christine Lian. Brigham & Women's Hospital and Harvard Medical School, Boston, MA; Dana-Farber Cancer Institute, Boston, MA.
Background: Novel genomic sequencing technologies have advanced our understanding of the mutations underlying human malignancy. Melanoma is a prototype of a virulent, genetically heterogeneous cancer that is notorious for developing chemoresistance. In addition, evidence is accumulating that dysregulated epigenetic mechanisms may play an important role in melanoma pathogenesis. We sought to characterize the frequency of mutations in epigenetic regulators in clinical melanoma specimens obtained at our institution.
Design: 26 metastatic melanoma tissue samples were obtained from 26 treatment-naive patients. Targeted next generation sequencing detected mutations in the exons of 275 known cancer genes with clinically actionable potential or significant scientific prominence.
Results: 20.2% (107 of 530) of all mutations affected an epigenetic regulator. The most frequently mutated genes were *MECOM, FLT3, and NRAS.*



Of the 40 most frequently mutated genes, 12 (30%) encoded epigenetic regulators, including histone modifying (*MLL, CREBBP*), chromatin remodeling (*ARID1B, ARID2*), active DNA demethylation (*TET2, IDH1*), and microRNA processing (*DICER1*) enzymes. Among the 26 melanoma samples, 25 (96.2%) harbored at least one mutation in an epigenetic regulator. In addition, mutations to epigenetic genes had the highest frequency of C to T transitions, reflecting putative UVB damage.

Figure 2. Percent of mutations to gene attributable to UVB by gene



Conclusions: Our study provides direct genomic evidence that epigenetic regulators involved in various mechanisms and processes may be involved in the pathobiology of melanoma. In addition we identified that *MECOM*, a novel chromatin remodeling/transcription modifying enzyme, and *TET2/IDH1*, critical enzymatic and metabolic regulators of DNA demethylation, are more frequently mutated in patient metastatic melanoma samples than previously reported.

1842 Profile: Exome Sequencing of 4027 Cancer Samples in a Clinical Laboratory

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Background: The PROFILE program between the Brigham & Women's Hospital (BWH), Dana Farber Cancer Institute (DFCI), and Boston Children's Hospital (BCH), performs genomic analysis for all cancer patients presenting to the three hospitals. Here are the results from the 275-gene next-generation sequencing (NGS) assay performed in the BWH clinical laboratory during the first year of the program.

Design: Cancer samples from all consenting patients presenting to the BWH/DFCI/BCH cancer center with a block, smear, or fresh sample with >2mm/>10% invasive cancer yielding >50 ng of DNA were tested. NGS (Illumina 2500) was performed on custom hybrid capture (Agilent) libraries constructed from all 4430 exons from 275 selected genes. A custom bioinformatics suite of open source and internally developed tools was used to detect, display, annotate, interpret, and report single nucleotide variants, insertions/deletions, gains/losses, and structural variants. Alterations were classified by clinical significance, from Tier 1 (established predictive/prognostic/diagnostic marker) to 5 (benign polymorphism). Tiers 1 and 2 were considered actionable.

Results: Between 9/1/13-8/18/14, 4027 samples were tested, while 157(4%) failed. All cancer types were tested, led by lung (661), CNS (437), heme (322), and breast (289). The most commonly altered genes were (a) mutation: *TP53, MLL2, APC, PIK3CA;* (b) large gain: *EGFR, MDM2, MYC, ERBB2, CCND1*, *CDK4* (c) biallelic loss: *CDKN2A, CDKN2B, PTEN, STK11*, *RB1*; subtle gains and losses were seen in many genes. 19% of samples had an actionable Tier 1 mutation, and another 42% of samples had a Tier 2 mutation (actionable in clinical research).

Conclusions: Massively mutliplexed genetic sequencing of every cancer patient presenting to a tertiary care medical center, performed within a clinical laboratory that also performs other routine testing, is feasible and useful. ~80 analyses/week were performed, assessing full coding sequence of 275 genes, with pathologist review and interpretation. ~60% of samples had at least one result that could lead to a change in medical treatment, either by directing an established therapy, a therapeutic trial, refining prognosis or diagnosis, or referral for genetic counseling related to hereditary cancer risk. The spectrum of mutations discovered was largely consistent with previous knowledge. Anecdotally, several instances of dramatic shifts in therapy and outcome have arisen as a result of these tests, although a systematic assessment of the impact on patient care is forthcoming.

1843 Dealing With the Incidental: Experiences Implementing the ACMG Guidelines for Reporting Germline Findings in a Comprehensive Somatic Mutation Assay

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Background: Sequencing paired tumor and germline DNA facilitates identification of somatic mutations, but raises the prospect of revealing germline mutations of potential significance. The American College of Medical Genetics (ACMG) has made a series of recommendations regarding reporting of these findings.

Design: The Ion AmpliSeq™ Comprehensive Cancer Panel (Life Technologies, Carlsbad, CA), a whole exon next generation sequencing assay of 409 cancer related genes, was validated with an algorithm for germline variant subtraction. Custom software was used to create a database of germline and somatic variant calls and annotate against the COSMIC and dbSNP databases.

A multidisciplinary Incidental Results committee was formed to review the literature and develop a process for patient consent and consensus review of germline findings, and to develop a set of filters restricting an initially large population of germline variants to a number amenable to clinicopathologic correlation.

Results: 141 comprehensive cancer panels with paired tumor/normal samples were analyzed.

Germline variants were identified in all samples, covering 401 of 409 analyzed genes. 126,784 germline calls (mean: 899; range 759-1162) passed a set of basic quality filters. Filtering against the 1000 Genomes data set and a local database of common polymorphisms and sequencing artifacts reduced this to 17,974 which was further reduced to 435 using the the ACMG group of actionable germline genes. Finally, excluding silent and intronic variants and those present at >2% population frequency resulted in 44 germline variants.

Manual review of this final set against public knowledgebases and predictive analysis identified 3 germline mutations warranting further investigation: an *APC* deletion, an *MSH6* deletion, and a *TP53* p.R280S mutation which is well-described as somatic but has not previously been shown to be germline. Further investigation confirmed the presence of familial adenomatous polyposis in the *APC* mutant patient, and strongly implicated Li-Fraumeni syndrome in the *TP53* mutant patient, who had developed sarcoma and lymphoblastic leukemia before age 30.

Conclusions: Sequential computational filters can effectively limit the germline variants identified during paired tumor/normal sequencing to levels amenable to clinicopathologic correlation. We suggest a proactive and interdisciplinary approach to address the issues of informed consent, patient education, and consensus review for incidental germline findings of potential importance.

1844 Androgen Receptor Signaling Mediated By a Novel Fusion Mutant Discovered in Men With Castration-Resistant Prostate Cancer

Changxue Lu, Yan Chen, Mary Nakazawa, Jun Luo. Johns Hopkins University, Baltimore, MD.

Background: Sustained androgen receptor (AR) signaling is a key determinant of castration resistance in men with advanced prostate cancer. Although multiple mechanisms underlying aberrant AR signaling have been characterized, a better appreciation of the diversity and heterogeneity of resistant mechanisms will require additional investigation. RNA sequencing has emerged as a major discovery platform for novel transcripts that may mediate aberrant AR activation in the castrate setting.

Design: We performed RNA-Seq using metastatic castration-resistant prostate cancer (mCRPC) samples along with normal and tumor samples derived from untreated radical prostatectomies.

Results: Here, we report the identification and characterization of a novel AR mutation identified in a metastatic tumor sample. This newly discovered AR mutant transcript is a fusion molecule containing AR exons 1, 2, and 3, followed by a 200bp sequence matched to a long interspersed elements L4 (LINE4) sequence distant from the AR gene locus. As a result, this novel fusion transcript encodes a truncated AR (called

AR-L4) consisting of AR N-terminal domain, DNA-binding domain, and a variant-specific peptide sequence. We have extanded the expression analysis of this novel fusion transcript in a set of mCRPC specimens. Although the overall frequency is low, AR-L4 is a recurring mutant. AR-L4 is mainly localized in the nuclei but also detected in the cytoplasm. By microarray analysis, AR-L4 is associated with at least 100 AR-related genes (including KLK3, NKX3.1, FASN, and TMPRSS2) in the absence of AR signaling mediated by the full-length AR, implicating a role in activating AR signaling when the full-length AR is potently inhibited by existing therapies.
Conclusions: These findings underscore the importance of laboratory discovery efforts in uncovering mechanisms of drug resistance pertaining to the novel AR-targeting therapies including abiraterone and enzalutamide.

1845 Over-Expression of S100A4 as Regulated By ß-Catenin Is Associated With a Higher Risk of Metastasis in Patients With Signet-Ring Cell Carcinoma

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Background: Aberrant expression of β-catenin, a key factor in Wnt signaling, plays a pivotal role in tumor progression and metastasis. S100A4, a downstream gene in the β-catenin/TCF transcription factor pathway, has well-established metastasis-promoting activity. As a small calcium binding protein, S100A4 drives metastasis by affecting the tumor microenvironment while stimulating cancer cell motility and invasion. It has been demonstrated that aberrant expression of β-catenin is associated with a higher risk of metastasis in signet ring cell carcinoma. The role of S100A4 is now further examined.
Design: Forty-five cases of SRCC or carcinoma with prominent signet-ring features from various organ systems were retrieved from the authors' institution. The immunoexpression of β-catenin and S100A4 was examined. Nuclear/cytoplasmic subcellular localization of β-catenin was scored as demonstrating aberrant expression. At least a 25% increase in intensity of S100A4 was considered as positive when compared to normal tissue.
Results: Among the 45 cases, 24 out of the 32 (75%) with aberrant expression of β-catenin had lymph node and/or distant metastasis while 5 out of 13 (38%) with membranous expression had metastasis ($p<0.05$). Thirty cases expressed aberrant β-catenin combined with increased S100A4 and 14 of these (47%) had metastasis. This is in contrast to the remaining 15 cases without aberrant β-catenin plus increased S100A4 where only 2 (13%) had metastasis ($p<0.05$).
Conclusions: SRCCs with combined aberrant β-catenin and increased S100A4 had a higher rate of metastasis (Pearson Chi-Square p=0.0463). This finding not only provides new insight into the role of the Wnt/β-catenin pathway in the progression and metastasis of SRCCs but also has implications for therapeutic targeting and functional blockade.

1846 Precision Cancer Medicine Program for Whole Exome Sequencing (WES) of Metastatic Tumors Reveals Biomarkers of Response

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Background: Next generation sequencing (NGS) has provided enormous insight into the genomic landscape of several tumor types, illuminating molecularly-defined subtypes, identifying new druggable targets and providing insights into heterogeneity and evolution of many tumors. Various NGS assays are starting to translate into clinical practice. We describe our multi-disciplinary WES-based Precision Medicine program designed to aid in therapeutic decision making and fuel translational research.
Design: Metastatic and treatment resistant cancer patients are prospectively enrolled for paired metastatic tumor and germline WES. Using a comprehensive computational pipeline, point mutations, indels and copy number alterations are detected. Mutations are categorized in Category I-III based on actionability, and a report is generated and discussed in tumor board. Patients are followed prospectively for correlation of molecular information with clinical response and patient outcomes. A rapid response functional team was established to follow up on key findings to expand actionability based on the WES results.
Results: In the first year, 156 tumor-germline pairs from 97 patients were sequenced with an average coverage of 95x and 16 mutations detected per patient. Among these, 16 were Category I, 98 were Category II and 1,474 were Category III. Tumor purity ranged from 14-100%. An unexpected finding, a prostate cancer "exceptional responder" was identified that harbored a somatic hemizygous deletion of the DNA repair gene *FANCA* and likely loss of function of the second allele through germline missense variant. Follow-up experiments established that loss of FANCA function was associated with platinum hypersensitivity *in vitro* and in a patient derived xenograft, thus providing biologic rationale for his extreme clinical response.
Conclusions: Understanding molecular mechanisms of response and resistance to anticancer therapies requires prospective patient follow-up and clinical and functional validation of both common and low frequency mutations. We describe a multi-disciplinary precision medicine program designed to bring WES into clinical practice and illustrate how this can be utilized to identify novel predictive biomarkers associated with exceptional systemic response.

1847 Adenomatous Polyposis Coli (APC) Promoter Hypermethylation in Pulmonary Hamartomas Contributing To a Malignant Phenotype

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Background: Pulmonary hamartomas (PHs) are benign clonal neoplasms. Although PHs do not transform to malignancy, high frequency of coexistence of PHs and lung cancer has been reported. Epigenetic alterations such as abnormal DNA methylation are associated with many human cancers. In previous study, we found that *APC* and *RASSF1* methylation levels were generally higher in tumors without associated PHs than tumors with PHs, but not significantly. The aim of the present study is to investigate *APC* promoter hypermethylation in PHs coexistent with lung cancer.
Design: We evaluated *APC* promoter methylation levels in 52 patients diagnosed with PH from 1993 to 2014. *APC* gene promoter methylation was quantified using pyrosequencing.
Results: Among 52 patients, there were 26 males and 26 females with mean age of 63 (range 35- 90 years). Thirty of 52 (58%) PHs were without associated cancer, 13 of 52 (25%) PHs had coexistent lung cancer and 9 of 52 (17%) PHs had cancer in other organs other than the lung. The coexistent lung cancer includes: adenocarcinoma (8), squamous cell carcinoma (2), mesothelioma (1), small cell carcinoma (1), and lymphoma (1). Ten of 13 (77%) patients who had PHs with lung cancer showed same lobe involvement and 11 of 13 (85%) had synchronous lung cancer. The average percent promoter methylation in *APC* was higher in PHs with associated cancer than those without (p= 0.002). A significant level of hypermethylation is found at CpG sites 148 (p=0.02), 129 (p=0.001) and 113 (p=0.02) bp upstream of exon 1. At the CpG site 129 bp upstream of exon 1, 8 of 13 (62%) PHs with associated lung cancer showed hypermethylation as compared to 9 of 30 (30%) PHs without associated lung cancer. We also found an interesting association of *APC* hypermethylation in PHs with other cancers including known breast (2), colon (1), prostate (1), kidney (1), liver (1) and sarcoma (2). All the patients (9/9) among this group showed hypermethylation of *APC* (p= 0.002).
Conclusions: Although pulmonary hamartomas are benign neoplasms, there is an associated increased risk of cancer and warrants thorough clinical work-up. To our knowledge, these are the first studies to evaluate the epigenetic characteristics of PHs and association with cancer. A significant percentage of PHs associated with cancer including lung and other organs show *APC* promoter hypermethylation at specific CpG sites. Promoter methylation of *APC* in PHs provides the tumoral background possibly by contributing to a general loss of cell cycle control and aberrations in other cell functions.

1848 BK Virus PCR Testing in Kidney Transplant Patients

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Background: BK virus associated nephropathy (BKVAN) develops in 8-10% of renal transplant recipients. Although often asymptomatic, signs and symptoms may include fever, hematuria or elevated creatinine. Fifty to 80% of those who develop BKVAN progress to graft dysfunction or failure. The gold standard for diagnosing BKVAN is the kidney biopsy. Immunohistochemistry for Simian Virus 40 (SV40) can be performed for confirmation in difficult cases. Unfortunatley histologic changes may be evident only after infection is well established. The presence of virus in the urine and blood often precedes the nephropathy by several months, can be easily detected by polymerase chain reaction (PCR), and may predict the development of BKVAN.
Design: The Wake Forest University Baptist Medical Center kidney transplant records were reviewed from January 2013 to April 2014 to identify a correlation between elevated viral levels in urine (>10,000 copies per milliliter) or blood (>500 copies per milliliter) and BK virus activity in kidney biopies. SV40 immunohistochemistry was performed on formalin fixed parafin embedded (FFPE) tissue in search of viral replication not identifed by routine light microscopy.
Results: Twenty kidney transplant recipients who developed BK viuria ranging from less than 500 to greater than 50 million copies per milliliter or viremia ranging from less than 500 to greater than 200,000 copies per milliliter were identified. None of the identified cases demonstrated evidence of BK virus replication in the renal parenchyma either by conventional histology or immunohistochemistry.
Conclusions: Despite development of viremia or viuria no evidence of viral activity could be found via routine histologic examination or immune methods. The most common diagnosis at kidney biopsy was acute tubular necrosis and preexisting disease (e.g. diabetes, hypertension). PCR results did not correlate with histology and could not predict development of BKVAN.

1849 Subtle Microsatellite Instability in Endometrial Cancer Is a Potential Diagnositc Pitfall for Lynch Syndrome

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Background: Loss of mismatch repair mechanisms (MMR) and microsatellite instability (MSI), features of Lynch syndrome (LS) associated tumors, occur in 15-30% of colorectal carcinomas (CRC) and endometrial carcinomas (EC). Immunohistochemical (IHC) staining for MMR proteins & PCR analysis of MSI are commonly used to screen patients for further LS testing. It is standard practice to designate a specimen as MSI when PCR identifies ³3 base pair shifts (BPS) in ³2/5 microsatellite loci. We observe subtle MSI in EC with loss of MMR by immunohistochemistry (IHC) that are easy to misinterpret as microsatellite stable (MSS). In order to characterize this deficit, we reviewed PCR data in CRC & EC and correlated with IHC.
Design: We analyzed 238 consecutive CRC (109) & EC (129) specimens by IHC for loss of MMR (MLH1, MSH2, MSH6, and PMS2) and by PCR for MSI (NR21, NR24,

NR27, BAT25, BAT26). IHC revealed loss of MMR in 23% of CRC and 29% of EC. PCR results were correlated with IHC findings to identify the optimal criterion for identifying MSI in EC.

Results: Median cumulative BPS for all markers were 2 and 6 in MSI positive EC and CRC, respectively. BAT26 was the most sensitive (100%) and specific (95%) microsatellite for both EC and CRC. Defining MSI as ³1 BPS in ³3 loci demonstrated optimal sensitivity (97%) and specificity (99%) for detecting MSI in EC. This criterion also demonstrated 100% sensitivity and 99% specificity in CRC. Using ³3 BPS in ³2 loci failed to identify 16/37 EC and 1/25 CRC with loss of MMR, with four of the 16 EC showing loss of MSH6. Our revised criteria for MSI correctly identified all but one EC with loss of MRR.

Conclusions: We have found that the accepted threshold for classification of MSI is sufficiently sensitive and specific in CRC. However, based on our findings small BPS in EC often do correlate with loss of MMR proteins by IHC, and may be missed by laboratories only utilizing PCR to screen for LS. Based on our data, this practice may result in erroneously designating 43% of EC as MSS. Many of these cases showed loss of MSH6 by IHC, characteristic of LS. This represents a pitfall in the screening for LS by MSI evaluation. We speculate that the decreased sensitivity of MSI evaluation in EC results from the earlier presentation and biopsy of these tumors over CRC, although further studies and clinical correlation are required.

1850 COMPAREDx: A Prospective Study Comparing Molecular Versus Histopathologic Accuracy in the Classification of Metastatic Tumors

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Background: Accurate tumor classification is essential to the application of biomarker testing and individualized care. Alongside the reported limitations of traditional methods of tumor classification such as IHC, there is an increasing requirement for molecular characterization of cellular context to enable precision medicine. Gene expression-based profiling is a validated technology for molecular classification of tumors, with reported accuracies in the range of 80% -90%, and initial studies have demonstrated its improved diagnostic accuracy versus IHC. In addition, inter-pathologist concordance has not been comprehensively characterized across a wide range of tumors in a systematic manner. The aim of COMPAREDx is to characterize key issues along the spectrum of diagnostic ambiguity such as inter-pathology concordance, limitations of IHC, and the integration of molecular profiling, with the goal to improve diagnostic certainty.

Design: This is a prospective, blinded study of approximately 400 cases, designed with two main objectives: 1) Determining the non-inferiority, and potential superiority, of molecular tumor classification by the 92-gene assay versus IHC across a study population of tumor types that consists of 96% of cancers based on incidence and 2) To characterize inter-pathologist concordance in diagnoses.

Results: The study utilizes a landmark approach through the creation of a web-based, digital pathology interface linked to a scalable database containing the results of 76 different IHC markers per case. The ability to interpret the IHC results in real-time will maintain the diagnostic continuity and permit the accurate capture of diagnostic certainty data through survey-based questions at the end of each case. The COMPAREDx web interface allows for remote access and review, and will evaluate the impact of differences in clinical experience, region, and practice setting on diagnostic accuracy.

Conclusions: COMPAREDx is a comprehensive comparator study measuring the diagnostic accuracy of immunohistochemistry vs a molecular 92-gene RT-PCR assay for the classification of a broad range of solid tumor types. The COMPAREDx web portal is a novel and scalable resource for assessing important parameters in diagnostic accuracy and diagnostic decision-making for poorly differentiated and metastatic tumors.

1851 BRAF Gene Fusions Occur Across a Diverse Subset of Human Solid Tumors and Respond To RAF Kinase Inhibitors

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Background: *BRAF*, a member of the RAS/RAF/MEK signaling pathway, has emerged as a major oncogenic driver and a potential target of therapy in a wide variety of solid tumors and hematological malignancies. *BRAF* V600E is an approved target for the BRAF inhibitors dabrafenib and vemurafenib in melanoma. *BRAF* gene fusions, which have been described in several solid tumor types, however, represent a different mechanism of BRAF activation. The use of targeted therapies against these *BRAF* fusion alterations has been limited despite their potential as a promising drug target.

Design: To determine the prevalence of *BRAF* fusions, a diverse series of 20,573 malignant tumors, including both solid tumors and hematological malignancies, underwent comprehensive genomic profiling for hundreds of known cancer genes using the FoundationOne™ or FoundationOne Heme™ next generation hybrid capture-based sequencing assays.

Results: *BRA*F fusions involving the intact BRAF kinase domain were observed in 57 (0.3%) of 20,573 tumors, across 15 distinct tumor types. These comprised 35 unique 3' fusion partners, of which 24 (43%) were known and 32 (57%) novel. The most common tumor types harboring *BRAF* fusions included 14/701 (1.9%) in gliomas, 12/531 (2.3%) in melanomas, 8/4,013 (0.2%) in NSCLC, 4/2,154 (0.2%) in CRC, 3/1,062 (0.3%) pancreatic carcinomas, and 3/214 (1.4%) thyroid cancers (3 cases). Pilocytic vs non-pilocytic gliomas ($p<0.001$), Spitzoid vs non-Spitzoid melanomas ($p=0.0015$), acinar vs non-acinar pancreatic cancers ($p<0.001$) and papillary vs non-papillary thyroid cancers ($p<0.001$) were all enriched for *BRAF* fusions. A *GTF2I-BRAF* fusion detected in a case of multiple myeloma, the only BRAF fusion identified to date in a hematolymphoid malignancy was not included in this study. Preclinical studies demonstrate that *BRAF* fusions result in activation of the mitogen activated protein kinase (MAPK) pathway which can be abrogated with MEK and CRAF inhibition. Clinical evidence of sensitivity of these *BRAF* fusions to the pan-kinase inhibitor sorafenib and other targeted therapies in combination with chemotherapy will be presented.

Conclusions: *BRAF* fusions are a rare driver alteration that develop in a wide variety of malignant neoplasms. *BRAF* fusions are most commonly encountered in gliomas, melanomas and NSCLC and appear to be associated with specific histologic subsets. Preliminary evidence suggests that *BRAF* gene fusions can be successfully targeted with pan-kinase and MEK inhibitors.

1852 POLE Expression and Integrative Genomic Characterization in Colorectal Carcinoma (CRC)

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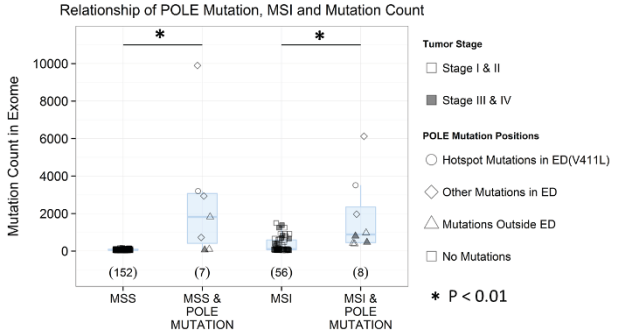
Background: Somatic and germline mutations in POLE, a DNA polymerase involved in replication and proofreading of DNA, have recently been detected in a subset of CRC. A subgroup of cases with POLE mutation originally classified with microsatellie instable (MSI) tumors as hypermutators by The Cancer Genome Atlas (TCGA) is now separated out as ultramutator (UM), with heightened mutation rates compared to MSI cases. These account for 6% of CRC. In other cancer types, UM have been associated with a favorable prognosis. We examine the role of POLE mutations as a prognostic biomarker in CRCs and their association with mutation rates, tumor stage and overall survival (OS).

Design: Tissue microarrays including 76 CRC and normal tissues were stained using a monoclonal anti-POLE antibody (ab134941, Abcam, Cambridge, MA, USA). The cBioportal for Cancer Genomics was used to access and visualize genomic data on 224 CRC, including 8 UM cases, and 7 non-UM POLE mutated cases, for point mutations, mutation frequencies, MSI and OS. The Wilcoxon rank-sum test was used to calculate the significance of associations.

Results: All CRC and normal tissues showed nuclear positivity, with decreased nuclear staining in only one cancer. The UM cancers uniformly possess POLE mutations within the exonuclease domain (ED) and are MS stable (MSS) or MSI-low. Both MSS and MSI cancers show a significant increase in mutation rates when POLE is mutated.



There is a trend towards lower stage in UM tumors. While the average mutation counts in the MS stable (MSS) cancers (n=152) are 4.8 fold lower than in the MSI cancers (n=56, $p=1.10\times10^{-7}$), no difference exists when POLE is mutated ($p = 1$). The POLE mutation status does not improve OS in the MSS or MSI groups ($p=0.8$).

Conclusions: POLE is constitutively expressed in CRC. The survey IHC suggests that POLE may not cause loss of the POLE protein; however, this requires further study of more tumors of known POLE status, using additional antibodies. POLE mutations increase mutation rates in both MSS and MSI tumors, but not all POLE mutated tumors are UM. Further study is needed on the impact of POLE mutation on prognosis.

1853 Differences in Biological Properties and Clinical Features of KRAS and GNAS Oncogenes in Pancreatic Cystic Disease

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Background: Pancreatic cysts, a potential early stage of pancreatic neoplasia, are increasingly recognized by radiologic imaging. Molecular testing of cyst fluid is an ancillary test that can help guide clinicians into appropriate management and treatment. Mutations of GNAS and KRAS are commonly seen in pancreatic cysts and are considered markers associated with potential malignant behavior. The aim of the study was to identify pathobiological differences in pancreatic cysts harboring GNAS and/or KRAS mutations.

Design: We analyzed data on pancreatic cyst fluids that were initially classify as indeterminate by cytology and radiology. We examined the associations between the presence or absence of KRAS and/or GNAS mutations and the occurrence of other indicators suggestive for carcinoma: 1)high CEA; 2)imagining with worrisome features for malignancy; 3)high DNA quantity; 4)non-degraded DNA; 5)cytology reported as "atypical" and 6)loss of heterozygosity (LOH). A pancreatic cyst with mutations of KRAS and/or GNAs and two or more additional indicators for malignancy were considered highly suggestive for aggressive behavior.

Results: We reviewed a total of 1036 pancreatic cyst fluids received in the last two years. Among these patients, 210 (16%) revealed mutations of KRAS, 84 (6%) mutations of GNAS and 119 (9%) mutations of both KRAS and GNAS. Pancreatic cysts with KRAS mutation were more often associated with presence of two or more additional criteria for aggressive behavior (table 1). This association was not identified

with GNAS mutations, and interestingly, neither with the presence of both GNAS and KRAS mutations. Pancreatic cysts with KRAS mutations were more likely to exhibit high DNA content and presence of LOH.

Indicators Suggestive for Malignancy	KRAS + GNAS n = 119 n (%)	KRAS (*)n = 210 n (%)	GNASn =84 n (%)	NONE n = 893n (%)
0 - 1	102 (86)	163 (78)	74 (88)	788 (88)
2 or more	17 (14)	47 (22)	10 (12)	105 (12)

(*) p value

Conclusions: To the best of our knowledge, this is the first study to provide evidence that suggests pancreatic cysts with GNAS and KRAS mutations are biologically and clinically different, with cysts having GNAS mutations behaving in a less aggressive fashion. We believe that our findings could guide clinicians into more personalized management of patients with pancreatic cysts based on the molecular findings. However, further studies including patient outcomes would be helpful in confirming these findings.

1854 EBV Infection of Oral Keratinocytes Leads To a Cellular Epigenetic Alteration That Is Retained After Loss of the Virus

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Background: Clinical observations raise the question whether EBV can contribute to the oncogenic process in the context of viral redundancy and loss. Various EBV genes are known to manipulate the host epigenetic machinery and cause epigenetic reprogramming of the host chromatin. We establish a transient model of EBV infection of immortalized normal oral keratinocytes (NOK) to investigate whether these virally induced epigenetic changes would be stable and heritable alterations to gene expression that would be maintained in the absence of the virus.

Design: A clonal human oral kerotinocyte cell line is infected with recombinant Akata EBV. EBV infectivity is monitored by in situ hybridization for EBERs. Then selective pressure is removed for additional passages to allow loss of EBV. Cells that lost EBV are single-cell cloned. For global DNA methylation profiling, Reduced Representation Bisulfite Sequencing (RRBS) was carried out on uninfected, vector control, an EBV-positive clone, and three EBV-negative transiently-infected clones. Transcriptional profiles were determined by microarrays. qRT-PCR was performed following treatment of cells with the DNMT inhibitor to determine if repression of gene expression was due to the acquisition of methylated CpGs.

Results: Analysis of global cellular DNA methylation identified over 13,000 differentially methylated CpG residues in cells exposed to EBV compared to uninfected controls. 65 cellular genes that acquired CpG methylation showed altered transcript levels. Genes with increased transcript levels frequently acquired DNA methylation within the gene body while those with decreased transcript levels acquired DNA methylation near the transcription start site. Treatment with the DNA methyltransferase inhibitor restored expression of some hypermethylated genes. Cytogenetic analysis does not show chromosomal alterations in EBV-positive and EBV-negative transiently-infected clones compared to uninfected controls.

Conclusions: Our observations suggest that EBV infection resulted in a cellular epigenetic reprogramming that was retained after loss of the virus. EBV infection is able to reshuffle the cellular epigenome and have long lasting consequences as a mechanism for hit-and-run oncogenesis. Unlike genetic alterations, these epigenetic changes can be reversed pharmacologically, providing therapeutic interventions to EBV-associated malignancies.

1855 Platelet Cloaking of CTCs Is a Key Driver in the Metastatic Cascade

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Background: Systemic spread of primary carcinoma resulting in metastatic disease is the main cause of death from solid tumours, yet the molecular mechanisms driving metatstasis are poorly understood. This study focused on the intermediate cells in the metastatic cascade, CTCs. Previously we showed that platelets, through direct interaction, aid survival and drive the metastatic profile of ovarian cancer cells. Here we sought to build on this work by examining if platelet cloaking of cancer cells is universal, whether previously seen EMT changes are a constant result and if we can identify genes that would characterise the interaction.

Design: We examined by flow cytometetry the interaction *in vitro* between platelets and 15 human cancer cell lines of different origin and metastatic potential. The EMT profile of cells 24hr post platelet exposure was assessed by morphology and gene expression analysis (RT-PCR). Whole transcriptome analysis (WTA) was performed using Affymetrix arrays on 2 ovarian cancer cell lines, SKOV3 and 59M, which have different EMT profiles; intermediate mesenchymal v's mesenchymal.

Results: We showed that platelet cloaking of cancer cells is universal, occurring across all 7 tumour types examined. However, it is heterogeneous with adhesion rates varying both across and within tumour types, from 34% (C33a-primary cervical cancer) to 83% (SKMES1-metastatic lung cancer). Changes indicative of EMT were seen in all cell lines. However, again they were heterogeneous in nature; with morphology changes akin to EMT observed at varying degrees across the cancer types. Also, there was no consistent pattern to the EMT-like gene expression changes seen. WTA showed that while there was a greater number of gene expression changes in SKOV3 cells (>32%), the biological processes affected in both cell lines were similar. We identified 30 genes that were commonly affected in both cell lines. Many of these 30 genes form part of an interlinking pathway, that is regulated by TGFß1 and associated with cell adhesion and ECM remodelling.

Conclusions: For the first time, we describe the universal nature of platelet cloaking and its role in driving metastasis. Additionally, we have identified a set of 30 genes that hold potential to be *in vivo* markers of this interaction and also therapeutic targets, allowing the establishment of therapies tailored to inhibit metastasis.

1856 CD133 Is a Putative Cell Surface Marker of Cancer Stem Cells (CSC) in Squamous Cell Carcinoma of the Uterine Cervix

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Background: Cervical Cancer is the leading cause of cancer related mortality in women in India with an overall survival of around 50-60%. Cancer Stem Cells (CSC), defined as self-renewing population of cells within a tumor that are able to initiate and maintain a tumor have been demonstrated in several solid cancers with implications for tumor biology. Our aim was to identify the surface immunophenotypic marker of Cancer stem cells in human tissue samples of squamous cell carcinoma of the uterine cervix.

Design: A portion of cervical biopsy from 26 patients with invasive squamous cell carcinoma of the cervix was taken in DMEM-F12 with 10% fetal bovine serum with antibiotics for establishing short-term primary cultures on which experiments were performed. Cells were grown under differentiating and under 'stemness' conditions. The Aldehyde dehydrogenase (ALDH) assay and cervicosphere assay were performed for identification of CSC. Fluorescence activated cell sorting (FACS) was performed for screening for various candidate cell surface markers including CD49f, CD133, CD44, CD105, and CD90. Immunocytochemistry was performed on cytospin smears, adherent cover slips, cell blocks and histological sections with the antibodies against pancytokeratin and CD133.

Results: Four short-term primary cultures were successfully established from biopsies of squamous cell carcinoma of the cervix. Under differentiating conditions, the cells grew as adherent monolayers with a doubling time of approximately 48 hours. Cervicospheres were generated and they showed upregulation of *VIMENTIN, NANOG, TWIST, SNAIL* and *SLUG* transcripts as compared to differentiated cells indicating that they were enriched for CSC. The cervicospheres showed 2-6 fold upregulation of CD49f and CD133. Cells were sorted into ALDH+ and ALDH- cells by FACS followed by screening for cell surface markers. CD133 was >20 fold upregulated in ALDH+ cells. In 16 tumor samples of cervical squamous cell carcinoma, CD133 positivity by flow cytometry ranged from 1.3-23% of the total population.

Conclusions: CD133 is a putative marker of Cancer stem cells in squamous cell carcinoma of the uterine cervix. Identification of a surface marker will enable further studies to discern the clinical significance of CSC in cervical cancer.

1857 Genomic Profiling of Adult Ewing Sarcoma Using Multiplex Targeted Next Generation Sequencing

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Background: Ewing sarcoma is a small round blue cell tumor characterized by chimeric fusions of *EWS* and *ETS* family transcription factors. While *EWS* translocations have been extensively studied, the somatic mutational landscape in adult cases remains largely unknown. We therefore sought to determine the frequency of recurrently mutated cancer genes in adult Ewing sarcomas using multiplex targeted next generation sequencing.

Design: We selected a cohort of 5 adult patients and macro-dissected areas of high tumor cellularity from FFPE blocks. Extracted DNA was then captured using a custom panel of 151 genes implicated in cancer and sequenced on an Illumina 2500 sequencer or MiSeq on a single lane using 2x101 bp reads; this assay averages a read depth of approximately 1000x. Data were analyzed for non-synonymous single nucleotide variants (SNVs) and indels using the Genome Analysis Toolkit. Probable somatic mutations were determined by removing all variants present in the NHLBI Exome Sequencing Project, 1000 Genomes Project, or dbSNP databases with mean population allele frequencies >1%; the COSMIC database was also reviewed to determine recurrence across other cancer types.

Results: Multiple mutations were identified in 4/5 cases (80%) with approximately 6 non-synonymous somatic alterations identified on average in each case. Identified mutations included predominantly SNVs (26/29 variants (90%)), and 3 frameshift substitutions; 6 variants were listed in the COSMIC database. Mutations were most frequently identified in *TP53* (3/5 cases (60%)). Mutations in *JAK1, JAK2*, and *JAK3* were identified in 2/5 cases (40%). Mutations in *DNMT3A*, *IDH1*, and *TET2* were also separately seen in 3/5 cases (60%).

Conclusions: *TP53* was the most frequently mutated gene among sequenced cases. This finding is consistent with recently published studies of pediatric Ewing sarcoma which also demonstrated a high frequency of *TP53* mutations where they were associated with an increased rate of tissue metastasis and adverse prognosis. The finding of common *TP53* mutations suggests that cooperating mutations in addition to *EWS* rearrangement are required for oncogenesis. Our analysis did not identify any currently therapeutically targetable mutations using standard agents for Ewing sarcoma, however the findings raise the possibility that agents directed against JAK-STAT signaling or epigenetic modifiers involved in regulation of DNA methylation may be of interest in some cases.

1858 Differentially Expressed miRNAs Implicate Pathways in Sebaceous Carcinomas and Adenomas

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Background: miRNAs are non-coding RNAs that regulate gene expression. Tumor-specific changes in miRNA expression implicate biological pathways driving tumorigenesis thereby identifying biomarkers informing diagnosis and prognosis and

candidates for targeted therapy. Sebaceous carcinoma (SC) is an aggressive cancer. Its benign counterpart, sebaceous adenoma (SA), is a cutaneous marker of microsatellite instability (MSI) in some patients with Muir-Torre syndrome (MTS). The molecular-genetic alterations contributing to the pathogenesis of SC and SA (sporadic versus MTS) remain poorly understood, but given the high morbidity and mortality of SC and MTS, there is a critical need to delineate the pathways driving these tumors and candidates for targeted therapy. We describe differentially expressed miRNAs in a series of periocular SC compared to SA and among SA (MTS versus sporadic).

Design: 30 SC and 23 SA (including 8 confirmed MTS and 8 confirmed sporadic SA) were profiled. Using total RNA from formalin-fixed paraffin embedded tissue, real-time RT-PCR was performed in a microfluidics card containing 378 unique miRNAs. Fold-change was determined using ΔΔCt method (reference: RNU48). Median centering normalized the data. Two-sample t-tests identified differentially expressed miRNAs. False discovery rate was assessed by beta uniform mixture analysis of p-values from the t-statistics. Significance was defined by this estimated false discovery rate.

Results: Serial testing and validation confirmed a statistically significant over-expression of oncogenic miRNAs in SC versus SA: miR-486-5p (3.8 fold; $p=1.7e^{-6}$) and miR-184 (2.8 fold; $p=3.0e^{-4}$), and down-regulation of tumor-suppressive miRNAs: miR-211 (-6.8 fold; $p=5.8e^{-10}$) and miR-195 (-2.5 fold; $5.2e^{-7}$). In MTS-SA versus sporadic SA, we show over-expression of miR-10b (2.1 fold; $p=6.2e^{-3}$) and miR-10a (2.6 fold; $p=1.1e^{-2}$) and down-regulation of miR-518f (-5.32 fold; $p=7.4e^{-3}$), miR-192 (-0.95 fold; $p=1.4e^{-2}$) and miR-126 (-1.2 fold; $p=2.8e^{-2}$).

Conclusions: SC exhibits a distinctive miRNA expression profile from SA, implicating dysregulation of NFkB and PTEN (targets of miR-486-5p) and epithelial to mesenchymal transition (target of miR-211) in the genesis of SC. Further, MTS-SA show down-regulation of miR-192 and miR-126—both similarly changed in MSI-high colorectal carcinomas. miRNAs provide a novel entry point for a comprehensive understanding of the molecular-genetic alterations central to the development of SC and SA.

1859 Molecular and Clinical Characterization of 1,577 Primary Prostate Cancers (PCa) Reveals Novel Clinical and Biological Insights into Subtypes

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Background: Prostate cancer molecular subtypes based on ETS gene fusions and SPINK1 were originally identified through distinct gene expression profiles. Such molecular subtypes may have utility in disease stratification and clonality assessment, complementing available purely prognostic tests. Hence, we determined the validity of molecular subtyping using global gene expression profiles.

Design: We analyzed 1,577 patient Affymetrix Human Exon 1.0ST GeneChip expression profiles from 8 RP cohorts. Multi-feature random forest classifiers and outlier analysis were used to define molecular subtypes.

Results: A random forest (RF) classifier was trained and validated to predict ERG status (m-ERG) using atraining subset with known ERG rearrangement status, achieving >95% sensitivity and specificity in the validation subset. The model was then applied to 7 independent RP cohorts to predict ERG rearrangement status. Less frequent rearrangements involving other ETS genes (ETV1,4,5, FLI1) or SPINK1 over-expression were predicted based on gene expression outlier analysis. Across cohorts, 45%, 9% 8% and 38% of PCa were classified as ERG+, ERG-ETS+, ERG-SPINK+, and Triple Negative (ERG-/ETS-/SPINK1-), respectively. Results show that the four subtypes could be collapsed into three entities (ERG+, ERG-/ETS+ and SPINK+/Triple Negative) based on shared expression patterns and clinical characteristics. Based on MVA logistic regression analysis, m-ERG+ status was significantly associated with lower pre-operative serum PSA ($p<0.001$), lower Gleason score ($p=0.001$), the presence of extraprostatic extension ($p<0.001$) and white Caucasian race ($p=0.001$).

Conclusions: The DECIPHER test platform can accurately determine ERG status and PCa molecular subtypes. Inclusion of molecular subtyping, such as m-ERG status, may enable additional precision medicine opportunities in prognostic tests.

1860 Molecular Traits of Tumors From Patients Status Post Solid Organ Transplant

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Background: The immunosuppression associated with solid organ transplant (SOT) is known to increase the risk for developing malignancy. While infection-related malignancies are relevant to the increased risk, the rate of certain epithelial malignancies without known infectious etiology is also increased in SOT patients. This observation provoked us to hypothesize that epithelial tumors arising in SOT patients have unique molecular profiles.

Design: To identify SOT patients, we queried the clinical laboratory data warehouse to pinpoint patients who had been tested for serum tacrolimus from the period of April 2010 through August 2014. We then cross referenced these patients with the patients who had molecular testing performed between January 2012 and August 2014.

We identified the molecular and clinical characteristics of the SOT cohort patients. We also compiled a control group (N=82) consisting of patients with targeted next generation sequencing (TNGS) performed on colorectal carcinoma (CRC).

Results: The molecular testing performed was a combination of targeted single gene sequencing (TSGS) (57%) and TNGS (43%). TNGS yielded an increase in mutation frequency compared to TSGS: 4 mutations per tumor tested versus 0.25. The mutation frequency of the patients with SOT tested with TNGS was increased 33% over the control group (4.0 vs. 3.0, p=NS). Notably there was one SOT patient with CRC yielding 11 mutations, the highest number of mutations for any tumor tested in our laboratory.

Conclusions: This study shows that cross-referencing separate databases is a high-yield method for identifying specific patient populations. While the sensitivity is unknown the specificity is 100%.

Our results point toward a trend in increased mutation frequency in SOT patient malignancies. The results support our hypothesis that the mutation profile of tumors arising in patients with SOT is unique and that TNGS can be utilized to further characterize differences in these tumors.

1861 Differentially Expressed MicroRNAs and Their Targeted mRNAs in Ovarian Carcinoma and Associated Endometriosis

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Background: In a previous study, we identified significantly distinct oncogenic miRNAs and tumor suppressor (TS) miRNAs distinguishing ovarian cancer (OC) from endometriosis (EM) by comparing the comprehensive miRNA profiling of OC and associated EM. The relative changes of these miRNAs are verified using qRT PCR. However, the affect of miRNAs on ovarian carcinogenesis remains unclear. The study aimed to identify the downstream mRNA targets of these miRNAs and verify their alterations by real time PCR.

Design: Foci of EM and concurrent OC in 19 cases were microscopically selected and macro-dissected from FFPE sections, after review of the corresponding H and E slides. The comprehensive miRNA expression profiling of OC and EM were performed initially from pooled RNA samples from all 19 cases through LC Sciences using array technology. This was followed by quantitative real time RT-PCR (qRT-PCT) to verify the abnormal expression of selected miRNAs. The downstream mRNAs of these miRNAs were identified with ingenuity pathway analysis. The 2 most important mRNAs(PTEN and NTKB) were further analyzed by RT-PCR.

Results: The miRNA profiling demonstrated deregulation of over 1000miRNAs in OC of which, the top seven were further validated by qRT-PCR. The expression of TS miRNAs miR-1, miR-133a, and miR-451 were reduced significantly in OC while in oncogenic miRNAs miR-141, miR-200a, miR-200c, and miR-3613 was elevated significantly in OC as shown in fig 1 and 2. After IPA (ingenuity pathway analysis), the 2 most significant target mRNAs of these miRNAs: PTEN and NFKB were selected. The expression of their mRNAs were verified by qRT-PCR. PTEN mRNA was decreased significantly in the OC compared to the EM foci while NFKB mRNA has no statistically significant changes in these two entities as shown in fig 3.

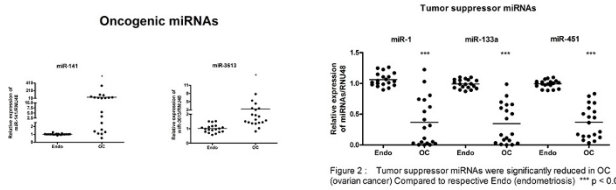

Figure 2 : Tumor suppressor miRNAs were significantly reduced in OC (ovarian cancer) Compared to respective Endo (endometriosis) *** p < 0.0001

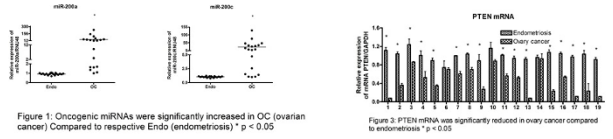

Figure 1: Oncogenic miRNAs were significantly increased in OC (ovarian cancer) Compared to respective Endo (endometriosis) * p < 0.05

Figure 3: PTEN mRNA was significantly reduced in ovary cancer compared to endometriosis * p < 0.05

Conclusions: Significant differences exist between the malignant OC and its benign associated EM at the level of miRNA and mRNA transcription. Both TS miRNA(1,133a,451) and oncogenic miRNAs(141,200a,200c,3613) are possible molecules to distinguish OC from its associated EM. These miRNAs should be studied further to identify their roles as possible biomarkers in the diagnosis of OC. These miRNAs and mRNAs could also serve as therapeutical targets due to its distinctive expression patterns in malignant OC.

1862 FOXO1/Sprouty2 Pathway Regulates Vascular Tumor Growth

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Background: Vascular tumors are neoplasms of endothelial cells with a wide clinical presentation, ranging from benign infantile hemangioma and low-grade malignant hemangioendothelioma in children to malignant angiosarcoma in adults. To date, the molecular basis of vascular tumor pathogenesis is poorly understood and standard therapies, particularly those for malignant lesions, have limited clinical efficacy. Forkhead box protein O1 (FOXO1) is transcription factor with tumor suppressor function and is dysregulated in human cancer. In this study, we showed that FOXO1 suppressed vascular tumor growth, and mechanistically, the inhibitory effects of FOXO1 were mediated by the downstream effector Sprouty2.

Design: The expression of FOXO1 and Sprouty2 was examined in infantile hemangioma, hemangioendothelioma and angiosarcoma tissues by immunohistochemical stains. Loss- and gain-of-function studies of FOXO1 and Sprouty2 were performed by using short-hairpin RNA to knockdown gene expression, and by transfecting cells with cDNA plasmids to overexpress a gene. Cell migration and proliferation were assessed by scratch area closure and cellular DNA content, respectively.

Results: FOXO1 expression was reduced in a variety of human vascular tumors examined (infantile hemangioma, hemangioendothelioma and angiosarcoma) as

compared with normal blood vessels. Knockdown of FOXO1 gene expression resulted in increased vascular tumor cell migration and proliferation in vitro and in vivo. Conversely, over-expression of FOXO1 in these cells suppressed cell growth. We found that FOXO1 interacted with Sprouty2 promoter in situ in chromatin immunoprecipitation assay and increased Sprouty2 gene expression in tumor cells. Similar to FOXO1, Sprouty2 expression was reduced in vascular tumors. Over-expression of Sprouty2 decreased tumor cell growth and migration. Conversely, knockdown of Sprouty2 increased tumor growth in vitro and in vivo. Knockdown of Sprouty2 in cells with over-expression of FOXO1 resulted in reduced tumor growth and "rescued" the FOXO1 phenotype, indicating that Sprouty2 is an important mediator of the FOXO1 effects. Microarray gene expression profiling of human angiosarcoma cells with Sprouty2 knockdown revealed important Sprouty-2-regulated genes that are involved in angiogenesis, apoptosis and growth signal transduction pathways.

Conclusions: These findings demonstrate important growth regulatory roles of the FOXO1/Sprouty2 pathway in vascular tumors, and reveal potential new therapeutic strategies for vascular tumors by targeting this pathway.

1863 Immuno-Proteomic Discovery of Cancer Biomarkers: OLFM4 Is a Crypt Stem Cell and Colon Cancer Biomarker With Prognostic Relevance

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Background: Colon cancer is a leading cause of cancer death. We developed cancer tissue-reactive serum antibody screening (immuno-proteomics) to identify novel tumor-specific antigens in colorectal adenocarcinoma.

Design: Proteins expressed in fresh-frozen matched normal and tumor tissue pairs were detected by Western blot using patient sera as antibody source. Protein bands of interest were analyzed by nano-HPLC and LTQ-Orbitrap mass spectrometry. MS data were searched with Mascot and proteins were investigated using NCBI Blast searches. Identified cancer biomarkers were validated by Western blot and IHC using matched pairs of FFPE colon cancer, liver metastases, and a colon cancer tissue microarray (TMA) from 44 patients. We investigated the prognostic value of OLFM4 expression in stage II colon cancer patients.

Results: 54 proteins were initially identified as expressed only in tumor tissue in either of two independent immune-proteomic experiments. 5 of these were present in both independent experiments, including olfactomedin-4 (OLFM4), integrin alpha-M (CD11b), and integrin alpha-2. By Western blot and TMA IHC, the three proteins expressed significantly stronger in both colon primary cancer tissue and colonic liver metastasis relative to paired normal tissue. Semi-quantitative (0-3+) assessment of OLFM4 expression (IHC) showed that the average score was 2.1 in colon cancer tissue and 1.6 in colonic liver metastases compared with 0.5 in normal colonic tissue and 0.0 in normal liver tissue. We discovered that, in normal colonic mucosa, OLFM4, an anti-apoptotic glycoprotein, is expressed exclusively within the basal crypt enterocytes (colon stem cell niche) with no expression towards the luminal surface. Furthermore, stage II colon cancer patients with high OLFM4 expression in their tumors had reduced recurrence free survival of 33.3% at 5 years compared with 70.0% with normal or low OLFM4 expression (OLFM4 status emerged as the only significant parameter in a multivariate model that included age, gender, grade, or tumor size).

Conclusions: Immuno-proteomics is a novel powerful technique for cancer marker discovery. OLFM4, CD11b, and integrin alpha-2 are promising colon cancer biomarkers. Our clinical data showed that OLFM4 is a strong prognostic marker of recurrence-free survival in stage II colon cancer. OLFM4 serves also as a novel colonic crypt stem cell marker that may provide information about colon cancer development and the similarity between cancer and stem cell phenotypes.

1864 Next Generation Pathology: The Intergation of Next Generation Sequencing With Glass-Based Histomorphology and Immunohistochemistry

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Background: Traditional pathologic diagnosis relies on the visual recognition of cellular, tissue, and organ-based morphology supplemented by immunohistochemistry (IHC). Recent integration of molecular biomarkers, that imbue classical pathology with powerful prognostic, predictive, and diagnostic information, has altered its practice in an era of precision medicine.

Design: The BCCA Personalized OncoGenomics (POG) program has sequenced the genomes and transcriptomes, using next generation sequencing (NGS), of over 130 matched pairs of pre/post- treated cancer samples in the past two years. POG explores the utility and potential shortfalls of NGS as diagnostic tool. We leveraged the transcriptomic data into a form of "virtual IHC" to validate the origins of malignancies, especially metastatic tumors of unknown primary site by comparing gene expression profiles against an ever-growing rank of publicly available reference cancers. The pathological diagnosis and IHC results of each case were reviewed and RNA sequencing data was used to quantitate the mRNA expression, in the form of RPKM (reads per kilobase per million mapped reads) values, of the corresponding genes. In cases of unknown primaries, the RPKM values are used for comparison with gene expression values of known cancers in the TCGA database to identify a "best match" using Spearman correlation.

Results: We have investigated concordance of IHC results of the primary tumour with RPKM values of the matched metastatic lesion. This addresses whether gene expression can recapitulate protein expression across space and time for molecules most relevant to diagnostic pathology. Also, comparison of RPKM profiles against those of known cancers in TCGA has validated the pathologic diagnoses. Furthermore, this method has proven to be an extremely useful clinical tool in the workup of patients with tumors of unknown origin.

Conclusions: Given the proper comparator tissue, whole transcriptome sequencing can inform on the expressions of all 22000 genes from the input library. This is superior than the limited repertoire of antibodies in most clinical laboratories and does not consume additional tissue. Also, the ability to perform complex gene expression correlations against "reference cancers" from established databases has the potential to significantly improve the accuracy of pathologic diagnoses especially in the identification of tumors of unknown origin.

1865 Differential Expression of DNA Polymerase eta mRNA and Protein in Head and Neck Squamous Cell Carcinoma and Breast Ductal Carcinoma

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Background: DNA polymerase eta (Polη, POLH) is a crutial eukaryotic DNA polymerase in DNA repair by allowing accurate translesion synthesis of damaged DNA ultraviolet radiation. We have previously reported that protein expression level of POLH is significantly higher in head and neck squamous cell carcinoma(HNSCC) compare to normal squamous mucosa. The expression level was inversely correlated with disease free survival. In this study, we compared POLH expression level in breast ductal adenocarcinoma to HNSCC and its correlation with clinical outcome.

Design: Fifty breast invasive ductal adenocarcinoma were retrospectively analyzed. The protein levels of POLH were determined by immunohistochemical staining (IHC) of anti-POLH antibody and intensity were scored from 1-3 by two independent scorers. qRT-PCR was carried out to determine the gene expression level of POLH in 30 cases of HNSCC and 50 cases of breast cancer. Public microarray data were downloaded from Gene Expression Omnibus (GEO) for validation. Statistical analysis was performed using JMP.

Results: The protein expression levels of POLH were positively correlates with POLH mRNA level (trends p=0.019). The Kaplan-Meier analysis revealed that higher expression of POLH was related with lower disease free survival in HNSCC. In contrast, higher expression of POLH was significantly related with better overall survival in Luminal A and B subtypes but not Triple negative subtype breast ductal carcinoma (HR=0.155 95% CI 0.04-0.62). In order to determine that the difference is not due to tumor heterogeneity, we validated our results using public array database. After normalization, POLH expressions were significantly higher in lung SCC than adenocarcinoma (P=0.03). In Valk's dataset, increased POLH mRNA in lung SCC were associated with higher stage and positive nodal status. While in Ivshina's breast cancer set, higher POLH mRNA level were correlated with progression free survival (trend P=0.04).

Conclusions: Our results suggest that POLH expression levels are of prognostic value, but are differently associated with tumor type (squamous vs adenocarcinoma) and location. Further studies are needed to investigate its potential role as a biomarker.

1866 WWTR1 (TAZ) and YAP Expression in Normal Endothelium and Vascular Tumors

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Background: WWTR1 (also known as TAZ) and YAP1 are involved in recurrent translocations, namely WWTR1-CAMTA1 (95%) and YAP1-TFE3 (5%) in epithelioid hemangioendothelioma (EHE). They constitute the amino terminus of the fusion genes and share significant homology in both sequence and function. The expression of WWTR1 and YAP1 in normal endothelium and other vascular tumors has not been studied.

Design: Tissue microarrays were constructed with from normal tissues (10 placentas, 10 lungs, 5 kidneys, 9 spleens), hemangiomas (9 soft tissue, 7 skin, 12 liver, 2 kidney, 4 breast, 3 spleen, 6 intramuscular), 3 littoral cell hemangiomas, 13 lobular capillary hemangiomas, 7 lymphangiomas, 2 spindle cell hemangiomas, 2 infantile hemangiomas, 1 epithelioid hemangioma, 1 Kaposi sarcoma, 28 EHE and 23 angiosarcomas. Tissue microarrays were evaluated by immunohistochemistry for antibodies to YAP1 (rabbit monoclonal EP1674Y, Abcam, Cambridge, MA) and WWTR1 (rabbit polyclonal, ab93362, Abcam, Cambridge, MA). Membranous, cytoplasmic and nuclear staining patterns were all classified as positive. The extent of staining was scored as none (0), < 25% (1), 26-50% (2) and > 50% (3). The intensity of staining was scored as no staining, faint, and strong.

Results: YAP1 was expressed more extensively than WWTR1, essentially showing at least some expression in all normal vessels and tumors. WWTR1 was more selective. No vessels within kidney and placenta, only 6% of blood vessels in spleen, and 37% of vessels in lungs had >50% cells positive and strong staining. EHE and spindle cell hemangioma had the highest percentage of tumors with >50% cells positive (95% and 100% respectively) and strong immunoreactivity (92% and 100% respectively).

Conclusions: YAP1 is more extensively expressed in normal endothelium and a variety of benign and malignant vascular tumors than WWTR1. WWTR1 has a narrower distribution and is almost uniformly and strongly expressed in EHE, where it is involved in a WWTR1-CAMTA1 translocation in approximately 95% of cases. Among normal vessels, endothelium in lung has a higher percentage of immunoreactivity than vessels in kidney, placenta or spleen. This suggests that endothelial cells or their precursors in selective organs could be the cells of origin of EHE. Interestingly, lung is one of the most common sites for EHE and EHE does not arise in kidney, placenta, or spleen.