Conclusions: Our data point to GLUT1 as a possible key discriminator between benign and malignant adrenocortical tumors in children, and this suggests that metabolic reprogramming of cancer cells towards aerobic glycolysis can be explored for development of therapeutic strategies.

618 Long Non-Coding RNA MALAT1 Expression in Thyroid Tissues and Tumors

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Background: Long non-coding RNAs (lncRNAs) participate in transcription and in epigenetic or post-transcriptional regulation of gene expression, and may contribute to carcinogenesis. MALAT1 (Metastasis Associated Lung Adenocarcinoma Transcript 1), a lncRNA that participates in the regulation of cell cycle and migration, is known to be deregulated in multiple cancers. Some studies suggest MALAT1 may function as both an oncogene and a tumor suppressor. We analyzed the expression of the MALAT1in thyroid tumors and compared its expression to miR-146b-5p, a microRNA known to be deregulated in papillary thyroid cancer.

Design: Tissue microarrays (TMAs) were constructed with formalin-fixed paraffin-embedded (FFPE) tissues of normal thyroid (NT, n=10), nodular goiters (NG, n=10), follicular adenoma (FA, n=32), follicular carcinoma (FCA, n=28), papillary thyroid carcinoma (PTC n=28), follicular variant of papillary thyroid carcinoma(FVPTC, n=29), poorly differentiated thyroid carcinomas (PDC, n=21) and anaplastic thyroid carcinoma (ATC, n=35). TMA sections were analyzed by in situ hybridization (ISH) using RNAscope technology with a MALAT1 probe (Advanced Cell Diagnostics). ISH for miR-146b-5p was also performed on the same set of TMAs (Exiqon). qRT-PCR was performed on a subset of the TMA cases (n=16). The results of the MALAT1 TMA ISH were analyzed with Vectra imaging technology, Nuance® and inForm ® software.

Results: MALAT1 was highly expressed in NT, NG and in benign and malignant thyroid tumors predominantly in the nucleus, but also in the cytoplasm. The highest levels of MALAT1 wwere observed in PTCs which was significantly higher than in NT ($p=0.014$) and FVPTC ($p=0.016$). In contrast NT expressed higher levels of MALAT1 than PDC ($p=0.015$) or ATC ($p<0.001$). qRT-PCR analyses supported the ISH findings. Expression of miR-146b-5p was highest in PTC (89%) followed by FVPTC (41%) and was lowest in ATC (8%).

Conclusions: MALAT1 is highly expressed in NT tissues and thyroid tumors with increased expression during progression from NT to PTCs. However both MALAT1 and miR-146b-5p are downregulated in ATC compared to PTCs, suggesting that MALAT1 may function both as an oncogene and as a tumor suppressor in different thyroid tumors and that non-coding RNAs may regulate the development of PTCs and ATCs.

Gastrointestinal Pathology

619 CD66b-Positive Tumor-Associated Neutrophils in Epstein-Barr Virus Associated Gastric Carcinoma: A Comparative Study with CD8-Positive Cytotoxic T-Lymphocytes

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Background: Epstein-Barr virus-associated gastric carcinoma (EBVaGC) is one of four molecular subtypes of gastric carcinoma. EBVaGC is characterized by prominent intratumoral lymphocyte infiltration, with some infiltration by other types of inflammatory cells, such as neutrophils. In this study, the significance of tumor-associated neutrophils (TANs) was investigated in EBVaGC and compared to CD8-positive cytotoxic T lymphocytes (CTLs).

Design: After immunohistochemistry of CD66b and CD8, specific markers of TAN and CTL respectively, whole sections of EBVaGC were analyzed with digital image analysis system (Tissue Studio), and their clinicopathological significance was analyzed.

Results: There was no correlation between CD66b- and CD8-positive areas in the tumor (correlation coefficient: $R^2=0.0075$, $P=0.453$), suggesting an independent mechanism of both types of inflammatory cell infiltration. Forty-two of 77 cases of EBVaGC (55%) had some or considerable infiltration of TANs (CD66b-positive areas>0.5%, TAN^+) and 35 (45%) had no or scant infiltration (TAN^-). Thirty-two cases (42%) had more CTLs (CD8-positive area>20%, CTL-high) and 45 (58%) had fewer (CTL-low). The cases of EBVaGC TAN^+ showed correlation with intestinal type histology ($P=0.048$) and absence of lymph node metastasis ($P=0.023$), while cases of EBVaGC CTL-low with upper location ($P=0.033$) and advanced invasion depth (pT2 or more) ($P=0.045$). Neither TAN^+ nor CTL-low was associated with disease-specific survival in EBVaGC. Multivariate logistic regression analysis revealed that TAN^- was independently associated with lymph node metastasis ($P=0.036$). None of the 21 cases of EBVaGC TAN^+ with submucosal invasion showed lymph node metastasis, and such probability was estimated to be extremely low (95% confidence interval: 0-13.3%).

Conclusions: TANs in EBVaGC may suppress lymph node metastasis through anti-tumor effect. Presence of TANs in the sites of submucosal invasion indicates absence of lymph node metastasis, which may be useful for pathological diagnosis of endoscopic submucosal dissection of gastric carcinoma.

620 High Expression of the Leaky Protein Claudin-2 in Esophageal Carcinoma and Precancerous Lesions Is Significantly Associated with the Bile Salt Receptors VDR and TGR5

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Background: Claudins are a family of integral membrane proteins and are components of tight junctions (TJs). Many TJ proteins are known to tighten the cell structure and maintain a barrier. In contrast, Claudin-2 is a leaky protein that plays an opposing role and increases cell permeability. Recently, we found that VDR enhanced Claudin-2 expression in colon and that bile salt receptors VDR and TGR5 were highly expressed in esophageal adenocarcinoma (EAC) and precancerous lesions. Here, we examined the expression of Claudin-2 in EAC and precancerous lesions and its association with VDR and TGR5 expression.

Design: Claudin-2 expression was examined by immunohistochemistry on tissue microarrays, containing EAC, high grade dysplasia (HGD), low grade dysplasia (LGD), Barrett's esophagus (BE), columnar cell metaplasia (CM), squamous cell carcinoma (SCC), and squamous epithelium (SE) cases. Intensity (0 to 3) and percentage were scored for each case. High expression was defined as 2-3 intensity in ≥ 10% of cells.

Results: Claudin-2 was highly expressed in 77% EAC (86/111), 38% HGD (5/13), 61% LGD (17/28), 46% BE (18/39), 45% CM (29/65), 88% SCC (23/26), and 14% SE (11/76). It was significantly more highly-expressed in EAC, SCC and glandular lesions than in SE and more in EAC than in BE and CM. No significant difference in Claudin-2 expression was found between CM, BE, LGD, and HGD. A significant association was found between Claudin-2 expression and VDR and TGR5 expression. No significant association was found between expression of Claudin-2 and age, gender, grade, stage, or patients' survival time in EAC and SCC.

Conclusions: We conclude that Claudin-2 might play a novel role in the development and progression of esophageal mucosal metaplasia, dysplasia and carcinoma. Claudin-2 expression is significantly associated with VDR and TGR5 expression. The functional relationship between Claudin-2, VDR and TGR5 will be studied in future.

621 Doxycycline Induced Gastrointestinal Injury: Case Series with New Sites of Involvement

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Background: Doxycycline induced gastric injury is a rarely recognized side effect of this common medication. Tetracyclines, especially doxycycline, are a widely accepted cause of pill-induced esophagitis, but little attention has been given to its association with other sites of gastrointestinal injury. In the stomach, a distinctive superficial capillary degeneration with fibrinoid material is seen in a background of a reactive gastropathy. Previously, only two cases have been reported describing this gastric finding. We have encountered additional examples of this injury pattern and sought to expand the limited information by describing the associated length of drug ingestion, original and follow-up endoscopic findings, sites of involvement, gender, and age.

Design: All gastrointestinal biopsy material for cases indexed with the word "doxycycline" were retrieved and the histology reviewed and recorded. The associated medical record was used to obtain pertinent clinical findings.

Results: Four cases were identified from the indexed search with available clinical and biopsy material, including two males and two females ranging in age from 39-80 years. Doxycycline ingestion could be confirmed in three of the four cases with the length of time from start of drug to initial endoscopy ranging from 5-54 days. Gastric endoscopic findings included a non-removable white coat, a greater curvature erosion, a superficial pyloric ulcer, and linear fundic ulcers. All the patients' gastric biopsies had reactive gastropathy, variable neutrophilic infiltrates in the superficial lamina propria and marked superficial small vessel injury with fibrinoid material in a concentric fashion around the interior of the vessel. A single patient, of three in which the duodenum was biopsied, had similar vascular changes in the duodenum. One patient underwent follow-up endoscopy after drug cessation and was found to have normal gastric mucosa endoscopically and histologically.

Conclusions: Doxycycline induced gastrointestinal injury that comes to clinical attention prompting endoscopy is rare given the frequency the drug is used. Previous to this report only two cases were documented with histologic findings. Thus, our series adds to the literature by confirming the distinct vascular injury and describes the finding in the duodenum, a site not previously known to be effected. Recognition of this evolving drug-specific injury pattern is important for patient management.

622 Histologpic Spectrum of Ipilimumab Associated Colitis Is Narrow and Resembles Inflammatory Bowel Disease

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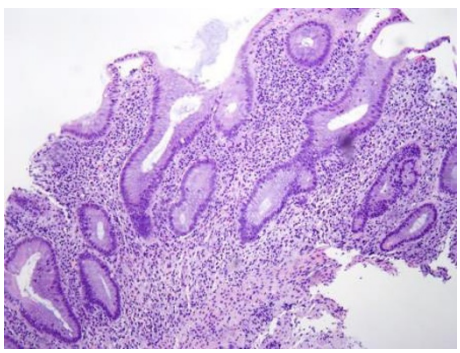
Background: Ipilimumab (AntiCTLA-4), a standard of care option for metastatic melanoma, renal cell carcinoma and lung cancer, has potentially lethal side effect of enterocolitis. Histopathologic features of Ipilumimab induced colitis are not well described.

Design: In a retrospective search of the institutional pathology database (2001-2014), we identified 22 patients on ipilimumab who underwent colonoscopy for diarrhea clinically suspected due to Ipilumimab. Patients' electronic medical records were reviewed for colonoscopic findings and to exclude other etiologies of colitis. In all patients all the segments of colon and rectum were biopsied. Hematoxylin and Eosin

stained sections were independently reviewed by two pathologists applying conventional histopathologic features of colitis including extent and type of inflammation, granuloma, features resembling lymphocytic colitis, ischemic type injuries and infectious colitis.
Results: The patient population included 14 men and 8 women with an age range of 30-80 years. Ipilimumab induced colitis involved right and left colon in all patients and commnest histologic findings was diffuse chronic active colitis.

Endoscopy	Patients (n)
Normal	2
Edema/erythema	9
Colitis with or without ulcer	11
Distribution	
Patchy	6
Diffuse	16
Abnormal epithelial architecture	
Absent	2
Mild	13
Moderate to severe	7
Cryptitis	
Present	18
Absent	4
Intraepithelial lymphocytosis	
Present	4
Absent	18
Intra epithelial eosinophils	
Present	3
Absent	19
Basal Lymphoplasmcytosis	
Present	16
Absent	6
Granuloma	
Present	3
Absent	19
Paneth cells in left colon	
Present	2
Absent	20
Epithelial apoptosis >2/HPF	
Present	18
Absent	4



18/21 patients who were treated with steroids along with cessation of Ipiliumab had symptomatic relief within 3-7 weeks.
Conclusions: Histopathologic changes with ipilimumab induced colitis are pancolitis with diffuse chronic active inflammation resembling active inflammatory bowel disease. Features resembling lymphocytic colitis were uncommon in this population.

623 Prospectively Reported Histologic Tumor Viability Score and Factors Predicting Long Term Survival in Patients Treated with Trimodality Therapy for Primary Esophageal Adenocarcinoma

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Background: The histopathological assessment of the resected specimen in primary esophageal adenocarcinoma (PEA) treated with chemotherapy combined with radiotherapy followed by surgical resection (trimodality therapy) includes a histologic tumor viability score. The aim of study was to assess if there were survival differences among pathologists who provided the histologic tumor viability (HTV) score.
Design: In a wide search of the institutional database (2001-2014), we identified 830 patients with biopsy proven PEA who received trimodality therapy. Pretreatment clinical staging was confirmed by endoscopic ultrasound and/or CT/PET CT scans. Esophagectomy specimens after preoperative chemoradiation were assessed and reported by 14 pathologists (range 11-142 specimens). The histopathological assessment included presence and depth of residual tumor, grade, lymphovascular and perineural invasion, lymph node and margin status, and pathologic stage. HTV was reported in all 830 patients and was classified as 0%-10%, 11%-50% and >50%. Univariate and multivariate Cox regression analyses were performed to identify variables associated with prognosis and survival. Pairwise comparisons among pathologists were performed by Log Rank (Mantel-Cox) method.
Results: The patient population included 749 men and 81 women with an age range of 21-84 years. Tumor was located in esophagus in 183 and gastroesophageal junction in 647 patients. HTV of 0%-10% was reported in 507 patients, 11%-50% in 186 patients, and >50% in 137 patients. Stage 0 was present in 175 patients, stage I in 136, stage II in 279, stage III in 225, and stage IV in 15 patients. At least one margin was positive in 100 patients and negative in 730 patients. By univariate analysis only HTV (p=0.001), post-therapy stage (p=0.001), margin status (p=0.001), and lymph nodes status (p=0.001) were significant. There were no significant differences in survival among pathologists (p=0.38). By multivariate analysis HTV, post-therapy stage, number of lymph nodes assessed and positive lymph nodes were significant. Pairwise comparisons among pathologists did not show any significant differences.
Conclusions: After trimodality therapy, long term outcomes of PEA depend on HTV, post-therapy stage, and lymph node status. Histologic tumor viability is easily reproducible amongst pathologist and should be included in all cases prospectively.

624 Implementation of a Pathology-Driven Lynch Syndrome Screening Program

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Background: Lynch Syndrome (LS) is a genetic syndrome driven by germline mutations in mismatch repair (MMR) protein complexes which result in an increased risk for developing numerous neoplasms including colorectal carcinoma (CRC). Multiple laboratory methodologies can be used for the screening and confirmation of LS, including immunohistochemistry (IHC), molecular, and germline mutation analysis. As pathologists are intimately associated with the LS screening process, we are presented with the opportunity to develop comprehensive LS screening programs that go beyond the reporting of IHC and laboratory results. This study outlines the steps taken at an academic hospital to create a comprehensive LS screening program spearheaded by pathologists.
Design: Since May 2012 we have performed MMR IHC with MHL1, PMS2, MSH2, and MSH6 antibodies on all resected CRCs, with reflex BRAF V600E mutation analysis beginning in July 2013. We divided our process into three phases: Phase 1 involved MMR IHC testing without further follow-up or action by pathology; Phase 2 began with the implementation of a monthly Pathology-Genetic Correlative Conference (PGCC) to review all abnormal cases, providing written/verbal follow-up to the providing physician, and implementation of reflex BRAF testing; Phase 3 involved the addition of presenting cases with abnormal MMR IHC staining at our weekly GI Tumor Board (GITB) meeting. The number of abnormal MMR IHC cases subsequently referred to our genetic counseling service (GCS) was examined.
Results: In Phase 1 we demonstrated abnormal MMR IHC staining in 11 (12.5%) of 88 CRCs; follow-up to our GCS was unknown for this phase. In Phase 2, 32 (12.6%) of 254 CRCs had abnormal MMR IHC profiles requiring clinical follow-up; two (6.3%) of these cases were referred to our GCS. In Phase 3, 14 (8.6%) of 166 CRCs had clinically actionable abnormal MMR IHC profiles; three (21.4%) of these cases were subsequently referred to our GCS.
Conclusions: Through molecular and immunohistochemical testing, pathology plays a critical role in the LS screening process. We face the challenge of ensuring that our results are appropriately acted upon by our clinical counterparts. Through the step-wise implementation of a monthly PGCC and presentation of possible LS cases at GITBs we have been able to improve the referral of possible LS patients to our GCS, although there remains room for improvement.

625 Clinicopathologic Predictors of Long Term and Disease-Free Survival in Esophageal Squamous Cell Carcinomas with Complete Pathologic Response to Neoadjuvant Chemoradiation

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Background: Neoadjuvant chemoradiation treatment has been shown to improve outcomes for patients with esophageal squamous cell carcinoma (ESCC). Patients with pathologic complete response (pCR) to therapy have shown improvements in long term and disease-free survival, but there is significant heterogeneity with regards to outcome within this distinct patient group. The aim of this study was to evaluate possible clinicopathologic predictors of long term and disease-free survival among patients with ESCC who had pCR to neoadjuvant chemoradiation.
Design: 70 patients with ESCC who had pCR (ypT0N0) to neoadjuvant chemoradiation were identified. A pre-determined set of clinicopathologic variables was examined for each patient such as: age, gender, tumor grade (pre-treatment biopsy), tumor location, tumor size (pre-treatment endoscopy), pre-treatment endoscopic ultrasound (T & N) stage, extent of pathologic sampling, presence and depth of tumor bed, lymph nodes with evidence of tumor regression, and number of lymph nodes evaluated. Follow up data (overall mortality (OM), disease specific mortality (DSM), and disease recurrence (DR)) were analyzed by a Cox Proportional Hazards Model.
Results: Of the 70 ESCC patients with pCR (mean age 61.8 years, M:F ratio 1.3:1), DSM was 37% and DR was 26% (mean follow up time: 6.2 yrs). The most significant predictors of favorable outcome were complete histologic examination of the entire tumor bed (Hazard Ratio [HR]=0.39 for DSM and HR=0.47 for DR; p-values 0.02 and 0.11, respectively) and sampling of at least 10 lymph nodes (HR=0.29 for DSM and HR=0.49 for DR; p-values 0.01 and 0.14, respectively). Similarly, a minimum of 4 tumor

bed sections and a minimum of 1 section per cm of tumor bed were also significantly associated with reduced DSM ($p<0.05$). Finally, age>65 years and male gender were also associated with DSM (HR=2.28 and 3.13, respectively; both $p<0.05$). None of the other clinicopathologic parameters examined were related to any of the outcome variables.
Conclusions: Complete histologic examination of the entire tumor bed and sampling of at least 10 lymph nodes from the resection specimen are important predictors of disease specific survival and disease recurrence in patients with ESCC and pCR, and are necessary to identify the subset of complete responders with a more uniformly favorable outcome.

626 HPV is Not Involved in the Pathogenesis of Esophageal Squamous Cell and Adenocarcinomas; Analysis Based on a Highly Sensitive and Highly Specific RNA In-Situ Hybridization Technique

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Background: The role of Human Papilloma Virus (HPV) in the development of esophageal squamous cell carcinoma (SCC), and even adenocarcinoma (adenoCa), is controversial. Prior PCR-based studies of HPV DNA have shown conflicting results. Biologically, it is believed that HPV-related cancers are driven by overexpression of E6 and E7 viral oncoproteins, which leads to deregulation of cell cycle control by degradation of P53 protein and overexpression of P16. The main limitation of PCR-based assays is its inability to detect transcriptionally active virus, since the assays are DNA-based. The aim of our study was to evaluate a wide variety of high risk HPV viruses by using a highly sensitive and highly specific method of HPV RNA in-situ hybridization which can detect transcriptionally active virus by direct visualization of signal within tumor cells.
Design: Paraffin-embedded tissue from 50 patients with SCC and 22 with adenoCa, all from North America, were retrieved from two large academic medical centers. All tumors were stained for high risk HPV using a branch chain in-situ hybridization platform (viewRNA, Affymetrix, California). The assay uses a cocktail of oligonucleotides that targets the E6 and E7 transcripts that hybridize to the following high risk HPV viruses: types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. The assay was performed in an automated format (Leica Bond RX). Thirty-four head and neck SCCs known to be positive for HPV by PCR served as positive controls, and DapB as a negative control. A DNA assay was also performed on the head and neck SCCs using the Inform HPV III Family 16 Probe (Ventana Medical Systems), a cocktail with affinity for a similar set of high risk HPV genotypes.
Results: 30 of the SCCs involved the proximal esophagus, 15 the mid esophagus and 3 the distal esophagus. All adenocarcinomas involved the distal esophagus. All 50 (100%) SCC and all 22 (100%) adenoCa were negative for all high-risk HPV types via RNA in-situ hybridization. There was 100% agreement between our RNA-based assay and PCR positive head and neck SCC controls. The DNA ISH performed on the head and neck SCCs was positive in 21 cases (60%).
Conclusions: Our findings of a complete absence of transcriptionally active HPV in esophageal SCC and adenoCa indicates that HPV is not a factor underlying the development of these esophageal malignancies in North America.

627 Molecular Classification in Gastric Cancer Using Immunohistochemistry

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Background: The Cancer Genome Atlas Research (TCGA) and the Asian Cancer Research Group (ACRG) recently proposed molecular classification of gastric cancer based on clinically relevant genomic alterations and suggested their clinical validity by allowing better patient stratification for prognostication and targeted therapies. However, the use of sophisticated technologies and cost remain obstacles to their adoptions.
Design: We aim to reproduce the molecular classifications using immunohistochemical and in situ hybridization techniques.
Results: 337 gastric adenocarcinomas were classified according to the TCGA and ACRG. For each classification, we identified groups corresponding to the molecular subtypes, along with similar clinicopathologic characteristics. According to the TCGA and based on the expression of EBER-ISH, MLH1, and E-cadherin and p53, 4 subtypes were respectively determined: EBV positive (7.7%), microsatellite-unstable (MSI) (7.1%), genomically stable (GS) (31.5%), and chromosomal instability (CIN) subtypes (53.7%). The GS subtype showed the worst survival, more female patients and was associated with diffuse type of Lauren classification. According to the ACRG and based on the expression of E-cadherin, MLH1 and p53, the cases could be classified into MSI (7.7%), microsatellite-stable/epithelial-to-mesenchymal transition(MSS/EMT) 20.5%, MSS/p53(-)(52.8%), and MSS/p53(+)(19.0%), respectively. MSS/EMT type had the worst prognosis and was associated with diffuse type morphology. MSI type which included intestinal type cancers and MSS/p53(-) variant and are associated with EBV positivity both showed overall better prognosis.
Conclusions: Molecular classifications of gastric cancer could be validated using mmunohistochemistry and ISH. The applicability of these simple modalities,suggest that once validated they can use in practice as alternates to expensive comprehensive genomic profiling.

628 Histopathologic Features for Prognostication of Superficial Barrett's Carcinoma: Data from a Multi-Center Study in Japan

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Background: Endoscopic resection (ER) has become the standard therapy for mucosal Barrett's carcinoma (BC) in Japan and the USA. Histopathologic evaluation of the ER specimen is considered important for prognostication.
Design: We attempted to clarify the risk factors for lymph node metastasis and local recurrence, and the importance of evaluating mucosal BC. This multi-center study included leading institutions for the diagnosis and therapy of superficial BC belonging to the Japan Research Society for Early Esophageal Cancer and Chromoendoscopy (http://sikiso.kenkyuukai.jp/about/index.asp?). Using a questionnaire, each collaborating institution entered details about the features of T1 BC cases they had treated, except for high-grade dysplasia (BC *in situ* in Japan). Tumor size, gross type, depth, histology, infiltrative pattern, lymphovascular invasion (LVI), resection margin, metastasis, and outcome were recorded. For T1b cases, depth and width of submucosal tumor components were also recorded.
Results: Seventy-four surgical specimens and 201 ER specimens were analyzed. In most cases showing recurrence (surgical: 7/7, ER: 6/8), the outcome was death due to the original disease. Significant risk factors for recurrence were tumor size, poor differentiation, depth and width of the submucosal component, a marked infiltrative pattern, LVI (+), and residual tumor. For recurrent cases, the cut-off values for tumor size was 17.5 mm. In addition, the cut-off values for depth and width of submucosal invasion were 990 mm and 4300 mm, respectively. Recurrence or metastasis was seen only for tumors with infiltration to the deep muscularis mucosae (DMM), or for T1b tumors.
Conclusions: Tumors infiltrating the DMM should be distinguished from other T1a tumors because their prognoses are different. Detailed histopathologic evaluation of ER specimens is necessary for decision-making about post-resection therapy.

629 Mcl-1 (Myeloid Cell Leukemia-1) as a Biomarker and Prognosticator in Colorectal Adenocarcinomas (CRCs): Protein Expression and Molecular Profiling

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Background: Mcl-1 is a Bcl-2 family-member protein that is highly expressed in a variety of human cancers including CRC. Mcl-1 differs from other family members in its high turnover rate, and regulates numerous processes associated with apoptosis. We studied the pattern of Mcl-1 protein expression by immunohistochemistry (IHC) and *MCL1* gene amplification in CRC.
Design: Formalin-fixed paraffin-embedded tissue sections from 104 CRCs were immunostained by an automated method (Ventana/Roche, Tucson, AZ) using rabbit polyclonal Mcl-1 (clone S-19, Santa Cruz). Nuclear (nMcl-1) and/or cytoplasmic (cMcl-1) immunoreactivity was scored based on intensity and percentage of positive tumor cells in both the tumor and adjacent benign epithelium in each case. Scoring was based on staining intensity (weak, moderate, intense) and percentage of positive cells (focal <= 10%, regional 11-50%, diffuse >50%). Each case was then assessed as tumor=benign (T=B), tumor>benign (T>B), tumor<benign (T<B), or negative (N). Results were correlated with histologic and prognostic variables. *MCL1* gene amplification was determined by hybrid capture based comprehensive genomic profiling (CGP) on a separate cohort of 4,627 CRC.
Results: Mcl-1 immunoreactivity was noted as nuclear and/or cytoplasmic. nMcl-1 was noted as follows: T>B 20/104 (19%), T<B 17/104 (16%), N 67/104 (65%) cases; nMcl-1 positivity correlated with negative lymph node status [T>B 26% LN- versus 13% LN+, T<B 22% LN- versus 11% LN+, N 52% LN- versus 76% LN+, ($p=0.039$)] and AJCC clinical stage within the LN- subgroup [T>B 0% advanced [AJCC stage III/IV] versus 27% low [AJCC stage I/II], T<B 75% advanced versus 18% low, N 25% advanced versus 56% low, ($p=0.029$)]. cMcl-1 expression was as follows: T>B 5/104 (5%), T<B 4/104 (4%), T=B 95/104 (91%) cases and correlated with shortened survival on Cox univariate analysis ($p=0.035$) with 3/5 (60%) T>B cases, 4/4 (100%) T<B, 57/95 (60%) T=B patients expired. *MCL1* gene amplification was identified in only 65 (1.4%) of 4,627 CRCs on CGP. On multivariate analysis, advanced tumor stage independently predicted shortened survival ($p<0.0001$).
Conclusions: Localization of the Mcl-1 protein is variable in CRCs. Aberrant nMcl-1 expression is associated with depth of invasion in LN- cases and cMcl-1 with overall shortened survival. Mcl-1 protein over-expression is independent of *MCL1* gene amplification; however, may be used as a sensitive marker to predict treatment response in CRCs. Further study of the mechanisms by which the Mcl-1 protein contributes to colon carcinogenesis is warranted.

630 Large Size and High Grade Dysplasia Are Strong Predictors of Mismatch Repair Deficiency in Lynch Syndrome-Associated Colorectal Adenomas

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Background: Lynch syndrome (LS) tumors are characterized by the presence of DNA mismatch repair deficiency (MMR), defined as microsatellite instability and/or loss of protein expression by immunohistochemistry (IHC). The prevalence of loss of protein expression by IHC in LS polyps remains poorly studied. We aimed at describing the prevalence of loss of MMR protein expression in LS-associated polyps and identifying independent predictors of MMR deficiency.

Design: A total of 171 colorectal polyps (121 adenomas, 50 serrated lesions) from 53 LS patients (18 MLH1, 22 MSH2, 10 MSH6, 3 PMS2) were tested for expression of MLH1, MSH2, MSH6 and PMS2 proteins by IHC. Clinical and pathological variables associated with the presence of pathological IHC were analyzed.
Results: The mean size of adenomas was 5.2 mm, standard deviation: 4.7 mm; 15 (12.4%) showed high-grade dysplasia (HGD), 11 (9.1%) were villous, and 19 (15.7%) were ≥10 mm. Loss of MMR protein expression was seen in 69 (57%) adenomas. In univariate analysis, variables associated with loss of protein expression were: female (62.8% vs 37.2%; p=0.04), *MLH1* mutation (71.4% vs 28.6%; p=0.03), HGD (100% vs 0%; p<0.001), size ≥5mm (75% vs 25%; p=0.001), size ≥10 mm (84.2% vs 15.8%; p=0.009) and rectal location (82.4% vs 17.6%; p=0.02). *PMS2* germline mutation was inversely associated with loss of protein expression (22.2% vs 77.8%; p=0.03). In multivariate analysis independent predictors of loss of protein expression were: HGD (OR=24.3; 95%CI: 1.5-378.8; p=0.02), size ≥5 mm (OR=2.55; 95%CI: 1.06-6.18; p=0.03), and *PMS2* germline mutation (OR=0.08; 95%CI: 0.08-0.82; p=0.03). All serrated lesions (49 hyperplastic polyps and 1 sessile serrated adenoma) retained protein expression.
Conclusions: Lynch syndrome patients with large adenomas and those with HGD are associated with loss of protein expression. *PMS2* germline mutation carriers may retain protein expression more frequently than other mutation carriers. Serrated lesions in LS patients do not display MMR deficiency. Although testing colorectal carcinoma is of value in the diagnostic evaluation of patients with suspected LS, retained protein expression in an adenoma should not be considered against LS diagnosis.

631 Utilization of High Throughput Cancer Hotspot Mutations Screening for the Molecular Profiling of Colorectal Cancer

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Background: Colorectal cancer (CRC) is a heterogeneous disease with different molecular characteristics. Tumor heterogeneity is caused or associated by several genetic and epigenetic tumor passenger or tumor driver variations. Known tumor driver mutations usually affect the APC gene, KRAS oncogene and then tumor suppressor TP53. Identification of such mutations could affect the clinical management of the tumor. The development of next generation sequencing has simplified the high-throughput mutational screening of tumors.
Design: We used the Ion Ampliseq Cancer Hotspot panel (version 2) in combination with the Ion Torrent personal genome platform from Life Technologies that allows the simultaneous screening of 2,800 COSMIC (catalogue of somatic mutations in cancer) mutations in 50 oncogenes and tumor suppressor genes in 10 nanograms of DNA extracted from FFPE tissues. We utilized this panel to screen 50 CRC cases from Saudi Arabia in order to better understand the molecular signature of such tumors.
Results: Pathogenic mutations affecting TP53 were identified in 57% of the cases. The most abundant mutation was at position 248 of the protein, while mutations affecting APC were identified in 46% of the cases analyzed. KRAS mutations (mainly affecting position 12) accounted for 41% of the cases with the slightly higher than average percentage is probably due to the increased sensitivity of the assay compared to other KRAS mutation detection methods. SMAD4 mutations were identified in 22% of the cases while PIK3CA mutations were detected in 15% of the cases analyzed. Other mutations were identified at lower frequency affecting 27 other genes. KRAS mutations are significantly associated with the proximal origin of the tumor (Fisher's exact t-test; p=0.037) and lymph node metastasis (Fisher's exact t-test; p=0.037). SMAD4 mutations are significantly associated with relatively young age at diagnosis (below 50 years) with p value of 0.012 (Fisher's Exact t-test).
Conclusions: High throughput cancer hotspot mutations screening provides a cost-effective and quick turnaround method for the molecular profiling of CRC which will aid the application of individualized therapeutic regimes and application of precision medicine to treat this disease.

632 Colorectal Carcinomas with Isolated Loss of PMS2 by IHC: An Unusual Subset of MSI-H Tumors

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Background: Recent studies estimate that isolated loss of PMS2 expression by immunohistochemistry (IHC) with preserved expression of MLH1, MSH2, and MSH6 occurs in 0.4-1.5% of all colorectal carcinomas (CRCs) and accounts for ~4% of CRCs with DNA mismatch repair (MMR) protein abnormalities. However, few studies have analyzed the pathologic features of these tumors or compared their behavior to other CRCs with high levels of microsatellite instability (MSI-H).
Design: Twenty-one cases of CRC with isolated loss of PMS2 expression detected by universal IHC testing for microsatellite instability (MSI) were identified from the surgical pathology files of three institutions. Pathologic and clinical features were analyzed and compared with data from a recently published large series of MSI-H CRCs (AK Merok *Annals of Oncology* 2013).
Results: Patients ranged in age from 20-85 years, with 9 of 21 patients age 50 or younger at diagnosis. Results of germline mutational analysis were available for 11 patients, of whom 5 had germline *PMS2* mutations, 3 had germline *MLH1* mutations, and 3 had no germline mutation detected. Results of MSI testing by PCR were available for 17 cases, all of which showed high levels of microsatellite instability. Of the 19 patients with a pathologic T stage available, 2 patients had T2 disease, 13 patients had T3 disease, and 4 patients had T4 disease. Nine of nineteen patients with lymph nodes retrieved had lymph node metastases, and two patients had distant metastases at presentation. Recurrent disease was detected in six patients during clinical follow-up. Compared to published data on MSI-H CRCs, cases with isolated PMS2 loss by IHC were significantly less likely to be right-sided (57.1% vs. 85.7%; p=0.005) and were more likely to present with stage III or IV disease (57.1% vs. 35.7%; p=0.089).
Conclusions: To our knowledge, this study represents the largest series of CRCs with isolated loss of PMS2 expression by IHC. These tumors appear to be a distinct subset of MSI-H CRCs that are more likely to be left-sided, present at a higher stage, and often have lymph node or distant metastases. The findings suggest that CRCs with isolated loss of PMS2 expression may behave in a more aggressive manner than other MSI-H tumors. Further study of additional CRCs with isolated PMS2 loss is necessary to determine the impact of germline mutational status on the behavior of these tumors.

633 Lymphocytic Colitis: Are There Pathologic Predictors of Response to Therapy?

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Background: While intraepithelial lymphocytosis is a unifying feature of lymphocytic colitis (LC), there is significant morphologic heterogeneity between cases. Limited data are available on the significance of this heterogeneity, and particularly on how it relates to response to therapy.
Design: Cases of LC diagnosed between 2002 and 2013 were identified using the Research Patient Data Registry of a tertiary referral center. H&E slides from diagnostic biopsies were reviewed and evaluated for histologic features of LC. Clinical data including type of therapy and response to treatment were collected. Fischer's exact test was used to compare histologic features with response to treatment.
Results: Thirty-six cases of LC with slides available for review were identified. The mean age was 61 years (range 18-90 years), and the female-to-male ratio was 2.6:1. Fourteen patients responded to steroid therapy, 12 patients responded to antibiotics, mesalamine, or bismuth subsalicylate, and 10 patients did not respond to therapy. Response to steroid therapy was significantly associated with the presence of "trapillaries" (entrapped capillaries in a collagenous meshwork without diagnostic features of collagenous colitis; p=0.006), the absence of eosinophilic clusters (p=0.013), and the absence of subepithelial histiocytes (p=0.015). Response to antibiotics, mesalamine, or bismuth subsalicylate was significantly associated with the presence of eosinophilic clusters (p=0.009) and the absence of trapillaries (p=0.010). Patients who did not respond to any treatment were significantly more likely to exhibit crypt architectural distortion in their biopsies (p<0.001). The extent of involvement by LC and the degree of surface epithelial damage, crypt epithelial damage, and neutrophilic inflammation did not differ significantly between treatment response groups. Demographic features were also similar between groups.
Conclusions: This study indicates that certain histologic features of LC can be used to triage patients towards appropriate therapy. In particular, the presence or absence of trapillaries, eosinophilic clusters, subepithelial histiocytes, and crypt architectural distortion are most likely to impact response to therapy. The significant variation in histologic features and their correlation with treatment response may also provide insight into etiologic differences between subgroups of LC.

634 An Optimized Strategy for Candida Detection: Insights from a Morphologic and Cost Comparison Study

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Background: A review of consult material found *Candida* was frequently overlooked. We undertook a retrospective review of esophageal *Candidiasis* (EC) to provide helpful clinicopathologic clues to its timely diagnosis.
Design: A computer search from 07/2012-02/2015 identified a total of 1011 esophageal biopsies, including 40 EC (40 unique patients) and 20 negative controls (20 unique patients). Clinicopathologic features were compared using Fisher's exact two-tailed *t* test. PAS/D cost analysis was performed based on current Medicare/Medicaid reimbursement rates.
Results: The incidence of EC was 5.7% (mean age=56 years, 45%=men). Statistically significant features of EC (p<0.05) included immunosuppression and abnormal endoscopy. Of those with available clinical impressions (n=37), 45.9% were incorrect: reflux and eosinophilic esophagitis represented frequent alternate impressions. Pseudohypha were seen on H&E in 92.5% (n=37/40) of positive cases, with a PAS/D needed in 2 EC with ulcers, and 1 EC where the single pseudohyphae was seen only on the deeper section. More than 95% of pseudohyphae were in the superficial desquamated and/or hyper-pink parakeratosis or detached debris. Other significant features of EC included lymphocytosis, dead keratinocytes, intra-epithelial neutrophils, and bacterial overgrowth. Neutrophils were usually focal, superficial, and ranged from 3-200/HPF. Bacterial colonization was significantly seen in EC, and was usually comingled with the pseudohyphae. "Up-front" PAS/D on all esophageal biopsies would have generated $68,333.49 in patient charges; our targeted PAS/D strategy resulted in $10,476.45 in patient charges (cost saving=$57,857.04).
Conclusions: While acute inflammation was generally a helpful clue to EC, we report up to 30% had no neutrophilic infiltrate, underscoring an important diagnostic pitfall. We propose efficient *Candida* screening target the superficial and detached fragments of desquamated and hyper-pink parakeratosis, with additional (less reliable) clues including lymphocytosis, intra-epithelial neutrophils, dead keratinocytes, and bacterial overgrowth. We recommend limiting PAS/D to cases where the organisms are not readily identifiable on H&E and with at least one of the following: 1) Cases with the above morphology, 2) Esophageal ulcers. This targeted approach results in an 84.7% patient charge reduction compared with "up-front" PAS/D staining.

635 The Impact of Deeper Levels on the Diagnosis of Hyperplastic Polyps and Sessile Serrated Adenomas

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Background: Sessile serrated adenomas (SSAs) and hyperplastic polyps (HPs) require different colonoscopic follow up.

Design: Retrospective review of sessile serrated adenomas and hyperplastic polyps diagnosed at a community hospital by a single pathologist in a two-year period. The distribution across colon subsites was recorded. Cases that required additional histologic levels for diagnosis were noted. Cases with conventional tubular adenomas were excluded.

Results: There were 295 hyperplastic polyps and 223 sessile serrated adenomas diagnosed in the two year period. Of the former, 79% were located in the sigmoid colon and rectum. Of the latter, only 2.2% were in the sigmoid colon and none were in the rectum. Notably, 38 (17%) SSAs were diagnosed only on deeper levels, as the initial sections showed changes that would have been classified as hyperplastic polyps. Another 38 polyps located proximal to the sigmoid colon had deeper levels obtained and remained classified as hyperplastic polyps.

Conclusions: On deeper sections, 50% of hyperplastic appearing polyps occurring proximal to the sigmoid colon will prove to be sessile serrated adenomas. Therefore, we recommend that deeper levels be routinely obtained on hyperplastic appearing lesions proximal to the sigmoid colon, since the colonoscopic follow up for SSAs is at a shorter interval than for HPs.

636 Concurrent Biopsies Improve Diagnosis of Malignancy over Bile Duct Brushings (BDBs) Alone

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Background: During evaluation of biliary pathology BDB is sometimes performed with concurrent bile duct biopsy(CBX).

Design: Agreement between paired BDB and CBX diagnoses (Dxs) was evaluated in 66 cases, 50 with followup (30 malignant, 20 benign).

Results:

Level of Agreement between the 2 Tests							
		Bx Dx(N)					
		Be-nign	Atypi-cal	Suspicious	Malignant	Total	% Agree-ment
BDB(N)	Benign	27	8	**2**	**3**	40	68
	Atypical	5	5	**1**	**4**	15	33
	Suspicious	0	0	0	3	3	0
	Malignant	0	0	0	8	8	100
	Total	32	13	3	18	66	**61**
	% Agreement	84	39	0	44		

Overall agreement between the two tests was 61%. Malignant BDB Dx had the highest agreement with CBX (100%) followed by benign BDB Dx (68%) (p=0.05). Atypical and suspicious BDBs showed the least agreement with CBX (p=0.002); 10 BDBs (5 benign/5 atypical) were upgraded to suspicious/malignant on CBX (6 BDB and 7 CBX were available for rereview (by 2 reviewers) and the original Dx was confirmed in all except 1 BDB which was originally called "benign" but proved to be malignant on rereview and followup). CBX had higher sensitivity (69%) and accuracy (80%) than BDB (42% and 69%, respectively) in predicting malignant outcome.

Comparison of Test Performance in 50 Cases with followup						
Dx	(%)	Sensitivity%	Specificity%	PPV%	NPV%	Accuracy%
Cytology	Malignant(18)	42	100	100	59	69
	Suspicious(6)	21				63
	Atypical(24)	42	80	67		62
Biopsy	Malignant(36)	69	100	100	65	80
	Suspicious(4)	20				68
	Atypical(14)		75	29		57
Cytology	Stent present(16)	0	86	0	86	75
	No stent(84)	66	77	86	50	69
Biopsy	Stent present(16)	0	86	0	86	75
	No stent(84)	76	69	85	56	74

Conclusions: Concordance between BDB and CBX is fair(61%) however CBX is better at identifying malignancy than BDB. The lower sensitivity BDB is likely attributable to procedural undersampling since a suspicious/malignant Dx was achievable on CBX in several cases. Despite this, both tests have 100% specificity and PPV for malignancy (0 false positives). Interestingly in both tests stented specimens had higher specificity, suggesting that cytopathologists and GI pathologists only call such cases malignant when the evidence is overwhelmingly convincing. When possible, cyto-histologic correlation should be performed during evaluation and signout of biliary tract specimens.

637 Nuclear Maspin Expression Is an Independent Prognostic Marker in Microsatellite Stable Stage II Colorectal Carcinoma

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Background: Maspin is a serine proteinase inhibitor that acts as a tumor suppressor protein by mediating apoptosis and inhibiting motility, invasion, and tumor metastasis. Proteomic work from our group has shown that maspin is a key protein that is differentially expressed between normal colonic mucosa and colorectal cancer (CRC). Here, we investigate maspin as a potential prognostic marker in stage II colorectal cancer and correlate its expression with microsatellite instability status.

Design: Colon cancer patient serum was used as autoantibody source to screen for novel cancer-specific antigens using mass spectrometry-based proteomics. Maspin was one of the biomarkers identified, and we investigated maspin expression by immunohistochemistry in a well-characterized tissue microarray cohort of 322 colon cancer patients. A purified mouse antihuman maspin monoclonal antibody was used, and maspin expression was grouped into high and low based on median nuclear expression (H score) observed in the tumor. Microsatellite instability status was assessed by IHC using a mismatch repair protein panel of MLH1, MSH2, MSH6, and PMS2. Maspin expression was correlated with tumor pathological features including TNM staging and microsatellite status as well as clinical outcomes such as overall survival.

Results: High nuclear maspin expression was observed in 40.9% (132/322) of stage I-IV colon cancers and correlated with poor differentiation (p=0.0481), pT (p=0.0145), and nodal involvement (p=0.0132). High nuclear maspin expression colorectal cancers had reduced overall survival in all stages I-IV (p=0.0002), in the microsatellite stable (MSS) subgroup (n=273; p=0.0002), in the MSS stage II subgroup (n=66; p=0.0374), and in the MSS stage III subgroup (n=114; p=0.0275). In a multivariate analysis in the MSS stage II subgroup, there was no association of maspin with age, gender, pT and tumor size.

Conclusions: High maspin expression is an independent prognostic marker of poor overall survival in the MSS stage II CRC subgroup. Our results highlight subgroups of MSS stage II CRC patients whose tumors are clinically most aggressive, and development of a proteomics-based biomarker panel could act as an adjunct to decide on additional adjuvant treatment strategies in these high-risk stage II patients.

638 Gastric Intestinal Metaplasia: Is It a Worrisome Feature Warranting Follow-Up?

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Background: The significance of intestinal metaplasia (IM) in the absence of more worrisome pre-neoplastic lesions in gastric biopsy tissues is not entirely elucidated in the United States. In fact, national consensus guidelines are not yet available for the diagnosis and management of intestinal metaplasia of the stomach. This study aims to determine what changes are present in the follow-up gastric biopsy tissues of patients whose initial samples showed IM.

Design: 411 patients containing intestinal metaplasia in their initial gastric biopsy tissue were selected from the files archived in the institution Co-Path® system. Cases were selected from the time frame of 1998 to 2014. In addition to IM, these biopsy tissues may have had inflammation, low grade dysplasia (LGD), high grade dysplasia (HGD), intramucosal carcinoma (IMC), or invasive carcinoma (IC) as well. Of these 411 cases, 107 had follow-up biopsy tissues. 18 of the 107 samples had HGD, IMC, or IC and they were discarded for further analysis. Thus, a total number of 89 eligible patients were studied. No distinction between incomplete and complete IM was made.

Results: 84 of the 89 patients had only IM and inflammation in their initial samples. The other 5 had LGD in addition to IM. The follow-up biopsies for the 89 patients ranged from 0.1 to 144 (30.1) months. None of the biopsies showed HGD, IMC or IC at follow-up. 54 had persisting IM and 35 had no IM. One of the 5 patients with LGD had persisting LGD, 3 had persisting IM and 1 lacked both at 41 months (range 13 to 62 months).

Conclusions: 60% of patients whose gastric biopsy tissues initially showed IM alone or with LGD had persistent IM. None of the patients with IM or IM/LGD had progression towards HGD or carcinoma at follow-up between 30.1 and 41 months. Unless patients' clinical background and/or symptomatology dictates otherwise, endoscopic follow-up for IM sooner than that period of time may not be not warranted. The longer follow-up of 41 months for IM with LGD dysplasia in comparison to the 30.1 for IM without dysplasia may be explained by a gastroenterologist's concern to follow these lesions more intensively.

639 miRNA Analysis Identified PTEN Loss in Goblet Cell Carcinoid of Appendix in Comparison to Well Differentiated Neuroendocrine Tumors

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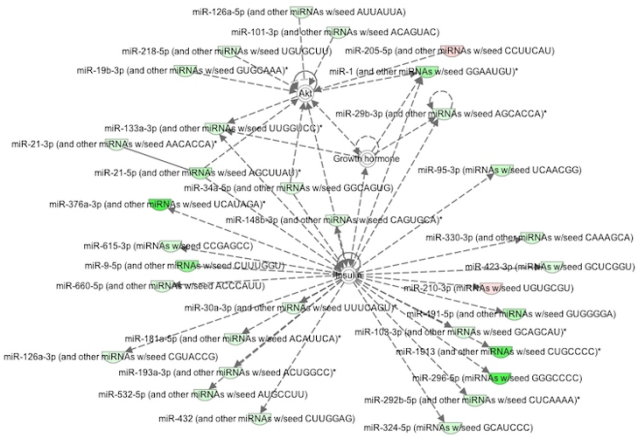
Background: Well-differentiated neuroendocrine tumors of appendix (WDNET) and goblet cell carcinoid (GCC) are distinct tumor entities. However, a significant number of cases may be diagnostically challenging. We explored differences in microRNA (miRNA) expression in these two tumors.

Design: 14 cases of WDNET and 10 GCC of appendix with unequivocal histomorphologic features were selected. miRNA expression profiling was performed for miRNAs (n=2555) using microarray (miRbase v20) on total RNA from formalin fixed paraffin embedded (FFPE) tumor blocks. The results were validated by real time qRT-PCR. The data was analyzed with Ingenuity® software to identify putative target gene pathways. PTEN expression was evaluated in 10 cases from both groups.

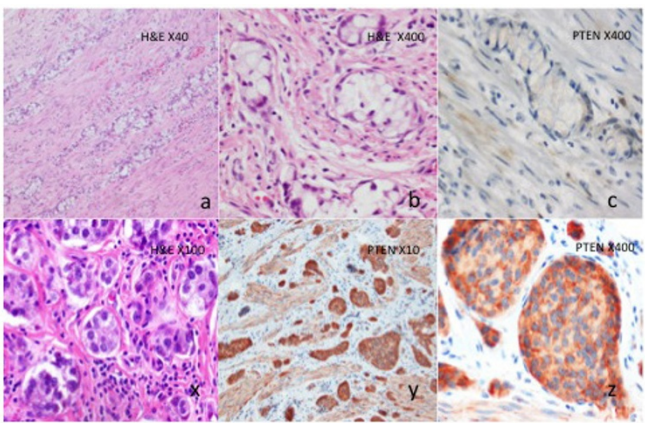
Results: miRNA profiling revealed significant differences in the two groups.

miRNA	Appendix WDNET	Goblet cell carcinoid	log G2/G1	p value
Tumor suppressor gene				
miR-375	23640	1363	-4.12	2.92E-06
miR-642b-5p	962	0	-17.28	5.89E-06
miR-642a-5p	1118	0	-17.49	6.48E-06
miR-488-3p	528	0	-16.66	9.14E-06
miR-29b-3p	10298	1678	-2.62	1.84E-05
miR-148b-3p	1703	331	-2.36	2.04E-05
Oncogene				
miR-7843-5p	99	1694	4.09	9.96E-06
miR-6075	373	2736	1.70	2.56E-05

Ingenuity pathway analysis revealed involvement of akt-mTOR pathway and insulin gene.



10 GCC showed loss of PTEN expression, a downstream effect of akt-mTOR pathway involvement (c) [figure 2], while 10 WDNET were immunoreactive for PTEN (y,z).



Conclusions: PTEN immunostaining can distinguish between WDNET and GCC. In addition, akt-mTOR pathway involvement may have therapeutic potential.

640 IMP3 Is Similarly Expressed Immunohistochemically in Intrahepatic and Extrahepatic Cholangiocarcinomas

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Background: Insulin-like growth factor-II (IGF-II) messenger RNA binding protein-3 (IMP3), is a 580 amino acid oncofetal RNA binding protein encoded by the *IGF2BP3* gene. IMP3 has been demonstrated to be overexpressed in multiple human malignancies, including pancreatic adenocarcinoma and hepatocellular carcinoma. Limited studies have been performed on IMP3 expression in intrahepatic cholangiocarcinomas (ICCs). The aim of this study was to examine IMP3 expression in cholangiocarcinomas, including comparison of ICCs and extrahepatic cholangiocarcinomas (ECCs).

Design: Biopsy or resection specimens of 43 cholangiocarcinomas, including 23 ICCs and 20 ECCs, were immunohistochemically studied using a monoclonal antibody against IMP3. Cytoplasmic staining was considered positive. The percentage of positively stained tumor cells was recorded and the staining intensity was graded as weak, moderate, or strong. Prognostic and survival factors were examined in relationship to IMP3 expression.

Results: Six (14.0%) of the cholangiocarcinomas were negative for IMP3 staining, while 10 (23.3%) demonstrated staining in 5-50% of neoplastic cells, and 27 (63.0%) demonstrated staining in greater than 50% of neoplastic cells. Staining intensity paralleled percentage of positive cells, including strong staining in 22 cases, all of which had IMP3-positivity in at least 80% of cells. When stratified by location, there was no significant difference in the staining profiles of ICCs and ECCs. Lymphovascular and perineural invasion were most commonly associated with ECCs with strong and diffuse IMP3 staining. When examining patients who died secondary to their malignancy, IMP3-negative ICCs averaged 1.22 years of survival (0.57-1.59) as compared with 1.71 (0.3-4.58) years if IMP3-positive ICC. In comparison, IMP3-negative ECCs averaged 2.34 years of survival (2.26-2.42) as compared with 1.40 (0.07-3.98) years if IMP3-positive ECC.

Conclusions: Among the 43 examined specimens, IMP3 immunohistochemical staining patterns were similar in ICCs and ECCs, with the majority of tumors demonstrating strong and diffuse staining. Previous reports of IMP3 expression in ICCs have demonstrated that IMP3 expression is associated with poor outcome. This study lends support to the prior findings despite a longer average survival time for IMP3-positive ICCs as compared with IMP3-negative ICCs in this limited study. Additionally, our finding suggest that ECCs behave similarly to ICCs in regards to IMP3.

641 Increased Mucosal IgG4 Infiltration Is Associated with Poor Clinical Outcomes in Ulcerative Colitis

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Background: Ulcerative colitis (UC) can have a highly variable clinical course. Pancolitis will occur in as many as 50% of patients and a substantial proportion of patients will go on to hospitalization or colectomy. There is a need to better predict disease course to tailor clinical treatment. Several recent studies have explored the correlation of mucosal IgG4 cell counts to disease severity, but they are limited by small sample sizes. This study was undertaken to assess established diagnostic variables and to examine the predictive role of histologic IgG4 counts.

Design: A retrospective review (2008-2014) of rectal biopsies and clinical data was performed in patients with verified UC. Poor clinical outcome was defined as one of the following: (1) hospitalization for UC, (2) colectomy, and (3) use of tumor-necrosis-factor inhibitors (TNFi) or thiopurine therapy. Additional secondary outcomes included disease flares at both 6 and 12 months. Biopsies were evaluated by two pathologists for disease severity as well as total IgG4+ cells and maximum count per high power field by IHC. Consonant with prior studies, IgG4+ cells over 10 per high power field were considered significant.

Results: We evaluated 174 rectal biopsies in UC patients and correlated with multiple clinical outcome variables. Ninety-seven patients (56%) had a poor primary clinical outcome, including 27 patients (16%) with colectomy. Clinical factors predictive of worse outcomes included younger age (p=0.0002), absence of smoking history (p=0.03), and higher Mayo endoscopic score (p=0.002). On biopsy, 92 patients (53%) had mild chronic active colitis while 82 patients (47%) had moderate-to-severe chronic active colitis. Seventy-four patients (43%) had maximum IgG4 count greater than 10 per high power field. By multivariate analysis, these patients were more likely to have a poor clinical outcome (adjusted OR: 2.34 [1.02,5.37], p = 0.04). The correlation coefficient of pathologists inter-observer reproducibility of IgG4 counts was R=0.98 (p=<0.0001).

Conclusions: IgG4 staining is associated with worse outcomes in patients with UC, with very little inter-observer variability in assessment. Together with patient age, smoking history, and endoscopic appearance, IgG4 levels on colonic biopsies may be used by clinicians to individualize therapeutics.

642 Are the Genomics in the Cancer Genome Atlas Representative of Colorectal Cancer Patients with Recurrent Disease Refractory to Front-Line Therapy?

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Background: The Cancer Genomics Atlas (TCGA) provides a genomic fingerprint of various primary, untreated cancers. It is possible that primary cancers from patients with recurrent disease refractory to standard therapy have different molecular biology compared to patients with responsive disease. 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 considered actionable, defined as genes for which a matched genotype selected trial exists at our institution. Three hundred patients have been tested on this protocol to date; 57 of these have colorectal cancer (termed MDA patients). All colorectal cancer patients had MSI testing as standard of care. TCGA sequencing data for the same 409 genes from 212 colorectal cancer patients were extracted using cBioportal.org.

Results: MSI-high was more common in the TCGA cohort compared to the MDA patients (14% vs. 4%). The MDA group had a higher incidence of *TP53* mutations (68% vs. 52%). There were fewer mutations in actionable genes in the MDA group (1.8 mutations per patient) compared to TCGA (3.7 mutations per patient). Twenty-one genes (21/409; 5%) showed significantly different mutation frequencies comparing MDA to TCGA. Six of these genes (*SRC*, *AURKA*, *ATM*, *ATR*, *MTOR*, and *PIK3CA*) are actionable and have lower mutational frequencies in the MDA group.

Conclusions: Overall, the sequencing data between the two patient groups were similar. There were some important exceptions. Colorectal cancers from the MDA cohort were associated with molecular changes associated with an aggressive clinical course (less MSI-high cases) and had higher frequency of *TP53* mutation. In addition, MDA patients had significantly fewer mutations in 6 clinically actionable genes. These data support

the idea that colorectal cancer patients with recurrent disease refractory to standard therapy have molecular changes that are somewhat distinct from those represented in TCGA. This needs to be considered when designing targeted therapy clinical trials.

643 Distinct Expression of MACC1 and MET in Esophageal Squamous Cell Carcinoma and Esophageal Adenocarcinoma

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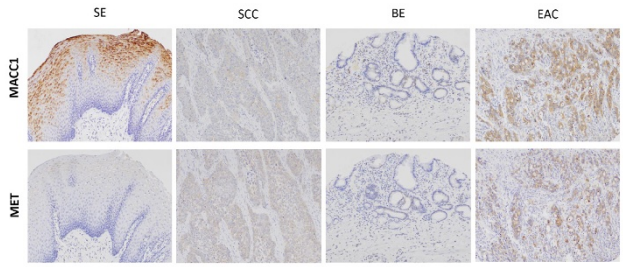
Background: Metastasis-associated in colon cancer-1(MACC1) is a key regulator of the HGF/MET pathway. High expression of MACC1 has been identified in many malignancies including colon, lung, and pancreas cancers, and generally associated with metastasis and poor prognosis. However, there are very few studies on its expression and role in esophageal cancer development and prognosis. We studied MACC1 and MET protein expressions in esophageal squamous cell carcinomas (SCC) and adenocarcinomas (EAC).

Design: Tissue microarrays (TMAs) were constructed from of 45 normal squamous epithelium (SE), 48 Barrett's esophagus (BE), 27 SCC, and 98 EAC cases. Expression of MACC1 or MET was detected by immunohistochemistry. The stained TMAs were evaluated for staining intensity (0-3) and percentage of positive cells using H score method. The mean value of H-score of MACC1 and MET in SE, BE, SCC and EAC were analyzed by one-way analysis of variance or *t* test.

Results: Positive cytoplasmic MACC1 staining was observed in SE, BE and esophageal cancer cells. Certain tumor cells in EACs also showed membranous MACC1 staining. In normal squamous epithelium, MACC1 staining in parabasal keratinocytes was stronger than basal cells. The expression of MACC1 in SCC was significantly decreased compared to SE (150.70±51.27 vs 89.83±51.11, p<0.001). Expression of MACC1 in BE was low. However, in EAC, MACC1 expression was greatly increased from 75.19±39.72 in BE to 116.00±66.69 (p<0.01). In contrast, MET expression was significantly increased in SCC as well as in EAC when compared to SE or BE respectively.



Conclusions: MACC1 expression patterns may be dependent on the tissue types that esophageal cancer derived from. Downregulation of MACC1 in SCC and upregulation of MACC1 in EAC may suggest the distinct roles that MACC1 plays in different esophageal cancer development and progression. High MET expression is associated with SCC and EAC progression.

644 Gastrointestinal Graft Versus Host Disease in Allogeneic and Autologous Stem Cell Transplant Patients: Does Cytotoxic, Helper, or Regulatory T Cell Infiltration Distinguish Alloimmunity from a Failure of Self Tolerance?

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Background: Graft-versus-host disease (GVHD) is a known complication of autologous stem cell transplantation (AuSCT) that arises in the gastrointestinal tract, skin, and liver. We previously reported greater crypt apoptosis and earlier onset in AuSCT patients compared to allogeneic stem cell transplant (AlloSCT) patients. The mechanism of GVHD in AuSCT patients is unclear, but is generally regarded as a failure of self-tolerance. Regulatory T cells (Tregs) are major agents of self-tolerance, and are readily characterized by FoxP3 immunohistochemistry (IHC). Our aim was to compare the presence, number, and localization of Tregs in AutoSCT and AlloSCT patients, and to correlate any differences with additional clinicopathologic variables.

Design: A tissue microarray was constructed using colon biopsies taken to evaluate for GVHD in 14 AuSCT and 56 AlloSCT patients. 17 normal colon biopsies from non-SCT patients comprised the control group. IHC for CD4, CD8, and FoxP3 was performed, and the number of positive intraepithelial (IEL) and lamina propria (LPL) lymphocytes was counted and averaged by high powered field. Histologic information, including Lerner grading and maximum apoptosis per 10 crypts was determined by retrospective review of H&E stained slides. Clinical information was gathered through chart review.

Results: AuSCT biopsies show T cell LPLs not statistically different from normal colon biopsies. AuSCT biopsies demonstrate a strong trend of greater LP Tregs compared to AlloSCT biopsies (9.2 vs. 5.3; p=0.057). AuSCT biopsies demonstrate increased CD4+ and CD8+ T cell populations compared to AlloSCT (32.6 vs. 25.3; p=0.024 and 30.3 vs. 23.0; p=0.063, respectively). No differences were observed between groups in terms of FoxP3, CD4, or CD8 positive IELs.

Conclusions: LP Tregs are similar in number between normal colon controls and AuSCT biopsies, suggesting that the failure of self-tolerance observed in AuSCT GIGVHD is not mediated by a decrease in the resident Treg population. Interestingly, AlloSCT biopsies appear to have fewer FoxP3, CD4, and CD8 positive LPLs, suggesting a unique attenuated T cell population in the setting of alloimmune GIGVHD. These trends warrant further investigation, including evaluation of Treg function in the AutoSCT setting.

645 Clinicopathologic and Outcome Study of Sessile Serrated Adenomas/Polyps with Serrated Versus Intestinal Dysplasia

Odise Cenaj, Robert Odze, Monika Vyas, Joanna A Gibson. Brigham and Women's Hospital, Boston, MA; Yale-New Haven Hospital, New Haven, CT.

Background: Many microsatellite unstable colorectal cancers arise from sessile serrated adenoma/polyps (SSA) via a dysplasia-carcinoma sequence. Dysplasia in SSA may be serrated or intestinal type, the former characterized by cells with oval-shaped hyperchromatic nuclei, slight stratification, eosinophilic cytoplasm, and a luminal saw-tooth growth pattern, and the latter by elongated, stratified pencillate nuclei with stratification, few or no goblet cells and no serrated growth pattern. Little is known about the clinicopathologic features and outcomes of SSA with serrated (SSA-S) vs intestinal (SSA-I) dysplasia, thus, this was the aim of our study.

Design: Biopsies from 86 patients with dysplastic SSA [SSA-S N=50, SSA-I N=22, SSA-S+I N=14] treated by polypectomy were retrieved from our archives. Patients were evaluated for demographics, location, size and distribution of their dysplastic SSAs, presence of carcinoma, and number and type of prior, synchronous and metachronous precursor lesions. Pathologically, dysplasia within SSA was categorized as low or high grade based on well-defined and objective criteria.

Results: Patients with high-grade SSA-S were significantly older than patients with low-grade SSA-S (75 vs 58 yrs,p=0.01) and patients with high-grade SSA-S were significantly older than patients with high-grade SSA-I. Patients with SSA-S+I and SSA-I showed a significantly higher rate of carcinoma (43% and 23%, respectively) within the polyp compared to patients with SSA-S (6%,p=0.01). Patients with either SSA-S or SSA-I showed a significantly higher rate of prior serrated (SSA, hyperplastic polyp, TSA) polyps (89% and 90%, respectively), and a higher but not significant rate of synchronous (30% and 45%, respectively) and metachronous (67% and 25%, respectively) serrated polyps compared to adenomas. There were no significant differences among the three groups with regard to gender, mean age at diagnosis, lesion size, or anatomic location (right vs left colon). Finally. no significant differences were seen with regard to synchronous or metachronous cancer elsewhere in the colon upon follow-up (SSA-S 7%, SSA-I 0%, SSA-S+I 12%).

Conclusions: SSAs with intestinal dysplasia may progress faster than SSAs with serrated dysplasia, and may be at higher risk for carcinomatous transformation. In all three groups, carcinoma only occurred in lesions with high-grade dysplasia, which confirms the dysplasia-carcinoma sequence in SSAs regardless of the type of dysplasia.

646 Immunohistochemical and Molecular Characterization of Colonic Sessile Serrated Adenomas/Polyps with Serrated Versus Intestinal Dysplasia

Odise Cenaj, Robert Odze, Monika Vyas, Joanna A Gibson. Brigham and Women's Hospital, Boston, MA; Yale-New Haven Hospital, New Haven, CT.

Background: The serrated pathway of colon carcinogenesis proposes that cancers develop via a sessile serrated adenoma (SSA)-dysplasia-carcinoma sequence. Dysplasia in SSA may be serrated (SSA-S) or intestinal type (SSA-I). However, the molecular signatures of the types and grades of dysplasia are poorly defined. The aim of this study was to evaluate selected immunohistochemical and molecular markers in SSAs with dysplasia to better define the biology of their neoplastic progression.

Design: 86 formalin-fixed paraffin-embedded SSAs with dysplasia (50 SSA-S, 22 SSA-I, 14 SSA-S+I) were evaluated with a panel of immunohistochemical markers. The presence and extent of staining was scored separately in dysplastic foci of both types (serrated vs intestinal) and grades (low vs high), based on quantitative criteria specific to each marker.

Results: Overall, SSA-I show a significantly higher rate of expression of p53 (45%) and downregulation of p21 (37%) compared to SSA-S (20% and 81%, respectively, p=0.01). Similarly, beta-catenin expression and p21 downregulation are significantly more frequent in low grade SSA-I vs SSA-S (33% vs 9%, 29% vs 94%, respectively, p=0.01), but not high grade SSA-I vs SSA-S. Irrespective of SSA type, there is a significant stepwise and parallel increase of beta-catenin, p53, p16 expression and a simultaneous downregulation of p21 expression between low to high grade foci (p<0.05). Interestingly, although BRAF-V600E expression is higher in low grade SSA-S vs low grade SSA-I (96% vs 81%, p=0.05), this significance is lost in high grade foci. In contrast, the rate of PMS2 loss increases with dysplasia grade, both in intestinal and serrated SSAs (p=0.01). MSH6 was universally intact. Markers of gastric (MUC6, MUC5AC) and colonic differentiation (DAS1) show statistically non-significant trends of reduced expression between types and grades of dysplasia.

Conclusions: The development and progression of intestinal and serrated dysplasia in SSA are associated with altered expression of cell cycle and proliferation proteins, namely upregulation of beta-catenin, p53, p16 and downregulation of p21. These changes occur in a background of BRAF-V600E mutation and loss of mismatch repair protein PMS2. Our findings not only confirm the role of microsatellite instability in the serrated pathway of colorectal cancer arising from dysplastic serrated polyps, but also expand our understanding of the cellular mechanisms involved in the process.

647 Characterization of Colorectal Cancer Metastasis-Associated Genes Using Next-Generation Sequencing

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Background: Cancer metastasis is the main cause of colorectal cancer-related death. Histologic grade and T stage cannot predict the metastatic potential of a tumor. We hypothesize that colorectal cancer metastasis is driven by underlying genetic mutations, which may reveal therapeutic targets and prognostic biomarkers. We routinely perform next-generation sequencing (NGS) on colorectal cancers, which provided the material

for our study. In this study, we performed a retrospective analysis of colorectal cancer NGS data with respect to the presence or absence of metastasis and characterized specific underlying mutations that may "drive" metastasis.
Design: A 1-year retrospective review of all colorectal cancers analyzed at our institution by NGS was performed. An extensive review of the electronic medical record yielded multiple clinical data points, including the presence or absence of metastasis, T and N stage, primary colonic site, histologic grade, MSI status, age, and sex. Tumors were localized, macrodissected, and sequenced using the Ion AmpliSeq™ Cancer Hotspot Panel v. 2. In this particular study, our analysis was focused on the genetic mutations associated with the presence or absence of metastasis. A critical review of the NGS data for each sample was performed and apparent false positives, such as SNPs and samples with low variant frequency, were excluded.
Results: Our total sample size was n=109, of which 64 tumors were metastatic and 45 were not. Overall, we identified 1071 unique mutations in 33 genes within the tumors. Of these, JAK3, ATM, and MET genes emerged with patterns of recurring mutations with increased metastatic potential.

Gene	Total samples with mutation	Metastasis	No metastasis	Chi-square
ATM	7	2	5	0.4228276
JAK3	3	3	0	0.5380654
MET	9	2	7	0.1453921

All of the JAK3 mutations occurred at the same hotspot, resulting in a p.P132T missense mutation. We also identified several genes that had a statistically significant association with histologic grade.
Conclusions: This pilot study identified JAK3, ATM, and MET as genetic mutations associated with increased metastatic potential in colorectal carcinomas. While the initial sample size was large, this number decreased when pared down to specific mutations. As the sample size continues to grow, our study will reveal a more extensive genomic landscape associated with colorectal cancer metastasis.

648 Diagnostic Utility of Keratin and Elastic Stains in evaluation of Tumor Invasion of Peritoneal Membranes

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Background: Invasion of the peritoneal surface by tumors of the gastrointestinal system is a recognized adverse prognostic feature, but the clinical significance of peritoneal invasion in the absence of surface involvement is less clear. This may be due in part to the difficulty in identifying the peritoneal elastic reticulum with significant tumor-associated retraction and destruction. Because serosal injury is associated with proliferation of keratin-expressing stromal cells, we examined the diagnostic utility of a keratin immunostain in conjunction with an elastic stain in identifying tumor invasion of the peritoneum.
Design: The peritoneum in 90 primary tumors and 19 non-neoplastic portions of the stomach, small intestine, colon, appendix, and gallbladder were examined with hematoxylin and eosin, elastic Verhoeff-Van Gieson, and an immunostain for pan-keratin. Peritoneal invasion was defined by tumor that penetrates the elastic reticulum and colocalizes with keratin-positive stromal cells.
Results: 65 primary tumors of the GI tract and gallbladder invaded the peritoneum that ranged from focal to widespread with visceral surface involvement. In most cases, there was retraction of the elastic reticulum toward the tumor with variable attenuation and destruction. There was concomitant expansion and proliferation of serosal connective tissue with stromal cells that showed cytokeratin expression. These cells were sharply delimited from the subserosa by the peritoneal elastic reticulum. In all tumors with peritoneal invasion, the neoplastic cells invaded through the elastic reticulum and abutted or were surrounded by keratin-positive stromal cells. The degree of keratin reactivity appeared to correlate with the severity of serosal injury. By contrast, all 25 tumors that invaded the subserosa without involvement of the peritoneum showed no breach of the peritoneal elastic reticulum and no tumor cell that colocalized with keratin-expressing stromal cells. The association between tumor invasion of the peritoneum and tumor cells that colocalized with keratin-positive stromal cells was statistically significant.
Conclusions: Evaluation of keratin and elastic stains for tumor involvement of the peritoneum is a reliable, sensitive, and facile methodology in assessing invasion of the peritoneal elastic reticulum and connective tissue. This analytic method will enable a more definitive diagnosis of tumor invasion of the peritoneum in gastrointestinal organs and correlation with patient outcome.

649 Objective Measurement of HER2 in Colorectal Adenocarcinomas Reveals Heterogeneity of the Protein's Extracellular Domain

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Background: HER2 overexpression/amplification has been identified in 5-8% of colorectal adenocarcinomas (CRC). In the metastatic setting, approximately one third of HER2-positive patients treated with dual HER2 blockade show clinical benefit. Previous quantitative studies in breast cancer by our group have shown that the intracellular domain (ICD) of HER2 is expressed in some cases that do not express the HER2 extracellular domain (ECD). Here, we measured both the HER2 ICD and ECD in a CRC cohort and determined its relationship with key clinico-pathological characteristics and survival.
Design: We measured HER2 ICD and ECD in a cohort including 716 CRC patients, represented in tissue microarray format, using the AQUA® method of quantitative immunofluorescence (QIF). Previously validated and standardized HER2 protein assays targeting the ICD (CB11) and ECD (SP3) were used. The upper 5th percentile of QIF scores was used to stratify patients into high or low HER2 ICD/ECD status, based on previous studies reporting the prevalence of HER2 overexpression/amplification in CRC.
Results: Of 716 patients measured, 35 had HER2 expression at a level that would be considered 2+ or greater in breast cancer. In this subgroup of patients with high expression, 71.4% with high HER2 ICD had low levels of ECD expression (25/35 patients). There was no association between HER2 ICD/ECD status and sex, age, histological grade, tumor size, nodal status, TNM stage, or survival.
Conclusions: Using objective, domain-specific HER2 measurement in CRC, approximately 70% of patients with HER2 ICD overexpression showed low levels of the ECD. This may be related to the expression of p95 HER2, an oncogenic fragment generated by full protein cleavage or alternative initiation of translation. This observation raises the possibility that HER2-positive CRC patients with low levels of ECD might benefit more from therapies targeting the intracellular domain (tyrosine-kinase inhibitors) instead of agents directed towards the extracellular domain (e.g. trastuzumab).

650 Detection of Mismatch Repair Protein (MMR) Loss by Immunohistochemistry (IHC) in Colorectal Adenomas from Young Patients: An Appraisal of Its Utility in Lynch Syndrome Screening

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Background: Colorectal adenomas may develop in patients with Lynch syndrome at young age. In the context, they likely to show loss of MMR proteins, particularly in large ones from right colon. Although not a recommended screening approach, MMR testing has been routinely performed in adenomas from young patients (<40 years) to exclude potential Lynch syndrome. We decide to formally evaluate the clinical utility of this approach.
Design: All cases with MMR tests performed on colorectal specimens from 2013 were retrieved and reviewed. Patient ages, diagnoses, reasons for MMR testing and test results were recorded. Accompanied endoscopic reports were also reviewed for adenoma cases to document locations, sizes and indications of endoscopy. Statistical analysis was performed using Fisher exact test.
Results: Total of 43 adenomas (40 tubular adenomas, 2 tubulovillous adenomas and 1 sessile serrated adenoma) were tested from 43 patients with a mean age of 32 (13- 40) including 21(48.8%) adenomas from right colon. The mean size of the adenomas is 8.5 mm (2 -35 mm). Endoscopy was performed for high risk colon cancer surveillance in only 5 (11.6%) patients including 1 with family history of familial adenomatous polyposis (FAP). It was done for melena in 17 (39.5%) patients and for abdominal pain and diarrhea in 21 (48.8%). None had history or diagnosis of inflammatory bowel disease. All these adenomas showed normal MMR expression profile. In contrast, 21 adenomas with serrated morphological features were also tested in the same period. They were from older patients with a mean age of 59 (44-78). Abnormal MMR expression was detected in 4 (19%) with 2 exhibiting MSH2/6 loss ($p<0.01$). In addition, 245 colorectal adenocarcinomas were also tested and 67 (27%) were found to have abnormal MMR expression ($p<0.01$).
Conclusions: Detecting MMR loss by IHC in colorectal adenomas from young patients has no clinical use as an approach for Lynch syndrome screening. Not only are positive results exceedingly rare, but also negative results can not completely exclude risks of the disease.

651 Development and Validation of a Prognostic Nine Gene Expression Signature for Stage II/III Colorectal Cancer Patients

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Background: Several clinical and pathological factors have an impact on prognosis in colorectal cancer (CRC), but as yet they are inadequate for risk assessment. The present study aimed to develop a prognostic algorithm for patients with CRC.
Design: 554 CRC samples (stage II/III) were included in the study. A two-step gene expression profiling study was conducted. First, gene expression measurements from 81 CRC fresh frozen samples were obtained using Affymetrix Human Genome U133 Plus 2.0 Array. Second, a focused gene expression assay including prognostic genes was validated from 473 formalin-fixed paraffin-embedded (FFPE) samples using a NanoString-based assay. A 9-gene assay was trained using 237 FFPE samples; the locked assay was then validated using another independent cohort of 236 FFPE samples.
Results: An optimal 9-gene expression classifier (C9orf116, HS2ST1 /// LOC339524, MEG3, MMP3, NME1, NUP210, PROS1, TIMP1, and VEGFC) for prediction of relapse free survival among patients with CRC was developed using a test series ($n=237$, $p<0.01$, HR=4.01), and its predictive value was validated for patients with CRC in a second series ($n=236$, $p<0.01$, HR=2.05). Multivariate Cox regression analysis, including all two sample series and various clinicopathological variables, confirmed the independent prognostic value of the 9-gene classifier ($p<0.01$).
Conclusions: The 9-gene expression classifier is an independent prognostic factor for stage II/III colorectal cancer.

652 Multivisceral Transplant Is a Viable Treatment for Patients with Unresectable Intra-Abdominal Fibromatosis

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Background: Intraabdominal fibromatosis (desmoid tumor), especially in patients with familial adenomatous polyposis (FAP), often involves the bowel or mesentery root. Additionally, the recurrence rate after conventional surgery is reported as high as 77.7% with a median followup of 61 months (7 out of 9 cases, Roswell Park Cancer

Institute, 1994). Unresectability and recurrence pose a clinical challenge. Multivisceral transplant (MVT), as a potential cure to unresectable fibromatosis, has rarely been reported and the prognosis not assessed.
Design: Six patients who underwent MVT for unresectable intraabdominal fibromatosis, from 2005 to 2015, were reviewed. Clinicopathological features, immunohistochemistry for β-catenin, p53, and Ki67 proliferation index, and followup were evaluated.
Results: Four of the six patients had FAP. Four presented with unresectable short gut syndrome, and the other two had recurrence post intestinal transplantation. One patient died of peri-operation hemorrhagic stroke. Five patients (83.3%) survived with a median followup of 53 months (range, 2–116 months). The 1-year and 5-year survival rates were both 83.3% with no recurrence. Among these five patients, three had positive surgical margins; one had negative margins; and one cannot be determined. The mean Ki67 proliferation index was 3.4% (range, 0.7–5.7%). The mean percentage of tumor cells positive for nuclear β-catenin expression was 77.3% (range, 41.0–92.3%) and the mean percentage of tumor cells positive for p53 was 10.2% (range, 0.4-20.9%).
Conclusions: MVT is a viable option for patients with unresectable intraabdominal fibromatosis. Surgical margin, Ki67 proliferation index, β-catenin, and p53 expression do not seem to be related to tumor recurrence in MVT. If patients can survive MVT-related complication, recurrence rate is much lower in MVT compared to that of conventional surgery.

653 The Prognostic Significance of Peritumoral Chronic Inflammation in Rectal Adenocarcinoma after Neoadjuvant Treatment

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Background: Peritumoral chronic inflammation (PCI) is a favorable prognostic indicator in breast carcinoma, with and without neoadjuvant treatment (NT), and in untreated colorectal adenocarcinoma; however, the significance of PCI in rectal adenocarcinomas post NT is relatively unexplored. The aim of this study is to determine the association of PCI with validated prognostic factors in post-NT rectal adenocarcinoma patients.
Design: We searched our pathology database for diagnoses of rectal adenocarcinoma between 2008-2015 and identified 117 rectal resections, of which 52 cases met inclusion criteria for residual tumor post NT and resection. Tumor slides were reviewed and stromal chronic inflammation was defined as percentage of peritumoral stroma containing inflammatory infiltrate and was graded as follows: 0=none, 1-30%=mild, 31-60%=moderate, 61-100% =extensive. Areas of necrosis, ulceration and acute inflammation were avoided. Group 1 included patients with grade 0-1 inflammation; Group 2 had grade 2-3 inflammation. Chi square and ordinal logistic regression analysis were used to evaluate association between PCI and size, perineural (PNI), differentiation, lymphvascular invasion (LVI), pre and post NT stage, positive lymph nodes (LN) tumor deposits (TD), distant metastases (DM), positive margins and recurrence.
Results: Of 117 resections, 65 patients (55%) received NT and subsequent resection. 7 (10%) achieved complete response and 8 were excluded (incomplete data). Of the 52 remaining patients, 61% were in group 1. High PCI (group 2) was associated with favorable prognostic factors including low rates of PNI (p=0.03), smaller tumor size (p=0.03; 2.43 ±1.82 cm vs. 3.77 cm ±2.73), better tumor differentiation (p=0.04), trended toward lower LVI (p=0.07), and negative margins (p=0.06). Although not statistically significant, group 2 also had lower recurrence rates (p=0.2). There was no significant difference between the groups regarding pre and post NT stage change, LN, TD, or DM (p>0.05).
Conclusions: PCI involving >30% of residual post-NT rectal adenocarcinoma is associated with favorable tumor characteristics and could serve as an additional pathologic prognostic marker to document. Larger studies are needed to confirm our findings.

654 Spectrum of Pathologic Findings in Clinical Liver Cirrhosis without Confirmed Cirrhosis on Histology

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Background: Imaging modalities such as ultrasound, CT, and MRI in conjunction with clinical findings are used in the clinical diagnosis of liver cirrhosis. However, few contemporary studies have reported findings in discrepant cases where histology did not confirm cirrhosis. We aimed to review the spectrum of pathologic findings in the liver when a clinical impression of cirrhosis is not confirmed.
Design: Our pathology database was searched for cases of clinically suspected cirrhosis from 2010 to 2015. A total of 141 cases were retrieved and among them, 31 cases were confirmed to have a clinical impression of cirrhosis with no cirrhosis examined on histology. Pathology reports and slides from the corresponding liver biopsies were retrieved. Pathology diagnoses were recorded and slides were selectively reviewed. The corresponding medical records were also reviewed to interrogate the basis of the diagnosis of cirrhosis. Specifically, the presence or absence of ascites, varices, splenomegaly, hypoalbuminemia, thrombocytopenia and increased portal pressure measurements were ascertained. The radiologic assessment of the liver was also recorded.
Results: A spectrum of pathologic findings was seen in a total of 31 cases. Five cases showed features compatible with cardiac disease, 4 had granulomatous disease, 1 had hepatoportal sclerosis and 4 had features of nodular regenerative hyperplasia (NRH). Four cases had advanced fibrosis (stage 3) in the setting of fulminant liver injury with extensive parenchymal dropout and ductular proliferation. Four cases also showed significant siderosis. Two cases were inadequate for evaluation (4-6 portal tracts present) and had focal nodule formation suggestive of cirrhosis. Five cases had minimal changes without suggestive pathologic diagnoses; clinical features of cirrhosis were diverse (portal hypertension, imaging studies, fibrospect results, and clinical history) among these without any trends. Clinically 18 of the 31 cases had portal hypertension; among these were most (5/7) patients with stage 0 fibrosis, all cases of NRH and most cases of granulomatous disease (3/4) including a case of cryptosporidiosis.
Conclusions: In addition to the usually reported etiologies of portal hypertension and clinically apparent cirrhosis, acute liver injury with marked ductular proliferation and granulomatous liver disease may present clinically with portal hypertension and imaging characteristics of cirrhosis. Intriguingly, marked siderosis may also be the only significant pathologic finding in the liver biopsy of patients with clinical evidence of cirrhosis.

655 Immune Complexes and Eosinophil Activation in Eosinophilic Esophagitis

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Background: We previously demonstrated the presence of abundant soluble IgG4 in the esophageal mucosa in eosinophilic esophagitis (EoE), and the presence of granular IgG4 deposits resembling immune complexes in 83% of active EoE cases. Solid phase deposits of IgA and IgG are known to activate eosinophils, inducing degranulation. However, IgG4 is not believed to induce inflammation. Antibodies to the common trigger foods are often present in the sera of EoE patients. Immune complexes other than IgG4 have not been studied in the esophageal tissue in EoE.
Design: We studied frozen sections of esophageal mucosal biopsies from 21 subjects with active EoE and 11 age and gender matched normal controls, and formalin-fixed, paraffin-embedded esophageal biopsies from 20 subjects with active EoE and 16 age and gender matched normal controls. These were immunofluorescent stained for IgA, IgM, and total IgG. Most of the frozen section cases were also stained for IgG4 and complement C3. Chromotrope 2R staining was used to minimize nonspecific immunostaining of eosinophil granules.
Results: In both the frozen sections and paraffin sections, moderate to abundant granular immunoglobulin deposits resembling immune complexes were found in 90 and 95% active EoE cases, respectively. Such deposits were absent or sparse in all of the normal controls ($p<0.001$ for both comparisons). Granular C3 deposits were found in 81% of frozen section cases, but none of the 11 frozen section control tissues ($p<0.001$). Usually, either IgG or IgA granular deposits were dominant, but this varied from case to case and in different fields in the same case. Total IgG immunostaining was substantially more extensive and brighter that IgG4 staining.

The granular immunoglobulin and C3 deposits were frequently present on the surface of eosinophils, particularly in areas of eosinophil degranulation. Eosinophil microabscesses contained the granular immunoglobulin deposits between the eosinophils.
Conclusions: Immune complex-like granular deposits with IgA, IgM, and IgG other than IgG4 are common in the esophageal mucosa in EoE. Their presence on the surface of eosinophils, particularly in areas of degranulation, suggests a possible mechanistic role for food-antibody immune complexes in EoE by inducing eosinophil degranulation.

656 Genta Stain Necessity in Diagnostic Accuracy of Detecting Helicobacter Pylori in Gastric Biopsies

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Background: Detection of Helicobacter pylori (H pylori) is essential as eradication alleviates symptoms and prevents malignancy. Histologic examination is the most useful diagnostic tool, with 95% sensitivity and 99% specificity. Genta stains are universally performed on gastric biopsies at our institution; our aim is to determine which cases truly need the Genta, and cost implications of changing this practice.
Design: This is a case series study consisting of a retrospective review of pathology reports and H pylori-positive slides of gastric biopsies from June 1, 2014 to May 30, 2015. Two pathologists reviewed the H pylori-positive H&E slides with the option of viewing the Genta.
Results: During the 12-month study period, 3428 cases were reviewed, and 219 (6%) were positive for H pylori. The pathologists agreed on the necessity of the Genta in 85%, with 21% Genta necessity rate. Mild H pylori cases were significantly more likely than severe cases to need a Genta. The quantity of polymorphonuclear cells (PMNs), mononuclear cells, and intestinal metaplasia (IM) did not change with Genta necessity. The quantity of PMNs, mononuclear cells, and IM was significantly higher in H pylori-positive than negative cases.

Table 1. Genta necessity for H pylori detection,*p<0.05		
	Genta Stain	
	Necessary(n=45)(%)	**Unncessary(n=174)(%)**
H pylori		
Mild	31(69)	8(5)*
Moderate	13(29)	77(44)
Severe	1(2)	89(51)*
PMNs		
None-Mild	14(31)	42(24)
Moderate-Severe	31(69)	132(76)
Mononuclear Cells		
None-Mild	2(4)	3(2)
Moderate-Severe	43(96)	171(98)
IM		
None-Mild	36(80)	152(87)
Moderate-Severe	9(20)	22(13)

Table 2. H pylori positive versus negatve cases,*p<0.05		
	H pylori	
	Positive(n=219)(%)	**Negative(n=3209)(%)**
PMNs		
None-Mild	56(26)	3108(97)*
Moderate-Severe	163(74)	101(3)*
Mononuclear Cells		
None-Mild	5(2)	2504(78)*
Moderate-Severe	214(98)	705(22)*
IM		
None-Mild	188(86)	3003(94)*
Moderate-Severe	31(14)	206(6)*

Conclusions: In sum, if Genta was ordered on cases with only moderate-severe mononuclear inflammation, 2680 (78%) of cases would not have had a Genta. This would result in a potential cost savings of $295,000; 2 of 219 (1%) cases of H pylori would have been missed.

657 Pancreatic Mesenchymal Chondrosarcoma Harboring HEY1-NCOA2 Gene Fusion

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Background: Mesenchymal chondrosarcomas are rare tumors that typically arise in the bony sites such as the pelvis, ribs, and jaw. Approximately 30% can occur primarily in somatic soft tissue. Recently, the *HEY1-NCOA2* gene fusion was identified as a characteristic genetic abnormality of mesenchymal chondrosarcoma. Suspected cases of visceral, including pancreatic, mesenchymal chondrosarcoma have been reported based on histomorphology, but genetics of these tumors have not been characterized. We investigated whether tumors that histomorphologically resemble mesenchymal chondrosarcomas, but involving the pancreas, are genetically similar to their somatic soft tissue and bone counterparts.

Design: We identified eight cases of mesenchymal chondrosarcoma in departmental archives from 1990-Present. Of these, there were two cases that had been morphologically diagnosed as pancreatic mesenchymal chondrosarcoma. Clinicopathologic features were reviewed. mRNA was extracted from formalin-fixed paraffin embedded tissue sections, and reverse transcriptase polymerase chain reaction and Sanger sequencing for the *HEY1-NCOA2* fusion transcript were performed.

Results: Two mesenchymal chondrosarcomas (2.9 and 9.5 cm) were considered as primary pancreatic tumors based on clinical and imaging work-up. Microscopically, both tumors exhibited biphasic appearance with lobules of neoplastic hyaline cartilage alternating with sheets of primitive small round cells with scant cytoplasm and so-called "hemangiopericytoma-like" vasculature. Both tumors demonstrated fusion of *HEY1* exon 4 to *NCOA2* exon 13.

Conclusions: Mesenchymal chondrosarcomas can involve the pancreas and show the same *HEY1-NCOA2* rearrangement found in tumors involving bone and somatic soft tissue. As the differential diagnosis of such tumors in the pancreas includes sarcomatoid carcinoma and other primary and metastatic sarcomas, demonstration of HEY1-NCOA2 rearrangement can help to confirm the diagnosis, especially on small CT-guided or endoscopic ultrasound-guided biopsies.

658 The Role of Immunohistochemistry in Subtyping Ampullary Adenocarcinoma

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Background: Individual outcomes from ampullary adenocarcinomas (AACs) are difficult to predict and can pose a significant challenge in both management and prognosis. A suggested predictor of outcome and treatment response is histologic classification of AAC into intestinal, pancreatobiliary and other subtypes. Immunohistochemistry (IHC) may aid distinction between subtypes however the added value of IHC over H&E evaluation is unclear.

Design: 60 cases of resected AAC (pancreatico-duodenectomies) were identified from a cancer database in a tertiary center. All tumor slides were subtyped first by H&E evaluation and then by evaluation of an IHC panel composed of CK7, CK20, CDX2, MUC1 and MUC2. Cases were re-evaluated by a second pathologist using a double-headed microscope. Cases were then assigned to one of three categories (intestinal, pancreatobiliary or ambiguous) according to an IHC subtyping schema proposed by Ang *et al* in 2014. The relationship between H&E and IHC typing was examined and correlated with detailed clinical and pathological features.

Results: See table.

	H&E typing (n[%])			
	Intestinal	***Pancreatobiliary***	***Mixed***	***Total***
IHC typing				
Intestinal	3(17)	0	2(17)	5(8)
Pancreatobiliary	4(22)	26(87)	9(75)	39(65)
Ambiguous	11(61)	4(13)	1(8)	16(27)
Total	18	30	12	60

Conclusions: Our results suggest that IHC may not have a primary role in subtype classification. Immunohistochemistry was useful in defining the mixed morphological group. The majority (92%) of our 'mixed' H&E group was defined as either 'intestinal' or 'pancreatobiliary' by IHC. We conclude that H&E subtyping is a reliable method of subclassification in the majority of AACs. IHC may be a useful adjunct in tumors with mixed morphology.

659 Myointimal Hyperplasia of the Mesenteric Veins: A Rare Disease with Distinct Histopathologic Features in Colonic Biopsy Specimens

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Background: Idiopathic myointimal hyperplasia of mesenteric veins (IMHMV) is an uncommon cause of ischemic colitis. The disease is characterized by myointimal proliferative changes with obliteration of mesenteric veins. All reported cases to date have been diagnosed following surgical resection. Most biopsy samples obtained before resection are diagnosed as IBD or ischemic colitis, NOS. The aim of this study was to define specific features, if any, present in biopsy samples that allow recognition of IMHMV and distinguish it from other colitides.

Design: The study group consisted of 9 patients with IMHMV: 8 underwent surgical resection and 1 was diagnosed based on mucosal biopsy. Five study patients had biopsy samples prior to colectomy that were also reviewed. The control group included 30 patients with radiation, ischemic, and pseudomembranous colitis (10 each). Cases and controls were assessed for specimen site, fibrinoid deposits in vessel walls, arterialized capillaries and venules, myointimal hyperplasia of submucosal and/or extramural veins, and fibrin thrombi.

Results: Most study patients were elderly (mean: 74 years) males (89%) with disease confined to the rectosigmoid colon (89%), and symptoms of abdominal pain (89%) and/or bloody diarrhea (67%). Cases from all 9 patients with IMHMV showed myointimal hyperplasia of veins; most cases showed features of ischemic colitis with hyalinization and hemorrhage in the lamina propria, atrophied microcrypts, and ulceration. Fibrinoid vascular deposits and arterialized vessels were present in the mucosae of both resection (7 and 8, respectively) and biopsy (5 and 5, respectively) specimens from study patients and were significantly more common in IMHMV than in radiation-induced (1 and 0, respectively), ischemic (1 and 0, respectively), and pseudomembranous colitis (0 and 0, respectively) controls ($p<0.0002$ for all comparisons). Fibrin thrombi were also present in the mucosae of IMHMV (67%), and ischemic and pseudomembranous colitis (18% and 14%, respectively), but not in cases of radiation colitis ($p=0.01$).

Conclusions: Fibrinoid deposits in vascular walls and arterialization of vessels are commonly present in mucosal biopsies from patients with IMHMV and these changes are quite specific. Recognition of these features may enable pathologists to render a definitive diagnosis, especially in the appropriate clinical scenario.

660 Patterns of Lymphocytic Gastritis May Reflect the Underlying Etiology

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Background: Lymphocytic gastritis may occur in isolation, or in association with other disorders such as celiac disease, H. pylori infection, and Menetrier disease. It is characterized by numerous (>25/100 epithelial cells) intraepithelial lymphocytes (IELs), variably intense lamina propria inflammation, or even neutrophils and eosinophils. We performed this study to evaluate the morphologic spectrum of lymphocytic gastritis and determine whether any features are specifically associated with other inflammatory conditions of the gastrointestinal tract.

Design: Fifty-eight gastric biopsy samples that showed >25 IELs/100 epithelial cells were identified, including 18 with H. pylori infection, 26 with celiac disease, and 14 with no other gastrointestinal disorders (idiopathic). All cases were evaluated for the number and distribution of IELs, composition and severity of lamina propria inflammation, pit or gland destruction, and degree of foveolar hyperplasia.

Results: Most patients were adults (mean: 42 years) and 67% were women, although patients with celiac disease were significantly younger (mean: 31 years) and usually (85%) women ($p<0.05$ for both comparisons). The number of IELs/100 epithelial cells was significantly higher in idiopathic cases (mean: 80) than celiac disease (mean: 64,

p=0.03) and H. pylori infection (mean: 48, p<0.001). Cases of H. pylori infection tended to show focally increased IELs in the surface epithelium (39%) with a superficial band of inflammation in the lamina propria (56%). Intraepithelial and lamina propria neutrophils were common (94%), as were eosinophils (44%); 72% of cases showed IELs in the deep mucosa. In contrast, cases associated with celiac disease showed diffusely increased IELs (92%, p=0.02) with evenly dispersed mononuclear cell-rich inflammation in the lamina propria (73%, p=0.03). Rare cases contained neutrophils (12%, p<0.001) or eosinophils (12%, p=0.03), and all showed IELs in the deep mucosa (p=0.008). Idiopathic cases resembled those associated with celiac disease, but tended to contain more plasma cells in the lamina propria (50% vs. 8%, p=0.006) and showed foveolar hyperplasia in the surface epithelium (21% vs. 0%, p=0.02).
Conclusions: H. Pylori infection may be associated with increased IELs, but the finding is usually focal and accompanied by superficial lamina propria inflammation with neutrophils and eosinophils. A diffuse, lymphocyte-predominant infiltrate is typical of celiac disease and idiopathic lymphocytic gastritis.

661 Loss of Hes1 Expression Is Associated with Microsatellite Instability in Human Colorectal Carcinoma

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Background: Notch signaling plays an important role in enterocytes development and differentiation, and Hes1 is the major downstream transcriptional factor of Notch signaling pathway. In colon, Hes1 is normally expressed in crypt cells and lamina propria inflammatory cells. Current literature on Hes1 expression in the human colorectal carcinoma (CRC) is sparse and puzzling, with some paper reporting nuclear expression of Hes1 with higher expression level in poorly differentiated carcinoma, and some paper reporting loss of Hes1 nuclear expression in carcinoma cells. This study is performed to explore Hes1 expression in a variety of human CRC and its association with clinical and pathological features of the tumor.
Design: 60 cases of CRC from a single tertiary hospital, 33 from right colon, and 27 from left colon were studied. The patients' ages range from 23 to 93 years old with mean of 68 years; M/F ratio was 1.4:1 (35 male, 25 female). Immunohistochemistry of MSH1, MSH6, MLH3 and PMS6 were performed on 55 cases.
Results: Hes1 immunohistochemistry show three distinct expression patterns in CRC. Loss of nuclear Hes1 expression in invasive carcinoma is observed in majority of the right sided CRC (28/33, 85%), and is less commonly found in the left sided tumor (7/27, 26%). Uniformly increased nuclear expression of Hes1 in invasive carcinoma is mostly observed in left sided CRC (14/27, 52%) and rarely seen in right side tumor (3/33, 9%). Heterogenous intratumor immunostaining is present in a subgroup of right (2/33, 6%) and left sided CRC (6/27, 22%). There is significant correlation of loss of Hes1 with loss of MSI (microsatellite instability) marker by immunohistochemistry (90% in MSI *vs* 29% in MSS, r=0.63, P<0.0001) and right sided location of the tumor (85% right *vs* 26% left, r=0.59, P<0.0001). No significant correlation was found between loss of Hes1 and gender, mucinous differentiation, tumor grade or lymph node metastasis.
Conclusions: In CRC, loss of Hes1 expression is associated with MSI status and right-sided location, while positive nuclear Hes1 expression is associated with MSS status and left-sided location. There is a third group of CRC with heterogenous intratumor Hes1 expression, which suggests that dysregulation of Notch signaling pathway in some of the CRC is fairly complex. Our study demonstrates that Hes1 is not uniformly lost or expressed across all the CRC, and its expression is significantly associated with both MSI status and anatomic location of the tumor.

662 Overall Tumor Budding and Hotspot Scores in Colorectal Cancer: A Simplification of the 10 HPF Method

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Background: In colorectal cancer (CRC), tumor budding is conventionally defined as single tumor cells or clusters of up to 5 tumor cells at the invasive front (peritumoral budding, PTB). However, intratumoral buds (ITB) may also be seen and are highly correlated with PTB. The aim of this study was to assess the performance of an overall tumor budding score (OTB) using 10 high-power fields (HPF) or a single hotspot method.
Design: Specimens from 156 patients with Stage I-IV CRC were included. On pancytokeratin stained slides, OTB was calculated from budding scores in the 10 densest of 20 high power fields (40x, 0.24mm2, 10 HPF each ITB and PTB). Hotspots were defined as the initially identified intra- and peritumoral HPF with the highest bud count. A previously defined cutoff of 10 buds/HPF was compared to OTB as a continuous variable in correlation with clinicopathological features and survival.
Results: OTB highly correlated with PTB and ITB (both r=0.95, p<0.0001). For PTB and ITB, the hotspot method highly correlated with 10HPF (r=0.93, p<0.0001). Using the cut-off, high grade OTB was associated with higher pT, the presence of lymph node and distant metastases and higher TNM-stage (all p<0.05). Continuous OTB scores correlated with higher pT, lymph node and distant metastasis, venous invasion and high tumor grade (all p<0.01), lymphatic invasion (p<0.0001) and higher TNM-stage (p<0.05). OTB maintained its adverse prognostic effect after adjusting for TNM-stage and adjuvant therapy (HR (95%CI): 1.02, CI: 1.002-1.04, p= 0.03).
Conclusions: This study demonstrates the significance of OTB as a strong adverse prognostic factor and simplifies scoring by incorporating the densest areas of tumor budding irrespectively of the tumor area. The hotspot method was comparable to the 10HPF method, indicating that assessing further areas may add only marginal value and highlighting the potential utility of the hotspot method in specimens with limited material, such as malignant polyps or pre-operative biopsies. OTB as a two-tiered system was outperformed by OTB as a continuous variable, which may be integrated a in a nomogram-type prognostic model for CRC.

663 VE1 Immunohistochemistry in Colorectal Cancer Is Homogeneously Expressed and Is a Predictor of BRAF Mutational Status and Worse Survival

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Background: BRAFV600E mutations occur in about 10% of colorectal cancers (CRC) and have been identified as a driver mutation of the serrated pathway. Currently, the main use of BRAF mutational analysis in daily practice is to exclude Lynch syndrome. However, targeted anti-BRAFV600E therapies have shown promising results in improved combined therapy regimens, indicating that the role of BRAF mutational status in CRC is likely to grow. Recently, the VE1 monoclonal antibody was developed to identify the mutant protein. The aim of this study was to validate the VE1 antibody as a predictor of BRAF mutational status and investigate its prognostic value and heterogeneity of expression within primary CRCs and metastases.
Design: VE1 was examined on multiple-punch tissue microarrays (TMA) in three cohorts with known mutational status: A pilot cohort consisting of CRC (n=34), melanomas (n=23), thyroid cancers (n=8) and eight cell lines, in addition to two prognostic cohorts (cohort 1, n=259 and cohort 2, n=226). In a subset of cohort 2 (n=118), VE1 expression was also assessed in pre-operative biopsies to determine concordance with resections. Primary tumors and metastases from 13 patients were tested for VE1 heterogeneity on a TMA, including punches from all primary and metastatic tumor blocks (n=100).
Results: The concordance between VE expression/mutation was 98.5% in tissues and 100% in cell lines. Interobserver agreement (3 observers) was 100% (kappa=1.0). The frequency of VE1 positivity in cohort 1 was 13.5% and associated with older age (p.0.0175) and MLH1 deficiency (p<0.0001). In cohort 2, VE1 was positive in 12.8% of cases and associated with female gender (p=0.0016), right-sided tumors, higher tumor grade and mismatch repair deficiency (each p<0.0001). In survival analysis, VE1 had an independent adverse prognostic effect in postoperatively untreated and non-metastatic patients. Staining in preoperative biopsies matched resections in all cases except one. No heterogeneity was found across primary/metastatic tumors.
Conclusions: VE1 is homogenously expressed and concordant with mutational status. It is easy to assess, yielding high interobserver agreement and may be used in preoperative biopsies, resection specimens or metastatic lesions.

664 Size Does Matter: Tumor Diameter Predicts Lymph Node and Distant Metastases in Gastric Adenocarcinoma

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Background: Modern therapy protocols for advanced gastric cancer combine neoadjuvant therapy and radical surgery. Therefore, pre-operative assessment of tumor extent, especially tumor depth and lymph node involvement, is critical for further treatment. Imaging has only limited value in preoperative staging of gastric cancer and the most accurate, albeit not perfect methods are endoscopy and endo-luminal ultrasound. This study examined the role of macroscopic tumor features as predictors of tumor stage, nodal metastasis (NM) and distant metastasis (DM).
Design: 2071 Stage I-IV therapy-naïve gastric cancer resection specimens from 4 institutions between 1983 and 2013 were included. Tumor size, Borrmann classification, histological tumor type (WHO), Lauren classification, lymphovascular invasion and TNM stage were retrieved from pathology reports. Associations between tumor size and histopathological features were assessed. Tumor size cut-offs for predicting tumor stage and NM were determined by ROC analysis.
Results: Increasing tumor size was highly correlated with higher tumor stage, the presence of NM and DM, diffuse type cancers, Borrmann types 3-4 and lymphatic invasion (all p<0.001). The best cutoff of tumor size for predicting pT3-4 tumors was 6.5 cm (AUC: 0.8, OR 1.397, 95% CI 1.35-1.446) and 6 cm for NM (AUC: 0.775, OR 1.389, 95% CI 1.338-1.442). The 6 cm cutoff yielded a specificity and positive predictive value (PPV) of 0.8201 for NM and a negative predictive value of 0.880 for DM. The PPV for Borrmann 3-4 type tumors for lymph node metastases was 0.807.
Conclusions: This study highlights the significance of gross features as indicators of aggressive tumor behavior in a large collection of primary resected gastric cancers. The critical tumor size for predicting advanced tumor stage and NM appears to be around 6 cm. Tumor size, which can be assessed by endoscopy, may be a valuable indicator of tumor extent as an additional aid in pre-operative assessment.

665 Circulating Tumor Cells and Circulating Tumor DNA in Patients with PseudoMyxoma Peritonei

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Background: PseudoMyxoma Peritonei (PMP) is a rare disease in which histopathological characteristics and optimal management remain under discussion. The initial tumor is, in the vast majority of cases, a Low grade Appendiceal Mucinous Neoplasm (LAMN), which then extends to the peritoneal cavity. The imperfect understanding of PMP physiopathology, and lack of prognostic markers limit the

optimal management of these patients. Circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) have shown potential in many solid cancers, and their amounts appear to correlate with the overall tumor burden. These circulating markers have not been investigated in patients with primary PMP. The main objective of this work was to look for and isolate CTCs by filtration method, and to quantify ctDNA (*KRAS, NRAS* or *GNAS* mutations,) in patients with PMP.
Design: *KRAS, NRAS GNAS* mutations were sought on appendiceal and/or peritoneal tumor samples by SNaPshot and in free circulating DNA by digital PCR. CTCs were isolated from peripheral blood by filtration on track-etched filters. P53 immunocytochemistry was performed on appendicular and/or peritoneal tumor samples and on isolated CTCs.
Results: Fourteen patients with PMP were prospectively included: 15 somatic mutations (6 *KRAS* and 9 *GNAS* mutations) in 10 (71%) tumors were found. ctDNA search was performed in 9 patients (carrying 12 mutations, GNAS mutations are non exclusive): 5 (56%) patients had ctDNA in an amount ranging from 0.442% to 1.309% of total circulating DNA, with no statistical difference between low-grade and high-grade PMP (trend towards high grade PMP). CTCs were present in 7 (50%) patients (5 low-grade and 2 high-grade), with a median of 113 [69-496] CTC / 3mL of blood. p53 immunostaining on filter was performed in 5 patients: 4 patients (80%) had positive p53 CTCs, providing additional arguments in favor of the tumoral peritoneal origin of these CTCs.
Conclusions: This study is the first to highlight CTCs and ctDNA in patients with PMP. This discovery allows insights into the carcinogenesis of this tumor: PMP cells derived from LAMN cells are able to survive and proliferate in the peritoneal cavity, but also seem able to circulate in the blood, hence providing additional arguments for the terminology "low- or high-grade peritoneal mucinous adenocarcinoma" proposed by the latest WHO. These results should encourage further research on the characterization of CTCs and ctDNA isolated from patients with PMP for the identification of new prognostic markers.

666 Dominant Overexpression of Programmed Death-Ligand 1 in MSI-Unstable Colorectal Adenocarcinoma

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Background: Immune checkpoint inhibitors against programmed death-1 (PD-1) pathway and its ligand (PD-L1) have significant potential for tumor treatment by allowing tumor-infiltratring lymphocytes (TIL) to exert anti-tumor action against cancer cells. With immunotherapy options available, it is important to identify potential clinical responders. This study identified a framework for classification of colon cancers based on the presence or absence of TIL and PD-L1 expression and compared PD-L1 expression with key oncogenic alterations.
Design: 46 patients with a recent diagnosis of colonic adenocarcinoma were identified and further categorized based on mutational pathway present, specifically microsatellite instability (26/46) and KRAS (20/46). Immunohistochemistry (IHC) for PD-L1, p53, CD-3 was performed on randomly selected slides from each tumor with appropriate controls. Nuclear positivity of p53 in epithelial cells from 5 high power fields was calculated and >50% positivity was considered as mutant p53. PD-L1 staining was evaluated based on the intensity and proportion of membranous and cytoplasmic staining in tumor cells, and scored as 0 (negative), 1 (weak intensity, <10%), 2 (high intensity, >10%). CD-3 labeled T cells in the tumor nests or in the stroma surrounding the tumor were further semi-quantitatively analyzed as minimal, focal intense, or diffusely intense.
Results: Overall, PD-L1 expression was identified in 30/46 colorectal adenocarcinoma cases (65%). For MSI-unstable tumors, 22 of 26 cases (84%) expressed PD-L1, including 12 cases with over-expression (score 2) and 10 cases with weak expression (score 1). In carcinoma with either KRAS or p53 mutation, only 8 of 20 cases (40%) displayed weak expression of PD-L1 and no high intensity PD-L1 expression ($p<0.05$, compared to MSI-unstable tumor). All 12 PD-L1 over-expression MSI-unstable tumors showed diffuse/intense TIL. Weak PD-L1 expression with corresponding focal/intense or diffuse/intense TIL was identified in 18/46 cases (39%).
Conclusions: Our results demonstrated that PD-L1 over-expression is only dominant in MSI-unstable tumors and significantly correlates with intense TIL, indicating its potential as biomarkers to predict immunotherapy response against checkpoint molecular blockage and potential for PD-L1 expression as a surrogate IHC marker for MSI-unstable tumors. Weak PD-L1 expression with TIL can be found in multiple mutational pathways, including KRAS and p53 mutations, suggesting further immune-regulation is necessary for potentially enhancing immunotherapeutic effect.

667 LGR5 Expression in Barrett's Esophagus and Associated Neoplasia: Support for Its Role as a Cancer Stem Cell and Diagnostic Marker

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Background: Leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5) is the most reliable intestinal stem cell marker and has been implicated as a cancer stem cell marker. Maintenance of a viable intestinal stem cells is dependent on LGR5 and its ligand, R-spondin 1.We examine the expression of LGR5 and R-spondin 1 in the normal gastrointestinal tract, Barrett's esophagus (BE) and BE-related neoplasia.
Design: RNA ISH for LGR5 and R-spondin 1 was performed on paraffin embedded biopsies from 54 patients with esophageal columnar metaplasia, either with or without dysplasia (low grade dysplasia (LGD) n= 16, high grade dysplasia (HGD) n=23) and 15 invasive adenocarcinomas using a branch chain RNA ISH (ViewRNA, Affymetrix, CA). The location of transcripts (individual red dots) in the epithelium was noted i.e. basal vs. surface involvement. The percentage of crypts positive for LGR5 transcripts as well as the number of transcripts/cell was calculated. Normal small bowel and colon were used as controls.
Results: In the normal small bowel and colon LGR5-positive cells were located exclusively at the base of the crypts. Cells co-expressing LGR5 and R-spondin 1 were identified in gastroesophageal junctional (GEJ) mucosa and in the esophageal submucosal glands. In non-dysplastic BE, LGR5 transcripts were present only in the base of the crypts, whereas in dysplasia LGR5+transcripts were identified in the crypts in all cases, but also in the surface epithelium in the majority of cases (LGD:60%, HGD:83%). The LGR5 basal dominant polarity seen in normal intestinal crypts was maintained in dysplasia, but lost in invasive carcinomas. In contrast to non-dysplastic BE, dysplastic biopsies showed a significantly higher number of LGR5-positive transcripts/cell ($p=0.02$) and a larger percentage of LGR5-positive crypts ($p=0.001$). A statistically significant stepwise increase in these parameters was seen from non-dysplastic BE biopsies to LGD, HGD and invasive carcinoma ($p=0.02$). Based on the presence of LGR5-positive cells on the surface epithelium, or the presence of > 60% LGR5+crypts, the specificity of the LGR5 assay was 94% and the sensitivity was 79%; sensitivity was higher for HGD (92%) than LGD (72%).
Conclusions: Our findings support the origin of BE from proximal migration of LGR5+ cells in the GEJ and/or submucosal glands. LGR5 cells are expanded in neoplasia and this support it as a marker of cancer stem cells in BE-related neoplasia. LGR5 reactivity within surface epithelial cells constitutes a specific marker of dysplasia.

668 Brincidofovir (CMX001) Toxicity: Another Potential Mimicker of Gastrointestinal Graft Versus Host Disease

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Background: Brincidofovir (CMX001) is a new oral nucleotide analog medication with activity against DNA viruses, including cytomegalovirus (CMV), adenovirus (ADV), BK virus, and herpes simplex virus. Clinical trials are currently using this drug in immunocompromised hosts including hematopoietic stem cell transplant (HSCT) patients. It is known to cause gastrointestinal (GI) toxicity, most commonly diarrhea.
Design: We report 2 patients clinically diagnosed with GI toxicity related to brincidofovir. Review of the patients' charts and histology were performed.
Results: *Case 1:* A 2 year old male with medulloblastoma treated with chemotherapy and autologous HSCT developed bacteremia and ADV viremia. He received 2 doses of cidofovir and was then started brincidofovir. ADV became undetectable. 6 weeks later he had a second autologous HSCT. He began vomiting 5 weeks after transplant and underwent GI endoscopy which showed marked gland drop out and moderate epithelial apoptosis in the sigmoid colon. Molecular testing and/or stains for CMV, ADV, and *C. difficile* were negative. The pathologic diagnosis was drug related injury versus infection. He was not on mycophenolate mofetil (MMF). Brincidofovir toxicity was diagnosed clinically and the drug was held. His GI symptoms slowly improved. 2 weeks later, follow up GI biopsies showed reparative epithelial changes and crypt distortion in the sigmoid colon and rectum. To date, he has not had GI symptoms.
Case 2: A 59 year old female with chronic lymphocytic leukemia treated with chemotherapy and allogeneic HSCT started brincidofovir for CMV prophylaxis 3 weeks after transplant. 9 days later she presented with abdominal pain, vomiting, and diarrhea. GI biopsies 18 days into therapy showed reactive gastropathy and reactive epithelial changes, increased eosinophils, and minimal epithelial apoptosis in the duodenum and rectum. The pathologic diagnosis was possible graft versus host disease (GVHD) with concern for drug related injury. She was not on MMF. Low grade brincidofovir toxicity was diagnosed clinically; however, the drug was continued due to the mild symptoms. There were no clinical concerns for GVHD. Her symptoms improved over her remaining course of therapy.
Conclusions: Brincidofovir is a new medication that can cause GI toxicity and histologically may demonstrate epithelial apoptosis and crypt injury, similar to GVHD and MMF toxicity. The possibility of brincidofovir toxicity should be included with this histologic differential.

669 Combined GATA3 and BRST2 Immunohistochemistry Is Both Sensitive and Specific in the Identification of Metastatic Signet Ring Cell Carcinoma of Breast Origin in the Upper GI Tract

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Background: Metastatic invasive lobular carcinoma (mILC) of the breast has a tendency to involve the GI tract. Distinguishing mILC and primary diffuse gastric carcinoma (PDGC) is challenging on histology alone, as both may assume a signet ring cell morphology. Also, mILC may occasionally be negative for estrogen receptor, necessitating the use of other markers. There are few studies evaluating GATA3, a breast carcinoma marker, in PDGC. We sought to determine if GATA3 and BRST2 can be used to differentiate mILC to the stomach and PDGC.
Design: Cases with tissue available for immunohistochemistry were retrospectively identified in our pathology database from 2004-March 2015. The intensity and the proportion of signet ring cells staining with GATA3 (nuclear) and BRST2 (cytoplasmic) were evaluated by a pathologist blinded to the primary site. A modified Allred score (0-8) was generated. Two-tailed Student's t-test and Fisher's exact test were used for continuous and categorical variables respectively.
Results: 42 PDGC (sex ratio: 22 M: 20 F; average age: 67.3 years) and 10 mILC cases (all female; average age: 64.2 years) in the upper GI tract (stomach/esophagus) were identified.
GATA3:
10/10 mILC cases stained with GATA3 vs 10/42 (23.8%) PDGC cases. All mILC had Allred scores (AS) of 8, intensity scores of 3 (maximal) and staining in ≥ 80% of tumour

cells. In comparison, PDGC cases had an average AS of 0.9 (range 0-7), intensity of 0.3 and staining in average 4.2% of tumor cells (p<0.0001 for all 3 values). GATA3 was 100% sensitive and 76% specific for mILC.
BRST2:
10/10 mILC cases vs 4/42 (9.5%) PDGC cases stained with BRST2. The average AS was 6.8 (range 3-8) for mILC and 0.4 (range 0-5) for PDGC (p<0.0001). 9 mILC cases had an AS cutoff of ≥5 vs 3 PDGC cases. BRST2 sensitivity and specificity were 100% and 90% respectively.
No cases of PDGC stained for both GATA3 and BRST2.
Conclusions: All cases of mILC were correctly identified by intense GATA3 staining in the majority of tumour cells. In addition, GATA-3 in combination with BRST2 distinguished all mILC from PDGC. Our findings show that GATA3 is extremely useful as part of a panel of markers in the evaluation of signet ring cells in the upper GI tract of a female patient.

670 Low Frequency of Epithelial PD-L1 Staining in Esophageal and Other Gastrointestinal Adenocarcinomas

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Background: To investigate the expression of PD-L1 in esophageal adenocarcinomas (EAC) and other gastrointestinal adenocarcinomas.
Design: 116 primary resected EACs were included. A next generation tissue microarray (ngTMA) contained 6 tissue cores of the tumor as well as 3 tissue cores of corresponding lymph node (n=56) and distant metastases (n=18). Immunohistochemical staining for PD-L1 was established for two clones (SP142 and E1L3N). Intratumoral CD3+ and CD8+ T-lymphocytes (TIL) were determined using image analysis. A different TMA with one core/tumor comprising 125 unselected gastric and 377 colon carcinomas and 30 non-gastrointestinal tumors was used for comparison.
Results: Membranous epithelial PD-L1 expression in carcinoma tissue was detected in only 3 cases (2.6%) of primary EAC, moreover in one lymph node metastasis (1.8%) and two distant metastases (11.1%), which was unrelated to the primary tumor. PD-L1 expression in the tumor stroma and infiltrating inflammatory cells was observed in 35 cases (30.2%). Both epithelial and stromal PD-L1 positivity was predominant focal and generally very weak compared to our positive controls and tumors that have been reported to show high PD-L1 expression such as non-small cell lung cancers and seminomas. PD-L1 positive EAC (any) had higher CD3+ and CD8+ TIL counts (p<0.001 and p=0.001) but there was no association with pathological features (pTNM categories; grading) or prognosis. Similar, only 10/125 gastric carcinomas (8.0%) and 23/377 colon carcinomas (6.1%) showed membranous PD-L1 staining. Comparable to EAC, PD-L1 expression was detected to a higher degree in the accompanying inflammatory infiltrate in 138/502 cases (27.4%).
Conclusions: EAC and other gastrointestinal adenocarcinomas, such as gastric and colon carcinomas, expressed membranous PD-L1 only in a minor percentage of cases while stromal PD-L1 expression could be observed more often. However, both staining patterns were very weak, focal and with high intratumoral heterogeneity, which may influence the results of PD-L1 testing in tissue, especially in smaller biopsies. The rationale of epithelial PD-L1 expression alone for PD-L targeted therapy in gastrointestinal adenocarcinomas has to be considered questionable.

671 Concordance of HER2 Status between Local and Central Review in Gastric (GC) and gastroesophageal Junction Cancers (GEJC): A French Observational Study of 394 Specimens: HERable Study

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Background: As some GC and GEJC overexpress HER2, HER2 testing should be part of routine assessment to decide target treatment initiation. Thus, the study objective was to assess the concordance of HER2 status between local and central laboratories.
Design: From July 2012 to February 2014, this observational study tested tumor samples from patients (pts) with GC or GEJC, regardless of disease stage. HER2 positive (+) status was defined as immunohistochemistry (IHC)3+ or IHC2+/*In Situ* Hybridization (ISH)+. The concordance was analyzed between HER2 status assessed by local laboratories (any technique) and centralized laboratory (IHC 4B5 and silver (S) ISH for all specimens).
Results: 394 specimens from 367 pts were analyzed by 19 local laboratories. Pts' characteristics were: mean age 66±13 years, male 69%, GC 60%, intestinal type 55%. The specimens were surgical resections (52%), biopsies (41%), adenopathy (4%) and distant metastasis (3%). HER2+ status was found in 18% of locally tested specimens (69/394: 52 IHC3+; 17 IHC2+/ISH+) and in 19% of centralized specimens (73/394: 56 IHC3+; 17 IHC2+/SISH+). Among HER2+ specimens, 53% (39/73) were GC and 73% (53/73) were of intestinal histological type. Regarding amplification, 20% of centralized SISH tests (79/393) were amplified.
The concordance between HER2 status (n=393) assessed by local and centralized laboratories was acceptable according to Landis and Koch classification with a kappa coefficient of 0.69 (95%CI [0.60-0.78]).
The discordance between HER2 status assessed by local and centralized laboratories was 9%. The rate of false negative was 27% and false positive 5%

	Centralized laboratory		
	HER2+ N=73	HER2- N=320	All N=393
Local HER2+	53 (72.6%)	16 (5.0%)	69 (17.6%)
Local HER2-	20 (27.4%)	304 (95.0%)	324 (82.4%)

Conclusions: The HERable study is the first large French study to evaluate concordance between HER2 status assessed by local and centralized laboratories. The cumulative rate of false negative and false positive in GC and GEJC is 9% (95% CI [6%; 12%]). Quality control should be setup to improve the quality of this test.

672 Role of Mucosal Glands in the Progression of Neoplasia in Barrett's Esophagus

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Background: There is histologic heterogeneity in the type and extent of glands in the base of columnar mucosa in Barrett's esophagus (BE). We have noted, anecdotally, loss of mucosal glands in BE patients with carcinoma, and hypothesized that loss of glands may contribute to progression of neoplasia in BE. The aim of the study was to evaluate the presence, type, and extent of mucosal glands in BE, and in BE-associated neoplasia, by evaluating large endomucosal resection specimens (EMR) acquired as part of a prospective study on volumetric laser endomicroscopy.
Design: 72 esophageal EMR specimens (average size: 2.0 x 1.5 cm) of BE patients without dysplasia (NDBE) (N=22), low-grade dysplasia (LGD) (N=9), high-grade dysplasia (HGD) (N=19), or adenocarcinoma (CA) (N=22) were examined in their entirety using a digital platform. The following parameters were measured in each case: highest grade of neoplasia; type (mucous, oxyntic, mixed) and extent of mucosal glands (<25%, 25-50%, 50-75%, or >75% of horizontal mucosa length with glands); proportion of surface/crypts with goblet cells in NDBE; and the minimum and maximum thickness of the mucosal gland compartment. All histologic findings were correlated with maximum level of neoplasia.
Results: Mucosal glands were present in 73% of NDBE, but in only 40% of neoplastic (dysplasia or CA) BE (P=.020). In terms of extent, the glands were present in >25% of the mucosa in 69% of NDBE cases (with 50% of the NDBE cases having glands present in >75% of the mucosa), but only 20% of neoplastic cases (p=.006), with a mean maximum thickness of 729 pixels in NDBE and 415 pixels in neoplasia (p=.038). In NDBE, glands were mainly of the mixed type (81% of cases), whereas in dysplasia and CA they were mainly of the pure mucous type (90% and 100% of cases, respectively). No significant differences were identified in the presence, type, or extent of mucosal glands in LGD vs HGD or dysplasia vs CA. A decrease in the extent of glands was noted in NDBE with abundant goblet cells (>50% of surface/crypts) compared to NDBE with no goblet cells (p<.001).
Conclusions: There is a marked decrease in the quantity of mucosal glands, and a change in the type, with progression of BE to dysplasia and carcinoma, which suggests that mucosal glands may play a role in BE-associated neoplasia development. A decrease or change in the type of glands may lead to increased risk of exposure of epithelium to the noxious influence of luminal refluxate.

673 KRAS Mutations Are Frequently Identified in Colorectal Carcinoma with BRAF Wild-Type Sporadic MLH1 deficiency from MLH1 Promoter Hypermethylation

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Background: Between 10 to 15% of colorectal carcinomas (CRC) can demonstrate sporadic microsatellite instability (MSI-H) as a result of *MLH1* promoter hypermethylation. While the presence of the *BRAF* V600E mutation is indicative of a sporadic cancer, up to 30-40% of CRC with *MLH1* promoter hypermethylation will lack a *BRAF* mutation. We report the clinicopathologic and molecular features of sporadic MSI-H CRC with wild-type *BRAF* and *MLH1* promoter hypermethylation.
Design: A consecutive series of MSI-H CRC with sporadic MLH1 deficiency, wild-type *BRAF*, and positive *MLH1* promoter hypermethylation (MSI-H wt-*BRAF*, n=36) was compared with MSI-H CRC with *BRAF* mutation (MSI-H mut-*BRAF*, n=113) and CRC in patients with confirmed LS (LS, n=31) for patient demographics, tumor stage, histological features, precursor polyps, and *KRAS* exons 2 and 3 mutations.
Results: *KRAS* mutations were identified in 31% of MSI-H wt-*BRAF* CRC compared to 0% of MSI-H mut-*BRAF* CRC and 37% of LS CRC (p<0.0001). MSI-H wt-*BRAF* CRC frequently arose from precursor polyps resembling conventional tubular/tubulovillous adenomas (TA/TVA) (25% of cases) compared with MSI-H mut-*BRAF* CRC which arose from precursor sessile serrated adenomas (SSA) (p<0.001). Signet ring cell differentiation was more frequently identified in MSI-H wt-*BRAF* CRC compared with MSI-H mut-*BRAF* CRC (28% vs. 8%, p=0.004). MSI-H mut-*BRAF* CRC more frequently occurred in women (82%) compared with MSI-H wt-*BRAF* CRC (61%) and LS CRC (35%) (p<0.001). There was no significant difference in patient age, tumor location, tumor stage, mucinous differentiation, tumor infiltrating lymphocytes, Crohn's-like reaction, or medullary differentiation between MSI-H wt-*BRAF* CRC and MSI-H mut-*BRAF* CRC (p>0.05). In contrast, LS CRC more frequently involved the left colon/rectum (58%) compared with MSI-H wt-*BRAF* CRC (14%) and MSI-H mut-*BRAF* CRC (8%) (p<0.001).
Conclusions: Our results indicate that MSI-H wt-*BRAF* CRC with *MLH1* promoter hypermethylation and MSI-H mut-*BRAF* CRC have different pathways of tumorigenesis. In contrast to MSI-H mut-*BRAF* CRC which arises from the serrated pathway, MSI-H

wt-*BRAF* with *MLH1* promoter hypermethylation frequently harbors *KRAS* mutations, arises from precursor polyps resembling conventional TA/TVA, and frequently demonstrates signet ring cell differentiation.

674 Are Mesenteric Tumor Deposits in Small Intestinal Neuroendocrine Tumors a Stronger Indicator for Liver Metastasis and Poor Prognosis Than Nodal Metastasis?

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Background: Mesenteric tumor deposits (MTDs) are frequently seen adjacent to small intestinal neuroendocrine tumors (SI-NETs) but are not included in the current AJCC staging system for SI-NETs. We have demonstrated that MTDs may indicate poor prognosis for patients with SI-NETs. In this study, we further explored whether MTDs are associated with liver metastasis and consequent poor prognosis.

Design: We searched our pathology database for SI-NETs resected between 1997 and 2015 and identified 121 cases with/without liver resection. We reviewed slides to record primary tumor stage, lymph node (LN) metastasis, MTD, liver tumorlets (defined as tumor cell nodules ≤1 mm within portal tracts), and liver metastasis. Electronic medical records were reviewed for follow-up data (clinical or pathological evidence of metastases and overall survival).

Results: The 121 cases included 66 with both MTD and LN metastasis, 27 with LN metastasis only, 10 with MTD only, and 12 without MTD or LN metastasis; MTD status could not be determined in 6 other cases. The odds ratio for liver metastasis in the presence of MTD was 5.60 (95% CI 2.17-15.22; P=0.0001) compared to 2.17 (95% CI 0.77-6.47; P=0.10) for LN metastasis. Similarly, the odds ratio for tumorlets with MTD was 18.5 (95% CI 2.63 to >100; P=0.0003) and 2.23 (95% CI 0.55 to 13.0; P=0.23) for LN metastases. The hazard ratio for overall survival in cases with MTD was 6.27 (95% CI, 0.75 to 52.42) and for cases with LN metastases was 1.22 (95% CI, 0.27 to 5.49). Among the 10 cases with MTD only, 7 (70%) had multiple liver lesions (6 with numerous lesions) in follow-up imaging studies, 2 died of disease, and 6 were alive with disease. Among the 27 subjects with positive LN only, 9 (33%) had multiple liver lesions (2 with numerous lesions) in follow-up imaging studies, 1 died of disease, and 8 were alive with disease.

Conclusions: MTDs are associated with liver tumorlets and liver metastasis, which may be a source of clinically detectable liver metastasis. Moreover, the presence of MTD appears to be a stronger predictor of hepatic tumorlets and metastases than LN metastasis. MTD therefore should be considered as a staging criterion for SI-NETs.

675 HTRA3 Stromal Expression Is Correlated with Tumor Budding in Stage II Colorectal Cancer

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Background: Tumor budding is a well-established adverse prognostic factor in colorectal carcinoma (CRC); however, very little is known about its pathogenesis. High-temperature requirement A3 (HTRA3) is a bone morphogenic protein inhibitor which we previously demonstrated to be highly expressed in the desmoplastic stroma at the invasive front of CRCs. The purpose of this study was to determine if HTRA3 expression in the tumor body and/or invasive front of CRCs correlated with high-grade tumor budding and other clinicopathologic characteristics including outcome.

Design: Immunohistochemistry for cytokeratin and HTRA3 was performed on 172 stage II CRCs. Representative areas of the tumor body and invasive front, including budding and non-budding areas, were selected on cytokeratin (CK) stained slides, and then assessed on corresponding HTRA3 stained slides. HTRA3 expression in tumor (tHTRA3) and stroma (sHTRA3) was assessed for staining percentage and intensity (the product yielding a final score). High grade tumor budding (HGTB) was defined as ≥100 CK+ buds/10 HPF using the 10 HPF method. Tumors with ≤25 CK+ cells/10 HPF were considered to have minimal budding. The Student's t-test was used for group comparisons and to assess correlation between HTRA3 expression and clinicopathologic parameters; the log-rank test was used for survival analyses.

Results: Tumors with HGTB showed significantly higher expression of sHTRA3 at the invasive front compared to tumors with minimal budding (P=0.028). In addition, tumors with HGTB showed increased expression of sHTRA3 in budding areas compared to non-budding areas (P<0.001). sHTRA3 expression in the tumor core (but not at the invasive front) was significantly associated with decreased 5-year overall survival (P=0.031). No significant associations were found between HTRA3 expression and gender, tumor location, tumor grade, lymphovascular invasion or lymph node yield.

Conclusions: HTRA3 stromal expression is significantly associated with high-grade tumor budding in stage II CRC and may be a marker of poor outcome.

676 MCM2 and Chromogranin Are Markers of Serrated Polyp Progression

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Background: Serrated polyp (SP) pathophysiology is of great interest, and examination of colonic stem cell (SC) differentiation and crypt cell proliferation can provide insight into the natural progression of SPs. In normal colonic mucosa, SCs can differentiate into neuroendocrine cells (NEC) that reside in the crypt-base stem cell niche. SCs also produce rapidly proliferating cells located in the proliferative compartment in the lower half of the crypt. Staining for minichromosome maintenance 2 (MCM2) protein that marks proliferation in all cell cycle phases and chromogranin (CGA) that marks differentiated NECs, we examined these cell types in SPs (hyperplastic polyps (HP) and sessile serrated adenomas (SSA) ± dysplasia).

Design: Immunohistochemistry was performed on 46 paraffin-embedded SPs (11 HPs, 31 SSAs, 4 SSAs with dysplasia) and sections of normal colon using anti-MCM2 antibody (Santa-Cruz, Dallas, TX) and CGA (Dako, Carpinteria, CA). Staining positivity and distribution in both SP and adjacent normal colonic mucosa was assessed.

Results: Sections of normal colonic mucosa showed the expected proliferation/differentiation patterns: positive nuclear MCM2 staining of the lower crypt and cytoplasmic CGA staining mainly in the crypt bottom. 100% of HPs, SSAs, and SSAs with dysplasia showed areas of positive MCM2 staining throughout the entire crypt (full crypt staining). Diffuse polyp involvement was seen in 100% of SSAs with dysplasia, 81% of SSAs and 64% of HPs. Surface staining was focal/absent in all polyps. When present, areas of histologically normal mucosa adjacent to SPs showed aberrant, full crypt staining of MCM2 in 100% of SSAs with dysplasia and 58% of SSAs. Adjacent normal mucosa of HPs showed the expected to slightly expanded pattern of MCM2 staining. CGA staining in HPs and SSAs showed strong positivity of individual cells in the crypt bases (>5 cells per crypt base). 100% of SSAs with dysplasia, along with a subset of SSAs (30%), showed rare to absent CGA positive cells.

Conclusions: SPs are highly proliferative lesions which lose NEC differentiation along the serrated pathway. HPs and SSAs show a similar proliferative profile. Aberrant proliferative cell staining patterns in adjacent normal colonic mucosa seen in SSAs with dysplasia and a subset of SSAs suggest a field effect of microenvironment changes which may promote SP formation and predisposition to malignancy. Increased proliferation in background normal mucosa and NEC loss in SSAs are changes suggestive of progression.

677 Tumor Expression of C-Met and Plexin B1 May Predict Peritoneal Carcinomatosis Recurrence in Colorectal Cancer

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Background: Colon cancer is a major health problem due to its high incidence in western countries. It is estimated that 10-20% of patients treated with curative handling can develop peritoneal recurrence, considered incurable in a high percentage of cases. This data suggests the need of investigation around risk factors which predict recurrence, in order to detect the optimal candidates for subsidiary carcinoprofilaxis.

Different biomarkers have been described as useful in peritoneal carcinomatosis. The establishment of carcinomatosis include (in this order) exposure of tumor cells to peritoneum, cell viability in this new environment and the mesothelial colonization. In this context C-MET, Plexin B1, COL11a1 and IGF1 have been involved in various of this carcinomatosis-development stages.

Our hypothesis is that the presence of any of these biomarkers in the primary tumor can predict the recurrence of colon cancer as peritoneal carcinomatosis.

Design: Eighty-seven colorectal adenocarcinomas samples have been selected with a minimum follow-up of the patients of 5 years. Forty-eight out of 87 presented subsequent peritoneal carcinomatosis.

Immunohistochemistry for anti C-MET, IGF-1, Plexin B1 and COL11A1 antibodies was performed in all samples according to previously published criteria. Cell positive staining percentage was separately evaluated by two independent pathologists

Results: Association (logistic regression) between the percentage of cell with positive expression (in percentage of labeled cells) and the presence or absence of subsequent carcinomatosis was performed. Both Plexin B1 and IGF-1 showed a significant association (p = 0.021 and 0.022 respectively), C-MET presented statistical trend (p = 0.074), while COL11A1 presents no association. Multivariate analysis of Plexin B1, IGF-1 and C-MET showed a model of two factors (CMET and plexin) with sensitivity above 70% when cutoff is set at 50% of stained cells.

Conclusions: Our data suggest that the expression of both C-Met and Plexin B1 could predict the risk of recurrence of colon cancer as peritoneal carcinomatosis with a sensitivity level above 70%. We propose, therefore, its use as a peritoneal carcinomatosis risk prognostic tool for clinical routine.

678 The Immune Microenvironment of Medullary Colon Cancer Differs from Other Microsatellite Unstable (MSI) Tumors: A Gene Expression and Immunohistochemical Analysis of 105 Cases

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Background: Medullary carcinoma (MC) is a unique subtype of MSI colorectal carcinoma (CRC) due to its poor differentiation and frequent tumor infiltrating lymphocytes (TILs). Despite its association with improved survival and a robust immune response, many of these tumors progress. Using gene expression analysis and immunohistochemistry (IHC) we identified key immune modulating proteins differentially expressed in MC.

Design: Six cases of MC were laser capture microdissected and analyzed by gene expression microarray. In addition, we performed a bioinformatics analysis of differentially expressed genes in MC compared to other CRCs using The Cancer Genome Atlas (TCGA) data. Immune regulation genes commonly expressed in both analyses were chosen for IHC. In addition, PD-1, PDL-1 expression and T lymphocyte subsets were evaluated. Tissue microarrays were constructed from MC (n=105), MSI well differentiated (MSI WD) CRC (n=22), microsatellite stable well differentiated

(MSS WD) CRC (n=30), and poorly differentiated (PD) CRC (n=47). IHC was scored in epithelium and stroma based on percentage of cells with moderate-to-strong staining (0%,1-25%,26-50%,and 51-100%). TILs were scored as average number of positive lymphocytes in 2 cores. Statistical analysis was evaluated using the Chi-square test as appropriate.

Results: The interferon gamma pathway related genes *IDO1*, *WARS* (TRP), and *GBP5* were significantly overexpressed in MC in both our expression array and TCGA datasets. IHC confirmed strong epithelial expression of the corresponding proteins in MC compared to other MSI, MSS and PD CRCs.

Protein	MC (%)	MSI WD CRC (%)	MSS WD CRC (%)	PD CRC (%)	p
IDO1	63	13.6	6.7	19.2	<.0001
TRP	95.2	81.8	43.3	61.7	<.0001
GBP5	33	0	0	6	<.0001

TILs expressing PDL-1 were also higher in MC (p=.003) but there was no statistically significant difference in epithelial expression of PDL-1. Finally, intraepithelial CD8 and FOXP3 positive TILs were more prevalent in MC versus other tumor types.

Conclusions: We identified several key immune regulatory proteins such as IDO1, TRP and GBP5 that are significantly upregulated in MC compared to other MSI and MSS CRCs. IDO1 activation in particular has been shown to alter inflammation and favor T-cell tolerance in cancer. MC may show a particular sensitivity to immune checkpoint inhibitors currently being investigated in clinical trials.

679 Appendiceal Goblet Cell Carcinoid Tumors – Findings after Definitive Resection

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Background: Goblet cell carcinoid tumors (GCC) of the appendix are rare tumors and as such are the cause of much confusion amongst pathologists and surgeons alike as to their nomenclature and management. In particular, there is debate within the surgical and oncologic fields regarding the appropriate management of these tumors, which are often incidental findings on appendectomies that have been performed for other reasons, usually acute appendicitis. Therefore, we decided to investigate the outcomes of goblet cell carcinoids to better guide management.

Design: The pathology database from 1999 to 2015 was searched for all cases of GCC. Cases with admixed adenocarcinoma (adenocarcinoma ex GCC) were excluded. Clinicopathologic features were assessed.

Results: Thirty-six patients met criteria for inclusion. Three (8%) patients underwent a right hemicolectomy, 5 (14%) underwent an appendectomy only, and 28 (78%) patients initially underwent an appendectomy followed by completion right hemicolectomy. In the patients who underwent a completion hemicolectomy following appendectomy, all 12 (43%) patients with negative surgical margins on the appendectomy specimen had no residual disease in the cecum/pericecal adipose tissue. Of the 11 (39%) patients with positive surgical margins on the appendectomy specimen, there was residual disease in the cecum/pericecal adipose tissue in 8 (73%) patients. In the remaining 5 (18%) patients, the margin status on the initial specimen could not be assessed. Three (11%) patients had nodal disease in the subsequent colectomy specimen; however, only 2 (7%) patients were upstaged from stage II to stage III as one of the appendectomy specimens contained a positive lymph node. Overall, 18 (of 31 cases assessed, 58%) had lymphovascular invasion and 29 (of 30 cases assessed, 97%) had perineural invasion. Thirty-one patients could be staged and stratified into stage II (27 [87%]) and stage III (4 [13%]). Follow up data (range, 2 mos. - 8 yrs.) was available in 27 patients. Recurrence was identified in 2 (7%) patients (4.3 and 5 years) whom were stage III with lymph node involvement; stage III was significantly associated with recurrence (two-tailed Fisher's exact, p = 0.017)

Conclusions: In spite of poor prognostic features such as lymphovascular and perineural invasion, appendiceal GCCs without a frankly carcinomatous component, i.e. adenocarcinoma ex GCC, behave in a relatively indolent fashion with lymph node status/stage being an important feature for recurrence. Hemicolectomy is curative in most cases.

680 Microsatellite Instability Is Associated with Reduced Disease Specific Survival in Stage III Colon Cancer in a Series of 1250 Colorectal Cancers Prospectively Tested for MSI

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Background: Up to 15% of colorectal cancers exhibit microsatellite instability (MSI), where errors in replication go unchecked due to defects in the mismatch repair system. This study aimed to investigate the association of MSI with lymph node counts, pathological features and survival in a series of 1250 consecutive colorectal cancers subjected to universal MSI testing.

Design: Retrospective analysis of clinical and pathological features of patients with colorectal cancer identified on prospectively maintained colorectal and pathology databases at St. Vincent's University Hospital from 2004 to May 2012. Immunohistochemistry was used to identify mismatch repair (MMR) status. Survival analysis was performed using Kaplan-Meier curves, the log-rank test and Cox regression.

Results: Of the 1250 colorectal cancers in the study period, 11% exhibited MSI (n=138). MSI tumours occurred predominantly in female patients, and in the right colon. (MSI: 70% (n=97) female, 80% (n=111) right colon). MSI tumours had significantly lower rates of lymph node and distant metastases (MSI N+ rate: 24.8% compared with MSS N+ rate: 46.2%, p<0.001); The MSI M1 rate was 1%(n=2), compared with an MSS M1 rate of 9%(n=104), p<0.001) MSI tumours had a higher number of negative and total node counts. MSI was associated with improved disease free survival (DSS) compared with MSS Stage I and II colon cancer, but Stage III MSI colon cancers had a worse DSS than MSS tumours. Stage III MSI tumours exhibited higher rates of lymphovascular invasion and perineural invasion than Stage I/II MSI tumours.

Conclusions: MSI is associated with a reduced risk of nodal and distant metastases, with an improved DSS in Stage I/II colon cancer. However, MSI tumours who progress to nodal disease may be biologically distinct from early MSI tumours, as demonstrated in this study where Stage III colon cancer had worse outcomes and pathological features. Targeted therapy for this cohort may be needed to improve outcomes.

681 Gastrointestinal Tract Histologic Findings in Patients with Systemic Lupus Erythematosus

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Background: Systemic lupus erythematosus (SLE) is an autoimmune disorder that can affect multiple organs, mainly in the form of vasculitis and immune deposits. Gastrointestinal (GI) manifestations in SLE patients are mostly due to vasculitis and immune-complex mediated changes in the superior mesenteric arteries leading to ischemia, infarction or bowel perforation. There are limited data on other GI manifestations in SLE patients.

Design: We searched our laboratory information system for patients with SLE who had upper and/or lower GI biopsies and identified twenty-nine samples from six individual patients. We analyzed these biopsies for the presence/absence of active colitis, increase in eosinophils, increased apoptosis, and increased mitotic activity. We excluded patients with a history of renal transplant.

Results: Reasons for endoscopy included diarrhea (3), abdominal pain (3), nausea/vomiting (1), reflux (1), rectal bleeding (1), and unknown (1). Endoscopic findings were reported as normal (5), "colitis" in the setting of a Hartman pouch (1) esophageal white plaques (1), "peptic" changes (1), and gastritis (2). The most common histologic findings were prominent and increased mitotic activity (9, 56% in colonic crypts; 3, 50% in gastric pits) and apoptosis (5, 31% in deep colonic crypts; 2, 33% in gastric pits). Additional findings included acute colitis (5), loss of colonic surface epithelial mucin (2), mild lymphoplasmacytic expansion of the colonic lamina propria (2), gastric *Sarcina ventriculi* organisms (1), and *Helicobacter pylori* gastritis (1). No significant architectural distortion or ischemic changes were observed in any of the samples. Common drugs in this cohort included hydroxychloroquine (n=4), predisone (n=5) and mycophenolate (n=2).

Conclusions: Histologic findings in this small group of patients with SLE were varied and non-specific. Prominent, increased mitotic activity/apoptosis in the proliferative compartment is a common finding and may be related to medication use or to ongoing injury and regeneration as a result of the systemic autoimmune process.

682 Solid-Type Gastric Carcinomas: Categorization and Comparison with Solid-Type Colonic Carcinomas

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Background: Carcinomas with a solid growth pattern present diagnostic challenges in the gastrointestinal tract. Our goal was to examine features in solid-type gastric cancers that allowed distinction between medullary carcinoma (MC) [linked to microsatellite instability (MSI)], lymphoepithelial carcinoma (LC) [Epstein-Barr virus-positive], and conventional poorly differentiated carcinoma (PDC). A secondary goal was to compare clinicopathologic features of gastric and colonic MC.

Design: We identified 15 stomach carcinomas with solid growth pattern and 25 colon carcinomas originally diagnosed as MC and gathered clinicopathologic data. All cases were tested for MSI, and all stomach and 16/25 colon cases were stained for EBER. Sixteen colon cases and all stomach cases were stained for CDH17 and CDX2.

Results: MSI and EBER results categorized cases as stomach MC (5), stomach LC (7), stomach PDC (3), colon MC (22), and colon PDC (3). No case was both MSI-high and EBER-positive. All categories showed predominantly nested growth pattern, pushing borders, ulceration, vesicular nuclei, nucleoli, and tumor-infiltrating lymphocytes. Other inflammatory cells were often present, including neutrophils (sometimes forming microabscesses). Table 1 shows clinicopathologic findings. Tumor budding was common in colon (14/25, 56%) but not stomach (4/15, 27%) cases. Most tumors were CDH17-positive, and about half in each organ were CDX2-positive. Median overall follow-up was 1063 days. Combined, 2 of 34 MC or LC patients died of disease (6%). In contrast, 2 of 6 PDC patients (33%), all of whom were originally diagnosed with MC or LC, died of disease.

	Caucasian	Male	Average size (cm)	Necrosis	Germinal centers	Neutrophils	Eosinophils
Stomach MC	4/5	2/5	**9.2**	4/5	0/5	1/5	**0/5**
Stomach LC	**3/7**	**6/7**	5.0	**1/7**	**4/7**	4/7	5/7
Stomach PDC	3/3	2/3	5.2	2/3	0/3	1/3	1/3
Colon MC	22/22	**5/23**	7.2	15/23	1/23	10/22	6/22
Colon PDC	3/3	1/3	5.6	3/3	0/3	**0/3**	2/23

Conclusions: Gastric cancers with morphology suggestive of MC or LC should be investigated with EBER, and such gastric or colon cancers with medullary appearance should undergo MSI testing. EBER-positive gastric tumors should be called LC, not MC (colonic EBER-positive LCs have very rarely been reported). In the stomach, large

tumor size and lack of eosinophils may favor MC, and non-Caucasian race, male sex, intratumoral germinal centers, and lack of necrosis may favor LC. PDC mimicking MC or LC can arise in the stomach and colon but has a worse prognosis.

683 Lanthanum Deposition Is Frequently Observed in the Gastric Mucosa of Dialysis Patients with Lanthanum Carbonate Therapy: A Clinicopathologic Study of 13 Cases Including One Colon Case and Two Non-Granulomatous Gastric Cases

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Background: Lanthanum carbonate (LC) is available as a phosphate binder agent for hyperphosphatemia of dialysis patients. Recently, four papers regarding lanthanum deposition in the gastroduodenal mucosa have been published. The aim of this study is to evaluate the precise incidence rate based on pathologic investigation.

Design: We surveyed 153 pathological specimens of the digestive tract, which were biopsied or resected from 103 dialysis patients, including 19 patients treated with LC, from May of 2009 to May of 2015 in a single institute, Kainan Hospital, Japan.

Results: Out of 153 total endoscopic examinations from 103 dialysis patients, our microscopic survey revealed lanthanum depositions in the gastrointestinal mucosa of 18 lesions among 13 patients; although the extent of deposition varied, all 13 patients were treated with LC. With regard to the involved organ sites, the stomach was involved in 12 cases, including one case wherein the duodenum was also involved; in the remaining case, the transverse colon was involved. We were unable to find any similar histologic findings in the other 84 dialysis patients who did not receive LC therapy. Histological examination revealed that gastric deposition was observed in 12 (85.7%) of the 14 patients who received LC therapy. The sampled lesions included benign polyps and erosion. The deposition material was often observed in the cytoplasm of multinucleated giant cells, however, only two gastric cases showed the deposition on the surface of the mucosal erosion with no granulomatous reaction. The total LC dosage of gastric cases with and without deposition prior to the biopsy was 1,448,375 mg and 579,000 mg, respectively. In contrast, only one (11.1%) of nine colon cases showed the same findings, which sampled lesion was a tubular adenoma. The total LC dosage of the colon case with deposition was 1,050,000 mg, and that in the other colon cases without deposition was 1,087,500 mg. The presence of lanthanum and phosphorus in the lesion was confirmed using a field emission-scanning electron microscope with an energy dispersive x-ray microanalyzer in one gastritis sample.

Conclusions: This study demonstrated that lanthanum deposition was histologically quite frequently (85.7%) in the gastric mucosa and infrequently (11.1%) in the colonic mucosa of patients who received LC therapy (12/14 vs. 1/9, respectively, $p < 0.001$).

684 Spectrum of Non-Synonymous Variants in Appendiceal Epithelial Neoplasms Identified by Targeted Clinical Next-Generation Sequencing

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Background: Appendiceal epithelial neoplasms are a rare and heterogeneous group of tumors with a variable clinical course. There are a limited number of studies investigating these tumors at the genomic level. Using a next generation sequencing (NGS) based panel of common cancer-associated genes, we explored the genetic landscape of 13 appendiceal epithelial neoplasms.

Design: 13 appendiceal epithelial neoplasms (5 well-differentiated mucinous appendiceal adenocarcinomas (MAC), 2 moderate- to poorly-differentiated appendiceal adenocarcinomas (AAC) with mucinous features, 2 signet ring cell adenocarcinomas, 2 goblet cell carcinoids (GCC), 1 low-grade appendiceal mucinous neoplasm (LAMN), and 1 mixed adenoneuroendocrine carcinoma (MANEC)) were retrospectively identified from our archives. DNA, extracted from formalin-fixed, paraffin-embedded tissue blocks using standard methods, was used to construct Illumina paired-end libraries. These libraries were then captured using a panel of 25 genes (3 cases), 40 genes (5 cases), 42 genes (3 cases), and 49 genes (2 cases) that have been implicated in cancer and sequenced using 101bp paired-end reads. In-house sequencing analysis pipelines were used to identify and characterize single nucleotide variants (SNV) and insertions/deletions (indels). Variants were filtered to restrict to non-synonymous variants (NSV) with a population minor allele frequency of <1% as reported in the NHLBI Exome Variant Server or 1000 Genomes databases.

Results: After filtering, analysis revealed 5 non-SNP NSV in 25 gene targets (3 cases), 23 non-SNP NSV in 40 gene targets (5 cases), 5 non-SNP NSV in 42 gene targets (3 cases), and 5 non-SNP NSV in 49 gene targets (2 cases). 13 of the 38 variants had been previously identified as predictive or prognostic in other cancers. Of those, the most frequently altered genes were *KRAS* (7/13), *EGFR* and *P13KCA* (2/13), and *KIT* and *NRAS* (1/13). *KRAS* mutations were found in 5 MACs and 2 AACs with mucinous features. 6/7 *KRAS* variants were in codon 12. *EGFR* mutations were found in 2/5 MACs. *P13KCA* mutations were found in 2/2 AAC with mucinous features. *NRAS* and *KIT* mutations were identified in 1 GCC. No such variants were identified in the other case of GCC, 2 signet ring cell adenocarcinomas, 1 LAMN, and 1 MANEC.

Conclusions: Our data demonstrates the heterogeneous mutation spectrum associated with appendiceal epithelial neoplasms and corroborates previous studies wherein *KRAS* mutations have been reported in mucinous appendiceal adenocarcinomas.

685 CK17 Is a Marker of Anal Transition Zone and May Aid in Distinguishing Reactive Anal Transition Zone from High-Grade Squamous Intraepithelial Lesions (AIN II/III)

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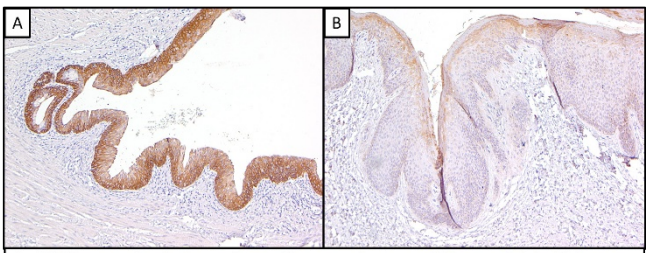
Background: The anal transitional zone (ATZ) is composed of a mixture of glandular, transitional and squamous mucosa that may appear complex histologically. When inflamed, reactive changes may mimic high-grade squamous intraepithelial lesions (AIN II/III); immunohistochemical aids that could differentiate reactive ATZ from HSIL would be extremely helpful in these cases. P16, a surrogate marker for high-risk HPV infection, is expressed in the vast majority of anal HSIL cases. CK17 has been implicated as a marker of immature squamous metaplasia in the female genital tract. Novel features of this study include the description of the CK17 staining pattern in the anal canal and its putative role in distinguishing reactive ATZ from anal HSIL.

Design: 8 cases of anal HSIL were re-reviewed by 3 gastrointestinal pathologists and compared with 10 cases with reactive ATZ obtained from hemorrhoidectomies. P16 and CK17 immunostains were performed. P16 positivity was defined as diffuse, "block" staining. CK17 positivity was defined as diffuse, full-thickness staining.

Results: P16 was positive in 100% of HSIL cases and 0% of reactive ATZ. Diffuse, full-thickness CK17 expression was seen in 12.5% of HSIL and 100% of reactive ATZ. Interestingly, patchy, superficial staining with CK17 was seen in 62.5% of HSIL cases. This pattern of CK17 staining has been described in vulvar HSIL, in which CK17 expression is thought to be a marker of immature squamous metaplasia. This staining pattern also supports a recently proposed model of HPV-related anal carcinogenesis (Yang EJ, et al.) where predominantly superficial metaplastic cells initiate malignant transformation in a top-down manner.

	P16	CK17
Anal HSIL (n=8)	100%	12.5%
Reactive ATZ (n=10)	0%	100%
P value	<0.0001	0.0003



A. CK17 expression in the anal transition zone is strong, diffuse, and full-thickness.
B. In anal HSIL, CK17 expression is patchy, weak and superficial.

Conclusions: Diffuse CK17 expression is a reliable marker of ATZ. Supplementing p16 with CK17 staining may be useful in cases where anal HSIL and reactive anal transition zone are difficult to distinguish histologically. Lastly, the patchy, superficial staining pattern of CK17 in anal HSIL mirrors that seen in vulvar HSIL, and may lend further insight into anal HPV-related carcinogeneisis.

686 Treponema pallidum Immunohistochemistry Is a Sensitive Diagnostic Adjunct for Intestinal Spirochetosis

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Background: Intestinal spirochetosis (IS) is caused by *Brachyspira* species and has been recognized for decades, but whether it represents commensalism or a pathogenic process is still debated. It has been suggested that the presence of invasion indicates symptomatic infection, but this has not been examined in a large series. The diagnosis of IS is made on routine sections with confirmation typically by silver stain. The presence of tissue invasion has only been examined by ultrastructural study. We evaluated the *Treponema pallidum* immunostain, which cross-reacts with *Brachyspira,* as a diagnostic tool for IS.

Design: We reviewed biopsies diagnosed as IS to confirm the diagnosis, assess for tissue invasion and concomitant pathology. Each case was tested by Warthin-Starry (WS) and *T. pallidum* immunohistochemistry (IHC). Species specific genotyping was performed in 3 cases. Colonoscopy reports, clinical follow up and follow up biopsies were examined blinded to the results of ancillary tests.

Results: Patients (n=33) with IS ranged from 22-82 years with no sex predilection. IS involved normal (n=15), and inflamed (n=5) mucosa and the non-neoplastic mucosa next to adenomatous (n=8), serrated (n=3) and hyperplastic polyps (n= 2). WS (n=33 of 33) and *T. pallidum* IHC (n=31 of 31) were positive in all cases including both species of *Brachyspira*. *T. pallidum* IHC confirmed the presence of tissue invasion into epithelium and lamina propria in 97% cases. Six (18%) patients were treated for IS, and all had symptom resolution. Follow up biopsies in treated (n=2) IS patients did not show residual IS. In those with untreated incidental IS on cancer screening (n=5), follow up biopsies were negative for IS. *T. pallidum* IHC required 75 minutes less hands-on technical time than WS for performance and was also faster to interpret (average 46% less time) by 2 pathologists.

Conclusions: *T. pallidum* IHC is 100% sensitive for diagnosis of IS and is easier to perform and interpret than WS. It highlights invasive organisms in almost all IS cases suggesting that invasion does not indicate symptomatic infection. Treatment of patients with IS and otherwise unexplained diarrhea led to resolution.

687 Osmoprep-Associated Gastritis: A Histopathologic Mimic of Iron Pill Gastritis and Mucosal Calcinosis

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Background: The gastrointestinal tract is subject to unique injuries as one of the primary routes of pharmacologic therapy. As the number of medications approved for use increases, iatrogenic drug injury will likely increase as well, requiring pathologists to be on alert for foreign materials as a source of tissue reaction. In such cases, correct identification of the inciting agent can prevent clinical confusion and unnecessary additional patient work-up.

Design: We have identified seven cases of gastritis characterized by the presence of dark purple-black granular deposits in the superficial mucosa associated with marked reactive epithelial changes. In each case, the patient had taken Osmoprep, a tablet form of sodium phosphate used for bowel preparation just prior to upper endoscopy and had undergone concurrent colonoscopy.

Results: Endoscopic findings ranged from normal gastric mucosa to severe inflammation, congestion, and friability. No other gastrointestinal sites were noted to contain the deposits or show similar mucosal injury. On initial histologic review, the deposits raised the differential diagnosis of elemental iron and mucosal calcinosis. However, none of the patients was noted to be taking iron supplements, and none had a history of renal disease or other cause of calcium dysmetabolism. Histochemical stains revealed the deposits were negative on Perls' iron stain (7 of 7 cases), positive on von Kossa stain (6 of 7 cases), and negative on Alizarin red stain (7 of 7 cases) – a histochemical profile compatible with sodium phosphate but inconsistent with mucosal calcium. A crushed Osmoprep tablet was subjected to routine and special stains and demonstrated similar histologic features and histochemical profile. Additionally, biopsies of 20 consecutive patients who did not take Osmoprep and who underwent concurrent endoscopy and colonoscopy with biopsy were reviewed, and no deposits with similar histochemical profile were identified.

Conclusions: In summary, we have characterized a unique form of gastritis associated with Osmoprep use. Attention to clinical history and use of a select panel of histochemical stains allow for accurate diagnosis.

688 Transcription Factor E3 as a Novel Diagnostic Marker for Solid Pseudopapillary Neoplasm

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Background: Solid pseudopapillary neoplasms (SPNs) are rare malignant neoplasms with an indolent disease course, accounting for 1-2% of all exocrine pancreatic tumors. The diagnosis of SPN can be straightforward in cases with typical morphology and immunohistochemical profile, however, morphological variants, such as solid variant and clear cell variant, have been reported, which make the histologic diagnosis more challenging. Other pancreatic tumors, including acinar cell carcinoma, neuroendocrine tumor, and pancreatoblastoma, may share similar morphological and immunohistochemical features as SPNs. Therefore, a definitive diagnosis can be difficult to make in selected cases. In this study, we propose transcription factor E3 (TFE3), a member of the Microphthalmia family of transcription factors (MiT), as a novel diagnostic marker for SPNs. Overexpression of MiT proteins have been shown to alter the Wnt/ β-catenin signaling pathway, the latter of which is vital in the pathogenesis of SPN.

Design: Pathology databases were searched from 2000 to 2015 and 16 cases of resected SPNs were retrieved. Additionally, 21 cases of other pancreatic tumor types (17 neuroendocrine tumors, 2 acinar cell carcinomas, and 2 pancreatoblastomas) were used as controls. Immunohistochemistry for TFE3 was performed on selected tumor blocks and scored independently by at least 2 pathologists in a masked manner. Tumors with positive nuclear immunoreactivity for TFE3 were scored as weak (1+), moderate (2+), or strong (3+) based on the intensity of labeling, and absence of nuclear immunoreactivity was scored as negative (0).

Results: Nuclear immunoreactivity for TFE3 was detected in 15/16 of the SPNs and none of the control tumors, resulting in a sensitivity of 94% and specificity of 100% (positive predictive value 100%, negative predictive value 95%). Weak (1+) and moderate (2+) nuclear immunoreactivity for TFE3 was seen in 7/16 and 8/16 SPN cases, respectively.

Conclusions: TFE3 is a highly sensitive and specific marker for SPN, and might be of value in distinguishing between SPN and other pancreatic tumors. Our results also indicate that TFE3 may be involved in the pathogenesis of SPN, and further studies of the mechanisms of TFE3 nuclear overexpression in SPN are warranted.

689 Is Classification of T cell Subsets Useful in the Evaluation of Gastrointestinal Graft Versus Host Disease?

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Background: Gastrointestinal graft versus host disease (GIGVHD) is a complication of stem cell transplantation (SCT). Biopsy findings at the lower diagnostic threshold are nonspecific, consisting solely of crypt apoptosis. Some grading systems consider a single apoptotic body sufficient for diagnosis, but low specificity may contribute to overtreatment. Using a threshold of >6 apoptotic bodies per 10 crypts (>6ap), a recently proposed method, increases the specificity of histologic evaluation. Our aim was to determine whether localization and quantity of T cell subsets in GI biopsies by immunohistochemistry (IHC) correlates with >6ap and evidence of GVHD at other organ sites.

Design: A tissue microarray was constructed from 68 colon biopsies taken to evaluate for GIGVHD, 13 consecutive normal colon biopsies, and 6 consecutive cases of CMV colitis. IHC for CD4, CD8, and FoxP3 was performed to identify helper, cytotoxic, and regulatory T cells (Treg). The number of positive intraepithelial (IEL) and lamina propria (LPL) lymphocytes was counted and averaged by high powered field. Original H&E stained slides were reviewed for maximum number of apoptotic bodies per 10 contiguous crypts. Diagnostic biopsies (Ydx) were defined as presence of symptoms and histology showing >6ap. Non-diagnostic biopsies (Ndx) were defined as presence of symptoms and histology showing ≤6ap, and absence of crypt drop out and ulceration. Clinical information was collected through chart review.

Results: The mean number of CD4+ and FoxP3+ LPL was significantly higher in Ydx versus Ndx biopsies (34.1 vs. 22.2; $p<0.001$ and 8.6 vs. 4.5; $p=0.03$, respectively). CD8+ LPL were also higher in Ydx than Ndx biopsies (32.9 vs 19.1; $p=0.003$). Multivariate logistic regression showed that combined elevation of CD4+ and FoxP3+ LPL was negatively correlated with the presence of GVHD at other sites ($p=0.03$, OR=0.89). CMV positive cases showed the least LPL Tregs (mean=3.2) compared to normal (mean=6.4;$p=0.11$), Ndx (mean=4.3; $p=0.45$), and Ydx(mean=8.2;$p=0.02$) biopsies.

Conclusions: Differing proportions of T cell subsets in Ydx and Ndx biopsies provide immunobiologic support for the division of these two groups at the threshold of >6ap. CMV positive cases show significantly fewer LPL Tregs compared to Ydx, a potentially helpful feature in the differential diagnosis of apoptotic colopathy. Combined elevation of helper and Treg populations is a quantitative correlate of a low pre-test probability of GVHD. Further analysis of this group may identify patients with Ndx histology that might be spared increased immunosuppression.

690 High Expression of EphA4 Predicts Lesser Degree of Tumor Regression after Neoadjuvant Chemoradiotherapy in Rectal Cancer

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Background: Numerous receptor tyrosine kinases have been found to play an important role in tumor progression in a wide variety of cancers. The aim of this study is to evaluate the clinical impact of the transmembrane receptor protein tyrosine kinase, EphA4 in patients with rectal cancer treated with neoadjuvant concurrent chemoradiotherapy (CCRT) followed by surgery, with special emphasis on tumor regression.

Design: We initially analyzed the public expression profiling dataset of rectal cancer, particularly focusing on genes associated with signaling pathway of transmembrane tyrosine kinase receptors. Among the significantly altered genes, *EphA4* was found to be the significantly top-ranking, upregulated gene in the non-responders to CCRT, compared with the responders. Immunohistochemical study was conducted to assess the expression of EphA4 in pretreatment biopsy specimens from 172 rectal cancer patients without distant metastasis. Furthermore, the relationships between EphA4 expression and various clinicopathological factors or survival were analyzed.

Results: High expression of EphA4 was significantly associated with increased vascular invasion (P=0.015), post-treatment depth of tumor invasion (P=0.006), pre-treatment and post-treatment lymph node metastasis (P=0.004 and P=0.011, respectively). More importantly, high EphA4 expression was significantly predictive for lesser degree of tumor regression after CCRT (P=0.031). In univariate analysis, high EphA4 expression predicted inferior disease-specific survival (P=0.0009) and metastasis-free survival (P=0.0001). In multivariate analysis, high expression of EphA4 still served as an adverse prognostic factor for disease-specific survival (Hazard ratio=2.528, P=0.024) and metastasis-free survival (Hazard ratio=3.908, P=0.003).

Conclusions: High expression of EphA4 was associated with an aggressive phenotype and lesser degree of tumor regression after CCRT in patients with rectal cancer. It acted as not only a predictive biomarkers for CCRT response but also a negative prognostic factor in rectal cancer.

691 Stress-Induced Phosphoprotein 1 (STIP1) Promotes Tumorigenesis and Predicts Poor Survival in Gastric Carcinoma Through Induction of the Phospholipase C-γ 1 and ERK Signaling Pathway

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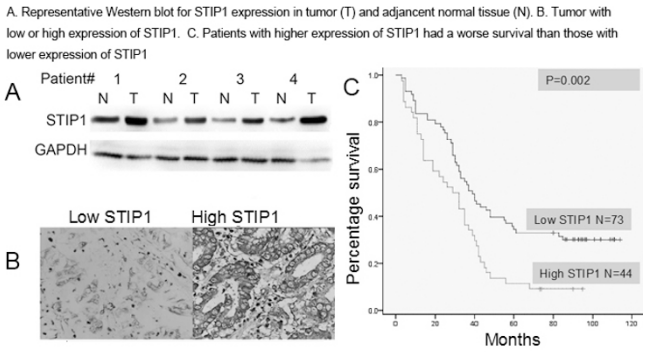
Background: Stress-induced phosphoprotein 1 (STIP1) is an adaptor protein that coordinates the functions of HSP70 and HSP90 in protein folding. Recently, STIP1 has been identified as a novel biomarker in human cancers. This study was designed to assess the role of STIP1 in the tumorigenesis and prognosis of gastric carcinomas (GC).

Design: STIP1 protein was measured in GC tumor and adjacent normal tissues from 30 GC patients by Western blot. Serum STIP1 was measured in 10 GC patients and 10 age-matched health controls by ELISA. A total of 117 paraffin-embedded GC tissues were evaluated for STIP1 expression by immunohistochemistry and analyzed for a possible correlation with patient characteristics and survival. We used Kaplan-Meier plot to compare GC patient survival between low and high tumor STIP1 groups. Two GC cell lines were treated with recombinant STIP1 or anti-STIP1 antibody to study the effects of STIP1 signaling on cell proliferation, apoptosis and 5-Fluorouracil (5-FU) resistance.

Results: STIP1 protein expression was much higher in GC tumors than that in normal tissues (80% increase, $P<0.01$, Figure 1A). Serum STIP1 was higher in GC patients

than age-matched health controls (56% increase, p<0.001). STIP1 expression in GC tumor tissue was significantly correlated with Bormann classification and TNM staging. Patients with high-level STIP1 expression had a significantly poor survival, as the median survival time was 56 month in low STIP1 group (N=73) and 32 months in high STIP1 group (N=44), respectively (Figure 1B&C).



The 5-year overall survival rate was 34.25% in low STIP1 group and 11.36% in high STIP1 group. Multivariated analysis demonstrated that STIP1 expression status is an independent prognostic predictor for GC patients. STIP1 increased GC cell proliferation and survival through activating PLCγ1-ERK1/2 pathway. Blocking STIP1 by neutral antibody promoted GC cell apoptosis and increased 5-FU induced cell death.

Conclusions: STIP1 is an independent prognostic predictor for poor survival in patients with GC. STIP1 promotes GC tumorigenesis through induction of phospholipase C-γ1 and ERK pathway.

692 Histologic Predictors of Endoscopic Treatment Failure in Barrett's Esophagus-Associated Dysplasia

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Background: The histologic diagnosis of dysplastic Barrett's Esophagus (DBE) is the best known predictor of progression to esophageal adenocarcinoma (EAC) in BE. Radiofrequency ablation (RFA) is an efficient, safe, and cost-effective treatment for BE with low (LGD) and high-grade dysplasia (HGD). A subset of patients, however, will eventually fail RFA with re-growth of DBE. Potential histologic predictors of RFA failure have not been studied to date. We aimed to determine which histologic features present at baseline biopsy were predictive of future RFA failure.

Design: Initial endoscopic mucosal biopsies of the gastroesophageal junction were examined in 25 patients (mean age 65 yr, range 51-82; M:F=5:1) identified clinically as failing RFA and in 57 age and diagnosis matched controls (mean age 65 yr, range 42-88; M:F=3:1) who responded. RFA failure was defined by persistent dysplasia following ≥4 sessions of ablation. Baseline data including presenting symptoms, endoscopic findings, Barrett's length, BMI, smoking status, and alcohol use were assessed by chart review. The mean follow-up period was 47.5 months (range 6-180 mo) vs 45.4 mo (4-168 mo) for the failure and responder groups, respectively. Two gastrointestinal pathologists blindly evaluated cases and controls for the following histologic parameters: nondysplastic Barrett's esophagus (NDBE), buried BE, crypt dysplasia, LGD, HGD, and intramucosal carcinoma (IMC), acute neutrophilic inflammation within glandular mucosa, ulceration, and active esophagitis.

Results: Ulceration and LGD were identified in 7 (28%) and 10 (40%) RFA failure pts, respectively, at baseline biopsy versus in 5 (8.8%) and 2 (3.5%) pts in the RFA responder group (p=0.0051 and 0.0031, respectively, by chi-square analysis). There was no difference between groups with regard to the histologic features of neutrophilic inflammation within glandular mucosa (44 vs 28%), HGD (16 vs 33%), and IMCA (16 vs 7%, respectively). Clinical and demographic data collected revealed no differences between patient groups.

Conclusions: The presence of ulceration or low grade dysplasia at presentation in patients who either presently have, or go on to develop DBE, are independent predictors of future RFA failure. Although further studies are needed to confirm these associations, aggressive early interventions may be warranted for BE patients presenting with ulceration or low grade dysplasia on index biopsy.

693 EBV Status and Molecular Alterations in Gastric Carcinomas with Lymphoid Stroma

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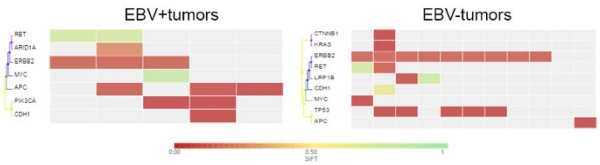
Background: The Cancer Genome Atlas identified four molecular signatures among gastric carcinomas based on EBV status, microsatellite status, and chromosomal instability. However, that study did not include uncommon cancer subtypes, such as gastric carcinomas with lymphoid stroma (GCLS), which are enriched in tumors that contain EBV encoded RNA (EBER), suggesting a pathogenic role of the virus. We performed this study to evaluate the clinicopathologic and molecular features of GCLS with respect to EBV status.

Design: The study group consisted of 23 GCLS that were mismatch repair proficient by immunohistochemistry (MLH1, MSH2, PMS2, and MSH6). All cases were subjected to in situ hybridization for EBER and immunostains for β-catenin and HER2. DNA from 16 cases (5 EBV+ and 11 EBV-) was extracted from tumors and matched normal controls and subjected to next generation sequencing (NGS) using a targeted panel interrogating 400 cancer-related genes. Somatic single nucleotide variants, indels, and copy number alterations (CNAs) were evaluated.

Results: There were no clinical differences between EBV+ (n=8, male/female: 3/1, mean age: 67 years) and EBV- (n=15, male/female: 11/4, mean age: 68 years) tumors. Most GCLS (75% of EBV+ and 73% of EBV- cases) were Stage I or II at resection. Nuclear β-catenin staining was more common in EBV+ (63%) than EBV- (13%) cases (p=0.04), and HER-2 overexpression was infrequent in both groups (0% and 13%, respectively). NGS revealed genomic differences between EBV+ and EBV- GCLS. The former showed frequent *CDH1*(20%)and *PIK3CA* (60%) mutations compared to EBV- tumors (10% and 36%, respectively), but less common *ERBB2* mutations (40% vs. 82%, respectively). Only EBV- GCLS had *TP53* and *LRP1B* mutations (46% and 18%, respectively). CNAs in *PIK3CA*and *MYC* were more prevalent in EBV- tumors (40 and 60%, respectively) than EBV+ GCLS (25 and 42%, respectively).



Conclusions: GCLS negative for EBV show frequent *ERBB2, TP53, and LRP1B* variants reflecting enhanced chromosome instability. The relatively high prevalence of *PIK3CA* variants and CNAs among GCLS raises the possibility that some may be amenable to treatment with PI3-kinase inhibitors.

694 Mutational Landscape of Anal Melanoma

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Background: Anal melanoma is a rare and aggressive tumor, representing <5% of all anal tumors. Patients typically present in the 5th/6th decade, often with metastatic disease. Prognosis is poor with a 5y survival rate of <20%. Limited genetic analyses show infrequent BRAF/NRAS mutations in anal as compared to cutaneous melanomas, and suggest activating KIT mutations play a role as driver events, similar to other mucosal melanomas. Anal melanomas harboring KIT mutations are reported to respond to cKit inhibitor therapy. Meanwhile, emergence of targeted therapies accelerated development of molecular diagnosis and next generation sequencing (NGS) technologies as new tools in oncology and personalized medicine. We performed expanded molecular profiling of anal melanomas, with the aim to identify new genetic markers and ultimately discover targets for therapy.

Design: 15 cases of anal melanoma were collected from the United States, Canada and Germany. After microdissection to enrich for lesional FFPE tissue, DNA was isolated and analyzed by NGS using the Columbia Combined Cancer Panel, a 467-gene panel of cancer-related genes.

Results: Driver mutations were identified in 14/15 cases. In most cases (10/14), a single driver mutation was identified, with 2-3 driver mutations in the remaining cases. Oncogenic KIT mutations were found in 33% (5/15 cases), which involved exons 11 (juxtamembrane domain) and 17 (tyrosine kinase domain) and are expected to confer sensitivity to imatinib. NRAS or HRAS mutations were seen in 13% (2/15), and one tumor harbored a BRAF mutation (p.T599dup). Mutations in the tumor suppressor NF1, described in desmoplastic melanomas and potentially affecting PI3K/AKT as well as MAPK pathway activity, were identified in 20% (3/15) of cases. Of note, three cases showed mutations of SF3B1, previously described in uveal melanoma, but not, to our knowledge, in anal melanoma. SF3B1 mutations were seen in combination with other driver mutations (KIT/NF1). Future studies will determine associations between SF3B1 mutations and prognosis.

Conclusions: Mutational profiling of cancer-related genes by NGS represents a powerful approach to improve our understanding of the genetic landscape and possible evolution of poorly-characterized melanoma subtypes. The identification of previously unknown mutational events in anal melanoma may help to identify new targets for therapy of this lethal disease.

695 Rare Sarcina Ventriculi of Human Gastrointestinal Tract: Six New Examples

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Background: Sarcinous vomiting (1842) first led to microscopic discovery of human gastrointestinal (GI) *Sarcina ventriculi* (SV). This obligate-anaerobic gram-positive tetrad-forming coccus has been identified in air, soil, polluted streams, numerous animals, and human body fluids, feces (mostly vegetarians), GI tract, and solid organs, reported only as rare cases or small series**.** SV can ferment glucose over a wide pH range. When found in rumen or stomach, SV has been associated with bloating, distention, perforate ulcer, and gastric outlet obstruction, although causation is controversial.

Design: Slides, charts and clinical follow-up data of GI SV cases from two institutions were reviewed.

Results: Six cases of SV in GI biopsies were identified (stomach, n=4 [antrum, fundus, GE anastomosis]; duodenum, n=2) in 5 patients. There were 4 males and 1 female (age 20 to 81 years, median 68), none known vegetarians. Patients underwent routine or

surveillance endoscopy for symptoms of epigastric pain and nausea. PMH included esophageal carcinoma s/p esophagectomy, gastric band procedure, primary gastric signet ring cell carcinoma with metastases to bone, and cervical carcinoma s/p multiple abdominal and pelvic procedures. Endoscopic findings included gastric erythema, hiatal hernia, and residual gastric food. Biopsies ranged from 2 to 9 mm in aggregate. Two to >100 clusters of tetrad-forming cocci, measuring 7-10 microns (tetrads), were identified in the lumen and on the surface of the mucosa. One case demonstrated epithelial involvement and gastric erosion. All biopsies demonstrated active and/or chronic inflammation, 1 with lamina propria eosinophils, 1 with dilated duodenal lacteals suggestive of obstruction. No intestinal metaplasia, dysplasia, or carcinoma were identified. SV cocci exhibited peripheral staining by GMS and were negative for PAS, H. pylori, HSV1, CMV, and Fe stains. One patient's symptoms improved after 4-weeks of Ciprofloxacin and Flagyl.

Conclusions: SV has a distinctive appearance in gastric or duodenal biopsies, with associated mucosal inflammation and variable numbers of tetrad-forming coccoid organisms that may involve mucosa or luminal contents. Most patients had history of carcinoma and possible compromised immune system, GI obstruction, and/or history of regional surgery, a route to introduce this ubiquitous organism. In the absence of other identifiable cause, patient symptoms and resolution following antibiotic treatment lend support to characterization of SV as a disease-causing organisms, not just an innocent-bystander.

696 Human Papillomavirus Genotyping of Unexpected Malignant and Pre-Malignant Lesions on Hemorrhoidectomy Specimens

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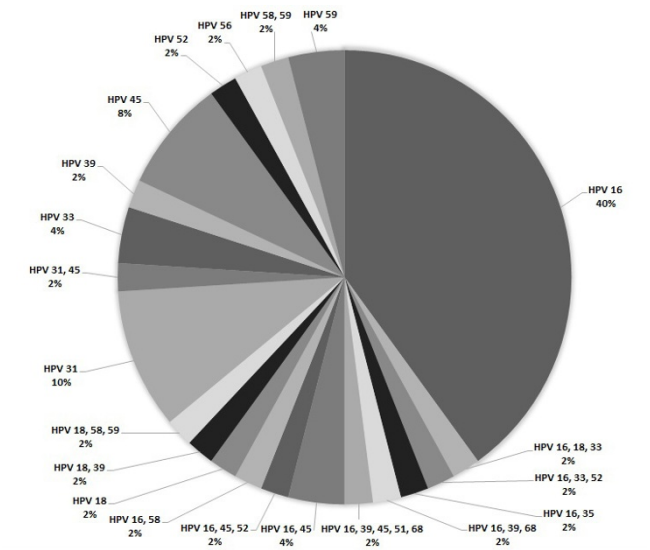
Background: High-risk human papillomaviruses (HR-HPV), commonly HPV types 16 and 18, are implicated in the development of anal intraepithelial neoplasia (AIN) and squamous cell carcinoma (SCC) of the anus. Routine histopathologic examination of these pre-malignant and malignant lesions may lead to unexpected identification of these lesions. The HR-HPV types associated with these incidentally identified lesions is presently unknown. We sought to identify cases of incidental high-grade AIN and SCC, genotype HR-HPVs in these lesions, and assess the potential need for histopathologic examination of hemorrhoids.

Design: Institutional records from 1995 to 2015 were reviewed to identify appropriate cases. Demographics and potential risk factors were also collected. Representative lesional tissue was dissected from unstained tissue sections for each case. Tissue DNA was extracted and HR-HPV genotyping for 14 types was performed using multiplex PCR followed by signature Tag/Capture probe hybridization. Results were assessed against the potential risk factors using univariate Fisher's exact test.

Results: From 2663 hemorrhoidectomy specimens, 62 (2.1%) high grade AIN and 6 (0.2%) SCC were identified of which 62 were available for genotyping. In these, 80.6% (50/62) were HR-HPV positive while 19.4% (12/62) were negative. Among positive cases, multiple HR-HPV types were found in 26% (13/50) of cases. Both HPV 16 and 18 were negative in 36% (18/50) of cases. Figure 1 illustrates the HPV subtype distribution. HPV 39 was associated with IV-drug abuse history (p=0.0020) and HIV-positive status (p=0.049). HPV 58 detection correlated with chemotherapy-induced immunosuppression (p=0.035).

Conclusions: In the largest series of AIN and SCC in hemorrhoidectomy specimens to date, we found that HPV 16 is the most common type in high-grade AIN and SCC and that multi-type infections are common. A significant subset of these lesions is attributable to non-HPV 16 and 18 types that may not be covered by current vaccines. IV-drug abuse, HIV status, and chemotherapy may increase the likelihood of acquiring some uncommon high-risk types. Our findings support the use of routine histopathologic examination of hemorrhoidectomy specimens.



697 Prediction of Clinical Outcomes of Stage 3 Colorectal Carcinomas with FOLFOX Adjuvant Chemotherapy According to Molecular Subtypes

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Background: Colorectal cancers show diverse clinicopathologic characteristics. Recently, most patients with stage 3 colorectal cancer receive standard FOLFOX adjuvant chemotherapy after curative resection; however, clinical outcomes are variable. These findings indicate that colorectal cancer is a heterogeneous disease. The aim of this study was to identify the molecular subtypes of the good prognosis group of stage 3 colorectal carcinomas after FOLFOX adjuvant chemotherapy.

Design: A total of 101 fresh-frozen primary colon cancer samples and 35 matched non-neoplastic mucosa tissues from 101 patients with stage 3 colorectal cancer were included in this study. All patients underwent chemotherapy using the *FOLFOX* regimen after curative surgery between 2006 and 2012. We used consensus-based unsupervised clustering to analyze the gene expression profiles.

Results: We identified four molecular subtypes on gene expression analysis (1764 genes, fold change (FC) cut-offs of >2, and p-values of <0.01). Among the four subtypes, we found that two subtypes showed significantly better disease-free and overall survival than the other two subtypes. Particularly, one subtype (subtype 2) showed no disease recurrence or death. The pathologic characteristics of this subtype showed higher levels of MSI phenotypes (8 of 21) and mucin production (12 of 21) and more instances of a right-sided location (12 of 21) than the other subtypes. We performed a g*ene set* enrichment analysis (GSEA) on the subtype-specific gene expression data. We found that all four subtypes showed increased expressions of genes that encode PI3K/AKT/MTOR signaling, MTORC1 signaling, and MYC Targets. Gene sets of Wnt/β-catenin signaling and notch signaling were significantly over-expressed in three subtypes, though not in subtype 2.

Conclusions: We identified a molecular classification of colon cancers that can predict clinical outcomes after standard adjuvant chemotherapy. This molecular classification can be used for specific patient selection of stage 3 colon cancers for standard adjuvant chemotherapy or for developing a new adjuvant chemotherapy regimen.

698 Conventional Colonic-Type Adenocarcinoma of the Appendix: Clinicopathologic Comparison with Cecal Adenocarcinomas

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Background: Non-mucinous colonic-type adenocarcinoma (NCTA) of the appendix accounts for approximately 25% of all malignant appendiceal neoplasms (SEER 1973-2007). We studied clinicopathological factors of these tumors and compared them to their cecal equivalents, in order to determine clinical implications.

Design: 33 patients with the diagnosis of moderately differentiated NCTA were identified from a surgical database of non-carcinoid appendiceal malignancies (2001-2014). Only tumors with conventional desmoplastic stromal invasion and without significant mucinous differentiation were included. 33 moderately differentiated cecal adenocarcinomas (CA) resected from 2001-2011 were compared. Clinicopathologic findings were assessed by chi-square and t-test. Survival was assessed by Kaplan-Meier log-rank method.

Results: 82% of NCTA presented with acute appendicitis while presentation of CA was variable (anemia/bleeding-33%, abdominal pain-24%, colonoscopy-18%). 23 (69%) NCTA patients underwent appendectomy with only 19 (82%) receiving more definitive resection. Of these, 12 (63%) patients had residual tumor in the resection specimen (predominantly nodal disease). See Table 1 for clinicopathologic features. 5 (15%) NCTA received heated intraperitoneal therapy (HIPEC); only 1 (3%) CA had HIPEC. Survival was similar in the two groups (P=0.541).

Table 1. Features of non-mucinous colonic-type appendiceal adencoarcinoma and cecal adenocarcinoma

	Appendix (n=33)	Cecum (n=33)	P
Female	13 (39%)	19 (58%)	NS
Age in yrs; mean (range)	56 (32-73)	63 (44-90)	0.010
Perforation	12 (36%)	1 (3%)	0.001
pT1/pT2	1 (3%)/3 (9%)	1 (3%)/3 (9%)	
pT3/pT4	11 (33%)/18 (55%)	23 (70%)/6 (18%)	
Precursor lesion	20 (61%)	6 (18%)	
Stage IV	9 (27%)	0 (0%)	0.003
Follow-up in mos; mean (range)	43 (1-110)	51 (2-113)	
Dead of disease/dead	10 (30%)/12 (36%)	12 (36%)/13 (39%)	

Conclusions: NCTA is seen in a younger age group and tends to present acutely with advanced disease; however, overall survival in similar to CA.

699 Yttrium-90 Microspheres as a Cause of Non-Healing Gastric Ulcer: Histologic Features Often Mimic Chemical Gastropathy

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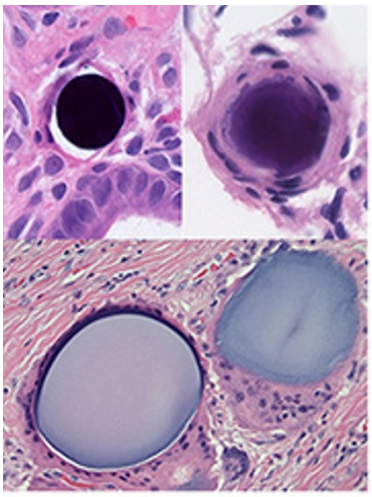
Background: Transarterial radioembolization (TARE) with Yttrium-90 (Y90) microspheres is used to treat liver metastases. Aberrant celiac and hepatic vasculature or procedural complications may lead to unintentional embolization of the gastroduodenal artery (GDA), which supplies blood to the gastric antrum/pylorus. The Y90 spheres

occlude vessels and emit continued radioactivity ($t^{1/2}$=64.1 hours), with resultant injury of the gastric mucosa. We report the clinical and pathologic features in 4 patients who underwent TARE and developed gastric ulceration with key caveats for proper diagnosis.
Design: A Cerner database search was performed between 2010 and 2015, identifying four patients with a history of TARE for liver metastases who subsequently developed gastric ulceration. Histologic features and clinical follow-up were noted.
Results: Features noted in the gastric biopsy specimens included ulceration, foveolar hyperplasia, mucin depletion, reactive atypia, fibropurulent debris, and mucosal erosions. Occasionally chronic inflammation (1/4) or giant cell reaction (2/4) was seen. The number of spheres in each tissue fragment (average surface area 1mm^2) ranged from 0-46 (median = 2), were found in the lamina propria, had a median size of 32.5 microns, and appeared dark purple and round.



From clockwise: TARE, psammoma body, TACE

Non-healing antral/pyloric ulcers were identified in all 4 patients at an average of 10 weeks after the last IR procedure. In one patient, the non-healing ulcer necessitated distal gastrectomy and the other 3 patients had biopsy proven ulceration at last follow-up.
Conclusions: Due to inadvertent embolization of the GDA, Y90 microsphere-induced gastric ulceration preferentially affects the antrum/pylorus. The histologic features often mimic chemical gastropathy. The number of spheres per tissue fragment may vary greatly, and often none are present. The spheres are morphologically distinct from TACE beads, but may mimic psammoma bodies and calcifications and can be easily missed. When presented with an antral/pyloric ulcer, with or without identifiable microspheres, it is important for the pathologist to consider the patient's clinical history and to keep this entity in mind.

700 Role of Submucosal Glands in the Development and Progression of Adenocarcinoma in Barrett's Esophagus

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Background: Esophageal submucosal glands (SMG) secrete mucous and other chemicals that are believed to serve as protection of the mucosal surface from luminal noxious agents, either digested or refluxed. We hypothesized that changes in the type, distribution or number of SMG may contribute to, or are associated with, the development of Barrett's esophagus (BE) and progression to adenocarcinoma. The aim of this study was to investigate the morphologic and anatomic characteristics of SMG in BE-associated neoplasia.
Design: H&E stained slides from 64 esophageal resections for BE-associated adenocarcinoma and 32 squamous cell carcinomas (SCC)(as controls) were retrieved from the surgical pathology archives. All patients did not receive neoadjuvant chemotherapy. The resections were reviewed for a variety of features related to SMG including gland density (# glands/cm), type (mucinous, oncocytic, serous, atrophic), degree of inflammation in and surrounding the glands (0-3+) and the type of overlying epithelium (squamous, nongoblet columnar, BE with <50% goblet cells (GC), BE with >=50% GC, low grade dysplasia, high grade dysplasia, and adenocarcinoma). The histologic features of the SMG were compared between BE-associated adenocarcinoma and SCC and between the various types of mucosa overlying the glands.
Results: A total of 865 submucosal glands were evaluated. Overall, the mean number of glands/cm was 0.88/cm. Between BE-associated adenocarcinoma and SCC, there were no significant differences in the glandular density (0.91/cm and 0.81/cm, p=0.74), and components of mucinous (78%), oncocytic (4%), atrophic (17%) and serous (1%) morphology, and overall, 37% of glands had moderate-to-severe inflammation. However, SMG beneath dysplasia or adenocarcinoma showed a significant decrease in mucinous glandular component (74.3% vs 83.2%, p=0.027) and concomitant increases in atrophic glands (23.8% vs 13.7%, p=0.011) and in the degree of inflammation (53% vs. 35% moderate-to-severe inflammation, p= 0.002) compared to BE cases without neoplasia.
Conclusions: Our results show a difference in the characteristics of SMG underneath BE-associated neoplasia compared to SMG associated with non-neoplastic mucosa. This suggests that these structures may play a role in the progression of neoplasia in BE, possibly by offering less protection to the mucosal surface of the esophageal epithelium from chemical injury.

701 Clinical Utility of Tumor Hotspot Sequencing in Colorectal Cancer

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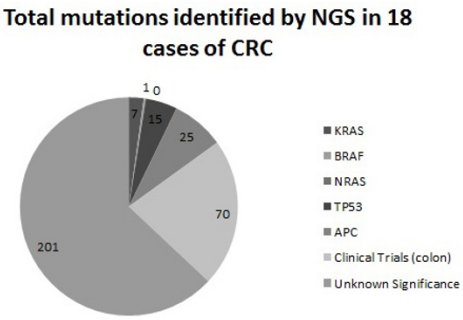
Background: Colorectal cancer (CRC) has effective treatments for early stage disease; however, metastatic CRC has an abysmal 11% 5-year survival. The basic molecular carcinogenesis pathway is known in CRC, and mutations in some genes can guide therapy. National Comprehensive Cancer Network (NCCN) guidelines recommend mutation analysis for codons in three genes (KRAS 12, 13, 61, 146; NRAS 12, 13, 61; BRAF 600). This study examines the clinical utility of extensive targeted genomic profiling by next generation sequencing (NGS) in the current and future management of CRC.
Design: DNA was extracted from 18 cases of relapsed/metastatic CRCs (40 mm of FFPET). NGS was performed on hybrid-captured libraries to a mean coverage of >500X for 315 cancer-related genes and 37 introns from 14 genes which can be rearranged. Genomic alterations (GA) included base substitutions, INDELs, copy number alterations and fusions/rearrangements.
Results: In the 18 CRCs, 319 mutations were found in 175 genes, and included 8 mutations in KRAS (7) and BRAF (1). NGS also identified other genes with well-established roles in the pathogenesis of CRC including TP53 (15), APC (25), and PIK3CA (5). 65 mutations in additional genes (excluding the NCCN genes, TP53, APC, and PIK3CA) under study and eligible for open clinical trials related to CRC were identified. Adding PIK3CA (required WT for many clinical trials) the total comes to 21.9% of mutations being relevant to clinical trial enrollment. Taken together, 24.5% of GA found in this study are clinically relevant when including GA linked to drugs on the market or under evaluation in mechanism-driven clinical trials.



Conclusions: This study shows that NGS identified a high percentage of GA pertinent to open clinical trial enrollment, which would not have been identified by the minimum NCCN testing requirements. Significantly, the percentage of RAS mutations in this population is lower than expected because patients with known RAS mutations may not have been selected for NGS. All 18 CRCs had a mutation in at least one NCCN gene and/or one clinically relevant gene. While costs must be considered in healthcare decisions, NGS is justified by the current low survival in metastatic CRC and high yield of mutations relevant to clinical trial enrollment.

702 Extended RAS Mutations and Microsatellite Instability in Patients with Colorectal Cancer

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Background: Colorectal cancer is one of the most commonly diagnosed malignancies worldwide and it is a second cause of cancer death. With the new medications like anti-vascular endothelial growth factor (VEGF) and anti-epidermal growth factor receptor (EGFR), survival has improved. Numerous trials have pointed that anti-EGFR therapy is not effective in colorectal cancer with KRAS mutations. However, in all of these studies, only mutations in exon 2 were evaluated. Recent investigations demonstrate patients with additional RAS mutations (other KRAS mutations and NRAS mutations) also do not benefit from anti-EGFR therapy. According to these data, it has been recommended to test patients with metastatic colorectal cancer for expanded RAS mutation rather than only KRAS mutation in exon 2.
Design: In this study we evaluated 425 patients with colorectal cancer who had expanded RAS mutation between 2013 and 2015. Demographic and tumor data of patients with KRAS exon 2 were compared with patients with other RAS mutations and patients with wild type RAS.
Results: Analysis of data showed that microsatellite instability is significantly higher in patients with additional RAS mutations in comparison with patients who have KRAS mutation on exon 2 or do not have any mutations.

	negative for mutation	KRAS mutation on exon 2	Other RAS mutations (Other KRAS mutations and NRAS mutation	P-value
Sex male female	146 (58.4%) 104 (41.6%)	69 (48.6%) 73 (51.4%)	12 (54.4%) 10 (45.5%)	0.172
Distant metastasis Absent Present	159 (63.6%) 91 (36.4%)	95 (66.9%) 47 (33.1%)	12 (54.5%) 10 (45.5%)	0.695
regional lymph node metastasis absent present	119 (47.6%) 131 (52.4%)	80 (56.3%) 62 (43.7%)	12 (54.5%) 10 (45.5%)	0.236
tumor grade low grade high grade	178 (74.2%) 62 (25.8%)	111 (81.6%) 25 (18.4%)	13 (65%) 7 (35%)	0.126
microsatellite instability stable instable	147 (84.5%) 27 (15.5%)	93 (95.9%) 4 (4.1%)	14 (73.7%) 5 (26.3%)	0.004
age (mean ± SD)	65.5 ± 13	63.5 ± 15	65.5 ± 17	0.392
tumor size (mean ± SD)	5.2 ± 3.0	5.0 ± 2.5	5.3 ± 3.0	0.764
tumor stage (pT)	3.2 ± 0.8	3.1 ± 0.7	3.1 ± 0.8	0.552

Conclusions: These findings demonstrate that the chance of finding additional RAS mutation in colorectal cancer patients with microsatellite instability is higher and this group of patients should specifically evaluated for expanded RAS mutation as the anti-EGFR therapy will not be helpful in these patients.

703 Clinicopathologic Study of the Ipilumimab-Induced Colitis Compared to Emerging Inflammatory Bowel Disease

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Background: Ipilumimab (Ipi) is an anti-cytotoxic T-lymphocyte antigen-4 (CTLA-4) monoclonal antibody used in the treatment of different cancer types. Ipi has shown significant clinical benefit, but there is a risk of developing an immune associated colitis. Ipi-associated colitis (Ipi-AC) histologically can mimic emerging inflammatory bowel disease (eIBD). In this study, we compared the clinical, endoscopic and histopathologic findings of Ipi-AC to those of eIBD.

Design: We identified 23 Ipi-AC in patients treated for metastatic (met) melanoma (n=15), met pancreatic cancer (n=4), met lung cancer (n=3) and met renal cell carcinoma (n=1). The eIBD controls were chosen as the first biopsy of patients with established ulcerative colitis. The number of intracryptal, pericryptal and superficial apoptotic cells were counted in 10 high power fields (hpf). Biopsies were scored for the presence of cryptitis, dilated crypts with inspissated mucin, normal or distorted crypt architecture, ulceration, intraepithelial lymphocytes and mitoses. A students T test was used to test for significance.

Results: The most common clinical symptom of Ipi-AC was watery diarrhea (n=22, 95%) in contrast to the most common symptom in the control group which was hematochezia (n=9, 81%). Symptoms developed within a few weeks to > 1 year after starting Ipi therapy (mean of 60.1 days) and after a median of 3 cycles. Endoscopically, Ipi-AC appeared normal (n=5; 21%) to edematous and erythematous (n=8; 34%) and ulcerated (n=7; 30%). The most common endoscopic findings in the eIBD group were left sided erythematous friable and ulcerated mucosa (n=9; 81%). Both groups showed cryptitis and increased lymphoplasmacytic expansion in the lamina propria. Histologic features that differentiated Ipi-AC from eIBD were an increase in apoptotic crypt nuclei (18.3 vs 8.3) and intraepithelial lymphocytes (6.3 vs 4.4). Intracryptal apoptotic cells per 10 hpf in the Ipi-AC were significantly higher than eIBD ($P<0.05$). Pericryptal and intracryptal apoptosis, dilated crypts with inspissated mucin, increased lymphocytes and normal crypt architecture had higher association with the Ipi-AC. Ulceration or erosion (n=7; 63%) and crypt distortion (n=8; 72%) were more frequent in eIBD.

Conclusions: Routine H&E histology can separate Ipi-AC from eIBD. Increase in intracryptal and pericryptal apoptosis is a reliable indicator of the Ipi-AC compared to eIBD. Future efforts on these samples will be directed at quantitative IHC analysis of mucosal lymphocytes subsets.

704 Utilization of H. Pylori Immunostaining in Gastric Biopsies: A Cost-Effective Diagnostic Algorithm

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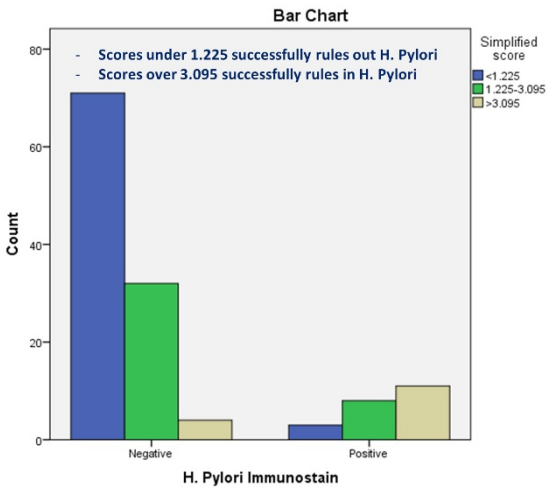
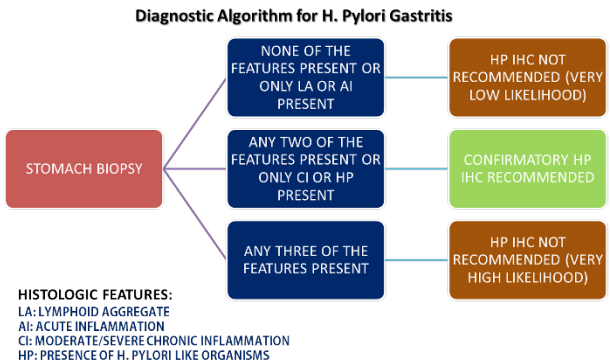
Background: Emphasis on immunohistochemistry (IHC) to confirm the presence of H. Pylori (HP) has become routine for evaluation of gastric biopsies. It is unclear if this is a necessary and cost-effective test. We performed a retrospective study of gastric biopsies in order to address diagnostic accuracy and cost-effectiveness.

Design: 129 H&E stained gastric biopsies with concomitant HP IHC were independently evaluated by two pathologists and rated for acute & chronic inflammation, erosion/ulceration, edema, lymphoid aggregates, intestinal metaplasia, foveolar hyperplasia, atrophy and presence of HP on H&E. HP status was confirmed by IHC. A binary logistics regression analysis was performed with HP IHC results as the dependent variable. Regression coefficients (β) of the significant variables were assigned as weights and an algorithm for predicting HP results was designed. The cutoff points for the score with the highest specificity and highest sensitivity were decided utilizing ROC curve.

Results: Logistic regression identified HP organisms on H&E (p value<0.01, β: 1.32), moderate/severe chronic inflammation (p value<0.01, β: 1.29), acute inflammation (p value<0.05, β: 1.16) and lymphoid aggregates (p value<0.05, β: 1.13) as features to be included in the diagnostic algorithm. The scoring system (AUC: 0.842) assigns scores between 0 to 4.9 with scores below 1.225 ruling out HP in 95.9% of cases (sensitivity) and scores above 3.095 corresponding to a positive HP rate of 96.3% (specificity).

Conclusions: Features in our diagnostic algorithm for HP gastritis are HP on H&E, moderate/severe chronic inflammation, acute inflammation and lymphoid aggregates. Scores below 1.225 rule out HP in 95.9% of cases eliminating the need for HP IHC. Scores above 3.095 rule in HP in 96.3% of cases. The cost of a single HP IHC is $175. Using this algorithm HP IHC can be avoided in 2/3 of cases; this translates to millions of dollars of savings without loss of diagnostic accuracy.





705 Distinct Protein Expressions of the Key Molecules in the Activated Signaling Pathways of Solid-Pseudopapillary Neoplasm of the Pancreas

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Background: Solid-pseudopapillary neoplasm (SPN) is an uncommon pancreatic tumor with distinct clinicopathologic and genetic features. We previously reported that Wnt/β-catenin, Hedgehog, and androgen-receptor signaling pathways were activated in SPNs based on a DNA microarray analysis. We aimed to characterize the protein expressions of SPNs for the key molecules of these three activated signaling pathways.

Design: A total of 14 SPNs and seven non-neoplastic pancreatic fresh tissue samples were used to compare mRNA expressions and protein expressions for the key molecules of SPN-specific signaling pathways. For the protein expression analysis by quantitative proteomics, seven SPNs and seven non-neoplastic pancreatic tissues were pooled and examined with high-resolution mass spectrometry. We used six pancreatic adenocarcinomas (PCAs) and six neuroendocrine tumors (NETs) as controls.

Results: On quantitative proteomics analysis, we found differentially expressed proteins in SPNs. Among these proteins, 444 were up-regulated and 418 were down-regulated. Many proteins interacting with the key proteins of Wnt/β-catenin, AR, Notch, and Hedgehog signaling pathways were up-regulated. Several Wnt signaling proteins, including DKK4, APC, RUVBL1, and β-catenin, were up-regulated in SPNs versus non-neoplastic pancreatic tissue ($p < 0.05$). Additionally, two up-regulated proteins, FUS and NONO, bound directly to β-catenin. We also identified 37 up-regulated proteins, including DDX5, FUS, ALB, GSN, NOV, UBE2I, and RAN, that bind directly to androgen receptor. We validated the expressions of beta-catenin, WIF-1, GLI2, androgen receptor, FUS, and NONO by western blotting and immunohistochemistry. Among them, androgen receptor was highly expressed in all SPNs, though not in the PCAs, NETs, and non-neoplastic pancreatic tissues.

Conclusions: SPNs show characteristic protein expressions of the genes in the activated Wnt/β-catenin and androgen-receptor signaling pathways. These molecules can be used for developing additional diagnostic biomarkers and molecular targets. Particularly, androgen receptor can be used as an additional diagnostic marker for SPNs.

706 SSBP2 Are Associated with Patient's Survival in Hepatocellular Carcinoma

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Background: SSBP2, single-stranded DNA binding protein 2, is known as a subunit of ssDNA-binding complex that is involved in the maintenance of genome instability. Most previous studies have suggested a tumor suppressive role of SSBP2, which is silenced by promoter hypermethylation in several human cancers, such as hematologic malignancies, gallbladder, and ovarian cancers. However, oncogenic role of SSBP2 has been suggested in glioblastoma patients. We investigated the clinicopathologic significance of SSBP2 expression in hepatocellular carcinoma.

Design: We constructed tissue micro-arrays consisting of 21 normal, 49 non-neoplastic liver tissue adjacent to tumor, and 213 hepatocellular carcinoma tissue. The SSBP2 expression was inves-tigated by immunohistochemistry, and more than 10% of positive tumor cells with nuclear staining were defined as positive expression. SSBP2 expression correlated with various clinicopathologic parameters, including tumor size, tumor focality, histologic grade, vascular invasion, AJCC stage, Ki-67 labeling index, serum alpha-fetoprotein (AFP), and patient's survival.

Results: Normal hepatocytes were negative for SSBP2, whereas nuclei of normal bile duct epithelium and sinusoidal endothelium were immunoreactive. Positive immunoreactivity was found in 2 (4.08%) out of 49 non-neoplastic liver tissue adjacent to tumor, and in 16 (8.46%) out of 189 hepatocellular carcinomas. Positive SSBP2 expression was significantly correlated with multifocality (p=0.027, chi-square test), high histologic grade (p=0.003, chi-square test) and frequent vascular invasion (p=0.001, chi-square test). Kaplan-Meier survival curves revealed that patient with SSBP2 expression had poor prognosis on both overall and disease-free survival (p=0.023 and p=0.003, repectively, log-rank test). Furthermore, SSBP2-positive tumors had higher Ki-67 proliferation index (p<0.001, t-test).

Conclusions: Minority of hepatocellular carcinoma expressed SSBP2 by immunohistochemistry, whereas normal hepatocytes were completely negative. SSBP2-positive hepatocellular carcinomaswere significantly associated with aggressive phenotypes and poor clinical outcome.

707 Pathologic Assessment and Clinicopathologic Correlation of Tumor Microenvironment in Colorectal Carcinomas

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Background: The importance of the tumor microenvironment has been emerged in progression and survival of cancers. There has been diverse pathologic methods to assess the tumor microenvironment, such as tumor necrosis, host immune response and tumor stroma percentage. In this study, we analyzed the relationship between tumor microenvironment and clinicopathologic factors including patients' survival in colorectal carcinomas.

Design: One hundred and seventy two patients with colorectal carcinomas were retrieved and pathologically assessed with tumor infiltrating lymphocytes (TILs), Crohn-like lymphoid reaction (by using Graham-Appleman criteria and Ueno criteria), Klintrup-Mäkinen (KM) grade, and tumor stroma percentage (TSP). The prognostic value and correlation with other clinicopathologic factors were evaluated in colorectal carcinomas.

Results: TILs and Graham-Appleman score were correlated with lower pT stage. KM grade was correlated with lower T stage, absence of lymphovascular invasion and perineural invasion while TSP was associated with higher T stage, presence of lymph node and distant metastasis, presence of perineural invasion and invasive tumor margin. TILs were associated with Ueno and KM grade, and TSP was associated with KM grade. High Graham-Appleman score and low TSP were significantly associated with better overall survival and high Graham-Appleman score was an independent good prognostic factor in colorectal carcinoma patients.

Conclusions: Inflammatory response and TSP are variably associated with clinicopathologic factors in colorectal carcinomas. In addition, Crohn-like lymphoid reaction assessed by Graham-Appleman criteria and TSP are important factors in predicting outcome of colorectal cancer patients.

708 See a "Sleeve" Today … Save a Life Tomorrow?

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Background: Sleeve gastrectomy (SG) is quickly becoming a preferred bariatric surgery. According to the American Society for Metabolic and Bariatric Surgery guidelines, routine preoperative upper gastrointestinal endoscopies are not recommended universally for bariatric surgery and are at the discretion of the surgeon. Furthermore, some studies have shown the histologic examination of SG specimens as insignificant and not a cost-effective practice. However, some speculate SG examination may unveil pertinent pathology and prevent further progression of precursors. Identifying precursors such as atrophic gastritis is important as it can progress to gastric adenocarcinoma. Gastric intestinal metaplasia is considered as a pre-malignant condition leading to a tenfold increase in the risk of developing gastric cancer. Autoimmune atrophic gastritis may predispose to neuroendocrine hyperplasia/dysplasia with progression to neuroendocrine tumors. This study aims to reveal the possible precursors/lesions that can be identified with SG examination.

Design: We performed a retrospective analysis of SG specimens to evaluate for any histopathologic changes or findings.

Results: We analyzed 511 partial gastrectomy specimens obtained during bariatric surgery. The majority of histopathology results after SG showed no significant changes or chronic inactive nonspecific gastritis. Incidental findings were grouped into two categories – clinically significant/actionable and minor.

Incidental findings	Number of cases	Percentage
Clinically significant/actionable:	29	5.7
Gastrointestinal stromal tumor (GIST), grade 1	5	
Helicobacter pylori (HP) negative MALT lymphoma	1	
Autoimmune atrophic gastritis with neuroendocrine hyperplasia or dysplasia	2	
Autoimmune atrophic gastritis	2	
Intestinal metaplasia without dysplasia	3	
HP associated gastritis	14	
Iron pill induced gastritis	1	
Gastric glandular siderosis	1	
Minor:	32	6.3
Fundic gland polyps	19	
Hyperplastic polyp	1	
Leiomyoma	1	
HP negative active gastritis	11	

Conclusions: There were clinically significant findings in seemingly healthy and young patients, which altered their postoperative management and prompted surveillance protocols. For example, the lymphoma case is cured, GIST patients are on endoscopic 6-12 months surveillance, and HP patients are treated. In view of these results, routine histological examination of SG specimens is highly recommended as it can identify clinically significant findings that can provide long-term benefits.

709 A Clinicopathologic Study of Collagenous Enteritis

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Background: Collagenous enteritis is traditionally classified as a form of advanced celiac disease, associated with poor outcome secondary to intractable malabsorption. While a recent study suggests that exposure to olmesartan medoxomil, an angiotensin receptor blocker, may be associated with upper tract collagenous deposition, the etiopathogenesis of collagenous enteritis remains largely unknown. We aim to characterize the clinical and pathologic features of collagenous enteritis.

Design: A 20-year (1994 to 2014) retrospective review pooled from two, tertiary care centers was performed on all surgical pathology specimens from which a pathologist diagnosed a collagenous enteritis. Patient demographic characteristics, olmesartan medoxomil exposure, HLA haplotype, celiac serologies, and clinical outcomes were recorded. Ten available cases from one institution were also evaluated for intraepithelial lymphocytes and villous blunting.

Results: A total of 28 cases of collagenous enteritis were included. The mean age at diagnosis was 68 years old (range: 33-80) and the majority (19/28) of patients were women. Only two patients (2/28) carried a prior diagnosis of celiac disease. Of the remaining patients on whom genetic susceptibility loci typing (n=4) and serologies (n=15) were performed, all were HLA-DQ2 or HLA-DQ8 positive, but none had positive celiac serologies. Four patients (4/28) had a history of olmesartan medoxomil exposure. Histologically, in addition to subepithelial collagen deposition, all of the cases examined (10/10) showed villous blunting, and 6/10 cases showed increased intraepithelial lymphocytes. Among patients with follow up information, a symptom-free course was rarely achieved (3/12) despite various medication and dietary changes.

Conclusions: Collagenous enteritis is an intractable disease most commonly occurring in older women. Affected patients have genetic susceptibility loci and histologic features similar to celiac disease, but have negative celiac serologies. Our data suggests that an alternative environmental trigger other than gluten (e.g. medication) may be important in the pathogenesis of collagenous enteritis.

710 Molecular Analysis of Neurofibromatosis Type 1 Associated Gastrointestinal Stromal Tumors

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Background: Neurofibromatosis type 1 (NF1) is clinically characterized by the presence of café au lait spots and neurofibromas. Approximately 7% of NF1 patients are found to develop gastrointestinal stromal tumors (GISTs). GISTs in NF1 patients are often multiple, located in the small bowel, spindle-cell type, and low risk tumors. Unlike most sporadic GISTs, those arising in the context of NF1 have been reported to lack mutations in *KIT* and *PDGFRA* genes. Some show loss of SDH expression by immunohistochemistry but detailed molecular studies have not been performed. Our study is designed to further elucidate the molecular profile of NF1 associated GISTs using next generation sequencing (NGS).

Design: Five GISTs in NF1 patients were found in the pathology archives. Immunohistochemistry (IHC) stains with CD117, DOG-1, and CD34 antibodies were performed on all cases. DNA was isolated from paraffin embedded tissue and used as substrate for library preparation. Libraries were amplified, pooled and subjected to hybrid capture (NimbleGen SeqCap, Roche) to enrich for 1211 cancer-related genes. Following hybridization, the target-enriched library pool was amplified and sequenced

via the Illumina HiSeq 2500 system. The data were analyzed using custom-designed NGS analytical pipelines with resulting variants annotated for interpretive review using Alamut Batch software (Alamut, Rouen, France).
Results: All 5 tumors were spindle-type, low grade GISTs located in the small bowel, with 2 of 5 cases having multiple GISTs. IHC demonstrated tumor immunoreactivity for CD117, DOG-1, and CD34 in all cases. No pathogenic *KIT* or *PDGFRA* mutations were detected in any of the 5 cases. No deleterious SDH subunit mutations were identified. Variants predicted to be deleterious based on in-silico analysis were found in *JAK2* (2 cases). Variants found in individual cases include an R132H IDH1 mutation, CHEK2 mutation, DAXX mutation and NOTCH2 mutation. These variants require further confirmation. No pathogenic mutations shared by all of the NF1 associated GISTs were identified.
Conclusions: Our study supports an alternative pathogenesis for NF1 associated GISTs, with all cases lacking deleterious mutations in *KIT* or *PDGFRA*. The tested cases did not show any mutations in the genes encoding SDH subunits. Previously reported changes in SDH expression in NF1 associated GISTs may therefore be the result of epigenetic alterations. Additional cases need testing to further characterize the pathogenesis of NF1 GISTs.

711 Association between HER2 Status in Gastric Cancer and Clinicopathological Features: A Retrospective Study Using Whole-Tissue Sections

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Background: Gastric cancer is usually diagnosed in an advanced stage of disease and treatment options are sparse. Trastuzumab was recently approved for metastatic or locally advanced carcinomas arising in the stomach or in the gastroesophageal junction in patients with HER2-positive tumors. However, data on the frequency of HER2-positive cases among Brazilian patients are limited. Our aim was to characterize HER2 protein and gene statusin a series of Brazilian patients with gastric cancer and to evaluate its association with clinicopathological data.
Design: Histological slides from 124 primary gastrectomies were reviewed and their pathological reports were retrieved from the files at a Brazilian university hospital. Automated immunohistochemistry for HER2 was performed on whole-tissue sections from each tumor. HER2-equivocal cases by immunohistochemistry were submitted to automated dual in situ hybridization for gene amplification evaluation. HER2 status was confronted with clinicopathological parameters in order to assess statistically significant associations.
Results: Immunohistochemistry analysis revealed that 13/124 cases (10.5%) were HER2 positive (3+), 10/124 cases (8.1%) were equivocal (2+) and 101/124 cases (81.4%) were negative, being 7 cases 1+. None of the equivocal cases showed gene amplification. The overall HER2 positivity rate was 10.5%. There was an association between HER2 expression and Laurén's intestinal histological subtype (P=0.048), well to moderately differentiated tumors (P=0.004) and presence of lymphovascular invasion (P=0.031). No association was found between HER2 status and tumor topography.
Conclusions: Confronted with data published by other authors, the lower percentage of HER2-positive cases found in our series might be partially explained by the lower frequency of tumors arising at the gastroesophageal junction in comparison with distal gastric carcinomas in Brazilian patients. This could also account for the lack of statistically significantassociation between HER2 status and tumor topography in our study.

712 The Duodenal Microbiome in Refractory Celiac Disease

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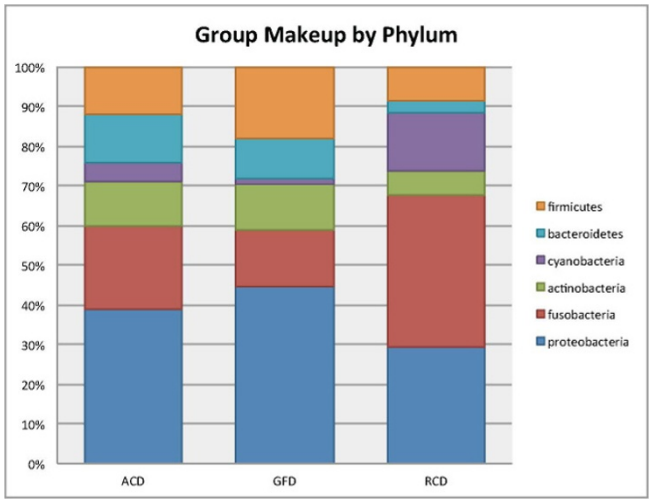
Background: Gastrointestinal (GI) symptoms, histopathology and serologic abnormalities (tissue transglutaminase IgA, TTG) of most patients with active celiac disease (ACD) resolve on a gluten-free diet (GFD). A subset (5-10%) have persistent symptoms and histopathologic abnormalities despite GFD and normalization of TTG, and they are classified as having refractory celiac disease (RCD). Rare patients with RCD have clonal expansions of phenotypically aberrant small intestinal intraepithelial lymphocytes (IELs) and severe malnutrition (RCD II). The etiology the refractory state in patients manifesting normal IEL phenotype (RCDI), is unknown. We hypothesized that there may be differences in the small intestinal microbiome of RCD1 patients, compared to ACD or patients on GFD, and that this may contribute to the refractory state.
Design: DNA was extracted from routinely processed formalin-fixed paraffin embedded duodenal biopsies of 9 celiac patients without RCD, including 4 pairs of ACD/GFD patients and 10 patients with RCD1 for next-generation sequencing of bacterial 16S RNA. The biopsies were graded for celiac features.
Results: All ACD patients had villous atrophy and increased IELs and the 4 patients with follow up biopsy on GFD showed resolution of these findings. The mean TTG was 133 units (3 patients) for the ACD group. RCD patients had villous atrophy (7) or severely increased IELs (3). The mean TTG of the RCD cohort was 5.7 units (9 patients). Analysis of the microbiome showed increased frequency of *Fusobacterium* and *Cyanobacteria* species and decreased frequencis of *Bacterioides*, *Firmicutes*, and *Proteobacteria* in RCD1 compared to ACD and GFD samples.



Conclusions: Alterations in the microbiome, specifically an increase in potentially pathogenic bacteria (Fusobacterium) and decreased beneficial bacterial species in patients with RCD1 are recognized for the first time, whether these changes are contributing to the refractory state, or are the result of it, needs to be determined.

713 PD-L1 Expression Correlated with Lymphocytes Infiltrating in Gastric Cancer

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Background: Recently, a molecular classification for gastric cancer has been published. Epstein-Barr virus positive gastric cancer is special type of tumor display recurrent PIK3CA mutations, extreme DNA hypermethylation and amplification of programmed cell death protein one ligand-1(PD-L1) and PD-L2.This study aim to analyze the relationship among PD-L1 expression, Epstein-Barr virus infection, Lauren classification, lymphocytes infiltrating and other clinical-pathological factors.
Design: Totally 137 cancer patients who underwent curative gastrectomy for stage II/III gastric cancer were involved in this study. PD-L1 expression and Epstein-Barr virus infection were examined by immunohistochemistry and in situ hybridization respectively. For statistical analysis, Logistic regression and multivariate analysis were used.
Results: Univariate analysis identified that PD-L1 expression was correlated with young age, diffused type, Epstein-Barr virus infection and lymphocytes infiltrating. Multivariate analysis confirmed that only Lauren classification ($p = 0.013$) and lymphocytes infiltrating ($p < 0.001$) have statistical significances.
Conclusions: PD-L1 expression was correlated with lymphocytes infiltrating, this may give new sight to select suitable patients for immune checkpoint inhibitors treatment in gastric cancer in near future.

714 Sporadic Gastric Well-Differentiated Neuroendocrine Tumors Have a Higher Ki-67 Proliferation Index

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Background: The 2010 WHO classification divides gastrointestinal neuroendocrine neoplasms into grade 1 (≤3%), grade 2 (>3-20%) and grade 3 (>20%) based on the Ki-67 proliferative index (PI). Well-differentiated neuroendocrine tumor (WNET) of the stomach can arise in three distinct clinical settings: 1) in association with autoimmune atrophic gastritis, 2) in association with multiple neuroendocrine type I (MEN I) or Zollinger-Ellison syndrome (ZES), or 3) sporadic. The Ki-67 PI in gastric WNETs in these three distinct clinical settings has not been evaluated in detail.
Design: Forty-five gastric WNETs underwent polypectomy (n=4), endoscopic mucosal resection (n=12) and surgical resection (n=29) between 1994 and 2015 were included. H&E slides from each case were reviewed and Ki-67 immunostain was performed on one representative tumor block. Ki-67 PI was determined by quantitative Aperio image analysis in areas of strongest nuclear labelling ("hot spots"), and correlated with underlying clinical and pathological features.
Results: Forty-five cases (21 males and 24 females) were included with a median age of 57 years (range, 30 – 80 years). They were classified as type I (associated with autoimmune atrophic gastritis; n=17), type II (associated with MEN I or ZES; n=6), and type III (sporadic; n=22). WHO grade based on Ki-67 PI was higher in Type III WNETs [grade 1 (n=3), grade 2 (n=15), and grade 3 (n=4)] than in type I WNETs [grade 1 (n=5) and grade 2 (n=12)] and in type II WNETs [grade 1 (n=2) and grade 2 (n=4)] (p=0.05, overall). Ki-67 PI was significantly higher in type III WNETs (mean ± SD=13.0 ± 13.3 %) than in non-sporadic (type I and II) WNETs (mean ± SD=5.3 ± 3.3 %; p=0.02). There was no difference in Ki-67 PI between type I WNETs (mean ± SD=5.2 ± 3.5 %) and type II WNETs (mean ± SD=5.6 ± 3.0 %; p=0.82). Higher Ki-67 PI was associated with higher tumor T-stage [T1 (n=31), mean ± SD=6.5 ± 4.7 %; T2 (n=6), mean ± SD=8.0 ± 3.8 %; T3/4 (n=8), mean ± SD=19.8 ± 19.9 %; and p=0.003]. Ki-67 PI was higher in WNETs with lymph node metastasis (N1, n=14, mean ± SD=14.2 ± 16.2 %) than in WNETs without lymph node metastasis (N0; n=8; mean ± SD=6.1± 3.7 %), however, the difference was not statistically significant (p=0.10).

Conclusions: Sporadic (type III) gastric WNETs have a significantly higher Ki-67 PI than non-sporadic (types I and II) gastric WNETs. Gastric WNETs with higher tumor stage are also associated with higher Ki-67 PI.

715 Differences between Hereditary and Sporadic Diffuse Type Gastric Cancer Indicating Different Carcinogenetic Pathways

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Background: Hereditary diffuse gastric cancer (HDGC) is an inherited form of diffuse type gastric cancer (DTGC) related to germline mutations in CDH1 (E-cadherin) gene. We compared morphological and immunophenotypical features of HDGC to sporadic DTGC. Our findings suggest different carcinogenetic pathways for these two types of DTGC.

Design: 21 patients with genetically confirmed HDGC who underwent prophylactic total gastrectomy at our institute were included. 10 sporadic DTGC resections were used as controls. H&E sections were reviewed to identify the presence of in situ signet ring cell carcinoma and invasive signet ring cell carcinoma. Immunohistochemical stains for p16, CEA and CDX2, as well as mucicarmine stain, were performed on selected sections.

Results: The median age of 21 HDGC patients (7 male, 14 female) was 40 years (range, 15 - 60). The median age of 10 sporadic DTGC (2 male, 8 female) was 59.5 years (range, 44 – 77). Among 21 HDGC cases, 7 patients had multiple foci of invasive signet ring cell carcinoma in the superficial gastric mucosa. Two patients had a focus of in situ signet ring cell carcinoma. Two distinct signet ring cell populations were seen in HDGC. The larger signet ring cells beneath the surface epithelium contained abundant mucin and were positive for mucicarmine stain and CEA, negative for p16 and CDX2; on the other hand, groups of smaller signet ring cells, mostly seen at the neck region, had much less mucin and larger atypical nuclei; they were all positive for p16 and negative for CEA, CDX2, and mucicarmine stain. The sporadic DTGC cases showed typical features of poorly differentiated signet ring cell carcinoma. Tumor cells were positive for CDX2 (10/10, 100%), p16 (10/10, 100%), CEA (9/10, 90%), and mucicarmine (4/10, 40%).

Conclusions: About one third ofourHDGC patients were found to have intramucosal signet ring cell carcinoma after prophylactic surgery. In situ signet ring cell carcinoma is very rare and difficult to identify. Signet ring cells in HDGC do not express CDX2 and show two different cellular populations with different location, morphology and immunophenotype, featuring by gain of expression for p16 and loss of expression for CEA as cells become less well differentiated. The signet ring cells in HDGC show different morphology and immunophenotype from those of sporadic DTGC which are essentially all positive for CDX2, p16, and CEA. The morphologic and immunohistochemical differences between hereditary and sporadic DTGC may indicate different carcinogenetic pathways for these two types of gastric cancer.

716 Programmed Cell Death-Ligand 1 (PD-L1) in Mismatch Repair Protein Deficient Colon Cancer: An Immunohistochemical and Genomic Profiling Study

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Background: Programmed cell death-ligand 1 and 2 (PD-L1 and PD-L2) play a role in suppressing the host immune system thereby promoting the survival of cancer cells. Monoclonal antibodies against this immune checkpoint have demonstrated significant beneficial therapy responses in multiple malignancies including mismatch repair (MMR) deficient colorectal cancer (CRC). We studied the pattern of PD-L1 protein expression in MMR deficient CRC by immunohistochemistry (IHC), and the genomic profiling data from an extensive series of advanced stage relapsed/refractory CRCs.

Design: Thirty seven cases of MMR deficient CRCs were identified. The MMR status was determined by either IHC for 4 mismatch-repair proteins (MLH1, MSH2, MSH6 and PMS2), or multiplex PCR. PD-L1 IHC was performed on formalin-fixed paraffin-embedded tissue sections from representative tumor blocks, utilizing rabbit monoclonal PD-L1/CD274 (Spring Bioscience, clone SP142) and ultraView DAB detection (Roche/Ventana). PD-L1 immunoreactivity was evaluated as negative, low positive (1 – 24%) and high positive (= or > 25%) in both the tumor cells (membranous) and tumor infiltrating lymphocytes (TILs) (nuclear/cytoplasmic). Hybrid capture-based comprehensive genomic profiling of 315 genes was performed on 2,268 clinically advanced CRCs. Analytical sensitivity for amplification detection (8 or more copies) in the assay was >95%.

Results: Six (16%) MMR deficient cases were low positive (4) or high positive (2) for PD-L1 within the tumor cells; with MLH1/PMS2 loss (5), and MSH2/MSH6 loss (1). Seven (19%) additional MMR deficient cases with no tumor cell staining were high positive for PD-L1 within the TILs. In the series of 2,268 clinically advanced CRCs, only 5 (<1%) cases demonstrated amplification of *PD-L1* or *PD-L2* by hybrid capture-based genomic profiling.

Conclusions: PD-L1 protein expression by IHC may be used as a sensitive surrogate to identify PD-L1 positive tumors in MMR deficient CRCs (35% in this series). This study also supports previous observations in the literature that MMR deficient CRCs are enriched by PD-L1 overexpression compared to MMR proficient CRCs. *PD-L1/PD-L2* amplification is extremely uncommon in CRC indicating that PD-L1 overexpression occurs most often in the absence of increased gene copy number, e.g., by alterations of functionally related genes. Further study of PD-L1 status and response of advanced CRC to immune checkpoint inhibitors appears warranted.

717 Paired Box 5 (PAX5) Expression in Poorly Differentiated Neuroendocrine Carcinoma of the Gastrointestinal and Pancreatobiliary Tract: Diagnostic and Potentially Therapeutic Implications

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Background: PAX5 plays an essential role in B-cell development and is widely used as a B-cell marker. Immunohistochemical expression of PAX5 has been reported in poorly differentiated neuroendocrine carcinoma (PDNEC) of the lung including small cell carcinoma and large cell neuroendocrine carcinoma (LCNEC). PAX5 regulates the transcription of *c-Met* and Hepatocyte Growth Factor (*HGF*) receptor in small cell carcinoma of the lung, offering a potential for targeted therapy. However, its expression in gastrointestinal (GI) and pancreatobiliary (PB) PDNEC has not been systemically evaluated.

Design: Archived pathology materials from 32 cases of GI and PB PDNEC were retrieved and reviewed. Representative tumor sections were subject to immunohistochemical stain for PAX5 and CD20. The staining extent in tumors was recorded as focal (<10%), patchy (10 to 50%), and diffuse (>50%). The intensity of staining was scored as 1+ to 3+. Seven cases of well-differentiated neuroendocrine tumors (WDNETs) from GI/PB tract served as negative controls.

Results: From the 32 patients [21 male, 11 female; mean age 69 years (range 45-87)], 23 resections, 7 biopsies, and 2 autopsies were included. The primary sites were colorectum (10), gastroesophageal junction (GEJ)/esophagus (7), ampulla (5), pancreas (4), bile duct (3), stomach (1), duodenum (1), and anus (1). Seventeen were LCNEC; 8 of them were combined with adenocarcinoma and 1 with squamous cell carcinoma. Six were small cell carcinoma. Nuclear PAX5 staining was observed in 14 (44%) cases, with diffuse (7), patchy (3) or focal (4) staining. The intensity was 3+ (2), 2+ (6), and 1+ (6). The common patterns were diffuse/ 1+ (4) and patchy /2+ (3). Among the PAX5+ PDNECs, 11 were LCNEC and 3 were small cell carcinoma; the common locations were ampulla (4) and GEJ/esophagus (4). CD20 was negative in all tumors. All 7 WDNETs were negative for PAX5.

Conclusions: PAX5 is commonly expressed in PDNEC of the GI/PB tract (44% in this series), including small cell carcinoma. This observation warrants a cautious approach when interpreting small biopsy of poorly differentiated neoplasms. PAX5 expression is common in ampullary (4 of 5) and GEJ/esophageal (4 of 7) PDNEC. Further study of PAX5-HGF/c-Met signaling pathway and its potential therapeutic value in GI/PB PDNEC is warranted.

718 An Intimate Dance: Patterns and Clinical Relevance of PD-L1 and PD-1 Expression in Colorectal Carcinoma

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Background: Immune checkpoint-blockade targeting the programmed death 1 (PD-1) pathway is an effective treatment for cancer including mismatch repair deficient (dMMR) colorectal cancer (CRC). Whether MMR status is the optimal criteria for anti-PD-L1/PD-1 therapy is unclear. Efficacy of targeting this pathway correlates with the degree of PD-L1 staining in several tumor types. Our study aims to evaluate 1) the frequency and pattern of PD-L1 and PD-1 expression by immunohistochemistry (IHC) in CRC, and 2) their association with clinicopathological features.

Design: The study included 447 CRCs with clinicopathological data, including MMR status by IHC. IHC for PD-L1 (Cell Signaling/E1L3N) and PD-1 (Cell Marque/NAT105) were performed on tissue microarrays. Expression of PD-L1 on tumor epithelium and PD-1 on tumor infiltrating lymphocytes (TILs) was assessed based on the intensity and proportion of cells stained, and converted into a score defined as 0, 1+ or 2+. Associations between PD-L1 and PD-1 expression, MMR status, and clinicopathologic features were assessed by Pearson correlations, Chi-square tests, and Kaplan-Meier estimates.

Results: Of the cases, 56.8% were high stage (III-IV) and 17.0% were dMMR. High levels (2+) of PD-L1 in tumor cells was observed in 19/394 cases (4.8%) and high levels (2+) of PD-1 in TILs was in 76/392 cases (19.4%). PD-L1 levels correlated with PD-1 levels ($p<0.001$). Higher rates and stronger staining intensity for both PD-L1 and PD-1 were observed in dMMR tumors ($p<0.001$ for both). PD-L1 and PD-1 expression were associated with various histologic features related to MMR deficiency, even when controlling for MMR status. Notably, among dMMR CRCs, high PD-L1 expression was associated with medullary type morphology ($p<0.001$) and poor differentiation ($p=0.003$). PD-L1/PD-1 levels stratified recurrence free survival (RFS) only among dMMR tumors: PD-L1 high tumors showed decreased RFS ($p=0.026$). This difference was maintained when tumors were also PD-1 high ($p<0.001$) but lost when they were PD-1 low. PD-1 high tumors showed improved RFS ($p=0.041$). This difference was maintained when tumors were also PD-L1 low ($p=0.006$) but lost when they were PD-L1 high.

Conclusions: High levels of PD-L1 and PD-1 occur in a subset of CRCs that are often dMMR and show specific clinicopathological features. There exists an interdependence between PD-L1 and PD-1 for their negative and positive prognostic impact respectively. Our data shed light on the role of PD-L1/PD-1 in CRC and may guide patient selection for trials aimed at evaluating the effectiveness of anti-PD-L1/PD-1 therapy.

719 Diagnosing Colorectal Medullary Carcinoma: Interobserver Variability and Clinicopathological Implication

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Background: Colorectal medullary carcinoma (MC) has emerged as a histological subtype of favorable prognosis largely due to the frequent presence of mismatch repair deficiency (dMMR). While the World Health Organization (WHO) has diagnostic criteria for MCs, it is not specific. In practice and in the published literature, criteria employed are variable and often subjective, resulting in frequencies ranging from <1% to 5%. We aim to evaluate the interobserver variability of the current WHO criteria in distinguishing medullary carcinoma from non-medullary poorly differentiated carcinomas, and to assess the associated clinicopathological characteristics.

Design: Eighty cases of colorectal adenocarcinomas with ≥50% of the tumor lacking overt gland formation, excluding poorly differentiated neuroendocrine carcinomas and signet ring cell carcinomas, were selected for study. Cases were classified by two methods into MC, indeterminate-type (IT), and poorly differentiated (PDC): 1) by WHO criteria (IT resembling MC but not fulfilling the criteria), and 2) refined criteria created based on thorough morphologic assessment. Five GI pathologists assessed the cases in two separate rounds, first using the WHO criteria, and later using the refined criteria. Results were statistically correlated with clinicopathologic factors.

Results: The WHO criteria resulted in poor inter-observer agreement (κ=0.157; 95%CI, 0.127 to 0.263; p=0.001). Our refined criteria yielded an improved agreement (κ=0.41; 95%CI, 0.274 to 0.497; p<0.001). Classifying our cases using the refined criteria resulted in 19 MCs, 26 ITs, and 35 PDCs. Correlation analyses revealed that 100% of MCs and 85% of ITs showed dMMR by immunohistochemistry, while only 14% of PDCs were dMMR. Similarly, MC and IT tumors were more likely to be right sided and of low stage compared to PDCs. A trend towards better overall survival was observed in both MC and IT tumors compared to PDC group, although this did not reach statistical significance.

Conclusions: Our data suggest that current WHO criteria for MC are inadequate. More specific criteria can classify colorectal adenocarcinomas with solid growth into 3 subtypes that correlate well with mismatch repair status.

720 Ideal Number of Biopsy Tumor Fragments for Predicting HER2 Status in Gastric Carcinoma Resection Specimens

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Background: Intratumoral heterogeneity of HER2 expression is common in gastric cancers and poses a challenge for identifying patients who would benefit from anti-HER2 therapy.

Design: The aim of this study is to compare HER2 expression in biopsy and resection specimens of gastric carcinoma by immunohistochemistry (IHC) and to find the ideal number of biopsy tumor fragments that can accurately predict HER2 overexpression in the corresponding surgically resected specimen. The HER2 IHC results of 702 paired biopsy and resection specimens of gastric cancer were compared.

Results: The mean number of biopsy fragments among all cases was 4.3 (range 1-11). HER2 was positive in 130 (18.5%) endoscopic biopsies and in 102 (14.5%) gastrectomy specimens. Intratumoral heterogeneity of HER2 was found in 80 (61.5%) biopsies and 70 (68.6%) resection specimens. Out of the 70 surgical specimens with intratumoral heterogeneity, 24 (34.3%) of the corresponding biopsies were categorized as negative (positive conversion). In the 86 (12.3%) discrepant cases, negative conversion was observed in 57 (66.3%) cases and positive conversion in 29 (33.7%). The fragment numbers were significantly correlated with the discrepancy of results and positive predictability (P = 0.0315 and P = 0.0052). ROC curve analysis and positive predictability showed that 4 fragments should be obtained to minimize the differences in HER2 scores between biopsy and resection specimen.

Conclusions: In gastric carcinomas with discrepant HER2 results between biopsy and surgical resection specimens, intratumoral heterogeneity is common with most of them showing positive conversion. To predict HER2 status precisely, at least 4 biopsy fragments containing tumor cells are required.

721 Mutation Profile of Colorectal Neuroendocrine Neoplasm

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Background: High grade neuroendocrine carcinoma (NEC) is a rare histologic subtype of colorectal carcinoma (CRC), accounting for <1 % of all tumors of the large intestine. However, colorectal NEC is a highly aggressive malignancy that does not response well to conventional chemotherapy. Although allelic imbalance involving *TP53, DCC* and *APC* has been reported, the distinct underlying molecular alteration for neuroendocrine differentiation (NED) of CRC have not been identified. We investigated overall mutational profile of NEC, mixed adenoneuroendocrine carcinoma (MANEC) and adenocarcinoma with NED by next-generation sequencing (NGS).

Design: We screened 1802 consecutive primary and metastatic CRCs subjected to molecular profiling using NGS on the Ion Torrent PGM and Ion Proton (Life Technologies). We examined substitution and small indels in 46/50/409 cancer-related genes sequence alignment and analysis were performed using Torrent Suite software (Life Technologies) and lab-developmented software (OncoSeek). We examined the mutation status in respect to tumor histology.

Results: We found 24 cases (1.33%) of CRC with NED consisting of 13 cases of NEC, 8 cases of MANEC and 3 cases of poorly differentiated adenocarcinoma with NED. Mutations were detected in 21 (88%) cases of CRC with NED (table 1). In colorectal neuroendocrine neoplasms, *KRAS* gene was frequently co-mutated with *TP53* and/or *APC* genes (12 cases, 50%).

Conclusions: Overall, colorectal neuroendocrine neoplasm has shown frequent single or co-mutations of *APC, KRAS,* and *TP53* genes. Particularly, *KRAS* gene was frequently co-mutated with *TP53* and/or *APC* genes. Single or combined activating or inactivating mutation of these genes may be a main oncogenic mechanism of colorectal neuroendocrine neoplasm suggesting a promising consideration of targeted therapy by inhibiting related pathways.

	Total N=24 (100%)	NEC N=13 (54%)	MANEC N=8 (33%)	CA with NE N=3 (100%)
Mutations	21(88%)	10(77%)	8(100%)	3(100%)
Co-mutations	18(75%)	9(69%)	6(75%)	3(100%)
Tumor suppressor gene				
APC	12(50%)	7(54%)	3(38%)	2(67%)
TP53	9(38%)	3(23%)	3(38%)	3(100%)
RB1	2(8%)	2(15%)	1(13%)	0
PTEN	2(8%)	0	1(13%)	1(33%)
FBXW7	2(8%)	2(15%)	0	0
CDKN2A	1(4%)	1(8%)	0	0
Oncogene				
KRAS	12(50%)	6(46%)	6(75%)	0
BRAF	4(17%)	2(15%)	1(13%)	1(33%)
GNAS	3(13%)	2(15%)	1(13%)	0
ERBB2	1(4%)	1(8%)	0	0
PIK3CA	1(4%)	0	1(13%)	0
FGFR3	1(4%)	1(8%)	0	0
PTPN11	1(4%)	1(8%)	0	0
SMO	1(4%)	0	1(13%)	0

722 Positive Fungal Testing Associated with Higher Grade Graft Versus Host Disease and Increased Mortality in Stem Cell Transplant Recipients

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Background: Graft-versus-host disease (GVHD) is a major cause of morbidity and mortality in stem cell transplant recipients. Definitive diagnosis is based on clinicopathologic correlation. Bacterial and viral pathogens are known to lead to exacerbation of acute GVHD. Fungal polysaccharides in animal models were recently found to exacerbate GVHD in multiple organs, possibly through inducing production of IL-17A by donor T cells. We seek to answer whether fungal infection is associated with severity of GVHD in humans.

Design: The pathology database was searched for diagnoses of GVHD. Inclusion criteria included available biopsies of liver or digestive tract. Patients with positive cytomegalovirus immunostains, and mycophenolate toxicity were excluded. Remaining cases were divided into three groups: negative for GVHD, mild (I-II) GVHD, and severe (III-IV) GVHD. Fungal infection status was determined by concurrent (1,3)-beta-D-glucan assay, individual fungal antigen tests, fungal cultures, and histologic examination.

Results: Of 110 biopsies identified, 58 biopsies from 44 patients met inclusion criteria. Thirty-three of 58 biopsies were positive for GVHD. Negative biopsies served as a control group. Mean age was similar between test and control groups (52.6 vs 52 years). Race and sex varied between test and control groups (40% vs 62% Caucasian, 70% vs 48% female). Multi-organ GVHD was present in 18 patients. Primary diseases were hematologic with acute myeloid leukemia being the most common. Transplant types include: 7% autologous, 25% sibling, 68% unrelated. Fungal tests (FT) were positive in 4.0% of control patients, 0% of mild GVHD patients (p=0.34), and 45% of severe GVHD patients (p=0.002). Fungal organisms were not visualized in biopsy material. Clinical outcomes for the severe group were significantly worse when they had concurrent fungal infections. Eighty-three percent of FT positive patients in the severe group died from consequences of GVHD vs 50% of FT negative patients (p=0.04).

Conclusions: Positive FT has a strong association with severe GVHD when compared to mild GVHD and control patients and is associated with a high mortality. Fungal testing, including (1,3)-beta-D-glucan assay, may aid in determining prognosis in patients with severe GVHD.

723 An Unusual Case of Colitis Cystica Profunda Co-Existing with Adenocarcinoma: Literature Review and Case Comparison

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Background: Colitis cystica profunda (CCP) is a benign lesion characterized by the presence of intramural mucus-containing cysts in the colon and rectum. CCP has been associated with inflammatory diseases such as Crohn's disease. The invasion level of CCP varies from mucosa muscularis to submucosa to muscularis propria. CCP at all three levels is reminiscent of colonorectal adenocarcinoma (ColRecCA). Studies have shown that due to its unique histopathological features, CCP may be difficult to differentiate from ColRecCA. The task is more difficult when these two conditions coexist in one patient.

Design: Literature was reviewed, and only two cases of simultaneous CCP and ColRecCA were reported. These two cases were compared with a new case, in which a 71-year-old woman with a history of cervical cancer completed radiotherapy and chemotherapy and later was diagnosed with a rectal adenocarcinoma (RecCA). The subsequent rectal resection specimen revealed, besides the RecCA, a submucosal lesion (CCP) 2 centimeters from the primary malignancy, which had not been noticed by the surgeon.

Results: In the new case, histopathological examination showed mucus-containing cysts residing deep in the muscularis propria, whereas the level of CCP residing in the two case reports in the literature were much shallower (submucosa only). Stromal reactions such as stromal fibrosis or inflammatory cell infiltrates were not seen in any of the three cases. Further immunohistochemical (IHC) stainings, including p53, Ki-67, and MSH2/MLH1, were executed on the new case, and the results showed distinct differences between the CCP and the RecCA. No IHC studies were performed in either of the past case reports.
Conclusions: The striking invasion pattern of CCP mimics and may be mistaken for ColRecCA. With proper histopathological examination and IHC methods, differentiating these two disease entities may be less challenging.

724 Dual Stain with SATB2 and CK20 Is Useful to Distinguish Colorectal Carcinoma from Other Tumors

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Background: Small samples limit the number of IHC stains that may be attempted in colorectal carcinomas (CRC) biopsies. A transcription factor, special AT-rich sequence-binding protein 2 (SATB2), is a sensitive and highly specific marker for CRC. Since both SATB2 and CDX2 are nuclear markers, we evaluated their effectiveness in identifying CRC by a dual stain (DS) with a cytoplasmic marker CK20 or Villin.
Design: Tissue microarrays with 2 cores (1mm) of each222 CRC and 375 other carcinomas (45 breast, 67 hepatocellular, 50 lung, 32 neuroendocrine, 18 ovary, 40 pancreas, 45 prostate and 78 stomach) were built. DS were performed pairing nuclear stains CDX-2 or SATB2 with CK20 or Villin. Stains were graded as positive or negative, with a 5% threshold. DS were interpreted in 2 ways; both stains positive necessary for a positive result and either stain positive necessary for a positive result. Sensitivity and specificity of each dual stain were calculated with exact binomial 95% confidence intervals (CI), and statistical analyses were performed to compare sensitivity and specificity for paired comparisons by McNemar's test.
Results: All four DS showed excellent sensitivity (99-100%) if either stain positive was considered a positive DS. SATB2 DS showed a higher specificity than CDX2 DS with a comparable sensitivity. Sensitivities ranged from 90 to 96% if both stains positive were necessary for a positive DS. DS with villin showed the lowest specificity, regardless of the way DS results were combined.

	stain	sensitvity (95% CI)	specificity (95% CI)
dual stains - both positive	CDX2+CK20	0.92 (0.88, 0.95)	0.93 (0.89, 0.95)
	CDX2+villin	0.96 (0.93, 0.99)	0.88 (0.84, 0.91)
	SATB2+CK20	0.90 (0.86, 0.94)	0.98 (0.96, 0.99)
	SATB2+villin	0.93 (0.89, 0.96)	0.97 (0.94, 0.98)
dual stains - either positive	CDX2+CK20	1.00 (0.98, 1.00)	0.71 (0.66, 0.75)
	CDX2+villin	1.00 (0.98, 1.00)	0.53 (0.48, 0.58)
	SATB2+CK20	0.99 (0.96, 1.00)	0.69 (0.64, 0.74)
	SATB2+villin	0.99 (0.97, 1.00)	0.47 (0.42, 0.52)

Conclusions: SATB2 DS shows higher specificity than CDX2 DS with comparable excellent sensitivity. DS with CK20 performs better than DS with villin. Therefore, DS with SATB2 and CK20 may be helpful for small biopsies when the materials are limited.

725 Microscopic Crohn's Disease Activity at Resection Margins Is Not Significantly Associated with Post-Operative Clinical Outcomes

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Background: Most Crohn's disease (CD) patients eventually require at least 1 surgical resection in their lifetime; however effectiveness of this therapy is limited by high relapse rates. Prognostic parameters can be useful to help guide post-operative therapy; however, there are conflicting data and varying practice patterns centered around reporting and management of microscopic CD activity at resection margins. Therefore, the aim of this study was to evaluate the clinical significance of microscopic CD at surgical resection margins.
Design: 68 consecutive bowel resections for complicated Crohn's disease were retrospectively collected from Jan 2005-Jan 2015. Resection margin slides were reviewed and CD activity at margins graded as none, mild, moderate, severe. Microscopic CD activity at the margin – assessed as dichotomous (none vs. any) and ordinal (none vs. mild vs. moderate vs. severe) – was correlated with post-operative clinical recurrence (via Harvey-Bradshaw Index score), radiographic recurrence (via CT or MR enterography) and endoscopic recurrence (via colonoscopy, EGD, or capsule) using ordinal logistic regression and chi-square analysis.
Results: Ileocolonic resection was the most frequent surgery (88.2%) followed by ileum alone (5.8%). Stricture (63.2%) was the most common indication for surgery, and both stricture and fistula affected 16.2% of patients. Most patients (70.6%) failed pre-surgical anti-TNF and immunomodulator treatment, and almost half the patients (44.1%) received no prophylactic medication post-operation. Nearly half (47.0%) of original pathology reports did not mention presence or absence of CD activity at resection margins. Following review of all margin slides, 58.8% of cases were negative, 23.5% mild, 5.9% moderate, and 11.8% had severe CD activity. There was no significant correlation of post-operative clinical, radiographic or endoscopic recurrence with CD microscopic activity at the resection margins, regardless of presence or severity of CD activity ($p>0.05$).
Conclusions: Our study highlights differences in pathology practice patterns when reporting CD resection specimens, as disease activity at the margins was only reported in half of cases. However, microscopic CD activity at resection margins does not appear to significantly predict post-operative outcomes and therefore may not be important to assess and/or report.

726 Reduced Expression of Argininoscuccinate Synthetase 1 Has a Negative Prognostic Impact in Patients with Pancreatic Ductal Adenocarcinoma Treated with Neoadjuvant Therapy and Pancreaticoduodenectomy

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Background: Argininosuccinate synthetase1 (ASS1), the rate-limiting enzyme for arginine biosynthesis, is expressed in many malignant human tumors, including pancreatic ductal adenocarcinoma (PDAC). Recently, ASS1 deficiency was reported to be associated with clinical aggressiveness in nasopharyngeal carcinoma, myxofibrosarcoma and bladder cancer, which might be attributed to the newly identified tumor suppressor function of ASS1. The goal of this study was to evaluate the prognostic impact of ASS1 expression in PDAC treated with neoadjuvant therapy and pancreaticoduodenectomy (PD).
Design: The study population was comprised of 122 patients with PDAC who have completed neoadjuvant therapy and PD at our institution between 1999 and 2007 (49 women and 73 men with a median age of 62.7 years). Immunohistochemical staining for ASS1 was performed on tissue microarray slides, which contain two representative 1.0 mm cores from each tumor. The staining results are graded by combined score of the intensity of cytoplasmic staining (0-negative, 1-weak, 2-moderate, and 3-strong) and the percentage of positive tumor cells. The expression of ASS1 was categorized as ASS1-low and ASS1-high using the combined score 1.5 as a cutoff and was correlated with clinicopathologic parameters and survival using SPSS Statistics.
Results: Eighteen (15%) patients were ASS1-low and 104 (85%) patients were ASS1-high. Patients whose tumors were ASS1-high had better overall survival (35.28 ± 2.78 months) than those whose tumors were ASS1-low (16.47 ± 5.23, $P=0.04$). In multivariate analysis, expression of ASS1 (HR: 0.56, 95% CI: 0.32-0.97, $P=0.04$) and lymph node metastasis (HR: 1.57, 95% CI: 1.004-2.47, $P=0.048$) were independent prognostic factors for overall survival. However, we did not observe significant correlation between ASS1 expression and other clinicopathologic parameters ($P>0.05$).
Conclusions: Our study showed that 15% of treated PDAC has low expression of ASS1. ASS1-low is an independent prognostic factor for poor survival in patients with PDAC who completed neoadjuvant therapy and PD. Patients whose tumors are ASS1-low may benefit from targeted therapy using pegylated arginine deiminase.

727 Tumor Deposits and Venous Invasion in Colorectal Cancer: Clarification of Morphologic Criteria and Role of These Parameters in Staging

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Background: Tumor deposits (TD) are discrete tumor foci in peri-colic/rectal fat with no residual lymph node and should be separately recorded (AJCC 7th ed). The CAP/AJCC guidelines also recommend recording of venous invasion (VI) and perineural invasion (PNI), both of which are well-established adverse prognostic factors. Hence it appears that the AJCC had intended tumor foci representing extramural VI and PNI to be recorded separately, and not be considered as TD. Since the AJCC definition is somewhat unclear, it leads to variable interpretation of criteria for TD.
Design: Colorectal cancers (n=85) from 2000-2015 in which TDs had been reported were reviewed. Elastic stain was performed on all cases in which VI was not obvious on H&E. Tumor foci originally classified as TDs were reviewed and reclassified as venous invasion (VI), perineural invasion (PNI), positive lymph node (LN), or TD-not otherwise specified (NOS). Tumor foci were considered VI when the vein wall was discernible on H&E or elastic stain. The influence of revised categorizations on stage and outcome was examined.
Results: Of 294 TD originally reported in 85 patients, 150 (51%) were reclassified as VI, 9 (3%) as positive LN, and 8 (3%) as PNI. The remaining 127 (43%) were considered TD-NOS. 21/85 (25%) cases were originally staged as N1C; revised categorization led to reclassification of 9 cases (11%) to N0 with VI and change of pathologic stage from III to II. The 5-year survival in LN-negative VI cases was similar to N1a and N1c disease (75% vs 80% vs 75%,p=0.5). 5-year survival in VI cases (without TD-NOS) and TD-NOS cases (without VI) was similar (55% vs. 59%,p=0.5).

	Venous Invasion	TD(NOS)	PNI	LN
Tumor Deposits (n=294) Based on pathology report	150 (51%)	127 (43%)	8 (3%)	9 (3%)

Conclusions: Majority of TDs can be identified as VI on review by H&E and elastic stains. The way TDs are defined can influence stage II vs. stage III categorization in 11% of cases,which can have major implication on treatment. We advocate the use of a clear definition of TDs that excludes identifiable LN, VI and PNI. Vascular involvement reflects spread of primary tumor and we recommend updating the AJCC staging scheme for this feature to be included in T category (similar to other sites like liver, pancreas) rather than N1c. Since VI has an adverse influence on outcome similar to positive LN and TD-NOS, VI should be included as a criterion for stage III disease irrespective of N status.

728 A Prospective Evaluation of Incidental Fundic Gland Polyps with Epithelial Dysplasia: Implications for Clinical Management

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Background: Fundic gland polyps (FGPs) are the most common gastric polyp; rarely they exhibit dysplasia of the overlying foveolar epithelium. Based on retrospective data, FGPs with dysplasia (FGPDs) are thought to be a strong marker for familial adenomatous polyposis (FAP). Sporadic, non-syndromic FGPDs also occur (up to 3% of all FGPs). Due to the significant syndromic association, diagnosis of an apparently sporadic FGPD prompts additional clinical evaluation for FAP, especially its attenuated variant. The main purpose of this study is to prospectively evaluate a series of incidental FGPDs to determine the positive predictive value of this finding for FAP. In addition, we characterize the clinicopathologic features of these FGPDs to advance our understanding and management of these lesions.

Design: All FGPDs from our institutional archives were identified from January 2004-July 2015, defining incidental cases as those without a diagnosis of FAP at the time of the biopsy. H&E stained sections of each incidental FGPD were reviewed by three gastrointestinal pathologists for consensus diagnosis and inclusion into the study. Clinical chart review was conducted to determine the clinical presentation, associated endoscopic findings, and clinical management in each case.

Results: From a total of 2526 FGPs, we identified 212 FGPDs (8.4%), 28 of which were confirmed as incidental (13.2%), while 179 were syndromic (84.4%) and from our institution's large FAP registry. Most incidental FGPD patients presented with epigastric pain/dysphagia (71%), and were on proton-pump inhibitor therapy (61%). Three patients had a family history of polyps or other gastrointestinal cancers. Clinical management was variable and included repeat endoscopy for complete resection of the polyp (43%), endoscopic surveillance at 0.5-2 year intervals (57%), no specific recommendation (21%), colonoscopy (14%), genetic counseling referral (3%) and unknown (7%). None of the 28 patients were found to have clinical evidence of a polyposis syndrome.

Conclusions: Although most FGPDs are indeed associated with FAP, incidental, sporadic FGPDs are also rarely encountered (28 of 2526 total FGPs, or 1.1%). In our series, sporadic FGPDs were not a harbinger of FAP, and in the absence of other clinical features, this isolated finding does not appear to warrant follow-up genetic services or testing. Other current management strategies, including frequency of endoscopic surveillance, should also be re-evaluated.

729 Histologic Features of Gastrointestinal Tract Biopsy in Henoch-Schönlein Purpura

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Background: Henoch-Schönlein purpura (HSP) most commonly occurs in the pediatric population, with rare cases also occurring in adults. Gastrointestinal (GI) involvement is common. The typical histologic finding in HSP is leukocytoclastic vasculitis (LCV); however, the other histologic features in biopsies of HSP have only been rarely described.

Design: The pathology files at our institution were searched for GI biopsies from patients with HSP. Slides were retrieved and histologic and clinical features were reviewed.

Results: We identified 13 patients with HSP with GI biopsy series, including both adult and pediatric patients. The most common histologic finding was lamina propria hemorrhage (12/13 cases) with many cases also showing lamina propria fibrin deposition with red cell sludging and nuclear debris (7 cases). 10/12 patients with duodenal biopsies available showed acute duodenitis; 2 of these cases showed a severe erosive duodenitis. Several cases showed an eosinophilic infiltrate. 5/8 cases with jejunal or ileal biopsies showed acute jejunitis or ileitis. Out of 10 colorectal biopsies, and acute colitis or proctitis was observed in 6 cases. Six cases showed the presence of LCV; in each of these cases, the involved vessels were small capillaries within the lamina propria. Only 1 of these 6 cases showed deeper submucosal vessels within an involved biopsy; of note, the deep submucosal vessels were uninvolved. Sites involved by LCV included the colorectum (2 cases), colorectum and terminal ileum, terminal ileum only, duodenum, and jejunum (1 case each). All patients presented with abdominal pain, with 10/13 showing a rash. Other presenting symptoms included diarrhea and/or hematochezia (7 cases), nausea/vomiting (4 cases), and intussusception (1 case). 3 patients had concurrent skin biopsies showing LCV; only 1 of these patients had LCV on GI biopsy. Indications for biopsy included non-specific presenting symptoms, lack of rash, and/or failure to respond adequately to steroid therapy.

Conclusions: The histologic features of HSP, aside from LCV, are often thought to be non-specific. The presence of hemorrhage and lamina propria fibrin deposition with an associated neutrophilic infiltrate should prompt a diligent search for vasculitis. The duodenum is commonly affected and shows ulcerative duodenitis with necrosis in severe cases. LCV is present in a subset of cases; however, the findings are often focal and may be easily overlooked in a background of active duodenitis or colitis. Because the lamina propria vessels are commonly involved, HSP-associated LCV may be diagnosed on superficial biopsy specimens.

730 Gastrointestinal Tract Vasculopathy: A Single Institution Experience

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Background: Systemic vasculitic diseases may infrequently show gastrointestinal (GI) manifestations, and are often overlooked when forming differential diagnoses due to their rarity. Involvement of the GI tract may lead to serious complications, including ischemia and perforation; thus, awareness of the types of systemic vasculitis that may involve the GI tract is central to arriving at a correct diagnosis.

Design: 11 cases of GI tract vasculopathy (excluding Henoch-Schönlein purpura) were collected at our institution over a 20 year period and histologic and clinical features were reviewed.

Results: Out of the 11 cases, 10 presented with symptoms related to the vasculopathy (including 2 presenting with a mass on endoscopic exam); the remaining patient presented with an incarcerated hernia and vasculitis was an incidental finding. 6 cases were isolated to the GI tract, and 5 cases had accompanying systemic disease. For the latter 5 cases, the underlying diagnoses included systemic lupus erythematosus (2 cases), and 1 case each of granulomatous polyangiitis (GPA), dermatomyositis, and Churg-Strauss. Out of these cases, 3 patients had a known prior diagnosis. The patient with GPA and one of the two patients with lupus did not have a prior diagnosis; both initially presented with GI symptoms. The patient with GPA presented with a large cecal mass concerning for malignancy, which on biopsy showed necrotizing arteritis. Further workup showed bilateral otitis media and granulomatous pulmonary nodules, consistent with GPA. The patient with lupus presented with weight loss and bloody diarrhea; colon biopsies showed leukocytoclastic vasculitis. The 6 cases of isolated GI tract vasculopathy included 2 cases of fibroproliferative vasculopathy without inflammation (late stage mesenteric veno-occlusive disease), 1 case of granulomatous venulitis, 1 case of granulomatous arteritis, 1 case of necrotizing arteritis and venulitis, and 1 case of enterocolic (lymphocytic) phlebitis.

Conclusions: Gastrointestinal tract vasculopathies are rarely diagnosed on either biopsy or resection specimens; a large proportion of cases are isolated to the GI tract. Systemic vasculitis may uncommonly present with GI symptoms, and clinical correlation is required when vasculitis is identified histologically. Only when systemic involvement is excluded can a diagnosis of isolated GI tract vasculitis be rendered, which if truly localized should have a good prognosis. Knowledge of the spectrum of vasculopathies that may present in the GI tract is essential, particularly in cases where GI biopsy is the first step towards the diagnosis of a systemic disease.

731 The Notch Pathway Is Activated in Neoplastic Progression in Esophageal Squamous Cell Carcinoma

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Background: The Notch signaling pathway is integral to human development and homeostasis, where it has a deterministic function on cell differentiation. Activation of the transmembrane Notch receptor results in gamma-secretase enzymatic cleavage of the intracellular domain, which translocates into the nucleus to form a Notch transcriptional complex. Abnormalities in Notch signaling may contribute to neoplastic progression, a pathologic process for which targeted therapy by gamma-secretase inhibitors may prove efficacious. Exome sequencing studies of esophageal squamous cell carcinomas (SCCs) have identified NOTCH1 activating mutations in a subset of cases. We hypothesized that an immunohistochemical stain targeting the Notch1 intracellular domain (NICD) would reveal increased nuclear expression in esophageal SCCs compared to normal epithelium and an inverse relationship between NICD expression and differentiation in SCCs. We were also interested in the relationship between NOTCH1 activity in esophageal SCCs and survival.

Design: 61 biopsy cases of esophageal SCC and 40 cases of benign esophageal squamous epithelium were identified at our institution from 2007 through 2015. An immunohistochemical stain for NICD [Cleaved Notch1 (Clone D3b8) (Val1774); CELL SIGNALING 4147; Rabbit mAb; 1:20 dilution] was performed on all cases (formalin fixed paraffin embedded tissue), and nuclear staining intensity was graded using the Vectra imaging system and Inform 2.0.2 software (PerkinElmer). Clinicopathologic data (age at diagnosis, gender, smoking status, tumor grade, keratinization, tumor location, and survival) were collected for each SCC case.

Results: Compared to benign epithelium, esophageal SCC cases had 1.6-fold higher levels of nuclear NICD staining ($p<0.0005$). Among esophageal SCC cases, those with poor differentiation demonstrated 1.4-fold higher NICD expression than well-differentiated cases, and those with distant metastases at diagnosis demonstrated 1.4-fold higher NICD expression than those without ($p<0.05$). There was a trend between NICD expression and decreased survival.

Conclusions: NOTCH1 activation is an important mechanism in neoplastic transformation in esophageal SCC, where it may function to inhibit differentiation, accounting for the increased expression associated with poor differentiation. There was a trend toward decreased survival with increased NICD expression, a relationship that will be explored in future study. Our study raises the possible treatment of esophageal SCCs with therapies targeted toward the NOTCH pathway.

732 Goblet Cell Carcinoid of the Appendix – An Interobserver Variability Study Using Two Proposed Classification Systems

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Background: Goblet cell carcinoid (GCC) is an uncommon tumor of the vermiform appendix, with dual features of an endocrine and glandular neoplasm. Due to a broad spectrum of morphological differentiation, GCCs remain a challenging entity for pathological classification. Recently, two separate systems have proposed classifying GCC tumors into three (classical GCC; adenocarcinoma ex-GCC, signet ring cell type; adenocarcinoma ex-GCC poorly differentiated carcinoma) OR two subgroups (low and high grade GCC) based on morphological criteria. No study has independently compared the inter-observer variability associated with each new classification system.

Design: 6 pathologists (3 subspecialty trained GI and 3 general anatomical pathologists) reviewed one representative slide selected from each of 20 previously diagnosed GCC cases. Each participant was blinded to the original diagnosis and independently scored each case using both classification systems. Data was analyzed using SPSS software

to determine Fleiss' kappa (κ) and the average pairwise percent agreement for each scoring system. The κ scores were assessed as having slight (0.01-0.2), fair (0.21-0.40), moderate (0.41-0.60), substantial (0.61-0.80) or almost perfect (0.81-0.99) agreement between pathologists.

Results: Table 1 shows the percent agreement and kappa overall, and for subgroups of GI and non-GI pathologists. Overall, the two tiered system showed better agreement than the three tiered system. GI trained pathologists had substantial agreement for both two and three-tiered systems (0.65 vs. 0.65). Non-GI trained pathologists had lower overall agreement than GI trained pathologists, but their agreement was better using the two tiered system (0.44) than the three tiered system (0.22).

Rating System	% agreement	Kappa	95% CI
3 tier - All raters	62	0.42	0.34-0.50
2 tier - All raters	78	0.54	0.43-0.66
3 tier - GI subspecialty	77	0.65	0.47-0.83
3 tier - general	53	0.23	0.05-0.41
2 tier - GI subspecialty	83	0.65	0.40-0.91
2 tier - general	73	0.44	0.19-0.70

Conclusions: There is substantial agreement among GI pathologists using both two and three-tiered classification systems. However, agreement among non-GI trained pathologists, often the first to identify GCC in appendectomies, is less impressive. Research on methods to improve agreement is warranted.

733 A Modified Tumor Regression Grade Incorporating Lymph Node Involvement for Assessing Response to Neoadjuvant Chemotherapy in Oesophageal Cancer

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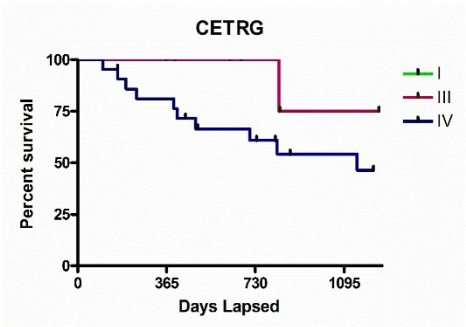
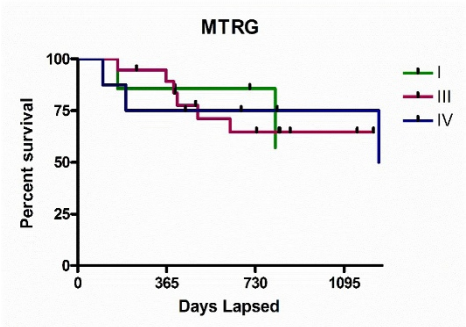
Background: Mandard tumor regression grade (MTRG) is widely utilized for evaluating pathological response to chemotherapy. Major limitation in its use is omission of lymph node metastases. We recently devised a modified tumor regression grade system incorporating lymph node metastases (Cork Tumor Regression Grade; CTRG) for use in breast cancer, where cases were separated into validated MD Anderson Residual Cancer Burden survival classes. The aim of this study is to develop a simple and easy-to use tumor regression grading system for oesophageal carcinoma (Cork Esophageal Tumor Regression Grade; CETRG), and determine its value in predicting survival following neoadjuvant chemotherapy, in comparison with MTRG.

Design: 33 cases of oesophageal cancer treated with neoadjuvant chemotherapy were retrieved. Pathological response to neoadjuvant therapy was evaluated as per the standard 5-tiered MTRG. CETRG was devised as a 5-tiered system, where cases with lymph node metastasis were upstaged by two grades. Clinicopathological correlation was performed for all cases. Kaplan Meier survival analysis was utilized to compare patients' survival.

Results: Cases with MTRG 1, 2,3,4,5 had a 3-year survival of 50, 65, 65, 85, 0% respectively. On applying CETRG, grades 1, 2, 3, 4, 5 had a 3-year survival of 100, 100, 75, 65, 45% respectively. Combining CETRG groups 1 and 2, and 4 and 5, gave three distinct survival classes; I (1, 2), II (3), (III) 4 and 5, which had 100, 75 and 50% 3-year survival respectively. Kaplan Meier analysis curves demonstrated better separation with CETRG (figure 1), as compared to MTRG (figure 2).





Conclusions: CETRG provides a simple and easily applicable grading system for pathological response to neoadjuvant in oesophageal carcinoma. Correlation with survival requires validation in a larger future cohort.

734 Prognostic Significance of Venous-Associated Colorectal Cancer Tumor Deposits Identified by Elastic Tissue Staining

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Background: Metastatic tumor deposits (TDs) in colorectal cancer resection specimens are clinically adverse but pathologically heterogeneous, corresponding variously to replaced lymph nodes, venous invasion (TD/v), neural invasion (TD/n) or unclassified metastases. We hypothesized that stratification of patients following primary cancer resection based on the type of TDs might correlate with the likelihood and anatomical patterns of metastatic progression, and in particular that subclassification of TDs with the help of elastic tissue staining might be more informative than H&E alone.

Design: 44 randomly selected primary colorectal cancer resections (34 colonic, 6 rectal, colorectal 4) with or without nodal disease but with presence of TDs recorded in the pathology reports underwent joint review of all available H&E slides by 2 observers. Discontinuous pericolorectal lesions located 5 mm or more from the leading tumor edge and lacking any evidence of lymph node architecture were evaluated for evidence of venous invasion in routinely stained and parallel EVG-stained slides and classified as TD/v, TD/n or unclassified TD.

Results: 84 TDs were identified, none of which were readily recognizable as TD/v or TD/n in routine stains. Following EVG staining, we identified 9 TD/v (11%) corresponding to 8 cases and identified 9 TD/n (11%) corresponding to 4 cases by differential counterstaining. The remaining 66 TDs remained unclassified. Review of medical records (mean follow-up 16.25 mo, range 1-53) showed distant metastases in 5/8 (62.5%) patients with TD/v, 1/4 (25%) patients with TD/n and 9/67 (22%) patients with unclassified TDs (some of these cases overlapped), reflecting a trend toward more frequent distant metastases in the TD/v group (TD/v vs TD, p=0.09). There were no significant differences among the tumors with and without TD/v with respect to duration of follow-up (p=0.8) or to T and N stages (p=0.21 and 0.17, respectively).

Conclusions: Subclassification of TDs by means of selective tissue staining may afford prognostic information independently of T and N stage.

735 A Combined Deposition of Lanthanum and Hemodialysis-Associated Amyloid in the Gastroduodenal Mucosa: An Immunohistochemical, Electron Microscopic and Dispersive X-Ray Spectrometric Analysis

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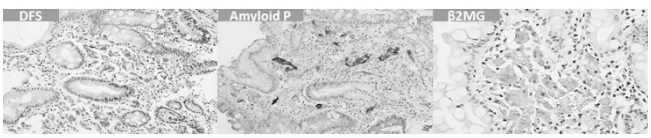
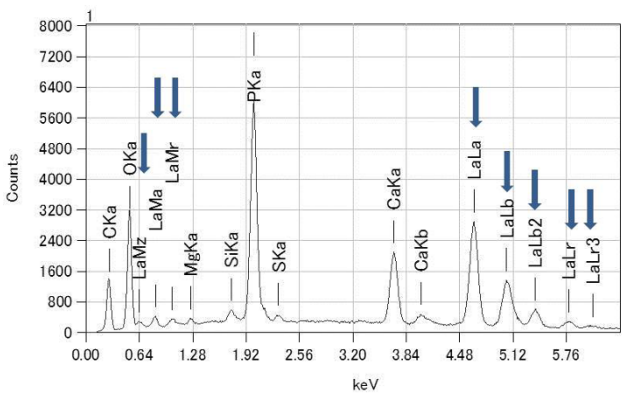
Background: Lanthanum carbonate (LC) is a new type of phosphate adsorbent used to treat patients with hyperphosphatemia. Recent studies have shown lanthanum deposition in the cytoplasm of histiocytes in the gastroduodenal mucosa of hemodialysis patients. On the other hand, it is well known that hemodialysis patients can develop deposition of b2-microglobulin-related amyloid (Ab2M). However, Ab2M deposition in gastrointestinal (GI) mucosa has been thought to be very rare, occurring only in vessel walls and muscularis propria. In contrast to AA amyloid, biopsy of GI mucosa has been considered to have little significance in detecting Ab2M deposition.

Design: Six hemodialysis patients taking LC for more than a year complained of upper abdominal discomfort and/or epigastric pain. All exhibited mild erosion with a finely granular/slightly nodular mucosal surface on endoscopy. Gastroduodenal biopsy showed accumulation of eosinophilic histiocytes in the mucosa. Immunohistochemistry (IHC), transmission and scanning electron microscopies (TEM and SEM) and electron dispersive spectrometry (EDS) were employed to investigate the cause of the unusual histologic findings.

Results: The histologic findings were due to a combined deposition of lanthanum and Ab2M in the cytoplasm of histiocytes. The deposition of Ab2M was confirmed by IHC and TEM, and that of lanthanum was by TEM and SEM/EDS.





Conclusions: This is the first report of such a peculiar combined deposition of lanthanum and Ab2M in the mucosa of hemodialysis patients with GI symptoms. Although the

exact mechanism of combination and pathogenesis is unclear, we believe that GI histologic examination should be considered in the careful follow-up and observation of hemodialysis patients taking LC.

736 Conventional Risk Stratification Fails to Predict Progression of Succinate Dehydrogenase-Deficient Gastrointestinal Stromal Tumors

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Background: Gastrointestinal stromal tumors (GISTs) that lack kinase mutations often show loss of function of the succinate dehydrogenase (SDH) complex, due to germline mutation or promoter hypermethylation. SDH-deficient GISTs are exclusive to the stomach and have a multinodular architecture. Conventional risk stratification criteria may not predict outcome, although data are limited. Here, we present the clinical, histologic, and genetic findings from a large cohort of SDH-deficient GISTs.

Design: SDH-deficient GISTs diagnosed from 2005-2015 were identified based on histologic features or family history. Immunohistochemistry for SDHB and SDHA was performed on all cases. Clinical history was recorded from electronic medical records.

Results: 71 cases were identified (10% of gastric GISTs; 42 female/29 male; mean age 32 yrs; range 12 to 71 yrs; 10 patients ≥50 yrs). SDHB was lost in all cases; SDHA was lost in 27 (38%) tumors (indicating *SDHA* mutation). In 31%, the primary tumor was multifocal; tumors ranged from 1.9 to 22.5 cm. Mitotic rate ranged from 1 to >80 per 5 mm^2. Lymph node metastases were found at primary resection in 13 (18%) patients; 22 (31%) had distant metastases at presentation, and 46 (65%) on follow-up, most often to liver, but also bone, lungs, breast, and brain. Applying conventional criteria (size and mitotic rate), 60-65% of patients with tumors ranging from very low risk to high risk for progressive disease developed distant metastases, regardless of category. Carney-Stratakis syndrome (CSS) and Carney triad (CT) were diagnosed in 6 and 7 patients, respectively. Of 35 patients tested, 25 harbored SDH mutations (11 *SDHA*, 7 *SDHB*, 6 *SDHC*, 1 *SDHD*). In CSS patients, 3 of 4 tested had germline mutations (1 each *SDHB*, *SDHC*, *SDHD*). In CT patients, 1 of 3 tested had a germline *SDHC* mutation; 3 other tumors showed loss of SDHA. Follow-up was available for 63 patients, ranging from 1 month to 39 yrs (mean 8.6 yrs); 19 patients had no evidence of disease (mean 6 yrs), 30 were alive with metastases (mean 11 yrs), and 14 died of disease (mean 7 yrs; range 9 mos-24 yrs).

Conclusions: SDH-deficient GISTs account for 10% of gastric GISTs, can be reliably diagnosed based on multinodular architecture, and are associated with a high rate of distant metastasis, regardless of conventional risk category. Many affected patients have germline SDH mutations (most often *SDHA*). Identification of SDH-deficient GISTs is critical for prognostication and genetic counseling.

737 CDX2 as a Prognostic Marker in Gastric Adenocarcinoma

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Background: There is considerable evidence in the literature to suggest a role for CDX2 in intestinal metaplasia and in the development of gastric cancer, but its prognostic implications in gastric cancer continues be a matter of debate. We conducted this study to assess the prognostic significance of CDX2 in gastric adenocarcinoma.

Design: We retrospectively reviewed our pathology database for gastric adenocarcinomas (well, moderate and poorly differentiated) diagnosed at our hospital from 2004 to 2008. These were subsequently stained with CDX2 (Leica®) immuno-histochemical stain. CDX2 positive and negative groups were then compared for overall survival.

Results: A total of 101 patients (mean age 50y; 60% male) were included in the study. 31/101 (30.7 %) cases were CDX2 positive. Of these, 23 (74%) patients underwent curative surgical resection. In the CDX2 negative group, only 12 (17%) patients underwent curative surgery (p=.0001). Of those who underwent surgical resection, 9% had stage I, 37 % had stage II, 43 % had stage III, and 11% had stage IV tumors on TNM staging of the resected histological specimens. The mean overall survival of the CDX2 positive group was 17 months, while that of the CDX2 negative group was 6 months (p= 0.0001).

Conclusions: Patients with gastric adenocarcinoma positive for CDX2 are more likely to have resectable tumors and have significantly better survival.

738 Lymphatic Invasion and Nodal Metastasis in Dysplasia and Carcinoma of the Stomach: Examination of 183 Cases Undergoing Endoscopic Submucosal Dissection Using D2-40 Immunostaining

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Background: Gastric dysplasia was defined as being localized in the mucosa, but Sakurai *et al.* reported submucosal (SM) invasion of high grade dysplasia (HGD) based on 125 endoscopic submucosal dissection (ESD) tissues (AJSP 2014). In their study, lymphatic invasion was evaluated by HE stain, and nodal metastasis was not examined. Therefore, we examined lymphatic invasion of dysplasia and/or carcinoma of the stomach by D2-40 immunostaining including nodal metastasis.

Design: 183 cases of ESD of the stomach were examined. The ESD tissues consisted of 15 low grade dysplasia (LGD), 84 HGD, 61 HGD + carcinoma (admixture of HGD and carcinoma), and 23 carcinoma. Carcinoma is defined as a tubular and/or papillary structure without a poorly differentiated structure. In 12 cases (3 HGD + carcinoma and 9 carcinoma), gastrectomy and nodal dissection tissues after ESD were examined for nodal metastasis. The resected tissues were examined with HE and EVG stains and also with D2-40 immunostaining for the evaluation of lymphatic invasion (ly0 – ly3).

Results: The SM invasion, lymphatic invasion, and nodal metastasis are shown in Tables. In HGD, 4 cases (4.7%) showed less than 0.2 mm of SM invasion without lymphatic invasion. In HGD + carcinoma, 7 cases (11.4%) showed SM invasion with a less than 0.9 mm depth, and none of the 3 examined cases (absence of lymphatic invasion in 2 cases) had nodal metastasis. In carcinoma, 9 cases (39.1%) showed SM invasion with various distances of SM invasion and degrees of lymphatic invasion.

Conclusions: SM invasion of HGD shows a low incidence and shallow SM invasion without lymphatic invasion. In HGD + carcinoma, SM invasion of less than 0.9 mm without lymphatic invasion is a low risk indicator of nodal metastasis. This indicator is useful for the decision regarding post-ESD treatment.

Neoplasms	No. of cases	SM invasion	Lymphatic invasion in SM
LGD	15	0	0
HGD	84	4	0
HGD and carcinoma	61	7	1
Carcinoma	23	9	7

Neoplasms	Nodal metastasis	No. of cases	Depth of SM invasion (mm)/lymphatic invasion in SM in each cases
HGD	No examination	4	0.1/ly0, 0.1/ly0, 0.2/ly0, 0.2/ly0
HGD and carcinoma	No examination	4	0.2/ly0, 0.4/ly0, 0.5/ly0, 0.8/ly0
HGD and carcinoma	Absence	3	0.4/ly1, 0.6/ly0, 0.9/ly0
Carcinoma	Absence	7	0.3/ly2, 0.4/ly1, 0.5/ly1, 0.5/ly0, 0.7/ly1, 0.8/ly1, 2.3/ly2
Carcinoma	Presence	2	1.1/ly3, 1.2/ly0

739 Utility of Longitudinal Slicing of Rectal Cancer Specimen in the Evaluation of Resection Margins

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Background: Pathological assessment of the resection margin of rectal cancer is known to be important. However, the best method of evaluating the surgical margin remains unclear. We studied the utility of the longitudinal slicing method for evaluating the resection margin of rectal cancer specimen.

Design: We retrospectively investigated 197 consecutive patients with primary rectal cancer who underwent intersphincteric resection (ISR) without neoadjuvant therapy between January 2000 and December 2013. The resected specimens were opened along the anti-tumor or in the anterior side, and cut into 12 slices in the direction of the long axis. The closest distance from the outermost part of the tumor or tumor deposit to the resection margin was measured as the circumferential resection margin (CRM). CRM was considered positive when the resection margin was less than or equal to 1 mm. CRM-positive cases were classified into two groups, namely the DEEP group, where CRM was positive in the deepest tumor invasion area, and the ENTRY group, where CRM was positive in the initial cutting point of the anal canal.

Results: The median tumor distance from the anal verge was 4.0 cm (range, 0.6–9.5 cm). The clinical stages were I, II, and III in 56 (27%), 78 (37%), and 73 patients (35%), respectively. CRM was positive in 23 patients (12%), of whom 16 (70%) belonged to the DEEP group and 7 (30%) belonged to the ENTRY group. The median tumor distance from the anal verge was 4.0 cm in the CRM-negative group, 4.1 cm in the DEEP group (p = 0.916), and 3.0 cm in the ENTRY group (p = 0.021). Poorly differentiated tumor was found in 7 patients (4%) in the CRM-negative group, in 2 patients (12%) in the DEEP group (p = 0.288), and in 2 patients (29%) in the ENTRY group (p = 0.013).

Conclusions: CRM-positive areas were found in the deepest tumor invasion area and in the initial cutting point of the anal canal. The longitudinal slicing method may contribute to precise assessment of resection margins, especially in cases of tumors located near the anus or poorly differentiated tumors.

740 Extra-Ileal Manifestations of Crohn's Disease at Initial Presentation

Gregory Miller, Ian Brown. Envoi Specialist Pathologists, Brisbane, Queensland, Australia.

Background: Crohn's disease (CD) most commonly presents with ileal inflammation which is seen in up to 2/3 of cases at diagnosis. The extra-ileal gastrointestinal manifestations of CD have been well described but are not well documented in a consecutive cohort of treatment naïve patients at their initial diagnostic endoscopy.

Design: Retrospective analysis of 111 cases of CD at first presentation, prior to any immunosuppressive or immunomodulatory treatment, were independently reviewed by 2 pathologists. Biopsies from the oesophagus (n=32), stomach (n=66), duodenum (n=69), colon (n=98) and rectum (n=47) were examined. They were assessed for the presence of and pattern of any inflammatory infiltrate, granuloma formation and any other pathology. Cases were also analysed to see if any differences were present between children (<17 years) and adults (≥17 years).

Results: Extra-ileal inflammatory changes were seen throughout the gastrointestinal tract. The oesophagus showed lymphocytic oesophagitis in 13/32 (37%) biopsied cases, the stomach showed focally or diffusely enhanced gastritis in 37/66 (56%) biopsied cases, the duodenum showed active duodenitis in 23/69 (33%) biopsied cases and in the colon there was a focal or diffuse colitis pattern in 63/98 (64%) biopsied cases. Children were more likely to show inflammatory changes outside the ileum at diagnosis (100% vs 66%; p = 0.0002), particularly in the colon (p=0.016). Granulomas were seen in up to 39% of cases. They were most commonly seen in the stomach and colon and were much more common in children than adults (77% vs 26%; p = 0.0001).

Conclusions: CD will often have extra-ileal gastrointestinal manifestations at initial presentation and these changes can be present throughout the gastrointestinal tract. This

is particularly the case in children where, in our cohort, they will almost invariably show extra-ileal gastrointestinal inflammation. The presence of granulomas and inflammatory changes outside of the ileum helps in the diagnosis of CD in both adults and children.

741 Comparison of the Extra-Ileal Manifestations of Crohn's Disease and Non-Specific Ileitis

Gregory Miller, Ian Brown. Envoi Specialist Pathologists, Brisbane, Queensland, Australia.

Background: The ileum is the most common site of involvement in Crohn's disease (CD), however, without clinical context the microscopic features are not entirely specific. Extra ileal gastrointestinal manifestations CD are well described and used as a helpful clue in the diagnosis. There is little data currently on whether the extra-ileal changes allow separation of CD associated ileitis from non-specific ileitis due to another cause, such as infection or medication use.

Design: A retrospective analysis of the extra-ileal biopsies from 111 patients with ileal CD at first diagnosis and 151 cases of non-specific ileal inflammation without documented CD at follow up (of up to 8 years). Biopsies from the oesophagus, stomach, duodenum, colon and rectum were examined. They were assessed for the presence of inflammation and the pattern of involvement in the biopsy.

Results: Biopsies from the upper gastrointestinal tract were more likely to be normal in the non-specific ileitis cohort than the CD associated cohort (85.5% versus 24.3%; p = 0.0001). Colonic and rectal biopsies were also more likely to be normal in the cases of non-specific ileitis than in the cases of CD (91.4% versus 34.0%; p = 0.0002). In the CD cohort there were 14 cases of lymphocytic oesophagitis, 32 cases of focally enhanced of diffuse active gastritis and 22 cases of active duodenitis whereas the non-specific ileitis cases showed chemical gastritis changes in 6 cases only. The CD associated cases showed a focal active chronic colitis pattern of injury in 44 cases whereas this was present in only 4 cases of non-specific ileitis. Granulomata were present in 39% of cases associated with CD while none were seen in the non-specific ileitis cases.

Conclusions: CD will often have extra-ileal inflammation at presentation whereas cases of non-progressive non-specific ileitis will usually have mucosal changes limited to the ileum. This indicates it is reasonable to downplay the significance of isolated non-granulomatous inflammation in the ileum as almost all cases of Crohn's disease will show inflammation elsewhere within the gastrointestinal tract.

742 HMGA2 Expression Correlates with High Tumor Stage, Nodal Metastasis, and Poor Outcomes in Patients with Esophageal Adenocarcinoma

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Background: Esophageal adenocarcinoma (EAC) tumorigenesis has been primarily linked to loss-of-function mutations in tumor suppressor genes but knowledge of specific oncogenes that drive progression and their relationship to patient outcomes is limited. High Mobility Group A2 (HMGA2) was recently reported to be amplified in a subset of EAC but the clinicopathologic and prognostic implications of HMGA2 expression in EAC are unknown. In this study, we analyzed the significance of HMGA2 protein expression and *HMGA2* amplification in EAC.

Design: 91 primary EAC resections without neoadjuvant treatment were identified from the surgical files of the Brigham and Women's Hospital and immunohistochemistry for HMGA2 was performed. The presence and absence of nuclear staining in the invasive tumor component was evaluated and correlated with clinicopathologic parameters by Chi-square test and with patient outcomes by both Kaplan-Meier and Cox proportional hazard regression analysis. FISH for *HMGA2* amplification was successfully performed in 7/17 tumors in which it was attempted.

Results: HMGA2 expression was present in 25/91 (27.4%) tumors (3 diffuse, 22 focal/multifocal). HMGA2 expressing cells were often present in poorly differentiated solid areas (n=12/25) or at the invasive front of tumors (n=14/25). 3-4 copies of *HMGA2* were frequently present by FISH irrespective of HMGA2 protein expression (n=6/7). High level *HMGA2* amplification was seen in only one case (n=1/7) as a distinct clonal population in a background of tumor cells with 3-4 *HMGA2* copies. HMGA2 expression was associated with numerous adverse clinical parameters including high T ($p<0.02$) and N ($p<0.001$) stage, presence of lymphovascular invasion ($p<0.01$), and also correlated with poor outcomes including recurrence free ($p<0.05$) and overall survival ($p<0.001$) by Kaplan-Meier analysis. A trend towards poor overall survival was also seen on multivariate analysis but this did not reach statistical significance (p=0.08, HR 1.8, 95% CI: 0.92-3.6).

Conclusions: HMGA2 expression appears to be regulated through non-chromosomal level alterations and high-level *HMGA2* amplification is a rare event in EAC. HMGA2 protein expression in EAC correlates with high tumor stage and worse overall and recurrence free survival.

743 Intestinal Helminths in a Vey Large US Patient Cohort- A Retrospective Review of over 81,000 Coloscopy Samples

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Background: *Ascaris lumbricoides* is the most common intestinal helminth worldwide. Children, immunosuppressed and residents with poor sanitation are considered at greatest risk of harboring parasites. Little is published about intestinal helminths in the US population.

Design: Retrospective review of a large national referral gastrointestinal pathology practice received 499,904 samples between 2012-2105, including 81,249 (36.28%) from large bowel.

Results: Of all the large bowel samples, 32 (0.02%) harbored intestinal helminths including *Strongyloides stercoralis* (15 cases), *Trichuris trichura* (9 cases) and *Enterobius vermicularis* (8 cases). Not a single case of *A. lumbricoides, Necator americanus or Ancylostoma dudonale* was received. All patients were >30 years in age, averaging 56.4 years, men aged 50-59 years being most common (28%). 13 patients (41%) reported vague symptoms including abdominal pain (most frequent), constipation or diarrhea, rectal pain or bleeding, dysphagia and weight loss (least frequent). Helminth was present within a polyp in 7 (22%) cases. Travel history was not available. Diagnosis was made at routine microscopy, through identification of parasitic body parts or ova in the tissue.

Conclusions: This large review reconfirms that colonic helminths are not a significant cause of human morbidity in the US, are typically asymptomatic or cause vague symptoms which are unlikely to be linked to the presence of helminths. Because of their low incidence, pathology training programs should keep study material to be shared with residents to ensure competency in diagnosing intestinal helminths.

744 "Peptic" Duodenitis Lacks Gastric Histologic, Infectious, and Pharmocologic Correlates: A Review of 225 Consecutive Biopsies

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Background: Foveolar metaplasia (FM) of the duodenum is a sign of chronic injury, primarily thought to be related to peptic injury secondary to H. pylori infection. With the decreasing incidence of H. pylori infection and widespread use of proton pump inhibitors (PPI), the prevalence of duodenal FM would be expected to decrease. The goal of this study was to evaluate a consecutive series of duodenal biopsies for the presence of FM and to correlate these findings with evidence of H. pylori infection and other clinicopathologic variables.

Design: 225 consecutive duodenal biopsies were retrospectively reviewed by one pathologist for the presence and extent of FM as well as presence of gastric heterotopia, acute inflammation, Brunner gland hyperplasia (BGH), lamina propria expansion, villous blunting, and intraepithelial lymphocytosis. 168 corresponding gastric biopsies were also reviewed for presence of gastritis, parietal cell hyperplasia (PCH), and fundic gland polyps (FGP). Warthin-Starry stains were evaluated for presence of H. pylori. Clinical information was gathered through chart review.

Results: The study group consisted of 224 patients (M:F 1:1.9; mean age 50.7 yrs). The most common indications for endoscopy were abdominal pain (26.7%), diarrhea (16.9%), and reflux/heartburn (14.2%). 59 patients (26.3%) had FM and 10 patients (4.5%) had evidence of H. pylori on gastric biopsy. The number of biopsy fragments in patients with and without FM was similar (5.5 vs. 5.0, p=0.06). Patients with FM were significantly older (54.7 vs. 49.2, p=0.02) but there was no difference in gender. FM was not significantly associated with H. pylori infection (p=0.81), and only two patients (0.9%) had coexisting FM and H. pylori. 2 patients with FM had Crohn's disease and 3 had celiac disease (8.5% of FM patients). FM was significantly associated with presence of acute inflammation, BGH, villous blunting, and lamina propria expansion ($p<0.01$). FM was not associated with NSAID use (p=1), PPI use (p=1), or presence of PCH/FGP on gastric biopsy (p=0.46). On multivariate analysis, FM was significantly associated with older age (p=0.048), BGH ($p<0.01$), and villous blunting ($p<0.01$).

Conclusions: FM is seen in a significant proportion of our patient population (26.3%) but is not associated with H. pylori infection. The incidence of FM is not decreased in patients on PPI or those with evidence of PPI effect on gastric biopsy. H. pylori and "peptic" injury are likely not the most common cause of duodenal FM. Further studies are necessary to determine the etiology of duodenal FM.

745 Retrospective Review of Colectomy in Patients with Pre-Pouch Ileitis after Surgery for Ulcerative Colitis

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Background: Biopsies from pre-pouch ileum may be obtained in patients after proctocolectomy with ileal pouch for ulcerative colitis (UC). Pre-pouch inflammation raises consideration for Crohn's disease (CD), but there are other possible causes. We re-reviewed colectomies from patients with pre-pouch biopsies, aiming to find features predictive of either pre-pouch ileitis or a change in the clinical diagnosis from UC to CD.

Design: We re-reviewed colectomies from 28 patients operated at our institution and diagnosed as ulcerative colitis. These patients were a subset of 89 previously studied patients with pre-pouch biopsies. The clinical diagnosis had been changed to CD in 23 of those 89. Two pathologists independently re-reviewed each case, blinded to clinical outcomes. Colons with extensive, deep ulceration were classified as fulminant (and therefore indeterminate). We classified colons with neural hyperplasia or transmural lymphoid aggregates in the absence of deep ulcers as CD.

Results: All 28 colectomies had been diagnosed as UC (9 severe, 15 moderate, 3 mild and 1 inactive). Fourteen patients had pan-colitis, 9 subtotal and 5 left-sided. In retrospect, we diagnosed fulminant colitis in 4 of 28 patients and recognized features of CD in 7. Eight patients now carry a clinical diagnosis of CD. We identified CD features in 4 of 8, but we also suggested CD in 3 other patients who have not developed evidence of CD. All 4 patients with fulminant colitis continue to be classified as UC. Pre-pouch pathology varied slightly by patient group: patients we diagnosed as fulminant colitis or CD had at least one pre-pouch biopsy with active chronic ileitis, as did the 4 patients that we called UC but clinicians now call CD. In contrast, 6 of 14 patients with UC pathology in the colectomy and continued clinical diagnosis of UC have never had a pre-pouch biopsy with active chronic ileitis.

Conclusions: Neural hyperplasia and transmural lymphoid aggregates in the absence of deep ulcers are known features of CD that must be assessed in order not to miss CD limited only to the colon. We correctly identified 4 such patients in our retrospective review, although 3 other patients that we diagnosed as CD have no clinical evidence

of CD after 20 years of follow-up, and four patients who did develop CD had no morphologic clues in the colectomy. There is no single finding in a pre-pouch biopsy that is diagnostic of CD, but patients who are eventually changed to CD have fewer normal biopsies and more biopsies with active chronic ileitis.

746 Characterization of the Effect of the Tumor Epigenetic Clonality in the Evolution of Colorectal Cancer

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Background: The clinical behavior of a colorectal cancer (CRC) depends of many pathological, cellular and molecular factors, however, there a few validated biomarkers to predict the outcome of the disease. Part of the explanation resides in the recognition that CRC are heterogeneous between patients, but probably also at the intratumoral level. We have explored this last question in CRC at the epigenetic level: 1) to identify epigenetic changes at inter-tumor and intra-tumor level, and 2) to relate intra-tumor clonality to clinical, molecular and histopathologic parameters.

Design: From 79 FFPE tumors, 3 different regions were macrodissected: invasive front (IF), digestive tract surface (DTS) and central bulk (CB). Clinical, molecular, and histopathologic parameters were stablished. Epigenetic analysis was performed using Infinium 450K beadchip (Illumina) and R statistics.

Results: Intra-tumor regions clustered together by patient. The biggest epigenetic changes were in IF vs DTS/CB. By patient, the most often divergent region was IF (49.4%) comparing with DTS and CB (25.3% in both). Interestingly, the region closer to the gastrointestinal transit (DTS) shares a DNA methylation signature with the corresponding liver metastases (n=23). When we calculated individual coefficients of clonality to determine in a quantitative manner heterogeneity, we found that epigenetic clonality was significantly associated with short relapse free survival (Log Rank P = 0.037) and overall survival (Log Rank P = 0.026) in CRC patients.

Conclusions: These results indicate the existence of DNA methylation intratumoral heterogeneity in CRC that it has a biological and clinical impact in the natural history of the disease.

747 Regression Grading in Pancreatic Cancer: An Interobserver Study

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Background: Several regression grading systems have been proposed for neoadjuvant chemoradiation treated pancreatic ductal carcinoma (PDC). This is the first study to examine the utility, reproducibility and degree of concordance in three common grading systems.

Design: Four gastrointestinal pathologists from a single centre graded representative slides [7-20] from 14 cases of PDC treated with neo-adjuvant chemoradiation using the College of American Pathologists (CAP), Evans, MD Anderson Cancer Centre (MDACC) regression grading systems. A post scoring discussion with each pathologist was conducted. The results were entered into a standardised data collection form and analysed using Kendall coefficient of concordance (KCC) (standard criteria for interpretation of KCC scores: <0.20=poor; 0.21-0.4=fair; 0.41-0.6=moderate; 0.61-0.8= good; 0.81-1.00= very good).

Results: Grading performed by RC and SNK served as the study standard. KCC scores showed highly variable levels of concordance.

Pathologist	KCC for CAP	KCC for Evans	KCC for MDACC
A	0.18	0.66	0.00
B	0.29	0.56	0.41
C	0.32	0.36	0.41
D	0.40	0.74	0.67

The agreement scores for pathologists are as follows: CAP: 2-poor, 2-fair; Evans- 1-fair, 1-moderate, 2- good; MDACC- 1-poor, 2- moderate, 1-good. After analysis and discussion, CAP showed greatest discordance in distinguishing Grade 2 (minimal residual tumour) and Grade 3 (tumour outgrown by fibrosis). In the Evans system, quantifying percentage of tumour destruction between grades IIa (10-50%) and IIb (51-90%), presented the greatest discordance. Although the MDACC system generated greatest concordance, we feel that this is due to "oversimplification" surrounding arbitrarily assigned thresholds of </> than 5% of tumour. Furthermore, assessing fibrosis is contentious, which may be either secondary to desmoplasia and/ chronic pancreatitis.

Conclusions: These systems are definitionally flawed resulting in disparate interpretations. The prognostic value is questionable and essentially does not contribute significantly to patient management. While the degree of regression may be indicative of the biology of the tumour, a rough approximation would suffice for clinical purposes. Finally, pathologic regression grading in PDC is fraught with interpretative error, has limited clinical value and calls into question its use in current practice.

748 Esophageal Squamous Papillomas: Incidentaloma or Harbinger of Human Papilloma Virus Infection?

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Background: Esophageal squamous papillomas (ESP) are a rare entity with a long-debated significance and pathogenesis, including possible association with human papilloma virus (HPV). The recent introduction of a sensitive and specific immunohistochemical target for HPV, the L1 capsid protein, provides a mechanism for detecting associated viral infection in patients with ESP. We retrospectively identified a series of ESP cases to study clinical associations and implications of the diagnosis while investigating associated HPV infection using immunohistochemical methods.

Design: We performed a review of our institutional pathology database to identify cases of ESP from January 2000-June 2011. 50 patients (mean age 53+/-15 y, 51% F) had 52 ESP (size of 5.8+/-4.4 mm) over this period. A single gastrointestinal pathologist blinded to clinical information reviewed slides to confirm the diagnosis and record associated histopathologic features. Baseline data such as indication for EGD, location of ESP, and concomitant esophageal disorders were recorded by chart and follow-up data was evaluated over a mean follow-up period of 4.9 y (range 1-16 y). A second blinded GI pathologist evaluated each tissue sample for immunoreactivity for the HPV L1 capsid protein as a surrogate for HPV infection.

Results: 0/52 cases demonstrated immunoreactivity with immunostain for HPV L1 capsid protein, supporting the lack of dysplasia and viral cytopathic effect on H&E-stained sections. Diffuse positive staining in anal neoplasia with cytopathic effects of HPV was used as a positive control. 31 pts (62%) had concomitant gastroesophageal reflux disease and 5 patients (10%) had a history of Barrett's esophagus (BE) or BE at diagnosis. The most common location for ESP was the distal esophagus (48.1%), followed by mid esophagus (38.5%), and upper (13.5%). ESP in the lower third were larger with an average size of 7.2 vs 4.5 mm in the upper and mid esophagus (p=0.037). 38 pts (72%) had long term follow-up. No carcinoma or dysplasia developed over the follow-up period, but esophageal symptoms were persistent with reflux and dysphagia affecting 22 (58%) and 6 (16%) patients, respectively.

Conclusions: ESP are rare, benign neoplasms with little long-term consequence or risk for malignant progression. Despite previous studies reporting the contrary, no link between ESP and HPV infection was found in our study.

749 L861Q EGFR Mutation in a Metastatic Solid-Pseudopapillary Neoplasm of the Pancreas

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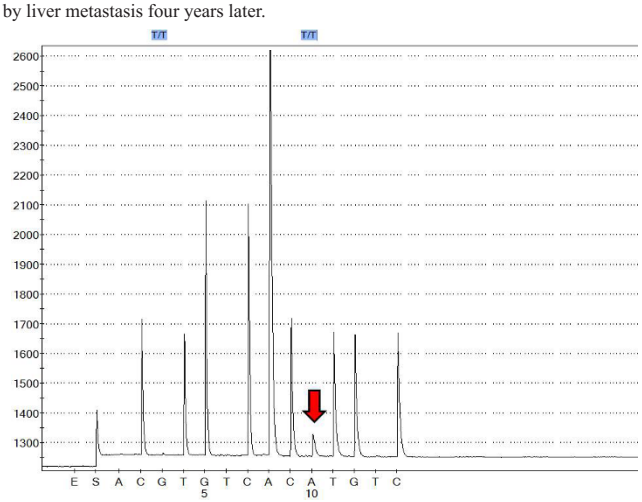
Background: Solid-pseudopapillary neoplasm of the pancreas (SPN) is a rare neoplasm that is typically indolent in nature. Surgical resection is the preferred method of treatment and is often associated with a good prognosis. Local invasion and metastasis have occurred in a small subset of patients. Currently, there are no studies on molecular mutations that may predispose SPNs to become locally aggressive and metastasize. In this study we analyzed the molecular differences between an aggressive SPN with metastasis to the liver versus an indolent SPN without local extension or metastases.

Design: Pyrosequencing mutational analysis for EGFR, KRAS, NRAS, PIK3CA and BRAF was performed on formalin fixed paraffin embedded tissue from two cases of SPN prior to treatment. Both neoplasms were similar in size, patient age and initial clinical presentation. The first case had an indolent clinical course followed by curative surgical resection. The second case was locally invasive, unresectable and progressed to liver metastasis despite aggressive chemoradiotherapy.

Results: A baseline EGFR mutation c.2582T>A (L861Q) in the kinase domain (exon 21) was identified in the SPN that showed initial unresectable local progression followed by liver metastasis four years later.



There were no baseline EGFR mutations or other mutations identified in the indolent case of SPN that was cured by routine surgical resection.

Conclusions: A L861Q EGFR mutation increases kinase activity and has been shown to confer increased sensitivity to EGFR tyrosine kinase inhibitors in non-small cell lung cancer and could possibly benefit SPN patients carrying such mutation. EGFR mutations may be responsible for the more aggressive behavior of SPNs, however, a larger subset of aggressive SPNs need to be analyzed.

750 The Prognostic Significance of Infiltrating Lymphocytes in Patients with Pancreatic Ductal Adenocarcinoma Treated with Neoadjuvant Therapy

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Background: Tumor microenvironment plays an important role in chemoresistance and tumor progression of pancreatic ductal adenocarcinoma (PDAC). The role of tumor infiltrating lymphocytes in PDAC is not clear. This study is to examine the intratumoral CD4, CD8, and FOXP3 positive lymphocytes and their correlation with survival and clinicopathologic parameters in patients with localized PDAC treated with *neoadjuvant therapy and pancreaticoduodenectomy (PD)*

Design: 140 patients with PDAC who completed neoadjuvant therapy and PD between 1999 and 2007 were studied (mean age: 63.3 years, 54 women and 86 men). Immunohistochemistry (IHC) for CD4, CD8, and FOXP3 were performed on tissue microarray (TMA) slides. Digital images of IHC-stained TMA slides were obtained at 20 x magnifications using a whole slide scanner (ScanScope, Aperio AT Turbo). The tumor regions were hand-annotated. Computer assisted quantitative analysis was performed by using Aperio's ImageScope software with their custom-made algorithm "Color Deconvolution v9". The tumor infiltrating lymphocytes were calculated as the percentage of positive staining area versus the total tumor area.

Results: The average percentage for CD4, CD8, and FOXP3 positive cells were 0.71 ± 0.06, 1.13± 0.10, and 1.47± 0.38 respectively. Using the 75 percentile value as a cutoff, patients with high CD4+ lymphocytes had better overall survival (49.48±8.49 months) than those with low CD4 positive lymphocytes (28.44±8.49 months, P=0.004). High CD4+ lymphocytes were also associated with better differentiation (P=0.02) and lower recurrence (P=0.02). There were no significant correlations between CD8+ or FOXP3+ lymphocytes and survival and other clinicopathologic parameters (P>0.05). In multivariate analysis, high CD4+ lymphocytes (HR: 0.49, P=0.004) and lymph node metastasis (HR: 1.68, P=0.02) were independent prognostic factors for overall survival.

Conclusions: Our study showed that the presence of high CD4+ lymphocytes is associated with better tumor differentiation, lower recurrence and is an independent prognostic factor for overall survival in PDAC patients treated with neoadjuvant therapy and PD.

751 Prognostic Value of a lncRNA Signature in Gastric Cancer: A lncRNA Expression Analysis

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Background: Long non-coding RNAs (lncRNAs) can be used as prognostic biomarkers in many types of cancer. We aimed to identify lncRNAs that were prognostic in patients with gastric cancer (GC)

Design: We retrospectively analysed lncRNAs expression profiles in 8 pairs of fresh frozen GC tissues and adjacent normal tiuuses. Potential core regulative lncRNAs were identified by integrating bioinformatic, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses and then validated by qRT-PCR in the training group (n=95). Kaplan-Meier method and log-rank tests were used to estimate correlations of the lncRNA signature with disease-free survival (DFS) and overall survival. A prognostic lncRNA signature was developed from the training group using univariate and multivariable Cox regression analyses, which were then validated in a test group (n=95) and an independent group from TCGA (n=223).

Results: LncRNAs showed significantly altered expression levels in the GC tissues. A signature of four lncRNAs, each significantly associated with DFS, was identified in the training set. We calculated a risk score from the signature and classified patients as high risk or low risk. Compared with patients with low-risk scores, patients with high risk scores in the training set had shorter DFS (hazard ratio [HR] 2.498, 95% CI 1.783–3.501; p=0.000), and overall survival (2.716, 95% CI 1.909–3.865; p=0.000). We noted equivalent findings in the test group for DFS (1.247, 1.002–2.768; p=0.003) and overall survival (1.186, 95% CI 1.178–3.324; p=0.005) and in the independent group for DFS (3.216, 1.165–6.404;p=0.007) and overall survival (3.107, 1.134–7.201; p=0.008). The four-lncRNA signature was an independent prognostic factor. A combination of this signature and TNM stage had better prognostic value than did TNM stage alone in the training set (area under receiver operating characteristics 0·72 [95% CI 0·65–0·96] vs 0.68 [0.51–0.87]; p=0.033), the test group (0.68 [0.60–0.78] vs 0.67 [0.56–0.72]; p=0.021), and the independent group (0.69 [0.61–0.81] vs 0.65 [0.58–0.72]; p=0.042).

Conclusions: Identification of patients with the four-lncRNA signature might add prognostic value to the TNM staging system and inform treatment decisions for patients at high risk of progression.

752 DNA Mutational Signature in Colorectal Cancer Predicts Mismatch Repair Pathway Status

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Background: Universal evaluation of colorectal carcinoma (CRC) for mismatch repair (MMR) pathway status requires multiple testing modalities for complete clinical analysis. The aim of this study was to determine whether DNA mutational signature, as evaluated by next-generation sequencing (NGS), could serve as a predictor of MMR pathway status in colorectal carcinoma, potentially serving to streamline MMR pathway testing algorithms.

Design: DNA was extracted from FFPE or fresh frozen tissue samples of CRC and analyzed by an NGS assay that interrogates 275 cancer-associated genes for mutations, copy number variations and structural alterations. Immunohistochemistry for MSH2, MSH6, MLH1 and PMS2 was performed on all cases, with subsequent MSI testing and *MLH1* promoter methylation testing when needed. Separate training and validation sets were used to generate mutational signature profiles and subsequently test criteria for identifying MMR-deficient cases. Wilcoxon rank-sum test was used to test for significant differences in mutational signature measures.

Results: MMR deficiency, as assessed by standard criteria, was present in 7.8% (12 of 153) of the training cases. Compared to MMR-proficient CRCs, MMR-deficient CRCs had a significantly higher overall mutational burden (p<0.001), including a higher burden of single nucleotide variations (p<0.001) and insertions/deletions (p<0.001), including those involving homopolymers (p<0.001). MMR-deficient CRCs also had a significantly higher ratio of transitions to transversions in SNVs (p=0.003) and a higher proportion of genes with multiple mutations (p<0.001). A combination of high overall mutation rate (≥29 per Mb) and high rate of insertions/deletions (≥5 per Mb) optimally segregated MMR-proficient from MMR-deficient cases in the training set. Application of these criteria to the validation set resulted in 100% sensitivity and 100% specificity in identifying the 10.4% (10 of 96) of cases that were MMR-deficient. The criteria successfully detected MMR deficiency in CRCs with either *MLH1* promoter methylation or a mutation in any of the four core MMR pathway genes. Notably, the criteria were also successful in separating MMR-deficient cases from two *POLE*-mutant cases in the validation set, which were characterized by a very high mutational burdens but low numbers of insertions/deletions.

Conclusions: Our findings demonstrate that the DNA mutational signature in CRC can serve as an accurate predictor of MMR pathway status. NGS-based MMR pathway evaluation may serve to streamline CRC molecular testing and efficiently guide downstream clinical decisions.

753 Overexpression of POSTN in Cancer-Associated Fibroblasts Is a Poor Prognostic Indicator of Colorectal Cancer

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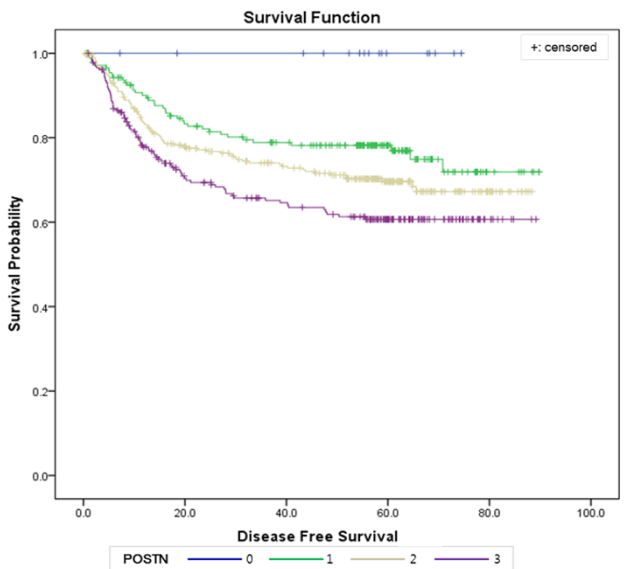
Background: Recent studies reveal that cancer-associated fibroblasts (CAFs) are associated with subgroups of colorectal cancers (CRCs) with poor prognosis. Several proteins are overexpressed in CAFs, however, the predictive value of these proteins as prognostic marker of CRCs are not yet to be proven.

Design: We evaluated stromal POSTN expression using immunohistochemistry in 734 surgically resected CRCs. Then, we compared clinicopathologic and molecular characteristics of cancers with stromal POSTN expression and investigated prognostic implication of stromal POSTN expression.

Results: 17 (2.3%), 178 (24.3%), 307 (41.8%) and 232 (31.6%) of patients showed stromal POSTN expression from grade 0 to 3, respectively. Stromal POSTN expression was positively correlated with distal tumor location, higher TNM stage, poor differentiation, tumor budding and luminal necrosis. Stromal POSTN expression showed no association with CpG island methylator phenotype, microsatellite instability, *KRAS* and *BRAF* mutation. In univariate survival analysis, stromal POSTN expression was gradually associated with poor overall survival ($p = 0.007$) and progression-free survival (PFS) ($p < 0.001$). Especially, patients with no stromal POSTN expression (grade 0) did not recur nor expire during follow-up period. In multivariate survival analysis, stromal POSTN expression was an independent prognostic marker for PFS (hazard ratio: 1.22, 95% confidence interval: 1.03 – 1.44, $p = 0.021$).

POSTN expression	0	1	2	3	*p* value
	17 (2.3%)	178 (24.3%)	307 (41.8%)	23 (31.6%)	
Stage					<0.001
I, II	16 (94.1%)	113 (64.5%)	135 (44.0%)	85 (36.6%)	
III, IV	1 (5.9%)	65 (36.5(%)	172 (56.0%)	147 (63.4%)	



Conclusions: Our study demonstrated that stromal POSTN expression could be a marker of aggressive clinical behavior in CRCs.

754 CD44 Expression and Its Clinical and Histologic Associations in Gastric Cancer: A Tertiary Care Center Experience

Stephen Olsen, Ilke Nalbantoglu. Washington University in St. Louis School of Medicine, Saint Louis, MO.

Background: CD44 is a glycoprotein that facilitates adhesiveness and cell-cell interaction. It is postulated to be a stem cell marker and is expressed in many tissue types including gastric, pancreatic, and colon cancers. In some series, expression of CD44 in gastric cancer has been reported to be associated with lymph node metastasis (LNM), higher tumor (tm) grade and stage, and decreased survival. We evaluated CD44 expression in gastric cancers and its associations with histologic and clinical parameters.

Design: Total of 104 gastric cancers were identified from Dept of Pathology database. Cases with prior chemo-radiation were excluded. Histopathologic features including tm type (intestinal vs diffuse), T stage, grade, and lymph node status were evaluated and recorded. Clinical data including tm recurrence and survival were collected. Representative sections of each tm were stained with CD44 antibody with appropriate positive and negative controls. The expression pattern was recorded based on percentage of tm cell staining as negative (<5%), low (>5% but <40%), or high (=>40%).

Results: 62 patients (pts) were male and 42 female. The mean age was 67.5 years. 24 pts had recurrence during a mean follow up of 55 months. Median time to recurrence was 36 months. 34 pts had T1, 14 had T2, 24 had T3 and 32 had T4 disease. 48% had intestinal type, whereas 37% were diffuse and 15% were mixed. 72 (69%) of cases were poorly differentiated. In 57 cases (55%) CD44 was negative. Low CD44 expression was seen in 32 (31%) and high CD44 expression was seen in 15 (14%) cases. Pts with high CD44 expression had a lower rate of recurrence during follow up (7%) compared to low or negative CD44 expression (29%), though this did not reach statistical significance. However, no association between negative, low, or high CD44 expression and any clinical or histologic features reached statistical significance.

Conclusions: Although several studies with similar sample sizes in gastric carcinoma reported CD44 expression to be associated with higher tm grade, LNM, and decreased survival, we do not detect any significant associations. Although not significant, we did find that patients with high CD44 expression had a lower rate of recurrence than those with low or negative expression, a finding similar to at least one other study that showed improved survival correlated with increased CD44 expression. Given the conflicting results, more studies with larger samples sizes are required before the utility of CD44 expression in gastric adenocarcinoma is established.

755 Synaptophysin-Ki67 Double Stain: A Novel Technique That Can Improve Inter-Observer Agreement in the Grading of GI Neuroendocrine Tumors

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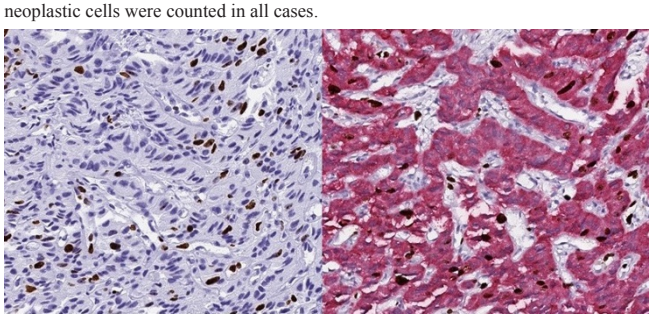
Background: A common problem in the assessment of proliferative index in well-differentiated GI neuroendocrine tumors (NETs) is distinguishing tumor from non-tumor. Both the delicate vascular network (characteristic of neuroendocrine tumors) and background lymphocytes frequently contain a subset of proliferating cells. If these non-neoplastic cells are mistakenly included in the Ki67 count, they can artificially elevate the proliferative index. Furthermore, in small biopsies, crush and cautery artifact can alter the morphologic appearance of tumor cells, making the Ki67 proliferative index more difficult to assess.

Design: To address these issues, we developed a synaptophysin-Ki67 double stain (Syn-Ki) using the DAKO EnVision DuoFLEX kit, allowing simultaneous visualization of tumor and proliferating nuclei. Three GI pathologists individually graded 50 GI NETs (1 slide/tumor) using the Syn-Ki double stain. After a wash-out period, they graded the same 50 GI NETs using a Ki67 only stained slide and routine H&E. As a gold standard, one pathologist separately graded the tumors using the Ki67 only stain by photographing the tumor area of highest Ki67 activity (on Ki67 only stained slides), printing the image, and manually ticking off each neoplastic cell. A minimum of 500 neoplastic cells were counted in all cases.



Results: Per gold standard, 39 tumors were grade 1; 11 were grade 2; and none was grade 3 (≤2% cut-off for grade 1). Inter-observer agreement was 74% using the Syn-Ki double stain and 64% using the Ki67 only stain. Grade concurrence among all 3 pathologists AND the gold standard occurred in 64% of cases using the Syn-Ki double stain and 60% using the Ki67 only stain. In addition, variance in Ki67 index between pathologists was lower using the Syn-Ki double stained slides compared to the Ki67 only slides in a majority of cases (32/50).

Conclusions: The Syn-Ki double stain is the first technique to specifically address the histomorphological challenges of evaluating Ki67 proliferative index in GI NETs. Although further validation is needed, this study provides preliminary evidence that the Syn-Ki double stain can improve inter-observer agreement.

756 Lymphocytic Esophagitis (LE) with CD8 T-Cell Predominance May Be Associated with Gastroesophageal Reflux Disease (GERD)

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Background: LE is thought to be associated with GERD. Also, GERD is known to frequently overlap with non-achalasia esophageal primary motility disorders (PEMD), which can contribute to the severity of GERD. We have recently reported that LE with CD4-predominant T-cells is associated with PEMD (Xue et al. AJSP, 2015, Sep 16. Epub ahead of print). Thus, the real association with LE observed in patients with GERD may be between LE and PEMD, and not between LE and GERD. The goal of this retrospective study was to test the hypothesis that LE with normal esophageal motility may be linked to GERD.

Design: LE was defined as overtly increased intraepithelial lymphocytes (IEL) with absent or rare granulocytes. 63 biopsy cases of LE were diagnosed during routine diagnostic work from 2010-2015. Motility testing, either by standard manometry or barium esophagram, was performed in 26/63 patients with LE. An additional 4 cases of LE were identified in biopsies from 70 patients with severe GERD and manometry-proven absence of PEMD who were referred for a Nissen fundoplication during the same time period. IEL were counted in the most affected field of view (x400). CD4 and CD8 T-cells were analyzed by immunohistochemistry. The CD4:CD8 ratio > 1 and ≤ 1 indicated predominance of CD4 and CD8 T cells, respectively. Data is presented as mean ± SD.

Results: Of the 26 cases of LE with motility testing identified during routine sign out, 7 had CD8-predominant IEL and 19 patients had CD4-predominant IEL. A significantly higher proportion of patients with CD8-predominant LE, 5/7 (71%; CD4/CD8 ratio 0.45 ± 0.18), had normal esophageal motility in comparison to 3/19 (16%) patients with CD4-predominant LE (CD4/CD8 ratio 3.2 ± 2.6) (P= 0.014). Clinically, all 5 patients with CD8-predominant LE and normal motility (age 60 ±15 yrs, M:F=4:1) complained of dysphagia. Three patients had a history of GERD. Endoscopically, 4/5 patients had features compatible with GERD, such as esophagitis (1/5), benign stricture (1/5), Schatzki ring (3/5), hiatal hernia (1/5), rings and furrows (3/5). Histologically, 3/5 patients had separate areas of reflux esophagitis. In addition, 3/4 (75%) LE cases from patients with severe PEMD-negative GERD, who were referred for a Nissen fundoplication (age 59 ± 16 yrs, M:F=1:3) also showed CD8 T-cell predominance.

Conclusions: Taken together, the findings suggest that LE may be associated with GERD in the absence of PEMD. Moreover, CD8 T-cell predominance appears to be characteristic for this type of LE.

757 Pediatric Focal Active Colitis. A Retrospective Review

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Background: Focal active colitis (FAC) is a histopathologic diagnosis of uncertain clinical significance in individual patients. In adults, infection accounts for approx. 50%, Crohn's disease (CD) for 0-13%, and 20-30% are idiopathic (likely prep related). A previous study reviewed 29 cases of pediatric FAC with 28% diagnosed as CD. Histologic features to distinguish between idiopathic FAC and inflammatory bowel disease (IBD) related FAC have not been elucidated. The present study reviewed a larger cohort of pediatric patients to determine what proportion with FAC had IBD and whether there was an amount or pattern of inflammation that predicted IBD.

Design: One hundred patients aged ≤18 years with FAC were identified (2002-2015). Patients with a prior diagnosis of IBD or chronic colitis in the index biopsies were excluded. Original slides were assessed for number of colonic sites, fragments and crypts involved, max. number of neutrophils per crypt, crypt abscesses, aphthous and surface inflammation, and associated terminal ileal (TI) and upper GI inflammation. Comprehensive clinical data were recorded and final diagnoses were grouped as IBD, infection, allergic colitis, other definitive diagnosis and 'idiopathic FAC' (irritable bowel syndrome, functional abdominal pain, no clinical diagnosis). Data were analysed using Pearson correlations and Fisher's exact chi-square analyses.

Results: Sixty-eight biopsy sets and follow-up data from 68 patients were reviewed. Seventeen patients (25%) had a final diagnosis of IBD, 1% had infectious colitis, 9% allergic colitis, 10% another definitive diagnosis to explain FAC and 54% idiopathic FAC. Four patients had colonic granulomas; 3 of these had a final diagnosis of CD. Twenty-two patients had either focal surface active inflammation only or a single inflamed crypt; 8 of these (36%) had CD. A final diagnosis of IBD was significantly associated with the presence of TI inflammation (p<0.001), and both TI and upper GI inflammation (p=0.043). There were no significant relationships between IBD and amount or pattern of colonic inflammation.

Conclusions: This study demonstrated a 25% rate of IBD in pediatric patients with FAC, similar to the previously reported rate. There was no correlation between the degree or distribution of colonic inflammation and subsequent diagnosis of IBD and, in this patient group, even when very focal and minimal, FAC always requires clinical follow-up.

758 Comprehensive Genomic Profiling of Advanced Stage Colorectal Carcinoma Reveals Alterations in Epigenetic Regulators

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Background: Epigenetic processes, including chromatin remodeling, DNA methylation and histone modification are involved in tumorigenesis. Genomic alterations (GA) in epigenetic regulators have been described in microsatellite unstable (MSI) colorectal cancer (CRC); however, their spectrum and significance in microsatellite stable (MSS)

CRC is unclear. Our goal was to identify GA in epigenetic regulators in clinical samples of MSS CRC and delineate clinicopathologic features of CRCs harboring these mutations.
Design: Forty nine samples of advanced stage, treatment resistant CRCs were assayed by hybrid capture-based comprehensive genomic profiling (CGP) including 315 cancer-related genes and introns from 28 genes frequently rearranged in cancer. GA were correlated with clinicopathologic data.
Results: There were 30 males and 19 females with a median age of 57 years (range 40-83 years), all with a stage IV metastatic CRC at the time of CGP. A total of 261 GA were identified by CGP (mean 5.3; range 0-10) in 108 genes. Most common GA were *TP53* (89.8%), *APC* (79.6%), and *KRAS* (40.8%). Twenty six mutations involved nine epigenetic regulators *ARID1B, ARID1A, ARID2, MLL3, MLL2, DNMT3A, TET2, KDM5A,* and *NSD1* (mean 0.53, range 0-2). The most common GA in epigenetic regulators were identified in the chromatin remodeling genes *ARID1B* (16.3%), *ARID1A* (10.2%), and the histone modifier *MLL3* (10.2%). Rare GA were present in DNA methylators *DNMT3A*, *TET2*, chromatin remodeling gene *ARID2*, histone modifiers *KDM5A, MLL2* (all at 2%), and histone modifier *NSD1* (4%). Nineteen patients (38.8%) featured GA in at least one of 9 epigenetic regulators. There was no difference in age, sex, tumor grade, budding, or peritumoral lymphocytic response between patients with and without GA in epigenetic regulators. However, there was a trend of a stronger intratumoral lymphocytic (ITL) response with ≥3 lymphocytes/HPF in cases with GA in epigenetic regulators (p=0.06). This trend was also observed when ITL were analyzed in tumors harboring *ARID1B* or *ARID1A* mutations (p=0.05).
Conclusions: Genomic alterations in epigenetic regulators, particularly *ARID1B, ARID1A, and MLL3* were identified in up to 39% of advanced stage CRC. Alterations in the tumor suppressor activity may play a role in carcinogenesis of MSS CRC and represent a therapeutic target. Deregulation of gene expression through mutations of epigenetic regulators may be associated with an increased lymphocytic tumoral response.

759 Comparison of Morphology and Proliferation Markers of the Primary vs. Metastatic Lesions of Gastroenteropancreatic Neuroendocrine Tumors

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Background: Mitotic count and Ki67 index are validated prognostic markers for gastroenteropancreatic neuroendocrine tumors (GEP-NETs), and are used in the 2010 WHO three-tier grading system. Depending on the clinical situation, pathologists may be asked to grade both the primary tumor and a metastatic lesion for unclear reasons. Given the potential impact of grading on treatment decisions, we sought to compare the morphology and WHO grade of primary GEP-NETs to their metastatic liver lesions.
Design: We retrospectively identified patients at 3 geographically diverse academic medical centers who had a primary GEP-NET and liver metastasis resected between 2000 and 2015. Morphological features were compared and mitotic counts and immunostaining for Ki67 (with digital analysis) was performed on both the primary and metastasis to assign grade according to WHO criteria.
Results: Of 39 cases, 51% were female and the median age was 59 years (IQR, 51-62). Primary sites included small bowel (19), pancreas (16), ileocecal junction (3), and large bowel (1). Markedly different morphology and size between the primary and metastatic GEP-NET occurred in 7.7% of cases (figure 1).

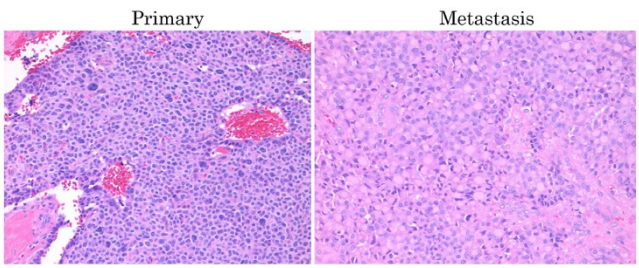

Figure 1 VIPoma (0.7 cm) arising in the duodenum with areas of markedly different morphology in the metastatic liver lesion (14.0 cm) resected from the same patient.

WHO grade was concordant by mitotic count in 78.1% (25/32) and by Ki67 index in 75.0% (27/36) of cases with complete data. Of discrepant cases, WHO grade was more often higher in the metastasis than the primary by both mitotic count (12.5% (4/32)) and Ki67 index (19.4% (7/36)). However, lesion size, mitotic count, Ki67 index, and proportion of necrotic lesions did not statistically differ between the primary and metastasis (p>0.4, all comparisons).
Conclusions: Primary GEP-NETs not infrequently have different morphologic patterns from their liver metastases. Although such discrepancies can present a diagnostic challenge to the surgical pathologist, we demonstrate that the proliferation markers used to determine WHO grade do not significantly differ between primary GEP-NETs and metastatic liver lesions. These findings support standardizing the evaluation of GEP-NETs based on the WHO grade of the primary lesion, in order to conserve surgical pathology resources.

760 Identification of RhoA and Related Gene Mutation in Diffuse Type Gastric Cancer: Distinct Clinicopathological Features and Mucin Expression

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Background: Gastric cancer is one of important malignancy in its prevalence and impact on survival probabilities. Recently, whole exome or genome sequencing revealed recurrent mutations of *RHOA* in diffuse type gastric cancer. However, detailed nature or clinical significances of *RHOA* mutated gastric cancer are still unclear. We have tried to validate *RHOA* mutation in diffuse type gastric cancer and their association with clinicopathological significance mucin expression.
Design: *RHOA* mutation were confirmed and extended by target sequencing analysis of 103 diffuse gastric cancer cases. We included *RHOA* and *RHOA* effector genes (*ROCK*1/2 and *PKN*) as well as frequently mutated genes in the previous reports (77 genes in total). We also did immunohistochemistry for mucin (Muc2, Muc5AC, Muc6) and CD10.
Results: We found 16 cases *RHOA* mutation (15.5%), 2 cases of *ROCK* mutation (1.9%) among 103 diffuse type gastric cancer. *RHOA/ROCK* mutated gastric cancer revealed distinct clinicopathological features, 1) body and fundic location (p=0.003), 2) poorly non-cohesive carcinoma (p=0.001), and 3) diffuse infiltrative growth pattern (p=0.001). *RHOA/ROCK* mutated gastric cancer showed Muc6 expression compare to wild type. Interestingly, *RHOA/ROCK* mutated gastric cancer showed distinct histologic pattern, featuring superficial signet ring cell carcinoma with deep invasive non-cohesive poorly carcinoma (11/18 cases, 61.1%), whereas pure signet ring cell carcinoma showed no *RHOA/ROCK* mutation. There were no relationships between depth of invasion, lymph node metastasis, perineural invasion, lymphovascular emboli and *RHOA* mutation. Also overall survival was not different between *RHOA* mutated and wild type diffuse gastric cancer.
Conclusions: Taken together, these data identify the distinct clinicopathological features and mucin expression of *RHOA/ROCK* mutated gastric cancer, implying that *RHOA* and *ROCK* mutation is involved in carcinogenesis of diffuse type gastric cancer and candidate for therapeutic target.

761 Clinicopathologic Features of Tumor Infiltrating Neutrophils in MSI-H Colorectal Carcinoma

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Background: The interaction between tumor cells and neutrophils is becoming increasingly recognized. Studies show neutrophils are cytolytic and remove tumor cells. While others suggest neutrophils stimulate the invasive potential of tumors. Tumor infiltrating neutrophils (TINs) have been evaluated in pancreatic and gastric carcinomas, however whether they portend a worse prognosis remains unclear. The significance of TINs in colorectal carcinomas (CRCs) and whether their presence harbors an effect on prognosis remains controversial. Of particular interest are MSI-high (MSI-H) CRCs, which have varying clinopathololgic features but differ in disease progression and treatment response. The goal of this study is to investigate TINs involvement of CRCs and to assess if MSI-H neutrophil-rich (NR) CRCs have worse clinicopathologic features compared to MSI-H neutrophil-poor (NP) CRCs.
Design: The pathology database was searched to identify CRCs between 2011-2015. The parameters recorded included age, gender, histologic type, grade, TNM staging, tumor site, extra-colonic carcinoma and MSI status. The presence >15 TINs per 100 tumor cells was considered as NRCRCs. The Fisher's exact and t-test were used with P<0.05 considered to be significant.
Results: Among 237 cases reviewed, 72 were MSI-H tumors of which 21 were NR (21 women, average age 75.2, range 50-92) and 51 NP (36 women, 15 men, average age 70.1, range 26-92). 4 microsatellite stable (MSS) NR cases were identified (4 men, average age 45, range 28-58). The NR MSI-H group compared to NP MSI-H group showed a difference in terms of gender (100% vs 70% female, p=0.004) but no difference in age (75 vs 70, p=0.2). All MSI-H NRCRCs were compared to MSI-H NPCRCs for T, N, size and AJCC clinical stage to show no statistical difference (p=0.62, 0.53, 0.2, and 0.4, respectively). There was a difference between NR MSI-H cases and NP MSI-H cases in terms of tumor grade (67% vs 39% high grade, p=0.04). Between the MSI-H NRCRCs and MSS NRCRCs there was a difference in nodal status (p=0.01) but no difference in T or clinical stage.
Conclusions: Our findings reveal that MSI-H NRCRCs have a high grade morphology but show no difference in other clinicopathologic factors compared to MSI-H NPCRCs. To our knowledge this is the first study to evaluate TINs in MSI-H CRCs. Among the NRCRCs, MSI-H cases have a significant difference in nodal status, however no difference in tumor size or stage. These findings suggest that TINs in the setting of MSI-H tumors are not a predictor of tumor aggressiveness.

762 Clinicopathologic Findings in Gynecologic Proliferations of the Appendix

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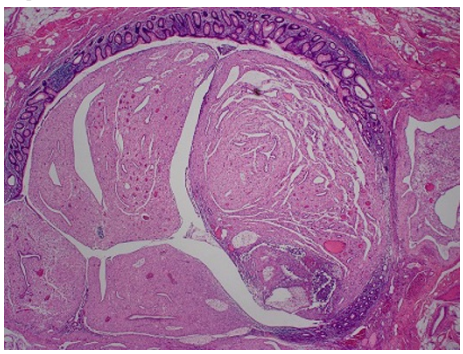
Background: Appendiceal endometriosis is uncommon but well-described; its clinical presentation can vary. Similar appendiceal lesions, such as endosalpingiosis and decidual lesions, are less studied.
Design: We identified 67 cases of appendiceal gynecologic proliferations with available slides. Clinical presentation was recorded when available, and histologic findings were correlated with clinical data.

Results: Cases included conventional endometriosis (55), endosalpingiosis (6), and decidual lesions (6). Endometriosis patients presented with known endometriosis (23/47), acute appendicitis (11/47), ovarian mass (7/47), intestinal mass (2/47), and other complaint (4/47). Only one patient presenting with appendicitis was ever diagnosed with extra-appendiceal disease. All 55 cases had glands, 43 (78%) had endometrial stroma, and 23 (42%) had hemosiderin. Disease was confined to the appendix tip in 22 cases (40%); none involved the appendix diffusely or the lumen. Stroma, hemosiderin, and tip confinement were equally prevalent in patients presenting with acute appendicitis and with known endometriosis. Only one case progressed to endometrioid adenocarcinoma. Endosalpingiosis patients presented with acute appendicitis (2), pelvic mass (2), and other complaint (2). Endosalpingiosis was an incidental finding in all cases, confined to the serosa in 5 and extending intramurally in 1. The decidual cases included florid decidualized endometriosis (3), deciduosis (2), and pseudodeciduosis (1). Two decidualized endometriosis cases presented as acute appendicitis in pregnant patients, diffusely involved the appendix, and obliterated the lumen (image); the third arose in a nulligravid woman with endometriosis and involved the serosa and outer wall. The deciduosis and pseudodeciduosis cases were serosa-confined.



Conclusions: Conventional appendiceal endometriosis can have several clinical presentations. Patients with it who present with acute appendicitis rarely develop it elsewhere. Otherwise, microscopic appearance and extent do not appear to correlate with symptomatology. Appendiceal endosalpingiosis is rare and effectively incidental. Decidualized endometriosis may overtake the entire appendix (unlike conventional endometriosis), whereas deciduosis and pseudodeciduosis are serosal lesions.

763 Clinical and Pathologic Characteristics of Incidentally Discovered Dysplasia in IBD Patients Resected for Medically Refractory Disease

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Background: Dysplasia in IBD is sometimes detected in surveillance biopsies (bx) with the exception being patients who present initially with cancer. In contrast, only a subset of IBD patients have dysplasia discovered incidentally, for the first time, in resections performed for medically refractory disease. As this form of "incidental" dysplasia (ID) has never been studied, we performed a case-control study to compare clinicopathologic features of patients with ID, and compare them with patients who had dysplasia detected by routine surveillance (SURV).

Design: 27 IBD patients with ID diagnosed between 2000 to 2013 were compared to 50 IBD SURV patients (controls) with dysplasia . Demographics, duration and location of disease, no.of previous bx, dysplasia focality (unifocal vs.multifocal), gross appearance (visible vs invisible), grade (low-grade, high-grade, invasive adenocarcinoma) and morphologic type (intestinal, serrated, mucinous, or mixed) were statistically analyzed.

Results: The study group consisted of 23 UC (86%), 2 CD (7%) and 2 (7%) indeterminate colitis patients (M:F:1.7,mean age:50yrs, range 17-83). Most patients had pancolitis (93%). Grossly, dysplasia was unifocal in 19 (70%) and multifocal in 8 (30%). The distribution of dysplasia was as follows: right colon (44%), left colon (44%), right and left colon (4%) and small bowel (8%). Of the 29 dysplastic foci, 13(45%) were visible and 16(55%) were invisible. Microscopically, all dysplasia was of the intestinal-type (100%). None of the patients had serrated, mucinous, or mixed dysplasia. 88% was low-grade, 12% was high-grade, and one case had invasive adenocarcinoma. 11 patients (41%) did not have any prior bx, 15 (56%) had 1-5 prior bx, and 1 (3%) had >5 bx in which dysplasia was undetected. In comparison to SURV group, those with ID dysplasia showed a significantly higher rate of unifocal dysplasia (70% vs 40%; p=0.02), invisible dysplasia (27% vs 55%; p=0.018), intestinal-type dysplasia (100% vs 32%; p<0.001), low-grade (88% vs 55%; p=0.003), and a significantly shorter duration of disease (8 vs 16 years, p=0.0045). No other clinical or pathologic differences were noted between the groups.

Conclusions: Patients with ID dysplasia show distinctive clinical and pathologic characteristics, including propensity to be unifocal, invisible, low-grade and of intestinal type. This suggests that in its earliest form, dysplasia may be exclusively of the intestinal type, and other non-intestinal types of dysplasia may develop further in the course of progression.

764 Morphologic Heterogeneity of Dysplasia in Inflammatory Bowel Disease: A Clinicopathologic Study

Deepa Patil, Erica Savage, John Goldblum, Robert Odze. Cleveland Clinic, Cleveland, OH; Brigham and Women's Hospital, Boston, MA.

Background: Since the initial description of the classification and criteria for IBD dysplasia in 1983, morphologic subtypes other than the intestinal type have been recognized. However, the clinical, pathologic, and biologic characteristics of these subtypes is unknown. The aim of this study was to evaluate the prevalence and clinicopathologic features of these recognized subtypes of dysplasia in an effort to better understand their characteristics and identify areas in need of future study.

Design: The study consisted of 50 pts who had resection for IBD dysplasia (UC=43, CD=7)between 2000-13. Clinical charts, gross and microscopic characteristics of the mucosa and dysplasia were evaluated according to recently published SCENIC classification of IBD dysplasia. Dysplasia was categorized as intestinal, serrated, mucinous, or mixed (mixture of ≥1 type). Age, gender, location and extent of colitis, gross findings (visible vs invisible), location and extent of dysplasia (unifocal vs multifocal) were also analyzed.

Results: Of the 50 IBD pts (M/F ratio:1.2,mean age: 54 yrs), 17 (34%) had serrated,16 (32%) intestinal, 4 (8%) mucinous, and 13 (26%) mixed dysplasia. Of the 67 foci of dysplasia identified in these 50 pts, there were 24 (36%) intestinal, 23 (34%) serrated, 4 (6%) mucinous, and 16 (24%) mixed dysplasia. In the serrated dysplastic foci, 15 (65%) showed intestinal differentiation but with serrated architecture, 3 (13%) showed features of a hyperplastic polyp or sessile serrated polyp only, and 5 showed serrated adenoma-like features (eosinophilic cytoplasm, lack of goblet cells, but oval-shaped non-stratified nuclei) (22%). The most common mixed pattern was intestinal plus serrated (N=9/16;56%). Two of the 16 mixed cases (13%) showed a combination of all 3 histologic types of dysplasia. Overall, dysplasia was most often visible (64%) vs invisible (36%), present on the left vs the right side (61% vs 37%), and more often multifocal vs unifocal (52% versus 47%). There were no differences between these dysplasia subgroups with regard to type of IBD, extent of colitis, or any other clinical or pathologic features.

Conclusions: Dysplasia in IBD is morphologically heterogeneous, occurs mostly on the left side, is visible, and more often multifocal. Dysplasia with serrated features, either pure, or mixed with other types, is the most common type of IBD dysplasia. Further pathology, biology and outcome studies should utilize a subclassification system of dysplasia that may help identify different molecular pathways of IBD carcinogenesis.

765 Dysplasia in Inflammatory Bowel Disease: Correlation of Biopsy and Resection Findings

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Background: Management of dysplasia in IBD depends on the endoscopic appearance, focality, and grade of dysplasia. Multifocal high-grade dysplasia (HGD) detected on surveillance biopsies is an indication for colectomy, while unifocal low-grade dysplasia is managed conservatively. In this study, we perform a detailed clinical and pathologic analysis of dysplasia detected in biopsy (bx) specimens and corresponding resections to determine the concordance between grade and focality of dysplasia.

Design: Biopsy specimens and corresponding resections from 50 IBD patients diagnosed between 2000-2013 formed the study group. Age, gender, duration and location of disease, location and focality (unifocal vs multifocal) of dysplasia, gross appearance, and grade (low-grade - LGD, high-grade-HGD, invasive adenocarcinoma-CA) and morphologic subtype (intestinal, serrated, mucinous, or mixed) were recorded and statistically analyzed.

Results: The study group consisted of 43 UC and 7 CD patients (M:F:1.2; mean age-54 yrs,range:22-84). Unifocal dysplasia was noted in 34 (68%) patients while multifocal dysplasia was noted in 16 (32%) patients. Comparison of unifocal versus multifocal dysplasia showed concordance between bx and resection findings in 38 (76%) cases (concordant group) and 12 (24%) discordant cases (discordant group) (kappa=0.448). There was a significant difference in mean age of patients between the concordant and discordant group (56 vs 44 yrs, p=0.02). Of the 12 discordant cases, 8 showed unifocal dysplasia on bx and multifocal dysplasia on resection, and 4 showed multifocal dysplasia on bx and unifocal dysplasia on resection. Majority of these 8 unifocal dysplasia patients had visible, multifocal lesions (5), with intestinal (3) or mixed type (3) dysplasia on resection. 28/35 LGD cases showed LGD on resection, while 7/35 showed HGD/CA (kappa=0.65). One case of unifocal LGD on bx showed multifocal HGD on resection, while 5 showed multifocal LGD on resection. There was no change in diagnosis in HGD cases, while 3 HGD bx showed cancer on resection. There were no significant differences in other clinical features between the concordant and discordant groups.

Conclusions: There is moderate concordance between bx and resection findings of unifocal and multifocal dysplasia. Patients with LGD that harbor HGD/CA are more likely to have visible, multifocal lesions on corrresponding resections. Up to 17% of patients with apparently unifocal LGD show multifocal LGD or HGD on resections, and are thus potential candidates for surgical therapy rather than conservative management.

766 Histopathological Findings in Gastrointestinal Tractus in Patients with Common Variable Immunodeficiency (CVID)

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Background: Histopathological diagnosis of common variable immunodeficiency (CVID) is challenging as it may mimic several other chronic inflammatory entities and yet is not well documented as the changes have a wide spectrum.

Design: Endoscopic and/or colonoscopic biopsies of 25 patients with CVID obtained in a 15 year-period including biopsies from esophagus (9), stomach (86), small intestine (77) and colon (13) were re-evaluated.

Results: More than half of the patients were female (60%). Age at the time of diagnosis ranged from 16 to 72 years (median, 35 years). Only 2 patients (8%) had esophagitis. Fifty-six percent of them had Helicobacter Pylori infection, 40% had atrophic gastritis with intestinal metaplasia and atrophic changes were seen in both antrum and corpus in half of them. None showed neuroendocrine cell hyperplasia. Nodular lymphoid hyperplasia was present in 40% and it was more common in antrum samples. There was

a paucity of duodenal/bulbar plasma cells in the vast majority of patients (92%). There was nodular lymphoid hyperplasia in duodenal/bulbar samples in 68%. Duodenitis/bulbitis and increased intraepithelial lymphocytes (IELs) was seen in 52% and 28%, respectively. Villous abnormalities were found in 44% and 7 patients (28%) had Giardiasis. Only 10 patients (40%) had colonoscopic biopsy samples and the most common finding was absence/paucity of plasma cells (16%), followed by cryptitis (12%) and nodular lymphoid hyperplasia (8%). None of the patients showed granulomatous reaction, however one patient had granulomatous hepatitis.
Conclusions: CVID enteropathy is a heterogeneous disease. In addition to absence/paucity of plasma cells, it may present with nodular lymphoid hyperplasia, mucosal architectural abnormalities and increased intraepithelial lymphocytes. Presence of Giardiasis in almost one third of patients was a notable finding in our series. These findings may be challenging in differential diagnosis with diseases such as celiac disease, lymphocytic gastritis, etc. and must be interpreted carefully to avoid misdiagnosis and unnecessary treatment.

767 Vasodilator-Stimulated Phosphoprotein (VASP) Immunoreactivity Is Associated with Lymphovascular Invasion of Colorectal Adenocarcinoma (CRC) Leading to Liver and Lymph Node Metastases

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Background: Vasodilator-stimulated phosphoprotein (VASP) is an actin binding protein that regulates cell shape and motility. The role of VASP in Colorectal Cancer (CRC) pathogenesis is unknown. In this study, we hypothesized that the role of VASP in CRC pathogenesis may be associated with the development and metastasis of CRC.
Design: A total of 141 tissue samples from 77 CRC patients were analyzed, which includes 117 tissue arrays (20 mm^2/tissue) from 63 patients, and 24 whole sections from 12 patients with paired primary and liver metastatic CRCs. 8 of the 63 patients had distant metastases to the liver or other organs. Immunohistostains of VASP and four Mismatch Repair (MMR) proteins (MLH1, MSH1, MSH6 and PMS2) were performed. BRAF and KRAS mutations were evaluated in patients with loss of MMR and/or liver metastasis. VASP immunoreactivity was double blindly scored as 0, 1+, 2+ or 3+ in density with the correlating percentage of positive cells. Statistical analysis was performed using Fisher exact test and statistical significance was set at a P value less than 0.05.
Results: VASP immunoreactivity was essentially negative in the non-neoplastic colonic mucosa. However, it was upregulated in the carcinoma cells in 81% of the CRC patients, and in 55% of the CRC adjacent adenoma of the same patients. VASP was negative in 7/63 CRCs (11%), 1+ in 22/63 CRCs (35%), 2+ in 23/63 CRCs (36%), and 3+ in 11/63 CRCs (17%). 19 of the 20 CRCs with liver or other distant metastases were strongly positive for VASP (2~3+). High level of VASP (2-3+) had significant positive correlation with lymphovascular invasion (n=58, P=0.0158), lymph nodes metastases (n=57, P<0.0001), distant metastases (n=77, P<0.0001), and particularly, liver metastases of CRC (n=71, P<0.0001). VASP was not associated with gender, age, BMI, tumor grade, tumor stage, loss of MLH1 and PMS2, BRAF mutation, or KRAS mutation (p>0.05).
Conclusions: VASP was upregulated in carcinoma cells of primary CRCs. High level of VASP immunoreactivity was positively correlated with lymphovascular invasion leading to liver and/or lymph node metastasis. Moreover, VASP had an increased expression in adenoma. Thus, VASP might be used as a predictive biomarker for the development and metastasis of CRCs.

768 Intestinal Follicular Lymphoma: Outcomes of Low Versus High Stage Disease

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Background: Primary follicular lymphoma (FL) of the gastrointestinal tract is known to be an indolent process with excellent long-term survival. This study compares the outcomes of patients with lymphoma limited to the intestinal tract (Stage I) to patients with extra-intestinal spread at the time of diagnosis.
Design: The pathology database was searched (2001-2014) for patients with a diagnosis of follicular lymphoma involving the small or large intestine. Clinical histories were reviewed and correlated with outcome.
Results: Specimens from 109 patients with a diagnosis of FL involving intestinal mucosa were identified. Since the majority of these specimens (88%) were seen in consultation, complete follow-up was available on only 35 (32%) of patients. Of these 35 patients, 18 (51%) had FL limited to the intestinal tract on initial diagnosis (Stage I). The remaining 17 (49%) patients had FL first diagnosed in the intestinal tract but then found to involve the lymph nodes (88%), liver (6%) and/or bone marrow (12%) (stage II-IV: "systemic" disease). The median age was 52 in the Stage I group and 51 in the systemic group, and women were more common than men in both: 83% in Stage I, 59% in systemic. The majority of patients had small intestinal rather than colonic disease, with small intestinal involvement in 17 (94%) of the Stage I group and 15 (88%) of the systemic group patients. Two patients in each group were diagnosed on a small bowel resection to relieve obstruction; the rest, on endoscopic biopsy. None of the patients in the Stage I group experienced B symptoms prior to diagnosis, while 4 (22%) patients with systemic disease endorsed fevers, night sweats, and/or weight loss. Six (33%) patients with Stage I disease received treatment: three had abdominal symptosm, and one presented with Grade 3B disease. One Stage I symptomatic patient who was treated with rituximab had nodal spread and large cell transformation at 2 and 6 years, respectively, after initial diagnosis. Ten (56%) patients with systemic disease received treatment, one of whom presented with grade 3A disease but did not progress. Two patients initially treated with Rituximab progressed to diffuse large B-cell lymphoma, as did 2 patients who did not receive initial therapy. Time to progression ranged from 1 month to 9 years (median 63 months).
Conclusions: Although FL limited to the intestinal tract appears to be an indolent disease, the importance of fully staging these patients and arranging for long-term follow-up cannot be overstated. Even patients with Stage I disease may undergo large cell transformation years after initial diagnosis.

769 Identification of Helicobacter pylori in Routine Gastric Biopsies without Reflex Ancillary Stains

Meredith E Pittman, Laura Wood, Elizabeth A Montgomery, Lysandra Voltaggio. Johns Hopkins University, Baltimore, MD.
Background: Despite the recommendation of expert gastrointestinal pathologists, private and academic centers (including our own) have continued to use ancillary stains for identification of *Helicobacter pylori*. The purpose of this study was to investigate if any cases of *H. pylori* would be missed with the removal of an up-front Diff-Quik stain and to determine the impact of discontinuing this special stain on the work-flow of the histology laboratory.
Design: Gastric biopsies were prospectively collected for one month. When *H. pylori* were identified, it was noted if the organism was seen on the hematoxylin and eosin stain, on the Diff-Quik stain, or both. Biopsies were correlated with demographic and laboratory data from the electronic medical record.
Results: From May to June 2014, 379 gastric biopsies were collected on 326 patients. The median age was 50 years (range 1-89), and 55% of patients were women. *H. pylori* organisms were identified in 23 patients (7%). Only one (4%) of these 23 cases was negative by H&E but positive on Diff-Quik; however, the H&E stained slide in this case showed the classic histologic finding of acute gastritis with a superficial lymphoplasmacytic infiltrate expanding the lamina propria. All patients with H. pylori showed either marked acute gastritis (87%) or marked inactive chronic gastritis (13%). No patients with normal gastric histology (47%), chemical gastritis (16%), or chronic inflammation (27%) were found to have *H. pylori*. On the other hand, there were 10 patients (3%) who had acute gastritis without identifiable *H. pylori* (confirmed retrospectively by immunohistochemistry). Two of these patients had a history of *H. pylori* and had been treated with antibiotics within the last 4 months. Two had chronic liver disease with varices, one had celiac disease, one had a pancreatic head mass, and four patients had no documented etiology for their acute gastritis. During the study month, 9 immunostains for *H. pylori* were performed in addition to the 380 Diff-Quik stains. After discontinuation of the Diff- Quik stain in our lab, approximately 30-40 immunostains are performed for *H. pylori* each month, which decreases overall technical time spent on gastric biopsies in the laboratory.
Conclusions: *H. pylori* organisms can usually be identified on routine H&E stained slides. The organisms are associated with marked superficial expansion of the lamina propria with or without epithelial neutrophils. Discontinuation of up-front ancillary studies saves laboratory technician time without compromising patient care.

770 Yap1 Oncogene Overexpression in Colerectal Polyps and Association with Nuclear ß-catenin

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Background: Yes-associated protein 1 (Yap1), an effector of the Hippo signaling pathway, promotes cellular proliferation when overexpressed. Yap1 overexpression and ß-catenin mutation are oncogenic events downstream from the APC tumor suppressor gene, reported commonly in colorectal cancers. Systemic evaluation of Yap1 in precursor colon polyps has not been performed. Our aim was to evaluate colon polyps for overexpression of Yap1 via immunohistochemistry (IHC) and correlate with polyp morphology, location, and ß-catenin expression.
Design: A tissue microarray was constructed with 16 tubulovillous adenomas (TVA), 27 tubulovillous adenomas with serrated features (TVASF), 10 traditional serrated adenomas (TSA), 18 sessile serrated adenomas (SSA), and 12 hyperplastic polyps (HP). IHC for Yap1 (H-125; sc-15407; Santa Cruz Biotechnology) was performed on all polyps, and nuclear and cytoplasmic staining was graded as negative, low, or high as described in prior studies. IHC for ß-catenin was performed on all TVA, TVASF, and TSA. Clinical information was collected through chart review.
Results: 15 polyps showed negative, 61 low, and 7 high cytoplasmic staining. 8 polyps showed negative, 6 low, and 69 high nuclear staining (see table 1). Right-sided polyps showed a nonsignificant trend of greater nuclear Yap1 overexpression (47.8% vs. 21.4%, p=0.08) as did serrated polyps compared to TVA (79.1% vs 46.1%, p=0.051). TVASF showed significantly more Yap1 nuclear overexpression compared to TVA (74.3% vs. 12.5%, p=0.001). Cores with Yap1 overexpression were significantly more likely to have nuclear ß-catenin on IHC (43.2% vs. 30.3%, p=0.02).

Polyp Type	(C) Neg	(C) Low	(C) High	(N) Neg	(N) Low	(N) High
HP	0/12 (0%)	11/12 (91.7%)	1/12 (8.3%)	0/12 (0%)	1/12 (8.3%)	11/12 (91.7%)
SSA	9/18 (50%)	9/18 (50%)	0/18 (0%)	1/18 (5.6%)	1/18 (5.6%)	16/18 (88.8%)
TSA	2/10 (20%)	8/10 (80%)	0/10 (0%)	2/10 (20%)	1/10 (10%)	7/10 (70%)
TVASF	1/27 (3.7%)	22/27 (81.5%)	4/27 (14.8%)	0/27 (0%)	1/27 (3.7%)	26/27 (96.3%)
TVA	3/16 (18.7%)	11/16 (68.8%)	2/16 (12.5%)	5/16 (3.2%)	2/16 (12.5%)	9/16 (56.3%)
Total	15/83 (18.1%)	61/83 (73.5%)	7/83 (8.4%)	8/83 (9.7%)	6/83 (7.2%)	69/83 (83.1%)
(C)=cytoplasmic, (N)=nuclear						

Conclusions: Yap1 nuclear overexpression in a majority of polyps suggests that aberrant expression occurs early in colorectal carcinogenesis. Frequent nuclear ß-catenin in association with Yap1 overexpression supports reported interaction with the APC pathway.

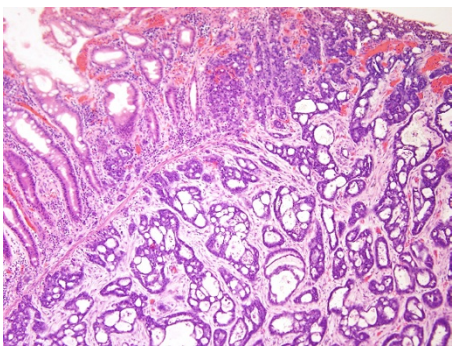
771 Germ Cell Tumor Metastases to the Gastrointestinal Tract: A Diagnostic Challenge

Justin Poling, Arunima Ghosh, Whitney Green, Maryam Kherad Pezhouh, Meredith E Pittman, Lysandra Voltaggio, Elizabeth A Montgomery. Johns Hopkins Medical Institutions, Baltimore, MD.

Background: Metastases to the GI tract often present diagnostic difficulty, especially in mucosal biopsy specimens. Germ cell tumors (GCT) display wide morphologic variation and can mimic primary GI cancers, especially if the primary tumor is occult at the time of biopsy. We reviewed biopsy and resection specimens with GI tract involvement by GCT of the testes, ovaries, or retroperitoneum to define the morphologic spectrum and clinical outcome of these cases.

Design: We searched our Pathology Data System, which include in-house and consult cases since 3/20/1984 for all GCT types involving the GI tract. We reviewed available slides. Follow-up data were collected.

Results: There were 11 cases of GCT involving the GI tract; 8 (73%) of these were in males and 3 (27%) were in females. Ages were 12-56 years (median 30 years). There were four (36%) yolk sac tumors (as pictured below); 4 (36%) seminomas; 1 (9%) mixed yolk sac tumor and dysgerminoma; 1 (9%) teratoma; and 1 (9%) choriocarcinoma; 1 case (9%) was initially diagnosed as carcinoma of unknown primary and the patient was later found to have a mixed GCT of the retroperitoneum morphologically consistent with the small bowel lesion. One of the seminomas had been diagnosed as small bowel Ewing sarcoma and treated accordingly; a testicular primary was later detected prompted by re-review of the initial slides. Five (45%) cases involved the small intestine; 5 (45%) the colon; and 1 (9%) involved both sites; no cases of gastric or esophageal involvement were found. Six cases (55%) with GI involvement were diagnosed prior to orchiectomy/oophorectomy; 3 (27%) were diagnosed concurrently with primary resection; 2 (18%) were diagnosed following primary resection. Two (18%) patients died (at 134 and 508 days post-operatively, respectively) following the GI diagnosis. IHC or FISH studies were done to support the diagnosis in 9 (82%) cases.



Conclusions: GCT metastases to the GI tract, although rare, can be a diagnostic pitfall, especially when the primary tumor is occult. These lesions should be considered in younger patients without risk factors for primary GI cancers. IHC and other ancillary studies can help resolve diagnostic difficulties.

772 Significant Prognostic Factors in Invasive Ampullary Adenocarcinoma (AC): Clinicopathologic Analysis of 367 Cases

Michelle Reid, Serdar Balci, Yue Xue, Nobu Ohike, Takuma Tajiri, Olca Basturk, Takashi Muraki, Grace E Kim, Alyssa M Krasinskas, Brian Quigley, Burcu Saka, Pelin Bagci, Bassel El-Rayes, David Kooby, Juan Sarmiento, Shishir Maithel, Volkan Adsay. Emory University, Atlanta, GA; MSKCC, NY, NY; UCSF, San Francisco, CA.

Background: The literature regarding the prognosis of AC is highly conflicted. Recent studies even suggest that the distinction of AC from pancreatic ductal adenocarcinoma (PDAC) is unnecessary because of their reportedly similar prognosis. This controversy is partly attributable to persistent confusion in the definition of AC, its' frequent inclusion with non-ampullary and non-invasive cancers in study cohorts, many of them from surgical databases lacking pathologic verification.

Design: Based on newly improved definitions the prognostic relevance of pathologic parameters was investigated in 367 stringently-defined, pathologically verified ACs. Only ACs with at least 3 mm of invasion were analyzed. Non-ampullary cancers (of CBD, extra-ampullary duodenum and PDACs) and non-carcinomas were excluded.

Results: M:F,1.4, mean age 65 (22-89); median overall size 21 mm, invasion size 17 mm (1-95). Overall 3, 5 and 10-yr survival were 62, 48 and 36%, respectively (vs 11, 7, <1, for pathologically verified PDACs). While AJCC Tstage did not correlate linearly with survival, size of invasion (for 1cm/2cm cutoffs) did (p=.001 and 0.03 respectively). Site-specific classification (ref, AJSP, 2012; PMID:23026934) also had significant prognosis: Median survival of amp-ductal cases was 38 mos vs 133 for intra-ampullary/IAPN-associated, and 103 for (peri)amp-duodenal AC (p=0.004). Among tubular type carcinomas, those with intestinal dominant pattern had better median survival than pancreatobiliary dominant type (101 vs 42 mos; p=0.015). Independent factors were age, PNI, LVI, margin and nodal status.

Independent Prognosticators on Multivariate Analysis					
Factor(%)		**Median Survival(mths)**	**HR**	**95%C.I.**	**P value**
Age			1:03	1:02-1.05	
PNI(34)	0:absent 1:present	81 vs 26	1.84	1.32-2.56	<0.001
Margin Positivity (5)		61 vs 16	3.8	2.0-7.4	
LV1(64)		106 vs 33	1.62	1.12-2.35	0.01
Node Metastasis(41)		107 vs 33	1.6	1.17-2.3	0.004

Conclusions: For invasive ACs, node metastasis, margin status, LVI and PNI are independent prognosticators. Site-specific classification of ACs is also important, because amp-ductal type tumors have significantly worse prognosis than amp-duodenal or intra-amp AC. Current T-stage system does not have significant value, whereas, size of invasion appears to be a prognostic factor and thus should be reported separately.

773 Adenocarcinoma Ex-Goblet Cell Carcinoid (XGC) Is a Morphologically Distinct "Appendiceal-Type Crypt Cell" Adenocarcinoma with Highly Aggressive Behavior and Frequent Association with Peritoneal/Intra-Abdominal Dissemination: An Analysis of 77 Cases

Michelle Reid, Olca Basturk, Walid Shaib, Serdar Balci, Hyejeong Choi, Gizem Akkas, Brian Robinson, Bahar Memis, Bassel El-Rayes, Charles Staley, Christopher Staley, Joshua Winer, Maria Russell, Jessica H Knight, Michael Goodman, Alyssa Krasinskas, Volkan Adsay. Emory University, Atlanta, GA; MSKCC, NY, NY.

Background: The clinicopathologic characteristics of XGC, a rare malignant appendiceal neoplasm, remain to be fully characterized.

Design: 77 XGCs were analyzed.

Results: Tumors occurred predominantly in females (73%) of mean age 55 years (29-84), most presenting with disseminated abdominal carcinoma (77% peritoneal, 59% gynecologic tract) and stage IV (65%) disease. Many first presented to GYN oncologists, and 9 had a working diagnosis of ovarian ca. Hematogenous metastasis (to liver(n=3) and lung(n=1)) was uncommon. A combination of distinctive histologic patterns rendered these tumors recognizable even at metastatic sites: I: Ordinary GCC/crypt pattern (rounded, non-luminated acini of goblet cells). II: GCC pattern but with high-grade morphology III: Poorly cohesive cell pattern (PCC), with diffusely-infiltrative cords/single signet-ring-like cells. IV: PCC with non-mucinous cell diffuse-infiltrative pattern. V: Microglandular pattern (relatively small round, rigid, rosette-like tubules) without goblet cells. VI: Rare patterns including other focal mixed carcinoma subtypes (intestinal/mucinous areas).VII: Solid sheet-like pattern punctuated by goblet cells/microglandular units. Ordinary carcinoid pattern (well-differentiated NET) with nested/trabecular growth was seen only in 2. Median survival was 38 mos, 5 yr survival 32%, and 33(52%) died of disease. On multivariate analysis perineural invasion (p=0.01, HR 0.12,C.I. 0.02-0.63) and younger age (<55 vs ≥55) (p<0.01,HR 0.06, C.I. 0.02-0.25) were independently associated with worse outcome. Female gender, LVI, clinical stage and nodal status showed a trend, but were not statistically significant.

Conclusions: XGC is an appendix-specific, high-grade malignancy with distinctive morphology recapitulating crypt cells in different formats, that is recognizable as appendiceal even at metastatic sites. It occurs predominantly in women, and is often disguised as GYN malignancy. Unlike intestinal-type adenocarcinomas, XGC typically spreads along peritoneal surfaces, and infrequently displays hematogenous metastasis. Unlike appendiceal mucinous neoplasms, XGC is infinitely more aggressive.

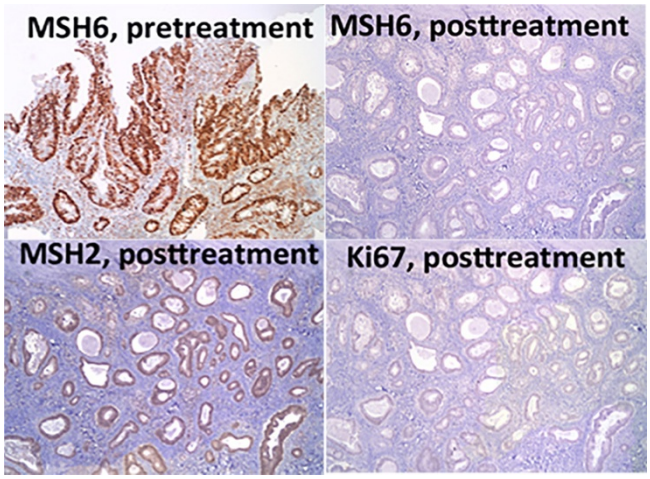
774 Concomitant Loss of MSH6 and PCNA Expression: A Putative Mechanism of Reduced MSH6 Expression in Microsatellite Stable Colorectal Cancer after Neoadjuvant Therapy

Bing Ren, Reetesh K Pai, Shih-Fan Kuan. University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: Immunohistochemistry of mismatch repair proteins (MMRP) is used for the universal screening for Lynch syndrome in colorectal cancer (CRC). Recent literature has shown that MSH6, but not MSH2, contains N-terminal sequence motifs characteristic of proteins that bind to PCNA (proliferting cell nuclear antige, or Ki67). Reduced expression of MSH6 is often identified in microsatellite stable (MSS) CRC after neoadjuvant therapy with cell-cycle specific reagents such as capecitabine and 5FU-containing chemotherapies, such as FOLFOX. The mechanism remains unclear. We correlated the expression of MSH6 and Ki67 in attempt to determine the mechanism of reduced MSH6 expression in MSS CRC after neoadjuvant therapy.

Design: 30 resected MSS CRCs with preoperative neoadjuvant therapy along with 10 cases of normal colon were retrieved for MSH6 (test) and MSH2 (control) staining and correlated with Ki67 staining. The percentage of cancer cells with loss of nuclear positivity were scored for each stain.

Results: All 30 CRCs showed MSH2 nuclear staining in all cancer cells. 8 of 30 (27%) cases revealed reduction of MSH6 nuclear staining ranging from 10 to 100%. The loss of MSH6 coincided with loss of Ki67 in the individual cells for all cases. Reduction of MSH6 expression was mostly associated with capecitabine and high cycles of FOLFOX while 5FU or radiation alone did not reduce MSH6 expression. Normal colon expressed both MSH6 and Ki67 in the proliferation zone at lower crypts. The maturation zone in the upper crypts was negative for MSH6 and Ki67.



Neoadjuvant treatment	Total case #	No. of cases with reduced MSH6 expression (percent of loss)
Capecitabine	7	4 cases (100%, 90%, 50%, 10%)
FOLFOX (>6 cycles)	4	3 cases (90%, 60%, 20%)
FOLFOX (up to 6 cycles)	11	1 case (10%)
5FU only	5	0
Radiation only	3	0

Conclusions: We demonstrate an MSH2-dissociated, PCNA(Ki67)-related loss of MSH6 expression in MSS CRC after high cycles of cell cycle specific chemotherapy and in normal colon. Our results indicate that MSH6 expression is closely linked to Ki67 expression and that cell cycle specific chemotherapy results in reduced expression of both proteins.

775 Programmed Death Ligand-1 (PD-L1) Expression in Mismatch Repair Deficient Gastric Tumors and Gastric Tumors Positive for Epstein-Barr Virus

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Background: Recent clinic studies showed mismatch repair deficient (MSI) colorectal tumors were more responsive to Programmed death 1 (PD-1) blockade than mismatch repair proficient (MSS) tumors. Aberrant expression of PD-1 and its ligand 1 (PD-L1) has been demonstrated in gastric cancer. The TCGA gastric adenocarcinoma study also demonstrated gene amplification and mRNA over expression of *PD-L1/L2* in Epstein-Barr virus positive (EBV+) gastric tumors. However, little is known if the PD-L1 expression in gastric cancer is associated with MSI and EBV infection. In this study we sought to correlate PD-L1 expression in gastric cancer with mismatch repair and EBV status.

Design: Thirty-eight resected gastric cancer cases including 6 EBV+, 12 MSI and 20 MSS tumors were stained and scored for the intensity and proportion of tumor infiltrating lymphocytes (TILs) and expression of PD-L1. Positive PD-L1 expression in tumor cells and TILs was defined as 5% or more cells with membranous staining. The intensity of TILs was graded as none, focal, moderate, and severe. The proportion of CD4-positive and CD8-positive TILs was determined as CD4 < CD8 and CD4 ≥ CD8.

Results: The mean age of the 38 patients was 72 years; 63% (24/38) were men. Medullary histology was significantly enriched in EBV+ tumors (6/6, 100%) compared with that in MSI (4/12, 33%) and MSS tumors (0/20) ($P < 0.0001$). The remainder MSI (8/12, 67%) and 75% (15/20) of MSS tumors were adenocarcinoma, intestinal type. Both EBV+ and MSI tumors had a significantly greater amount of TILs and a greater density of CD8-positive TILs than did MSS tumors ($P < 0.0001$ and $P = 0.0032$, respectively). Membranous PD-L1 expression was observed predominantly in tumor cells and TILs located at the invasive fronts of tumors. Of the 38 cases, 15 (39%) had positive PD-L1 expression in tumor cells and 13 (34%) in TILs. Compared with MSS tumors, significantly higher proportions of EBV+ and MSI tumors were positive for PD-L1 expression in both TILs (EBV+: 5/6, 83%; MSI: 5/12, 42%; MSS: 3/20, 15%; $P = 0.0369$) and tumor cells (5/6, 83%; 8/12, 67%; 2/20, 10%; $P = 0.0041$).

Conclusions: Our results show positive PD-L1 expression was predominantly seen in MSI and EBV+ gastric tumors. These findings suggest an immune-active tumor microenvironment and PD-L1 expression are associated with EBV infection and microsatellite instability in gastric tumors. Our study provides further support for the application of immune checkpoint inhibitors for gastric cancer treatment.

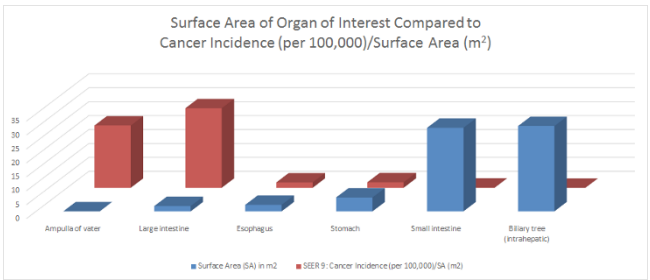
776 The Ampulla of Vater, a Potential Cancer "Hot Spot": Higher Cancer Incidence Per Surface Area in Comparison to Other Sites of GI Tract in the Last 4 Decades of SEER 9 Registry

Elizabeth Richards, Matthew Yeh. Montefiore Medical Center, Bronx, NY; University of Washington, Seattle, WA.

Background: The ampulla of vater is a unique anatomic area with the convergence of the pancreatic and common bile ducts and transition between foregut and midgut. Though ampullary cancer is rare, accounting for 0.5% of GI malignancies, more cancers arise in the ampulla than the rest of the small intestine (SI). We propose that relative to its small surface area (SA), the ampulla has a disproportionately high cancer incidence in comparison to other sites in the GI tract.

Design: To determine the SA of the GI organs we pooled data available from the literature which analyzed SA utilizing light and electron microscopy, autopsies, endoscopic ultrasound, and computer-aided 3D imaging techniques. To define cancer incidence per anatomic site per 100,000 people we used SEER*Stat Software 8.2.1 to pull data from the SEER Registry 9 database from the National Cancer Institute. SEER 9 contains epidemiologic data from 1973-2012 and includes approximately 9.4% of the United States population. We then calculated a ratio of cancer incidence/SA for the esophagus, stomach, SI, ampulla, intrahepatic biliary tree (IHBT) and colorectum.

Results:



The ampulla and colorectum had the highest cancer incidence/SA, of 22.36 and 28.56 (per 100,000/m^2) respectively. When compared to the SI, which had an incidence/SA of 0.07 (per 100,000/m^2), the ampulla had a more than three hundredfold increase. The IHBT, which has a similar SA to the SI (30.64 m^2), also had a low incidence/SA of 0.02 (per 100,000/m^2)- more than a thousandfold less than the ampulla. The esophagus and stomach had rates of 1.93 and 1.97 (per 100,000/m^2) respectively, more than tenfold lower than the ampulla.

Conclusions: Previous studies have examined the relationship between SA and cancer incidence in other organs, however, to our knowledge this is the first study that focuses on the ampulla of vater and GI tract. We propose that the high incidence of cancer in the ampulla relative to its SA points to it being a potential cancer "hot spot". This implies that there may be unique pathways towards tumorigenesis in the ampulla of vater in comparison to the rest of the SI and GI tract which warrant further investigation.

777 Digital Image Analysis of Serrated Lesions of the Colorectum

Brian Robinson, David Martin, Alton B Farris. Emory University, Atlanta, GA.

Background: Discrimination of serrated lesions in the colon, in particular sessile serrated adenomas (SSAs) and microvesicular hyperplastic polyps (HPs), has proven difficult given the morphologic overlap of these lesions. Consequently, several studies have highlighted substantial interobserver variability among pathologists in diagnosing these lesions in everyday practice. Architectural features are often used in the discrimination of SSAs from HPs; however, investigators have also pointed out cytologic differences. Given apparent limitations in correlation between pathologists for the determination of SSAs and HPs, we investigated the nuclear morphometric features of serrated lesions using digital image analysis.

Design: Archived cases representing normal colonic mucosa (n=10), HPs (n=8), SSAs (n=10), and conventional tubular adenomas (TAs) (n=8), were digitally scanned with a whole slide scanner. Ten representative nuclei from the crypt, mid-villus, and surface sections in areas exhibiting the diagnostic pathologic change for each case were then circumscribed at 40x-magnification. Parameters for the discriminant analysis between groups included: nuclear circumference (um), area (um2), and 13 different nuclear staining measurements from a positive pixel count (PPC) algorithm.

Results: Average nuclear area and circumference were significantly different (p<.0001 by Student's t-test) between all groups at the mid crypt region and between several groups in the crypt and surface regions (Figure 1). The PPC algorithm also demonstrated significant differences between groups in different regions when evaluated for: number of total pixels, total area in of all pixels, and average staining intensity. For the PPC algorithm, the average intensity appeared to be the most discriminative and was significantly different between several groups.

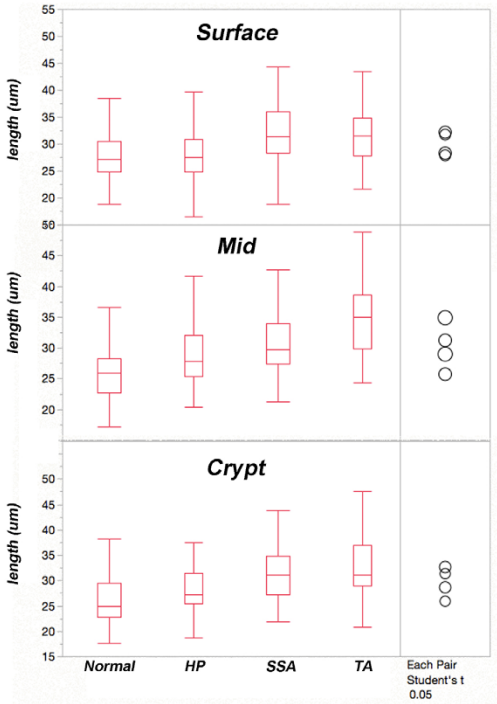


Figure 1. Box Plots (left) depict the distribution of data for each diagnostic category. Circles (right) graphically depict the t-test with non-intersecting circles statistically different. Intersecting circles are not statistically different.

Conclusions: Our results indicate that image analysis of nuclear features shows promise in helping to delineate SSAs from HPs. Nuclear area, circumference, and findings seen with the PPC algorithm distinguished serrated lesions from one another with statistical significance. Future studies will be useful in further automating the nuclear analysis and in validating these findings, particularly in polyps where there is diagnostic ambiguity.

778 Sleeve Gastrectomy: Unanticipated Findings in the Pathology Review. More Than Anyone Expects!

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Background: Sleeve gastrectomy is a surgical weight-loss procedure which is rapidly becoming the most common treatment option for bariatric patients with a BMI of 60 or higher. This procedure is usually performed laparoscopically and involves the removal of about 75-80% of the stomach along the greater curvature creating a narrow gastric tube or "sleeve". The University of Miami Hospital (UMH) is the only university-based bariatric center in the region and one of the most sought after destination for bariatric surgery. The patients that undergo these surgeries are evaluated extensively and presumed not to have any significant gastric pathology. The aim of this study is to review the pathologic findings seen in sleeve gastrectomy cases over a 2 year period.

Design: A retrospective review of the pathology database at University of Miami Hospital (UMH) from 2013-2015 was performed for all sleeve gastrectomy cases including the pathologic diagnosis, gross description and demographic information.

Results: There was a total of 524 specimens identified in the two year period. The most prevalent diagnosis was congestion seen in 42.7% of cases (224/524) follow by chronic gastritis; 40.6% (213/524) and active chronic gastritis; 7.5% (40/524). Additional findings included Helicobacter pylori infection, 3.2% (17/524) gastric fundic gland polyps; 2.8% (15/524), intestinal metaplasia without dysplasia in 2.26% (10/524) and autoimmune atrophic gastritis; 0.3% (2/524). Neoplastic tumors were identified in 1.7%, 8 GIST and 1 leiomyoma. No malignancies were identified. Non-specific histopathologic changes were found in 12% of the specimens (66/524). Female patients were more likely to have this procedure (378) than males (146). The patients ranged from 16 to 79 years (mean age of 44 years), 14 patients were teenagers.

Conclusions: The demographic population that is seen at UMH is diverse and there are a variety of additional or incidental findings which usually requires follow up. The most frequent diagnosis is chronic gastritis and congestion. The neoplastic unexpected findings include 8 gastrointestinal stromal tumors and 1 leiomyoma. No malignancies were identified.

779 Completion Resections in LAMN with Extra-Appendiceal Mucin and Appendiceal Margin Involvement

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Background: The management of patients with low grade appendiceal mucinous neoplasms (LAMNs) who have undergone an appendectomy is unclear. There are no clear guidelines regarding appropriate management of patients where the appendiceal margin is positive. Recently some authors have shown the presence of neoplastic epithelium/acellular mucin at the appendiceal margin does not predict recurrence in patients with LAMNs confined to the appendix nor the presence of residual disease in the subsequent resection specimen. These authors advocate conservative management in these patients. It is unclear if other patients could also be spared additional completion surgeries. This study aims to evaluate the status of subsequent resections in patients with LAMN, extra-appendiceal mucin, and an involved appendiceal margin.

Design: The pathology database was searched to identify patients with appendectomies and subsequent completion resections between 2007 and 2014. Cases with LAMN, including acellular mucin outside the appendiceal wall with or without perforation, and the presence of neoplastic epithelium/mucin at the appendiceal margin were included. Cases with LAMN and neoplastic cells outside the appendix and other appendiceal malignancies, e.g. mucinous adenocarcinomas, were excluded.

Results: Nine patients (4M, 5F) with a mean age of 52 years (range 28-68 yrs.) were identified in the database. Neoplastic epithelium was present at the appendiceal margin in 6 (67%) patients, and acellular mucin was present at the appendiceal margin in 3 (33%) patients. LAMN with acellular mucin outside the appendix was identified in 8 (89%) patients, and perforation was seen in 2 (22%) patients. In the subsequent completion resections, there was no residual disease at the appendiceal stump and cecum adjacent to the appendiceal orifice in 7 (78%) patients. Residual disease at the appendiceal stump was seen in 2 (12%) patients who had neoplastic epithelium on the appendiceal margin. No nodal disease was identified in any of the patients. However, peritoneal dissemination was identified in 7 (78%) patients. All patients were alive at last follow up (mean 4 years; range 1.6-7 yrs.).

Conclusions: Similar to patients with confined LAMN, there may be limited utility of a subsequent resection of the appendiceal stump/cecum in patients with positive appendiceal margins at the time of surgical intervention. While these patients may have disseminated disease, the likelihood of residual local disease is low.

780 Alterations of the Genomic Landscape in Inflammatory Bowel Disease-Associated Neoplasia

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Background: Inflammatory bowel disease (IBD)-related dysplasia has a higher incidence of concomitant neoplastic lesions and a faster progression rate compared to sporadic lesions. Previous studies have highlighted increased chromosomal instability in IBD-related neoplasia. In addition to genetic alterations shared with sporadic colorectal carcinomas (i.e., gains of 8q, 13q, 20q and losses of 17p and 8p), IBD-related neoplasms are also associated with gains of 5p and losses of 5q and 17q. Screening for IBD-associated colorectal neoplasms is based on increased frequency of colonoscopic examinations in patients with long-standing disease. Genetic alterations implicated in IBD have been identified mainly using fresh tissue samples, with only partial validation for limited gene panels on paraffin-fixed tissue.

Design: Formalin-fixed IBD biopsy samples were analyzed for genome-wide copy number alterations and loss of heterozygosity using Affymetrix OncoScan Molecular Inversion Probe (MIP) arrays. Nine low-grade dysplasia (LGD), two high-grade dysplasia (HGD), one adenocarcinoma (AC) and nine non-dysplastic matched control cases were included. DNA was extracted from formalin-fixed samples after microdissection for epithelial enrichment. After processing, the results were analyzed with NexusExpress software.

Results: The HGDs and AC shared gene copy number gains on 5p, 19q, chromosomes 7 and 20, and losses of 17p, in addition to other individual sample genetic alterations. The LGD cases showed a much lower fraction of genome alterations (mean of 0.87%) compared to HGD (36.3%) ($p<0.01$ on T-test, unpaired). Four LGD cases showed large regions of genomic alterations including loss of heterozygosity at 5q, 17q, 4q, 10q and gene copy number gains at 13q and chromosome 20.

Conclusions: Genome-wide SNP genotyping by MIP arrays, a method that requires low DNA sample input, is useful for gene copy number detection in formalin-fixed biopsy samples. Genomic alterations, including some that are similar to previously characterized ones, have been detected in a subset of the LGD cases and, with higher frequency and complexity, in the AC and the HGD cases analyzed. This method may develop as a valuable tool for integrating molecular analysis in the pathology diagnosis and potentially help detect IBD-associated dysplastic lesions at higher risk of progression to carcinoma.

781 Carbonic Anhydrase IX (CA9) Expression in Adenocarcinoma of the Stomach and Esophagus

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Background: Carbonic Anhydrase IX (CA9) is a biomarker for clear cell renal cell carcinoma and only positive in very few malignancies. Among normal tissue, only gastric mucosa and bile duct are strongly positive for CA9. In this study, we would like to evaluate CA9 expression in adenocarcinoma of stomach (ADCS) and esophagus (ADCE).

Design: We examined a total of 91 Cases, including: 42 cases of ADCS, 27 cases of ADCE/high grade dysplasia (HD), and 22 cases of esophageal low-grade dysplasia (LD). Among these, 39 cases contained Barrett's esophagus (BE). All the cases were subjected to CA9 immunostaining (Sigma, 1:100 dilution). For each case, CA9 expression was carefully examined for intensity and pattern.

Results: The normal gastric mucosa, BE and BE with LD showed strong membranous staining for CA9. Most ADCS (81%) demonstrated strong membranous CA9 staining, even in signet-ring cell carcinoma.

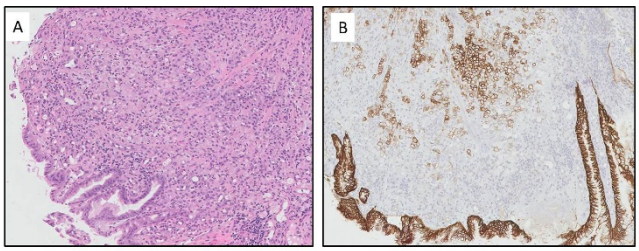

Fig. 1. Signet ring cell carcinoma of the stomach.
(1A) H&E. (1B) CA9 staining is retained in signet ring cells.

On the other hand, a significant portion of ADCE/HD (44%) lost CA9 membranous staining. Interestingly, a large portion of ADCE/HD (60%) showed nuclear CA9 staining.

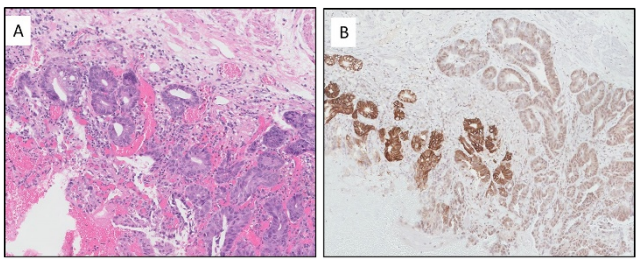

Fig. 2. Adenocarcinoma of the esophagus with Barrett's mucosa.
(2A) H&E. (2B) Loss of membranous and gain of nuclear CA9 staining in adenocarcinoma of the esophagus.

Conclusions: Since only few malignancies are positive for CA9, CA9 could be a novel ADCS biomarker in the right clinical context. The loss of membrane/gain of nuclear CA9 staining in ADCE can be used to distinguish it from ADCS or BE with low grade dysplasia.

782 Line-1 Methylation Status Predicts Aggressive Behavior in Pancreatic Endocrine Neoplasms

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Background: Global DNA hypomethylation is associated with activation of transposable elements, which results in genomic instability and tumorigenesis. Long interspersed nuclear element-1 (LINE-1), a retrotransposon comprising approximately 17% of the human genome, serves as a surrogate marker for global DNA methylation. A prior study from our institution demonstrated that LINE-1 RNA expression is a marker of LINE-1 methylation status (i.e. low LINE-1 RNA correlates with hypermethylation of genomic LINE-1. Moreover, LINE-1 hypomethylation has been independently correlated with poor prognosis in several malignancies, including colon cancer. LINE-1 methylation status may therefore serve as a robust marker of aggressiveness for tumors in which prognosis is indeterminate. Initial studies report that LINE-1 is hypomethylated in pancreatic neuroendocrine neoplasms; however, the clinicopathological significance remains to be studied in a large cohort of patients.
Design: In situ hybridization (ISH) using an RNA probe aligned to the open reading frame 1 of LINE-1 was performed on formalin-fixed, *paraffin-embedded tissue* from pancreatic neuroendocrine tumors. The intensity of LINE-1 staining in malignant cells was then compared with staining in stromal cells. Tumors in which malignant cells showed a signal low or equal to non-neoplastic cells were considered LINE-1 hypermethylated, while tumors with relatively high staining were considered LINE-1 hypomethylated. Ki67 proliferative index, and mitotic index were recorded. Disease specific survival was obtained, with a mean follow-up for this cohort of 80 months.
Results: The mean age of the cohort was 58 years; 48% of patients were male. The mean tumor size was 3.6 cm. At presentation, 54% of patients were stage I and 46% stage II. 87 tumors were evaluated for LINE-1 status, with 26 (29%) tumors staining high for LINE-1 (LINE-1 hypomethylated) and 61 (68%) tumors staining low**.** The survival rate of the high LINE-1 (hypomethylated) group was significantly lower than the rate of the low LINE-1 group ($p = 0.04$). The low LINE-1 group maintained a 100% survival rate with no tumor related deaths, while the high LINE-1 group had 11 (42%) deaths.
Conclusions: LINE-1 hypomethylation, a finding indicative of global genomic hypomethylation, predicts a higher risk of tumor related deaths in pancreatic neuroendocrine neoplasms. We demonstrate a LINE-1 status assays that is easily-implemented in the clinical laboratory.

783 Histopathologic Features of Colorectal Carcinoma in Patients with HIV Infection

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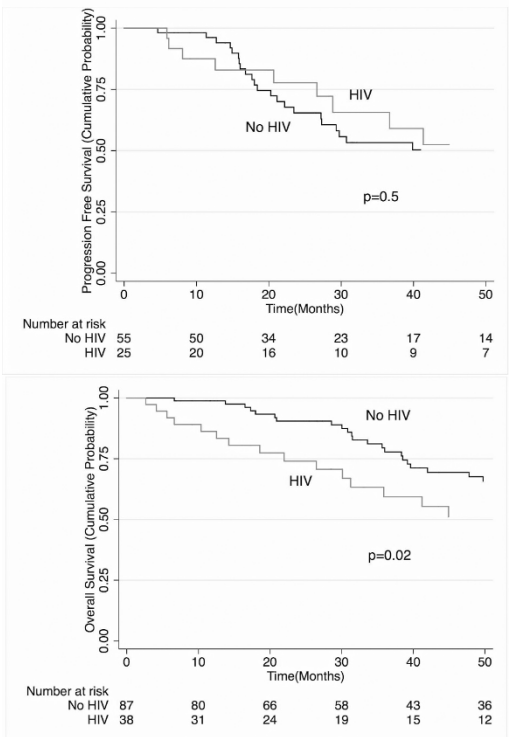
Background: Non-AIDS-defining cancers have become a major cause of mortality in patients with HIV infection due to effective antiretroviral therapy and aging of the HIV infected population.Emerging evidence suggests clinical differences in HIV+ patients with colorectal cancer (CRC) compared to HIV- patients including earlier onset, higher tumor grade and stage, and rapid disease progression.We compared clinicopathological variables and outcome in HIV+ and HIV- CRC from a single large cancer hospital.
Design: A nested case-control study was conducted within our hospital-based cohort of 14,116 patients treated for CRC between 1993-2014.Cases were identified by query for the ICD-9 code for HIV infection and confirmed by chart review.Control patients were selected randomly from all patients treated for CRC.Patients were matched by age, gender, year of CRC, and race/ethnicity.Histology was assessed for presence of mucin, tumor infiltrating lymphocytes (TIL) per 10 high power fields (HPFs), and tumor grade.Clinical and tumor characteristics were compared between cases and controls using fixed effects logit modeling to account for matching.
Results: A total of 184 CRC (38 HIV+, 146 HIV- control) were included.Median patient age at CRC onset was 55.The average duration of HIV infection prior to CRC was 15 years and 66% of patients had well-controlled HIV at CRC diagnosis.Compared to HIV- CRC, HIV+ patients were more likely to have smoked (P=.001); their CRCs were more likely to be right-sided (37% vs 14%; P=.003), had a higher frequency of TILs above 50/10 HPFs (21% vs 7%) and a lower frequency of absent TILs (14% vs 36%; P=.02).No differences in tumor grade, stage, mucinous histology or peritumoral lymphocytes were detected.HIV+ CRC patients had reduced overall survival (OS) (P=.02) but no difference in progression free survival (PFS).

Figure 1. PFS (top) and OS (bottom) in HIV+ patients with CRC



Conclusions: CRC onset in HIV+ is at a lower median age than population-based (SEER) estimates (median 69 years) and with a higher frequency of right-sided disease which should inform screening.Lower OS is likely due to HIV-related morbidity.Our study is the first to establish a higher frequency of TILs in HIV+ CRC suggesting altered tumor biology and host-immune interaction.

784 Mismatch Repair Protein Deficiency Is Not Present at a Higher Rate in HIV-Infected Patients with Colorectal Carcinoma

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Background: Microsatellite instability (MSI) has been shown to be present at a higher rate in Kaposi sarcoma, lymphomas, and lung cancer in HIV infected patients (HIV+) when compared to patients without HIV infection (HIV-). It is not known whether this difference exists in colorectal cancers (CRCs), a tumor type with a subset harboring MSI. We recently observed that CRCs in HIV+ patients have early onset (median age, 55 years) and show elevated tumor infiltrating lymphocytes (TILs), both characteristic of CRCs with MSI. To explore whether MSI is causally related, we systematically evaluated for MSI in CRCs arising in HIV+ patients using matched HIV- CRC cases as control and immunohistochemistry (IHC) for mismatch repair (MMR) proteins as the detection method.
Design: We conducted a nested case-control study using our hospital-based cohort of 14,116 patients treated for CRC between 1993-2014. Cases were identified by query for the ICD-9 code for HIV infection. Control patients were selected randomly from all patients treated for CRC. Patients were matched by age, gender, race/ethnicity, and CRC site (right/left). IHC for MLH1, MSH2, PMS2, and MSH6 was performed. Histology was reviewed for tumor grade, TILs, and mucinous histology. MMR expression and histology were compared using fixed effects logit modeling to account for matching.
Results: 83 CRC (21 HIV+, 62 HIV- control) were included. The median age of onset after matching was 55 years. When compared to the HIV- CRC group, HIV+ CRC had no significant difference in loss of MMR protein expression; 11% (HIV- n=7) vs 14% (HIV+ n=3); P=.6. The three HIV+ patients with MMR abnormalities comprised:

MLH1/PMS2 loss with corresponding *MLH1* germline mutation, PMS2 loss with MSI-high, and MLH1/PMS2 loss with unknown genetic status. Histologic comparison of HIV+ CRC to HIV- CRC confirmed higher frequency of TILs above a threshold of 50 TILs per 10 high power fields in HIV+ patients (>50TILs/10HPF: 29% vs 6%) and a lower frequency of absent TILs (12% vs 69%; *P*=<.001). No differences in grade, mucinous histology, or peritumoral lymphocytes were detected.
Conclusions: A higher rate of MMR protein expression was not detected between matched CRC with and without HIV suggesting the presence of TILs may be related to mechanisms other than MSI.

785 Mismatch Repair-Deficient Neuroendocrine Carcinomas of the Lower GI Tract

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Background: Mismatch repair deficient (dMMR) adenocarcinomas (ACA) of the colorectum are known for better outcome and different response to chemotherapy, as well as differing clinicopathologic features, when compared to MMR proficient (pMMR) ACAs. Studies attempting to establish the same association in high-grade neuroendocrine carcinomas (NECs) of the GI tract are few and have shown conflicting results. We collected poorly differentiated NEC cases of the lower GI tract (LGI) from our institution to investigate the frequency of dMMR tumors, their clinicopathologic features and outcome.
Design: Pathology database was searched for pure or combined NECs (associated with adenoma, ACA or squamous cell carcinoma) of the LGI. Features evaluated included patient demographics, tumor histology (NEC morphology [small cell or non-small cell], presence, type and grade of exocrine component, presence of tumor infiltrating lymphocytes [TILs, ≥4/HPF] and peritumoral lymphoid aggregates [PTLA]), TNM staging, and clinical outcome. MMR status was determined by immunohistochemistry for MLH1, MSH2, MSH6 and PMS2 in all cases.
Results: Fifty LGI NECs with available material were included. Mean age at diagnosis was 62 years, with no gender or site predilection. Non-small cell morphology was seen in 76% of cases and an exocrine component in 64%. Five cases (10%) were dMMR: 4 lost MLH1/PMS2 and 1 lost MSH6 alone. Compared to the 45 pMMR cases, dMMR were larger in size (mean 6.9 vs 4.1 cm, p=0.004), were more likely to have TILs (60% vs 11%, p=0.005), and were more often node-negative (100% vs 27%, p=0.01). dMMR tumors also showed trends toward right-side dominance (80% vs. 35%, p=0.1), older age at diagnosis (mean 73 vs 60 years, p=0.062), presence of PTLA (60% vs 11%, p=0.057), and lower overall stage (node and distant metastasis-negative) (60% vs 18%, p=0.057). Follow-up information was available for 4 of 5 dMMR patients, none of which died of disease (median follow up 28.8 months; range 8-60), whereas 32% (12/38) of pMMR patients did (median follow up 18.3 months; range 1.2-129.3).
Conclusions: Ten percent of poorly differentiated NECs of the LGI were dMMR and showed overlapping clinicopathologic features with dMMR ACAs. Occurence at an older age and higher frequency of MLH1/PMS2 deficiency suggests dMMR is more likely a sporadic event related to *MLH1* promoter hypermethylation. Although limited, our data suggests dMMR NECs may represent a subset of these highly aggressive tumors that have a better prognosis and that would potentially benefit from arising treatment options for dMMR tumors, specifically immune therapy.

786 Pathologic Features and Pouch Outcome in Patients with Ulcerative Colitis and "Extended Backwash" Ileitis

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Background: Studies have shown that terminal ileum (TI) inflammation ("backwash" ileitis;BWI) in UC or indeterminate colitis (IC) patients is usually restricted to 2-5cm, and has no impact on pouch outcome. Anecdotally, we have seen cases of ≥ 5 cm of resected TI with histologic evidence of BWI ("extended backwash" ileitis;EBWI). The clinicopathologic features of this subgroup have not been studied. Here we compare the clinical, pathologic, and pouch outcome data of this cohort to UC patients with "regular" BWI and those without BWI.
Design: Between 1992-2015, 18 resections with EBWI were compared to 20 with BWI and 20 without BWI. Age, gender, duration and distribution of disease, pouch outcome, degree of TI and colonic inflammation (grade 0-none,1-lamina propria (LP),2-cryptitis,3-crypt abscess,4-ulcer), villous atrophy (none, subtotal, total), LP mononuclear inflammation (LPMI), muscularis mucosae thickening (MMT), pyloric gland metaplasia (PGM), granulomas, transmural lymphoid aggregates (TLA) ,and dysplasia were recorded and statistically analyzed.
Results: The mean age of the study group (M:F:3:2) was 35 yrs (range:12-73). The indication for resection was medically refractory disease (50%) or dysplasia (44%), with a mean disease duration of 10 yrs (range:1-31). The mean length of resected TI was 13.3 cm (range:5-22.5). Most cases had grade 3/4 TI inflammation (88%), increased LPMI (89%), subtotal villous atrophy (83%), MMT (83%), PGM (67%), and diffuse TI involvement (89%). No patients had granulomas or TLA. All cases had pancolonic grade 3/4 colonic inflammation. 7/18 (39%)patients had dysplasia (4-LGD, 2-HGD and 1-invasive adenocarcinoma). In contrast to BWI patients, those with EBWI had a higher rate of LPMI (89% vs 55%;p=0.03), MMT (83% vs. 10%;p<0.001), and PGM (67% vs 15%;p=0.002). No clinical differences were seen between EBWI patients and BWI patients. Pouch procedure was performed in 14/18 cases of EBWI, 18/20 cases of BWI and 20/20 cases of non-BWI. After a mean follow-up of 4.4 yrs, most patients from the study (83%) and control group (BWI- 89%, no BWI- 90%) had no pouch-related complications.
Conclusions: "Extended" BWI is rare and is characterized by inflammatory changes that are slightly more severe than "regular" BWI, with prominent PGM, MMT, and LP inflammation. As there was no significant difference in pouch outcome between the groups, EBWI should not be considered a contraindication to pouch procedure or alter the surgical management of UC or IC patients.

787 Clinical Significance of Fibroblast Growth Factor Receptor 2 Expression on Residual Disease after Preoperative Chemoradiotherapy for Rectal Cancer: A Retrospective Single Institution Study

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Background: Aberrations of the fibroblast growth factor/fibroblast growth factor receptor (FGFR) signaling has been found in colorectal cancer, and blocking of this pathway has been considered as a potential candidate target. The purpose of this study was to explore the prognostic value and clinical significance of FGFR2 on residual disease after preoperative chemoradiotherapy (CRT) in patients with rectal cancer.
Design: The surgical specimens of 141 patients with residual rectal cancer after preoperative CRT were analyzed. Immunohistochemistry was performed to evaluate FGFR2 expression using whole section tissues. Because FGFR2 protein was strongly expressed in the membrane of normal non-neoplastic colonic epithelium, all cases with no, weak, and moderate reactivity in tumor cells were considered low-expression, whereas cases with strong reactivity were high-expression.
Results: Of the 141 cases,low-expression of FGFR2 was observed in 24.8% (n=36). Low-expression of FGFR2 significantly correlated with absence of neural invasion (p=0.012), superior tumor regression (p=0.028), and lower clinical stage (p=0.023). However, FGFR2 expression showed no relationship with *KRAS* or *BRAF* mutation. On univariate analysis, low-expression of FGFR2 was significantly or at least tentatively associated with better rectal cancer-specific survival (RCSS) (p=0.009) and disease-free survival (DFS) (p=0.076). Unfortunately, multivariate analysis revealed that low-expression of FGFR2 was not independently associated with RCSS and DFS (p>0.05).
Conclusions: Although FGFR2 protein expression is not an independent prognostic marker, the low-expression of FGFR2 can predict a better prognostic outcome. Our results may aid in understanding the therapeutic approaches targeting FGFR2 in patients with residual rectal cancer after preoperative CRT.

788 Immunohistochemistry for the Mismatch Repair Proteins is Concordant between Colorectal Cancer and Corresponding Metastasis

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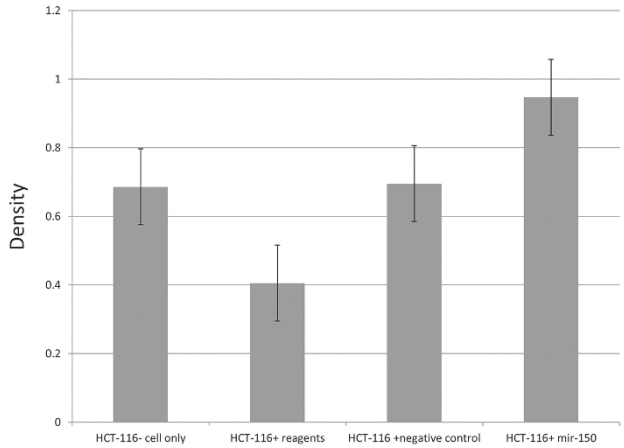
Background: Universal screening for mismatch repair (MMR) deficiency in cancer is increasingly being implemented to detect Lynch syndrome and aid in treatment decisions. The MMR immunohistochemistry concordance rate between primary colorectal cancer (CRC) and metastasis has not been documented. At times, only metastatic tumor is available for screening rather than the primary tumor. Therefore, it is important to confirm that tissue from metastases can be used to screen for deficient MMR. We evaluated immunohistochemistry for MMR proteins in primary and metastatic tumor to assess concordance between the two.
Design: We identified MMR deficient CRC from 1999-2013 and found corresponding metastases from lymph nodes and other sites. Tissue microarrays were constructed using two, 1 mm cores from each metastasis and stained for all four MMR proteins (MLH1, MSH2, MSH6, PMS2). Expression was considered intact if any convincing nuclear staining was identified. Patient records were reviewed for the etiology of the MMR deficiency, and the molecular findings were noted when available.
Results: A total of 50 cases had MMR deficient CRC and available regional lymph nodes (26 cases) or other metastatic tissue (24 cases). Of those cases, molecular workup revealed that 13 had *MLH1* hypermethylation, 10 had Lynch syndrome, two had somatic MMR mutations, and the etiology for MMR deficiency was unknown in the other 25 cases. All cases showed concordance in immunohistochemical staining for the MMR proteins between the primary tumor and corresponding metastatic tissue. In 36 cases, metastatic lymph nodes and/or other metastases were resected at the same time as the primary tumor. In 14 cases, time lapsed (median 16.5 months; range 3-69 months) from the primary resection until the resection of the metastases.
Conclusions: There is 100% concordance of MMR immunohistochemistry results between MMR deficient primary CRC and corresponding regional lymph nodes or other metastases, confirming that metastatic tissue can be used to screen for Lynch syndrome or MMR deficiency to aid in therapeutic choices.

789 MicroRNA 150-5p Has a Role in Progression and Possibly Therapy in Colorectal Cancer

Aisha Sethi, Stefan Costinean, Prasanthi Kumchala, Veronica Balatti, Luciano Cascione, Carlo Croce, Wendy L Frankel. The Ohio State University, Columbus, OH; Institute of Oncology, Bellinzona, Switzerland; University of Nebraska Medical Center, Omaha, NE.
Background: Colorectal cancer (CRC) is the third most common cancer in the US. Despite rapid advances in multimodal therapeutic options, prognosis remains poor with metastatic disease. Identifying which patients are likely to develop metastasis, would assist in targeted treatment to improve outcomes. MicroRNAs (miRs), small non-coding RNAs, play a role in post-transcriptional regulation by inhibiting or degrading mRNA. They have prognostic and therapeutic potential. Our group has previously shown that lymph node metastasis in CRC show up-regulation of miR150-5p. We assessed miR150-5p transfected CRC cell lines to evaluate metastatic behavior, and the role of TP53 as a gene target of miR150-5p.
Design: Colorectal cancer cell lines (SW620, SW480 and HCT116) were cultured and transfected with miR precursor 150-5p (Ambion) and miR precursor negative control

using Lipofectamine 2000 (Invitrogen Life Technologies). We assessed mobility of transfected cell line HCT116 using Abnova method based gel migration study to assess mobility and thus metastatic potential. Cell lines (SW620 and SW480) were harvested at 24, 48 and 72 hours and protein was extracted to evaluate p53 using Western blot.
Results: HCT116 cell line transfected with miR150-5p showed significantly increased mobility compared to controls ($p < 0.05$). Cells with highest density coefficient, indicate those with greatest mobility in the gel.



SW620 and SW480 cell lines transfected with miR150-5p showed decreased p53 expression by Western blot.
Conclusions: MiR150-5p transfected cell lines showed increased mobility, suggesting this miR has a role in progression and metastasis of CRC. MiR150-5p transfected cell lines were associated with decreased p53, confirming TP53 as a target. Since TP53 is a well-known tumor suppressor gene, miR150-5p is presumed oncogenic. MiR150-5p expression in CRC could be useful to indicate increased risk of progression and lymph nodal metastasis. Additionally, targeted therapy with anti-miR150-5p may have a therapeutic role in CRC by affecting the tumor suppressor gene TP53.

790 PTEN- Key Molecule in Lynch Syndrome Related MicroRNA Deregulation in Colorectal Cancer

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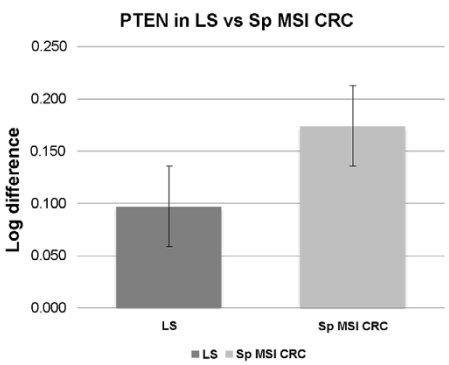
Background: Lynch Syndrome (LS), the most common hereditary colorectal cancer (CRC) syndrome, results from germline mutations in mismatch repair genes. In contrast, sporadic microsatellite unstable (Sp MSI) CRC, usually contains BRAF mutations and is due to epigenetic silencing of the *MLH1* mismatch repair gene. MicroRNAs (miRs), noncoding RNAs, are a fairly new class of genes that play a role in post-transcriptional regulation by inhibiting or degrading mRNA. We previously identified miR494, miR26b-5p and miR186 as up-regulated in LS vs. Sp MSI CRC, but targets have not been studied. We investigated *PTEN*, a tumor suppressor gene, as a possible target of these miRs, by evaluating expression in LS and Sp MSI CRC.
Design: 24 cases of MSI CRCs were identified from the archives including 12 with confirmed LS and 12 with BRAF mutation and therefore presumed Sp MSI. In-silico studies were performed to identify the most likely targets of miR494, miR26b-5p and miR186. Representative areas from CRCs were marked and cored for RNA. *PTEN* mRNA was measured by RT-PCR and protein expression was evaluated with immunohistochemistry for PTEN.
Results: In-silico studies showed *PTEN*, a well-known tumor suppressor, to be a target for the altered miRs. RT-PCR showed significantly decreased expression of *PTEN* in LS compared to Sp MSI CRC.



There was absent cytoplasmic staining for PTEN in 90% of LS CRC and in 12.5% of Sp MSI CRC.
Conclusions: Up-regulation of miRs in LS vs. Sp MSI CRC was associated with decreased expression of *PTEN* mRNA and protein (by immunohistochemistry) in the same cases, confirming *PTEN* as an important target. This is the first study to show that miR deregulation in LS acts, at least in part, via *PTEN*. Decrease of *PTEN* expression promotes cellular proliferation, survival and tumorigenesis. Additional studies are necessary to determine if anti-miR and/or *PTEN* restorative targeted treatment could play a role in the therapy of LS patients.

791 Comprehensive Multiplex Molecular Genomic Signature in Barrett's Carcinogenesis: Potential Predictive, Prognostic, Early Diagnostic & Therapeutic Role

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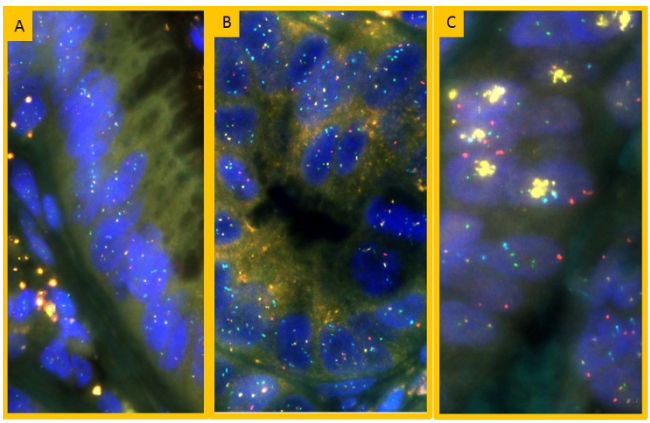
Background: Histologic assessment of dysplasia is the most reliable marker of assessing Barrett's-related neoplastic progression. However, there is considerable variability among pathologists in recognizing and classifying dysplasia. Additionally, clinical response to endoscopic therapy for high-grade dysplasia (HGD) is also quite variable. We explore genomic alterations in histologically normal biopsies (bx) of patients with normal initial bx who developed dysplasia (progressors, P) & those who did not develop dysplasia (non-progressors, NP) during endoscopic surveillance.
Design: Biopsies from BE patients with follow up [initial & paired bx after (3-5years)] & controls [no dysplasia, HGD & intramucosal adenocarcinoma (IMC)] were interrogated for *CDKN2A* (9p12), *HER2* (17q11.2-12), *CMYC* (8q24.12-13), *ZNF217(*20q13) & *p53* alterations using fluorescence in-situ hybridization (FISH). Differences between dysplastic & non-dysplastic mucosa in P (n=3) versus NP (n=7) were statistically analyzed [Chi^2 test, logistic regression & receiver operator characteristic (ROC)].
Results: In BE-P group, compared to initial non-dysplastic bx (A) (figure 1), follow-up bx with HGD (B) showed gains of *CMYC, HER2* or *ZNF217* in 2/3, 2/3 & 1/3 cases, respectively (p value=0.002); less than the positive control IMC (C) and none in the NP group.



CDKN2A was lost in P group (2 initial non-dysplastic bx and 1 follow-up dysplastic bx)but not in NP group. No case showed *p53* alteration.ROC analysis for accuracy of genes to predict HGD showed area under curve (AUC) 0.833, 0.833 & 0.667 for gain of *HER2, ZNF217* & *CMYC,* respectively. Amplification of combination of any of these genes perfectly predicted HGD with AUC of 1.0.
Conclusions: Significant gains of *CMYC, HER2* or *ZNF217* were identified in dysplastic mucosa of BE-P as compared to BE-NP, indicating their predictive & prognostic role in BE carcinogenesis. *CDKN2A* loss in both non-dysplastic & dysplastic mucosa of BE-P indicates an early molecular event that could be detected before phenotypic expression of dysplasia. This multiplex molecular genomic signature of actionable gene targets has clinically useful predictive, prognostic & early diagnostic role in BE carcinogenesis, providing a platform to tailor multitargeted therapy.

792 WHO Morphologic Classification of Gastric Cancer Correlates with Protein Expression Based Classification

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Background: Gastric carcinoma (GC) is a heterogeneous disease, composed of distinct biologic subtypes, as proposed by the recent TCGA. The molecular taxonomy can be reproduced clinically using a panel of immunohistochemical and in-situ hybridization markers (EBER ISH, MLH1, E-cadherin and p53). However, the correlation with WHO histological classification-if any- has not been evaluated.
Design: Protein expression classification of GC was performed on a tissue microarrays of 146 tumor resections based on the algorithm proposed from unsupervised cluster analysis. GC were subdivided into EBV-related (gp1), MSI (gp2), aberrant e-cadherin (gp3), abnormal p53 (gp4) and normal p53 (gp5) subgroups; this was followed by comparison with the WHO histologic type.
Results: Tubular GC was the most common morphologic type in the entire cohort (39.7%); it is seen commonly, but not limited to, abnormal p53 GC (gp4) [p0.04, PPV 0.48, CI 0.36-0.60 and NPV 0.72, CI 0.61-0.81]. Papillary and mucinous GC types are uncommon (8%) but were seen predominantly in abnormal p53 GC(Gp4) and MSI GC (Gp 2) [PPV 0.92, CI 0.62-0.99]. Poorly cohesive/signet ring cell morphology is significantly associated with aberrant e-cadherin GC (gp3) (27/30, 90%, $p < 0.001$). However, 23% of abnormal p53 GC (gp4, 17/75 cases) also show this morphology, the positive predictive value improves if this morphology is taken as an indicator for both aberrant e-cadherin GC (gp3) and abnormal p53 GC (gp4) [PPV 0.91, CI 0.77-0.97].

Carcinoma with lymphoid stroma phenotype is significantly associated with, but not exclusively seen in, EBV GC (gp1) [4/7, 57%, p<0.001, NPV 0.97, CI 0.92-0.99, PPV 0.5, CI 0.17-0.82].
Conclusions: The current clinical classification system is heavily dependent on histological typing; this system has functional value and broadly correlates with molecular subtypes determined by protein expression. We suggest that the morphologic classification can be further enhanced by judicious incorporation of the protein expression profile to develop a comprehensive classification system.

793 Immunostaining of Tyrosine Kinase PTK6 Decreases in the Colonic Epithelium Ranging from Normal, Hyperplasia, Dysplasia and Carcinoma, While Stromal Immunostaining Increases as Tumorigenesis Progresses

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Background: Colon cancer, the third most frequently diagnosed neoplasm, often arises from a benign mucosal polyp that originates as a result of either genetic mutations or other stimuli. Protein tyrosine kinase 6 (PTK6), also known as Breast Tumor Kinase (BRK), is an intracellular non-receptor tyrosine kinase involved in signal transduction in epithelial tissues. Recently, a tumorigenic role has been demonstrated for PTK6 in ERBB2/HER2 positive breast cancers (Tyner, Cell Death and Dis., 2015). In pancreatic cancer, the overexpression of PKT6 may contribute to tumor development by increasing cellular migration and invasion. In the colon PTK 6 has demonstrated a dual role, acting as a tumor suppressor in the normal state, and as an oncogene in cancer.
Design: Aim: To clarify the role of PTK6 in colon cancer development, we investigated the cytoplasmic immunohistochemical expression of PTK6 in colon mucosa and adjacent stroma ranging from normal, hyperplasia, dysplasia, and carcinoma in 131 patients who underwent colectomy for colon cancer.
Following IRB approval, a progression colon tissue array was designed and created from 131 colectomy specimens for colon cancer with a total of 752 cores sampled. Standard immunohistochemistry for PTK6 antigen was performed. Evaluation was based on published PTK6 immunostaining features for non-neoplastic colon and colon cancer as validated by the Human Protein Atlas. Staining was analyzed independently by 2 observers (AS, GG). Statistical analysis employing chi-square test was performed (YZ).
Results: Our data demonstrated a significant correlation between PTK6 staining intensity and tumor progression in both colon epithelium and stroma (p<0.0001). Even more interesting, PTK6 staining intensity decreases in the colon epithelium with tumor development (γ= 0.4825, moderate strength of relationship), while PTK6 staining intensity increases in the colon stroma with tumor progression (γ=-0.6267, strong strength of relationship).
Conclusions: Our results support the possible tumor suppressor role in normal colon tissue and the suspected oncogenic activity of PTK6 in colon carcinoma in humans. Moreover heightened immunoexpression of PTK6 in the stroma adjacent to cancer suggests a role for PTK6 in the mesenchymal phase of tumor development, necessitating further studies for confirmation.

794 Programmed Cell Death Ligand 1 (PD-L1) Expression in Pancreatic Ductal Adenocarcinoma

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Background: Immune checkpoint inhibitors have recently shown promising anti-cancer effects in a number of solid tumor types, typically through inhibition of the programmed cell death 1 (PD1) signaling axis. A predictive biomarker to this class of drugs has not been clearly identified; however, overexpression of the PD1 ligand (PD-L1) has shown particular promise in lung adenocarcinoma, and mismatch repair deficiency (dMMR) has been identified in colorectal adenocarcinoma. In this study, we explore the staining characteristics, prevalence, and clinicomolecular correlates of PD-L1 overexpression in pancreatic ductal adenocarcinoma (PDAC).
Design: A clinically annotated tissue microarray (TMA) was constructed using archival tissue from cases of resected PDAC. PD-L1 immunohistochemistry (IHC) was performed using the SP142 primary antibody (Spring Biosciences), additional IHC stains for MMR proteins (MLH1, MSH2, MSH6, PMS2), as well as CD3 and CD8 were reviewed. PD-L1 status was assessed independently by two anatomical pathologists and consensus achieved on all cases. Tumor cells were considered positive with membranous staining seen in ≥1% of tumor cells.
Results: 242 cases were included in the TMA and were evaluable by IHC. 31 (14%) cases were positive for PD-L1 overexpression. No statistically significant association was identified between PD-L1 status and MMR status or smoking history. Both CD3+ and CD8+ tumor infiltrating lymphocytes showed increased prevalence in the epithelial component of cases with PD-L1 expression (p=0.0015 & p=0.0415). Disease-specific survival was significantly shorter in PD-L1 positive patients (0.9 vs 1.5 years, p<0.0257).
Conclusions: IHC can identify PD-L1 positivity and MMR deficiency. Our data indicate that these properties are independent, but not necessarily mutually exclusive in the setting of PDAC. There is evidence to suggest that one, or both, may be useful predictors of response to PD-1 axis inhibition, but additional studies looking specifically at these markers in the context of treated patients are needed.

795 Comprehensive Morphological Assessment of Colorectal Cancers Characterized by The Cancer Genome Atlas (TCGA) Identifies Specific Morphology-Molecular Alteration Associations

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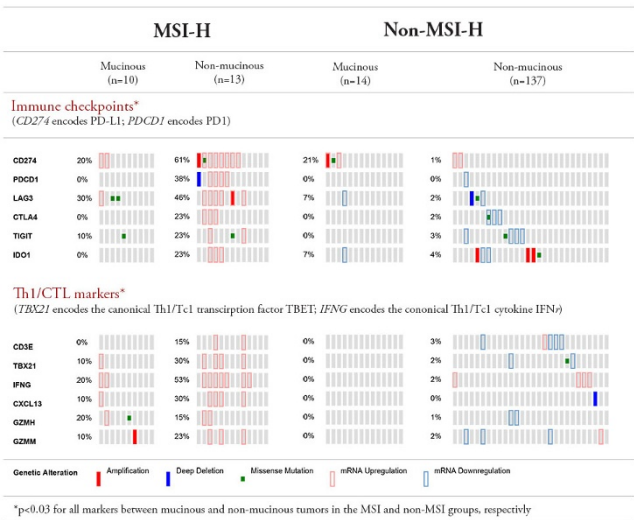
Background: The recent advancement in DNA sequencing technology has allowed a rapid outpouring of the tumor's genomic information. In this new light, we sought to further explore tumor morphology with the aim of detecting patterns that are molecularly unique.
Design: The 224 TCGA colorectal cancers (CRCs) that had whole exome sequencing data (*PMID 22810696*) were evaluated. Morphological assessment was performed on 207 tumors that had adequate virtual images on "http://www.cBioPortal.org/" and included: histologic type (conventional/NOS, medullary, mucinous, other), glandular architectural complexity (grade 1-5); and tumor-infiltrating lymphocytes (TILs)/medium power (0; 1= <10; 2=10-20; 3=>20).
Results: "MSI-H histology" (medullary, mucinous and high TILs) was significantly more frequent in MSI-H tumors (n=28) comparing to non-MSI-H (n=172) and POLE-mutated (n=7) tumors (p<.001). Association studies revealed several main findings. First, among all histologic types, NOS tumors had the lowest overall mutation count (p<.001 for entire cohort, <.03 for MSI-H); among MSI-H tumors, NOS also correlated with significantly fewer homopolymer mutations (p<.01). Second, mucinous histology showed frequent mutations/copy number alterations in *BRAF* (p<.001) and *PIK3CA* (p=.01) overall, and a rate of TGF-ß pathway alteration in non-MSI-H tumors (59% overall, 47% in *SMAD4*) comparable to that in MSI-H tumors and significantly higher than in other histologies (p<0.001). Third, the CRCs that exhibited upregulation of immune checkpoint genes (genes currently being explored for targeted therapy) were primarily MSI-H tumors, frequently associated with co-upregulation of anti-tumoral Th1/cytotoxic T cell (CTL) genes, and had distinctly non-mucinous histologies (mostly medullary/poorly differentiated) (Figure 1).



Conclusions: Molecular characterization is shedding new light on tumor morphology. While all revelations may reflect molecular impact on tumor morphogenesis, some may bear immediate clinical implications; the distinct molecular/morphological alterations associated with immune checkpoint genes carry the potential of allowing more informed patient selection in clinical trials that target immune checkpoints in CRC.

796 Hepatoid Carcinomas of the Gastrointestinal Tract: A Reappraisal of Morphological and Immunohistochemical Features

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Background: Hepatoid carcinoma of the GI tract is a subtype of adenocarcinoma variably classified on morphologic, immunohistochemical, or clinical bases, but diagnostic criteria are not well defined. Many cases lack classic hepatoid morphology but maintain positive staining for markers of hepatoid differentiation. This study explores the morphologic spectrum as well as the utility of special stains in classification of hepatoid carcinomas.
Design: A cohort of 51 GI hepatoid carcinomas from 4 continents was categorized as: type I, classic hepatoid carcinoma; type II, fetal-intestinal type (enteroblastic); and type III, adenocarcinoma, NOS. Clinical data was collected, and cases were evaluated by IHC for AFP, HepPar1, Glypican3, and Arginase1, and by ISH for albumin. Using as criteria positivity in at least 2 hepatoid markers in type II/III tumors and at least 1 marker in type I tumors, 45 cases were classified as hepatoid carcinomas. Tissue microarray controls (n=461), including carcinomas of the pancreas, colon, stomach, gallbladder, ampulla, and esophagus, were also evaluated.

Results: Of the 45 cases, 18 cases were classified as type I, 20 cases as type II, and 7 cases as type III. Type I tumors had unequivocal hepatocytic morphology; type II tumors had characteristic subnuclear vacuolization; and type III tumors were poorly differentiated carcinomas. Serum AFP was elevated in 92%. The most sensitive marker across all subtypes was Gly3 (91% sensitivity), followed by albumin (88% sensitivity) and AFP (82% sensitivity). Arg1, the least sensitive marker, was only positive in type I tumors (35% sensitivity in type I). Controls had positive staining in 5 cholangiocarcinomas and 1 gastric carcinoma, which were added to the cohort as type II and III tumors. Of the remaining controls, only 4 were positive for either Gly3 or AFP but lacked morphologic evidence of type I/II. All controls were negative for albumin. HepPar1 had poor specificity and was reactive in 15% of carcinomas.
Conclusions: Type I and II hepatoid carcinomas are morphologically distinct compared to type III tumors. Gly3, albumin, and AFP are sensitive and specific markers of hepatoid carcinoma in the GI tract, while HepPar1 lacks specificity. Type II hepatoid carcinomas are under recognized in Western countries.

797 Clinicopathologic Characteristics of Systemic Mastocytosis in the Intestine

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Background: The WHO diagnostic criteria for systemic mastocytosis (SM) combine clinical, morphologic, and molecular information, and include histologic evaluation for compact infiltrates of mast cells (> 15 cells) or atypical mast cells (> 25%) in extracutaneous organs. Clinical evidence of gastrointestinal (GI) involvement is nonspecific, and pathologic diagnosis can be difficult when only a scattered mast cell infiltrate in the lamina propria is present. This study aims to characterize the pathologic features of mast cell infiltrates in the GI tract.
Design: Seven patients with gastrointestinal biopsies showing an atypical mast cell infiltrate were identified, including three consultation cases in which mast cells were overlooked. The mast cells were evaluated for morphology and distribution as well as CD117 and CD25 expression.
Results: The patients (3 males, 4 females; aged 55-80 years, with a mean of 65 years) had an average serum tryptase level of 64.2 ng/mL (range: 15.6 to 199 ng/mL). Endoscopy was performed for chronic diarrhea (n=1, with normal findings) as well as anemia and routine screening (n=3, with small polyps). Biopsies showed involvement of the large bowel (n=5), small bowel (n=1), or both (n=1) by a wide morphologic spectrum of mast cells, including: bland spindle cells; small cells with slightly irregular nuclei, smooth chromatin, and variably abundant pale cytoplasm; and medium-sized monotonous cells with elongated nuclei, smooth chromatin, and moderately abundant pale cytoplasm. Mast cells in all cases showed strong positive staining for CD117 and CD25. The patterns of mucosal involvement included: a polypoid mast cell aggregate of 0.6 cm, admixed with numerous eosinophils (n=1); diffuse sheets of mast cells, admixed with eosinophils and plasma cells (n=2); and multifocal aggregates of mast cells (n=4). No crypt distortion was identified. All patients fulfilled WHO criteria for indolent SM. On follow-up in 4 cases, three patients had clinical symptoms (episodic diarrhea, pruritis, and cytopenia), while one patient was asymptomatic at 2 years.
Conclusions: Mast cell infiltrates in the intestine are often subtle and may be overlooked. Atypical mast cells have a wide morphologic spectrum, and recognition can be difficult. Clues to diagnosis may include expansion of the lamina propria by monotonous cells with pale cytoplasm admixed with numerous eosinophils in the absence of crypt distortion. While most patients with GI involvement by SM have clinical symptoms, a subset remain asymptomatic.

798 Helicobacter Heilmannii-Gastritis: Why It's Not Okay to Wash Your Cat's Bowl in the Kitchen Sink and Boys Shouldn't Kiss Their Pigs

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Background: *Helicobacter heilmannii* (*Hh*) frequently inhabit stomachs of domesticated animals, including dogs, cats, and pigs. Compared to *H. pylori* (*Hp*), they are longer, thicker, and more tightly spiraled. Although classic teaching is that *Hh*-gastritis is quite mild, we recently encountered a case indistinguishable from typical *Hp*-gastritis. Herein, we present a detailed clinicopathologic evaluation of *Hh*-gastritis.
Design: The pathology database was queried for all *Hh*-gastritis cases. We assembled a group of 50 consecutive *Hp* controls. Hypothesizing that these would not be age/gender-matched, we planned to assemble a second age/gender-matched *Hp* control group. For *Hh* cases and this second *Hp* group, we assessed the following, according to the Updated Sydney System: gastritis topography; germinal centers; organism burden, neutrophilic infiltrate, mononuclear infiltrate, atrophy, and intestinal metaplasia (these latter 5 scored 0-3). We also recorded the clinical indication for endoscopy and endoscopic findings. Fisher's exact and Mann-Whitney tests were used; $p<0.05$ was considered significant.
Results: We identified 22 *Hh* cases (20 with slides): mean age 31, 14M:8F. The initial 50 consecutive *Hp* cases were not age or gender-matched: mean age 45, 10M:40F ($p=0.0008$, $p=0.007$). In the age/gender-matched cohorts, *Hh*-gastritis demonstrated less organism burden, neutrophilic infiltrate, and mononuclear infiltrate; no other histologic variable was significant. Detailed data are presented in the Table. Common clinical indications, in descending order of frequency, included dyspepsia, N/V/D, melena, and GERD; common endoscopic findings included erythema/gastritis, normal, nodularity, ulcer, and erosion. None of these clinicoendoscopic variables significantly differed between the groups.

	Hh	*Hp*	P
Categorical: % (n)			
Antral-predominant Corpus-predominant or pangastritis	92% (11) 7% (1)	75% (15) 25%(5)	0.37
Germinal centers present	50% (10)	32% (10)	0.25
Ordinal: mean (median)			
Organism burden	1.43 (1)	1.9 (2)	**0.03**
Neutrophilic infiltrate	0.75 (1)	1.23 (1)	**0.02**
Mononuclear infiltrate	1.6 (2)	2.1 (2)	**0.02**
Atrophy	0.6 (1)	0.96 (1)	0.56
Intestinal metaplasia	0.06 (0)	0.33 (0)	0.35

Conclusions: *Hh*-gastritis is more common in younger men, likely due to environmental/behavior factors. Overall, cases are associated with less inflammation, likely due to lower organism burden. In an individual biopsy, however, *Hh*-gastritis may be indistinguishable from *Hp*-gastritis.

799 Incidental Finding of Terminal Ileitis (IFTI): Indication for Colonoscopy Does Not Predict Outcome

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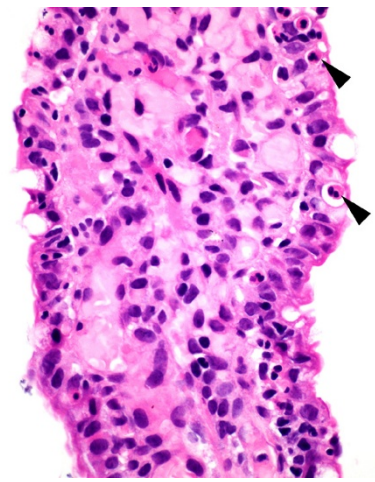
Background: IFTI has unclear clinical significance for patients who do not have known or suspected IBD. Symptoms are considered to be best indicator for progression to IBD. We aim to describe long term follow up of IFTI and define clinical characteristics of these patients.
Design: Pathology database was used to identify patients who had biopsy proven terminal ileitis in 2010 and 2011 with 5 yr followup.



Medical records were reviewed to exclude patients who were suspected of or had known IBD. Endoscopy and imaging data was recorded. Disease progression was defined as unresolving inflammation and/or persistent symptoms of IBD. Patients were divided into 2 groups based on the indication of colonoscopy. First group underwent colonoscopy for screening of colonic polyps called "Asymptomatic Group". The second group underwent colonoscopy for reasons other than known or suspected IBD, the"Symptomatic Group". The two groups were compared for outcomes.
Results: 41 patients met inclusion criteria with mean age 54. 17 were men. Mean follow up was 3.2 years. Common endoscopic findings were ulceration and erosions. 37%(15/41) were exposed to NSAIDS. 10%(4/41) progressed to IBD. Patients who did not progress were compared to patients who developed IBD.

Variable	**IBD**	**Did not develope IBD**	**P-value**
Mean Age (SD)	56.5 (17)	53.4 (13.5)	0.61
Asymptomatic (%)	2 (50%)	18 (48%)	0.95
Abnormal Imaging (%)	**4 (100%)**	**4 (17%)**	**0.007**

Patients who developed IBD had statistically significant abnormal cross-sectional imaging (p-value 0.0005). 20 subjects were included in asymptomatic group and 21 in symptomatic group. Clinical characteristics of the 2 groups were compared.

Variable	**Asymptomatic Patients**	**Symptomatic**	**P-Vlaue**
Mean Age (SD)	55.8 (8.9)	51.8 (17.0)	0.34
Progression to IBD (%)	**2 (10%)**	**2 (9%)**	**0.95**

The incidence of IBD was not different among two groups (p-value=1.0).
Conclusions: Indication for colonoscopy does not predict progression to IBD. In the absence of abnormal finding on cross sectional imaging, patients can be reassured that IFTI commonly does not progress to IBD. NSAIDs are associated with IFTI.

800 Comparative Study of Genome-Wide Copy Number Aberrations in Colonic Mixed AdenoNeuroEndocrine Carcinoma, Adenocarcinoma and Neuroendocrine Carcinoma

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Background: Colonic Mixed AdenoNeuroEndocrine Carcinoma (MANEC) is a rare neoplasm, characterized by coexisting exocrine and neuroendocrine neoplastic components, either as two distinct components (composite subtype, MANEC-C) or as individual neoplastic cell with features of both the components (biphenotypic/amphicrine subtype, MANEC-B). It is a very aggressive neoplasm that mostly present at high stage. The dilemma remains as to whether MANEC should be treated as adenocarcinoma (AC) or neuroendocrine carcinoma (NEC). The genomic study is the best way to understand the nature of these three colonic neoplasms. We believe this is the first high resolution genomic study in an effort to characterize the genomic alterations in MANEC, in which we also compared genome wide copy number aberrations (CNA) of MANEC with AC and NEC.

Design: We analyzed 23 samples from 19 colonic tumors (4 MANEC-C [one sample from each component, total 8 samples], 6 MANEC-B, 4 NEC and 5 AC) for CNA using Affymetrix OncoScan FFPE microarray. We then used the results of CNA to compare the specific genomic similarities and differences among MANEC, NEC and AC.

Results: MANEC shared specific genomic features of AC and NEC. AC showed extensive hyperploidy throughout the genome, with all cases (5/5) had gain in chromosomes (chr) 20q and 13q that was not observed in NEC. Conversely, NEC showed frequent deletions, specifically in chr 5q (4/4), which was not observed in AC. MANEC-B had frequent gains in chr 8q (5/6), 13 q (5/6), and 20q (4/6) and 3 of 6 cases showed chr 5q deletion. Both, exocrine and neuroendocrine, components of MANEC-C shared similar chromosomal alterations, with minor variation in a small subset of chr, suggesting their clonal evolution. Interestingly, both the components had frequent chr 13q (2/4) and 20q (3/4) gains.

Conclusions: MANEC is a distinct colonic neoplasm, with specific genomic alterations, some of which is shared with colonic AC and NEC. However, the genome in all MANEC cases were relatively stable and did not show extensive hyperploidy of AC and frequent deletions, as seen in NEC. Of note, gain in chr 20q and 13q in AC and deletion in chr 5q in NEC appeared to be mutually exclusive.

801 The Genetic Landscape of Interval Colon Cancers Is Similar to Sporadic Non-Interval Colon Cancers

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Background: The pathogenesis of interval colon cancers (iCRC) may be related to missed cancers and precursors on colonoscopy, or to a unique rapid progression pathway. We compared the molecular profiles between a series of well-characterized iCRC and matched sporadic non-interval CRC to determine the presence of any unique signatures specific to iCRC.

Design: iCRC was defined as CRC diagnosed prior to the next recommended colonoscopy and at least 1 yr after last colonoscopy. 59/1159 (5.1%) CRC diagnosed between 2007-2012 were classified as iCRC. High-stage (pT≥3) iCRC associated with large precursor lesions that were likely missed cancers were excluded and the remaining 21 iCRC were selected for molecular profiling. DNA from FFPE tissue was analyzed by a custom hybrid-capture NGS assay that interrogates full coding sequences of 309 genes for mutations and copy number variations (CNV), as well as 113 selected introns across 35 genes for rearrangements. iCRC findings were compared to 42 non-interval CRC matched 1:2 on age, gender and tumor location. Frequencies of genetic alterations in iCRC and non-interval controls were compared using conditional logistic regression with correction done for multiple comparisons when appropriate.

Results: 12/21 (57%) iCRC cases were located in the right colon and 14 (67%) were ≥pT3. DNA mismatch repair protein expression was similar between interval and non-interval CRC (14% vs. 20%; p=0.64). Mutations in iCRC were most commonly seen in *APC* (81%), *TP53* (62%), *KRAS* (38%) and *PIK3CA* (19%). Recurrent CNVs most frequently involved *TP53* (52%), *SMAD4* (52%), *SMAD2* (52%) and *SOX9* (48%). Frequencies of alterations in the 5 microsatellite-stable pathways most commonly involved in CRC were similar in iCRC and matched controls [Table 1].

Signaling pathway (CRC-related genes with alterations)	Interval cancer (n=21) %	Non-interval cancer (N=42) %	P (Matched comparison)
Wnt (*APC, CTNNB1, TCF7L2, TCF7L1, FBXW7, ARID1A, SOX9*)	95	93	0.73
P53 (*ATM, TP53, CDKN1A, CDKN2A*)	90	78	0.28
RTK/RAS (*EGFR, ERBB2, NRAS, KRAS, BRAF*)	86	76	0.33
PI3K (*IGF1R, PIK3CA, PIK3C2B, PTEN, PIK3R1, AKT1, PRKDC, MET*)	80	73	0.54
TGF beta (*SMAD2, SMAD4, MYC, MECOM*)	62	67	0.72

Conclusions: Genetic alterations in iCRC are similar to non-interval CRC matched for age, gender and tumor location. iCRC are likely to develop from missed precursors or cancers rather than a unique rapid progression pathway.

802 Comparison of Dysplastic Fundic Gland Polyps in Patients with and without Familial Adenomatous Polyposis

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Background: Dysplastic fundic gland polyps (d-FGPs) are rare. They typically arise in patients with familial adenomatous polyposis (FAP) but may occur in non-syndromic (NS) patients. They almost never progress to carcinoma, but their significance is unclear, especially in NS patients. The *APC*/β-catenin pathway has been implicated in their pathogenesis, and β-catenin immunohistochemistry (IHC) is occasionally positive. We compared the clinicopathologic and IHC findings of d-FGPs in FAP and NS patients.

Design: We identified 106 FGPs diagnosed with low- or high-grade dysplasia (LGD, HGD) or deemed indefinite for dysplasia (IFD) from 59 patients (27 with clinical FAP, 32 NS). We recorded patient sex, age at first d-FGP, time until subsequent d-FGP, history of non-gastric cancer (no patients had gastric cancer), and proton-pump inhibitor (PPI) use. β-catenin IHC was performed on cases with available blocks, with any nuclear staining considered positive.

Results: Mean age at d-FGP diagnosis was 36 years for FAP patients and 61 for NS patients (p<0.0001). Patients were commonly female (14/27 FAP [52%], 23/32 NS [72%], p=0.1764). Sixteen FAP patients (59%) developed at least one subsequent d-FGP, compared to 6 (19%) NS patients (p=0.0026). Mean time between d-FGP detection was 14 months in FAP patients and 7 months in NS patients (p=0.1212). PPI use (usually omeprazole) was seen in 12 FAP patients (44%) and 26 NS patients (81%) (p=0.0058). Six FAP patients (22%) and 13 NS patients (41%) had reported non-gastric malignancies (p=0.1676). After slide review, the 106 FGPs included 88 with LGD, 3 with HGD, and 15 IFD. β-catenin IHC was positive in 12/94 (13%), including 10/77 with LGD, 2/3 with HGD, and 0/14 IFD. It often highlighted small dysplastic foci and demonstrated non-dysplastic surface epithelium overlying dysplastic nests. Three of 24 FAP patients (13%) and 6/32 NS patients (19%) had an IHC-positive polyp (p=0.7176). Two FAP patients and one NS patient had more than one IHC-positive polyp.

Conclusions: FAP patients unsurprisingly are diagnosed with d-FGP earlier in life and are more likely to develop additional ones. NS patients with multiple d-FGPs may have a shorter time between their detection, possibly due to focused follow-up. If PPIs play a role in d-FGP development, this effect is more prominent in NS patients. Dysplastic FGPs in FAP and NS patients have similar low rates of β-catenin IHC positivity. NS patients developed non-gastric cancer more often, likely because FAP patients are typically younger and likely receive more thorough overall surveillance.

803 Evaluation of Ki-67 Index and Mitotic Count in the Assessment of Prognosis of Small Intestinal Neuroendocrine Tumor

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Background: The WHO classification system uses either Ki-67 index (Ki-67) or mitotic count (MC) for gastrointestinal neuroendocrine tumor (NET) grading. It is not clear whether this system and its specific cutoff points apply equally to all NET arising in different sites.

Design: 130 small intestinal NETs (SINETs) were included in this study. The hot spot method was applied to determine the Ki-67 using automated cellular imaging system. The Ki-67 was rounded to the nearest whole integer. MC was counted in 50 high power fields (HPF). Associations of Ki-67 and MC at different cutoff points, and other clinicopathological features, with patient survival were evaluated using Cox proportional hazards regression.

Results: The median age of 130 patients was 63.5 years. 94 patients had died at a median of 5.5 years following surgery. Overall survival rates (OSR) at 5, 10, and 15 years following surgery were 66%, 38%, and 28%, respectively. Nearly half of the cases had no mitosis or 0% Ki-67. There were no examples of WHO grade 3 SINET. On multivariable analysis, older age (HR 1.49), liver metastases (HR 1.89), Ki-67 >5% (HR 4.34), and MC >10/50 HPF (HR 5.25) were significantly associated with death. Only 9 cases (7%) had Ki-67 >5% and/or MC >10/50 HPF. OSR at 5, 10, and 15 years for 70 stage I, II, and III patients were 75%, 45%, and 36%, compared with 55%, 31%, and 18%, respectively, for patients with stage IV disease. Ki-67 >5% was associated with a nearly 4-fold increased risk of death (HR 3.97) for patients with stage IV disease. There was no significant difference in outcome between patients with Ki-67 ≤2% and those between 2-5%. Among patients with stage I, II, or III disease, older age (HR 1.63) and MC >1/50 HPF (HR 2.23) were significantly associated with death.

Conclusions: Our study demonstrates that Ki-67 and MC, along with older age and the presence of liver metastases, predict clinical behavior of SINET. However, the majority of SINETs have either zero or very low Ki-67 and MC. Using cutoff points of Ki-67 >5% or MC >10/50HPF, we were able to identify a small group of patients with significantly worse prognosis. For those patients without evidence of distant metastasis at the time of surgery, our data indicates that Ki-67 may not play a significant role due to overall lower Ki-67 in these tumors, unless a lower threshold is used (>5% indicating more aggressive disease). Different cutoff points for NETs at different sites may be needed in order to better use this prognostic tool for risk stratification and therapeutic management.

804 Analysis of PD-L1 Expression Pattern in Microsatellite Instability Colon Cancer

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Background: A recent *New England Journal of Medicine* study highlighted the importance of screening patients who carry defects in mismatch repair to predict the benefit of anti-PD-1 treatment. In the study, 2/3 of microsatellite instability (MSI) colon cancer responded to anti-PD-1 drug. However, no response was detected in microsatellite stable (MSS) colon cancer. They hypothesize that compared to MSS tumor, MSI tumor

has more than 20 times as many DNA mutations which increases the likelihood that immune cells will recognize MSI tumor as foreign. However, developing more accurate predictive biomarkers of response in these patients presents a challenge.

Design: In the current study, we reviewed 42 cases of MSI colon cancer. Tumor specimens were subjected to immunohistochemistry to evaluate PD-L1 expression (clone SP263 approved for clinical trial use). Tissue was subjected to PCR for BRAF/KRAS mutation.

Results: PD-L1 was expressed in 71% of MSI colon cancer. Expression pattern includes: diffuse (7%), scattered (12%), at tumor infiltrating border (52%), or negative (29%). To further evaluate the PD-L1 expression and host immune response, we identified four distinct groups of tumors as having the presence of both PD-L1 and tumor infiltrating lymphocytes (TILs) (64%), absence of both TILs and PD-L1 (19%), presence of TILs without PD-L1 (10%), or PD-L1 expression without TILs (7%). Our result suggests that PD-L1 expression is correlated with host immune response as indicated by 83% of the MSI cancer being either double positive or double negative.

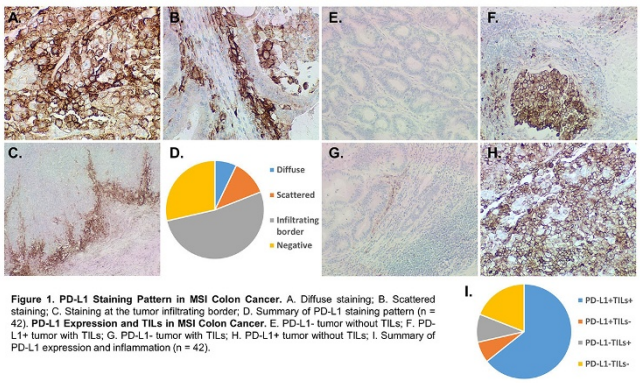

Figure 1. PD-L1 Staining Pattern in MSI Colon Cancer. A. Diffuse staining; B. Scattered staining; C. Staining at the tumor infiltrating border; D. Summary of PD-L1 staining pattern (n = 42). **PD-L1 Expression and TILs in MSI Colon Cancer.** E. PD-L1- tumor without TILs; F. PD-L1+ tumor with TILs; G. PD-L1- tumor with TILs; H. PD-L1+ tumor without TILs; I. Summary of PD-L1 expression and inflammation (n = 42).

Finally, we analyzed PD-L1 expression and BRAF/KRAS mutation. PD-L1 expression was identified in 89% of MSI only tumor vs 50% of MSI/BRAFmut tumor ($p = 0.01$) or 63% of MSI/KRASmut tumor ($p = 0.09$). However, no significant difference of PD-L1 expression was detected between the MSI/BRAFmut and MSI/KRASmut group ($p = 0.50$).

Conclusions: Our result suggests that 2/3 of the MSI colorectal carcinoma expresses PD-L1 with staining at the tumor infiltrating border being the predominant pattern. Second, expression of PD-L1 correlates well with host immune response. Finally, MSI tumor without simultaneous KRAS or BRAF mutation seems to have the highest level of PD-L1 expression. Further studies evaluating PD-L1 expression in MSS colon cancer are being investigated.

805 As an Independent Prognostic Factor, EZH2 Promotes Gastric Cancer Progression via Akt Pathway

Cong Tan, Mi-die Xu, Shujuan Ni, Weiwei Weng, Xiangjie Sun, Qiongyan Zhang, Dan Huang, Weiqi Sheng, Xiang Du. Fudan University Shanghai Cancer Center, Shanghai, China.

Background: EZH2 is known to play important role in the development and progression of human malignancies. However, the influence of EZH2 on the progression and prognosis of gastric cancer (GC) are still poorly understood. Here, we aimed at investigating clincopathological significance of EZH2 in GC and its underlying mechanisms.

Design: Quantitative real-time polymerase chain reaction (qRT-PCR) and immunohistochemical assays were performed in GC samples for detection of EZH2. Pearson analysis was used to calculate the correlation between clinicopathological features and the expression of EZH2. Kaplan–Meier curves with the log rank test and Cox proportional hazards analysis were used to analyze the overall survival (OS). We also evaluated influence and underlying mechanism of EZH2 on *in vitro* and *in vivo* biological charateristics, including cell proliferation, epithelial–mesenchymal transition (EMT) and cancer stem cell (CSC) phenotype, and metastasis, by using gain-or-loss function experiments, immunohistochemical and immunoblotting assays.

Results: We found that the expression of EZH2 was higher in gastric cancer tissues comparison with paratumorous epithelium. High expression of EZH2 mRNA was associated with a more aggressive biological behavior and poor prognosis in GC. Moreover, enforced overexpression of EZH2 in gastric cancer cells increased cell growth, clonogenicity and sphere-forming (pancreatospheres) capacity. Which also led to the acquisition of EMT phenotype and increased invasion. Finally, by immunohistochemical and immunoblotting assays and functional experiments, we found that the Akt pathway contributed to the effects of EZH2 in GC cells.

Conclusions: These results suggest that EZH2 overexpression is responsible for the acquisition of EMT and cancer stem cell (CSC) phenotype, which is in part mediated through the AKT signaling pathway. Taken together, EZH2 has a central role in regulating diverse aspects of the pathogenesis of GC, indicating that it could be an independent prognostic factor and potential therapeutic target.

806 PD-L1 Expression and the Tumor Immune Microenvironment of Esophageal Adenocarcinomas

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Background: Novel treatment strategies that synergize with traditional therapeutics are needed for esophageal adenocarcinoma (EAC). Immunomodulatory therapy targeting programmed death ligand-1 (PDL1) has shown promise in other tumors, but little is known regarding the tumor microenvironment of esophageal tumors. Here we report the status of PDL1 expression on EAC and associated tumor infiltrating lymphocytes (TIL), both pre and post neoadjuvant therapy.

Design: 18 surgical resections of invasive EAC and 16 pre-resection biopsies were stained for PDL1 and the tumors and associated TILs were scored for PDL1 expression. Greater than 5% membranous staining on tumor cells were considered PDL1 positive. TILs were scored as no significant staining (0), less than 50% (focal) or greater than 50% (high). TIL intensity was graded as 0 (no TIL), 1 (<5%, focal), 2 (5-50%, moderate) or 3 (>50%, diffuse). Clinicopathologic data were collected for all cases.

Results: Our EAC had an average patient age of 63 with 94% men and 6% women; all cases had been treated with neoadjuvant chemoradiation. At resection, 33% showed no residual invasive tumor, 28% were stage 1, 28% stage 2 and 11% stage 3. In pre-treatment biopsies, no tumors were PDL1+ and only one case had focal PDL1+ TIL. In post-treatment resections, 33% of EAC with residual invasive tumor were PDL1+ and 94% had PDL1+ TIL (53% focal, 47% high), often present in a dense lichenoid band in the area of treated tumor. TIL intensity was much greater in areas of treated non-viable tumor (6% no TIL, 11% focal, 50% moderate, and 22% diffuse TIL) than in areas of residual viable invasive tumor (22% no TIL, 33% focal, 11% moderate, and 0% diffuse TIL). Patients with only focal PDL1+ TIL or no/focal TIL associated with their residual invasive tumor were more likely to have died of disease than patients with high PDL1+ TIL or moderate/diffuse TIL intensity, with a median follow-up time of 28 months.

Conclusions: We demonstrate robust TIL associated with treated EAC. A subset of treated EAC also express PDL1 along with virtually all associated TIL, with apparent induction of PDL1 expression following neoadjuvant chemoradiation. These findings suggests the potential for synergy between anti-PDL1/PD1 therapy and traditional therapeutics and highlight the importance and potential clinical utility of further exploration of the tumor microenvironment both pre and post neoadjuvant chemotherapy

807 Ipilimumab Induced Perforating Colitis: Severe Cases Mimic Crohn Disease and Ulcerative Colitis

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Background: Ipilumumab (anti CTLA-4) is used to treat various cancers and is known to cause enterocolitis in up to 31% of patients, with serious complications including colonic perforation and death in <1%. The histologic, gross and endoscopic features of ipilimumab induced colitis (IIC), especially when severe, may mimic inflammatory bowel disease. Only rare case reports on the histopathologic findings seen in cases of perforation have been documented. We present our series of ipilimumab induced perforating colitis with key features to distinguish it from other colitidies such as Crohn disease.

Design: Histopathologic, endoscopic findings and clinical outcomes for patients with IIC between 8/2012 to 8/2015 were studied. Twelve specimens (resection(s) and/or biopsies) from a total of six patients were identified. The pattern of injury, presence of eosinophils, apoptoses, number and location of perforations, features of chronicity and concurrent diverticular disease were analyzed.

Results: All patients initially presented with diarrhea and pancolitis on endoscopy. 5 patients received ipilimumab for malignant melanoma and 1 patient for metastatic squamous cell carcinoma. 4 patients underwent biopsy and then subsequent resection(s). An extensive laboratory workup was negative for infectious etiologies. 3 patients had perforations in the sigmoid colon while 1 patient had 7 perforations in the right colon and 2 in the sigmoid colon. 2 of the 4 patients who underwent resection died.

Histologically, varying degrees of basal epithelial apoptoses were noted in all specimens. The pattern of inflammation varied, with 3 patients showing a lymphocytic autoimmune colitis like pattern, 2 patients with an infectious neutrophil-rich colitis like pattern, and 1 patient with a mixed pattern. The presence and number of eosinophils varied. Resection specimens revealed multifocal deep ulcerations with fissures penetrating into the muscular layer with adjacent pseudopolyp formation. There was no evidence of chronicity, vasculitis, thrombosis or granulomatous inflammation in any of the specimens.

Conclusions: IIC may lead to perforation and serious outcomes. In our study, 4 of 6 (66%) patients with biopsy proven IIC had subsequent perforation. Perforations in the sigmoid colon were prevalent and not associated with diverticular disease. The finding of basal epithelial apoptoses without eosinophils is a consistent feature. Perforation specimens may show deep knife like ulcerations and pseudopolyps that mimic Crohn disease, however other features of chronicity are absent.

808 Low Frequently Mutated Genes in Colo-Rectal Cancer: Evidences from Next-Generation Sequencing of 653 Routine Cases

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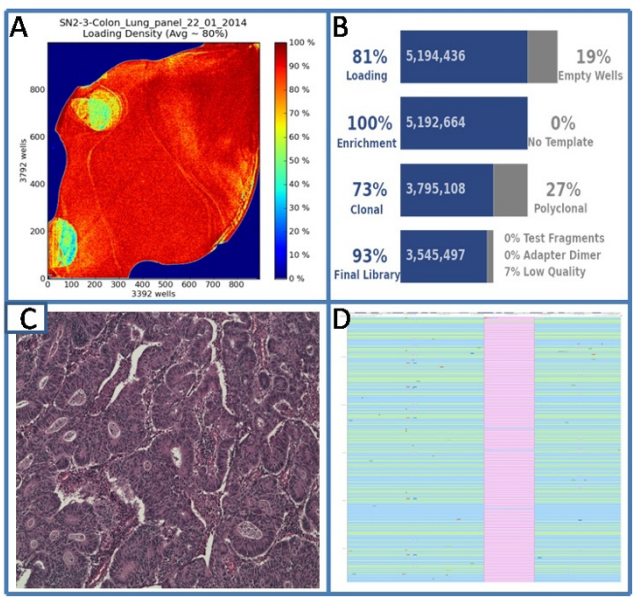
Background: The incidence of *RAS/RAF/PI3KA* and *TP53* gene mutations in colorectal cancer (CRC) is well established. Less information, however, is available on other components of the CRC genomic landscape, that are potential CRC prognostic/predictive markers.

Design: Following a previous validation study (Malapelle U, *et al. J Clin Pathol* 2014;68:64-8), ion-semiconductor next generation sequencing (NGS) was employed to process 653 routine CRC samples by a multiplex PCR targeting 91 hotspot regions in 22 colorectal cancer significant genes.
Results: A total of 1009 mutations in 564 (86.4%) tumors was detected. Besides *RAS/RAF/PI3KA* and *TP53* other 14 genes showed at least one mutation including *FBXW7* (6%), *SMAD4* (3,7%), *MET* (3,1%), *PTEN* (3,1%), *FGFR3* (2,3%), *STK11* (1,8%), *EGFR* (1,2%), *CTNNB1* (1,1%), *AKT1* (0,9%), *ERBB2* (0,6%), *ERBB4* (0,6%), *ALK* (0,2%), *MAP2K1* (0,2%) and *NOTCH1* (0,2%).

Total cases analyzed	n= 653
Wild type in all 22 gene analyzed	n= 89 (13.6%)
Mutated at ≥1 of 22 genes analyzed	n= 564 (86.4%)
Total mutations	n= 1009
Mutated genes	19/22

Genes	Number of mutated cases (%)
TP53	384 (58,8%)
KRAS	247 (37,8%)
PIK3CA	98 (15%)
BRAF	63 (9,6%)
FBXW7	39 (6%)
NRAS	30 (4,6%)
SMAD4	24 (3,7%)
MET	20 (3,1%)
PTEN	20 (3,1%)
FGFR3	15 (2,3%)
STK11	12 (1,8%)
EGFR	8 (1,2%)
CTNNB1	7 (1,1%)
AKT1	6 (0,9%)
ERBB4	4 (0,6%)
ERBB2	4 (0,6%)
NOTCH1	1 (0,2%)
ALK	1 (0,2%)
MAP2K1	1 (0,2%)

LEGEND TO FIGURE 1. Loading density (A) and performance parameters (B) of an Ion Torrent sequencing run, carried out using a 316 chip are shown. DNA extracted from the CRC shown in (C) harbored an *EGFR* p.E746_A750delELREA mutation. (D) was observed with an Genome Brower web app.



Conclusions: In a routine diagnostic setting, NGS had the potential to generate robust and comprehensive genetic information also including less frequently mutated genes potentially relevant for prognostic assessments or for actionable treatments.

809 Is Sloughing Esophagitis (SE) All That It's Cracked Up to Be? Clinicopathologic Comparison of SE in Adults and Children

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Background: Sloughing esophagitis (SE) is rare, characterized by white plaques, peeling membranes on endoscopy (EGD) and splitting of a necrotic superficial epithelium on histology. Whether SE is a specific entity associated with chronic debilitation and high risk of mortality or a manifestation of esophageal injury is controversial. Therefore, we performed a clinicopathologic review of SE cases in adults and children.
Design: Review of 34 cases of SE fulfilling EGD and histologic criteria. Based on outcome, patients were classified into 3 groups: 1) Favorable: symptoms (Sx) resolved or no sloughing on subsequent EGD/Bx 2) Intermediate: Persistent Sx or sloughing/stricture on subsequent EGD/Bx 3) Poor: Death or palliative care within 1 month of initial SE diagnosis. 45 factors were evaluated by chi-square analysis for prognostic significance.
Results: Favorable outcome was seen in 65.5% adults and 60% children, intermediate in 20.7% adults and 40% children, and poor in 13.8% adults only. History of cancer (Ca), readmission within 2 weeks of initial SE diagnosis and chemotherapy were significantly associated ($p<0.02$) with poor outcome. SE was associated with multiple clinicopathologic factors (table1).

	Adult (n=29)	Pediatric (n=5)
Age mean (range)	60.0 (33-81)	13.2 (10-17)
Gender (M/F)	10/19	2/3
Prognosis (%)		
Favorable	19 (65.5)	3 (60)
Intermediate	6 (20.7)	2 (40)
Poor	4 (13.8)	0
Microscopic co-finding (%)		
Total	14 (48.3)	5 (100)
Reflux	5 (17.2)	1 (20)
Eosinophilic esophagitis	1 (3.4)	3 (60)
Fungal infection	5 (17.2)	1 (20)
Foreign body	4 (13.8)	0
Hx of medical diseases (%)		
HTN	12 (42.6)	0
Psychiatric/neurogenic	12 (42.9)	2 (40)
Metabolic	7 (25.0)	1 (20)
Ca	7 (25.0)	0
CHF, valvular disease, arrhythmias	6 (21.4)	0
Total atherosclerotic disease	8 (28.6)	0
≥ 5 comorbidities	19 (67.9)	1 (20)
Medication (%)		
Meds causing esophageal injury/dysphagia/dry mouth	24 (88.9)	3 (60)
CNS depressants	8 (29.6)	1 (20)
Antiepileptics	8 (29.6)	0
≥ 5 medications	20 (74.1)	1 (20)

Conclusions: Despite an association with multiple comorbidities and polypharmacy, 65% of adults had resolved sloughing. No children had poor outcome, possibly due to a lower likelihood of association with Ca, comorbidities and polypharmacy. Because co-existing esophageal diseases are frequently found, we conclude that SE represents a pattern of esophagitis rather than a distinct entity.

810 Mucinous Neoplasms of the Appendix: Challenges in Grading and Staging with Proposal for a New System

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Background: Low grade appendiceal mucinous neoplasms (LAMN) are low grade carcinomas (WHO 2010). AJCC/CAP recommends staging LAMN as adenocarcinoma,which presents several problems:potential for being mistaken for conventional adenocarcinoma,difficulty in assigning T category due to lack of destructive invasion or presence of acellular mucin, and lack of prognostic value of T categories.Appendiceal mucinous adenocarcinoma(MC)with peritoneal involvement are graded as low or high(WHO 2010),but AJCC/CAP recommends 3-tier grading(well, moderate, poorly differentiated);prognostic significance of 3 grades is supported by NCDB database.However,degree of gland formation as criteria for 3 grades(AJCC/CAP) is not readily applicable to MC. This study examines problems in grading and staging, and recommends a scheme based on a small case series and detailed literature review.
Design: Clinicopathologic data for 31 LAMN and 27 MC involving peritoneum was reviewed. Published literature (1995-2015) was reviewed for significance of T category in LAMN and significance of 3-tier grading schemes for MC involving peritoneum.
Results: There was no recurrence for LAMN confined to muscularis propria(our series:n=21,literature:n=43).Recurrence was seen with extension beyond muscularis propria:acellular mucin(our series:n=0/5,literature:n=3/64,5%);cellular LAMN(our series:n=4/6,75%, literature:n=41/59,69%).All LAMN corresponding to T1 and T2 had benign outcome.Multiple studies show prognostic value of 3-tier grading scheme for MC involving peritoneum.

Study	3-tier grading scheme	5-year survival
Ronnett	DPAM, PMCA-I/D, PMCA	75%, 50%, 14%
Shetty	PMP1, PMP2, PMP3	86%, 63%, 32%
Bradley	DPAM, PMCA-I, PMCA	62%, 68%, 37%
Davison	G1, G2, G3	91%, 61%, 23%
NCDB database	WD, MD, PD	64%, 50%, 25%

Proposed grading system (modified from Davison *et al*):

G1	Low grade cytologic atypia (corresponds to LAMN in appendix), includes acellular mucin
G2	Mix of low and high grade cytologic atypia, or diffuse high grade cytologic atypia or architectural complexity, or destructive invasion of implants, or >20% cellularity
G3	Any component of signet ring cells

Conclusions: AJCC staging specific for LAMN is necessary in which tumors limited to muscularis propria should be Tis and acellular mucin or cellular LAMN beyond muscularis propria should be T4a.There is no relevance of T1-3 in LAMN.The proposed grading scheme for MC with peritoneal involvement provides definite criteria for 3-tier scheme,which is supported by NCDB data and recommended by AJCC.

811 Substantial Interobserver Agreement in the Assessment of Barrett's Esophagus within an Expert Panel of Gastrointestinal Pathologists

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Background: The presence of dysplasia determines treatment indication or surveillance interval in Barrett's esophagus (BE). Dysplasia assessment in BE is, however, hindered by significant inter- and intraobserver variability among general pathologists. Therefore, the new Dutch guideline advises histopathological review of all dysplasia diagnoses. To accommodate expected review requests, a national digital review platform for BE will be set up, consisting of expert gastrointestinal (GI) pathologists with extensive experience in evaluating BE dysplasia. Digital versus microscopic revision of BE samples has been validated previously on single slide images.

Design: We aimed to assess the inter- and intraobserver agreement of digital review by expert pathologists using whole-endoscopy slide sets, instead of single slides. Sixty cases (2-13 slides per case) were selected (non-dysplastic BE (NDBE); n=20, low-grade dysplasia (LGD); n=20, high-grade dysplasia (HGD) or cancer (EAC); n=20). All slides were digitally scanned at x20 magnification and made available on a digital server. Five expert GI pathologists independently assessed all digital cases twice in a random order, marking the highest histological grade (NDBE; IND; LGD; HGD or ca). Intra- and interobserver agreement were calculated using a custom weighted Cohen's kappa.

Results: Mean interobserver agreement for all assessments in three categories (NDBE; IND + LGD; HGD or ca) was K=0.69 (range 0.53-0.87). Mean intraobserver agreement was K=0.77 (range 0.65-0.87). A 4-pathologist consensus diagnosis was reached in 85/110 (77%) assessments.

Conclusions: Inter- and intraobserver agreement within this digital review panel of five expert pathologists is substantial. The panel homogeneity is further demonstrated by the 77% 4-pathologist consensus diagnoses. Hence, the panel seems well suited to provide advice on management decisions in BE. This study substantiates the upcoming national digital review panel for BE, which will be able to overcome the logistical problems associated with review of glass slides by multiple pathologists.

812 A New Immunohistochemistry Prognostic Score (IPS) for Recurrence and Survival in Pancreatic Neuroendocrine Tumors (PanNET)

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Background: We aimed to evaluate the expression and prognostic significance of N-myc downstream-regulated gen-1 (NDRG-1), O6-methylguanine DNA methyltransferase (MGMT) and Pleckstrin homology-like domain family A member 3 (PHLDA-3) by immunohistochemistry (IHC) and methylation analysis in resected pancreatic neuroendocrine tumors (PanNET).

Design: Ninety-two patients with resected primary PanNET and follow-up > 24 months were included in this study. Nuclear staining for MGMT and PHLDA-3 were scored as 0, 1-5%, 6-50% and ≥ 51%; cytoplasmic NDRG-1 staining was scored based on intensity and pattern from 0 to 2. We then grouped IHC scores for MGMT (absent versus any expression); for NDRG-1 (0 versus 1 versus 2) and for PHLDA-3 (<50% versus ≥ 51%). Finally, we developed an immunohistochemistry prognostic score (IPS) based on MGMT, NDRG-1 and PHLDA-3 IHC expression to predict disease free survival (DFS) and overall survival (OS). The discriminatory ability of multivariate models combining the IPS and important clinical variables was assessed with Harrel's c-index (HCI) and a modification of Harrell's c-index (mHCI).

Results: DFS was significantly worse in patients without any expression of MGMT compared with those with any grade of expression (HR: 2.21; 95%CI: 0.97-5.02; p=0.013), in patients with moderate or high score for NDRG-1 (p=0.005), and in those with high-expression for PHLDA-3 (HR: 1.94; 95%CI: 1.05,3.6; p=0.036). A significant difference in OS was observed based on NDRG-1 score (p=0.013). In multivariate analyses, ki-67 (HR: 2.45; 95% CI: 1.20-5.01; p=0.01) and IPS (HR: 2.68; 95% CI: 1.60,4.49; p=0.00018) were independent prognostic factors for DFS, while age (HR: 7.67; 95% CI: 2.14,27.45; p=0.0017) and IPS (HR: 2.67; 95% CI: 1.11, 6.41; p=0.03) were independent prognostic factors for OS. HCI for the multivariate DFS and OS models were 0.796 and 0.788, respectively.

Conclusions: Our IPS is a useful prognostic biomarker for recurrence and survival in patients following resection for PanNET. Prospective studies are warranted to validate our findings and determine its role for patients' selection to neo/adjuvant treatments.

813 Bile Reflux May Play an Important Role in the Development of Gastroesophageal/Gastric Cardia Intestinal Metaplasia and Carcinoma

Monika Vyas, Romulo Celli, Manpreet Singh, Natalie Patel, Harry Aslanian, James J Farell, Daniel Boffa, Dhanpat Jain. Yale School of Medicine, New Haven, CT.

Background: It is increasingly recognized that bile reflux (BR) may play a major role in gastric mucosal injury leading to adenocarcinoma of gastric cardia(GC), gastroesophageal junction(GEJ) and distal esophagus(DE). However, gastric BR is difficult to diagnose and investigate clinically. Reactive gastropathy (RG) in most cases represents bile-mediated gastric mucosal injury, once other known causes such as non-steroidal anti-inflammatory drugs (NSAIDS)} are excluded, and can be used as a histologic marker of BR. Also, bile-mediated mucosal injury may be enhanced by chronic acid suppression due to the absence of neutralization by gastric acid. The goal of this study is to explore the association between antral RG and mucosal inflammation and intestinal metaplasia (IM)/Barrett's esophagus (BE) in GC/DE.

Design: The pathology database was searched for patients with gastric biopsies with a diagnosis of antral RG and concurrent GC/GEJ/DE biopsy between 2013-15, and 115 patients were identified. Age and sex matched patients (n=97) with normal gastric antral biopsies were included as controls. The biopsies were reviewed and RG was confirmed and graded (1-3)(Wolf 2014). The biopsies from the GC/GEJ/DE were evaluated for a variety of histological changes, especially nature and grade of inflammation, RG, pyloric and pancreatic metaplasia, oxyntic gland atrophy, proton-pump inhibitor changes (PPIs), IM and dyspalsia. In addition, detailed clinical history including clinical presentation, prior history, endoscopic findings, PPI, NSAID or other drug use was recorded.

Results: See Table 1

	Test (n=115)	Control (n=97)	P value
Average age (years)	57.6	52.3	
Sex ratio (M:F)	1.3:1	1:1.2	
IM at GEJ/Cardia	37	5	0.0002
Antral IM	12	0	0.0005
Moderate-severe carditis	25	5	0.0006
PPI changes in body/fundus	57	18	0.0001
Prior history of BE	12	3	0.057

Conclusions: Our results indicate that chronic inflammation and IM are more frequently seen in the GC/GEJ/DE in patients who have antral RG compared to controls. The findings support the notion that BR may play a crucial role in the development of chronic mucosal injury/IM/BE and subsequent adenocarcinoma. Our study also supports the idea that PPI use may augment injury due to BR. Further studies are needed to study duodenogastric-esophageal BR and its role in development of proximal gastric, GEJ and distal esophageal adenocarcinomas.

814 Intestinal Metaplasia of Appendiceal Endometriosis Is Not Uncommon and May Mimic Appendiceal Mucinous Neoplasm

Monika Vyas, Serena Wong, Xuchen Zhang. Yale School of Medicine, New Haven, CT.

Background: Appendiceal endometriosis (AE) may be an incidental finding or the cause of appendicitis, intussusception, perforation or retention mucocele. Intestinal metaplasia (IM) of AE is rarely reported, which can lead to a misdiagnosis of low-grade appendiceal mucinous neoplasm (LAMN).

Design: A retrospective search of the pathology database from 2001 to 2015 was performed, and 78 cases of AE were identified. For each patient, age, clinical presentation, procedure and presence of endometriosis at other sites were recorded. Each case was reviewed for the extent of endometriosis, location, presence and focality of IM and type of stroma. Immunostains for CK7, CK20, CDX2, ER and CD10 were performed on representative cases.

Results: IM was found in 13% (10/78) of the AE cases, with an age range of 24 to 52 years (average 37 years). Of the 10 patients with IM, 5 presented with right lower quadrant pain, 3 presented with a pelvic mass, and 3 presented with an ileocecal/appendiceal mass. Endometriosis in other pelvic sites was seen in 5/10 patients. Treatment included appendectomy (4/10) and right hemicolectomy (6/10). All cases had extensive, transmural involvement of the appendiceal wall by endometriosis. The foci of IM were submucosal in 9/10 cases and within the muscularis propria in 1 case. Intestinal and endometrial hybrid glands were present in 9/10 cases. One case showed complete replacement of the endometriotic epithelium by intestinal epithelium. The stroma was markedly decidualized in 3/10 cases (2 of these patients were pregnant at the time of surgery).

Conclusions: In our review of 78 cases of AE, we found 10 cases (13%) with IM, indicating that IM in AE is not uncommon. These cases were often associated with marked appendiceal distortion, luminal obliteration and mass formation, causing concern for a LAMN clinically. Pathologically, findings that favor AE with IM over a LAMN include intestinal epithelium in continuity with endometrial epithelium surrounded by endometrial stroma and absence of luminal mucinous neoplasm. The mechanism of IM in AE is unclear. The foci of IM are predominantly located close to the appendiceal lumen, suggesting the possibility of colonization of endometrial epithelium by appendiceal intestinal type mucosa over a true metaplasia. Endometriosis is well

known as the origin of certain types of mullerian tumors. The possibility that a portion of appendiceal mucinous neoplasms may arise from AE with IM needs to be further studied. AE should be considered when a diagnosis of LAMN is made, especially in young women with a history of endometriosis.

815 Glucose Metabolic Reprogramming and Cell Proliferation Arrest in Micropapillary Colorectal Carcinomas

Monika Vyas, Natalie Patel, Margaret Cho, Narendra Wajapeyee, Dhanpat Jain, Xuchen Zhang. Yale School of Medicine, New Haven, CT.

Background: Micropapillary colorectal carcinoma (MPC) has been reported as an aggressive variant of carcinoma associated with frequent lymphovascular invasion and poor outcome. The micropapillary components (MC) are clusters of closely adherent neoplastic cells with inverted cell polarity and are located in distinct empty spaces. However, the mechanisms by which the MC obtain nutrients and gain aggressive biological behavior in the absence of a fibrovascular core and the formation of cell clusters surrounded by empty spaces are unclear. Altered cellular glycogen metabolism by metabolic reprogramming plays critical role for cancer cell growth and promotes survival. However, the role of altered glycogen metabolism and it's potential impact on the biology of MPC remain poorly understood. The goal of the study was to investigate the glycogen metabolism in MC of CRCs.

Design: 10 cases of CRC with MC were included in this study. Immmunostains for Ki-67 and glucose transporter 1 (GLUT1) were performed. Cultured monolayered and spheroid (a mimic of micropapillary carcinoma *in vitro*) HCT116 human colon carcinoma cells were used for *in vitro* study. Real-time PCR anaylsis of expressions of GLUT1 and glycogen metabolizing enzymes: glycogen synthase (GYS1) and glycogen phosphorylase (PYGL) were performed on monolayered and spheroid HCT116 cells.

Results: GLUT1 was strongly expressed in MC as compared to adjacent tumor cells of conventional glandular component in all 10 cases, and was also significantly increased in spheroid as compared to monolayered HCT116 cells (6 fold induction, $P<0.01$). Similarly, expressions of 2 fundamental glycogen metabolizing enzymes GYS1 and PYGL were markedly increased in spheroid as compared to monolayered HCT116 cells (2.6 and 2.3 fold induction, $P<0.01$). The proliferation index (Ki-67) of MC was significantly lower compared to adjacent tumor cells of conventional glandular component (19.36%±5.8 *vs* 77.0%±12.7, $p<0.01$).

Conclusions: High expressions of GLUT-1 and/or GYS1/PYGL have been correlated with tumor cell survival, tumor progression and poor survival in several cancer types. Our current study demonstrated increased expressions of GLUT1, GYS1 and PYGL in MC of CRCs as well as spheroid HCT166 cells, indicating the presence of altered glycogen metabolism. The reprogramming of glycogen metabolism provides a source of energy contributing to the tumor cell survival in a low proliferation state and aggressive biological behavior of MPC. Targeted inhibition of glycogen metabolism enzymes might warrant consideration as possible anticancer therapies in future studies.

816 H. pylori Infection Induces Early Expression of CD44 during the Progression of Gastric Cancer

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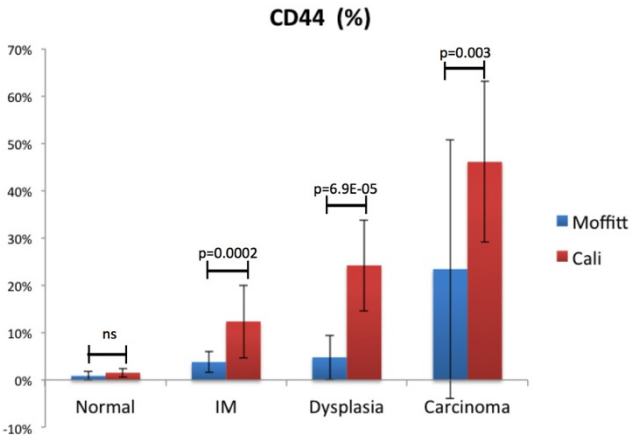
Background: Poor prognosis of gastric cancer (GC) patients with late-stage diagnosis necessitates the identification of markers for early detection. CD44 is involved in cell adhesion and migration and plays a role in carcinogenesis. We propose CD44 positive (CD44+) cells as a potential biomarker for early detection of GC. *Helicobacter pylori* (HP) infection is the leading cause of GC worldwide and is a pathogenetic factor involved in progression from normal mucosa (NM) to chronic gastritis, intestinal metaplasia (IM) and ultimately dysplasia (DS) and invasive adenocarcinoma (GC). We measured variations in the number of CD44+ cells during each of these stages of progression both with and without HP infection. We hypothesize that CD44 expression is associated with progression of GC and the presence of HP may contribute to this expression.

Design: Number of CD44+ cells was measured and compared in HP(+) gastric samples from Colombia (Cali) and in HP(-) gastric samples from Moffitt Cancer Center (MCC), Florida. We tested 63 samples from Cali [10 NM; 17 IM; 10 DS; 26 GC] and 48 from MCC [11 NM; 9 IM; 9 DS; 19 GC]. Cases were stained for CD44 using a rabbit polyclonal Ab (#HPA005785, Sigma Aldrich, St. Louis, MO and the Ventana automated immunostainer Discovery XT (Ventana, Tucson, AZ).

Results: A statistically significant increase in CD44+ cells was noted during all three stages of progression in the Cali HP(+) cohort: NM to IM (p=2.5E-05), IM to DS (p=0.004) and DS to GC (p=4E-05).



Interestingly, the number of CD44(+) cells for each stage was significantly higher in the HP(+) samples as compared to the HP(-) samples. In the latter, the increase in CD44+ cells between NM and IM was less prominent (p=0.006) and no increase in CD44+ cells was visible during the transition from IM to DS (p=ns). CD44(+) cells were, however, increased between DS and GC in the HP(-) samples (p=0.003).

Conclusions: CD44+ cells may represent a biomarker for early detection of the transition from DS to GC, particularly in patients at increased risk for GC such as those infected with HP. HP infection induces early expression of CD44 during the carcinogenic process of GC and leads to greater CD44 expression at each stage of disease than in comparable cases without HP.

817 Histopathologic Findings in Patients with Strongyloides Stercoralis and HTLV-1 Infection

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Background: *Strongyloides stercoralis* is an intestinal helminth infecting 50-100 million people worldwide with a higher prevalence in tropical areas. While infection is usually asymptomatic, the ability of the parasite to complete its lifecycle within a human host allows a severe form to develop in immunosuppressed patients (hyperinfection). Concurrent infection with human T lymphotrophic virus type 1 (HTLV-1) is a risk factor for hyperinfection and treatment failure. The purpose of this study was to analyze the epidemiologic and histopathologic features of *S. stercoralis* hyperinfection.

Design: Ten cases of intestinal strongyloidiasis hyperinfection were identified. H&E stained slides were reviewed and evaluated for a number of features including nature and distribution of the inflammatory infiltrate, number of eosinophils per high power field, number of parasites, and crypt architectural distortion. Pathologic findings were correlated with clinical features, namely age, gender, ethnicity, HTLV-1 infection, and nature of immunosuppression.

Results: All 10 patients were adults and 8 were of Hispanic descent. Serologic positivity for HTLV-1 was identified in 3 patients, 3 were negative, and 4 were not evaluated. Biopsy specimens from all patients showed diffuse expansion of the lamina propria by a mixed inflammatory infiltrate with neutrophilic infiltration of the epithelium. Eosinophils were numerous, but tended to be less prominent among HTLV-1 infected patients (median: 40 vs 81; mean: 43 vs 70), while the number of parasites tended to be higher in these patients (median: 22 vs 13; mean: 18 vs 12).

Conclusions: Most patients with *S. stercoralis* hyperinfection are of Hispanic descent likely reflecting the geographic distribution of disease. Patients with HTLV-1 infection and *S. stercoralis* hyperinfection tend to have a less robust immune response and higher parasite load than patients without HTLV-1 infection, possible reflecting the role of the virus in altering Th2-mediated immunity.

818 Potential Therapeutic Targets for Gastroenteropancreatic Poorly Differentiated Neuroendocrine Carcinoma

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Background: Gastroenteropancreatic poorly differentiated neuroendocrine carcinoma (GEP-PDNEC) is rare and aggressive. Patients often present with distant metastases at initial diagnosis. Treatment using cisplatin and etoposide is often recommended; however, the prognosis remains poor, with 2-year survival <20%. Somatostatin receptor type 2A (SSTR2A) has been used as a therapeutic target for well-differentiated neuroendocrine tumors. In this study, we examined SSTR2A expression in PDNEC and explored whether the epidermal growth factor receptor (EGFR) signaling pathway could be a potential therapeutic target as well.

Design: We evaluated 27 PDNECs by immunohistochemistry for expression of SSTR2A and some key components of the EGFR signaling pathway, including EGFR, phospharylated MAPK (pMAPK), and phospharylated AKT (pAKT). SSTR2A membranous positivity was graded from 0-3+. EGFR membranous staining, pMAPK nuclear staining, and pAKT nuclear/cytoplasmic staining were scored as positive if >1% of cells stained. Patient clinicopathologic data were obtained from Electronic Medical Records. Tumors with invasive adenocarcinoma component >10% were excluded.

Results: The PDNECs arose from the gastrointestinal tract (n=22), pancreas (n=2), and gallbladder (n=3). In situ glandular/squamous lesions were present in some colorectal, gallbladder and esophageal cases. Average patient age was 60 years (range 30-80 years).

Six patients (22%) presented with stage III disease, and thirteen (48%) with stage IV disease. Seventeen of 27 (63%) died of disease (median follow-up 8 months), with 11 (41%) dead within a year; seven (26%) were alive with disease (median follow-up 16 months). Forty-six percent (12/26) of tumors showed some degree of SSTR2A positivity, but only 8% showed strong (3+) positivity. EGFR positivity was seen in 19% (5/26), pAKT positivity in 42% (11/26), and pMAPK nuclear positivity in 62% (16/26) of tumors. Of note, pMAPK staining was usually most strongly positive along the leading/infiltrative edge of the tumor.
Conclusions: This study provides evidence of SSTR2A, EGFR, pMAPK, and pAKT expression in GEP-PDNEC. In particular, pMAPK is expressed in more than half of the tumors. This expression was usually strong and was concentrated along the leading edge of the tumor. We suggest an important role for MAPK activation in GEP-PDNEC tumor infiltration. Given the poor disease prognosis with standard chemotherapeutic regimens, investigation of targeted therapy may be promising.

819 Identification of Copy Number Variation in Chromatin Regulators in Microsatellite-Stable Hereditary Non-Polyposis Colorectal Cancer

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Background: Approximately half of hereditary non-polyposis colorectal cancers (HNPCCs) don't display evidence of mismatch repair defects. The pathogenesis of microsatellite-stable (MSS) HNPCCs, and their effects on patients' prognoses and responses to treatment, differ from those of other colorectal cancers (CRCs). We sought to identify genetic somatic alterations associated with MSS HNPCCs.
Design: We examined genomic DNA samples from 95 primary HNPCCs and adjacent normal appearing mucosa from patients undergoing surgery. We performed the copy number variation (CNV) of the entire genome using the Cytoscan HD Array in 20 MSS and 4 MSI HNPCC tumors and their adjacent normal tissues. Extensive comparison with somatic alterations in MSI HNPCCs allowed segregation of MSS HNPCC-exclusive alterations. The prevalence of CNVs in selected genes was determined from an independent cohort.
Results: We found that copy number of genes that regulate chromatin was altered in MSS HNPCCs; the most frequent CNV were observed in CHD6, CHD7 and CHD8, which encode members of the chromodomain helicase/adenosine triphosphate dependent chromatin remodeling family. Somatic genetic alterations in these 3 genes were detected in 13/10/5 of 20 MSS HNPCCs. A prevalence screen showed that copy number of CHD6, CHD7 and CHD8 altered significantly more frequently in cancers than in the normal mucosa ($P < .01$). Genes altered in patients with CHARGE syndrome who had CHD7 mutations were also altered in MSS HNPCCs with copy number alteration in CHD7.
Conclusions: Genetic aberrations in chromatin remodeling could contribute to the development of MSS HNPCCs.

820 Appendiceal Goblet Cell Carcinoid: Common Errors in Staging and Clinical Interpretation with a Proposal for an Improved Terminology

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Background: Goblet cell carcinoid(GCC)is a low-grade carcinoma(WHO2010).It is treated and staged like adenocarcinoma(AC),and not like neuroendocrine(NET)per AJCC & NCCN guidelines.GCC can have an AC component;this has been termed as mixed adenoneuroendocrine carcinoma(MANEC,WHO2010),AC ex GCC(Tang *et al*) or mixed GCC-AC.This study explores the pitfalls in staging and clinical interpretation of GCC and mixed GCC-AC,and proposes new terminology to avoid common errors.
Design: The terminology for diagnoses,staging protocol and clinical interpretation were obtained for 26 GCC and 31 mixed GCC-AC.Opinions were collected from 19 pathologists by a survey.
Results: Use of NET protocol for staging and Ki-67 for grading was common in GCC. 2 cases each of GCC and GCC-AC were interpreted by oncologists as NET and Ki-67 grade was requested.For mixed tumors,MANEC gives the mistaken impression of combined AC and NET;WHO2010 has used MANEC for both GCC(pg126)and GCC with AC(pg124).Tang *et al* categorized mixed tumors as AC ex GCC,signet ring or poorly-differentiated type.Of 31 mixed cases in our series,AC component was well(WD) or moderately differentiated(MD)in 4 cases and could not be categorized by this scheme.

	Terminology*	Staging protocol	Survey results (n=19)	Proposed terminology
GCC (n=26)	GCC:24 (92%) Adenocarcinoid:4(15%) MANEC: 2 (8%)	Done in 8 cases AC:6 (75%) NET:2(25%)	NET protocol for staging: 7 (37%) Ki-67 necessary for grading: 8 (42%)	Goblet cell carcinoma
GCC with AC (n=31)	AC ex GCC: 16 (52%) MANEC: 7 (23%) Mixed GCC-AC:11(35%) Signet ring/goblet cell AC: 1 (3%)	Done in 22 cases AC:21(95%) NET:1 (5%)	Preferred terminology: Tang *et al*: 9 (47%) WHO: 4 (22%) Mixed GCC-AC: 6 (32%)	Mixed goblet cell carcinoma-adenocarcinoma (state %age of each)

Conclusions: Incorrect use of NET staging was seen in 25% of cases,likely due to the word 'carcinoid' in GCC.Nearly half of the pathologists responded that GCC be staged like NET,and that Ki-67 is necessary for grading.Both GCC and mixed GCC-AC were misinterpreted by oncologists with plans to treat like NET. For mixed tumors, MANEC is a misleading term while AC ex GCC fails to capture cases in which AC is WD or MD. We propose replacing "goblet cell carcinoid" with "goblet cell carcinoma" and the use of "mixed goblet cell carcinoma-adenocarcinoma" for GCC with AC.These terms clearly convey that these tumors are carcinomas and avoid confusion with NET,enabling proper staging and treatment.

821 Epithelial to Mesenchymal Transition Predicts Distant Metastasis in MMR-Intact Colonic Adenocarcinoma

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Background: The epithelial to mesenchymal transition (EMT) has increasingly been recognized to play pivotal roles for epithelial cancer to penetrate the basement membrane and disseminate to distant sites. This study aims to evaluate the predictive value of a mesenchymal RNA signature in metastatic progression of colonic adenocarcinoma (CA), the major cause of death in patients with colorectal cancer.
Design: We identified 104 consecutive CAs resected from 2012 to 2013. Demographic data as well as the development of systemic metastasis (SM) were recorded. The tumors were staged- 7th edition of AJCC. EMT was evaluated by in situ hybridization (ViewRNA, Affymetrix, CA). Both epithelial (E) and mesenchymal (M) signatures were assessed on a single slide, with red and blue chromogens, respectively. E signature recognizable with a 2X, 4X, or 10X lens was recorded as high, with other cases recorded as low. M signature was recorded as absent or present on 40x. ViewRNA probes against E included CDH1, EpCAM, KRT5, KRT7, KRT8, KRT18, KRT19. M markers included FN1, CDH2, SERPINE1. Mismatch repair (MMR) status was evaluated using immunohistochemistry for MLH1, PMS2, MSH2 and MSH6. Sporadic loss of MMR proteins was confirmed on MLH1 methylation and/or BRAF mutations; patients with Lynch syndrome were excluded.
Results: The mean age of the cohort was 67 years with 52% of patients males. Mean follow-up duration was 27 months. In tumors with intact MMR, a positive M signature was identified in 46 (61%), and of these, 12 (26%) developed SM. Of the 30 (39%) that lacked M signature, only 2 (7%) developed SM. In tumors with intact MMR, M signature significantly correlated with the presence of SM ($p=0.03$), AJCC stage at diagnosis (0.01) and node metastasis ($p=0.01$). T stage did not correlate with M signature ($p=0.4$). There was no correlation between E signature and the presence of SM ($p=0.89$). In stage II tumors with intact MMR, M signature predicted SM with a sensitivity and specificity of 80% and 65%. Sporadic loss of MMR correlated with the absence of M signature ($p=0.0001$). In tumors with sporadic loss of MLH1/PMS2, M signature did not correlate with the presence of SM ($p=0.89$).
Conclusions: In MMR-intact CA, the presence of M signature predicts increased incidence of SM, notably in stage II tumors. This panel would likely be a valuable test for optimizing surveillance and treatment of patients with MMR-intact CA. There is further indication that MSI status defines distinct tumor entities, with the data suggesting that EMT plays a more prominent role in the development of SM in MMR-intact tumors.

822 Novel Mutations in African American Colon Cancers Define a Distinct Clinicopathological Entity Linked to Poor Outcome

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Background: African Americans [AAs] are more likely to be diagnosed with, and to die of, colorectal cancer [CC] than any other ethnic or racial group. The causes of these disparities are unknown. Using whole exome and targeted resequencing of 232 AA and Caucasian CCs, we have recently identified a unique 15-gene mutation signature [M+] present in 41% of microsatellite stable AA CCs. 14% of AA CCs harbor mutations seen exclusively in AAs, most prominently including the ephrin type A receptor 6 gene [EPHA6] – present in 6% of AA CCs. These genes are likely newly discovered CC driver genes. This study explores whether these mutations are linked to unique pathological features and poor outcomes in AA CC patients.
Design: A blinded pathology review for high-risk features was performed on primary M+ and M- AA CCs along with a retrospective chart review to determine patient outcomes.
Results: A pathological review of 34 M+ and 35 M- primary AA CCs was performed. M+ AA CC were associated with high tumor grade [$p=0.0046$] and infiltrating growth pattern [$p=0.0005$]. No differences in age at presentation, sidedness, tumor type, mucinous differentiation, angiolymphatic invasion, immune response and tumor budding between M+ and M- cancers were found. 69 AA CCs had available outcome data. M+ AA CCs were more likely to have poor outcome (i.e. present with either de novo or relapsed metastatic disease) than M- AACCs – [$p=0.04$]. This difference appears to be driven primarily by the worse outcome of Stage III M+ CCs [$p=0.05$].

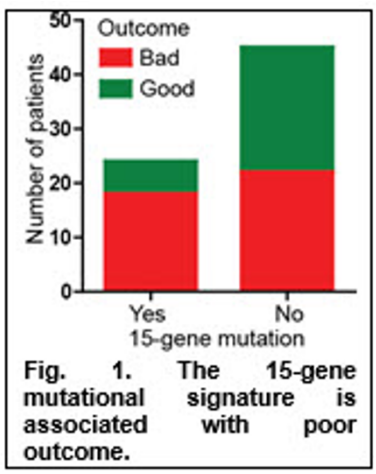

Fig. 1. The 15-gene mutational signature is associated with poor outcome.

Conclusions: This study identified that AA patients with M+ CCs are at increased risk of relapse after curative resection compared to M- AA CCs. M+ AA CCs also have morphological features known to be associated with poor outcome. This 15 gene set represents a unique resource to define new approaches in researching CC in AAs – to better define links to pathogenesis and potentially opportunities for new targeted therapies.

823 Fatty Acid Binding Protein1 (FABP1) Is Preferentially Lost in Microsatellite Instable and in Medullary Carcinomas of the Colon

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Background: Medullary carcinomas of the colon (MC) express few markers of intestinal differentiation and at times are difficult to separate from poorly differentiated (PD) colorectal carcinomas (CRCs) and other undifferentiated tumors. MCs almost exclusively exhibit microsatellite instability (MSI). Gene expression analysis was undertaken in an attempt to identify novel markers which may serve to better characterize MC. Preliminary gene expression analysis revealed loss of the *FABP1* gene in MCs. FABP1 is a cellular transport protein that has been linked to the peroxisome proliferator activated receptor (PPAR).

Design: Gene expression analysis was performed comparing the neoplastic epithelium of MC to laser capture dissected normal colonic epithelium from the same patient, and by bioinformatical analysis of The Cancer Genome Atlas database.

Immunohistochemical (IHC) analysis of FABP1, as well as markers of intestinal differentiation (SATB2, CDX2, and CDH17), was performed. TMAs were constructed from archived cases at Rhode Island Hospital, including MC (n=20), mixed MC (exhibiting medullary and glandular differentiation, n=18), well differentiated MSI CRCs (WD MSI, n=24), well differentiated microsatellite stable CRCs (WD MSS, n=30), and PD CRCs (n=46). IHC for all markers was scored in the tumor epithelium using a 4-tiered scoring system of percentage of cells exhibiting moderate/strong staining (0-4%, 5-25%, 26-50%, 51-100%). Statistical analysis was performed using the Chi-squared test.

Results: Preliminary gene expression analysis revealed 55- and 6- fold decreased expression of *FABP1* in MCs when compared to normal colonic mucosa and other CRCs, respectively. IHC FABP1 expression was significantly decreased in MCs (95%), mixed MC (94%), and in other MSI CRCs (88%) when compared to normal colonic mucosa and WD MSS carcinomas (20%, p = <0.0001). PD CRCs also showed decreased FABP1 IHC expression (78%). Loss of CDX2 and CDH17 was common in MCs (42% and 53%, respectively) and PD CRCs (33% and 22%), but not in WD MSI CRCs (4% and 8%). SATB2 was lost in 21% of MCs, 39% of PD CRCs, and 15% of WD MSI CRCs.

Conclusions: When compared to MSS CRC, FABP1 expression was decreased in MSI CRCs and in the MC subset. We also confirmed loss of other markers of intestinal differentiation in MC, though not with the previously reported sensitivity and specificity. Additional studies are underway to establish a link between FABP1 expression and the MSI pathway.

824 Gluten Free Diet Alleviates Irritable Bowel Syndrome through Intestinal Epithelial Tight Junction Regulation

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Background: Irritable bowel syndrome (IBS) is most common gastrointestinal disease. Although different hypotheses have been proposed to explain its pathogenesis, its underlying causes are not well defined and debatable. Recently, the contributive factor of diet to IBS has received more attention. In this study, we investigated the effects of gluten diet on IBS and its possible mechanisms in the development of IBS.

Design: In the database of over 800 patients with IBS diagnosed by staff at Mayo clinic, 45 patients were recruited into one center, prospective, double blind, 4 weeks, trial and allocated into two groups with matched ages, gender and HLA-DQ2/8 status. Among 45 patients, 28 patients were consented to undergo gastrointestinal endoscopy and flexible sigmoidoscopy procedures before and after the trial. Gluten free diet (GFD) or gluten rich diet (GRD) were prepared at the Mayo clinical research unit and distributed into the respective groups. The expression of claudin-2, claudin-8, claudin-15, MLCK, p-MLC from these biopsy samples were measured by immunohistochemical (IHC) stain. The AOV or ANCOVA analysis was used to assess the effects of diets. Non parametric Mann Whitney test was employed to examine the pre-post different scores between two groups.

Results: The stool frequency overall was decreased significantly in GFD group ($p<0.05$). No morphological abnormalities of intestinal mucosa was detected in all specimen examined. The expression of claudin-15 in colonic mucosa is significantly decreased in IBS patients after treatment with GFD compared to GRD group ($p<0.05$). MLCK, pMLC expression in colonic mucosa is also decreased in GFD group, however not statistically different. No statistical difference is detected in the expression of tight junction associated proteins, claudin-2, claudin-8, claudin-15, MLCK, pMLC, in the small bowel mucosa between the two groups.

Conclusions: The pore-forming tight junction protein claudin-15 expression is altered significantly in the colonic epithelium of IBS. Gluten free diet alleviates the symptoms of IBS through the remodulation of expression of pore-forming claudin-15.

825 Comparison of Universal Screening for Lynch Syndrome in Small Bowel and Colorectal Adenocarcinoma: Small Bowel Adenocarcinoma More Frequently Exhibits Lynch Syndrome-Associated Mismatch Repair Deficiency but Does Not Harbor Sporadic MLH1 Deficiency

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Background: Universal screening for Lynch syndrome (LS) has been advocated for colorectal carcinoma (CRC) but its utility in small bowel adenocarcinoma (SBA) has not been reported. We analyzed a consecutive series of SBAs for DNA mismatch repair (MMR) expression to (1) compare the clinicopathologic features of SBAs stratified by MMR status and (2) examine the patterns of MMR expression in MMR-deficient (MMRD) SBA compared to MMRD CRC.

Design: 72 surgically resected SBAs identified over an 8-year period were analyzed for MMR protein expression by immunohistochemistry and the results were correlated with clinicopathologic findings, histologic features, and *BRAF* mutation status. A consecutive series of MMRD CRCs identified during universal screening over a 3-year period was also analyzed.

Results: 9.7% (7/72) SBAs and 11.5% (149/1291) CRCs were MMRD. MMRD SBAs more frequently demonstrated abnormalities of MSH2 and/or MSH6 (5/7, 71%) compared with MMRD CRC (23/149, 15%) (p=0.002). The 7 MMRD SBAs had the following expression pattern: 4 with concurrent loss of MSH2 and MSH6, 1 with isolated loss of MSH6, and 2 with concurrent loss of MLH1 and PMS2 in patients with a family history strongly suggestive of LS. None of the MMRD SBAs harbored the *BRAF* V600E mutation while 60% of MMRD CRCs were positive for *BRAF* V600E with concurrent loss of MLH1 and PMS2 expression. Compared with MMR-proficient (MMRP) SBAs, MMRD SBAs more frequently demonstrated medullary differentiation (43% vs. 5%, p=0.001) and were larger in size (p=0.04). There was no significant difference in patient age, gender, stage, grade, location, mucinous / signet ring cell differentiation, tumor infiltrating lymphocytes, and Crohn's-like peritumoral reaction between MMRP and MMRD SBAs (all p>0.05). 12 patients with SBAs had Crohn's disease and 3 had familial adenomatous polyposis (FAP); none of the Crohn's disease or FAP-associated SBAs demonstrated MMRD.

Conclusions: SBAs more frequently harbor LS-associated MMRD than CRCs, providing support for screening of SBA to identify patients at risk for LS, particularly in the absence of Crohn's disease or FAP. In contrast to CRC, sporadic MLH1 deficiency associated with *BRAF* mutation is not seen in SBAs. With the exception of medullary differentiation, clinicopathologic and histologic features do not distinguish between MMRP and MMRD SBAs indicating that these features alone cannot reliably predict MMR status.

826 Immunohistochemical Typing of Ampullary Carcinomas May Not Be Ready for Prime Time: Analysis of 136 Cases Fails to Confirm Strong Prognostic Correlation with Recently Proposed Marker Panels, but Finds Strong Prognostic Value in MUC5AC

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Background: Recently, immunohistochemical (IHC)-based classifications of ampullary carcinomas (ACs) have been proposed (Ang, 2014, PMID: 24832159; Chang, 2013, PMID: 23439753) and are now being utilized by oncologists in management protocols.

Design: The prognostic value of Ang/Chang panel markers (CK20, MUC1, MUC2, CDX2) as well as CK7, MUC5AC and MUC6 were tested on ***full-faced sections*** of 136 stringently-defined AC resections with substantial (>5mm) ***invasive*** component. >20% labeling was considered "positive". IHC was correlated with histologic classification (intestinal [H-I], pancreatobiliary [H-P], or nontubular based on ≥3/5 observer agreement) and outcome.

Results: I. Assessment of Ang/Chang markers. None of MUC1/CDX2/MUC2/CK20 were found to have direct prognostic correlation despite testing with different cutoffs. CK7&20 were non-specific (59% of H-I cases were CK7+; 33% of H-P were CK20+), and CK20 determined the final Ang-category in only 2 cases. **II. Ang Panel.** Ang-classification correlated with pathologic signs of aggressiveness (+PNI, VI, LN) ($p< 0.05$) and approached but did not reach prognostic significance, even when the large (33%) Ang-"ambiguous" group was eliminated and only tubular cases were analyzed (Ang-I vs Ang-P; p=0.08). **III. Chang panel.** Comparison of MUC1+/CDX2- vs MUC1-/CDX2+ with survival was marginally significant (Log rank, p=0.1; Wilcoxon, 0.048). **IV. "Gastric" lineage markers, MUC5AC/MUC6.** MUC5AC was the only individual marker with direct and strong correlation with survival, with 5-yr survival of MUC5AC+ vs - cases as 73 vs 25% (p=0.0003). Additionally, MUC5AC stratified both Ang-Ambiguous and H-P into prognostic groups (both p=0.04).

Conclusions: In an analysis of well-characterized invasive ACs, we failed to identify direct/significant prognostic correlation of the putative lineage markers MUC1/MUC2/CK7/CK20/CDX2 and their corresponding panels (as proposed by Ang and Chang). CK7 and 20 lack specificity and may not worth continuing. Definition of P category by CDX2 negativity (a requirement both in Ang/Chang panels) may have to be reassessed because about a third of PDACs coexpress CDX2 (separate analyses). MUC5AC, on the other hand, proved to be a significant prognosticator of ACs and should be incorporated into future panels. More studies are needed before an IHC-based classification can be put into daily practice.

827 Colorectal Adenocarcinoma with ALK Rearrangement: Clinicopathologic and Molecular Characteristics

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Background: Chromosomal translocations in the anaplastic lymphoma kinase (*ALK*) oncogene have been identified as essential tumor drivers in lung adenocarcinomas and other tumors. Recently, *ALK* fusions have been described in rare cases of colorectal carcinoma (CRC). Here we present the largest series of *ALK*-rearranged CRCs with clinicopathologic correlation.

Design: CRCs were assayed by comprehensive genomic profiling (CGP) based on the FoundationOne NGS platform including 315 cancer-related genes and introns from 28 frequently rearranged genes. Four previously published CRCs with *ALK* rearrangements were also included. IHC, FISH, and MSI analyses were performed.

Results: Nine CRCs had *ALK* gene rearrangements including 6 cases identified by CGP (estimated incidence 0.2%), and 3 cases by IHC (estimated incidence 0.1%) and further confirmed by CGP. *ALK* gene rearrangements involved known fusion partners, such as *EML4* (recurrent), *STRN*, *C2orf44*, *CAD*, and the novel *PPP1R21*, *SENPF*, *MAPRE3*, and *PRKAP1B* genes. None of the *ALK*-rearranged tumors harbored mutations in other oncogenic drivers, including *EGFR*, *KRAS*, and *BRAF*, while *TP53* mutations coexisted in 7 of 8 cases. There were 6 females and 3 males with a mean age of 61.4 years (range 43 to 87). Eight patients had stage IV metastatic disease, and one had locally advanced tumor at the time of CGP. Eight tumors were right sided, while one was from rectum. Three cases tested positively for MSI with loss of MLH1 and PMS2 MMR proteins confirmed by NGS bioinformatics. Four tumors, including 2 MSI exhibited mucinous differentiation, one MSI case had medullary features, and four were moderately differentiated adenocarcinomas, morphologically typical of primary CRC. Four of 5 *ALK* rearranged CRCs with tissue available were ALK positive by IHC, while all 5 cases were *ALK* rearranged by FISH. All cases were TTF1 negative, whereas CDX2 was immunoreactive in 3 of 5 cases. One patient with *STRN-ALK* fusion had a dramatic response to the ALK inhibitor ceritinib.

Conclusions: *ALK* rearranged CRCs are uncommon, highly aggressive tumors with characteristic clinicopathologic features. They arise mostly in the right colon, and may exhibit MSI and mucinous phenotype. The lack of mutations in other oncogenic drivers suggests that the growth of these tumors is dependent exclusively on *ALK* fusions. Although rare, *ALK* fusions may represent a therapeutic target for CRC.

828 Predictive Factors for Curative Endoscopic Submucosal Dissection in Superficial Esophageal Squamous Cell Carcinoma

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Background: Without lymph node metastasis (LNM), superficial esophageal squamous cell carcinoma (SESC) can be cured by complete endoscopic submucosal dissection (ESD). ESD indication for SESC is confined to M1(squamous cell carcinoma in-situ) and M2 (involving lamina propria) tumors which are thought to have a low possibility of LNM. We tried to develop the pathological factors that can predict LNM and expand the ESD indication for SESC.

Design: We investigated the pathological factors in 292 cases of surgically resected SESC. The pathological factors include invasion depth, invasion width, tumor budding, lymphatic tumor emboli, tumor infiltrating lymphocytes, presence of lymphoid follicle, and lymph node metastasis. We divided M3 (RMM, reaching muscularis mucosa) into M3 (AMM, abutting muscularis mucosa) and M4 (IMM, invading muscularis mucosa) in the classification of invasion depth. The associtation between LNM and the other pathological factors were analyzed.

Results: About 26% (75/292) of the case had LNM. The rates of LNM according to invasion depth were 0% (0/5, M1), 3.9% (3/76, M2), 10.7% (3/28 M3), 19.0% (4/21 M4), 23.9% (11/46, SM1), 39.0% (16/41, SM2) and 50.7% (38/75, SM3), respectively ($p<0.001$). Any M4 tumor with muscularis mucosa (MM) invasion width less than 1 mm had no LNM (0/12) ($p<0.001$). The rates of LNM in cases with tumor budding and lymphatic tumor emboli were 49.3% (34/69) and 70.9% (39/55), respectively ($p<0.001$). Most cases had tumor infiltrating lymphocytes (98.6%, 288/292). The majority of them had no LNM (72.9%, 213/292) and a minority had LNM (25.7%, 75/292) ($P=0.198$). The rates of LNM according to the presence of lymphoid follicle were 30% (54/179, absent) and 18.6% (21/113, present) ($p=0.018$).

Conclusions: We thought that M3 (RMM) can be reclassified into M3 (AMM) and M4 (IMM) and the tumor with MM invasion width less than 1 mm can be included in ESD indication for SESC.

829 Comprehensive Proteomic Characterization of Pancreatic Adenocarcinoma

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Background: Pancreatic cancer is one of the most fatal cancers. Mass spectrometry-based proteomics is a recently developed powerful high-throughput analytic technique that can investigate global protein expression profiles to determine proteins that are differentially expressed between cancer and normal tissues. Human pancreatic cancer is histologically very heterogeneous and extremely difficult to interrogate proteomically due to very low tumor cell content per unit volume of involved tissue. We overcome this challenge by combining careful laser capture microdissection (LCM) with mass spectrometry that has been optimized to use an ultra-low input of cells.

Design: Samples were carefully laser capture microdissected (LCM) from fresh-frozen tissues. Proteins were extracted from less than 400,000 cells. Proteins were digested to peptides with trypsin/LysC. Label free protein quantitation was conducted on an Orbitrap Fusion tribrid mass spectrometer combined with nanochromatography. The MS data was searched with MaxQuant and Proteome Discoverer software against a human-specific database.

Results: Around 3000-4000 individual proteins were identified from each patient sample. These proteins were functionally assigend based on Panther classification, covering more than 120 cellular pathways. Proteomes isolated from pancreas cancer metastases clustered as close neighbours and show significant distance to non-neoplastic/reactive pancreatic ductal proteomes. 387 proteins were upregulated and 22 proteins downregulated in LCM-purified cancer. 257 proteins were uniquely detected in metastatic tissues (absent in normal ducts). Based on Panther pathway analysis, these proteins cover 44 protein pathways, including apoptosis signaling, p53 pathway, Wnt signaling pathway, TGF-beta signaling pathway, and FAS signaling pathway. We also combined proteomics with RNASeq transcriptomic results from the same patients. Preliminary data shows that upregulated proteins are frequently accompanied by concordantly upregulated RNA levels, such as concordant DKC1 (involved in telomere maintenance) protein and transcript overexpression. However, discordant protein expression and RNA transcript levels are also observed quite frequently for some genes.

Conclusions: Combining laser capture microdissection with label-free quantitative proteomics is a powerful technique to deeply characterize cancer proteomes from previously inaccessibly low tissue input levels. An integrated proteogenomics analysis of data from pancreatic adenocarcinoma is now possible and yields powerful new insights into protein signaling pathway aberrations of pancreatic cancer.

830 Discrete Punctate Nuclear Staining Pattern For MLH1 Protein Does Not Represent Intact Nuclear Expression

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Background: Immunohistochemical staining for the four DNA mismatch repair proteins (MLH1, MSH2, MSH6 and PMS2) is commonly used to screen for Lynch syndrome. We have noticed a discrete punctate nuclear staining pattern, in contrast to the conventional diffuse nuclear staining, which is only seen in MLH1. This staining pattern has caused confusion in interpretation. This study was designed to investigate whether this particular staining pattern represents intact nuclear expression of MLH1.

Design: One hundred and sixty-one colorectal adenocarcinoma cases with immunohistochemical staining for mismatch repair proteins were retrospectively reviewed, which included 86 biopsies and 75 resections. The medical record for each patient was also reviewed for other Lynch syndrome-related tests.

Results: Discrete punctate nuclear staining pattern for MLH1, in addition to conventional diffuse nuclear staining, was observed in the internal control (stroma or normal epithelium) cells in 76 biopsies and 27 resections; while only the conventional diffuse nuclear staining was seen in the remaining 10 biopsies and 48 resections (Fisher's exact test $p<0.0001$). In tumor cells, the discrete punctate nuclear staining pattern was typically seen along with the conventional staining pattern and those cases were interpreted as intact nuclear expression. When only the discrete punctate nuclear staining for MLH1 was observed, it was invariably associated with PMS2 loss of expression. In all 24 cases (21 patients) with PMS2 loss of expression, pure discrete punctate nuclear staining for MLH1 was seen in the tumor cells in 9 biopsies and 2 resections; while completely negative staining for MLH1 was seen in 2 biopsies and 11 resections (Fisher's exact test $p=0.003$). Three patients whose tumor biopsies showed PMS2 loss and discrete punctate staining for MLH1 underwent repeated testing on the corresponding resection specimens: two showed complete loss of MLH1 and one retained the discrete punctate staining. Seven patients who showed PMS2 loss and discrete punctate MLH1 staining underwent further testing: 3 had BRAF V600E mutation, 1 had MLH1 gene mutation, and 3 showed no MLH1 or PMS2 gene mutation.

Conclusions: Discrete punctate nuclear staining for MLH1 is more commonly seen in biopsy specimen, which may reflect differences in specimen handling. This staining pattern is often paired with PMS2 loss of expression and may be associated with BRAF mutation or MLH1 gene mutation, thus it should not be interpreted as intact nuclear expression.

831 WNT Signaling Markers, LGR5 and AXIN2, Show Unique Patterns of Expression in Colonic Polyps

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Background: Pre-neoplastic polyps of the colon, adenomas (TA), sessile serrated adenoma/polyps (SSA/P), and traditional serrated adenomas (TSA) have distinctive but occasionally overlapping morphological features. While TA are uniformly associated with high WNT signaling, SSA/P and TSA have been hypothesized to show low WNT signaling. We used two WNT reporters, LGR5 and Axin 2, the former also represents a stem cell marker. Our aims were twofold: 1) Do neoplastic colonic polyps show predictable WNT signaling and 2) Could this pattern of reactivity with WNT reporters assist in the histologic distinction of colonic polyps.

Design: We evaluated 94 colonic polyps including 18 TA, 21 SSA/P, and 10 SSA/P with dysplasia. We also evaluated 36 TSA that were classified into 2 categories: TSA with minimal atypia (n =18), and TSA with unequivocal dysplasia (n=18). Only polyps with characteristic histological features are included. In situ hybridization stains for LGR5 was performed on all cases, Axin 2 was performed on selected cases. High

reactivity was defined as chromogen visualized at 2 or 4X objectives, all other cases where categorized as low reactivity. Reactivity within the upper 3rd of the crypt was defined as full thickness staining. Accentuation within ectopic crypts was also evaluated.
Results: The vast majority of TA showed high reactivity with LGR5 as well as full thickness staining and, when present, lacked accentuation within ectopic crypts. Low LGR5 reactivity in SSA/P was confined to the basal crypt region. Low LGR5 reactivity was also seen in SSA/P with dysplasia, but full thickness LGR5 reactivity was seen in most cases (90%). TSA showed full thickness and low LGR5 reactivity; a minority of TSA with dysplasia showed high LGR5 reactivity (22%). Accentuation of LGR5 reactivity in ectopic crypts was seen in 45% of cases of TSA with minimal atypia, and 70% of cases of TSA with dysplasia. Axin 2 stain was performed on 33% of TSA with dysplasia, and 20% of SSA/P with dysplasia, and the findings mirror those observed with LGR5.
Conclusions: WNT signaling markers identify 2 classes of colonic polyps : TA show high and TSA and SSA/P with and without dysplasia show low WNT signaling. The presence of LGR5 reactivity in ectopic crypts supports the hypothesis that these structures recreate colonic crypts. The intensity of reactivity with LGR5 and Axin 2 as well an objective means of assessment of ectopic crypts, could assist in the distinguishing TA from TSA, and SSA/P with dysplasia from TA.

832 CALD1 and TAGLN Predict Poor Prognosis of Patients with Colon Cancer

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Background: We have shown that elastic laminal invasion (ELI) affects the prognosis of patients with colon cancer (CC), and the tumor microenvironment formed by ELI promoted tumor progression and metastasis through the interaction between subperitoneal fibroblasts (SPFs) and cancer cells. On the basis of these findings, we revealed that genes' altered expression in cancer cell-stimulated SPFs (*CALD1* and *TAGLN*) was associated with poor prognosis. We studied the reproducibility of these two genes as a clinically prognostic marker and correlated with the ELI in CC.
Design: A total of 112 curative resected CC specimens were immunostained with CALD1 and TAGLN. Each of 3 positive areas at the shallow and deep layers was calculated using morphometric software (WinROOF, Mitani Corporation, Tokyo). The association between expression level and recurrence-free survival (RFS) was analyzed by Log-rank test. Next, we assessed the expression level of two genes by the ELI status.
Results: Both CALD1 and TAGLN were strongly stained at the muscularis propria and tumor stroma. On the other hand, expression in tumor cells was weak (Fig. 1). High expressions of CALD1 and TAGLN at tumor stroma were associated with poor RFS both in the shallow and deep layers (Fig. 2a). The positive area of both genes was significantly higher in the deep area of ELI-positive cases than ELI-negative cases (Fig. 2b).

Fig. 1

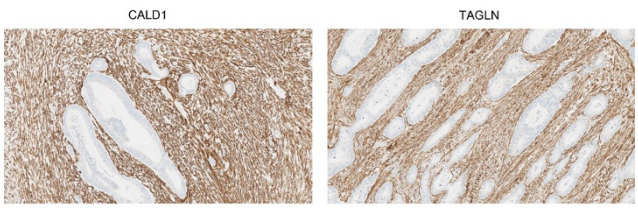
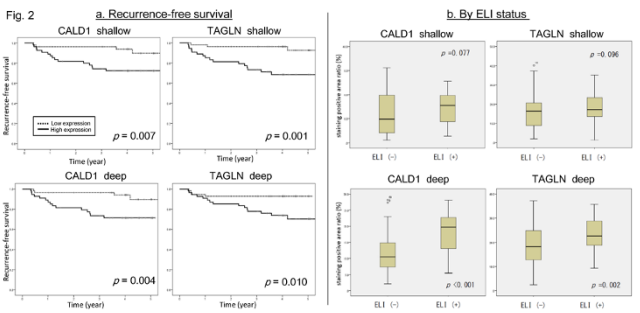


Fig. 2



Conclusions: We successfully identified new prognostic markers of colon cancer from global gene expression data *in vitro*. Alternation of these genes might be linked to the modification of the tumor microenvironment.

833 High PSB7 Expression in Colorectal Cancer Predicts Poor Overall Survival among Older Female Patients and Female Patients Treated with Adjuvant Chemotherapy

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Background: PSB7 encodes the beta-7 subunit of the 20S proteolytic core of the 26S proteasome that has been implicated in carcinogenesis. Previously, we had examined paired cancerous and normal clinical tissue specimens from patients with colorectal adenocarcinomas using heparin affinity fractionation enrichment (HAFE), which allowed for the enrichment of low-abundance proteins. PSB7 expression in colorectal cancer samples was validated by immunoblotting and immunohistochemistry. This is one of the first reports highlighting the utility of PSB7 as a prognostic marker in colon cancer.
Design: We investigated PSB7 expression by IHC (rabbit polycolonal Ab) in a well characterized cohort of 318 colorectal cancer cases (median follow-up, >60 months). PSB7 expression was grouped into high (>200) and low (<200) based on the median expression (H score) observed in the colon cancer tissue microarray. PSB7 expression was correlated with tumor pathological features including TNM staging as well as clinical outcomes such as overall survival and progression-free survival. Survival curves were generated using the Kaplan-Meier method, with significance evaluated using the Mantel-Cox log-rank test. Risk ratios were calculated using the Cox proportional hazard model in both univariate and multivariate analyses. The values of $p<0.05$ were considered statistically significant.
Results: High PSB7 was observed in 28.3% of colorectal adenocarcinomas in our large cohort. Colorectal cancer patients with high PSB7 expression had advanced stage (p=0.0004) and a trend was noted with poor tumor differentiation (p=0.0658). Interestingly, PSB7 expression progressively increased from normal colon to cancer to metastases and the difference in mean expression levels between the 3 groups was statistically significant. Colorectal cancer patients with high PSB7 expression showed trends for poor overall survival in all patients (p=0.0519), and this prognostic effect was accentuated in female patients who had received chemotherapy (p=0.0108).
Conclusions: High PSB7 expression is a prognostic marker for poor overall survival in elderly females (>60 years) who received chemotherapy. Our results highlight subgroups of CRC patients whose tumors may be driven by PSB7, and development of a proteomics-based biomarker panel may be able to be used as an adjunct to decide proteasome inhibitor based treatment strategies.

834 High FAK in the Background of Low c-MET and Low RON Levels Is Associated with Worse Survival in Stage I-III Colorectal Adenocarcinoma Patients

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Background: Focal adhesion kinase (FAK) is a key tyrosine kinase implicated in critical oncogenic processes of tumor invasion and survival. FAK is up-regulated at the protein level in various tumors including breast, thyroid, ovarian, melanoma, and colon. Although associations of FAK with p53, RIP, the death-receptor pathways and the apoptotic/survival nuclear pathways are well characterized, its interactions with downstream membrane receptors c-MET and RON are less studied. The prognostic significance of regulation of RON and c-MET through FAK in the colorectal cancer biology has been largely lacking.
Design: In this study, we studied the immunohistochemical expression of FAK, c-MET, and RON levels in a well-characterized cohort of 330 CRC patients. Quantitative H-scores were obtained using the Aperio color deconvolution method, and the obtained values were examined for their prognostic values as single and combined markers.
Results: We confirmed FAK to be overexpressed in colorectal adenocarcinomas, and high FAK expression was associated with poor overall survival (p=0.0343). c-MET and RON expression were not found to be of independent prognostic significance. FAK, c-MET, and RON levels showed weak to moderate levels of correlation with one another. Stratification of colon cancer samples (stages I-IV) based on expression signatures of the three markers resulted in significant differences in overall survival. The colon cancer subgroup with high FAK, low c-MET, and low RON showed an overall survival of 19.0% as compared to 55.5% for rest of colon cancer subgroups ($p<0.0001$). This difference was an independent prognostic marker for overall survival in stage I-III diseases in a multi-variate model that included patient age (>60 years at diagnosis), sex, tumor size (>5 cm), tumor grade, tumor site (right vs. left), and AJCC stage.
Conclusions: Colon cancer stratification based on expression of FAK, c-MET, and RON showed a colon cancer subgroup of high FAK in the background of low c-MET and low RON to be an independent prognostic marker for poor overall survival. These results suggest that a subset of patients with high FAK may have evolved a mechanism to activate FAK in c-MET/RON-independent manners, either contributing to or reflecting more aggressive disease.

835 Re-Appraisal of Clinical Features, Treatment, and Outcome in Patients with a Histologic Diagnosis of Collagenous Sprue

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Background: Collagenous sprue (CS) is characterized by abnormal subepithelial collagen deposition and inflammation in the small bowel. CS patients present with diarrhea and weight loss clinically and were reported to have poor prognosis historically. The aim of this study is to re-appraise clinical features, treatment, and outcomes in patients with a histologic diagnosis of CS.
Design: 21 patients with a histologic diagnosis of CS were identified from Cleveland Clinic Pathology database from 1990 to 2015. The demographics, clinical features, treatments, and outcomes of these patients were collected.
Results: CS patients were mostly elderly women (62% female, age 64±14 years) with body weight at presentation of 65.1±15.8kg and body mass index 23.6±5.7. 47% CS patients had coexisting collagenous gastritis and 57% CS patients had collagenous colitis. Common symptoms in CS patients included diarrhea (81%) and weight loss (71%), but one patient was asymptomatic. Common laboratory abnormalities in CS patients were anemia (40%) and hypoalbuminemia (50%). 52% CS patients were hospitalized for their disease; 38% CS patients needed temporary total parenteral nutrition (TPN). 24% CS patients used angiotensin receptor blocker (ARB) (4 patients on ARB at the time of diagnosis). 43% CS patients used nonsteroidal anti-inflammatory drugs. Positive celiac serology was only noted in 5 patients (23.8%) at the diagnosis of

CS. 6 CS patients were tested for celiac risk gene: 3 had high risk genes and the other 3 low risks (1 DQ2/other low risk gene and 2 DQ2-/DQ8-). One patient received no treatment due to a lack of symptoms. 13 CS patients were treated with gluten free diet (GFD) and corticosteroids, 5 patients with steroids only, and 2 patients with GFD only. For those 4 patients taking ARB at the diagnosis of CS, one was lost to follow-up, the remaining three stopped ARB. For those 19 patients with follow-up, all patients showed symptomatic reliefs after initial treatment and six (31.5%) were off GFD. During a mean follow-up of 26 months, six (31.5%) had symptom recurrence, three (15.7%) became steroid-dependent, but no disease-related mortality was observed.
Conclusions: Most CS patients are elderly female and symptomatic. About 50% CS patients have collagen deposition in other parts of the gastrointestinal tract. About half of them require hospitalization and one third need temporary TPN. With the use of steroid, removal of offending agents, attention to water and electrolyte balance and temporary nutrition support, CS patients have excellent prognosis and GFD may not be needed in a subset of patients.

836 Clinicopathologic Features of Interval Colorectal Carcinomas in Inflammatory Bowel Disease

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Background: Inflammatory bowel disease (IBD) patients may still develop colorectal carcinoma (CRC) while under regular surveillance, but little is known about the clinicopathologic features of such tumors compared to sporadic and interval CRCs in non-IBD patients.
Design: We searched our database for interval CRC cases (1995-2015), defined as those with non-malignant biopsies 0.5 to 5 years prior to diagnosis, excluding recurrent and intramucosal CRC. Randomly selected sporadic CRCs (N=100) served as controls. Microsatellite instability (MSI) was evaluated by immunohistochemistry. Student's *t*-test and one-way ANOVA was used to compare continuous variables (age, tumor size, and interval duration) and chi-square with Fisher exact test was used to compare categorical variables (tumor location, grade, stage, and MSI status). Two-tailed p value<0.05 were considered significant.
Results: Among 3917 total CRC cases, 132(3.4%) were interval CRCs, including 41 in IBD patients (15 Crohn, 23 ulcerative colitis, 3 indeterminate) and 91 in non-IBD patients. Mean intervals from index colonoscopy to CRC diagnosis and average tumor size were similar among IBD and non-IBD patients. Mean age at diagnosis was 10 years younger in IBD vs. non-IBD interval CRC patients (60.7 vs. 71.1; $p=7.9x10^{-5}$), but the latter was not different from sporadic CRCs(69.1). Interval CRCs in non-IBD patients occurred more frequently in the right colon compared to sporadic controls (52.7% vs. 27.0%, p=0.002). Interval CRCs in IBD patients did not show right-sided predilection (22.0%), but were more often multifocal (9.8%, p=0.0001). Interval CRCs in IBD patients were more often poorly differentiated (51.2%) compared to non-IBD and sporadic controls (29.9% and 20.0% respectively, p=0.001) and of higher stage (60.0% stage III or IV) compared to non-IBD and sporadic controls(43.5% and 29.3%, respectively, p=0.007). MSI was present in 1 of 11(9.1%) interval CRCs from IBD patients, similar to non-IBD and sporadic contorls(13.6% and 7.7%, respectively). While interval CRCs from non-IBD patients were commonly seen arising from villous/tubulovillous adenomas (51.7%), interval CRCs in IBD patients were instead prone to develop from low and high grade dysplasia (61.3%).
Conclusions: Our results show that, unlike non-IBD patients, interval CRC in IBD has no right colon predilection and no association with adenomas, but is of higher grade and stage, suggesting different underlying mechanisms. Rates of MSI were not higher in interval CRCs, suggesting missed lesions on colonoscopy rather than accelerated carcinogenesis.

837 Clinicopathologic Features and IgG4 Reactivity of Eosinophilic and Collagenous Gastritis in Adult and Pediatric Populations

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Background: Eosinophilic gastritis (EoG) and eosinophilic esophagitis (EoE) affect pediatric and adult age groups. Although their respective biopsies are histopathologically indistinguishable, they often have distinct clinical presentations and treatment responses. Likewise, collagenous gastritis (CG) affects both pediatric and adult age groups and is sometimes characterized by eosinophil infiltration of the gastric mucosa. We studied the clinicopathological associations and IgG4 reactivity among these entities.
Design: EoG was defined by ≥ 30 eosinophils per high power field (HPF) in gastric mucosa. EoE was defined by ≥15 eosinophils/HPF combined with characteristic endoscopic and clinical features. CG was defined by subepithelial collagen deposition >15 um. All slides of EoG cases from 2010-2015 and of CG cases from 2005-2015 at our institution were reviewed retrospectively. Clinicopathological data were collected from pathology reports and other information provided by the clinical staff. Plasma cell IgG4 reactivity was assessed immunohistochemically and defined as ≥10 positive cells/HPF. Student's *t*-test and one-way ANOVA were used to compare continuous variables (mean±SD) and chi-square with Fisher exact test was used to compare categorical variables. Two-tailed p values <0.05 were considered statistically significant.
Results: Sixty-seven EoG cases were identified, including 40 pediatric (59.7%, average age 9.5±5.6 y, range 3 m to 18 y) and 27 adult cases (40.3%, average age 37.2±13.7 y, range 24 to70 y). Twenty five (62.5%) pediatric EoG cases were associated with concurrent EoE, which was significantly more common than in the adult EoG group (10/27, 37.5%, p=0.049). In addition, only 1 (2.5%) pediatric EoG case was associated with CG, significantly less than the adult EoG group (6/27=22.2%, p=0.011). Fifteen cases of CG were identified (2 M /13 F). Four (26.7%) CG cases were associated with EoG, but none with EoE (p=0.099). Of 23 cases of EoG (17 adult and 6 pediatric) available for immunostaining, 13 (56.5%) were positive for IgG4. Eleven of 17 (64.7%) adult EoG cases were IgG4 positive, while only 2 of 6 pediatric EoG cases were IgG4 positive (33.3%, p=0.341). Of 2 CG cases available for immunostaining, both were negative for IgG4.
Conclusions: While pediatric EoG is more commonly associated with eosinophilic esophagitis, adult EoG is more commonly associated with collagenous gastritis and IgG4 reactivity. About two thirds of adult EoG cases are IgG4 positive, suggesting that a subgroup of EoG may involve non IgE-mediated processes.

838 Ectopic Crypt Formation Is a Common Histologic Feature in Large Tubular and Tubulovillous Adenomas, Not Specific for Traditional Serrated Adenomas

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Background: The term traditional serrated adenoma (TSA) refers to a distinct subtype of serrated polyps characterized by its overall protuberant exophytic configuration, complex villous growth pattern, and tall columnar cells with abundant eosinophilic cytoplasm. The ectopic crypt formation (ECF) is characterized with loss of crypt anchoring to the underlying muscularis mucosae and is considered as a specific diagnostic feature for TSA. The mechanism of ECF is unclear. We hypothesized that the ECF is associated with complex architecture and large size of polyps. This study aims to determine whether large tubular/tubulovillous adenoma also present with ECF.
Design: A total of 316 tubular, tubulovillous and villous adenomas were included in the study. Adenomas were grouped according to the size: < 0.5 cm (101); 0.5 to 1 cm (155); 1 to 2 cm (39) and > 2 cm (21); and as well as to the locations: right colon (144); left colon (73), rectosigmoid colon (100). All adenomas were removed with polypectomy and the specimens were formalin fixed and paraffin embedded. Three levels, each with 5-micron in thickness, were prepared for each blocks of polypectomy and stained with hematoxylin and eosin for histological examination. ECF was defined as abnormal crypts with loss of orientation and no abutting of crypt base from muscularis mucosae. A **chi-squared test** was used with a p value <0.05 considered as statistically significant.
Results: Overall, ECF was identified in 27.5% studied colonic adenomas (87/316), specifically, 8.9% in <0.5 cm group (9/101); 14.8% in 0.5-1 cm group (30/155); 84.6% in 1-2 cm group (33/39); 100% in >2 cm group (21/21). The presence of ECF is significantly correlated with larger size and villous architecture (P<0.001). There is no significant correlation between ECF and the locations of adenomas.
Conclusions: Our study showed that ECF is not specific for TSA. Rather, it is a common histologic finding in colonic adenomas, especially in those with larger size and villous architecture. Pathologist may avoid making a diagnosis of TSA solely based on the presence of ECF. Combination with other features such as eosinophilic cytoplasms and serrated luminal structures is needed before a diagnosis of TSA is rendered.

839 Portal Hypertensive Gastropathy or Gastric Antral Vascular Ectasia: Is an Accurate Diagnosis Possible on Histology?

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Background: Portal hypertensive gastropathy (PHG) and gastric antral vascular ectasia (GAVE) are mucosa-based vascular lesions leading to chronic gastric bleeding. Both has low incidence (12% and 1%, respectively) among the portal hypertension patients. Diagnosis of the two entities are mainly based on endoscopic patterns, specifically, GAVE is characterized by red spots organized in stripes (so called watermelon stomach) and PHG shows the presence of a mosaic mucosal pattern, usually in proximal stomach. Uncertain cases of GAVE and PHG may be confirmed histologically. The etiology of GAVE is unclear, however, it occurs more frequent in patients with portal hypertension. It is widely accepted that the presence of microthrombi in dilated vasculatures is the key histologic feature to distinguish GAVE from PHG. Here, we aim to determine whether these two entities could be separated solely based on histological findings.
Design: Fifteen PHG and 8 GAVE patients that underwent gastric biopsy were enrolled in our study according to their clinical and endoscopic findings. The histologic features (edema, vascular ectasia, active gastritis not associated with Helicobacter pylori, chronic gastritis, reactive epithelial changes, fibromuscular hyperplasia of the lamina propria, the presence of microthrombi in the dilated capillaries, and hyalinosis) were examined blindly by two GI pathologists without knowledge of clinical and endoscopic findings. Two-sided Fisher's exact test was used for statistical analysis.
Results: Compared to those in the PHG group, histologic finding of microthrombi in the dilated capillaries in the mucosa and submucosa is significantly more common in patients with GAVE (0% vs 50%, p=0.0079). Other common histologic findings in both PHG and GAVE are edema (60% and 100%, respectively), vascular ectasia (100% and 100%, respectively), chronic gastritis (46.7% and 62.5%, respectively), reactive epithelial changes (86.7% and 87.5%, respectively) and fibromuscular hyperplasia (66.7% and 87.5%, respectively). Active gastritis not associated with Helicobacter pylori (13% and 0%, respectively) and hyalinosis (6.7% and 0%, respectively) are rare findings in both PHG and GAVE.
Conclusions: Consistent with the notion that the presence of microthrombi is the single most helpful histologic feature in distinguishing GAVE from PHG. However, it is present in only 50% of cases with GAVE. Our data confirmed that the diagnosis of both GAVE and PHG are clinical-pathological correlation.

840 Post-Appendectomy Colorectal Adenocarcinomas Associate with Low Rates of Lymph Node Metastasis and High Rates of Mismatch Repair Deficiency

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Background: The vermiform appendix in humans is generally considered as a useless remnant. However, studies suggest that it serves as a "safe house" for biofilm formation to preserve and protect commensal bacteria needed for the epithelial mucosa and regulate immune response in colon. Also recent study suggests that an increased rate of colorectal adenocarcinoma (CRC) in associate with appendectomy. In this study, we tried to determine whether any histological features are specially associated with post-appendectomy CRCs.

Design: We reviewed all right side colectomy CRC specimens in one tertiary center between years of 2011-2015. Histological features including differentiation, mucinous components, Crohn's like reaction, angiolymphatic invasion, lymph node involvements were documented. MMR statuses have also been examined by IHC using a four-antibody panel (MLH1, MSH2, MSH6, and PMS2).

Results: 125 cases with appendix and 58 cases without appendix were identified. Mean age was 69.5 years for the cases with appendix and 71.6 year for the cases without appendix. Male verse Female ratio was 1:1.1 for the cases with appendix and 1:2 for the cases without appendix. The histological features with statistically significance are shown in the following table.

	Cases with appendix	Cases without appendix	
Mucinous components	23.8% (10/42)	75.8% (25/33)	P<0.05
Lymph nodes Involvement	44.2% (53/120)	38.9% (21/54)	P<0.05
MMR status	21.6% (16/74)	40.9% (9/22)	P<0.05

Conclusions: Though the data is not conclusive, we do observe a higher ratio of previous appendectomy in right colectomy CRC specimens comparing with that of in the general population. The CRCs status post appendectomy show higher rates of mucinous components, and MMR deficiency, but a lower rate of lymph node involvement. The study suggests that appendectomy may play a role in CRC development.

Genitourinary Pathology (including Renal tumors)

841 Racial Disparity in Papillary Renal Cell Carcinoma

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Background: Significant racial disparities exist in the incidence, distribution, and survival in renal cell carcinoma (RCC). Several studies have confirmed a higher distribution of papillary RCC (pRCC) in African Americans. The objective of this study was to evaluate if there were differences in pRCC subtypes between Caucasians and African Americans

Design: Using an IRB approved single institutional database, we retrieved all cases with previously designated pRCC. All individual cases were reviewed for designation of papillary subtype as well as clinico-pathologic features. Only those subjects that underwent nephrectomy were included for analyses. Clinical data was collected from electronic medical records. Comparisons were made between Caucasians and African Americans using chi squared and independent T-testing.

Results: A total of 122 subjects met the inclusion criteria (101 male and 21 female). The clinico-pathologic characteristics of all tumors are summarized in Table 1. African Americans were younger (p=0.009) at presentation and had larger tumors (p=0.052). There was no significant difference in the pRCC subtype distribution. Nodal metastasis occurred more frequently in African Americans (p=0.034) and there was a trend to advanced T stage (p=0.072) and distant metastasis (p=0.083). There was no significant difference in tumor grade, presence of necrosis or microvascular invasion.

		Caucasian n=80	African American n=42	p value
Age	Mean ± SD	60 ± 14.8	52.6 ± 12.8	p=0.009
Sex	Male Female	65 15	36 6	p=.535
Tumor Size	Mean ± SD	3.8 ± 2.6	4.8 ± 4.1	p=0.052
Histology	Type 1 Type 2 NOS	42 (54.5%) 27 (35.1%) 8 (10.4%)	22 (53.7%) 12 (29.3%) 7 (17.1%)	p=0.544
ISUP Grade	1 2 3 4	3 49 27 0	3 25 13 1	p=0.455
T Stage	T1 T2 T3 T4	64 12 3 0	32 4 3 3	p=0.072
N	N0/X N1	78 2	37 5	p=0.034
M	M0 M1	77 2	37 5	p=0.083
Necrosis	No Yes	43 32	22 16	p=0.955
Microvascular invasion	No Yes	75 1	41 1	p=0.668

Conclusions: In a single institutional series, we did not find racial differences in the subtype distribution of pRCC. However we found that African Americans with pRCC were significantly younger and had more nodal involvement. There was also a trend towards larger tumor size, higher stage and distant metastasis. Due to the low incidence of pRCC and a relatively low number of African American patients in this series, larger cohorts or multi-institutional efforts are needed to further analyze differences in pathologic characteristics in pRCC by race.

842 Neuroendocrine Carcinoma: A Genomically Distinctive Form of Prostate Cancer

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Background: Neuroendocrine carcinoma of the prostate (NCAP) is a rare, but highly aggressive form of poorly differentiated carcinoma of prostate (CAP). We compared the genomic landscape of NCAP with classic non-neuroendocrine CAP using comprehensive genomic profiling (CGP) focused on the search for genomic alterations (GA) that could influence therapy selection for patients with advanced disease.

Design: DNA was extracted from formalin fixed paraffin embedded tissue sections (40u total) from 37 consecutive cases of immunohistochemistry (IHC)-confirmed relapsed/metastatic NCAP and a control group of 509 cases of CAP. CGP was performed on hybridization-captured, adaptor ligation based libraries to a mean coverage depth of 583X for up to 315 cancer-related genes plus 37 introns from 14 genes frequently rearranged in cancer. GA included base substitutions (SUB), INDELs, copy number alterations (CNA) and fusions/rearrangements. Clinically relevant GA (CRGA) were defined as GA linked to drugs on the market or under evaluation in mechanism driven clinical trials.

Results: All 37 (100%) cases of NCAP were positive for neuroendocrine markers on immunohistochemical staining. The median age of the males in this study was 65.1 years (range 43 to 83 years). All (100%) of NCAP Cases were Stage IV at the time of CGP. Samples used for sequencing were obtained from the primary tumor in 9 (24%) NCAP and from metastatic sites in 28 (76%) (12 liver, 6 LN, 2 each from bladder, pelvis and soft tissue, and 1 each from rectum, bone, urethra and ureter). There were 213 total GA (5.8 GA/sample) and 47 CRGA (1.3 CRGA/sample). In comparison with CAP, NCAP featured an identical frequency of *TMPRSS2:ERG* fusions (32% vs 32%), a significantly higher frequency of GA in *TP53* (68% vs 42%) (p=0.011) and *RB1* (51% vs 7%) (p<0.0001) and lower frequency of GA in *AR* (8% vs 22%) (p<0.05) and loss of *PTEN* (32% vs 43%) (NS). The most frequent CRGA in NCAP involved *PTEN* (32%), *BRCA2* (14%), *FGFR1* (5%), PIK3CA (5%) and *AKT2* (3%). No alterations in *BRAF* were identified. Clinical responses to MTOR inhibitors in patients with MTOR pathway GA will be presented.

Conclusions: NCAP and CAP have similar *TMPRSS2:ERG* fusion frequencies, but differ in alteration frequencies for TP53, RB1, AR and PTEN. Multiple alterations in the MTOR pathway identified in this rare, but highly aggressive form of prostate cancer suggest that these patients may be candidates for MTOR inhibitor-based and other targeted therapies associated with *BRCA2* GA (PARP inhibitors) and *FGFR1* GA (FGFR inhibitors).

843 Prognostic Significance of Depth of Invasion in Transurethral Resection of Bladder Specimens

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Background: Depth of invasion in transurethral resection of bladder (TURB) biopsies of Stage T1 urothelial cancer has been evaluated using measured depth of lamina propria (LP) invasion or by muscularis mucosa invasion. These are affected by biopsy orientation, variation in LP, and inconclusive presence of muscularis mucosa. Identifying tumor adjacent to detrusor muscle (TADM) is not affected by orientation or size of the LP and may be a better histologic indicator for advanced T1 disease.

Design: A language search of pathology archives from 1995-2015 using "transurethral resection" and "bladder" identified 2786 TURB cases. Pathology reports and clinical information was reviewed, and only those with T1 cancer at initial resection, urothelial histology, detrusor muscle (DM) present in biopsy, and adequate clinical records were included. H&E and other available slides were reviewed, and patients were classified into two groups: LP invasion with TADM (group 1), or any other LP invasion without TADM (group 2). Further clinical information was obtained including, age, gender, treatment and disease course including all subsequent resections or cystectomy, and status of nodal or metastatic disease. Recurrence (subsequent resections with T1 or higher), rate of cystectomy, and progression (presence of T2 or higher or nodal/metastatic disease) were compared using Fisher's exact and chi-square test.

Results: 105 patients (M:F = 3.4:1, median age 74) met inclusion criteria. 20 patients had TADM (group 1), and 85 patients had no TADM (group 2). Disease recurrence was seen in 15 of 20 cases (75%) in group 1 and 32 of 85 cases (37%) in group 2, with significant difference (p=0.005). Disease progression was seen in 14 of 20 (70%) in group 1 and 19 of 85 in group 2 (22%), also statistically significant (p=0.04). Rates of lymph node positive disease at cystectomy or metastatic disease were significantly higher in group 1 (8 of 20, 40%) than in group 2 (9 of 85, 10%), p=0.003. The cystectomy rate was higher in group 1 than group 2, although the difference was not significant [8/20 in group 1 (40%) vs 22/85 in group 2 (25%), p=0.09].

Conclusions: This retrospective analysis of T1 disease in TURB specimens showed that LP invasion with TADM in TURB specimens is associated with higher rates of disease recurrence and progression. This means of subclassifying T1 bladder cancers is not affected by tissue orientation, variations in LP depth or presence of a muscularis mucosa layer, and may be a better prognostic tool.