	# Cases (% Total)		HGD Only		Invasion +/- HGD		Age		Size	
% Non-Mucinous/ Biliary Epithelium	Pan-creas	Liver	Pan-creas	Liv-er	Pan-creas	Liv-er	Pan-creas	Liv-er	Pan-creas	Liv-er
<5	6 (6%)	6 (19%)	1	0	4	3	57	58	9.4	12.8
5-10	24 (23%)	4 (12%)	3	0	6	0	45	56	9.3	11.0
15-50	22 (21%)	10 (31%)	3	1	1	0	40	44	7.7	11.4
55-85	27 (26%)	7 (22%)	0	0	0	0	47	50	4.8	12.5
90-100	25 (24%)	5 (16%)	0	0	0	0	45	48	4.1	6.2
			P=.007		P<.0001		P=NS		P<.0001	
P values are for ≤50% vs ≥55%.										

Conclusions: NM/B epithelium frequently occurs in MCNs of the pancreas and it can be the predominant component in some cases. Since mucinous and NM/B epithelia often occur together, there does not seem to be enough evidence to regard cases with predominantly NM/B epithelium as separate entities. Our study found that MCNs with abundant NM/B epithelium are significantly smaller than MCNs with abundant mucinous lining and we confirmed that these cases do not harbor HGD/malignancy. These findings suggest that mucinous change is a "progression" phenomenon in MCNs of the pancreas and liver and only when abundant mucinous epithelium is present is there a risk of progression to malignancy.

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

1785 Comprehensive Genomic Profiling of Lung Adenocarcinoma Identifies Patients with Genomic Alterations beyond Clinical Testing Guidelines Who May Benefit from Enrollment in Mechanism Driven Clinical Trials

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Background: The NCCN guidelines for lung cancer patients with metastatic disease recommend testing for *EGFR*, *BRAF* and *ERBB2* mutations, *MET* amplification and *ALK*, *ROS1* and *RET* rearrangements. *KRAS* is also a well-characterized driver of lung adenocarcinoma (AD) that predicts resistance to certain targeted therapies. However, many patients lack genomic alterations (GA) involving these established oncogenes. We therefore investigated the prevalence of other GA in lung AD using a comprehensive genomic profiling (CGP) test that can detect all classes of GA in hundreds of cancer-related genes.

Design: DNA was extracted from 40 microns of FFPE sections from 5371 consecutive clinical cases of lung AD from 2013-2015. CGP was performed using a hybrid-capture based next-generation sequencing assay to a median coverage depth of 554X. To identify patients who may benefit from investigational drugs that are available through clinical trials, we focused on genes enriched for GA in tumors with wild-type *EGFR*, *BRAF*, *ERBB2*, *MET*, *ALK*, *ROS1*, *RET* and *KRAS*.

Results: The mean age of patients was 63 years (range 13-88) and 55% were female. In this series, 5371 lung AD were tested by CGP and 2399 (45%) harbored at least one GA involving NCCN guideline genes while 1598 (30%) had mutated *KRAS*. Thirty-six genes were enriched for GA in the remaining cohort (1374 cases) without these known drivers. In this group, 402 (29%) harbored at least one GA involving known lung AD genes *NF1* (13%), *NRAS* (2.3%), *FGFR2* (1.3%), *HRAS* (0.7%), and *NTRK1* (0.7%) as well as potential drivers *RICTOR* (6.4%), *AKT2* (3.2%), *CRKL* (2.2%), *AXL* (1.6%), *MYCN* (1.5%), *MAP2K1* (1.2%) and *RAF1* (0.7%). Clinical responses to targeted therapies have been reported in patients with *NTRK1* and *FGFR* rearrangements and *CRKL* and *RICTOR* amplifications, among other GA listed above. Clinical trials that specifically target GA involving eight of these twelve genes are underway in lung AD.

Conclusions: Beyond detecting GA involving all seven driver oncogenes that are included in the NCCN guidelines and *KRAS* in 75% of lung AD patients, CGP identifies patients with other GA in a significant proportion of "pan-negative" cases who may benefit from enrollment in mechanism driven clinical trials for lung AD. Of note, there are currently hundreds of targeted therapies in development directed against GA in more than 150 distinct cancer-related genes.

1786 Integrative Immuno-Mapping of a Precision Cancer Medicine Cohort: Towards Establishing Predictive Molecular, Neoepitopes and TCR Signatures

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Background: Despite the clinical success of checkpoint blockade therapies, a systematic strategy to identify and evaluate fundamental determinants of clinical response to immunotherapy and its long-term prognosis is still lacking.

Design: To address this problem, we sought to generate a comprehensive immuno-mapping of genomically characterized metastatic biopsies from a precision cancer medicine (PM) cohort. The immuno-map of each tumor includes HLA restricted neoepitopes, immune-checkpoint modulators' expression, and cytotoxic T-cell (CTL) infiltration. Whole exome sequencing (WES) data was collected for ~350 PM samples across 32 different tumor types. Inferred HLA haplotypes and non-synonymous point mutations (NSPMs) were used to predict mutant neoepitope nanomers for MHC Class I molecules. A novel method to delineate and quantify the variable regions of T-cell receptors (TCR) in CTL's using RNA-seq data was applied for CTL infiltration and T cell repertoire assessment.

Results: In the PM cohort, approximately 50% of the global NSPMs (average=32 per tumor) resulted in at least one neoepitope. A significant correlation was found between the NSPMs and the neoepitope burdens ($R^2 \approx 0.99$), but this correlation did not extend to higher likelihood of strong binders (MHC Class I affinity <50nM). Neoepitope frequencies varied by tumor types, the highest median frequency was found in pancreatic tumors. A significant negative selection against the strong binding neoepitopes was observed in subsequent samples from metastatic tumors ($p<0.005$), suggesting possible immunoediting. A significant correlation between expressions of T-cell marker, CD8A, and the mapped TCR reads (density) was observed ($r=0.55$). TCR densities exhibited heterogeneity within and across tumor types (range=0.04-34 reads per million). Correlational clustering of TCR repertoires did not identify any enriched TCR clones to be tumor specific, highlighting widespread T cell clonal diversity. Although no strong correlation was found between the immunocheckpoint blockade proteins and TCR density ($r<0.50$), other immune response regulators were enriched e.g., leukocyte activation (FDR=0.05). No correlation was found between the number of neoepitopes and the TCR density.

Conclusions: Combining the three modalities mentioned above, our approach provides a panoramic immuno-map of patients with advanced cancer. We envision modeling a comprehensive immune-signature that would serve as a predictive biomarker to benchmark selection of patients best suited for immunotherapy.

1787 Frequency of KRAS Subtypes in Non-Small Cell Lung Cancer

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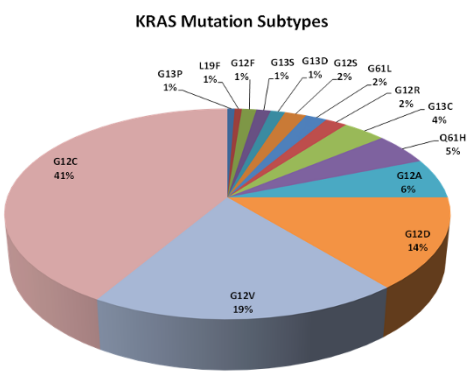
Background: It has been shown that KRAS mutant non-small cell lung cancers (NSCLCs) may vary in clinical outcome depending on which KRAS codon specific mutation is present. Shorter progression free survival has been associated with KRAS variants G12C and G12V, and cell lines with these variants depend to a greater extent upon the RAS/RAF/MEK/ERK signaling pathway. Consequently they may be more susceptible to MEK inhibition. Because different KRAS mutations are not biologically equivalent and may lead to different signal transduction cascades and altered drug sensitivity, we aimed to determine subtype specific KRAS mutation status in a NSCLC patient cohort at our institution.

Design: We tested 502 NSCLCs using a next generation sequencing 50 gene cancer hotspot panel that included the KRAS oncogene. DNA extracted from unstained tissue sections was used to prepare barcoded libraries and samples were multiplexed for sequencing on Ion Torrent 318v2 chips using the Ion PGM™ System. Variants were identified using the Variant Caller Plugin (v.4.0), and annotation and functional predictions were performed using Golden Helix SVS (v.8.3.4).

Results: The overall mutation rate in the KRAS gene was 32.7% among NSCLC cases studied (164/502). The most common KRAS mutations were G12C (41%) and G12V (19%) that are both considered RAS/RAF/MEK/ERK pathway dependent. The third most common subtype, G12D (14%), is known to act more through AKT phosphorylation. The remaining mutations included: G12A (6%), Q61H (5%), G13C (4%), and rarer subtypes: G12R, G61L, G12S (2% each), and G13D, G13S, G12F, L19F, G13P (1% each).



Conclusions: Based on this large mutation subtype-specific analysis, more than a half of KRAS mutant NSCLC patients could potentially benefit from the addition of a MEK inhibitor such as selumetinib to standard chemotherapeutic agents. It is especially important, considering that without MEK inhibition, G12C and G12V cancers seem to progress faster than all other KRAS subtypes or wild-type KRAS. Moreover, due to mutated KRAS, these patients will likely fail anti-EGFR therapies and have very limited therapeutic options.

1788 Are the Genomics in the Cancer Genome Atlas Representative of Patients with Recurrent Cancer That Is Refractory to Standard Therapy?

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Background: The Cancer Genomics Atlas (TCGA) provides a genomic fingerprint of various primary, untreated cancer types. Primary cancers from patients with recurrent disease refractory to standard therapy may have a distinct molecular biology compared to most primary cancers. Our institution initiated a protocol for the systematic identification of potential therapeutic targets in patients with advanced solid tumor malignancies, providing us with the opportunity to compare genomic data from these patients to those represented in TCGA.

Design: Patients eligible for the protocol had no remaining standard of care therapy anticipated to extend life by more than 3 months, ECOG performance status of ≤ 1, and a willingness to consider clinical trial enrollment. The patients' tumors and germline DNA were sequenced with a 409-full-length (Ion Proton) gene panel. Ninety-nine genes on the panel are actionable, defined as genes for which a matched genotype selected trial exists at our institution. Three hundred patients (termed MDA patients) have been tested on this protocol; 98 have cancer types that have been previously profiled by TCGA (57 colorectal adenocarcinoma (CRC), 21 lung adenocarcinoma (LADCA), and 20 ovarian high grade serous carcinoma (OHGSC)). Patients with CRC also had MSI testing as standard of care. TCGA sequencing data for the same 409 genes from 316 ovarian high grade serous carcinomas, 212 colorectal adenocarcinomas, and 230 lung adenocarcinomas were extracted using cBioPortal.org.

Results: For CRC, mutations in 6 actionable genes (*SRC*, *AURKA*, *ATM*, *ATR*, *MTOR*, and *PIK3CA*) were significantly less frequent in the MDA cohort compared to TCGA. CRC in the MDA group (4%) had significantly lower frequency of MSI compared to TCGA (14%). For OHGSC, mutations in the actionable genes *CCNE1* (20%), *NF1* (12%), *PIK3CA* (18%), and *PTEN* (8%) were the most common in the TCGA group; no mutations in these genes were observed in MDA patients. Wildtype *TP53* is associated with worse survival in OHGSC. *TP53* mutations were more common in the TCGA group (95%) compared to MDA (80%). In LADCA patients, mutations in *EGFR*, *KRAS*, and *BRAF* were comparable between MDA and TCGA. However, MDA patients had significantly fewer mutations in the actionable genes *CDKN2A* and *CDKN2B*.

Conclusions: The MDA group had significantly fewer mutations in actionable genes and, in some instances, more commonly had molecular changes associated with worse survival. These results have important implications for the design of targeted therapy clinical trials.

1789 Role of NOTCH Signaling Molecules in the Development of Barrett's Associated Adenocarcinoma

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Background: NOTCH signaling may be oncogenic in a variety of human tumors. For example, it is aberrantly activated due to chromosomal translocation of NOTCH3 in lung cancer and amplification and overexpression of NOTCH3 in ovarian cancer. Inactivating mutations of NOTCH1 are identified in 21% of esophageal squamous cell carcinoma and may be a tumor suppressor gene in esophagus. The function and expression of NOTCHs in Barrett's esophageal adenocarcinoma (EA) is not known. Therefore, in this study we examined the expression of NOTCHs and their roles in cell proliferation.

Design: Levels of NOTCH signaling molecules were examined in Barrett's cell lines (BAR-T and CP-A), Barrett's dysplastic cell lines (CP-B, CP-C and CP-D) and esophageal adenocarcinoma cell lines (FLO, OE33, SK-GT-500) by GeneChip® HumanGene 2.0 ST Array. CP-A Barrett's cell line was treated with acid (pH 6.5) for 24 hours or bile acid taurodeoxycholic acid (TDCA) 10^{-11}M for 24 hours, and then NOTCH signaling molecules were measured by PCR array.

Results: NOTCH1 was significantly decreased in dysplastic cell lines (5.1 fold decrease) and EA cell lines (3.8 fold decrease). NOTCH3 was significantly increased in dysplastic cell lines (2.5 fold increase) and EA cell lines (4.1 fold increase). NOTCH2, NOTCH4, DLL1, DLL3, DLL4, JAG1, JAG2, NUMB and NUMBL had no statistically significant difference among different cell lines. In addition, acid treatment significantly decreased NOTCH1 (2 fold decrease) and JAG1 (1.6 fold decrease), and increased NOTCH3 (1.7 fold increase) and NOTCH4 (3.9 fold increase) in CP-A Barrett's cells. Acid treatment did not cause significant changes of DLL1, DLL2, DLL4, JAG2, NOTCH2 and NUMB. TDCA significantly increased NOTCh4 (2.1 fold increase). Bile acid slightly increased NOTCH 3 and decreased NOTCH1, but did not reach statistical significance. Furthermore, Acid-induced increase in cell proliferation in CP-A Barrett's cells was significantly decreased by knockdown of NOTCH3 (from 285% control to 162% control, $p<0.001$) and NOTCH4 (from 236% control to 192% control, $P<0.001$) with their small interfering RNAs.

Conclusions: Downregulation of NOTCH1 and overexpression of NOTCH3 may play an important role in the development of Barrett's associated adenocarcinoma. It is possible that acid reflux present in BE patients may downregulate NOTCH1 and upregulate NOTCH3, increasing cell proliferation and thereby contributing to the progression from Barrett's esophagus to esophageal adenocarcinoma. Supported by NIH NIDDK R01 DK080703.

1790 Correlation between the Expression of Mismatch Repair Proteins (MLH1, MSH2, MSH6, and PMS2) and Cell Proliferation Status in Mismatch Repair-Proficient Tissues: Biological and Pathological Implications

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Background: Immunohistochemistry (IHC) for DNA mismatch repair (MMR) proteins, namely MLH1, MSH2, MSH6, and PMS2 has emerged as a simple and useful technique for colorectal and endometrial cancer screening for Lynch syndrome. However, IHC may be affected by many biological and technical factors, and the interpretation is not always straightforward. To facilitate the interpretation of IHC results, a non-neoplastic MMR-proficient tissue is routinely used as an internal positive control. However, the expression of MMR proteins ranges from completely negative to strong and diffuse nuclear expression. Understanding the cause of this variation will be very helpful for proper interpretation of MMR immunostaining. This study aims to explore the biological cause of different intensity and extent in MMR immunostaining in MMR-proficient tissues.

Design: The expression of MLH1, MSH2, MSH6, PMS2 and Ki67 were assessed using IHC in 65 cases of formalin-fixed paraffin-embedded tissues, including colon (30), uterus (9), lymph nodes (7), liver (6), skin (4), small intestine (3), stomach (3), and esophagus (3). Immunostaining results of MMR proteins were scored for intensity (0-3+) and extent (0-3+). Ki67 proliferation index was scored as percentage of total cells. Pearson test for correlation significance was performed using the GraphPad statistical software.

Results: The positivity of MMR proteins ranged from 0 to 100% among different tissues. Cells with low proliferative activities, e.g. smooth muscle, were either completely negative or only weakly stained. In contrast, actively proliferating cells, e.g. crypt base epithelium showed diffuse and strong nuclear staining. Statistical analysis revealed a significant correlation between Ki67 labeling index and MMR nuclear expression ($P<0.001$; $R=0.91$) in MMR-proficient tissue.

Conclusions: This study is the first to determine the direct relationship between the expression of MMR proteins and cellular proliferative status in both tumor and non-tumor MMR-proficient tissues. The finding is logic in biology since MMR proteins are required for correcting DNA mismatch errors associated with DNA replication. Pathologists should be aware of the variation in expression of MMR proteins in non-tumor MMR-proficient tissues and a negative stain is expected in mature non-proliferating tissues. Keeping this in mind is helpful for correct interpretation of IHC results of MMR proteins.

1791 Recurrent Chromosomal 8q24.21 Abnormalities and Their Functional Relevance in Pediatric Acute Leukemias

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Background: Our recent genome-wide SNP array analysis identified very few recurrent genomic alterations in pediatric AML genomes. However, one of the most recurrent lesions was focal amplifications on chr8q24.21 affecting a putative gene called CCDC26. Interestingly, this locus was identified in a retroviral forward genetic mutagenesis screen to identify genes required for retinoic acid-induced differentiation of a human AML cell line. It was found that one cloned retroviral integration within the CCDC26 gene partially blocked RA-induced differentiation. However, it's not quite clear whether the function affect was mediated by CCDC26 gene or an unknown transcripts or regulatory elements in this locus.

Design: In our study, we characterized the genomic structure and transcription profile of this locus through multiple strategies and then explored the functional relevance during leukemogensis.

Results: We found this focal genomic alteration in different cohorts of acute leukemias including pediatric ALL, CBF-AML, and secondary AML, with a consistent frequency of 3%-4%, in comparison to less than 0.4% in more than 2000 solid tumors (reanalysis of TCGA data). Moreover, our high resolution tiling array CGH assay further validated this focal CNAs mainly affecting CCDC26.We then went on to use cell fractionated transcriptome sequencing method to obtain the most comprehensive transcription profile of this locus. Surprisingly, we found multiple non-spliced and non-coding transcripts in the nucleus that were absent in the cytoplasm. One of the identified nuclear transcripts with 1.2 kb full length, was highly expressed in AML cell lines with focal copy number gains. Our data further indicated it was transcribed by RNA Pol II without metabolism into small RNAs. Knockdown of this transcript with two independent antisense oligonucleotides resulted in a subtle decrease in cell viability. However, the complexity of the Linc-RNAs encoded by this genomic locus, coupled with the lack of homology between mice and humans in this genomic region, makes it very challenging to define the role of this region in leukemogenesis.

Conclusions: Even though our priority hypothesis is that a lincRNA at this locus is the target of CCDC26 amplification, our study did not rule out the possibility of one or more unknown regulatory elements at this locus.

1792 Validation of a Clinical 1400-Gene Assay for Genomic Profiling of Cancer from DNA and RNA

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Background: Genomic assays are increasingly used in oncology to guide clinical management and assess tumor responsiveness to novel therapeutics. However, the number of cancer genes with clinical relevance continuously expands, necessitating

broader mutational profiling of tumors. Here we present the analytical validation of the ACE Cancer Panel, which is the largest cancer gene panel available to date for clinical use.
Design: We have targeted 1438 cancer and pharmacogenomic genes for enrichment and sequencing from DNA and RNA. Analytical validation of the ACE Cancer Panel, an augmented targeted sequencing platform, was accomplished across all variant types using 28 cancer cell lines and reference standards with known single nucleotide variants (SNVs) and small insertions and deletions (indels), 19 cancer cell lines with known copy number alterations (CNAs), and 17 cell lines with known gene fusions. We simulated tumor heterogeneity by mixing the cell lines at various ratios, generating variant allele frequencies down to 1%, and emulated reduced tumor purity by mixing cell lines with paired normals at ratios down to 10%. Data were analyzed using cancer bioinformatics pipelines in both tumor-only and tumor-normal modes. Additionally, the assay was validated for use in different clinical specimen types, including formalin fixed paraffin embedded (FFPE), fresh frozen (FF), and blood.
Results: Uniform DNA sequencing coverage was achieved at mean alignment depth of ≥500 reads. The sensitivity of the assay was 99.7% for SNVs at AF ≥ 5% (n = 16132), 99.4% for small indels at AF ≥ 10% (n = 671), 91.2% for CNAs (n = 34, copy number 0 or ≥8 in tumor-only cell-lines), and 95.0% for fusion transcripts (n = 20). The specificity of the assay was ≥99% for SNVs and indels. For cancer cell lines and clinical tumor specimens where a matched normal was available, tumor-normal analysis refined somatic variant calling in comparison to tumor-only analysis. The assay also performed robustly in real clinical specimens, including FFPE.
Conclusions: We have developed and validated a comprehensive cancer NGS panel, with highly uniform and deep coverage, ensuring high sensitivity and specificity for all variant types. This assay represents a versatile diagnostic tool that can be used not only to test a core set of clinically actionable genes, but also implicate new cancer genes as clinically relevant or facilitate discovery of novel therapeutic targets.

1793 Multiplex Gene Expression Analysis of Eosinophilic Esophagitis Refractory to Therapy Using Nanostring Technology

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Background: We have previously investigated the gene expression profile of esophageal biopsies of pediatric patients with eosinophilic esophagitis (EoE) and identified a set of genes that were differentially expressed before and after successful therapy with topical corticosteroids. However, for reasons that are unknown a significant fraction diagnosed with EoE do not respond to therapy. In this study, we aimed to investigate the gene expression profile of pediatric patients with EoE refractory to therapy with diet, proton pump inhibitors, and topical corticosteroids.
Design: Refractory EoE was defined as persistence of symptoms and biopsies with histologic features of EoE after at least 6 months of therapy. FFPE tissue samples from esophageal biopsies from 21 patients with refractory EoE or pediatric patients with normal endoscopy were sectioned and microdissected to isolate RNA. Gene expression analysis was performed using NanoString molecular barcode technology, which allowed for reverse transcription/PCR amplification-free multiplex testing of 249 genes involved in inflammation.
Results: The average age was 10 years old (range 1 to 17 years). There were 13 males and 8 females. The average followup time after initial diagnosis was 18 months. The average number of eosinophils/HPF was 66/HPF (range 25 to 125). Of the 249 inflammation genes tested, 21 were significantly differentially expressed by at least 4-fold compared to normal tissue, including downregulation of IL12A and IL18, and overexpression of CCL24 (eotaxin-2), CXCL1 and IFI44. Notably, ALOX15 was strongly and consistently overexpressed (mean 110-fold; range 9-405; p=0.0235). Conversely, ALOX12 expression was significantly decreased (mean 13-fold; range 4-33; p<0.0001) in EoE compared to normal esophageal tissue. Intriguingly, multiple members (C5, C6, C8A, C8B and C9) of the membrane attack complex, which is involved in the complement system, were coordinately downregulated in EoE.
Conclusions: A better understanding of the mechanisms of resistance to current therapies is critical in order to develop new approaches to refractory EoE. This is the first study to investigate the expression of a comprehensive panel of inflammatory genes in refractory EoE. These results provide valuable insight into the key molecular players in the pathogenesis of refractory EoE and suggest further research is needed on the role of the complement system in this disease.

1794 mTOR Pathway Genes Drive Intratumor Heterogeneity and Morphological Progression in Clear Cell Renal Cell Carcinomas

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Background: The meaning of morphological intratumor heterogeneity (ITH) in clear cell renal cell carcinomas (ccRCC) by Fuhrman grade (FG) has not been investigated at clinic-pathologic or genetic levels in the Cancer Genome Atlas (TCGA) set.
Design: A systematic evaluation of 401 ccRCC included: microscopic satellites, heterogeneous clones, FG (1-4), confluent necrosis, spindle cell presence, primary and secondary growth pattern (tubular-nested-thick trabecular-solid), tumor infiltrating lymphocytes, stromal reaction (none/myxoid/desmoplastic), and edges (pushing/infiltrative). Morphological heterogeneity (ITH+) was systematically assessed, and identified in 56 cases. Clinical data were also collected (gender, age, and stage). Whole-exome sequencing was performed on tumor and normal tissues from ccRCC available at the TCGA. We used a Random Forest machine learning approach comparing ITH- vs. ITH+ ccRCC.
Model Analysis
► Data was retrieved from the Pan-Cancer Analysis repository
► Functional somatic mutations unique to tumors were identified and represented as samples x genes mutation matrix (mutated=1, non-mutated=0).
► Pairwise Random Forest models were built for the low-intermediate-high CNAG subgroups
► Variable selection using Fisher's Exact test was conducted to reduce the number of predictors with 50 fold cross-validation design.
Random Forest models were based on the training set using the caret package in R, and predictive accuracy measured in an independent test set.
Results: Complete histological-molecular data were available in 313 cases (179 low FG, 19 ITH+; 139 high FG, 37 ITH+). ITH+ ccRCC were mainly defined by thick trabecular-solid secondary pattern (31/56 vs. 92/257 ITH-, P=0.007), and high FG (37/56 vs. 102/257 ITH-, P=0.001), with no differences on vascular invasion, satellites or multifocality. The age-gender-proteins model performed with an AUC of 0.875, being predictive features of ITH+ ccRCC: PBRM1 (OR = 9.562, P < 1.3E-17), BAP1 (OR = 9.144, P < 6.0E-16), TP53 (OR = 8.574, P < 4.7E-14), PTEN (OR = 0.661, P < 2.0E-10), PIK3CA (OR = 0.328, P < 5.38E-06). The proportion of C>T transitions at CpG sites increased from morphologically ITH- to ITH+ neoplasms and during grade progression.
Conclusions: Morphological heterogeneity in ccRCC represents a stepwise tumor progression defined by thick trabecular-solid growth and high nuclear grade, which is mainly driven by PI3K-mTOR pathway genes.

1795 Distribution of IDH1 and IDH2 Genomic Alterations across the Landscape of Relapsed and Refractory Solid Tumors and Hematologic Malignancies

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Background: *IDH1* and *IDH2* encode isocitric dehydrogenases, major players in normal and cancer cell metabolism and emerging targets for anti-cancer therapy. We surveyed an expansive series of solid tumors (ST) and hematologic malignancies (HM) by comprehensive genomic profiling (CGP) to identify the frequency of *IDH1/2* genomic alterations (GA) in a broad spectrum of malignancies.
Design: DNA was extracted from 40 microns of FFPE of 43,972 consecutive ST and 5452 HM clinical cases. CGP was performed on hybridization-captured, adaptor ligation-based libraries for up to 405 cancer-related genes plus 37 introns from 14 genes frequently rearranged in cancer to identify all classes genomic alterations (GA) including base substitutions, INDELs, copy number alterations and fusions in *IDH1*, *IDH2*, and other genes.
Results: GA in *IDH1* or *IDH2* were identified in 1165 ST and 134 HM from 691 (53%) male and 608 (47%) female patients with a median age of 51 years (range 7-88 yrs) and a mean frequency of 5.4 GA/sample. 999 (86%) ST featured *IDH1* GA, 164 (14%) *IDH2* GA, and 5 (0.4%) GA in both *IDH1* and *IDH2*. By comparison, 93 HM featured *IDH2* GA (69%; p < 0.0001), 48 *IDH1* GA (36%; p <0.0001), and 4 cases (3%; p =0.009) had both *IDH1* and *IDH2*. 98% of *IDH1* GA in both ST and HM occurred at R132, most often resulting in a change to H for ST (56%) and a change to C for HM (54%; p =.0004). ST subtypes with most frequent *IDH1/2* GA were: gliomas (56%), hepatic cholangiocarcinomas (12%), unknown primary carcinomas (9%), NSCLC (6%), additional pancreatobiliary carcinomas and melanomas (2%) and nasopharyngeal carcinomas (1%). The most frequent genes co-altered with *IDH1/2* in ST included *TP53* (54%) and *ATRX* (31%), compared to *DNMT3A* (30%) and *SRSF2* (29%) in HM. Notable clinically relevant GA co-altered in ST but not in HM included: *PIK3CA* (11%), *BRAF* (6%), *PTEN* (5%), *CDK4*, *PDGFRA* and *MET* (4%) and *EGFR* (3%).
Conclusions: Although concentrated in gliomas, cholangiocarcinomas and acute leukemias, *IDH1/2* GA are widely distributed across multiple cancer types. *IDH1* GA predominate in ST, whereas *IDH2* GA predominate in HM. Significant differences in the specific *IDH1/2* GA and co-occurring GA and CRGA observed between ST and HM could impact the relative efficacy of pharmacologic agents based on tumor type. As anti-IDH1/2 targeted therapies advance through clinical trials, further study of additional tumor types that feature *IDH1/2* GA for possible future basket trials appears warranted.

1796 Clinical Utility of a Next-Generation Sequencing Oncology Panel Designed for Low Input DNA

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Background: One challenge to next-generation sequencing (NGS)-based clinical oncologic testing is a high DNA input requirement. With our initial testing methodology (a 47-gene Illumina TruSeq Amplicon Cancer Panel® (TSACP)), this problem of insufficient quantity affected approximately 5% of all specimens we received. Hence, we developed a focused NGS panel of 20 cancer-associated genes requiring ≤10 ng input DNA. Here, we perform a retrospective review of this assay's clinical utility since implementation.
Design: Samples were reflexed to the 20-gene 'precision panel' (PP) if insufficient DNA was extracted or if TSACP failed. For both assays, DNA derived from FFPE samples (≥10% tumor) was amplified with content-specific PCR primer pools, then sequenced on a MiSeq® instrument (Illumina, Inc.) via paired-end, 2x185 base pair reads to an average depth of >1000x. Variants were detected using an in-house analysis pipeline. Sample data and variants reported were reviewed for all samples tested between July 2014 and June 2015.
Results: Over this time, we received a total of 1,323 solid tumor samples. Most specimens (n=1,128, 85.3%) were able to be tested by TSACP. However, 195 samples

(14.7%) were reflexed to PP; of these, 176 were reflexed up front (TSACP not run), whereas 19 failed TSACP and were then run successfully on PP. A total of 14 samples failed PP due to insufficient or overly degraded DNA. Overall, the PP assay provided results for 181 of 195 samples (92.8%) that otherwise would not have been tested. A total of 228 reportable variants were found in 145 (80.1%) of these specimens. These variants included 161 (70.6%) missense and 20 (8.8%) nonsense mutations, 10 (4.4%) splice site alterations, and 37 (16.2%) insertions/deletions. 207 (90.8%) variants were called 'pathogenic' and 21 (9.2%) 'variants of unknown significance'. Overall, 72 variants had direct therapeutic implications: 24 conferred increased susceptibility to specific therapies, whereas 48 conferred resistance.

Conclusions: We implemented a precision NGS panel optimized for ≤10 ng DNA input. In terms of clinical utility, this assay was able to test a significant number of samples (n=181) that otherwise would have been rejected. Furthermore, for a potential 70 patients, the results had a direct therapeutic impact. Our study demonstrates the value of implementing such testing in a clinical laboratory.

1797 Concordance of Anti-BRAF p.V600E immunohistochemistry with BRAF Gene Sequence in Solid Tumors Carrying Diverse BRAF Mutations

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Background: Determination of the *BRAF* mutation status is of great importance in management of patients with cancer. *BRAF* gene mutations analysis is performed using various DNA-based molecular assays, but cost, expertise and tissue requirements limit their widespread use. Recently, BRAF p.V600E-specific antibody has been developed for the use in immunohistochemistry (IHC) and shown to have a good concordance with detection of the BRAF p.V600E *(BRAF c.1799T>A)*() mutation, but ongoing investigations indicated presence of many different (non-V600E) BRAF mutations for which sensitivity and specificity of the IHC method is not fully known.

Design: Formalin-fixed paraffin-embedded tissue samples of 170 solid malignancies sequenced using the TruSeq Amplicon - Cancer Panel (TSACP, Illumina) were immunohistochemically (IHC) stained with BRAF p.V600E-specific antibody (VE1, Ventana Medical Systems). The cohort included 35 w.t. BRAF cases, 58 BRAFp.V600Eand 77 non-V600E BRAF mutations.

Mutation/IHC status	Negative	Positive	Concordance (%)
BRAF wild type	32	3	32/35 (91%)
Non-V600E BRAF V600K (18%) D594 (16%) G469 (10%) V600R (8%), G466 (8%)	76	1 (D594V)	76/77 (99%)
BRAFV600E	0	58	58/58 (100%)
Total	108	62	170

Results: Anti-BRAF p.V600E specific antibody exhibited an excellent concordance with TSACP result (100% sensitivity and 91% specificity). Three IHC positive/BRAFw.t. cases included 2 non-small cell lung carcinomas and one gastro-intestinal stromal tumor. Only 1 out of 77 cases (1.3%) with non-V600E mutations (D594V mutation, a case of colorectal carcinoma) was positive by IHC. *BRAF* gene sequences of all four IHC discordant cases were confirmed using Sanger sequencing (false positive IHC).

Conclusions: BRAF V600E specific antibody VE1 exhibits an excellent overall concordance with gene sequence and therefore may serve as a good screening tool for detection of BRAF V600E mutation in solid tumors.

1798 Metaplastic Breast Carcinomas and Gynecologic Carcinosarcomas: Variations of the Same Theme or Distinct Entities?

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Background: Metaplastic breast carcinoma (MBC) encompasses a heterogeneous group of tumors characterized by the presence of malignant cells that differentiate towards squamous epithelium or mesenchymal elements. A histologically similar counterpart in gynecologic organs is carcinosarcoma (CS), which is composed of carcinomatous and sarcomatous elements. Here we sought to compare the repertoire of somatic mutations in MBCs and gynecologic CSs.

Design: We sequenced the whole-exome of 26 MBCs. Somatic mutations and insertions/deletions were detected using state-of-the-art bioinformatics algorithms. Mutation data from 22 gynecologic CSs subjected to whole exome sequencing were retrieved from Jones et al. (Nat Commun 2014; PMID: 25233892). Overall non-synonymous (NS) mutation rates were compared using the Mann-Whitney U test, and the frequency of NS mutations in each gene was compared using Fisher's exact test. The mutational spectrum was compared for specific genes.

Results: 4 CSs had elevated mutation rates (904-5913 NS mutations) and were associated with bi-allelic inactivation of *MLH1*/*MSH6*, but none of the MBCs displayed mutations in mismatch repair (MMR) related genes. In both MBCs and CSs without MMR defects, *TP53* was the most frequently mutated gene (58% and 67%, respectively). Both MBCs (31%) and CSs without MMR defect (56%) harbored somatic mutations in genes involved in chromatin regulation (*ARID1A*, *ARID1B*, *BAZ1A*, *MLL3, SPOP*), and 46% of MBCs and 50% of CSs without MMR defects harbored likely pathogenic mutations in PI3K pathway genes (*PIK3CA*, *PIK3R1*, *PTEN*). MBCs and CSs devoid of MMR defects displayed genomic differences: i) 92% of *TP53*-mutant CSs had missense *TP53* mutations, whereas 53% of *TP53*-mutant MBCs had nonsense/frameshift/splice site mutations (p=0.02); and ii) *FBXW7* and *KRAS*, driver genes mutated in gynecologic cancers, were more frequently mutated in CSs without MMR defect than in MBCs (22% vs 0% and 28% vs 4%, respectively, p<0.05).

Conclusions: MBCs and CSs frequently harbored somatic mutations affecting *TP53*, and mutations in genes involved in chromatin regulation and the PI3K pathway. Importantly, however, MBCs and gynecologic CSs, albeit closely related, are not superimposable at the genetic level, given i) the presence of somatic mutations affecting MMR genes in 18% of CSs but not in MBCs, ii) their distinct *TP53* mutational spectra, and iii) the presence of somatic mutations affecting *KRAS* and *FBXW7* in CSs but not in MBCs.

1799 Proteomic Analysis of Formalin-Fixed Paraffin-Embedded Metastatic Well-Differentiated Pancreatic Neuroendocrine Tumors Yields Valuable Insights into Tumorigenesis, Biomarkers, and Therapeutic Targets

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Background: Well-differentiated pancreatic neuroendocrine tumors have relatively unpredictable behavior by current methods and do metastasize. In order to better understand these tumors we looked to new developments in mass spectrometry, which have allowed for a more global examination of protein expression in tissue. Our study sought to use this developing technology, through better understanding of tumorigenesis, to find prognostic markers and therapeutic targets for pancreatic neuroendocrine tumors.

Design: Protein extraction of two formalin-fixed paraffin-embedded tumor samples was executed using two redundant methods. Semi-quantitative label-free shotgun analysis by liquid chromatography-tandem mass spectrometry of synchronous liver metastases, one grade 1 and one grade 2, was performed and the data were used as a test set. Six grade 1 and grade 2 primary tumors were used as a validation set. Selected differentially expressed or overexpressed proteins were validated by immunohistochemistry. Signaling pathway analysis was performed using 482 differentially expressed proteins.

Results: The two extraction methods identified 1,056 and 1,041 proteins present in both grade 1 and grade 2 tumors, 866 proteins were common to the two methods, and 601 reached statistical significance for differential expression ($p < 0.05$). CLU, CK19, PR, CST3, and A1AT all showed lower expression in grade 2 tumors compared to grade 1 by MS and the predicted level of expression was confirmed by IHC to be a change within tumor cells, except for A1AT which showed a change in tumor the number of infiltrating mast cells. PARP1 was increased in both grade 1 and grade 2 tumors and also showed concordant IHC expression. Pathway analysis showed upregulation of proteins belonging to VEGF, death receptor, mTOR, and AMPK signaling pathways in G1 tumors.

Conclusions: Existing biomarker proteins CLU, CK19, and PR were confirmed, a novel presence of increased mast cells was identified, and therapeutic targets PR and PARP1 were identified in this study using mass spectrometry coupled with immunohistochemistry.

1800 TGFβ Is an Upstream Regulator of mTORC2-Dependent Bladder Cancer Migration and Invasion

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Background: Analysis of The Cancer Genome Atlas (TCGA) data identified frequent activating alterations and upregulation of mTOR and TGFβ related genes and mRNA in >90% of Urothelial Carcinoma (UC) specimens, raising the possibility that TGFβ/mTOR signaling may function cooperatively to regulate UC cancer-cell function. Prior studies have identified the mTOR complex 2 (mTORC2) as a key regulator of UC migration and invasion. We therefore tested whether TGFβ could regulate these processes via a non-canonical mTORC2-dependent mechanism.

Design: Data from TCGA related to UC was mined via cBioPortal. Patient specimens from the institutional registry were interrogated for phosphorylated SMAD2 (pSMAD2) expression by IHC and western blotting (WB). RT4, UMUC3, T24 and J82 UC cell lines (American Type Culture Collection) were used for *in vitro* studies. mRNA expression was determined through quantitative PCR and protein expression through WB and ELISA (TGFb1). SB431542 was used for TGFβ receptor inhibition and siRNA mediated ablation of Rictor was used to disrupt mTORC2. Functional assays included modified scratch wound assays and Matrigel invasion assays.

Results: In UC patient specimens, highest levels of pSMAD2, a TGFβ signaling intermediate, were present in high-grade and invasive UC. Elevated pSMAD2 was associated with more frequent recurrence (p=.01) and decreased disease-specific survival (p=.02). Higher expression of TGFβ isoforms, receptors and downstream signaling components was detected in multiple invasive high-grade UC derived cell lines. Application of TGFβ to these cells was functionally significant as it increased cell migration and invasion. These functional effects were correlated with TGFβ induced phosphorylation of the Ser473 residue of AKT, a selective target of mTORC2. The specificity of TGFβ signaling on migration and invasion was demonstrated by the use of a TGFβ receptor inhibitor as well as ablation of Rictor, a key mTORC2 component, both of which diminished TGFβ induced migration and invasion.

Conclusions: In summary, our study shows that TGFβ can induce UC cancer-cell motility and invasion with a requirement for mTORC2 activity and provides unique opportunities to expand prognostic therapeutic paradigms in bladder cancer.

1801 Recurrent, Truncating SOX9 Mutations Are Associated with KRAS and PI3K Pathway Mutations and May Be Oncogenic in Colorectal Carcinoma

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Background: *SOX9* is a transcription factor normally expressed in deep crypt nuclei of intestinal epithelium where it suppresses epithelial proliferation. Wild type *SOX9* has been shown to have oncogenic characteristics, and *SOX9* is recurrently mutated in colorectal carcinoma (CRC); yet the spectrum of mutations identified has lead to uncertainty as to its role as a tumor suppressor versus oncogene. We aimed to characterize the clinicopathologic and functional significance of *SOX9* mutations in colorectal carcinoma.

Design: Electronic medical records for CRC patients who received *SOX9* molecular analysis through MSK-IMPACT were reviewed. SOX9 mutation zygosity was estimated via microscopic tumor percent and driver allele frequencies. Immunohistochemistry for Sox9 expression was performed in subsets of *SOX9* wild type and mutated CRC FFPE tissues. Real time PCR for SOX9 mRNA (relative to internal beta actin) levels was performed in 7 CRC cell lines including 3 *SOX9* wild type lines, and one line each with the following mutations: p. T11A, p. R120L, p. Q265fs*30, and p. S484fs*? (stop loss).

Results: Of 409 CRC patients who received MSK-IMPACT testing, 35 (9%) had a somatic *SOX9* mutation. Among these 35 mutations, 86% (30) harbored a frameshift or nonsense mutation and 14% (5) were missense mutations. The presence of a *SOX9* mutation was significantly associated with a coexistent KRAS mutation (p=0.0012) and PIK3CA/ PTEN alterations (p=0.0001). Sox9 was overexpressed in 16 of 19 cases with truncating mutations, including 6 of 8 hemi/ homozygous *SOX9* mutant CRC (figure1); and 22 of 22 patients with wild type *SOX9*. Interestingly, mRNA levels were highest in the cell line with the distal frameshift mutation resulting in stop codon loss (figure2).

Conclusions: *SOX9* mutations occur in 9% of CRC, and are mainly truncating. They are associated with both *KRAS* and *PIK3CA/ PTEN* alterations. Sox9 is overexpressed in CRC with truncating mutations, suggesting that truncated Sox9 mRNA transcripts may escape nonsense or nonstop mediated decay and thus act as oncogenes.

Fig. 1

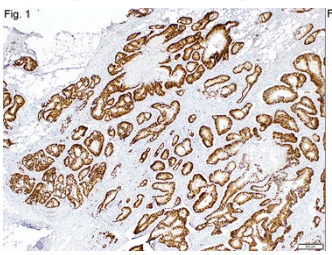
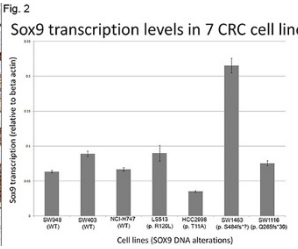


Fig. 2



1802 Copy Number Alterations in Primary and Recurrent Breast Tumors

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Background: Copy number alterations (CNAs) are a common feature of all breast cancers and vary among tumor subgroups. However, little is known regarding the role of CNAs in recurrent tumors of the breast and the extent to which CNAs in primary tumors are associated with estrogen receptor (ER) status of recurrent tumors. The major goals of the present study are to identify differences in CNAs between primary and recurrent tumors and to determine the extent to which CNAs in ER positive primary tumors predict ER status of recurrent breast tumors.

Design: The bioconductor packages *minfi* and *CopyNumber450K* were used to call CNAs from DNA methylation data obtained with Illumina 450k arrays. A total of 70 FFPE primary and local recurrent/second breast tumors from 40 women were analyzed. Hormone receptor status and clinicopathologic features were extracted from patient charts. Data from all samples passed quality controls. Initial analysis of 50 matched primary and recurrent tumors from three groups of patients (Group 1 = ER positive primary to ER positive recurrent/second; Group 2 = ER positive primary to ER negative recurrent/second; and Group 3 = ER negative primary to ER negative recurrent/second) is presented.

Results: Analysis of genome-wide CNAs using iClusterPlus R identified 3 clusters as optimal: a copy number loss cluster, a copy number gain cluster, and a mixed cluster. All three clusters included bother ER positive and ER negative tumors as well as primary and recurrent/second tumors. In general tumors from the same patients tended to cluster: eleven of the total 25 pairs, and four of the six pairs from group 3 (ER positive to ER negative) clustered together. Analysis of gene-specific CNAs identified 19 genes with significant differences in proportion between primary and recurrent/second tumors, and 769 genes with differences in proportions between ER positive and ER negative tumors. Analysis of the fraction of the genome altered, a measure of total CNN burden, revealed that recurrent tumors are associated with a significant increase CNAs as compared to their primary tumor (p =.03).

Conclusions: The initial analysis suggests that CNAs may prove useful in stratifying tumors beyond present subgroups and informing treatment options.

1803 Utility of Oral Fluid (Saliva) for Molecular Profiling in Hematologic Malignancy

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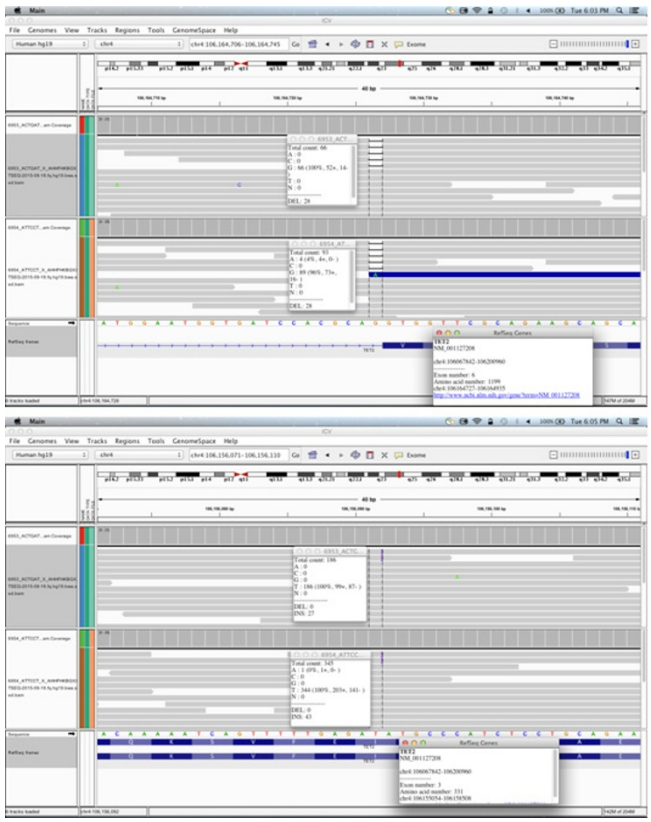
Background: Human saliva (a.k.a. oral fluid, spit) is a biological resource with a definable cell composition and copious DNA yields. Saliva molecular profiling captures the same predominant cell component as blood—the neutrophil-- while epithelial cells comprise ~20%, but lymphocytes are below a limit of detection of roughly 1-2% in healthy individuals. However, there are no established reference values for malignant cellular composition/frequency in saliva DNA extracts from patients with hematologic malignancy.

Design: We analyzed saliva from hematologic oncology patients. We measured genomic DNA yields, followed by cancer-gene mutation profiling by next-generation sequencing (NGS). Mutation allele frequencies were compared between saliva, blood and histologically involved anatomic collection sites (i.e. lymph node, bone marrow).

Results: Saliva mutation frequencies were substantially higher in myeloid versus lymphoid malignancy. Saliva from the latter had either no detectable mutations, or very low level detection beneath the cutoff for use as a matched normal. In contrast, driver mutations were readily detected in saliva from myeloid leukemia patients, above the limits of detection of routine molecular testing. Figure 1 shows inactivating TET2 mutations ranging from 10-20% allele frequency in saliva from a CMML patient (Figure 1).



Conclusions: We have demonstrated bimodal clinical diagnostic utility for saliva/oral fluid in the setting of hematologic malignancy. On one hand, saliva can be used as a matched normal specimen where blood may be involved by lymphoid malignancy. On the other hand, myeloid leukemic patients harbor substantially higher saliva mutation levels. Analyses of treatment-related changes as manifest in saliva are ongoing. The results support the hypothesis that oral mucosa is a selective "pump" that actively concentrates neutrophils in the oral cavity while excluding lymphocytes. This basic biology of oral mucosa underlies the differential manifestation of mutant DNA in saliva/ oral fluid from lymphoid versus leukemoid malignancy patients.

1804 Utility of a Rapid Turnaround, Clinically-Relevant Gene Panel for Next Generation Sequencing (NGS) Analysis of FFPE Solid Tumors

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Background: The widespread adoption of NGS technologies has redefined the ability to interrogate multiple gene mutations in complex tumor biopsies. However, many NGS cancer panels are complex, cumbersome, unoptimized, & slow to generate actionable results. Herein we present outcomes of an evaluation of a streamlined rapid-turnaround methodology for targeted NGS in cancer. This technology combines single-source reagents with a variant caller that is informed by pre-analytical QC data to accurately detect SNVs and indels in key cancer genes (Fig. 1).

Design: DNA was isolated from FFPE specimens of 16 archival cases of solid malignancies (colon, and melanoma, Fig 2) with known positive mutations identified

using qPCR methods. Each NGS library prep included a negative control and two positive controls (a residual clinical FFPE DNA control comprised of a 5% BRAF V600E variant, and a multi-variant synthetic control). Reagents for DNA quantification, 2-step targeted PCR enrichment, purification and library quantification (QuantideX™ Pan Cancer Kit, Asuragen Inc.) were used for all MiSeq runs. Variant calls and read metrics were linked to functional copy numbers analyzed per sample using the QuantideX Reporter™ standalone bioinformatics suite.

Results: Samples could be processed from DNA to sequence-ready library in a single shift, and a single training run was sufficient to achieve operator proficiency. All NGS data from training and proficiency test runs were 100% concordant across multiple variants and tumor types. For the 16 archival FFPE tumor biopsy specimens, results were 100% concordant across matched variant calls in 2 different cancer genes.

Gene	Codon Range	Gene	Codon Range
ABL1	249-258		9-20
AKT1	303-319	HRAS	59-76
AKT2	16-27		113-121
ALK1	16-26	IDH1	122-134
	1174-1196	IDH2	138-145
BRAF	1274-1278		163-174
	465-474	JAK2	607-620
	591-612	KIT	557-579
	486-493		815-826
EGFR	709-722		9-20
	737-761	KRAS	55-65
	767-798		104-118
	849-861		137-148
	755-769	MET	1245-1256
ERBB2	774-788		9-20
	839-847	NRAS	55-67
	877-883		110-119
FGFR1	123-136		144-150
	250-262	PDGFRA	560-572
FGFR3	247-260		840-852
	363-374	PIK3CA	540-551
	636-653		1038-1049
FLT3	829-840	RET	916-926

Fig. 1. Gene Panel Composition

Sample Type	Gene	Mutation
COL	KRAS	Mutation Gly13Asp (GGC->GAC)
COL	KRAS	Mutation Gly12Val (GGT->GTT)
COL	KRAS	Mutation Gly12Val (GGT->GTT)
COL	KRAS	Mutation Gly12Val (GGT->GTT)
COL	KRAS	Mutation Gly12Val (GGT->GTT)
COL	KRAS	Mutation Gly12Asp (GGT->GAT)
COL	KRAS	KRAS gene containing codons 12 and 13
COL	KRAS	Mutation Gly12Asp (GGT->GAT)
COL	KRAS	amplify the region of the KRAS gene containing codons 12 and 13
COL	KRAS	amplify the region of the KRAS gene containing codons 12 and 13
MEL	BRAF	BRAF V600E
MEL	BRAF	BRAF V600E
MEL	BRAF	BRAF V600E
MEL	BRAF	BRAF V600E
MEL	BRAF	BRAF V600E
MEL	BRAF	BRAF V600E

Fig. 2: Seventeen cases of Colonic adenocarcinoma and Melanoma which were tested for and positive for KRAS and BRAF gene mutations.

	Pan Cancer DNA							GRU		
Sample Type	Sample	Copies	Coverage	Gene	Cosmic	AA	Percent Variant	Sample_GRU	Gene	Variant
CC	ASU001	15370	25139	KRAS	c.38G>A	p.G13D	12.80%	1	KRAS	G13D
CC	ASU002	14520	22950	KRAS	c.35G>T	p.G12V	13.40%	2	KRAS	G12V
CC	ASU004	8890	23139	KRAS	c.35G>T	p.G12V	62.50%	4	KRAS	G12V
CC	ASU005	17890	23679	KRAS	c.35G>T	p.G12V	26.40%	5	KRAS	G12V
CC	ASU006	20640	20438	KRAS	c.35G>T	p.G12V	17.80%	6	KRAS	G12V
CC	ASU008	38710	47260	KRAS	c.35G>A	p.G12D	48.70%	8	KRAS	G12D
CC	ASU009	23970	42524	KRAS	c.35G>A	p.G12D	11.60%	9	KRAS	c 12/13
CC	ASU010	39690	32094	KRAS	c.35G>A	p.G12D	8.20%	10	KRAS	G12D
CC	ASU011	19560	11297	KRAS	c.34G>T	p.G12C	43.1%	11	KRAS	c 12/13
CC	ASU012	9480	39149	KRAS	c.35G>A	p.G12D	11.60%	12	KRAS	c 12/13

	Pan Cancer DNA							GRU		
Sample Type	Sample	Copies	Coverage	Gene	Cosmic	AA	Percent Variant	Sample_GRU	Gene	Variant
Mel	ASU013	12540	1174	BRAF	c.1799T>A	p.V600E	46.20%	13	BRAF	V600E
Mel	ASU014	30510	19011	BRAF	c.1799T>A	p.V600E	19.50%	14	BRAF	V600E
Mel	ASU015	1870	11103	BRAF	c.1799T>A	p.V600E	40.70%	15	BRAF	V600E
Mel	ASU016	32430	20525	BRAF	c.1799T>A	p.V600E	41.50%	16	BRAF	V600E
Mel	ASU017	20110	9773	BRAF	c.1799T>A	p.V600E	41.90%	17	BRAF	V600E
Mel	ASU018	42810	18414	BRAF	c.1799T>A	p.V600E	45.70%	18	BRAF	V600E

Fig. 3. Results: The FFPE positive control achieved a 100% pass rate across all operator runs with quantification of 4.5-7.5% BRAF V600E. All variant call percentages were within 15% CV. Variant calls had 100% PPV at > 4% variant. Finally, FFPE specimens were concordant to matched calls in KRAS, and BRAF variants.

Conclusions: While we recognize that this is a small cohort, our results show the value of integrating pre-analytical, analytical, and post-analytical steps within a targeted NGS workflow for clinically-relevant gene targets. Our data highlight the importance of unifying wet-lab procedures, validated controls, and the bioinformatics pipeline to enable accurate and timely results and address laboratory needs for facile training and expeditious validation of targeted NGS methods in oncology.

1805 Molecular Profiling Reveals That Multiple Merkel Cell Carcinoma Tumors May Represent Either Clonally Related Metastases or Multiple Clonally Distinct Primaries

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Background: Merkel cell carcinoma (MCC) is a highly aggressive cutaneous neuroendocrine malignancy, with pathogenesis likely driven by Merkel cell polyomavirus (MCPyV) or UV-mediated genomic damage. Approximately 1% of patients present with additional MCC tumors clinically consistent with a second primary instead of cutaneous metastasis. We utilized next generation sequencing (NGS) to evaluate clonal relatedness in MCC tumors clinically designated as multiple primary tumors.

Design: Eight MCC tumors (from 4 patients) clinically designated as multiple primaries were analyzed using targeted NGS on the Ion Ampliseq Comprehensive Cancer Panel, which assesses copy number alterations (CNAs) and mutations in the full coding sequence of 409 cancer-relevant genes. Overlap in CNAs and mutations was calculated for each pair. High-confidence somatic synonymous and non-synonymous mutations were considered for clonality analysis. MCPyV was detected by quantitative PCR.

Results: Tumors from each patient were consistently MCPyV-positive (3 patients, 6 tumors) or MCPyV-negative (1 patient, 2 tumors). Two tumor pairs displayed minimal to absent mutation overlap and no CNA overlap, suggesting these pairs were clonally unrelated. One pair displayed low but significant overlap in mutations (17%) and high CNA overlap (67%), consistent with clonality. Another tumor pair had rare overlapping mutations (1%), but high CNA overlap (80%); the high CNA overlap is suggestive of clonal relatedness. Random pairings of tumors from different patients displayed little overlap in mutations (average 0.01%) or CNAs (average 1.1%).

Conclusions: We evaluated 4 patients with multiple MCC tumors clinically designated as multiple primary tumors. Two cases were clonally unrelated by copy number changes, thus representing genetically distinct multiple primary tumors. MCPyV status was concordant for tumor pairs regardless of clonal relatedness. Our findings show MCC patients are at risk of a second genetically distinct primary tumor; second tumors likely develop through similar mechanisms of pathogenesis, either MCPyV-mediated or UV-mediated. Our findings also support clonality, and hence metastasis, in 2 cases of presumed multiple primary disease. The clinical implications of our findings and the use of NGS to assess clonality in such cases warrant additional investigation.

1806 Differential Expression of PDGFRB and EGFR in Microvascular Proliferation in Glioblastoma

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Background: Glioblastoma (GBM) is the highly malignant glioma and exhibits microvascular proliferation. The volume of the microvasculature is a small portion of GBM. It is necessary to start with isolated microvasculature derived RNA.

Design: We investigated the difference of mRNA expression profiles between GBM microvasculature and GBM tumor cells using Human Cancer Drug Targets PCR Arrays. The mRNA array results were validated by immunohistochemistry on tissue microarray. We also explored the reason that cause the high expression PDGFRB in GBM microvasculature and the high expression of EGFR in GBM tumor cells by genomic DNA analysis using The Cancer Genome Atlas data and microRNA(miRNA) array analysis.

Results: The mRNA level of PDGFRB in GBM microvascular proliferation was significantly higher than that in GBM tumor cells while the mRNA level of EGFR was higher in GBM tumor cells than microvascular proliferation. Immunohistochemical stains with anti- PDGFR antibody or anti-EGFR antibody were performed on tissue microarray, which demonstrated strongly positive expression of PDGFR in GBM microvascular proliferation, but was negative in GBM tumor cells and normal brain blood vessels. EGFR was positive in the majority of GBM tumor cells and normal brain blood vessels, but was negative in GBM microvascular proliferation. By analyzing The Cancer Genome Atlas (TCGA) datasets for GBM, It was found that genomic DNA alterations were the main reason for the high expression of EGFR in GBM tumor cells, but were less likely the reason for the decreased expression of PDGFRB in GBM tumor cells compared to GBM microvascular proliferation. Our miRNA microarray data showed that miRNAs (miR-193b-3p, miR-518b, miR-520f-3p and miR-506-5p) targeting PDGFRB were down-regulated in microvascular proliferation, which might be the most likely reason for the high expression of PDGFRB in GBM microvascular proliferation. The increase of these miRNA (miR-133b, miR-30b-3p, miR-145-5p and miR-146a-5p) targeting EGFR in GBM microvascular proliferation is at least one of the reasons for the lack of expression of EGFR in GBM microvascular proliferation.

Conclusions: These findings implicated that targeted therapeutic agents against both EGFR and PDGFRB can be developed and may effectively kill both tumor cells and microvascular proliferation in GBM. MiRNAs, such as miR-506, miR-133b, miR-145 and miR-146a, that target PDGFRB or EGFR, are potential therapeutic agents for GBM.

1807 Somatic Mutational Landscape of Inflammatory Bowel Disease-Associated Signet Ring Cell Colorectal Carcinoma

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Background: Inflammatory bowel disease (IBD) is specifically associated with increased risk of colorectal cancer (CRC), most often presenting with signet ring (SR) morphology and an aggressive clinical course. Differing pathways for sporadic CRC and IBD-associated CRC have been generally established; however fine grain resolution of the mutational landscape of these cancers remains unclear. To comprehensively characterize the somatic mutations that drive IBD-associated SRCRC we have whole exome sequenced (WES) and functionally analyzed five IBD-associated SRCRC cases with matched normal tissue.

Design: WES was performed on five IBD-associated SRCRC cases with matched normal colon tissue. The tumor and matched normal pairs were sequenced to an average depth of 233X and 183X, respectively. The captured exome reads were mapped to the human reference genome, and the tumor/normal pairs underwent somatic genotyping for single nucleotide variants (SNVs), insertions and deletions (INDELs), copy number alterations (CNAs) and structural variants (SVs). The somatic mutations were annotated for a series of functional predictions in order to identify deleterious coding mutations for subsequent downstream analysis.

Results: We identified a median of 196 SNVs and 276 coding INDELs across the samples in our cohort. No high confidence CNAs or SVs were identified. SNV mutational profiling identified five commonly mutated genes across our cohort: MUC16, TP53, MST1L, NEB, PCLO and DNAH17. MUC16 was the only gene commonly mutated when restricting to *de novo* SNVs. Further, analysis of somatic INDELs identified 71 coding variants with C11orf80, ODF1, OR6C76, KRTAP17-1, OR2T35, HRNR, KRTAP19-6 and NUDT11 harboring *de novo* INDELs shared across the cohort. Functional pathway analysis of the above somatically mutated genes found significant ($p < 0.01$) enrichment in cytoskeletal processes.

Conclusions: Our WES data provides the first broad analysis of the mutation landscape of IBD-associated SRCRC. In addition to common CRC mutations like TP53, there is evidence of Mucin gene family mutations and enrichment in cytoskeletal pathway alterations. Mucin gene family mutations are associated with poor prognosis in sporadic CRC and we provide the first molecular evidence that IBD-associated SRCRC may harbor mutations associated with prognostic significance. Our data extend the understanding of IBD-associated tumorigenesis, but confirmation in a larger cohort study is warranted.

1808 Up-regulated S100A4 Is Associated with Tumor Progression in Signet-Ring Cell Carcinoma

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Background: Membrane-bound β-catenin is a key component of the cadherin complex essential for cell adhesion. Aberrant expression of β-catenin, defined as loss of membranous expression and/or nuclear/cytoplasmic subcellular localization of the protein, is a central event in transducing Wnt signaling that plays a pivotal role in tumor progression and metastasis. S100A4, a downstream gene of the β-catenin/TCF transcription factor family, has a well-established metastasis-promoting activity. As a small calcium binding protein, it drives metastasis by affecting the tumor microenvironment and stimulating cancer cell motility and invasion. We have previously demonstrated that aberrant expression of β-catenin is associated with a higher risk of metastasis in signet ring cell carcinoma (SRCC). In this study, we sought to explore the potential role of S100A4 in the progression of SRCC.

Design: Consecutive SRCCs and adenocarcinomas with prominent signet ring features from various organ systems obtained from the authors' institution between 2004 and 2014 were retrieved. The immunoexpression of β-catenin and S100A4, along with the nuclear/cytoplasmic subcellular localization of β-catenin, were examined. Overexpression of S100A4 was defined as at least a 25% increased intensity when compared to adjacent normal epithelium.

Results: Among a total of 92 cases in the study period, 30 out of the 39 (77%) with S100A4 overexpression demonstrated aberrant β-catenin expression while 22 out of 53 (41%) without increased S100A4 had aberrant β-catenin ($p=0.0007$). Further, 48 out of the 52 (92%) cases with aberrant expression of β-catenin developed nodal or distant metastasis, whereas 4 out of 21 (19%) with membranous expression had metastasis ($p<0.0001$). Additionally, 28 of 30 cases (93%) with both aberrant expression of β-catenin and S100A4 overexpression had metastatic progression. This was in contrast to the remaining 62 cases with no simultaneous aberrant β-catenin or increased S100A4 expression. Of these only 43 (69%) had metastatic disease ($p=0.01$).

Conclusions: The observations that overexpression of S100A4 was associated with aberrant expression of β-catenin and that simultaneous increased S100A4 and aberrant β-catenin expression was significantly associated with a higher rate of metastasis strongly underlines the importance of Wnt signaling in the progression of SRCC. Further molecular investigation of this pathway may provide novel therapeutic targets for functional blockade in the subset of adenocarcinomas with prominent signet-ring features in the pursuit of precision medicine.

1809 Targeted Sequencing of Somatic Mutations in Plasma Circulating DNA of Patients with Primary Central Nervous System Lymphoma (PLCNS)

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Background: PLCNS are rare and aggressive tumors whose histological diagnosis can be difficult due to deep brain structure involvement. Circulating tumor DNA (ctDNA), a minimally invasive biomarker, could help for diagnosis. We characterized molecular abnormalities in PLCNS and demonstrated that targeted sequencing of ctDNA in blood at time of diagnosis could identify somatic mutations in PLCNS.

Design: We enrolled 30 immuno-competent patients with newly diagnosed PLCNS, without lesions outside the brain. High throughput sequencing was performed on primitive tumors using a panel of 34 genes relevant to lymphomagenesis. We next performed patient-specific targeted sequencing of identified somatic mutations in ctDNA.

Results: Twenty-nine tumors had at least one somatic mutation. The NF-kB pathway was the most affected by mutations: *MYD88* (n=23, 77%), L265P variant ($MYD88^{L265P}$) being the most frequent (n=20, 67%), *PIM1* (n=11, 37%), *TNFAIP3* (n=6, 20%), *IRF4* (n=3, 10%), *CARD11* (n=3, 10%), and *PRDM1* (n=3, 10%). One tumor harbored a single $MYD88^{L265P}$ mutation with no other detectable abnormality. The second most affected was the apoptotic pathway, including *GNA13* (n=6, 20%), *TP53* (n=2, 7%), *CDKN2A* (n=2, 7%), *MYC* (n=1, 3%), and *BCL2* (n=1, 3%) mutations. Other pathways were also affected: the B cell receptor with *CD79B* (n=10, 33%) and *ITPKB* (n=2, 7%) mutations; epigenetic regulation with *KMT2D* (n=10, 33%), *CREBBP* (n=3, 10%), *MEF2B* (n=2, 7%) and *EP300* (n=1, 3%) mutations; immunity with *B2M* (n=4, 13%) mutations; and JAK/STAT and MAPKinase pathways with *SOCS1* (n=2, 7%) and *BRAF* (n=1, 3%) mutations respectively. Eight tumors (27%) harbored a dual alteration affecting *MYD88* and *CD79B*. Of the 24 available plasmas, 13 patients (54%) had at least one somatic mutation detected in ctDNA, all comprising the $MYD88^{L265P}$ mutation without correlation to prognosis, tumor volume, or blood level of lactate dehydrogenase. Mutations in 5 other genes were also identified in ctDNA: *CD79B, CREBBP, PIM1*, *TNFAIP3* and *EP300.*

Conclusions: To our knowledge this is the first study highlighting the proof of concept that somatic mutations can be detected in ctDNA in patients with PLCNS, thereby constituting a minimally invasive tool for diagnosis. The recurrent mutation $MYD88^{L265P}$ detected in tumoral DNA and ctDNA might result in the introduction of targeted therapies.

1810 Clinician Experience with an NGS Clinical Observation Trial in Oncology

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Background: NGS assays are routinely executed in clinics, yet documented utility and experience with the assays is limited. In particular, the benefit of NGS tumor profiling in patient cohorts who have rare tumors, have exhausted standard of care, or have metastatic disease of unknown origin remains to be determined. The inherent complexity of the assay also creates challenges in generating a patient report that is comprehensive, but straightforward. An assessment via an observational trial of the clinical experience allows the opportunity to create a structured dialogue between the ordering physician and CLIA laboratory, which will enable well-documented feedback for improvement.

Design: An IRB approved observational clinical study was implemented to assess the use of a 358-gene targeted sequencing assay for patient tumor profiling. The study was designed to identify the clinical factors that are involved in the decision to utilize the assay, including the intent for ordering the test and the decision to use the resulting data to inform treatment. Case report forms were developed to obtain feedback from the clinician and to collect patient baseline and outcome data. The clinician utilization survey consisted of eighteen multiple-choice questions with the ability to provide additional comments.

Results: Data from nine de-identified patient samples were sent to the CLIA sequencing laboratory and all clinician utilization surveys were completed and returned. In 56% of the patients, the test was ordered because the patient exhausted standard of care. In all cases, an adequate specimen was available for testing, however only patients with suitable tissue were enrolled. Prior to ordering the test, 100% of clinicians expected the test to be somewhat likely to lead to the next treatment choice. 78% of survey responders indicated that the length of the clinical report was too long and correspondingly 89% found the front summary table to be the most useful.

Conclusions: Initial experience with an NGS observational trial in solid tumor profiling has provided invaluable feedback. First, it has become evident that the rate-limiting step to patient enrollment is the amount of available specimen for testing. Second, the data most likely to aid in treatment decision needs to be presented clearly and concisely on the NGS clinical report, but with the ability to probe deeper when warranted. Third, clinicians are enthusiastic and eager to try new technologies when traditional patient management strategies have been exhausted.

1811 Role of SOX4 in PTEN-Mediated Prostate Tumorigenesis

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Background: Large-scale gene expression studies have identified increased expression levels of the *SOX4* gene in a variety of human cancers. Deletion or mutation of the *PTEN* gene is one of the most frequent genetic alterations in many of the sporadic human cancers. To determine whether *SOX4* plays an important role in prostatic adenocarcinoma (PCA) initiation and progression, we developed a novel mouse model with prostate-specific conditional homozygous deletion of both *SOX4* and *PTEN*.

Design: To induce deletion of *Pten* and *Sox4* alleles, mice were injected intraperitoneally with tamoxifen at eight weeks of age after reaching sexual maturity. Six months after tamoxifen administration, animals were sacrificed, and prostate tissue samples were subjected to histological and molecular analyses.

Results: Seventy per cent (7/10) of the $PTEN^{-/-}$ mice developed solid prostate tumors that exhibited denser glands compared with those of wild-type mice. Strikingly, only 11% (2/18) of the double knockout $PTEN^{-/-}/SOX4^{-/-}$ mice had PCA ($p = 0.0028$). While $PTEN^{-/-}$ mice developed invasive PCA in the dorsolateral prostate, $PTEN^{-/-}/SOX4^{-/-}$ mice progressed primarily to high-grade PIN (HGPIN), but not invasive PCA. Notably, *SOX4* deletion significantly reduced Ki-67 positive cells (16% vs 28%). Immunohistochemical (IHC) staining of dorsolateral prostate lobes indicated that deletion of *Sox4* reduced the *PTEN* loss-induced phosphorylation of AKT at serine 473. Strikingly, a significant decrease of active β-catenin was also observed by IHC analysis of $PTEN^{-/-}/SOX4^{-/-}$ mice compared to $PTEN^{-/-}$ prostates. These findings were also confirmed by Western blotting analysis. Furthermore, we observed that homozygous deletion of *SOX4* reduced the expression of AKT1 in the mouse prostate, suggesting that SOX4 may have an important role in the regulation of AKT1.

Conclusions: Homozygous deletion of *SOX4* in the adult prostate epithelium strongly inhibits tumor progression initiated by homozygous loss of the *PTEN* tumor suppressor, demonstrating the key role of SOX4 in the development of PCA. Our findings indicate that SOX4 is a critical component of the PTEN-PI3K-AKT pathway, suggesting that SOX4 may be a potential molecular drug target for novel therapies of both primary and advanced PCA.

1812 Retrospective Review of Targeted Molecular Genotyping of Lung Adenocarcinomas at Baylor University Medical Center

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Background: A large urban hospital has been performing a clinical protocol-driven, cost-conscious, and patient care-oriented mutation analysis to identify disease relevant mutations in available tissue on essentially all newly-diagnosed lung adenocarcinomas since January 1, 2013. Mutation analysis consisted of targeted multiplex single nucleotide polymorphism (SNP) analysis and PCR for EGFR/HER2 insertions/deletions (indels). Mutation-negative samples were reflexively tested by fluorescence in situ hybridization (FISH) for ALK and ROS1 translocations.

Design: Retrospective review of 262 pathology reports was conducted over a 30 month period from January 1, 2013 to June 30, 2015. All cases of lung cancer where molecular testing was performed were included in the review. Data points, including age, gender, genotype, and smoking history were collected for analysis. Nineteen patients were further excluded from the study due to specimen/technical quality issues.

Results: Out of the 243 patients included in the retrospective review, 120 (49.4%) patients had missense mutations or indels in a cancer gene. Of the remaining 123 patients, reflex FISH for ALK and ROS1 demonstrated an additional three (2%) Alk(2p23)

rearrangements. The overall mutational profile was 64 (53%) KRAS, 37 (31%) EGFR, 6 (5%) BRAF, 6 (5%) PIK3CA, 3 (2%) HER2. In addition, there was 1 (1%) of each of the following: MEK, MAP2K1, ALK, NRAS. The most common KRAS mutation was G12C (53%). The two most common EGFR mutations were the exon 19 deletion (38%) and L858R (35%). One-hundred eighty-four patients had a history of smoking (75.7%) with a strong tendency to have KRAS mutations (32%), whereas 59 patients were non-smokers (24%) who tended to have EGFR mutations (46%).

Conclusions: This is a brief review of the experience of a large urban hospital that is using an algorithmic approach to mutation testing in lung adenocarcinoma. Overall, we find disease relevant mutations in approximately 50% of lung adenocarcinomas, similar to large academic institutions. Smokers tended to have KRAS mutations whereas non-smokers had increased frequency of EGFR mutations. The relatively small number of ALK and ROS1 rearrangements detected, raises the question of whether their acquisition was masked by exclusion of our testing algorithm; however, the substantial cost savings of the algorithmic approach may offset the difference.

1813 MET Molecular Epidemiology of Advanced Solid Tumors Candidate to Targeted Therapies

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Background: Aberrant MET activation occurs in many types of malignancies and includes protein overexpression, increased gene copy number, amplifications, mutations and deletions. The frequency of alterations in MET varies widely among solid tumors. The criteria for MET amplification and MET overexpression have not been established as well as the relationship between MET amplification, MET expression and additional genomic alterations important in tumor biology.

Design: Tumor samples(n=205) were from Colorectal(41%), Gastro-esophageal(12%), Lung(8%), SNC(7%), Breast(6%) and other cancers. Samples were 119 primary and 86 metastatic tumors. Fluorescence in-situ hybridization(FISH) for MET gene amplification and immunohistochemistry(IHC) for protein expression were used. MET protein levels were quantified by mass spectrometry(MS) in a subset of samples. Mutations in key oncogenes were determined using Mass Array and Amplicon-Seq.

Results: MET gene amplification (ratio MET/CEN7≥2) was found in 3.8% (7 of 180 evaluable samples) and copy number gain (≥5 copies) in 7%(n=13) of cases . Kras mutations were detected in 22% (30 of 137 evaluable samples, none harboring MET amplification) and PI3K mutations in 12% (n=16, 2 cases with MET amplification) of cases. c-MET high expression by IHC (≥50% with ≥2+) was found in 27% of cases (46 of 170 evaluable samples) with strong expression (≥50% with 3+) in 7% (n=12). Correlation between copy number and protein expression was significant(P<.0001). Higher expression of c-MET was observed in metastases compared with primary tumor (P=0.001). Differences in MET status according to tumor type are shown in table 1.

Histotype	H-score(av)	IHC ≥2+ (%)	IHC ≥3+ (%)	Ampl(%)	Gain(%)
Colorectal	97	40	6	0	4
Gastro-esophageal	37	11	0	0	10
Lung	126	50	25	19	25
SNC	38	14	14	14	14
Breast	13	0	0	0	0
H&N	30	0	0	0	0
Ovary	2	0	0	0	0
Soft tissue	50	0	0	0	0
Choroidal melanoma	155	67	67	33	33
Other	58	18	4	8	8

Conclusions: High c-MET expression was observed in advanced colorectal and lung cancers and in choroidal melanoma. Metastases expressed higher levels of c-MET compared to primary tumor. There was a significant correlation between c-MET expression and gene copy number. Correlation between MET status by IHC/FISH and quantitative MET protein expression by MS will be presented.

1814 20q Gain Is an Oncogenic Driver Event That Occurs Preferentially in KRAS/ BRAF Wild Type Colorectal Carcinoma

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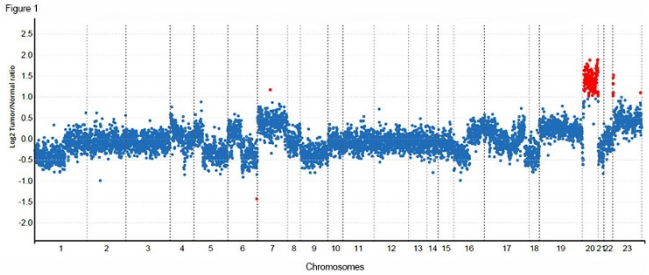
Background: Mutations in *KRAS*, *NRAS* and *BRAF* occur in approximately 50% of colorectal carcinomas (CRC) and are the only oncogenic driver alterations considered in decision for standard treatment. Chromosomal gain of 20q is an understudied, recurrent somatic genetic alteration in CRC. In this study, we characterize the clinicopathologic and molecular characteristics of CRC with 20q gain.

Design: Electronic medical records for all CRC patients with samples tested by MSK-IMPACT, a next generation sequencing assay which interrogates somatic mutations as well as copy number variations in 410 genes, between January 2014 to August 2015, were reviewed. Cases with low tumor content (no silent mutations and less than 10% maximum variant frequencies) were excluded from the analysis. A minimum 20q tumor: normal log2 fold change of 1.5 (i.e. 2.8X) was defined as a gain.

Results: Of 398 cases CRC, 96 (24.1%) had 20q gain (figure1). The most frequently amplified 20q genes within MSK-IMPACT were *BCL2L1*, *SRC*, *GNAS*, and *AURKA*. CRC with 20q amplification were significantly more frequent in the distal colon than in the proximal colon cases, (n=78, 81.2%. p<0.0001). No correlation was seen between 20q amplification and histologic grade and differentiation. 20q amplified CRC more frequently harbored mutations in *TP53* (n=90, 93.8%, p<0.0001), and *APC* (n=77, 80.2%, p<0.0001). Conversely, these tumors less frequently harbor mutations in *KRAS* (n=22, 22.9%, p<0.0001), *PIK3CA* (n=5, 5.2%, p<0.0001), *BRAF* (n=5, 5.2%, p<0.0037).

Conclusions: Our data show, for the first time, that 20q gain has a strong tendency to be mutually exclusive with known driver mutations in *KRAS*, *BRAF*, and *PIK3CA*, supporting 20q gain as a alternative oncogenic diver alteration in at least 17% of CRC where it occurs in the absence of *KRAS* or *BRAF* mutations. This subset of CRC with 20q gain remains to be characterized in terms of prognosis and response to standard and gene targeted therapies. Further assessment of driving genes within 20q may provide novel treatment opportunities.

Figure 1



1815 Next Generation Sequencing of Tumors: An Institutional Experience of 200 Consecutive Cases

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Background: For the last 4 years, a limited number of institutions around the country have offered clinical next generation sequencing (NGS) of tumor samples. This novel technology has generated great interest for its potential to open doors for advanced cancer patients who have failed standard chemotherapy. However, studies on the utility of NGS in actual clinical practice are limited. In the past two years, we have analyzed clinical patient samples for mutations in 2855 hotspots in 50 cancer-associated genes using the Ion AmpliSeq Cancer Hotspot Panel v2. Herein, we present our experiences with NGS in the first 200 patients and address its clinical utility in managing oncologic patients.

Design: Pathology reports and clinical records were reviewed on 200 patients who had NGS performed on formalin-fixed paraffin embedded tumor specimens at our medical center. A database was created to store clinically relevant data extracted from these reports. Key parameters included tumor type, clinical indication, and gene mutations. The mutations were categorized according to actionablity defined as one of three therapeutic categories: 1) determines eligibility for FDA approved drug for patient's tumor type, 2) predicts response to FDA approved drug for other indications (off-label use), or 3) determines eligibility for clinical trials.

Results: Of the 200 cases submitted to our department, the two most common indications were cancer patients refractory to standard therapy or to further search for genomically-guided therapeutic options when single gene tests were noncontributory. Lung (34%), breast (16%), colon (6%), and ovarian (7%) carcinomas were the most prevalent tumors in our series. In 140 cases (70%), a total of 238 mutations were identified in 28 of 50 genes with the most frequent mutations occurring in the TP53, KRAS, EGFR, APC, PIK3CA, and ATM genes. Three quarters of these mutations were actionable and were distributed over 111 cases (56%). About 5% of mutations determined eligibility for FDA approved therapy, 38% predicted response to off-label therapy, and 71% determined eligibility for clinical trials.

Conclusions: In over half of the cases at our institution, NGS was able to provide therapeutically relevant information. We conclude that NGS can be a useful tool for guiding cancer patient management for the preponderance of tumors. Further work is needed to determine whether NGS is changing patient management and improving outcomes in the clinic.

1816 Development of Patient Derived Tumor Organoids to Guide Precision Medicine

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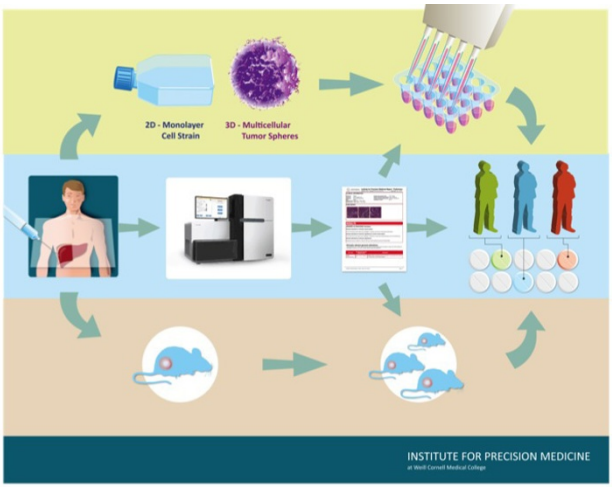
Background: Precision oncology is a clinical approach aimed towards tailoring treatment strategies for patients based on the genetic profile of each patient's cancer. Available cell line models alone often do not recapitulate the genetic profile of individual patient tumors and therefore limit preclinical evaluation of new targeted agents. A high failure rate of drug candidates can be attributed in part to the use of monolayer cultures as the initial screening method that is associated with highly variable responses and does not predict clinically observed chemoresistance. In our Institute for Precision Medicine we developed a program utilizing patient derived tumor organoids in combination with individualized genomic sequencing, to nominate drug candidates in a precision patient care setting. Utilizing these various genomic and biological platforms for pharmacological screenings, we can more closely recapitulate the in vivo tumor environment of individual patient tumors and can more accurately model personalized therapeutic response and resistance in vitro and in vivo.



Design: Fresh tissue samples were collected, washed, mechanically/enzymatically dissociated and then plated in Matrigel and cultured with primary culture media. Primary tumor organoids were characterized according to our cytology, histology and genomic platforms. Established and characterized tumor organoids were expanded, cryopreserved, used for in vitro studies and injected in mice for patient derived xenograft (PDX) studies.
Results: Our success rate in generating patient derived tumor organoids is ≈ 30%. Morphology and molecular profiles show good concordance among in vitro tumor organoids and native tumor tissues. In vitro studies show tumor specific drug sensitivity that can be further characterized in PDX models.
Conclusions: Cyto-, histo- and molecularpathology represent important platforms in our Precisicon Medicine Program. Tumor organoid development, characterization, patient's tumor specific pharmacological screening and drug validation in PDX models are effective models which can be used to tailor standard of care treatment, study drug resistance, and nominate novel therapeutic targets unique to the individual genomic landscape of each tumor.

1817 Utility of Genomic Analysis in Differentiating Synchronous Independent Lung Adenocarcinomas from Primary Adenocarcinomas with Intrapulmonary Metastasis

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Background: Distinguishing synchronous primary lung adenocarcinomas from adenocarcinomas with intrapulmonary metastasis is crucial for proper patient management. We used next generation sequencing (NGS) to compare the molecular signatures in multiple lung adenocarcinomas.
Design: The study group included 13 patients (29 lung adenocarcinomas). The histologic patterns of the invasive carcinomas included lepidic, acinar, papillary, micropapillary and solid. Tumors were diagnosed in resection specimens (n=26) or by fine needle aspiration (n=3). All tumors were subjected to ALK rearrangement testing (FISH) and targeted NGS (50 gene cancer panel).
Results: The cohort consisted of 9 females and 4 males. The mean age at diagnosis was 69 years. The synchronous lung tumors were located in the same lobe (n=6 patients), different lobes of the same lung (n=3) or contralateral lung (n=4). Three patients had 3 tumors and 10 patients had 2 tumors. The adenocarcinomas were non-mucinous and resected tumors were classified as invasive (n=22), minimally invasive (n=3) and in-situ (n=1). Morphological comparison was performed in resection specimens from 11 patients.
Tumors located in the same lobe were grossly and radiographically separate and histologically distinct in all 6 patients. Different genomic variants were identifed in 2 of the 6 patients. In contrast, all 7 tumors in contralateral lungs or different lobes of the same lung harbored different mutations. Overall, 9 of the 13 patients (69%) with synchronous tumors had different and potentially actionable molecular alterations in *EGFR, KRAS* and *PI3KCA* genes as well as alterations in *TP53, STK11* and *PTEN.* None of the specimens harbored ALK fusions.

	Tumor 1	Tumor 2
Tumors in the same lobe (n=6)	EGFR G719C, EGFR S768I KRAS Q61H TP53 G266* EGFR L747_T751del EGFR L858R None	KRAS Q61L None TP53 G266* EGFR L747_T751del EGFR L858R None
Tumors in different lobes or contralateral lung (n=7)	TP53 A159V EGFR L747_T751del, STK11 F354L KRAS G12V, PTEN L318fs*2 PIK3CA H1047L KRAS G12C KRAS G12V KRAS G12C	KRAS G12C EGFR L858R, STK11 F354L KRAS G12C EGFR E746_A750del TP53 R273C KRAS Q61H, TP53 G245V EGFR L747_T751del

Conclusions: Genomic profiles of synchronous lung adenocarcinomas complement the histological findings, improving accurate staging and identifying genetic alterations with therapeutic implications.

1818 Combined Molecular Testing (Mutational Analysis and MicroRNA Classifier Status) Advances Understanding of Molecular Pathobiology of Thyroid Follicular Neoplasia

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Background: The molecular pathobiology of thyroid neoplasia remains challenging as the detection of mutational change may not completely account for the benign versus malignant disease. While certain mutations (BRAF point mutation) are highly predictive of cancer, other forms of point mutation and translocation are not necessarily indicative of malignancy. Benign disease and hyperplasia can demonstrate mutational change. Biological aggressiveness can be independently classified by a panel microRNA (miRNA) expression profiling. We evaluated the utility of combining both approaches when evaluating thyroid nodule disease.
Design: Stained cytology slides and corresponding formalin fixed paraffin embedded (FFPE) tissue sections were collected on 15 patients with indeterminate thyroid nodule cytology. Total nucleic acids were extracted and evaluated by next generation sequencing (NGS) for point mutations in BRAF, HRAS, KRAS, NRAS, PIK3CA genes and translocations of PAX8/PPAR and RET/PTC genes. In parallel, 10 marker miRNA profiling, designed to classify benign vs. malignant disease, used miR-29b-1-5p, miR-31-5p, miR-138-1-3p, miR-139-5p, miR-146b-5p, miR-155, miR-204-5p, miR-222-3p, miR-375, and miR-551b-3p. Statistical analysis compared performance as determined by surgical pathology outcome.
Results: Microdissected fixative treated specimens provided adequate levels of good quality DNA for replicate NGS and miRNA analyses. Most papillary thyroid cancer specimens showed a range of mutation clonality ranging from 31-85% in keeping with a heterogeneous population of mutated and nonmutated cells. Analysis of miRNA status was consistently high in all papillary carcinomas irrespective of clonality. All benign lesions were categorized as such by miRNA analysis even though some of them demonstrated specific gene mutations.
Conclusions: Combined molecular analysis (mutation detection and microRNA classifier) can be accomplished on cytology and histopathology slides enabling close correlation of cellular microscopic and molecular changes. While mutation detection can predict malignancy, in many cases, the variability in individual mutational change and the need to account for complex interactions may not be clear. Correlative miRNA analysis has the ability to detect and characterize biological aggressiveness.

1819 The Ataxia-Telangiectasia Mutated and RAD3-Related Protein Kinase Regulates Intracellular Hydrogen Sulfide Concentrations

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Background: The endogenous gasotransmitter hydrogen sulfide (H_2S) regulates angiogenesis and cell proliferation, and is over-expressed in breast and colorectal cancers. The ATR protein kinase plays a central role in the stabilization of stalled DNA replication forks and checkpoint initiation. The role of ATR in regulating the enzymes involved in H_2S synthesis and cellular H_2S levels has not been examined, in part due to cell lines with ATR activity loss being inviable. Here we examined a colorectal carcinoma-derived cell line with either wild-type ATR expression or the same cell line carrying the Seckel syndrome hypomorphic ATR mutation.
Design: H_2S, acid labile, and bound sulfide pools where measured by HPLC. Western blotting quantified cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), 3-mercaptopyruvate S-transferase (3-MST), phospho-ATR(ser428), γH2AX, and nicotinamide phosphoribosyltransferase (Nampt) protein expression. The colony-forming efficiency assay (CEFA) measured cell colony-forming ability following treatment with oxidative stress and H_2S synthesis inhibitors.
Results: H_2S, the acid labile and bound sulfide pools where all lower in the cell line carrying the Seckel syndrome mutation. All three H_2S synthesizing enzymes, but not Nampt, were also significantly lower in the mutant cells, and in the CEFA the mutant cell line was significantly more sensitive to oxidative stress, especially in the presence of an H_2S synthesis inhibitor. The Seckel mutant cells showed elevated γH2AX, both with and without exogenous oxidative stress, indicating higher levels of endogenous and induced DNA damage. Phospho-ATR(ser428), a marker of ATR kinase activation, was also significantly lower in the mutant cell line and was not increased with cellular oxidant exposure, an event found in the ATR wild-type cells. Last, H_2S synthesis inhibitors induced ATR(ser428) phosphorylation in wild-type, but not mutant cells, indicating that cellular H_2S levels regulate ATR activation.
Conclusions: To our knowledge, this is the first time ATR has been shown to play a role in the regulation of cellular H_2S levels. Our findings show that stalled replication forks, which activate ATR, impinge on H_2S regulation and, as ATR influences the activities of many other proteins such as ATM, mTOR, RAD17, and BRCA1, suggests that the DNA damage response in general may alter cellular H_2S levels.

1820 Platelet Cloaking Maintains Migratory and Invasive Characteristics of CTCs

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Background: Metastatic disease is the main cause of death from solid tumours. During metastasis cancer cells gain migratory and invasive characteristics, through a process called epithelial–mesenchymal transition (EMT), in order to enter the blood. Our work focuses on circulating tumour cells (CTCs) and their interaction with components of the blood. Previously we showed that platelets, through direct interaction, aid survival and drive the metastatic profile of cancer cells. In this study, we looked at a more biologically relevant form of cancer cells, those undergoing EMT. Our aim was to examine how platelets affected these mesenchymal-like cells.

Design: To induce EMT *in-vitro* SK-OV-3 and 59M ovarian cancer cells were treated with epidermal growth factor (EGF) for 6 days. Cells were then either returned to normal culture media (NCM) or continued in EGF supplemented media for a further 6 days. To evaluate any EMT-like changes in the cells, we examined their morphology, migration rate and the expression of epithelial and mesenchymal related markers. Once validated, we assesed the effect platelets had on EMT-like cells.

Results: Treatment of cells with EGF for 6 or 12 days induced changes in morphology, characteristic of mesenchymal cells (elongated spindle shape, lack of cell-cell contact). There was a decrease in expression of the key epithelial marker E-cadherin and an increase in mesenchymal related genes, FN1 and VIM. Both cell lines demonstrated increased migratory capabilities when EGF treated compared to controls. However, when cells were returned to NCM their phenotype reverted to that of the control cells, i.e. epithelial-like, suggesting cells underwent mesenchymal-epithelial transition (MET). We next examined the effect of exposing 6 day EGF treated cells to platelets. These cells did not undergo MET like those returned to NCM but instead had a phenotype similar to those treated with EGF for 12 days.

Conclusions: This work suggests that *in-vivo* if EMT is induced in the primary tumour by environmental factors, that their loss, once the cell enters the blood, could result in the loss of this phenotype. As cancer cells still need to extravasate out of the blood to form a secondary tumour this would be problematic. This study shows the important role platelets could play by maintaining a mesenchymal-like phenotype. Thus, suggesting that targeting platelets may be a novel approach to inhibit metastasis.

1821 LINE-1 RNA and ORF1p Protein Are Broadly Co-Expressed in Colon Cancer

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Background: In an initial immunohistochemical survey, approximately half of human cancers express the ORF1p protein of the long interspersed element-1 (LINE-1) retrotransposon, with no detectable expression in corresponding normal tissues. Expression was correlated with higher grade malignancy and poor prognostic factors such as p53 mutation, suggesting that LINE-1 may be a useful biomarker in many tumor types, including colon cancers. LINE-1 is the only active human transposon and is a genomic parasite that comprises approximately 18% of our genome. 80-100 copies per individual are still capable of generating new insertions via a copy-and-paste mechanism, and are repressed by multiple mechanisms including DNA methylation. Increasing genetic evidence demonstrates that escape these repressive mechanisms in carcinogenesis generates a burden of somatic insertions, but the extent to which these insertions contribute to malignancy is unknown. Additionally, LINE-1 promoter methylation has been correlated with global hypomethylation, but the extent to which this correlates with LINE-1 expression or tumor microsatellite instability (MSI) is unknown, and ORF1p and RNA expression have not been correlated in cancers.

Design: We generated tissue microarrays from 162 colon cancers and evaluated LINE-1 RNA by 5' in situ hybridization, ORF1p expression using optimized immunohistochemistry with a monoclonal antibody, and MSI status by immunohistochemical expression of MSH2, MSH6, MLH1, and PMS2 proteins.

Results: ORF1p expression was found in 82% of colon cancers, higher than 50% previously reported, and higher expression of ORF1p correlated with LINE-1 RNA expression. No correlation with MSI status was identified.

Conclusions: These data suggest that LINE-1 may be a useful biomarker in colon cancer and have prognostic significance.

1822 Interim Analysis of 708 Patients Enrolled in the MOSCATO 01 (Molecular Screening for Cancer Treatment Optimization) Trial

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Background: Enrolling patients with ad hoc molecular alterations is one of the current challenges in early drug development. The MOSCATO 01 trial (NCT01566019) is one of the prospective trials ongoing worldwide using high throughput analysis of small tumor biopsies for characterisation of the genomic alterations in cancer patients (Hollebecque, ASCO 2013 ; Ferte et al, AACR 2014).

Design: An "on-purpose" tumor biopsy was collected from consenting patients with metastatic cancer referred for a phase I trial to our Drug Development Department at the Gustave Roussy Cancer Center. DNA extracted from frozen tumor biopsies with at least 30% of tumor cells were analysed by array comparative genomic hybridization (aCGH) and by hot spot mutations using Next Generation Sequencing (NGS) for 74 target genes when tumor cellularity was at least 10%. In addition, whole exome sequencing (WES) and RNA sequencing (RNAseq) were recently implemented in the MOSCATO trial. A weekly multidisciplinary molecular tumor board reviewed results to identify the molecular alterations and address such patients to relevant phase I trials or approved targeted therapies.

Results: Between 12/2011 and 11/2014, 708 patients were enrolled in the MOSCATO 01 trial, and 639 patients (90%) had undergone a tumor biopsy. Patient characteristics were as follows: median age at biopsy: 57 years (range, 18-78); main tumor types: lung (14%), head and neck (13%), prostate and bladder (11%), breast (10%), colorectal (6%); median of 3 previous lines of treatment.

Molecular profiling with aCGH and NGS was performed in 510 (80%) and 565 patients (88%) respectively, with median analysis turnaround time of 21 days. Clinical significant molecular alterations were found in 288 patients (45%), most frequently in PTEN/PI3K/AKT (n=84), FGFR/FGF (n=46), KRAS/NRAS/HRAS (n=24), and HER2/EGFR (n=22/18) pathways. Patients with rare genomic alterations were also identified. Therapy could be personalised in 141 patients (49%) with matched targeted therapy or dedicated phase I clinical trials. Preliminary results of WES and RNAseq as well as the response rate of patients with specific molecular alterations treated with matched targeted therapies will be updated and presented at the meeting.

Conclusions: Our updated results confirm in a larger cohort of patients the feasibility of molecular characterisation of cancer patients in routine clinical practice. Such approach underlines the critical role of the pathologist in helping oncologists to enrich early-phase clinical trials with specific and rare genomic alterations.

1823 Uterine Adenosarcoma and Phyllodes Tumor of the Breast Have Similar Repertoires of Somatic Genetic Alterations

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Background: Uterine adenosarcomas (UA) and breast Phyllodes tumors (PT) are biphasic lesions composed of mesenchymal and epithelial components. UAs are usually low-grade, but may display sarcomatous overgrowth. PTs are classified as benign, borderline or malignant based on histologic features. Here we sought to define whether UAs and PTs display similar repertoires of somatic genetic alterations.

Design: DNA from 19 UAs and 25 PTs (6 benign, 6 borderline and 13 malignant PTs) and matched normal tissues were subjected to either whole-exome sequencing (6 UAs) or targeted capture massively parallel sequencing (MPS; 13 UAs and 25 PTs) using the MSK-IMPACT assay, which targets all coding regions and selected intronic and promoter regions of 227 key cancer genes. Somatic genetic alterations were identified using state-of-the-art bioinformatics algorithms.

Results: Borderline and malignant PTs had a significantly higher somatic mutation rate (median 4, range 2-7 and 5, range 2-8, respectively) than UAs (median 1, range 0-5, $p<0.05$), whereas benign PTs (median 1, range 1-3) and UAs had similar mutation rates. *MED12* exon 2 and *TERT* promoter mutations were found exclusively in PTs (60% vs 0% in UAs, 52% vs 0%, respectively, both $p<0.05$). *RARA* and *SETD2* were mutated in 20% of PTs but not in UAs, whereas *RB1* and *EGFR* were altered only in malignant PTs (24% and 62%, respectively). *DICER1* and *FGFR2* were the only genes recurrently mutated in UAs (both 11%) and these genes were not altered in PTs. Although *TERT* gene amplification was observed in both UAs and PTs (21% and 4%), *MDM2* amplification was only detected in UAs (26% vs 0%, respectively, $p=0.01$). When UAs were compared to PTs stratified according to histologic grade, *MED12* exon 2 mutations were significantly more prevalent in benign, borderline and malignant PTs than in UAs (83%, 83%, 31% vs 0%, all $p<0.05$), and *TERT* somatic promoter mutations/ TERT gene amplification were significantly more frequent in malignant PTs than in UAs (69% vs 21%, $p=0.01$), but found at similar rates in benign PTs, borderline PTs and UAs.

Conclusions: The repertoire of somatic genetic alterations found in UAs is similar to that of benign and borderline PTs but differs from that of malignant PTs. *TERT* somatic alterations were significantly more frequent in PTs than in UAs, but UAs more frequently harbored *MDM2* amplification.

1824 PITPNC1 in Breast Cancer

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Background: Previously our lab has found that breast cancers with a 17q24.2 amplification were associated with the presence of sentinel or distal axillary lymph node metastases, larger tumor size and higher histological grade. PITPNC1 is an evolutionarily conserved gene within this region that binds 14-3-3, a protein kinase implicated in cancer. In addition, we looked into the NKI microarray expression dataset of 295 breast cancers and found PITPNC1 to be correlated with low survival, grade, ER and HER2 status. We want to develop a better understanding of the role of PITPNC1 in breast cancer by analyzing the status of PITPNC1 in breast cancer cell lines, its role in cell proliferation and its association to tumor characteristics in breast cancer patients.

Design: PITPN1C protein levels were measured in a panel of breast cell lines. PITPNC1-GFP and GFP vectors were transfected into MCF7 and MDA-MB-231 cell using endofectin transfection reagent according to the manufacturer's instructions. Proliferation was measured using the MTT assay. Cells were counted using a hemocytometer and placed into 96 well plates. Immunohistochemical analysis of PITPNC1 expression in breast cancers was performed using TMAs built from a consecutive series of invasive carcinomas collected from a single institution. Immunohistochemical staining was evaluated blinded using light microscopy.

Results: PITPNC1 protein expression by immunoblotting reveals its absence in the non-tumorigenic cell line MCF10A in contrast to high levels of expression in eight tumorigenic and invasive breast cancer cell lines evaluated. Transient overexpression

of the different isoforms into the MCF7 cell line did not increase cell proliferation. TMA analysis of patient's breast cancers reveals strong staining in myoepithelial cells and in situ duct carcinoma.

Conclusions: PITPNC1, a gene whose functional role is not well understood, is present in the17q amplicon which is associated with worse behavior of breast cancer. The gene plays a role in breast cancer although it does not appear to act through cell proliferation. Our initial data suggest that it may be one of the genes driving this amplicon. Additional analyses are ongoing.

1825 Identification and Validation of Differentially Expressed miRNAs in Appendiceal Mucinous Cystadenocarcinoma from Mucinous Cystadenoma

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Background: Mucinous cystadenocarcinoma of appendix is a rare entity and the differential diagnosis of its benign counterpart, mucinous cystadenoma can often be challenging on morphologic grounds only. Characterization of the molecular genetic profile of the two entities, particularly in cases with overlapping morphologically feature can help establishing the diagnosis with higher certainty. The objective of this study was to investigate the utility of differentially expressed miRNAs in the aiding the diagnosis of appendiceal mucinous cystadenocarcinoma and mucinous cystadenoma.

Design: All surgically resected mucinous tumors of the appendix diagnosed in WSU between 2000-2013 were considered for this study. The cases were microscopically reviewed by a senior pathologist with particular expertise in gastrointestinal pathology who classified the diagnoses into mucinous cystadenocarcinoma and mucinous cystadenoma. Paraffin blocks containing adequate diagnostic tissues of both entities were identified to extract total RNAs and the comprehensive miRNA expression profiling from pooled aliquots of samples were compared. The top seven differentially expressed miRNA were validated individually by reverse qRT-PCR. A student *t*-test was used to determine the significance of differential miRNA expression between the mucinous cystadenoma and mucinous adenocarcinoma groups. A level of $p < 0.05$ was regarded as statistically significant and $p < 0.01$ was regarded as statically very significant.

Results: Six mucinous cystadenocarcinoma and twelve mucinous cystadenoma of the appendix were classified on morphologic bases and included in the study. The microarray miRNA expression profiling analysis revealed that 646 miRNAs are differentially expressed in the mucinous cystadenocarcinoma. Among these differentially expressed miRNAs, the expression of 80 miRNAs shows statistical difference ($p< 0.01$). Real time RT-PCR revealed the expression of miR-1, miR-4328 is significantly down regulated in mucinous cystadenocarcinoma compared to the mucinous cystadenoma ($p< 0.05$). On the other hand, the expression of oncogenic miR-200c, miR-223, miR-21 is significantly increased in mucinous cystadenocarcinoma compared to the mucinous cystadenoma ($p<0.05$).

Conclusions: These data suggest that miRNA expression in mucinous appendiceal neoplasm can help supplement the morphological evaluation in distinguishing benign from malignant tumors. Our findings based on limited number of cases analyzed of these relatively rare entities can benefit from similar analyses of larger cohorts to help confirming these potentially promising results.

1826 Immuno-Proteomics of Colon Cancer: PSMA1 Is Implicated in Colorectal Carcinogenesis and Has Prognostic Significance

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Background: Discovery of tissue-based protein biomarkers for early diagnosis has the potential to improve targeted therapy through judicious selection of colon cancer patients in clinical trials. The immune system of cancer patients can sense aberrant proteins as tumor associated antigens and has the ability to produce autoantibodies against these antigens. We developed cancer patient serum antibody screening (immuno-proteomics) to identify novel tumor-specific antigens. We employed mass spectrometry-based proteomics to investigate global differential protein expression profiles.

Design: Proteins expressed in fresh-frozen matched normal and tumor tissue pairs were analyzed by 2-D SDS-PAGE. Potential cancer-specific biomarkers were detected by Western blot using cancer patient sera as autoantibody source. Protein spots were analyzed by nano-HPLC and Orbitrap mass spectrometry. Identified biomarkers were validated and characterized by Western blot and immunohistochemistry (IHC). We further investigated the prognostic value of PSMA1 in a tissue microarray cohort of 196 well characterized stage II-III colon cancers.

Results: Proteomic analysis identified PSMA1, maspin, annexin A3, and LAP3 as possible cancer markers. We validated these four markers in an independent cohort by Western blot and IHC using a TMA. Specifically, PSMA1 overexpression was observed in 44.3% (87/196) of the stage II-III colon cancer cohort, and loss of PSMA1 expression was associated with nodal metastasis (p=0.00003). Colon cancer with high expression of cytoplasmic PSMA1 had improved overall survival (p=0.0131). In a multivariate analysis in the stage II-III group, PSMA1 was predictive independently of age, gender, stage, tumor differentiation, site, histology, pT and tumor size.

Conclusions: Immuno-proteomics is a novel powerful technique for cancer marker discovery. PSMA1, maspin, annexin A3, and LAP3 are promising colon cancer biomarkers. PSMA1 is an independent prognostic marker for better survival, and loss of expression in the primary is associated with lymph node metastases. This is one of the first reports implicating PSMA1 in colorectal carcinogenesis and may have important implications for proteasome inhibitor based therapy.

1827 Cigarette Smoke Associated Genetic Alterations Found in Urine Specimens from Patients with Superficial Non-Muscle Invasive High Grade Urothelial Carcinoma

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Background: Studies show that cigarette smoking is the single greatest risk factor for bladder cancer, accounting for approximately 50% of bladder cancer cases. Smoking associated carcinogens and genetic alterations have been extensively studied in various human cancers. In particular, MDM2 SNP309 polymorphism and ataxia-telangiectasia mutated (ATM) gene have been reported to contribute to genetic susceptibility to lung cancer in smokers. Although various oncogenes and tumor suppressor genes have been found in bladder cancer, the role of smoking in the pathogenesis of superficial non-muscle invasive high grade urothelial carcinoma (NMIHGUC) with regards to genetic alterations is still unclear. The goal of this study is to evaluate genetic alterations associated with history of smoking using urine cytology specimens.

Design: Fifty-three slides from forty-two patients (14 never smokers and 28 ever smokers) with cytologic diagnosis and histopathologic confirmation of NMIHGUC were included for this study. All specimens were cellular and contained at least 1,000 cells neoplastic each and the tumor cells represented over 50% of the specimen cellularity. DNA was extracted from the ThinPrep cytologic slides and subjected to next generation sequencing (NGS) analysis using a customized targeted exome capture assay composed of 341 genetic abnormalities, including oncogenes, tumor suppressor genes, and components of pathways deemed actionable by targeted therapies.

Results: The average amount of DNA obtained from each slide ranged from 23 to 2080ng, with an average of 359ng. The number of genetic alterations ranged from 2 to 87 with an average of 18.4. Our results indicate the patients with smoking history had gene mutations in ATM and MDM2 amplification significantly more often than patients that never smoked ($P<0.05$). Interestingly, BAP1 gene mutation which has been found in malignant mesothelioma, was identified more frequently in never smokers (21.4%) than ever smokers (3.6%).

Conclusions: Urine cytology specimen is a suitable material for molecular analysis by NGS method, which provides excellent DNA quantity and quality. The significant high frequency of ATM and MDM2 genetic alterations in patients with smoking history and NMIHGUC suggests that urothelial carcinomas might share some oncogenetic pathways with lung carcinoma. The pathogenic role of BAP1 gene in the urothelial carcinoma of never smoking patients requires further investigation.

1828 Germline Variants and Secondary Findings in a Cancer Precision Medicine Cohort

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Background: Whole exome sequencing (WES) is rapidly gaining traction in precision oncology. To accurately identify somatic alterations, WES typically requires analyzing germline DNA from matched control samples. This creates an opportunity to identify and report cancer-associated germline variants and secondary findings that may impact disease surveillance and prevention. However, which variants to report and how to report them in the clinical setting are incompletely defined.

Design: To address these questions, we analyzed a cohort of 155 patients with advanced, metastatic tumors in which we have already performed tumor and germline WES. We performed germline variant calling and annotated variants using a variant categorization system based on evidence from ClinVar and OMIM. We focused the analysis on 91 reportable genes that include cancer risk genes and ACMG secondary finding genes. We also perform ethnicity inference using a statistical approach based on a panel of informative single nucleotide polymorphisms.

Results: We identify reportable variants in 1 out of 4 patients on average, including known and novel variants in APC, ATM, BRCA1, BRCA2, RAD51D and RET. We observe a statistically significant excess of germline cancer risk variants in our advanced cancer precision medicine cohort compared to two large control cohorts; p=8.8e-4 and p=0.021, respectively. On the other hand, we found that the number of non-cancer variants in ACMG genes was not significantly different in our cohort compared to the control cohorts. We however find that a link between cancer risk variants and diagnosis is not always obvious. For example, we observe BRCA1 and BRCA2 deleterious variants in prostate, brain cancer and sarcoma patients instead of only ovarian or breast cancer patients. Ethnicity inference reveals that our cohort consists of 30% Ashkenazi patients, which we show have a significant number of cancer risk variants likely due to founder effect. However, controlling for Ashkenazi individuals does not impact our statistically significant excess of germline cancer risk variants.

Conclusions: The global germline cancer risk variant enrichment pattern observed in this study has not been described before but is compatible with findings from a growing number of focused studies. Altogether these results further support the need to report germline results back to patients and their physicians.

1829 Shifts in Mutation Profiles between Primary and Synchronous Lymph Node Metastases (mLN) in Triple Negative Breast Cancer

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Background: Triple negative breast cancer (TNBC) has been subclassified into multiple distinct immunophenotypic subtypes with variable clinical features. For example, in our previous study, TNBC with overexpression of CD24 in tumor cells and CD10 in tumor stromal cells was associated with both nodal and remote metastases . Prior studies have also shown that TNBC breast biopsies and metastases are molecularly heterogonous (Eur J Hum Genet. 2010,18:560). Given that mLNs are a target for adjuvant therapy, we sought to assess the stability of the mutation profile in primary and metastatic tumors from the same patients.

Design: We studied paired breast primaries and synchronous mLN from 17 TNBC patients (pts, median ages: 51.5 years old). DNA from the paired samples was tested with a clinically-validated next generation sequencing (NGS) assay with 5% sensitivity for missense mutations, assessing mutations in 50 relevant target genes. BRAF V600E mutation was confirmed by Sanger sequencing.

Results: Among the 34 samples from 17 pts, 39 non-synonymous mutations were identified (2.3/pt), including 30 that were present at both sites (concordant). Mutations were seen in TP53 (22/39, 56.4%), BRAF V600E (10%), PIK3CA (10%), MET (8%), APC and KDR in 2 each, and ATM, FGFR3, JAK3 and KIT in one case each. In 6 cases (35.3%), a total of 9 mutations were present in the breast biopsy but absent in mLN. These absent mutations include BRAF (V600E, 4), TP53 (4), PIK3CA (1) and APC (1). The discordant TP53 mutants were p.V274_C275del (2), p.R273H (2), p.E339* (1) and p.H179Q (1). Sanger sequencing of the breast samples in BRAF-mutated cases showed that V600E levels were high in 2 cases and low-level in 2. Discordant mutation status was unrelated to CD10 or CD24 expression but was associated with cases with higher mutation burden (2.83±0.55 versus 1.81±0.77, $p<0.001$).

Conclusions: We noted a 35% incidence of discordant mutation profiles between breast and mLN sites in TNBC that preferentially affected cases with higher mutation burden. TP53 and BRAF V600E mutations were commonly absent in the metastasis, which could potentially affect the clinical management of these pts. Further work is needed to understand whether clonal heterogeneity or adverse selection might be underlying this phenomenon but multisite sequencing approaches may be warranted.

Pediatric Pathology

1830 Collagen XVIII and Procollagen-lysine, 2 oxoglutarate 5-dioxygenase 2 (PLOD2) Expression in Human Pediatric and Mouse Rhabdomyosarcomas: Exploring Potential Biomarkers of Therapeutic Relevance

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Background: Collagen XVIII (COL18A1) and Procollagen-lysine, 2 oxoglutarate 5-dioxygenase 2 (PLOD2) are recently described proteins associated with increased metastatic and local spread in carcinomas. Our aim is to study expression of COL18A1 and PLOD2 in pediatric rhabdomyosarcomas and mouse model rhabdomyosarcomas which are potential therapeutic targets.

Design: A tissue microarray (TMA) of 74 tumors was constructed with 4 human and 70 murine sarcomas. Human tumors included 4 pediatric rhabdomyosarcomas, including: 3 alveolar and 1 embryonal subtype. The TMA was stained with COL18A1 and PLOD2 immunohistochemistry yielding viable lesion for 47 COL18A1 and 48 PLOD2 cases. The TMA sarcomas are listed in table 1. The lesional cells were scored for intensity of staining (weak, moderate, strong) and percent of positive lesional cells (1-10; 11-50; 51-80; 81-100). In 30 cases, more than one section of viable tumor was available for scoring therefore averages of intensity and total percent were calculated. Any averaging less than 1 (mild) for intensity were scored as no staining.

Sarcoma Subtype	COL18A1 (47)	PLOD2 (48)
Alveolar Rhabdomyosarcoma	16	16
Embryonal Rhabdomyosarcoma	9	9
Pleomorphic Rhabdomyosarcoma	3	7
Mixed Alveolar/Embryonal Rhabdomyosarcoma	1	1
Undifferentiate Sarcomas NOS (plomorphic, spindle, epitheliod)	18	15

Results: 42 of 47 (89.4%) of the sarcomas stained positive for COL18A1, 26(55%) of which had a staining intensity of moderate or greater. The sarcomas that lacked COL18A1 expression were undifferentiated sarcomas NOS (4) and pleomorphic rhabdomyosarcoma NOS (1). 45 of 48 (93.8%) sarcomas expressed PLOD2, 27 (56%) of which had at least moderate staining intensity. The sarcomas that lacked PLOD2 expression were undifferentiated sarcomas NOS (2) and pleomorphic rhabdomyosarcoma NOS (1).

Conclusions: All human and mouse alveolar and embryonal rhabdomyosarcomas expressed significantly increased amounts of PLOD2 and COL18A1 relative to normal background tissue. While this pilot study analyzed only a small cohort of human tumors, it raises the possibility of eligibility for therapies targeting post-translational modifications of collagen, and suggests further human sarcoma subtypes to study for these markers.

1831 Will the Quest for a Novel Positive Marker for a Diagnosis of Hirschsprung Disease on Formalin Fixed Rectal Mucosal Biopsies End with a Glucose Transporter? A Detailed Dual IHC Panel Study of GLUT1 and Calretinin

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Background: Hirschsprung disease (HD) is characterised by the aganglionosis of the rectum and its diagnosis is challenging due to the lack of a specific positive marker on formalin fixed paraffin embedded sections (FFPES) of rectal mucosa. Neither is AChE histochemistry (gold standard for HD diagnosis) suitable for FFPES nor is calretinin IHC, as it is a negative marker. Hence this study, to search for a novel positive IHC marker on mucosal FFPES for a definitive diagnosis of HD.

Design: This cross sectional study was conducted on 20 full thickness FFPES of colon and 20 rectal mucosal biopsies; in 2 phases, each over a period of 6 months. In the first phase, 20 full thickness FFPES from resected ganglionic and aganglionic segments of HD were stained with H&E and GLUT1 (SPM498, Biocare) antibody and compared with the neuronal panel of Calretinin, Synaptophysin, CD56, NF, GFAP, S100 and PGP9.5. These were independently reported by 3 pathologist. The findings obtained were extrapolated to the second phase of the study for ratification, where subsequent 20 consecutive rectal mucosal biopsies, stained with H&E; GLUT1 and calretinin were studied (blind to the final diagnosis) and compared with the corresponding AChE stained sections.

Results: Phase 1: GLUT1 stained the perineurium (PN) of serosal nerves in all segments and the PN of both submucosal and myenteric plexus nerves in aganglionic segments but was absent/minimal in the PN of ganglionic/normal colonic segments; ganglion cells were negative. Phase 2: Of the 20 rectal mucosal biopsies (9 HD and 10 Non-HD cases; 1 case inadequate after FFP embedding) studied, all 9 HD cases were diagnostic on GLUT1 staining which showed strong PN staining of small and large fibres in the submucosa. On the other hand, of the 10 Non-HD cases, 2 cases yielded a false positive diagnosis of HD by showing a strong PN staining of small nerve fibres in the submuscosa. Thus, while the NPV of an independent GLUT1 IHC marker for HD was 100%, its PPV was only 80%. However, when the GLUT1 sections were read with calretinin, a correct diagnosis was obtained in 100% cases as ratified by the AChE staining.

Conclusions: The positive ring-like perineural staining of GLUT1 and negative staining of Calretinin are diagnostic of HD on FFPE rectal mucosal biopsies. A dual panel of GLUT1 and Calretinin IHC stains on FFPES could be as accurate as the gold standard AChE staining on frozen sections for the diagnosis of HD.

1832 A Strategy for Helicobacter Immunohistochemistry Utilization in the Pediatric Setting: Details from a Morphologic and Cost: Benefit Analysis

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Background: Dedicated cost-benefit analysis of universal *Helicobacter* immunohistochemical (IHC) staining in pediatric gastric biopsies has not been performed. As such, we undertook a morphologic study of pediatric *Helicobacter* cases with the intent to provide useful guidelines in the pediatric setting.

Design: All *Helicobacter* positive gastric biopsies were retrospectively collected from our primary and referral care center over twelve months (63 cases) and 122 *Helicobacter* negative gastric controls were randomly selected from the same time period. All cases were reviewed and scored according to the Sydney System for the severity of acute and chronic inflammation, as well as the location of inflammation and presence of lymphoid follicles.

Results: Within the reviewed 12 month period 1,979 total pediatric gastric biopsies were identified, including 63 *Helicobacter* positive cases (3.2%). We reviewed 185 biopsies (63 *Helicobacter* positive, 122 *Helicobacter* negative controls) and the corresponding *Helicobacter* IHC, which was performed in all cases. *Helicobacter* infection was significantly associated with lymphoid follicles (cases=42.9% vs. controls=4.1%, $p<0.0001$), active inflammation (cases=84.1% vs. controls=8.2%, $p<0.0001$), moderate or severe chronic inflammation in the oxyntic mucosa(cases=47.6% vs. controls=2.5%, $p<0.0001$) and any chronic inflammation in the antrum except inflammation that was both mild and superficial (cases=95.2% vs. controls=21.3%, $p<0.0001$). At least one of these inflammatory patterns was seen in all *Helicobacter* positive biopsies, compared with 34 of 122 control biopsies (100% vs. 27.9%, $p<0.0001$). Chronic active gastritis was present in 53 *Helicobacter* positive biopsies and 9 control biopsies (84.1% vs. 7.4%, $p<0.0001$).

Conclusions: We recommend performing *Helicobacter* IHC on pediatric gastric biopsies where organisms are not identifiable on H&E sections with any of the following inflammatory patterns: 1) lymphoid follicles, 2) any active inflammation, 3) moderate or severe chronic inflammation within the oxyntic mucosa, or 4) any chronic inflammation in the antrum except mild superficial inflammation. This approach can sensitively identify pediatric patients with *Helicobacter* gastritis while limiting IHC staining to approximately 30% of all gastric biopsies. In comparison with universal *Helicobacter* IHC, this approach would reduce patient charges at our institution by approximately $280,000 per year.

1833 TdT-Positive Cells in Inflamed Pediatric Kidney: A Potential Diagnostic Pitfall

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Background: We encountered a patient with infantile nephrotic syndrome associated with a dense mononuclear infiltrate and prominent extramedullary hematopoiesis (EMH). Immunohistochemical analysis revealed numerous Terminal deoxynucleotidyl