627 IQGAP1 Copy Number in Follicular-Patterned Lesions of the Thyroid – A Pilot Study

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Background: The accuracy and reproducibility of the diagnosis of follicular-patterned thyroid lesions (FPTL) is currently limited by subjective morphological criteria and difficulties in assessing capsular integrity and vascular invasion. IQGAP1 is a widely conserved multifunctionl protein currently thought to play an important role in cell polarity, adhesion, migration and proliferation through regulation of the actin microtubule cytoskeleton, transmembrane trafficking, and intracellular signaling. IQGAP1 genetic copy gain has recently been reported in association with increased invasiveness in some thyroid tumors. This pilot study was designed to explore the potential role of IQGAP1 copy number (CN) in the diagnosis of FPTL.

Design: 27 FPTL [10 follicular adenomas (FA), 10 follicular variants of papillary carcinoma (FVPTC), and 7 follicular carcinomas (2 invasive, 3 minimally invasive, and 2 metastases) (FC)] were retrieved from our files. After slides were reviewed and diagnoses confirmed, DNA was extracted from selected areas of two 10-micron sections of each lesion using standard Qiagen QiaAmp FFPE extraction (Valencia CA). Real time PCR detection of gene specific primers (IQGAP1 and a reference gene RNAase) was accomplished using MGB probes (Applied Biosystems). CN was calculated for each sample using the linear region of a standard curve established from a serial dilution and linear regression curve. Histological diagnoses were correlated with CN.

Results: IQGAP1 CN ranged from 1.7 to 3.9. Using a cut-off CN ≥2.5, 2 (20%) of 10 FA, 4 (40%) of 10 FVPTC, and 4 (57%) of 7 FC were positive. Although the percentage of cases with CN ≥2.5 increased from FA to FVPTC to FC, the differences in CN did not reach statistical significance ($p>0.05$) across the three diagnostic groups.

Conclusions: - Increasing percentages of cases with IQGAP1 CN ≥2.5 were observed when FA, FVPTC, and FC were compared.
- IQGAP1 CN may be helpful to stratify FPTL.
- Larger studies are warranted to further assess the potential role of IQGAP1 CN in the diagnosis of thyroid lesions.

628 Validation of BRAF V600E Mutation Using Qiagen Rotor-Gene Analysis System

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Background: Mutations in the BRAF oncogene are commonly seen in papillary thyroid carcinoma (PTC) and have been shown to be useful in cases of indeterminate cytology from thyroid fine needle aspirations (FNA). Mutation analysis can be performed on a variety of platforms with different diagnostic sensitivities. We present a validation study for BRAF mutational analysis of thyroid FNA samples on the newly introduced Qiagen Rotor-Gene Q platform which uses high resolution melting post-PCR analysis and compare it to results obtained by sequencing and final surgical specimen diagnosis.

Design: Ultrasound guided thyroid FNA samples were obtained. Non-enriched FNA samples were used. DNA was extracted from the specimens using the Roche MagNA Pure Compact extraction kit and was evaluated for the BRAF V600E mutation using the Qiagen PyroMark Q24 sequencer and Qiagen Rotor-Gene Q analysis system. The results were compared to the final surgical diagnosis (gold standard) to determine the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Results: 50 patients were identified for this study and 56 FNA samples were tested (Table 1). In this data set, 6 FNAs were indeterminate and 14 FNAs were diagnostic for PTC. 17 patients were diagnosed with PTC following total/partial thyroidectomy and 9 had metastatic disease. The FNA DNA quantity ranged from 0.34-32.07 ng/µL and the mutation prevalance ranged from 8%-34%.

Table 1: Comparison of Rotor-Gene, PyroMark Q24, and FNA to surgical diagnosis

	Rotor-Gene	PyroMark Q24	FNA
Sensitivity	14/19 (73.7%)	12/19 (63.2%)	17/19 (89.5%)
Specificity	37/37 (100.0%)	37/37 (100.0%)	34/37 (91.9%)
PPV	14/14 (100.0%)	12/12 (100.0%)	17/20 (85.8%)
NPV	37/42 (88.1%)	37/44 (84.1%)	34/36 (94.5%)

Conclusions: When compared to the PyroMark Q24 platform, the Rotor-Gene platform showed superior sensitivity and identical PPV. The Rotor-Gene is capable of successfully analyzing extremely small amounts of DNA and has an improved sensitivity when looking at the identification of metastatic disease. The results in this small cohort suggest that the Rotor-Gene platform BRAF mutation detection could be a useful tool in indeterminate FNA samples.

Gastrointestinal

629 Novel Chromosomal Abnormalities in Barrett's Esophagus and Esophageal Adenocarcinoma Identified by Array Comparative Genomic Hybridization (CGH)

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Background: Prior genome wide studies using biopsy samples of Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC) have shown gains of chromosomes 1q, 7q, 8p and losses of chromosomes 3p, 9p, 16q, 17p, and 21q22. Present study performed array CGH on carefully macro dissected fresh frozen tissue samples of BE and EAC and found novel regions of chromosomal alterations.

Design: The study comprised of 14 patients (M:F;11:3, average age 68 years) with BE associated EAC who underwent esophagogastrectomy without preoperative chemoradiation. Tumor stage was T1aN0 in 4, T1bN0 in 6, T1bN1 in 1, T2No in 2 and T4N2 in 1. H&E section of fresh frozen samples from squamous mucosa, non-dysplastic BE and EAC from the resection specimens were reviewed and area with more than 80% of lesion was macrodissected from the frozen block followed by DNA extraction using TRIzol Reagent (Life Technologies, CA) with post-PCR purification. Microarray analysis was performed using bacterial artificial chromosome (BAC) DNA microarrays (Constitutional Chip 4.0, PerkinElmer, Finland). This microarray has over 5200 BAC clones, including targeted coverage in well-characterized chromosomal regions, subtelomeric regions, and pericentromeric regions, as well as backbone coverage of the genome with an average resolution of 0.5 Mb. DNA extracted from either BE or EAC tissue was analyzed using DNA from squamous mucosa from the same patient as a reference. Arrays were scanned at 10 microns using a ScanArray Gx scanner (PerkinElmer) and analyzed using GenePix Pro 6.1 (Molecular Devices, CA).

Results: In BE, gains of chromosomal loci 1p36.33, 1p36.32, 3q21.3, 9q34.2, 9q34.3, 12q24.31, 12q24.33, 16p13.3, 16q24.3, 20q13.3, 21q22.3, 22q13.32 were present in two (18%) samples and losses of loci 9p21.3 in two (18%) samples. The remaining eight BE samples showed no chromosomal gains or losses. All EAC samples showed chromosmal gains. Six (43%) EAC showed gain of chromosomal loci 8p23.1 and 20q13.33, and four (37%) of 7p12.3 and 8q24.3. Other chromosomal gains included 5p15.33, 7p12.3, 7p11.2, 7q11.21, 7q21.13, 7q21.2-21.3, and 8q24.13 in 3 or less tumors. Loss of chromosome Yq11.22 was seen in all EAC from men.

Conclusions: There is marked increase in gains at multiple chromosomes in EAC as compared to BE. Present study supports prior studies showing gains of chromosomal locus 8p23.1 and identifies gain of chromosomal locus 20q13.33 and loss of locus Yq11.22 as novel abnormalities in EAC.

630 Immunohistochemical Features of Intestinal and Foveolar Dysplasia in Barrett's Esophagus

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Background: Recently, two major subtypes of dysplasia in Barrett's Esophagus (BE) have been identified, termed "intestinal" (INT) or "gastric", also referred to as "foveolar" (FOV). Some patients show a mixture of both types. Previous data have shown that these two subtypes of dysplasia may have different biological characteristics, arise from different types of BE mucosa, and may have different natural history and risk of malignancy. The aim of this study was to determine the immunohistochemical characteristics of these dysplasia subtypes in BE, with emphasis on the type of differentiation (intestinal vs. gastric) present in both.

Design: Thirty-eight BE-related endoscopic mucosal resections (EMR) were evaluated morphologically for the highest grade (low, high) and type of dysplasia (INT, FOV, mixed) by routine H&E histologic methods. Immunohistochemistry was performed for intestinal markers (CDX2, MUC2, and villin) and gastric markers (MUC5AC, MUC6) and for Ki67 and P53, and all markers were scored for the presence and degree of staining in a semiquantitative fashion (grade 0=negative, 1=focal, 2=multifocal, 3=diffuse staining). Staining was compared between the different types of morphologic dysplasia.

Results: By morphology, 11 (29%) were considered intestinal, 8 (21%) foveolar, and 16 (42%) mixed dysplasia. By immunohistochemistry, morphologically classified INT dysplasia showed significantly higher expression of INT markers, such as MUC2, CDX2, and villin, whereas FOV dysplasia showed significantly more MUC5AC and MUC6 expression. Mixed INT/FOV dysplasia showed an immunophenotypic pattern that matched, roughly, the morphologic areas of INT and FOV dysplasia. No significant differences were observed in P53 or Ki67 proliferative index. Interestingly, despite differences in quantity of the various INT and gastric immunomarkers in INT and FOV dysplasia, respectively, all cases of both types of dysplasia showed at least focal CDX2 staining.

Conclusions: Regardless of the morphologic phenotype of dysplasia, whether INT or FOV, all dysplastic epithelium shows some evidence of intestinalization characterized by at least focal CDX2 staining, similar to the background non-dysplastic metaplastic columnar epithelium as previously reported. In general, INT dysplasia shows more advanced INT immunophenotype, in contrast to FOV dysplasia which shows a more advanced gastric phenotype. Further studies are needed to determine whether dysplasia types with more advanced INT versus gastric differentiation have a different risk of malignancy.

631 Goblet Cells Are Depleted with Advancing Degrees of Preneoplasia in Barrett's Esophagus

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Background: A previous study by our group showed that the proportion of goblet cells in index biopsies of patients with Barrett's Esophagus (BE) is inversely proportional to the risk of malignancy, and this led to the hypothesis that goblet cells may represent a successful adaptive form of metaplasia that protects the mucosa from the carcinogenic effects of reflux. However, the topographic distribution of goblet cells in relationship to neoplasia is unknown, and no prior studies have evaluated resection specimens where anatomic distribution can be more accurately noted. The aim of this study was to evaluate the goblet cell density in adjacent non-neoplastic BE both adjacent to, near, and distant from areas of neoplasia in BE patients who had a resection for dysplasia or carcinoma without neoadjuvant therapy.

Design: Routinely processed resection specimens from 40 patients who had a distal esophagogastrectomy for high-grade dysplasia (N=1) or adenocarcinoma (N=39) were evaluated histologically for the mean number of goblet cells per crypt (GC/crypt) in areas adjacent to (<10 crypts), near (10-20 crypts) and distant from (>20 crypts) areas of neoplasia. Goblet cell counts were compared between different mucosal locations in proximity to neoplasia in all cases, and then also compared between areas of low vs. high-grade dysplasia.

Results: Overall, there was a significant decrease in goblet cell counts in areas of mucosa adjacent to neoplasia (mean GC/crypt = 3.8) vs. near (5.2) or distant from (5.5) neoplasia, (p=0.029). In all mucosal locations, there was a highly significant decrease in goblet cell counts in areas of high vs. low-grade dysplasia. For instance, mucosa adjacent to neoplasia showed a crypt count of 3.9 vs. 1.5 (p=0.05) for low vs. high grade dysplasia, respectively, values of 5.4 vs. 1.5 (p=0.005) for mucosa near low vs. high grade dysplasia, respectively, and values of 6.0 vs. 1.2 (p<0.001) for mucosa distant from low vs. high grade dysplasia, respectively. Goblet cell density did not change significantly with distance from invasive carcinoma.
Conclusions: In BE, goblet cells are depleted within the mucosal field in which neoplasia develops. Further studies are needed to determine whether goblet cell depleted mucosa is at increased risk for neoplasia, or neoplasia itself induces a shift in the columnar metaplasia that occurs in the background mucosa, perhaps due to changes in cell differentiation. Retention of goblet cells in areas adjacent to cancer was likely due to the destructive effects of cancer on the surrounding neoplastic precursor mucosa.

632 Magnification Endoscopic and Histologic Observations of Palisade Vessels at the Esophagogastric Junction, with Reference to Their Nature and Histologic Utility

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Background: Endoscopy is able to demonstrate palisade vessels at the level of the lower esophageal sphincter, but they are not present in the middle esophagus and stomach. We examined these vessels both endoscopically and histologically, clarified their density and caliber, and assessed their histologic utility for distinguishing samples of Barrett's esophagus (BE) from those of gastric cardia.
Design: We examined the density and caliber of palisade vessels by magnifying endoscopy with narrow-band imaging in 15 consecutive patients without GERD or BE, and histologically examined the incidence of veins >100 μm in diameter defined as palisade vessels in the middle and lower esophagus and stomach in 20 autopsy cases, and also in endoscopic mucosal resection (EMR) specimens from the gastric cardia (20 cases) and BE (66 cases). In the EMR specimens, we also examined 3 histologic markers of esophageal tissue: esophageal glands, squamous islands, double muscularis mucosae.
Results: Endoscopically, palisade vessels were not observed in the stomach and middle esophagus. The density of palisade vessels was highest near the squamocolumnar junction (SCJ), and gradually became more confluent and showed an increase in thickness towards their upper limit. The palisade vessels were 75 – 275 μm in diameter. Palisade vessels were evident histologically only in the mucosa of the lower esophagus in the autopsy cases, and were not observed in the gastric EMR specimens. However, 71% of the BE EMR specimens contained them. The 3 markers were not evident in the stomach EMR specimens, but present in 33%, 18%, and 71% of the BE EMR specimens, respectively.
Conclusions: We demonstrated that blood in palisade vessels flows orad from the SCJ, and that palisade vessels are a distinctive feature of esophageal tissue, in addition to the 3 markers already recognized.

633 Telomere Shortening in Esophageal Epithelium of Alcoholics: Differences in Terms of ADH-1B and ALDH-2 Genotypes and Chromoendoscopy Findings

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Background: Telomeres are repetitive G-rich DNA sequences and associated binding proteins found at the ends of eukaryotic chromosomes, and appear to play a key role in preventing genomic instability. Progressive telomere shortening with age or chronic inflammation may lead to genomic instability during the early stage of carcinogenesis. Squamous cell carcinoma of the esophagus is very rare in the young, but occurs frequently in alcoholics. It is known that the genotypes of ALDH-2 and ADH-1B are involved in carcinogenesis.
Design: All tissues were examined histopathologically. Using our Q-FISH measurement technique, we estimated telomere lengths of epithelial basal cells from 53 alcoholics and 20 age-matched normal controls. Biopsy specimens from alcoholics were obtained from areas unstained by iodine, but were not histologically distinct from control tissue. We then analyzed telomere lengths among patients grouped according to ADH-1B and ALDH-2 genotypes, and also examined the relationship between telomere length and chromoendoscopy findings.
Results: There was no histologic evidence of chronic inflammation in either alcoholics or non-alcoholics. Telomeres in basal cells were significantly shorter in alcoholics than in the controls. However, telomere lengths did not differ among patients grouped by genotype. Telomere lengths in biopsy specimens from unstained areas exceeding 10 mm in diameter (p = 0.0157), or those taken from cases showing multiple unstained areas (p = 0.0402), were significantly shorter than in other cases.
Conclusions: Telomeres in the esophageal epithelium appear to be shorter in alcoholics than in non-alcoholics, suggesting that telomere shortening may be associated with the frequent occurrence of squamous cell carcinoma in alcoholics. It is suggested that alcohol reduces telomere length, irrespective of genotype. Chromoendoscopy appears be provide evidence of telomere shortening in the esophageal epithelium of alcoholics, and may be predictive of carcinogenesis.

634 Comparative Study of Papillary Carcinomas and Conventional Adenocarcinomas of the Extrahepatic Bile Ducts. A Study of 5870 Cases from the SEER Program of the National Cancer Institute

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Background: Papillary carcinomas of the extrahepatic bile ducts are a controversial entity, because they have been compared to intraductal papillary mucinous neoplasms of the pancreas which are low-grade malignant tumors. However, some authors based on small series considered that both conventional adenocarcinomas and papillary carcinomas are high-grade malignant tumors.
Design: From 1973 to 2008 we reviewed all papillary carcinomas and conventional adenocarcinomas of the extrahepatic bile ducts collected by the SEER program of the National Cancer Institute and compared their demographics and long term survival rates.
Results: There were a total of 5870 cases of which 5582 (95.1%) were conventional adenocarcinomas and 288 (4.9%) were papillary carcinomas. Of these, 285 (99%) were invasive and 3 non-invasive carcinomas. There has been a decrease in the incidence of papillary carcinoma since 1973, while the incidence of conventional adenocarcinoma has been stable. There was a 77.2% decrease in the rate from 1973-2008, which is statistically significant. Overall, the 5-year survival rate of papillary carcinomas was 10%, similar to the 5-year survival rate of conventional adenocarcinomas. Localized papillary carcinomas (confined to the wall) had a 5-year survival rate of 30% while the localized conventional adenocarcinomas had a 20% 5-year survival rate. The difference was not statistically significant.
Conclusions: The vast majority of papillary carcinomas of the extrahepatic bile ducts are invasive (99%) and the 5-year survival rate is similar to that of conventional adenocarcinomas. Papillary carcinomas of the extrahepatic bile ducts are high-grade malignant neoplasms. The reason for the decrease incidence of papillary carcinoma is unknown.

635 Sexually Transmitted Disease (STD) Proctitis: Clues to a Frequently Missed Diagnosis

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Background: Recent European outbreaks of lymphogranuloma venereum (LGV) and the rising incidence of syphilis underscore the importance of recognizing these STDs, especially in HIV+ men who have sex with men (MSM). LGV and syphilis can present as isolated proctitis, providing an important diagnostic opportunity. We report a distinctive pattern of mononuclear inflammation, crypt distortion, and fibrosis in patients with STD proctitis.
Design: The syphilis proctitis group consisted of cases prospectively identified by a distinctive inflammatory pattern in patients later shown to be RPR+. LGV proctitis cases were retrieved from patients from an STD clinic known to have LGV proctitis who had undergone anorectal biopsies.
Results: We identified 12 cases from 10 patients whose anorectal biopsies showed STD-associated features. All patients were HIV+ men (average CD4 count 472/mm^3). All 6 with an available sexual history were MSM; 3 had documented receptive anal intercourse. The majority presented with rectal bleeding (8/10) and pain (7/10). Endoscopic impressions included rectal nodularity (10/12) and ulcerations (7/12). Three cases were previously misdiagnosed (3/10) as ulcerative colitis, mucosal prolapse, or nondiagnostic.
The syphilitic proctitis study group consisted of 10 colonic biopsies from 8 patients. The RPR titer ranged from 1:2 to 1:1024 (median 1:20). The majority of the biopsies had prominent plasma cell-rich mononuclear inflammation, perivascular plasma cells, crypt distortion, and fibrosis (8/10). Additional findings included ulcers (5/10) and poorly formed granulomas (2/10). The majority of cases containing squamous mucosa showed a plasma-cell rich, lichenoid pattern of chronic inflammation, perivascular plasma cells (3/4), and ulcers (3/4).
The LGV study group consisted of 2 colonic biopsies from 2 patients. Biopsies showed prominent chronic inflammation (including plasma cells) and fibrosis (2/2).
Conclusions: We report a unique histologic pattern associated with STD proctitis: prominent mononuclear infiltrates associated with crypt distortion, fibrosis, and lacking significant acute inflammation, features potentially mimicking IBD. Moreover, the histologic findings of STD proctitis differ slightly from the classic features described in the skin: plasma cells and perivascular lymphoplasmacytic infiltrates were less prominent in syphilis proctitis than that typically seen with cutaneous syphilis. This unique pattern is important to recognize, especially in MSM, to suggest additional testing for STD proctitis and to avoid the potential pitfall of misdiagnosing IBD.

636 Altered Intestinal Tight Junctions' Expression in Patients with Liver Cirrhosis: A Pathogenetic Mechanism of Intestinal Hyperpermeability

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Background: Increased intestinal permeability in cirrhosis exerts a pivotal role in the pathogenesis of spontaneous bacterial peritonitis and other complications of cirrhosis through promotion of systemic endotoxemia. The present study was designed to investigate whether alterations of tight junction associated proteins in the intestinal epithelium are involved, at the molecular lever, in intestinal hyperpermeabilty observed in cirrhotic patients.

Design: Twenty four cirrhotic patients at a decompensated (n=12, group A) or compensated condition (n=12, group B) and 12 healthy controls (group C) were subjected to duodenal biopsy. The expression of the tight junction (TJ) proteins occludin and claudin-1 in the intestinal epithelium was evaluated by immunohistochemistry. Plasma endotoxin concentrations were also determined.

Results: Cirrhotic patients presented significantly higher serum endotoxin concentrations as compared to healthy controls (P<0.001), whilst endotoxemia was higher in decompensated disease (P<0.05 vs. compensated cirrhosis). Patients with decompensated and compensated cirrhosis presented significantly reduced expression of occludin and claudin-1 as compared to controls (P<0.01, respectively). These alterations were significantly more pronounced in decompensated patients as compared to compensated (P<0.05). Regarding occludin, in cirrhotic patients a specific pattern of expression in the intestinal epithelium was observed, with a gradually increasing loss of expression from crypt to tip of the villi.

Conclusions: The present study demonstrates for the first time that human liver cirrhosis induces significant alterations of enterocytes' tight junctions. These changes might represent an important cellular mechanism for intestinal barrier dysfunction and hyperpermeability in patients with liver cirrhosis.

637 Characterization of Adenocarcinomas Arising in Sessile Serrated Polyps/Adenomas

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Background: In recent years, the topic of sessile serrated adenomas (SSA) has been the focus of much clinical and translational research. The significance of these lesions hinges on the risk of the development of adenocarcinoma, particularly for patients undergoing active endoscopic surveillance (so-called "interval" cancers). There is a paucity of research for these cancers that arise from SSAs (SSA-CA), primarily due to their rarity and under-recognition. We evaluated 33 biopsies of SSA-CAs in order to provide a detailed morphologic description, as well as review the demographic and endoscopic characteristics of the patients in which these neoplasms arise.

Design: 33 biopsies with both an invasive adenocarcinoma and either an adjoined or separate SSA on the slide were accepted into the study. When the tumor histology was not typical of conventional colorectal carcinomas (C-CRC), MSI related histologic patterns (mucinous, medullary, signet ring) and other distinct histologic patterns (serrated, percolating, microglandular) were used to characterize these lesions and then their percent composition quantified. Host responses such as intratumoral lymphocytosis, dirty necrosis, and neutrophilic infiltrate were also determined to draw further distinction.

Results: 21 of 33 cases were composed to at least some degree of MSI associated and other distinct histologic patterns, not typical for C-CRC. Four cases were composed entirely of patterns not seen in C-CRC, while 12 cases were composed entirely of C-CRC. The serrated pattern was seen in 12 cases and was the most common pattern seen in the study series. Of the host response features, 11 biopsies had a significant intratumoral lymphocytosis, 13 cases had a neutrophilic infiltrate and 28 cases had dirty necrosis. These carcinomas typically arose in polypoid lesions measuring 10 to 20 mm and located in the right colon in all but 5 cases. Twenty six of the patients were female, and the average age was 71 years.

Conclusions: Being a distinct type of adenocarcinoma by virtue of their pathogenesis, SSA-CAs share many morphologic characteristics commonly seen in C-CRCs and MSI associated cancers, as well as the recently described serrated adenocarcinomas. The identification of an SSA within in the same specimen is requisite for the diagnosis of SSA-CAs. A diagnosis of SSA-CA should be strongly suspected in elderly female patients with a right sided polypoid lesion, measuring between 10 and 20 mm, and an invasive adenocarcinoma. In addition, this is the largest case series of SSA-CAs to date.

638 Relevance of AKT Pathway Protein Expression in Gastrointestinal Kaposi Sarcoma

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Background: Gastrointestinal location of Kaposi sarcoma (KS) is classical but rare. Activation of the AKT pathway signaling pathway by KSHV/HHV8 encoded proteins plays a central role in Kaposi sarcomagenesis. There is increasing evidence that rapamycin, interfering with AKT pathway proteins, may be useful in the treatment of KS, including in HIV positive patients.

Design: The expression of AKT pathway proteins (AKT, 4EBP1, PTEN, mTOR) was assessed on tumor biopsies of 19 gastrointestinal KS (17 patients) by immunohistochemistry using antibodies directed against their activated forms. The percentage of positive cells was assessed in tumor and non-tumor tissues. The median was used as cutoff for classifying low/high expression. Protein expression was analysed with regard to patients' and tumor characteristics.

Results: Patients' age varied between 28-75 years (median 37), the gender ratio was women:men of 6:11 and, 16/17 patients were HIV positive. Twelve (12) tumors were gastric and 7 intestinal (duodenum and colorectum, 2 and 5 respectively). The tumor vascular component varied between 25-80% of tumor mass (median 45%), and comprised classical vessels as well as tumor slits (vascular spaces lined directly by tumor cells). Tumor hemoragia and hemosiderin were observed in 5 and 7 KS.

Tumor cell nuclear expression of AKT, 4EBP1, PTEN, and mTOR was observed in 42%, 88%, 65% and 100% KS and, vascular expression in 42%, 76 %, 53% and 94% KS. Nuclear AKT and 4EBP1 tumor and vascular expressions were coordinated (p<0.01 and 0.04). AKT was overexpressed in intratumor vessels as compared to extratumor normal vessels (p=0.04).

Tumor nuclear AKT and 4EBP1 were related to lack of hemoragia (0.04 and 0.07 respectively) whereas vascular AKT expression related to presence of a low vascular component (p=0.05). Expression of tumor nuclear 4EBP1 at high level related to a high slit component (p=0.04). Tumor nuclear PTEN correlated to a high vascular component and to presence of intratumor hemosiderin pigment (0.04 for both comparisons) while when expressed at high levels to hemorragia.

Conclusions: The results of our study suggest that activated forms of AKT pathway proteins are frequently expressed in gastrointestinal Kaposi sarcomas, explaining the response to drugs targeting this pathway, such as rapamicin. Interestingly, AKT pathway proteins have variate impacts on tumor angiogenesis and vascular permeability, AKT being associated with anti-angiogenic features whereas PTEN and 4EBP1 with pro-angiogenic tumor features.

639 High Grade Dysplasia (Intraepithelial Neoplasia) of the Gallbladder (GB): Patterns, Cell Lineages and Clinicopathologic Associations in an Analysis of 255 Cases

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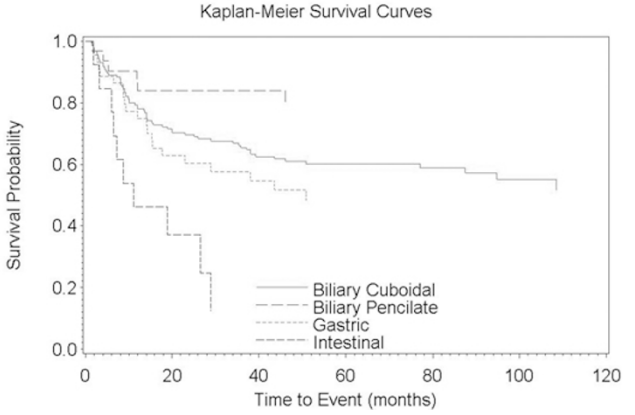
Background: Data on the morphologic spectrum and clinicopathologic associations of non-tumoral high-grade dysplasia (HGD) of the GB is highly limited.

Design: 255 cases with unequivocal HGD were analyzed. Excluded were indefinite cases, low-grade dysplasia and tumoral intraepithelial neoplasms (WHO's "adenomas" and "intracystic papillary neoplasms;" or as recently proposed, intracholecystic papillary tubular neoplasms).

Results: F/M=3. Mean age=60 (56 for non-inv, 66 for inv). Growth patterns encountered were flat (220), micropapillary/tufting (72), tall-papillary (without tumor formation; 54), denuding/clinging (47), glandular/cribriform (37). ***Different cell lineages that often occured in a mixture were recognized:*** **1.Biliary-cuboidal (223)**: Monotonous round cells with centrally or suprabasally located large nuclei (2-3x normal, typically >9.74μ), relatively fine chromatin, prominent nucleoli (mean 2.7μ), abundant cytoplasm (oncocytoid 19, chromophobe-like 38, or clear cell 29**). 2.Biliary-pencillate (44)**: Crowded, thin, elongated nuclei with no nucleolar prominence, minimal cytoplasm. **3.Gastric (58)**: Abundant apical pale cytoplasm, basally located enlarged nuclei, open chromatin, nuclear irregularities or nucleolar prominence. **4.Intestinal (22)**: Reminiscent of adenomatous GI epithelium. **5.Other patterns:** Squamous (2), signet-ring (2), and transitional (1). The overall frequency of encounter of the 4 main types and their clinicopathologic associations are in Table and Figure.

	Biliary Cuboidal (n=223)	Biliary Pencilate (n=44)	Gastric (n=58)	Intestinal (n=22)	p
Mean Age	60	61	63	67	0.185
F:M	3.8	3.8	4.2	2.2	0.652
Presence of invasion	106(50%)	15(35%)	34(62%)	15(79%)	0.005
Size of inv, cm	2.9	2.4	3.3	5.2	<0.001
Stage of inv tm					
T1	7(7%)	3(20%)	2(6%)	1(7%)	
T2	46(49%)	6(40%)	20(65%)	6(40%)	0.380
T3	41(44%)	6(40%)	9(29%)	8(53%)	



Conclusions: High-grade dysplasia of the gallbladder manifests in various thus far uncharacterized patterns with different biologic characteristics. Metaplastic phenotypes appear to reflect more aggressive biology, especially the intestinal group and (to a lesser degree) the gastric ones are associated with higher frequency of larger size, advanced stage invasive carcinoma, and poorer prognosis.

640 Loss of PTEN Immunohistochemical Expression in Patients with Advanced Colorectal Adenocarcinoma: Implications for Targeted Therapy

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Background: The advent of targeted therapy for advanced cancer offers unique challenges to pathologists to devise laboratory assays that are helpful in identifying patients who would benefit most from such therapies. Patients with advanced cancers only have a finite life-span, and targeted therapies are typically expensive. These 2 features highlight the importance of optimal patient selection. PTEN is a negative regulator of the PI3-kinase pathway that is activated in numerous types of cancers, including colorectal cancer. Loss of PTEN expression may potentially be predictive of response to drugs that target the PI3K/AKT/mTOR pathway.

Design: PTEN immunohistochemistry slides from 482 patients enrolled in Phase I clinical trials, which included 83 patients with colorectal adenocarcinoma, were

reviewed. We devised a 4-tiered scoring system to accommodate the heterogeneous nature of PTEN expression. Tumors were scored as positive, negative, reduced, and heterogeneous (positive and negative tumor foci). Stromal cells served as a useful internal positive control.

Results: Of the 482 cases reviewed, 46 patients had complete loss of PTEN IHC expression. Colorectal adenocarcinoma had the highest number with PTEN IHC loss (33%; 15/46). Additional colorectal adenocarcinomas had reduced (n=55) or heterogeneous (n=4) PTEN IHC expression which was less than that of PTEN positive cases (n=9) and may thus be amenable to inhibition of this pathway. For 5 of the PTEN IHC negative cases, sufficient tumor DNA was present for *PTEN* sequencing; no mutations were detected in these 5 cases. Only 3 of the PTEN negative colorectal carcinomas had mutations in *KRAS*, *NRAS*, or *BRAF*. This is important, as activation of the RAS/RAF/MEK pathway is associated with resistance to inhibition of the PI3K/AKT/mTOR pathway.

Conclusions: The 4-tiered scoring system for PTEN IHC identified a relatively high percentage of patients with colorectal adenocarcinoma with PTEN loss. IHC detected PTEN protein loss in cases with no *PTEN* gene mutation detected. Importantly, the advanced colorectal cancer patients did not have tumors with over-representation of *KRAS*, *NRAS*, or *BRAF* mutations, implying that most patients with PTEN IHC loss would be potential candidates for targeted therapy using inhibitors of the PI3K/AKT/mTOR pathway. This highlights the central role of the pathologist in identifying patients who would benefit most from targeted therapy.

641 Use of Immunohistochemical Expression of IMP3 in the Risk Stratification of Patients with Barrett's Esophagus-Related High Grade Dysplasia (BE-HGD)

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Background: The aim of our study was to determine whether the pattern of IMP3 expression in patients with BE-HGD is associated with a higher risk for an adverse clinical outcome.

Design: Immunostain for IMP3 was evaluated in 40 gastroesophagectomies [10 with HGD only, and 30 with HGD plus adjacent superficially invasive adenocarcinoma (HGD/T1)]. In the HGD/T1 group, IMP3 was assessed both in the invasive component and in the adjacent HGD. Absence of expression or focal positivity of IMP3 (5% or less) was considered negative. Our end-point was to establish if there is any difference in behavior and clinical outcome, assessed by presence of lymph node metastasis (LNM) and overall survival, between IMP3-positve and IMP3-negative HGD patients.

Results: None of the 10 cases with only HGD expressed IMP3, had LNM or DOD. 13/30 cases with HGD/T1 were IMP3 negative both in the HGD and invasive component, and none had LNM or DOD. Instead, IMP3 expressed strong cytoplasmic granular staining in the remaining 17 of 30 (56.6%) cases with HGD/T1 both in the invasive component and in the adjacent HGD, and of these, 2/17 (11.7%) had LNM and DOD. (See table) The difference in outcome between IMP3 positive and IMP3 negative HGD was statistically significant. Chi-Square p<.0001.

BE-HGD, expression of IMP3 and outcome

Category	# of cases	IMP3+	IMP3-	# Positive for IMP3 (%)	LNM (%)	DOD (%)
HGD only	10	0	10	0/10 (0)	0/10 (0)	0/10 (0)
HGD with T1	30	17	13	17/30 (56.6)	2/17 (11.7)	2/17 (11.7)
Total	40	17	23	17/40 (42.5)	2/40 (42.5)	2/40 (42.5)

DOD=Died of Disease

Conclusions: IMP3-negative HGD has a low-risk of association with a more advanced lesion and may be treated conservatively. Instead, IMP3-positive HGD has a significantly higher risk to be associated with a more advanced lesion and potentially may portend an adverse outcome warranting a more aggressive treatment.

642 A KRAS Mutation Profile in Colorectal Carcinomas: Mutation Detection Technique May Affect Patient Selection for Anti-EGFR Therapy

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Background: Mutant allele detection sensitivity and the tumor percentage of test material seem to have a crucial impact on KRAS mutation ratios in colorectal carcinomas. All FDA approved KRAS detection kits are very sensitive, and some even detect a single mutant allele in 10^6 wild-type background. On the other hand, commonly used and widely available conventional dideoxysequencing methods have limited sensitivity. Although there is no precise data about the presence of lower KRAS mutation ratios and the efficacy of anti-EGFR therapy, it is widely accepted to select only the wild type KRAS patients for rational therapy. In the present study, by using the same extracted DNA to exclude the possible effect of tumor content ratio, we performed both a sensitive pyrosequencing and a conventional dideoxysequencing technique to detect KRAS mutations to realize whether the detection methods effect the patient selection for anti-EGFR therapy.

Design: In a series of 227 colorectal carcinomas 121 mutant tumor were previously detected by a sensitive pyrosequencing method (Qiagen PyroMark Q24 system and KRAS Pyro Kit, CE, IVD). Mutations were located in codon 12 (n=56, 46,2%), codon 13 (n=26, 21,4%), codon 61 (n=26, 21,4%) and more then one codon in 13 cases (10,7%). Mutant allele ratios were between 3%-52 % and were <20% in 66 cases (54.5%). The same DNA samples of the 121 mutant cases were reanalyzed by conventional dideoxysequencing technique for KRAS exon 2 mutations (ABI 3730 DNA Analyzer).

Results: Reanalyzing the same DNA samples by dideoxysequencing technique revealed 49 cases with KRAS mutations out of previously detected 121 (40,5%). In the remaining 72 cases, mutant allele ratios were between 3%-23% by pyrosequencing. While KRAS mutation ratio by pyrosequencing method in our series of 227 colorectal carcinomas was 53,3%, dideoxysequencing technique detected only 49 of these 121 mutant tumor cases.

Conclusions: KRAS mutation analysis technique directly affects patient selection for anti-EGFR therapy. According to our experience, widely available conventional dideoxysequencing techniques can be applied as a first-line screening method. Detection of any activating mutation may exclude the patient from anti-EGFR therapy. If no mutation is detected, a second-line more sensitive method, such as pyrosequencing, should be applied for more reliable, targeted therapy decision.

643 Genomic Analysis of Esophageal Columnar Cell Metaplasia Reveals Less Frequent Changes in Non-Goblet Cell Metaplasia Than Intestinal Metaplasia

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Background: Gastroesophageal reflux disease (GERD) results in metaplastic changes in the esophagus from normal squamous epithelium to a columnar epithelium with (IM) or without goblet cells (NGM). While the presence of goblet cells is required in the United States for diagnosis of Barrett's esophagus (BE) and follow up surveillance, this is not the case in Japan and United Kingdom. BE/IM reportedly harbors frequent genetic alterations which are often observed in adenocarcinoma. The aim of this study is to investigate the genomic changes in NGM compared to IM and to assess if NGM is at direct risk for progression to cancer.

Design: Genomic DNA from 46 biopsies (36 patients) including 23 pure non-goblet metaplasia (NGM), 16 pure intestinal metaplasia (IM) samples, and 7 composite samples (one tissue piece is NGM and the other is IM from the same patient) were analyzed using Affymetrix SNP 6.0 arrays. Normal samples from the same patient population were used as the baseline reference. Analysis was performed with Nexus 5.0 Copy number software using SNPRank segmentation algorithm with log2 copy number thresholds for gains and losses set at +0.15 and -0.2 respectively while high level gains and homozygous loss were set at +0.5 and -0.8 respectively.

Results: IM samples display cancer associated changes including high frequency loss of *FHIT* (44%), *CDKN2A* (25%), and WWOX (19%) in addition to a low frequency gains at c-*MYC* (6%) and *GATA6* (6%). In contrast, one sample in the NGM samples displayed changes at *KRAS* (4%) and *CDKN2A* (4%). Genomic comparisons of the composite samples with available pure NGM and/or IM samples from same patients suggest that changes at *CDKN2A, FHIT,* and *WWOX* occur in the IM portion. Results from validation studies using FISH will also be reported.

Conclusions: Results from this study so far indicate that the NGM harbors genetic changes but at a much lower frequency than that observed in IM. These findings would therefore argue that NGM is at a much lesser risk for progression to cancer compared to IM, thereby supporting the US definition of BE.

644 Histopathology of "Cord Colitis Syndrome" in Umbilical Cord Blood Transplant Recipients

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Background: We recently described an antibiotic-responsive diarrheal syndrome in umbilical cord blood stem cell transplant recipients not attributable to acute graft-versus-host disease (GVHD), identifiable infection, or medication, which we termed "cord colitis syndrome" (CCS). This study describes the features (fxs) of endoscopic mucosal biopsy material from these patients.

Design: Existing diagnostic material from CCS patients, including H&E/histochemical/immunohistochemical-stained slides, was retrieved. Patient age/gender were recorded and the following assessed: activity (focal, mild, moderate, severe), lamina propria plasmacytic inflammation (for upper tract), basal plasmacytosis (colon), architectural distortion, Paneth cell/pyloric metaplasia, surface epithelial injury (SEI), viral cytopathic effect (VCE), granulomas (crypt/gland rupture, loose, Crohn-like), and GVHD-like fxs (apoptosis/dropout; graded 0-4). An overall pattern of injury (normal [nl], chronic and/or active disease, GVHD-like) was assigned to each biopsy.

Results: Forty-six biopsies (6 stomach, 6 duodenum, 3 terminal ileum [TI], 31 colon) from 11 patients (age 28-59; 5M, 6W) were reviewed. Four gastric biopsies demonstrated Helicobacter-negative chronic gastritis; 4 contained granulomas; GVHD-like fxs ranged from 0-1. One duodenal biopsy contained a granuloma and GVHD-like fxs ranged from 0-1; otherwise, the biopsies were nl. One TI biopsy displayed mild chronic active ileitis; GVHD-like fxs ranged from 0-1. Activity in the colon ranged from none to moderate-severe. Basal plasmacytosis was noted in 1 biopsy, and 1 patient demonstrated marked architectural distortion. Paneth cell metaplasia was common, present in 15 of 22 (68%) left-sided biopsies in 8 patients (73%). SEI was seen in 8 biopsies (26%). VCE was not seen, although rare CMV-positive cells were detected by immunostain in 1 biopsy (the only positive special study in the 46 biopsies). Granulomas were seen in 17 biopsies (55%). The median GVHD-like score was 1. Overall, granulomas were seen in 22/46 (48%) biopsies from 7/11 (64%) patients (crypt/gland rupture, loose, Crohn-like in 12, 9, and 6 biopsies, respectively).

Conclusions: The histopathology of CCS is typified by a chronic active colitis, often with a granulomatous component. Apoptosis is generally inconspicuous. Upper tract findings include non-specific chronic gastritis, and granulomas can be seen in otherwise normal biopsies. Recognition of this syndrome, and especially its distinction from GVHD, allows for initiation of appropriate antibiotic therapy.

645 A Muscular Abnormality: An Overlooked Cause of Intestinal Pseudo-Obstruction

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Background: Patients with chronic intestinal pseudo-obstruction can present with numerous types of malformations classically categorized into myopathies, neuropathies

or mesenchymopathies, although autoimmune causes have been described as well. Previously described structural muscularis anomalies include the presence of extra muscle layers, segmental absence of the muscularis layer and idiopathic fibrosis.
Design: Six cases of intestinal pseudo-obstruction with muscular abnormalities were identified in our files from 1997-2009. The cases were retrieved from storage, stained with desmin and smooth muscle actin and re-examined for characteristic features.
Results: The patients were five women and one man, with ages ranging from 49-78 years. The patients had a 2.5 month to 5 year history of abdominal discomfort, including abdominal distension, constipation, nausea and vomiting. Each patient was treated surgically with partial small bowel resection. Histologically each case had an area that was characterized by crisscrossing of smooth muscle fibers between the muscularis mucosae and muscularis propria, confirmed by strong staining with desmin and smooth muscle actin. This area was recognized grossly as a 0.2-2.0 cm long constriction in 3/6 (50%) of cases and was associated with ulceration in one case and perforation in another. In contrast to typically poor long-term outcomes in the majority of cases of chronic intestinal pseudo-obstruction, three patients remain without any symptoms of gastrointestinal discomfort 1-11 years after resection. One patient had a single episode of small intestinal obstruction two weeks after resection. One patient had a single episode of constipation, nausea and vomiting four years later and one patient suffers from irritable bowel syndrome.
Conclusions: We describe the first six cases of intestinal pseudo-obstruction due to criss-crossing of smooth muscle fibers between the muscularis mucosae and the muscularis propria. Whether this abnormality is congenital or acquired is unknown.

646 In Situ Contribution of Plasmacytoid Dendritic Cells in Gut Acute Graft Versus Host Disease: Relation with the Th17 Immune Response

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Background: Acute GVHD (aGVHD) after allogeneic stem cell transplantation (allo-SCT) is an exaggerated immune response against alloantigens involving dysregulation of inflammatory cytokine cascades. The role of Th17 cells in human aGVHD as well as the role of Plasmacytoid Dendritic Cells (PDC), which play an important role in triggering Th17-related cytokines and autoimmune diseases, is not yet established in the aGVHD setting. This prompted us to analyze Th17 cells and PDC in gastrointestinal biopsies taken from patients with aGVHD.
Design: Gastrointestinal biopsies of 23 patients who underwent allo-SCT for different hematological diseases were analyzed for the density of PDC and Th17 cells by immunohistochemistry. To identify the Th17 cells and PDC, biopsies were tested for the expression of CD161, CCR6, RORγ, and CD123 respectively. A quantitative evaluation of antigens expression was performed by counting the number of positive cells/field at 200 magnifications for each sample. The density of PDC and Th17 cells was correlated to the morphological grade of aGVHD.
Results: Eighteen patients had a histologically proven gastrointestinal aGVHD. The remaining 5 patients did not have clinical and histological signs of aGVHD, and thus were used as controls. Higher number of RORγ+ (p=0.02) and CD161+ (p=0.009) cells were counted in the intestinal mucosa of patients with aGVHD compared with intestinal mucosa of patients without aGVHD. In parallel, we found a significant increase of CD123+ PDCs in the intestinal mucosa of patients with aGVHD compared with intestinal mucosa of patients without aGVHD (p=0.012), this increased number of PDC paralleled the morphological grade of aGVHD.
Conclusions: The current study shed some light on the role of Th17 cells in the context of gastrointestinal aGVHD. We showed that Th17 cells infiltrate intestinal mucosa from patients with aGVHD and was associated with an increased number of PDCs, suggesting a potential new pathophysiological link between PDCs and Th17 immune response in the context of gastrointestinal aGVHD. This is consistent with studies showing that PDCs can drive the differentiation of Th17 cells. These data raise the prospect of future innovative approaches to optimize immunosuppression regimens for the treatment or prophylaxis of aGVHD by targeting PDCs and the Th17 immune response.

647 Inlet Patch in Children: Clinical and Pathological Characteristics of 18 Cases

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Background: Inlet patch (IP) is considered a congenital anomaly of the cervical esophagus consisting of gastric mucosa. We conducted this project to report the characteristics of IP in children. To our knowledge, IP in children has not been explored before.
Design: Children who underwent gastro-esophageal endoscopy at Arkansas Children's Hospital between July 2010 and April 2011 are included in this study. In each patient, the esophagus was carefully examined endoscopically with special attention to look for IP. Immunohistochemistry for Helicobacter pylori (Hp) was performed on both the gastric biopsies and IP biopsies when there was chronic and/or active gastritis in either location.
Results: A total of 393 patients (217 males and 176 females) underwent esophago-gastric endoscopy during this period (median age: 6 months-18 years). IP was identified in 18 patients (10 males and 8 females) with corresponding incidence of 4.58%. Median age was 12.1 years (range 5.3-18.0 years). No IP was identified in any patient younger than 5 years. Types of gastric mucosa included antral (11 patients; 61.1%), fundic (2 patients; 11.1%) and antral and fundic (5 patients; 27.8%). Chronic gastritis was present in 8 patients (44.4 %), chronic patchitis in 5 (27.8%), and concomitant chronic gastritis and patchitis in 3 (16.7%). Chronic active gastritis was present in 1 patient (5.6%) and chronic active patchitis was present in 1 patient (5.6%). In this patient, the stomach showed no abnormality. In five patients (27.8%), both the stomach and IP showed no abnormality. Hp immunostain was positive in both the stomach and IP in only one patient (5.6%). No other patient showed Hp positivity in neither the stomach nor the IP. Indications for endoscopic evaluation included abdominal pain (15 patients; 83.3%) and nausea and vomiting (3 patients; 16.7%). Associated symptoms were heartburn in 12 patients (66.7%), and dysphasia in 5 (27.8%). Six patients (33.3%) had history of cigarette smoking, 2 (11.1%) had history of marijuana smoking, 3 (16.7%) used alcohol for al least 18 months prior to presentation, and 10 (55.5%) had exposure to passive smoking. There was strong correlation between the presence of heartburn and IP (P=0.005).
Conclusions: Our results suggest that IP is an acquired condition and not a congenital anomaly. There was a strong correlation between IP and heartburn. Albeit statistically not significant, we found a correlation between IP and passive smoking. Because adults with IP have increased risk of intestinal metaplasia and neoplasia, these children may benefit from close follow up.

648 Poorly Cohesive Carcinomas of the Ampulla of Vater: Analysis of 9 Cases Identified among 249 Ampullary Carcinomas

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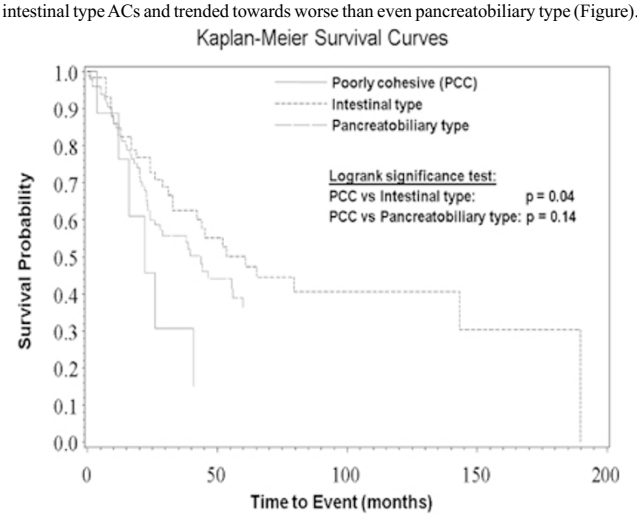
Background: There is virtually no data on the ampullary counterpart of carcinomas with single-cell or cord-like infiltration, previously called "diffuse-infiltrative" or "signet ring cell" (SRC), and now designated as "poorly cohesive carcinoma" (PCC) in the WHO 2010 classification.
Design: 249 resected ampullary carcinomas (ACs) were analyzed. 9 (3.6%) had a PCC pattern constituting >50% of the tumor (with or without SRCs) and were analyzed further. Excluded were 19 ACs with SR-like cells occurring in vacuolated type adenoca (infiltrating glandular/cribriform nests with prominent SR-like cells) and 4 with dyshesive SRCs floating within mucin but not infiltrating stroma.
Results: PCC cases were: M:F=5:4; median age=62 years (55-86); median size=2.0 cm (1.3-3.3). In most cases, the majority of tumor cells were pleomorphic with a minor component of SRCs. However, 2 were predominantly composed of small cells with bland cytology reminiscent of mammary lobular carcinoma. 2 cases had prominent plasmacytoid cells. 4 had a microglandular component including 2 with small goblet cell carcinoid-like foci. Conventional intraepithelial neoplasia (dysplasia) was minimal. No cases were associated with adenoma. IHC profile: 7/7 Galectin-3, 6/7 MUC5AC, 5/7 MUC6, 5/7 CK7, 4/7 MUC1, 3/7 e-cadherin loss, 2/7 MUC2 (focal), 0/7 CK20, 0/7 CDX2. MUC5AC helped highlight the subtle infiltration in the mucosa. Most were advanced stage at diagnosis (1 pT2, 6 pT3, 2 pT4). Lymph node metastasis was seen in 4/9 (44%), similar to other ACs (39%). 5/9 died of disease at a median of 22 months. 4 were alive at 4, 9, 15, and 53 months. Survival was significantly worse than intestinal type ACs and trended towards worse than even pancreatobiliary type (Figure).



Conclusions: PCCs are uncommon in the ampulla (3.6%) and are diagnosed at advanced stage. Some cases have a subtle infiltration pattern, which may present a diagnostic challenge; MUC5AC can be helpful in these cases. Ampullary PCCs do not show intestinal differentiation (MUC2/CDX2/CK20 negative). Overall survival is poor, significantly worse than intestinal type ACs and with a trend toward worse than pancreatobiliary type as well.

649 An Analysis of the Application and Reproducibility of the NIH Consensus Guidelines for the Histologic Diagnosis of Gastrointestinal Acute Graft Versus Host Disease

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Background: Following bone marrow transplant (BMT), acute graft-versus-host disease (aGVHD) of the gastrointestinal (GI) tract is a common complication associated with significant morbidity and mortality. Clinical symptoms often raise concerns for aGVHD; however, histologic confirmation and exclusion of other disease entities is critical as presentations can overlap. The histologic diagnosis of GI aGVHD can be difficult. As a result, in 2006, the NIH published consensus guidelines for the histologic diagnosis of aGVHD.
Design: A retrospective search of our LIS beginning in 2004 was performed. We identified 25 gastric and 25 colon biopsies that, based on the interpretation of the prior

pathologic diagnoses, represented an even distribution of potential NIH diagnostic categories (not aGVHD, possible aGVHD, consistent with aGVHD or unequivocal aGVHD). These biopsies represent 40 unique BMT patients (pts). Following review of the NIH Consensus guidelines, H&E and CMV immunohistochemical slides were blindly and independently reviewed by 3 pathologists and classified into one of the 4 NIH diagnostic categories. A chart review was performed to determine if there was a clinical diagnosis of aGVHD at the time of biopsy (based on the presence of skin or liver involvement or empiric treatment for GI symptoms without other known etiology).
Results: Of the 40 pts, 28 had a clinical diagnosis of aGVHD. These 28 pts had 37 biopsies (21 colon and 16 gastric). Twenty-five of the 37 biopsies were classified as either consistent with or unequivocal aGVHD by at least 2 of the 3 pathologists yielding a positive predictive value (PPV) of 96%, sensitivity (Sen) of 68% and specificity (Spec) of 92%. By site, 15 of 21 colon cases (PPV 100%, Sen 71%, spec 100%) and 10 of 16 gastric cases (PPV 91%, Sen 63%, Spec 89%) were diagnosed within the same categories. Of the clinically non-aGVHD pts, 6 were diagnosed with CMV or other infection, 3 with a potential drug toxicity, 1 with gastroesophageal reflux disease and 2 had resolution of symptoms without intervention. There was a single histologic false positive case within this group (presumed drug toxicity). The interobserver agreement for the classification of all 50 biopsies using the NIH guidelines was considered excellent (kappa 0.70-0.75) amongst the 3 pathologists.
Conclusions: Although the histologic confirmation of clinically diagnosed aGVHD can be elusive, the NIH diagnostic categories may aid in creating uniformity and diagnostic clarity.

650 Significant Operator Dependent Grossing Differences in Lymph Node Sampling from Esophageal Cancer Resections

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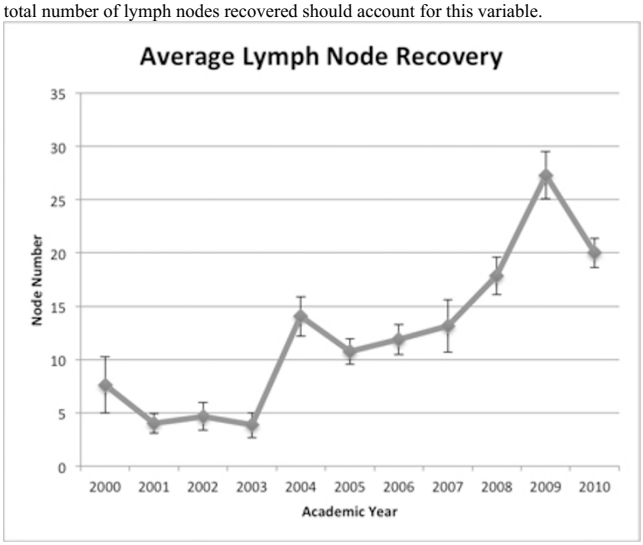
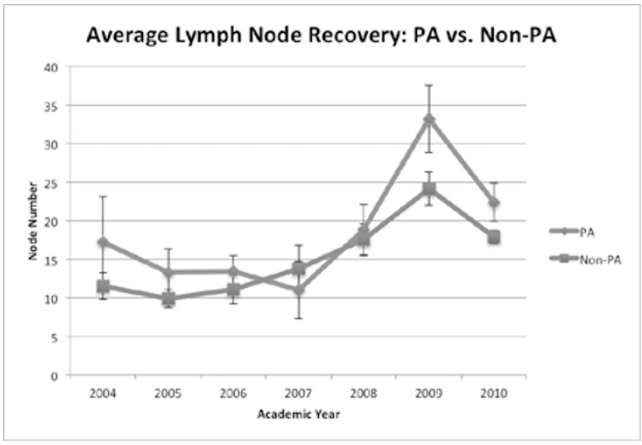
Background: It is currently unclear whether aggressive lymph node dissection improves patient survival after esophageal cancer surgery. Recent recommendations include minimum numbers of lymph nodes, both to ensure adequate staging and perhaps reflect adequate surgical treatment. Our objective was to identify potential sources of operator variance by personnel grossing esophageal cancer resections performed at our institution.
Design: We conducted a single center retrospective review of 178 patients with esophageal cancer treated by esophagectomy with and without neoadjuvant therapy between July 2000 and July 2011. The total number of dissected lymph nodes obtained by pathology assistants (PA), medical student fellows, and pathology residents (PGY1-4) were recorded for each academic year (July 1 through June 30). Preliminary trend analysis suggested potential differences between PA and non-PA personnel. Nonparametric statistics using the Mann-Whitney U-test were performed to evaluate for significance.
Results: Lymph node harvesting was relatively constant from 2000-2003 with a sharp upward trend starting in 2004 (fig 1). From 2004-2011, 148 esophagectomies were grossed revealing a divergence between PA (n=43) and those grossed by residents and pathology medical student fellows (non-PA, n=103) (fig 2). During this interval PAs recovered significantly more lymph nodes per specimen (mean=20.5) compared with non-PAs (mean=15.8) (Mann-Whitney U test p-value=0.0193).
Conclusions: The upward trend after 2004 may be due to a greater awareness of the significance of lymph node number by the pathology staff. Since more lymph nodes were recovered by PAs compared with non-PAs, we conclude that training and experience are important variables in total number of nodes submitted for microscopic examination and diagnosis. Future studies and recommendations about the biologic importance of total number of lymph nodes recovered should account for this variable.





651 A Clinicopathologic Review of Esophageal Candidiasis

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Background: Esophageal candidiasis (EsoCan) is one of the most commonly encountered esophageal infections in gastrointestinal pathology and is typically seen in immunosuppressed or predisposed patients because of administered medication. However, we have seen cases without any apparent predisposition. As far as we are aware, there has been no recent systematic study of EsoCan so we sought to establish characteristics of these patients.
Design: We reviewed the clinical, endoscopic and histopathologic features of EsoCan retrieved from our surgical pathology files for a ten year period. Cases were defined by clinicopathologic criteria in which classical endoscopic findings of white plaques, patches or furrows were correlated with the histologic demonstration of fungal pseudohyphae consistent with *Candida spp.* involving intact mucosa with an inflammatory response, or supported by positive esophageal brushing. Adult and pediatric (< 18 years; Pedi) cases were compared. All sections used in making the diagnosis including hematoxylin and eosin (H&E) sections or available Grocott methenamine silver (GMS) and periodic acid-Schiff with diastase (PAS-D) stains were reviewed.
Results: Of the cases retrieved, 13% (14/107) were Pedi (median: 14, range: 7-17 years; 13 females, 1 male) and 87% were adults (median: 64, range: 27-94 years; 60 females, 33 males). Six of 14 (43%) Pedi cases had no predisposing factors, of which 83% (5/6) were females. In the remaining 57%, underlying predisposition included immunosuppressive therapy, autoimmune disease (AuID), distorted anatomy and antibiotic use.
In 68 % (63/93) of adults there were no predisposing factors, of which 70% (44/63) were females. In the remaining 30%, underlying predisposition included (AuID), malignancy, AIDS, diabetes mellitus, COPD/asthma, ESRD, antibiotic use, SCD, foreign body, alcohol use and achalasia.
PAS-D was positive in 83% (89/107) of cases while GMS was positive in 68% (73/107) of cases. The staining pattern was similar in cases that were positive with both stains. Assessment of PAS-D was easier as removal of glycogen facilitated identification of pseudohyphae whereas on GMS background granular staining of squamous epithelium made interpretation more challenging.
Conclusions: In Pedi and adult cases >40% and >65% had no evident predisposition and females predominated (>70%). Although subtle immune abnormalities cannot be excluded, these findings raise the possibilty of hormonal influence. Overall we found PAS-D to be easier to interpret and cheaper than GMS, and therefore more cost-effective.

652 Utility of Immunohistochemical Investigation of SDHB and Molecular Genetic Analysis of *SDH* Genes in the Differential Diagnosis of Mesenchymal Tumors of GIT

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Background: In 2010 loss of expression of succinate dehydrogenase B (SDHB) was proved to be present in a subgroup of *KIT/PDGFRA* wt gastrointestinal stromal tumors (GISTs), mainly those associated with Carney trias or occurring in childhood or young adulthood. Later it was found out that these tumors may harbour inactivating germline mutations of genes encoding subunits od SDH (*SDHB-D*). To evaluate possible diagnostic utility of SDHB immunohistochemistry and molecular genetic analysis of *SDHB-D* we performed this study.
Design: Eleven cases of *KIT/PDGFRA* wt GISTs (7 gastric, 4 intestinal), 12 gastric schwannomas (GSs), 20 solitary fibrous tumors (SFTs), 4 leiomyomas (LMs), 16 leiomyosarcomas (LMSs), 5 synovial sarcomas (SSs), 3 endometrioid stromal sarcomas (ESSs), and 1 ileal inflammatory myofibroblastic tumor (IMT) were retrieved from our archives. Three recent cases of *KIT* or *PDGFRA* mut GISTs were used as control cases. SDHB immunoexpression of all tumors was compared with molecular genetic profiles of cases containing DNA of sufficient quality. In GIST cases, their pattern was compared with previous markers.
Results: Among the 11 *KIT/PDGFRA* wt GISTs, 5 tumors were SDHB+. Of these, 2 were of epithelioid type, and none showed mutations in *SDHB-D*. Among the 6 SDHB- cases, only 1 was of spindle cell type, and no *SDHB-D* mutations were identified. Interestingly, in 1 of the control GIST cases, which was located in the mediastinum, showed epithelioid morphology and was SDHB-, molecular genetic analysis revealed

an *SDHD* sequence change (G12S) with questionable pathogenicity in addition to W557_E561del of exon 11 of *KIT*. Five SFTs were SDHB-, one of them carried an *SDHB* sequence change (S163P) with questionable pathogenicity. Among the 15 SDHB+ cases of SFTs, one tumor also carried a G12S sequence change of *SDHD*. Two GSs, 1 LM, 2 LMSs, and 1 SS were SDHB-, but no *SDHB-D* mutations were identified in these types of tumor. All 3 ESSs and 1 IMT were SDHB+ and without identifiable *SDHB-D* mutations.

Conclusions: Based on the comparison of *SDHB-D* genotype and SDHB immunoexpression, it may be hypothesized that other additional genetic and epigenetic factors may play a role in regulating expression of SDHB. Furthermore, the study showed that SDHB immunohistochemistry alone is not sufficient to exclude other tumors than GIST (especially SFT) in the differential diagnosis of *KIT/PDGFRA* wt mesenchymal tumors of GIT.

653 Immunohistochemical Screening for Mismatch Repair Protein Deficiency in Colorectal Cancer – MLH1and MSH2 Stains Are Contributory in 10% of Cases with Equivocal or Deficient Protein Staining

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Background: Current data supports prospective screening of all cases of colorectal cancer (CRC) for Lynch syndrome using mismatch repair protein immunohistochemistry (MMR IHC), often in conjunction with microsatellite instability testing. A 2-stain MMR IHC panel (MSH6/PMS2) has been reported as equal in efficacy to the typical 4-stain panel (MSH2/MSH6/MLH1/PMS2) with the added benefit of decreased cost. Prior to establishing a screening protocol in a hospital laboratory serving a population with high incidence of CRC the efficacy of each panel was compared.

Design: Archival tissue from 200 consecutive CRCs was stained with the 4 stain MMR IHC panel. Cases with equivocal or deficient staining were identified and the efficacy of 2 and 4 stain panels was compared. In addition, histologic features of cases with equivocal staining were noted.

Results: All 4 proteins were intact in 160 (80%) cases; MSH2 and MSH6 were both deficient in 5 (2.5%); and MLH1 and PMS2 were concordantly deficient in 24 (12%). One case each of isolated MSH6 and PMS2 deficiency was identified, and in eight cases one stain was equivocal but its dimer pair was either intact or deficient with four of these being equivocal MSH6 or PMS2. Only one case had both proteins of a dimer pair with equivocal staining. Overall, a 2-stain panel would have produced the same result as a 4-stain panel in 196/200 (98%) of all cases, and 36/40 (90%) of cases with abnormal MMR-IHC. The commonest histologic features noted in cases of equivocal staining was solid tumour/poor differentiation with prominent intraepithelial lymphocytes.

Conclusions: A 4-stain panel provides contributory data in a small minority of cases (2%) but is useful in most cases where a 2-stain panel produces an equivocal result. In choosing an MMR IHC panel laboratories must determine whether the additional cost of an up front 4-stain panel is justified or ameliorated by the need to maintain technical expertise and other costs of maintaining infrequently ordered stains.

654 Expression of HER2 and GRB7 in Upper Gastrointestinal Tract Carcinomas

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Background: Overexpression of HER2 is identified in a subset of gastric and gastroesophageal junction carcinomas, leading to use of trastuzumab based therapy. GRB7, a signaling molecule implicated in tumorigenesis and trastuzumab resistance, is located on the 17q12 amplicon near HER2 and is frequently co-amplified with HER2. The aim of this study was to evaluate immunohistochemical expression of HER2 and GRB7 in upper gastrointestinal tract carcinomas and to correlate these with pathologic stage.

Design: 60 cases of gastroesophageal junction and 34 cases of gastric carcinoma between 1991 and 2006 were selected and immunostained for HER2 and GRB7. HER2 IHC scoring was per published FDA guidelines. 3+ score was considered positive, 0 and 1+ negative and 2+ equivocal. HER2 2+ cases and one GRB7 + HER2 1+ case were submitted for FISH for HER2 amplification. For GRB7 percentage of positively staining cells and intensity of staining were noted. Less than 10% cells with weak staining was considered negative. Results of HER2 and GRB7 were correlated with pathologic stage.

Results: Of the 60 GE junction carcinomas 6 cases (10.0%) were HER2 3+ positive and 7 (11.7%) were HER2 2+ equivocal. GRB7 was positive in 9 cases, distributed as follows: 6 HER2 3+, 2 HER2 2+, 1 HER2 1+. Of the 34 gastric carcinomas 2 cases (6.5%) were HER2 3+ positive and 1 case was HER2 2+ equivocal (2.9%). GRB7 was positive in one case which was also HER2 3+. Overall, GRB7 expression was only seen in conjunction with non-zero HER2 expression, typically at high level (3+, 70%). Of the remaining GRB7+ cases, FISH confirmed amplification in 2 (1 HER2 2+ and 1 HER2 1+, HER2/CEP17 3.79 and 9.36) and showed chromosome 17 polysomy in 1 (HER2 2+ CEP17 3.63). These results indicate that GRB7 is highly specific (90%) for HER2 amplification, with polysomy a possible cause of false positivity.

Overall, lymph node positivity was 6/7 (86%) in GRB7 positive cases versus only 37/74 (54%) in GRB7 negative cases, though not statistically significant (p=0.11), possibly due to the relative rarity of GRB7 expression.

Conclusions: GRB7 expression is detected in a subset of HER2-amplified cases of upper gastro-intestinal carcinoma, and is rare in unamplified cases. Used in a panel, GRB7 could be used to select cases for FISH when HER2 IHC is 2+ or 1+. Further, this study could be useful for identifying patients who might benefit from potential targeted anti-GRB7 therapy.

655 Optimal Immunohistochemical Panel for Distinguishing Esophageal Adenocarcinoma from Squamous Cell Carcinoma

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Background: Distinguishing between esophageal adenocarcinoma (ADC) and squamous cell carcinoma (SCC) is often based on standard morphologic criteria. In small biopsies, classification can be difficult, especially for poorly differentiated tumors. We analyzed commonly used immunohistochemical markers and two newly described markers, AGR2 and SOX2, in esophageal ADC and SCC to establish the optimal panel to distinguish these tumors.

Design: Tissue microarrays with 69 esophageal ADC and 41 whole sections of esophageal SCC biopsies (n=27) and excisions (n=14) were stained with p63, CK 5/6, CK7, CDX2, MUC2, MUC5AC, AGR2, and SOX2 and semiquantitatively scored. Sensitivities and specificities were calculated for individual markers and select combinations using the morphologic diagnosis as a gold standard.

Results:

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Marker	Diagnosis	Negative (%)	1-50% Reactivity (%)	>50% Reactivity (%)
p63	ADC	62 (90)	7 (10)	0
p63	SCC	0	3 (7)	38 (93)
CK5/6	ADC	60 (87)	9 (13)	0
CK5/6	SCC	1 (2)	1 (2)	39 (96)
SOX2	ADC	45 (65)	13 (19)	11 (16)
SOX2	SCC	6 (15)	7 (17)	28 (68)
CK7	ADC	6 (9)	9 (13)	54 (78)
CK7	SCC	27 (66)	9 (22)	5 (12)
MUC2	ADC	41 (59)	25 (36)	3 (4)
MUC2	SCC	33 (80)	8 (20)	0
MUC5AC	ADC	25 (36)	29 (42)	15 (22)
MUC5AC	SCC	40 (98)	1 (2)	0
CDX2	ADC	28 (41)	27 (39)	14 (20)
CDX2	SCC	30 (73)	5 (12)	6 (15)
AGR2	ADC	1 (2)	5 (7)	63 (91)
AGR2	SCC	26 (63)	11 (27)	4 (10)

Sensitivity and Specificity of Marker Combinations

Marker Reactivity	Predicted Tumor	Sensitivity (%)	Specificity (%)
p63 (any+)	SCC	100	90
CK5/6 (any+)	SCC	98	87
p63 (any+) and CK5/6 (any+)	SCC	98	99
MUC5AC (-) and p63 (any+) and CK5/6 (any+)	SCC	95	99
AGR2 (any+)	ADC	99	63
MUC5AC (any+)	ADC	64	98
AGR2 (any+) and p63 (-) and CK5/6 (-)	ADC	78	100

Among 55 poorly differentiated carcinomas (40 ADC, 15 SCC), the combination most specific (99%) and sensitive (98%) for SCC was p63 (any+) and CK 5/6 (any+) because p63 and CK5/6 in ADC were typically expressed independently of each other. Among poorly differentiated carcinomas, the combination of MUC5AC (-), p63 (any+), and CK5/6 (any+) was 87% sensitive and 98% specific for SCC and the combination of AGR2 (any+), p63 (-), and CK5/6 (-) was 85% sensitive and 100% specific for ADC.

Conclusions: A panel including p63, CK5/6, MUC5AC, and AGR2 is most helpful in differentiating esophageal ADC and SCC, especially in poorly differentiated lesions. The combination of p63 and CK5/6 offers the best sensitivity and specificity for SCC over ADC. AGR2 staining was highly sensitive but not specific for ADC. MUC5AC staining was highly specific but not sensitive for ADC.

656 Micropapillary Carcinoma Predicts Recurrence in Patients with Stage II Gastric Cancer and Treated with Surgery Only

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Background: Micropapillary carcinoma (MPC) of the stomach is a rare, newly recognized entity, and only few case series have been reported. Moreover, clinical significance of MPC in the stomach has yet to be established. The aim of the study is to identify prognostic significance of MPC for patients diagnosed with stage II gastric cancer and treated with surgery only.

Design: The eligible patients are those who received gastrectomy at Samsung Medical Center between the years 2000 to 2010, and diagnosed with stage II gastric cancer, and have not received any adjuvant chemotherapy, and have all tumor slides available for review. A detailed morphologic review was performed in all 432 cases and cut-off value of micropapillary pattern for the diagnosis of MPC was 5%, which is similar to prior reports of MPC in other organs. The primary end point was disease free survival (DFS), which was defined as time to first documented recurrence of gastric cancer or death from the date of surgery.

Results: MPC was observed in 17 cases (4%). During follow up, recurrence was observed in 177 patients (41%). In univariate analyses, pT stage (1b+2a vs 2b+3) (p=0.0003), host cellular immune reaction (p=0.0135), pN stage (0 vs 1+2) (p=0.0107) and MPC (p=0.0010) were significantly associated with DFS. However, Lauren type, WHO histologic grade, mucin contents, lymphovascular invasion and positive lymph node ratio were not significant. In multivariate Cox regression analyses with confounding factors with p-value <0.05, pT stage, host cellular immune reaction, pN stage, and MPC were significant (p<0.04). In multivariate Cox regression analyses with confounding factors with p-value <0.01, only pT stage (p=0.004) and micropapillary feature (p=0.007) remained significant.

Conclusions: In patients diagnosed with stage II gastric cancer and have not received any adjuvant chemotherapy, MPC is an independent prognostic factor to predict recurrence.

657 Loss of SDHB Expression Is Limited to a Distinctive Subset of Gastric Wild-Type Gastrointestinal Stromal Tumors: A Comprehensive Genotype-Phenotype Correlation Study

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Background: Gastrointestinal stromal tumors (GISTs) typically harbor activating mutations in *KIT* or *PDGFRA*; however, 15% of GISTs in adults and >90% in children lack such mutations ["wild-type" (WT) GISTs]. Pediatric WT GISTs, GISTs in patients with Carney triad and Carney-Stratakis syndrome, and some WT GISTs in adults show similar, distinctive clinical and pathologic features. These "pediatric-type" ("type 2") GISTs occur in the stomach, may be multifocal, show multinodular/plexiform architecture and epithelioid morphology, often spread to lymph nodes, are imatinib resistant, and tend to pursue an indolent clinical course even with metastatic disease. Recent studies have suggested that this distinctive group of tumors can be identified by loss of succinate dehydrogenase subunit B (SDHB) protein expression by immunohistochemistry (IHC). The aim of this study was to validate the predictive value of SDHB IHC for this subset of WT GISTs in a large genotyped cohort.

Design: SDHB protein expression was examined by IHC in whole tissue sections from 261 GISTs with known *KIT* and *PDGFRA* genotypes: 179 tumors with *KIT* mutations (154 in exon 11, 17 in exon 9, 4 in exon 13, and 4 in exon 17), 31 tumors with *PDGFRA* mutations (24 in exon 18, 4 in exon 12, and 3 in exon 14), and 51 WT tumors. IHC was performed following antigen retrieval using a mouse anti-SDHB monoclonal antibody (1:100; 21A11AE7; Abcam). Cytoplasmic staining was scored as "intact" or "deficient". Histologic features were recorded without knowledge of genotype or SDHB status.

Results: SDHB expression was deficient in 20 (39%) WT GISTs. All other tumors showed intact expression of SDHB, including 100% of *KIT* and *PDGFRA*-mutant GISTs and 31 (61%) WT GISTs. All SDHB-deficient GISTs with known primary site (N=19) arose in the stomach and had a multinodular/plexiform growth pattern and epithelioid or mixed morphology. Of the WT GISTs with intact SDHB expression, 10 arose in the small intestine, 7 in the stomach, 5 in the colon, and 1 in the esophagus; primary site was unknown in 8 cases. None showed a multinodular architecture, and only 4 (13%) had epithelioid morphology.

Conclusions: SDHB-deficient GISTs are WT gastric tumors with distinctive features that can be recognized histologically. IHC for SDHB can be used to confirm the diagnosis of this class of tumors, which has prognostic, therapeutic, and syndromic implications. SDHB expression is retained in all GISTs with *KIT* and *PDGFRA* mutations.

658 Raf Kinase Inhibitor Protein (RKIP), Lympho-Vascular Invasion and Peritoneal Invasion Can Be Used To Identify a High-Risk Group of Stage II Colorectal Cancer Patients

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Background: There is controversy regarding the use of adjuvant therapy in patients with Stage II colorectal cancer (CRC). It is agreed that patients with "high-risk" disease may benefit from adjuvant therapy. However, at present there is little consensus on how to define this group. New markers, identifying high-risk Stage II patients, are therefore needed. Here, we examine the utility of Raf Kinase Inhibitor Protein (RKIP) as such a marker.

Design: We stained a tissue microarray (TMA) of 220 patients with Stage II CRC with an anti-RKIP antibody. The TMA was scored using a semi-quantitative scoring system which assesses both staining intensity and percentage of tissue stained and has been validated in previous studies. The RKIP score was then correlated with survival in both univariate and multivariate analysis.

Results: RKIP expression correlated inversely with disease-specific survival in univariate analysis. In multivariate analysis, RKIP was found to be an independent prognostic indicator, along with lympho-vascular invasion (LVI) and peritoneal invasion (T4 disease). A Prognostic Index (PI) was developed, using these independent prognosticators by assigning a score of 1 to each of the poor prognostic indicators and summing these to give a PI for each tumor. The PI was highly predictive of disease specific survival in this cohort ($p<0.01$) (Table 1). Moreover, combining those patients with a PI of 2-3 identified a patient group with a 5-year survival of 44% (95%-CI 30-58%), which is similar to that of patients with lymph node positive (Stage III) CRC, a cohort in whom the benefit of adjuvant therapy has been conclusively shown.

Conclusions: RKIP independently predicts disease-specific survival in Dukes-B CRC. Moreover, when incorporated into a PI along with LVI and peritoneal invasion, a high-risk group of Stage II patients can be identified. These may be the patients who would benefit most from adjuvant therapy.

Table 1: 5-year disease-specific survival, stratified by PI.

PI	No of Patients	No Cancer Deaths (%)	5-year survival	Hazard Ratio
0	56	11 (20%)	89%	1.00
1	101	30 (30%)	70%	1.68
2	46	25 (54%)	48%	3.72
3	6	5 (83%)	17%	8.42

659 Evaluation and Prognostic Significance of Human Tissue Kallikrein-Related Peptidase 10 (KLK10) in Colorectal Cancer

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Background: Conventional prognostic markers for colorectal cancer (CRC) rely on clinicopathological parameters and are not always accurate. Biomarkers contribute additional information that may increase prognostic accuracy. Members of the human kallikrein-related peptidases gene family represent potential CRC biomarkers. The aim of this study was to investigate the expression of human tissue kallikrein-related peptidase 10 (KLK10) by immunohistochemistry in CRC, and to correlate this expression with clinical outcome data.

Design: Clinico-pathologic data was collected for 62 cases of colorectal carcinoma. Each tumor was immunostained for KLK10 and a proportion score (PS) and an intensity score (IS) was calculated as follows: PS 0: none, 1: <1%, 2: 1-10%, 3: 11-30%, 4: 31-75%, 5: >75%. IS 0: none, 1: weak, 2: moderate, 3: strong. A total score (TS) with a range between 0 and 8 was obtained by the addition of PS and IS. We then compared our data with traditional clinicopathologic prognostic parameters and survival data.

Results: A statistically significant positive association was observed between KLK10 and tumor stage and liver metastases ($p=0.015$ and $p=0.035$, respectively). Paradoxically, a negative association was observed between KLK10 and tumor grade ($p=0.009$). Kaplan-Meier survival curves and univariate analysis showed that both KLK10 expression and stage had statistically significant correlations with disease-free survival (DFS) ($p=0.030$ and $p<0.001$, respectively) and overall survival (OS) ($p=0.010$ and $p=0.001$, respectively). Cox multivariate analysis showed that both KLK10 expression and stage were independent predictors of unfavorable DFS ($p=0.057$ and $p=0.001$, respectively) and OS ($p=0.009$ and $p=0.001$, respectively).

Conclusions: Our results show that increased KLK10 expression in CRC was associated with higher tumor stage, increased liver metastases, and decreased DFS and OS. Our data suggest that KLK10 might be used as an adjunct prognostic marker in CRC and implies that kallikreins play a prominent role in the pathogenesis of cancer.

660 Arrest Defective 1 (ARD1) Protein Expression Is Associated with Clinical Outcome in Colorectal Cancer (CRC)

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Background: Arrest defective 1 (ARD1) protein is an acetyltransferase that has been linked with proliferation and apoptosis in mammalian cells. Although ARD1 expression has been demonstrated in CRC, the prognostic significance of ARD1 in clinical CRC specimens has not been previously studied.

Design: Formalin-fixed, paraffin embedded sections from 156 CRC were immunostained by automated methods (Ventana Medical Systems, Inc, Tucson, AZ) with mouse monoclonal ARD1 antibody (Abnova, Jhongli, Taiwan). Nuclear and cytoplasmic immunoreactivity was semiquantitatively assessed in invasive tumor cells for all cases. Scoring was based on staining intensity (weak, moderate, strong) and percentage of positive cells (focal <= 10%, regional 11-50%, diffuse >50%). Results were correlated with clinicopathologic variables.

Results: Any level of nuclear ARD1 expression was noted in 121/156 (78%) tumors. Intense diffuse nuclear ARD1 overexpression was noted in 38/156 (24%) tumors and correlated with advanced AJCC stage [23/74 (31%) stage III/IV and 14/79 (18%) stage I/II; $p=0.05$], mucin production [15/33 (46%) with mucin vs 15/60 (25%) without mucin, $p=0.043$; mucin status available in 93 cases] and shortened survival on Cox univariate analysis ($p=0.03$). Any level of cytoplasmic ARD1 expression was noted in 154/156 (99%) tumors. Intense diffuse cytoplasmic ARD1overexpression was noted in 73/156 (47%) tumors and correlated with advanced AJCC stage [41/74 (55%) stage III/IV and 31/79 (40%) stage I/II; $p=0.045$). There was no significant co-expression of subcellular localization patterns. On multivariate analysis, positive lymph node status ($p<0.0001$) and nuclear ARD1 overexpression ($p=0.05$) were independent predictors of overall survival.

Conclusions: Both nuclear and cytoplasmic ARD1 expression are biomarkers of adverse prognosis in CRC. Nuclear ARD1 overexpression is an independent predictor of overall survival in CRC. Further study of ARD1 expression and its potential as a target of therapy for CRC appears warranted.

661 Squamous Metaplasia in Residual Esophageal Adenocarcinoma after Chemoradiation

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Background: Esophageal adenocarcinoma is a tumor with increasing incidence worldwide, with approximately 460,000 new cases a year, and a less than 15% 5-year survival rate. In the past, the standard of care for esophageal adenocarcinoma was pre-operative staging followed by esophageal resection. More recently, pre-operative chemoradiation has been added in an effort to decrease the rate of recurrence. Chemoradiation alone has been suggested for treatment of esophageal adenocarcinoma in patients who are unable to undergo an esophageal resection. These patients are monitored with regular endoscopic biopsies for recurrence. Interpretation of follow-up biopsies after chemoradiation, with or without resection, must take into account the changes within epithelium in response to chemoradiation. This therapy results in squamous metaplasia of the surface epithelium, the cells lining the esophageal gland ducts, residual Barrett's mucosa, and deep glands. Previous studies have not evaluated the presence of squamous metaplasia within residual invasive adenocarcinoma.

Design: We evaluated 52 esophageal resections with pre-operative adjuvant chemoradiation for residual adenocarcinoma, Barrett's mucosa, and squamous metaplasia. Squamous metaplasia identified within residual carcinomatous glands was scored for focal, moderate, or diffuse involvement of the glands.

Results: Of the 52 specimens examined, 23% showed squamous metaplasia not only within residual Barrett's mucosa but also within nests of invasive adenocarcinoma; 15% focally, 6% moderately, and 2% diffusely.
Conclusions: Deep nests of squamous cells in biopsies from patients treated for esophageal adenocarcinoma are most likely squamous metaplasia in esophageal gland ducts or in residual Barrett's glands. However, residual nests of adenocarcinoma may also undergo squamous metaplasia which may obscure the residual adenocarcinoma or may be misinterpreted as invasive squamous cell carcinoma.

662 Low Grade Neuroendocrine Tumors Arising in Intestinal Adenomas: Evidence for Alterations in the Beta-Catenin/APC Pathway

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Background: Although high grade/small cell neuroendocrine tumors (NETs) arising from intestinal adenomas are well-described, intestinal adenomas rarely give rise to low grade NETs; the largest series to date includes only 4 cases. Such lesions have occasionally been reported in patients with familial adenomatous polyposis (FAP), suggesting a role for the APC/beta-catenin pathway in composite adenoma/low grade NET.
Design: We identified 18 cases of composite adenoma/low grade NET arising in the colorectum (15) and duodenum (3). These cases were evaluated for size, depth of invasion, presence of other invasive elements (adenocarcinoma, squamous carcinoma), lymphovascular invasion, survival, and nuclear beta-catenin expression by immunohistochemistry. Beta-catenin staining was scored as % nuclear positivity multiplied by intensity (weak=1, moderate=2, strong=3), for a total possible score of 300. Control groups included 63 adenoma/high grade NET and 32 sporadic intestinal NET.
Results: Adenoma/low grade NETs ranged from 0.01-0.9 cm. NET alone comprised the invasive component in thirteen (72%) cases, whereas the other 5 also contained adeno- or squamous carcinoma. The NET component was confined to lamina propria in 5 (28%), muscularis mucosae in 5 (28%), and submucosa in 8 (44%). Only one showed lymphovascular invasion by NET, and all were alive without evidence of disease at last follow-up. Four (22%) patients had FAP. In comparison to sporadic low grade NET, adenoma/low grade NET had significantly higher beta-catenin expression (mean score of 231 vs 48, p>0.001). High levels of nuclear beta-catenin expression were also found in adenoma/high grade NET (mean score, 173) but were not significantly different from adenoma/low grade NET.

Adenoma/NET Characteristics

	Adenoma/low grade NET (n=18)	Adenoma/high grade NET (n=63)	p
Age	63y (20-84y)	58y (30-88y)	NS
Gender (male)	11 (61%)	31 (49%)	NS
Size	0.4 cm (0.01-0.9 cm)	4.3 cm (0.2-18 cm)	0.004
Tumor components			
NET only	13 (72%)	45 (71%)	NS
+ACA	3 (17%)	12 (19%)	NS
+SCC	1 (6%)	5 (8%)	NS
+ACA and SCC	1 (6%)	1 (2%)	NS
Lymphatic invasion	1 of 16 (6%)	36 of 38 (95%)	0.001
Lymph node metastasis	0 of 5 (0%)	28 of 34 (82%)	0.001
Distant metastasis	0 (0%)	54 of 59 (92%)	0.001

ACA, adenocarcinoma; SCC, squamous carcinoma

Conclusions: Low grade NETs arising in intestinal adenomas are generally small and indolent lesions. In contrast to sporadic low grade NETs, the occurrence of these lesions in patients with FAP and their high levels of nuclear beta-catenin expression support a role for the beta-catenin/APC pathway in their histogenesis.

663 Image Cytometric HER2 in Gastric Carcinoma – Is a New Algorithm Needed?

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Background: Algorithms for quantitation of HER2 immunohistochemistry were developed for breast carcinoma, where the membranous stain must be entirely around the cell membrane. For gastric carcinoma, although assessment of intensity of immunostain (0-3) is similar, the site and percent of stain differs by lacking the requirement of entire cell membrane positivity (complete, basolateral or lateral membranous reactivity is sufficient for a positive result). We quantitated HER2 in gastric cancer specimens visually and by image cytometry, comparing results, and where available, with fluorescence in situ hybridization(FISH). The goal was to assess whether lack of concordance among results, might suggest a requirement for changing the image cytometric algorithm.
Design: All gastric carcinoma biopsies, resections and cell blocks studied for HER2 expression/amplification in the past 2 years were included. Immunostain intensity, percent and score 0-3+ (0, 1+ negative, 2+ equivocal, 3+ positive), were evaluated visually, and by image cytometry, with the ACIS score (DAKO) 0-3(0, 1 negative, 2 equivocal, 3 positive). FISH(<1.8 negative,>1.8-<2.2 equivocal,>2.2 amplified) was performed on all specimens with scores 2 and 3 by image cytometry.Results were compared.
Results: 41 specimens were studied, including 23(56%) biopsies, 13(32%) resections and 5(12%) cell blocks. 29(71%) were primary gastric, esophageal or gastroesophageal junction adenocarcinoma; 12(29%) were metastatic; 2(5%) were well, 8(20%) moderately, and 31(75%) poorly differentiated.

HER 2	Visual No (%)	Image Cytometry No (%)	FISH No (%)	p value
Negative(0,1)	31 (75)	32 (78)	3 (27)	<0.001
Equivocal(2)	2 (5)	2 (5)	1 (9)	0.85
Positive/Amplified(3)	8 (20)	7 (17)	7 (64)	0.87
Total	41	41	11	

Specimen Type (No)	HER2 Positive/Amplified No(%)	p value
Resection (13)	2 (15.4)	0.61
Biopsy (23)	4 (17.4)	
Cell Block (5)	2 (40)	
Tumor Type		
Primary (29)	5 (17)	0.73
Metastatic (12)	3 (25)	
Differentiation		
Well (2)	0 (0)	0.07
Moderate (8)	1 (12.5)	
Poor (31)	7 (22.6)	
Total (41)	8 (19.5)	

Conclusions: Despite different recommendations for interpretation of HER2 in gastric vs. breast cancer, equivocal and positive/amplified results visually, and by image cytometry, and where FISH was performed, are similar.
This concordance is noted for biopsy, resection and cell block specimens, for primary vs. metastatic, and for moderately vs. poorly differentiated carcinoma, although HER2 positivity/amplification is most frequent with poor differentiation.
There appears to be no need for the HER2 image cytometric algorithm used for breast cancer, to be changed when used for assessment of gastric cancers.

664 Assessment of MLH1 Promoter Methylation and BRAF Gene Mutation in Colorectal Carcinomas with Microsatellite Instability

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Background: Recent studies indicate that analysis of MLH1 promoter methylation and especially evaluation of BRAF gene mutational status can be employed to differentiate hereditary from sporadic MSI-H MLH1-negative colorectal carcinomas. In particular BRAF was demontrated to be frequently mutated in MSI-H sporadic but not in hereditary carcinomas.
Design: The study was conducted on a consecutive series of 2162 colorectal adenocarcinomas surgically resected from January 2004 to June 2010. Mismatch repair (MMR) status has been prospectively evaluated by immunohistochemical analysis of MMR protein expression (MLH1, MSH2, MSH6 and, in selected cases, PMS2) and microsatellite instability (MSI) analysis, using a fluorescent PCR method and the Bethesda panel markers (BAT25, BAT26, D2S123, D5S346, D17S250) plus BAT40. Tumors were classified as MSI-H, MSI-L and MSS according to the guidelines of the International Workshop of Bethesda. In MMR-deficient (MMR-D) tumors, analysis of MLH1 promoter methylation (C- region) was assessed by methylation specific PCR and evaluation of V600E BRAF mutation was investigated by direct DNA sequencing.
Results: 316 (14.6%) carcinomas were classified as MMR-D (loss of MMR protein expression and/or MSI-H). Most MMR-D tumors showed loss of MLH1 expression (256, 81%). MLH1 methylation was detected in 196/219 (89%) MLH1-negative carcinomas and in 2/50 (4%) MMR-D MLH1-positive carcinomas. V600E BRAF mutations were observed in 108/158 (68%) MLH1-negative and in only 1/42 (2%) MLH1-positive MMR-D cancers. BRAF mutations were identified only in tumors showing MLH1 promoter methylation (107/142, 75%). All the MLH1-negative carcinomas without MLH1 methylation examined (15 cases) did not demonstrate BRAF mutation. Both MLH1 promoter methylation and BRAF mutation were more frequently observed in older patients.
Conclusions: Our results confirm that MLH1 promoter methylation and BRAF mutation occur in a large fraction of MMR-deficient MLH1-negative colorectal carcinomas and are closely associated. Furthermore our data indicate that assessment of MLH1 promoter methylation and especially of BRAF mutation might be used in the selection of colorectal cancer patients with presumptive Lynch syndrome.

665 Intra-Tumoral Budding in Pre-Operative Biopsy Specimens Predicts Lymph Node and Distant Metastasis in Patients with Colorectal Cancer

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Background: Tumor budding (TB), the histological hallmark of the epithelial-mesenchymal transition (EMT) in colorectal cancer (CRC) is associated with tumor progression and is an independent prognostic factor. Alltough current definitions for TB are reserved for the invasive tumor front in CRCs, "tumor budds" can be observed in small preoperative biopsy specimens. Whereas the prognostic value of TB assessed in resection specimens has found wide acceptance, its value in preoperative biopsies, which normally do not encompass the invasive margins of the tumor and hence are better categorized as intra-tumoral budding, has not been systematically investigated yet. The aim of this study was to analyze the prognostic and predictive significance of ITB in preoperative biopsies.
Design: Preoperative biopsy samples, consecutive resection specimens from 72 patients were assessed for ITB in the biopsies and TB in the resection specimen. ITB and TB were scored semiquantitatively as high (detectable at low power magnification; 2.5x) and "not high" (occasional budding at intermediate magnification 10x, difficult to find or absent).
Results: High ITB was observed in biopsy samples of 12/72 patients (16.7.%). This was associated with high TB in resection specimen (11/12 patients, P=0.008), lymph node metastasis (10/12 patients, P=0.034) and distant metastasis (9/11 patients, P=0.007).
Conclusions: High ITB in preoperative biopsy specimen of CRC patients predicts high PTB at the invasive margin, lymph node and distant metastasis. Our data show that high ITB can be assessed in preoperative biopsy specimen of CRC and is of important prognostic and predictive value.

666 Massive Foveolar-Gland Polyposis of the Stomach: Clinicopathologic, Histologic, and Molecular Analysis of Three Cases with Gastrectomy

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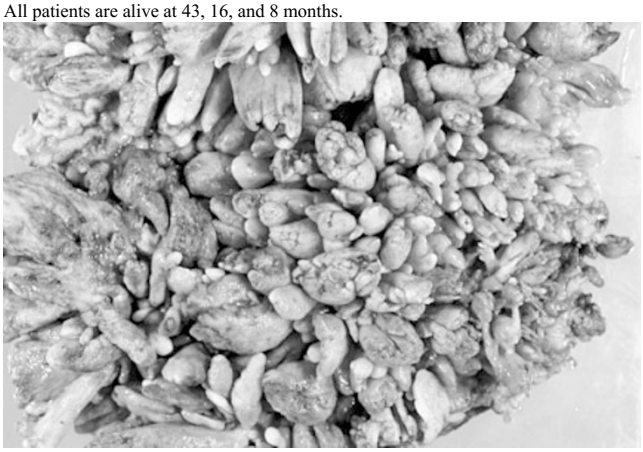
Background: A distinctive syndrome characterized by massive transformation of the gastric mucosa into innumerable hyperplastic/hamartomatous-like polyps has been sporadically described in the gastroenterology and surgical literature, but its pathologic features have not been fully characterized, and nomenclature has varied.

Design: Clinicopathologic and molecular characteristics of three such patients who underwent gastrectomy are described.

Results: Two men and one woman, aged 37, 50, and 53, had massive polyposis replacing the entire gastric mucosa (Image 1). The polyps ranged from a few mm to 9 cm in size. Microscopically, they had hyperplastic features with foveolar-type lining. They appeared to originate and protrude from surface epithelium, with lower aspects of the mucosa typically unremarkable. Cystically dilated glands were often present, suggesting a hamartomatous nature. All patients had fewer than five inflammatory-type colon polyps. One patient, with a history of microsatellite-stable right-sided colorectal carcinoma, had a 3 mm gastric invasive adenocarcinoma of diffuse-infiltrative ("poorly cohesive cell") type. The other patients had focal dysplastic changes but no carcinoma. The patient with cancer and her sister both had a heterozygous c.1139G>A SMAD4 germline mutation. Copy number variation analysis performed on tissue from another patient showed a gain in chromosome 18 at the locus containing SMAD4, again implicating the gene in the pathogenesis of this syndrome. Immunostaining for SMAD4 was noncontributory. All patients are alive at 43, 16, and 8 months.



Conclusions: Massive gastric polyposis is emerging as a distinct syndrome. Based on morphologic characteristics in this study, we propose the name Massive Foveolar-gland Polyposis Syndrome (MFPS). Germline SMAD4 mutation appears to be the key event, and this entity may be related to the subset of Juvenile Polyposis Syndrome (JPS) cases associated with SMAD4 mutation, which occasionally show malignant transformation. Unlike in JPS, polyps in MFPS are largely restricted to the stomach, with only rare non-adenomatous polyps in the lower GI tract. Appropriate genetic evaluation should be performed MFPS patients.

667 Gastric Biopsies Are Appropriate for Assessment of HER2: A Correlation Study with Resection Specimens

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Background: The survival benefit of trastuzumab in advanced HER2 positive gastric cancer has been demonstrated in a recent large prospective randomized multicentre trial (ToGA trial). Accurate assessment of HER2 status is essential to select patients who will benefit from this therapy. Biopsies are often tested, particularly in a neoadjuvant setting or when patients are inoperable, but little is known on the correlation of HER2 status in biopsy versus resection specimens. While biopsies may have more optimal tissue fixation than resections, gastric cancer has been shown to display significant tumor heterogeneity, which could lead to false negatives in small biopsy specimens. In this study, we sought to examine the diagnostic accuracy of HER2 testing in biopsy specimens.

Design: Forty patients with gastric carcinoma where both initial biopsy and subsequent resection specimen were available were evaluated for the expression of HER2 by immunohistochemistry (4B5, Ventana) by the criteria used in the ToGA trail. FISH (Abbott Molecular) was performed on cases equivocal (2+) by immunohistochemistry and on discrepant cases.

Results: The average number of biopsy fragments was 5.9 with an average of 3.2 fragments positive for carcinoma. Most tumors were intestinal (55%), 15% were diffuse and 30% mixed by the Lauren classification. Overall, 4 of 40 (10%) tumors were HER2 positive, defined as either 3+ by IHC or FISH HER2:CEP17 ratio ≥2.0. Seventy percent (28/40) of patient-paired biopsy and resection specimens showed concordant IHC scores. When 0+ and 1+ scores were grouped and defined as negative, 2+ as equivocal and 3+ as positive, concordance increased to 85% (34/40). Incorporating FISH results in equivocal cases further improved concordance to 95% (38/40). Two cases showed major discrepancies (positive vs. negative overall result), one of which would have resulted in a patient being denied trastuzumab if HER2 was evaluated on the biopsy alone.

Conclusions: Despite tumor heterogeneity in gastric cancer, biopsy specimens are appropriate for evaluation of HER2 when IHC is used along with FISH following the criteria used in the ToGA trial. In our study, there was excellent correlation for HER2 status between biopsy and resection specimens.

668 Brightfield Double *In Situ* Hybridization Is Comparable to Fluorescence *In Situ* Hybridization for Determination of HER2 Amplification in Primary Gastric Adenocarcinoma

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Background: HER2 is increasingly being assessed in gastric cancer to identify patients that may benefit from trastuzumab therapy; patients with 3+ HER2 staining on immunohistochemistry (IHC) or amplification by *in situ* hybridization show a significant survival benefit. Fluorescence *in situ* hybridization (FISH) is traditionally used as the gold standard to evaluate HER2 amplification. This method is labour intensive, requires a skilled technologist, and specialized fluorescent microscopy. An alternative is the newly developed automated Brightfield Double *in situ* Hybridization (BDISH) technique and uses differentially labeled Chromosome 17 and HER2 probes that can be read by light microscopy. The aim of this study was to assess the utility of BDISH in gastric cancer and to compare the results to those obtained by IHC and FISH.

Design: Cases were identified from a gastric adenocarcinoma database. Formalin-fixed, paraffin-embedded primary gastric adenocarcinoma specimens were analyzed by IHC (4B5, Ventana), FISH (Abbott Molecular) and BDISH (Ventana). The correlation between methods was calculated.

Results: Results for 48 patients (75% intestinal, 15% diffuse, 10% mixed) were available for analysis. There was a 98% (47/48) concordance rate between BDISH and FISH results (see Table 1). The one discrepant case was scored as 2+ on IHC, was non-amplified by FISH (1.4), but showed amplification by BDISH (2.17). This case displayed tumor heterogeneity, resulting in variable CEP17:HER2 ratios by BDISH depending on the area counted. 71% (5/7) of 3+ cases by IHC were amplified by both BDISH and FISH. All amplified cases were of intestinal type. None of the 0+ or 1+ cases showed amplification by FISH or BDISH (0/31).

IHC-FISH-BDISH correlation

IHC score	FISH amplified	FISH non-amplified	BDISH amplified	BDISH non-amplified	FISH BDISH concordance (%)
0 (n=20)	0	20	0	20	100
1+ (n=11)	0	11	0	11	100
2+ (n=10)	1	9	2	8	90
3+ (n=7)	5	2	5	2	100
Total	6	42	7	41	98

Conclusions: There is excellent correlation between BDISH and FISH for assessment of HER2 amplification in our cohort. BDISH is rapid, easy to interpret, and offers the added benefit of maintaining cell morphology, enabling assessment of variable amplification areas in tumors displaying heterogeneity. BDISH is a viable alternative to FISH and may identify amplified cases that could be missed by FISH due to heterogeneity in gastric adenocarcinomas.

669 Immunohistochemical Positivity for *Reg1* and *IL6*: Potential Markers for Distinguishing between Colitis-Associated Dysplasia and Sporadic Adenoma in IBD

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Background: Distinction between colitis-associated dysplasia (i.e. DALM) and sporadic adenoma in IBD (SA-IBD) is important but problematic. Discriminating markers are needed. We have investigated several proteins related to the link between chronic inflammation and carcinogenesis and/or preferentially altered in colitis-associated carcinoma, including *HSP47, Transgelin, Vanin1, Reg1, IL6, IL6Rα, STAT3* and *SOCS3,* for their potential of being such markers.

Design: In view of no gold standard for defining DALM and SA-IBD, only typical and convincing lesions were selected based on several strict criteria. 20 DALMs (M 16, F 4, 26 to 79 y/o; 7 resections, 13 biopsies; 7 with HGD, and 5 with carcinoma), 21 SAs-IBD (M 11, F 10, 32 to 71 y/o; 1 resection, 20 biopsies; all LGD), and 16 conventional adenomas in non-IBD (CAs) (M 10, F 6, 41 to 77 y/o; all biopsies; all LGD) were included. Tissues encompassing dysplastic epithelium (DE) and adjacent non-dysplastic epithelium (aNDE) were selected for immunostaining the 8 proteins. Immunopositivity was semiquantitated (as -, 1+, 2+, 3+). Comparisons of each protein expression between the 3 groups of dysplasia (on LGD) as well as between DE and aNDE in each group were analyzed.

Results: All proteins expressed similarly in DE in all groups but showed several differences: 1) Increase of *Reg1* immunopositivity in DE was less in DALMs than that in SAs-IBD (25% vs 61.9% for 3+, p = 0.024). 2) When using *Reg1* =3+ as a marker to discriminate SAs-IBD from DALMs, the sensitivity (SEN), specificity (SPE), and positive predictive value (PPV) was 61.9%, 75%, and 72.2%, respectively; when using *IL6* ≥2+ as a marker, the SEN, SPE and PPV was 76.2%, 45%, and 59.3%, respectively; when combining both together, the SEN decreased to 52.4% but SPE increased to 85% and PPV increased to 78.6%. 3) A weak expression of *Vanin1, IL6, STAT3* and *SOCS3* was commonly seen in aNDE in IBD as compared with that in non-IBD; however, no difference existed between DALMs and SAs-IBD. 4) A positive correlation between DE and aNDE for the intensity of expression of *Vanin1*, *IL6* and *STAT3* existed in DALMs (*Spearman's* rank correlation coefficient = 0.69, 0.70, and 0.73, respectively; all $p < 0.005$) but not in SAs-IBD.

Conclusions: Assessment for the immunopositivity of *Reg1* and *IL6*, in combination with the correlation between dysplastic and adjacent non-dysplastic mucosa for expression of *Vanin1, IL6* and *STAT3*, could be useful markers for distinguishing between DALMs and SAs-IBD. Further validation in a larger number of cases is needed.

670 Expression of Cancer Stem Cell Regulators, Twist-1 and Bmi-1, in Colon Cancer: Implications for Their Oncogenic Role

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Background: Bmi-1 has been implicated in the modulation of self renewal in several types of stem cells. It has been demonstrated that Twist-1 directly regulates Bmi-1 to promote epithelial-mesenchymal transition and tumor initiating capability. Bmi-1 is required for full manifestation of increased self-renewal initiated by active β-catenin. The role and the interaction of Twist-1 and Bmi-1 in the progression and metastasis of colon cancer is unclear. We evaluated the expression of Bmi-1, Twist-1, β-catenin and E-cadherin in colon cancer to investigate the role of Twist-1 and Bmi-1 in the neoplasia progression sequence.

Design: Resections of colonic adenocarcinoma (23 stage 0/I, 27 stage III/IV) were stained by immunohistochemistry for Bmi-1, Twist-1, Beta-catenin and E-cadherin on paraffin embedded sections. The percentage (%) of epithelial cells was determined by a 0-3 scale. The total staining score was calculated by the sum of staining intensity multiplied by % to give a score range of 0-300.

Results: Bmi-1 nuclear expression was progressively increased in the colon carcinoma sequence compared to adjacent non-cancerous tissues: normal (25.2 ± 8.5), adenoma (102 ± 9.29), cancer (151 ± 38.1). High Bmi-1 nuclear expression was correlated with advanced clinicopathologic classifications (T, N, and M) and clinical stages.

Table 1. Correlation between Bmi-1 expression and the clinicopathologic features of colon cancer

	Bmi-1 expression	P-value
Histologic grade		
Low grade	143 ± 38	0.0069
High grade	173 ± 27	
T Classfication		
T1	124 ± 23	<0.0001
T4	178 ± 31	
N Classification		
N0	125 ± 23	<0.0001
N1/N2	173 ± 35	
M Classificantion		
M0	144 ± 33	0.0002
M1	195 ± 27	
Clinical Stage		
0/I	125 ± 24	<0.0001
III/IV	173 ± 34	

Although Twist-1 expression showed no significant stage difference, it was relatively increased at the invasive tumor front in all stages as compared with the tumor center (P<0.01). At the invasive tumor front, there were increased nuclear expression of Bmi-1, Twist-1 and β-catenin, and decreased expression of membranous and cytoplasmic E-cadherin. Nuclear expression of β-catenin was less in stage 0/I adenocarcinomas compared to stage III/IV (p <0.01).

Conclusions: Bmi-1 and Twist-1 may be crucial for invasion and metastasis in colon cancer as their expresion was increased at the invasive tumor front and Bmi-1 expression was correlated with advanced clinical stage. Bmi-1 may also play a critical role in the pathogenesis of colon cancer as it showed increasing expression along the colon cancer progression sequence.

671 Autoimmune Gastritis Versus Severe Body Predominant *H.pylori* Gastritis: A Comparative Analysis of 88 Cases

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Background: Autoimmune gastritis (AIG) is a body predominant inflammatory process associated with mucosal atrophy and metaplastic changes. Although the features of AIG are reported to be distinctive from the common antral *H. pylori* gastritis (HPG), we are not aware of comparative analysis between AIG and severe chronic HPG involving the oxyntic mucosa.

Design: 58 consecutive cases of AIG diagnosed at MGH were compared to 30 consecutive cases of severe chronic HPG involving the oxyntic body mucosa obtained from the files of KUMC. In each group, the inflammatory pattern, i.e., distribution, degree and type of inflammation, including: neutrophilic and eosinophilic infiltrate, presence of lymphoid follicles, intraepithelial lymphocyte and granuloma were evaluated. Detection of glandular destruction, apoptosis, mucosal atrophy, intestinal, pyloric and pancreatic acinar cell metaplasia and parietal cell hyperplasia were also investigated.

Results: The average age of patients with AIG was 61.4 years and the ratio of female:male was 37:18. Neoplasia was observed in 11 patients including adenocarcinomas (n=7), adenomas (n=2) and neuroendocrine tumors (n=2). Within the AIG group, lymphoid follicles (p=0.019), intraepithelial lymphocytes (p=0.004), gland destruction (p=0.000), pancreatic acinar metaplasia (p=0.001) and nodular enterochromaffin-like cell hyperplasia (p=0.011) were more common in AIG cases with associated neoplasia. In the HPG group, the average was 54.2 years and there was no sex predilection. Comparing AIG to HPG, AIG patients were older (p=0.035). Lymphoplasmacytic predominant, basal inflammation with lesser neutrophilic (all p=0.000) but with more common eosinophils (p=0.009), lymphoid follicles (p=0.042) and intraepithelial lymphocytes (p=0.047) characterized AIG. In addition, severe glandular destruction, atrophy (p=0.000), apoptosis (p=0.003), intestinal metaplasia and marked pyloric metaplasia (p=0.000) were also more common in AIG. Pancreatic acinar cell metaplasia and enterochromaffin-like cell hyperplasia were only detected in AIG. There was no difference in the rate of parietal cell hyperplasia between both groups.

Conclusions: AIG with neoplastic lesions are associated with distinctive inflammatory pattern that need to be evaluated as surveillance tool. Furthermore, several mucosal stigmata of AIG remain distinct from those of severe long standing chronic HPG involving the oxyntic mucosa even in area of with heavy bacterial load like in Korea.

672 Polyglucosan Bodies Are an Overlooked but Prevalent and Diagnostically Useful Feature of Gastrointestinal Tract Leiomyomas

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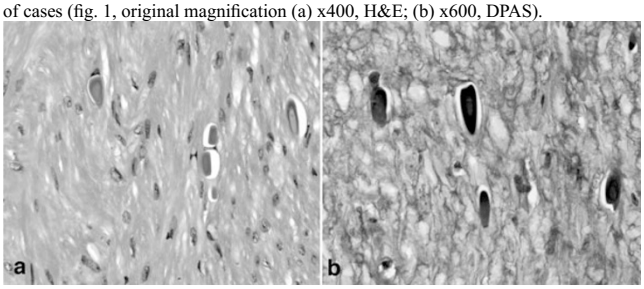
Background: Elliptical, basophilic intracytoplasmic inclusions termed polyglucosan bodies (PBs) are an enigmatic but histologically distinctive feature of some intestinal myopathies. We have observed similar inclusions in gastrointestinal (GI) leiomyomas (LMs). The goal of this study is to investigate their anatomic distribution, prevalence and diagnostic value in distinguishing LMs from GI stromal tumors (GISTs).

Design: All available H&E slides of 35 complete or partial LM resection specimens (8 esophageal, 7 gastric, 2 small intestinal, 18 large intestinal), 19 resected GISTs of low malignant potential (12 gastric, 7 small intestinal), and 10 uterine leiomyomas were examined for PBs. Identification of PBs was readily achieved with H&E and confirmatory positive staining with DPAS and Alcian blue was performed in a subset of cases (fig. 1, original magnification (a) x400, H&E; (b) x600, DPAS).



Diagnoses of LM and GIST had been routinely established immunohistochemically. Dimensions were obtained from clinical records, gross descriptions and microscopic measurements.

Results: The LMs and GISTs had mean diameters of 1.8 cm (range 0.05 - 9.0 cm) and 3.1 cm (range 1.2 - 6.0 cm), respectively. PBs were identified in 13/35 GI LMs (37%), including 7/14 LMs (50%) of diameter 1.0-9.0cm and 6/21 LMs (29%) of diameter 0.05-0.8cm. Their anatomical prevalence was esophagus, 4/8; stomach, 5/7; small intestine, 1/2; large intestine, 3/18. PBs were not identified in any GISTs {P=0.0029} or uterine LMs {P=0.0223}. In sections of LMs with PBs, the density ranged from 1 PB per 4 mm^2 to 1 PB per 200 mm^2. No size, gender or age differences were noted between resected LMs with and without PBs. PBs occurred in muscularis mucosae- and muscularis propria-based GI LMs.

Conclusions: PBs are a prevalent yet hitherto overlooked feature of GI tract LMs that are absent from GISTs. Their identification on routine histology permits distinction of LMs from GISTs of low malignant potential, obviating the need for immunohistochemical stains.

673 Hirschsprung's Disease and Calretinin in Inadequate Biopsies

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Background: Inadequate biopsies represent up to 20% of all rectal suction biopsies (RSBs) taken in the evaluation of Hirschsprung's disease (HD). Inadequate biopsies are generally either too superficial (insufficient submucosa to reliably confirm lack of ganglion cells) or too distal (taken from the 1-2 cm "physiologic" aganglionic zone). Calretinin immunohistochemistry highlights intrinsic nerves in the mucosa only in ganglionated bowel and has been shown to discriminate aganglionic rectum from normal rectum in adequate biopsy as well as resection specimens very reliably. However, the use of calretinin in inadequate biopsies is less well-characterized and to our knowledge, this is the first study that specifically targets inadequate biopsies in HD and the use of calretinin.

Design: A retrospective review of all RSBs taken in the evaluation of HD from 1999-2009 was performed. Those biopsies deemed inadequate for evaluation on the original pathology report were evaluated. Biopsies containing columnar mucosa with lamina propria and muscularis mucosae were included and stained for calretinin. The interpretation of the calretinin immunostains was performed independently and blinded to clinical outcome. Outcomes were determined by clinical and/or pathologic follow-up.

Results: A total of 15 cases were identified. The patients were aged from 3 days to 16 years (6 females and 9 males). Five HD cases were present, all of which were confirmed on resection pathology, and the remaining ten cases showed no clinical evidence of HD (mean follow-up: 4 years 8 months). The calretinin results were perfectly correlated with clinical and/or pathologic outcome (p=0.0003, Fisher's exact test).

Case	Age	Inadequate Reason	Follow-up	Calretinin Result	Calretinin match outcome?
1	4 years	S	N	+	Yes
2	3 years	D	N	+	Yes
3	9 years	D/S	N	++	Yes
4	3 years	S	N	+	Yes
5	5 years	D/S	HD	-	Yes
6	8 months	S	HD	-	Yes
7	3 days	S	HD	-	Yes
8	8 days	D	HD	-	Yes
9	2 months	D	N	+	Yes
10	16 years	D	N	+	Yes
11	1 year	D	N	++	Yes
12	5 months	D	N	+	Yes
13	7 years	D	N	++	Yes
14	16 years	S	HD	-	Yes
15	3 months	D	N	++	Yes

N=No HD, D= Too distal for diagnosis, S= Too superficial for diagnosis

Conclusions: Although confirming the lack of submucosal ganglion cells is the bedrock of HD diagnosis on RSB, many biopsies contain inadequate submucosa to be reliably assessed for ganglion cells. When an inadequate biopsy is received in pathology, calretinin stain can provide very useful information regarding the risk that the patient suffers from HD or not.

674 HER2/Neu Testing in 207 Gastric and Gastroesophageal Junction Adenocarcinomas: Immunohistochemistry and Silver In Situ Hybridization (SISH) Provide Effective Brightfield Methods for Clinical HER2 Testing

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Background: Advanced gastric and gastroesophageal (GEJ) adenocarcinomas have been shown to overexpress the HER2 receptor in a subset of cases, approximately 10-22%. ToGA, a phase III prospective randomized and multicentre trial, has demonstrated increased overall survival with anti-HER2 therapy, in patients with gastric/GEJ tumours overexpressing the HER2 receptor. Since then, status of the HER2 receptor has become an important biomarker for clinical management of these patients.

Design: Paraffin blocks from 187 primary and secondary gastric/GEJ adenocarcinoma biopsy specimens from 1999-2011 were retrieved from the archives of Sunnybrook Health Sciences Centre (SHSC), and were tested for HER2 expression using both immunohistochemistry (IHC) (Ventana PATHWAY anti-HER-2/neu 4B5), and silver in situ hybridization (SISH) (Ventana INFORM HER2 and chromosome 17 DNA probe kits). In addition, 20 consecutive outside referral biopsy specimens from 2011 were also tested, as above. IHC was scored from 0-3+ using published gastric cancer IHC interpretation criteria, with 0-1+ negative, 2+ equivocal, and 3+ positive. SISH positive (SISH+) was defined as HER2/chr.17 ratio ≥ 2.0.

Results: 28/187 (15.0%) SHSC cases were SISH+. Of these, 19/28 (67.9%) were IHC 3+ (positive) and 7/28 (25%) were IHC 2+ (equivocal). 2/28 (7.1%) were IHC 1+ (negative). No SISH+ cases were IHC 0. ($p < 0.0001$). For the outside referral cases, 3/20 (15.0%) had SISH overexpression, with all three cases IHC 3+. Overall 29/31 SISH+ cases demonstrated IHC 2+ or 3+ expression, with only 2/31 SISH+ cases demonstrating IHC 1+ expression. All SISH+ cases were of intestinal or mixed type. No pure diffuse type cancers had IHC 3+ expression, and all were uniformly SISH negative.

Conclusions: Since the approval of anti-HER2 therapy for gastric and GEJ cancer, HER2 testing has become necessary for patient management decisions. Traditional testing protocols have used IHC with secondary fluorescence based in situ hybridization testing when necessary. This study demonstrates excellent concordance of SISH+ and SISH- status with IHC 3+ and IHC 0 respectively, and very good concordance of SISH- status with IHC 1+. Thus, in the clinical setting, IHC can be used primarily, with reflex SISH testing of equivocal IHC 2+ cases and possibly IHC 1+ cases. This combination of IHC plus SISH provides an excellent brightfield-only alternative, allowing adoption in a greater number of pathology laboratories than is possible with fluorescence based techniques.

675 Carcinomas in the Distal Esophagus of Chinese Patients Are Heterogeneous in Histopathology but Adenocarcinoma Remains Rare

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Background: Columnar-lined esophagus (CLE) is a premalignant lesion to esophageal adenocarcinoma (EAC) in Caucasian patients. In our recent study, CLE was found in 68% of Chinese patients with cancer in the gastroesophageal junction (GEJ). This finding is to date of unknown clinical significance in Chinese. Herein, we systematically investigated the histopathology of distal esophageal neoplasms and CLE in resection specimens from a high-volume medical center in China.

Design: A computerized search of esophageal cancer resection was conducted in the pathology database over a 7-year period from 2004 to 2010 at Nanjing Drum Tower Hospital in China. Cancers with epicenters located within 5 cm above the GEJ were retained for analysis. Pathology reports were reviewed along with medical, radiologic, and endoscopic records. Cases with neoadjuvant therapy were excluded. All histology slides of qualified cases were evaluated (13/case). In addition to conventional EAC and squamous cell carcinoma (SCC), major esophageal cancers, ie, basaloid SCC (B-SCC), adenosquamous, mucoepidermoid, and neuroendocrine carcinomas were identified, according to the World Health Organization classification of esophageal cancers. In cases with residual esophageal squamous mucosa, CLE and other pathologic changes were investigated. GEJ was defined grossly as the proximal end of gastric longitudinal mucosal folds and microscopically as the distal end of squamous epithelium, deep esophageal glands and ducts, and multilayered epithelium.

Results: Among 1,105 consecutive resection cases of distal esophageal cancers, 204 (19%) were qualified for the study. The patient median age was 62 years (range: 38-91). The male/female ratio was 8.0. EAC, SCC, B-SCC, adenosquamous, mucoepidermoid, and neuroendocrine carcinomas represented 1%, 76%, 12%, 3%, 2%, and 6% of the cases, respectively. Synchronous cancers were found in 12% of the cases with SCC (50%), neuroendocrine carcinoma (8%), and leiomyosarcoma (4%) in the distal esophagus and proximal gastric adenocarcinoma (38%). CLE was detected in 16%, among which intestinal metaplasia was found in 30% of the cases and low-grade dysplasia in 7%.

Conclusions: In the distal esophagus of Chinese patients in China, SCC and B-SCC remain predominant (88%), but EAC is rare (1%). CLE appears insignificant for most distal esophageal cancers in this patient population. Further investigation of CLE in the general Chinese population is on-going.

676 Primary High-Grade Neuroendocrine Carcinoma of the Esophagus: A Clinicopathologic Study of 42 Cases

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Background: High-grade neuroendocrine carcinoma of the esophagus (HNCE) is rare and poorly understood. We aimed to systematically investigate clinicopathologic features of this fatal cancer in Chinese patients treated at a high-volume medical center in Nanjing, China.

Design: We searched pathologic files for esophageal cancer resections at the Nanjing Drum Tower Hospital in China over the period from January 2004 through August 2010. Pathologic reports were reviewed, along with medical, radiologic, and endoscopic files. HNCE was defined and classified with the same criteria for pulmonary counterparts. All histologic slides of selected cases were evaluated (14 slides per case on average). A corresponding archival tumor tissue block was used for immunostains with conventional methods.

Results: In 1,105 consecutive resection cases of esophageal cancer over the 7-year period, 42 (3.8%) were qualified for the study with strong immunoreactivity to synaptophysin. Patient median age was 62 years (range 47-79). The M/F ratio was 3.7. The epicenter of tumors located in the upper, middle, and distal esophagus was in 2%, 57%, and 40%, respectively. Tumor gross appearances were exophytic (31%), fungating (17%), ulcerated (48%), or flat (5%). The mean tumor size was 3.5 cm (range: 0.2-8). By histology, the small, large, and mixed small-large cell types accounted for 64%, 10%, and 26%, respectively. The majority (86%) of tumors were poorly differentiated. Focal squamous, glandular, and signet-ring differentiation were noted in 19%, 14%, and 2%, respectively. Squamous cell carcinoma in situ was found in 53%. Synchronous tumors (12%) included 3 squamous cell carcinomas, 1 gastrointestinal stromal tumor, and 1 mesothelioma. Lymphovascular (50%)/perineural (33%) invasion and metastasis to regional (48%) and celiac (29%) lymph nodes were common. Neoplastic cells exhibited strong immunoreactivity to chromograinin (67%), synaptophysin (100%), CD56 (93%), TTF1 (71%), CK8/18 (90%), p63 (55%), CD117 (86%), and p16 (84%) protein antigens.

Conclusions: In Chinese patients, most HNCE tumors were the small-cell type, located in the middle esophagus, associated with squamous cell carcinoma in situ, and showed extensive lymphovascular, perineural, and nodal metastasis as well as a high expression rate for p63, TTF1, CD117, and p16 proteins, suggesting an aggresive behavior.

677 Immunohistochemical Stains for CD3 and CD8 Do Not Improve Detection of Gluten Sensitivity in Duodenal Mucosal Biopsies

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Background: Patients with gluten sensitivity may have duodenal mucosal biopsies with preserved villous architecture and increased numbers of intraepithelial lymphocytes (IELs). Some authors advocate use of CD8 and/or CD3 immunostains to improve IEL detection, resulting in a growing trend to perform these stains “up front” on all duodenal biopsies. However, the added value of immunohistochemistry in detecting increased IELs when H&E stains are interpreted to be normal has not been systematically evaluated. The purpose of this study was to determine whether CD3 and CD8 stains improve detection of gluten sensitivity in duodenal samples compared to H&E stains alone.

Design: This retrospective study included 192 duodenal biopsies from 164 patients. All cases were accompanied by a clinical question of gluten sensitivity and originally interpreted to show normal numbers of duodenal IELs. We reviewed the H&E stained sections from each case and counted IELs and enterocytes in each of 5 villous tips. Cases were immunohistochemically stained with CD3 and CD8, and the number of positive cells in a similar distribution was noted. A mean of ≥12 IELs (or immunopositive cells)/20 enterocytes was considered increased, as described by others. A linear mixed-effects statistical model identified differences in the means determined based on review of H&E stained sections *versus* immunostained slides.

Results: The study included 52 men and 112 women (mean age: 49 years). Biopsies were interpreted to show no diagnostic abnormality (n=159) or features of peptic injury (n=33). Review of H&E stained slides revealed a mean of 2.09 +/- 0.07 (range: 0.4–5.8) IELs/20 enterocytes, compared to 3.2 +/- 0.1 (range: 0.2–9.6) CD3+ and 2.07 +/- 0.09 (range: 0–6.6) CD8+ cells ($p<0.001$ and $p=1$, respectively). This difference was not clinically relevant since none of the cases showed increased IELs. Five patients had elevated TTG antibodies, but mean numbers of IELs were within normal limits by H&E, CD3, and CD8 stains (2.7, 4.2, and 2.6, respectively).

Conclusions: Increased IELs are accurately assessed based on careful evaluation of H&E stained sections and immunostains for T cell markers do not unveil features of gluten sensitivity when routine sections are essentially normal. Thus, efforts should be directed at educating pathologists to recognize the spectrum of histologic features of celiac disease, rather than increasing health care costs with unnecessary stains.

678 Analysis of LGR5 Immunohistochemical Expression in Gastrointestinal Neuroendocrine Tumors

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Background: LGR5, a recently de-orphanized G-protein couple receptor (GPCR) and Wnt pathway regulator, was shown to be expressed in murine intestinal stem cells. In human small intestine, LGR5 stains rare crypt base cells with neuroendocrine features. The expression of LGR5 in tumors with neuroendocrine differentiation has not been reported yet. We therefore aimed to study the expression of LGR5 in gastrointestinal neuroendocrine tumors.

Design: A tissue microarray of 44 gastrointestinal neuroendocrine tumors and 57 hepatic neuroendocrine tumor metastases were stained for LGR5 by immunohistochemistry

(ab12827, Abcam; K5361, Dako). Thirty-four paired cases of primary tumors with their metastases were included. Normal pancreas and small intestine were used as control for staining. The LGR5 expression pattern was scored based on staining intensity and percentage of positive cells. The LGR5 staining was compared with the Ki67 index and the reported chromogranin and synaptophysin markers.

Results: In normal pancreas, LGR5 is strongly expressed in neuroendocrine (islet) cells. 88% of the primary (3/4 gastric, 23/24 intestinal, 13/14 pancreatic, 0/2 appendiceal) and 87% of the metastases stain positive for cytoplasmic LGR5. 67% of the tumors show similar LGR5 expression levels in their metastases. LGR5 is positive in the majority of the cases expressing chromogranin and synaptophysin (34/38). More interestingly, LGR5 was positive in the 7 cases negative for chromogranin and 5 negative for synaptophysin. In our study, twenty-three of the thirty seven cases with a Ki-67 index greater than 2% show weak or absent LGR5 expression. Only fourteen of the sixty two cases with a Ki67 index less than 2% have weak or absent LGR5. Thus, the partial or complete loss of expression correlates with an increased Ki67 index (>2%) (Fisher's exact test, $p<0.05$).

Conclusions: LGR5 is a novel immunohistochemical marker for gastrointestinal neuroendocrine tumors. LGR5 appears to also retain positivity in tumors that lost classical neuroendocrine marker expression. A possible explanation could be that LGR5 is a marker of early neuroendocrine differentiation that maintains its expression after other markers are lost. Interestingly, in our study, the loss of LGR5 expression correlates with an increased Ki67 index, therefore we suggest that it may be an indicator of poorer outcome.

Additional studies are needed to further confirm marker specificity. Given the widespread use of GPCR-targeting drugs in medicine, LGR5 may represent a novel target for neuroendocrine tumor chemotherapy.

679 Expression of Cancer Testis Antigens (CTAs) and Melanocyte Differentiation Antigens (MDAs) in Malignant Melanoma of the Ano-Rectal Mucosa (MMARM)

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Background: MMARMs are rare tumors carrying a poor prognosis. Recent data indicate that melanomas, not exposed to UV-light differ from their cutaneous counterparts on an antigenic and molecular level. MDAs such as Melan-A, gp100 and tyrosinase are expressed in cells and tumors of melanocytic lineage while CTAs such as MAGE and NY-ESO-1 are tumor-associated antigens which are expressed in various tumor types and in normal adult tissues solely in male germ cells. MDAs are standard diagnostic markers for melanocytic lesions. Due to their expression pattern, CTAs as well as MDAs are regarded potential vaccine targets for the immunotherapy of melanoma. However, little is known about the presence of MDAs and CTAs in MMARM.

Design: Paraffin blocks from 13 cases of MMARM were available. Standard IHC employing antigen retrieval techniques was done using mostly in-house generated(*) as well as commercial mAbs/to the following antigens: MA454*/MAGE-A1, 57B/MAGE-A4, CT7-33*/MAGE-C1, CT10#5*/MAGE-C2, #26/GAGE, E978*/NY-ESO-1; A103*/Melan-A, HMB45/gp100, and T311*/tyrosinase.

Results: MDAs Melan-A, tyrosinase and gp100 were all positive in all tested MMARM. Expression was mostly homogeneous; only one case showed solely focal expression of Melan-A and another case displayed focal presence of gp100.

All 13 MMARM cases showed expression of at least 1 CTA! MAGE-A4, MAGE-C1 and GAGE showed the highest incidence and were both positive in 10/13 (77%) cases, while MAGE-A1 showed the lowest expression (6/13 cases, 46%), NY-ESO-1 and MAGE-C1 were present in 9/13 (69%) and 7/13 (54%) cases respectively. Interestingly, 7/13 (54%) cases showed homogeneous CTA expression in more than 50% of the tumor while the presence in the remaining cases varied from <%5 to 50% of the tumor area.

Conclusions: Little is known about the presence of MDAs and CTAs in MMARM. Here we show that Melan-A, tyrosinase and gp100 show a high incidence and homogeneous expression in MMARM. CTAs show a lower incidence and a less homogeneous expression than MDAs. However, a majority of MMARMs show homogeneous presence of at least 1 CTA.

Our data indicate that unlike on a molecular level, protein expression of CTAs as well as MDAs in MMARM resemble the expression of their cutaneous counterparts. Moreover, due to their homogeneous presence in a majority of cases, MMARM may be susceptible to vaccine-based immunotherapy employing CTAs.

680 Colonic Dysplasia and Malignancy in Patients with SMAD4 Mutation-Associated Juvenile Polyposis-Hereditary Hemorrhagic Telangiectasia

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Background: Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant disease, usually associated with mutations in Endoglin and activin receptor like kinase 1 genes, characterized by telangiectasias and arteriovenous malformations. Juvenile polyposis (JP) is a syndrome characterized by hamartomatous polyps throughout the gastrointestinal tract, with increased risk of malignancy. SMAD4 and BMPR1A mutations can be identified in JP. A recently described SMAD4 mutation-associated syndrome combines the features of HHT and JP. In this study, we review the rate and types of dysplastic and malignant lesions in this population.

Design: 15 JP-HHT patients with confirmed SMAD4 mutation were identified from HHT clinic files. All available biopsies and resections were reviewed.

Results: The majority of the patients had only few juvenile polyps detected (3-5 polyps), but one patient had multiple (>100 polyps). Six of our fifteen patients (three male, three female) developed dysplastic and malignant colonic lesions; 26 dysplastic lesions and 2 adenocarcinomas were identified at a mean patient age of 27.5 years. Four patients had lesions developing within juvenile polyps, including low grade and high grade dysplasia and signet ring carcinoma, the remainder of the dysplastic lesions were tubular or villous adenomas. Two patients developed invasive adenocarcinoma one year after dysplastic lesions were identified on colonoscopy.

Conclusions: Though patient numbers are small, the rate of dysplasia and malignancy is elevated and the lesions are developing at an early age. Also, in two patients, the time period between the detection of colonic dysplastic lesions and adenocarcinoma was short (1 year). Patients with SMAD4 mutations and the combined JP-HHT syndrome develop colonic dysplasia and malignancies at an early age: this emphasizes the need for early, close surveillance of this population.

681 Time-Dependent Analysis of the Lymph Node Ratio and Its Prognostic Impact in Advanced Colorectal Cancer Stratified by Mismatch Repair Status

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Background: The aims of this study were (1) to evaluate the prognostic effect of the lymph node ratio (LNR: number of positive LN/number of LN harvested) in advanced colorectal cancer patients stratified by mismatch repair (MMR) status, (2) to compare the prognostic ability of this feature to the number of positive lymph nodes (LN) and (3) to identify a non-arbitrary cut-off score best discriminating high- and low-risk prognostic subgroups.

Design: The LNR was analyzed in 494 MMR-proficient and 86 MMR-deficient stage III-IV colorectal cancer patients. Cox regression and time-dependent receiver operating characteristic curve analysis (tROC) were carried out to analyze the prognostic abilities of each factor over time and for cut-point determination. Results were validated on a second cohort of 87 stage III patients.

Results: Relative risk of death with an elevated LNR was 6.91 (95%CI: 4.72-10.1) in MMR-proficient and 3.56 (95%CI: 1.1-11.53) in MMR-deficient cases. In multivariate analysis the LNR had a more unfavourable effect than the number of positive LNs (HR: 5.18 (95%CI: 3.5-7.6)) ($p<0.001$). The prognostic performance of the LNR was 10-15% greater than the number of positive LN regardless of MMR status. A threshold value of 0.231 was best for classifying patients into prognostic subgroups. This cut-off value was confirmed ($p<0.001$) using the validation cohort.

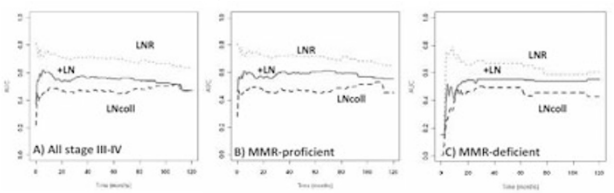

Figure 1

Conclusions: The LNR outperforms the number of positive LNs as a critical prognostic factor by 10-15% and is independent of MMR status. Patients with LNR >0.231 should be considered high-risk and may benefit from additional therapy. This cut-off score warrants validation in prospective studies.

682 "Mass-Forming" Ischemic Colitis Is a Distinctive Variant with Predilection for the Proximal Colon: A Clinicopathologic Study of 16 Cases

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Background: We have observed, anecdotally, mucosal biopsies with ischemic colitis (IC) in which the endoscopic impression is that of a mass lesion suspicious for carcinoma. The purpose of this study was to systematically evaluate the clinical and pathologic features, and outcome in patients with this unusual presentation of IC.

Design: A retrospective search was performed through the pathology archives of two participating institutions for a diagnosis of IC. Twenty patients were initially identified in whom the mucosal biopsies were reported to be from a colonic mass but the pathologic findings were those of an IC. Four of these cases were excluded because review of colonoscopic findings showed prolapse (n=1), polyp (n=2) and diverticular disease (n=1) without any concern for malignancy. The remaining 16 patients formed the final study group. Pathologic findings (biopsy=16 cases; resection =4 cases) were reviewed in all cases. Demographic, clinical, radiologic and follow-up data was obtained by medical chart review.

Results: The study group consisted of 5 males and 11 females with a mean age of 74.5yr (range 47-98 yr). Patients presented most often with abdominal pain (56.3%), either alone or associated with hematochezia (25%) or diarrhea (12.5%). CT scan was done in 8 patients and 6/8 showed segmental thickening suspicious for a neoplasm. Colonoscopic findings included an exophytic mass (n=13), circumferential firm constriction (n=2), or diffuse wall thickening with ulceration (n=1). The "mass lesions" were 1-8cm in size (mean: 4.7cm) and were located in the cecum (n=6), ascending colon (n=3), hepatic flexure (n=1), transverse colon (n=2), descending colon (n=2) and sigmoid colon (n=2). Mucosal biopsies in all 16 patients showed typical features of IC. 4/16 patients underwent segmental colectomy. The mass-like appearance in all 4 resections was due to marked submucosal edema. Follow-up was available in 14/16 patients and ranged from 1-150 mth (mean: 35.5 mth). No malignancy was identified on resection or follow-up in any case. 3 patients had follow-up colonoscopy 1-3 months after initial diagnosis and showed complete resolution of the mass lesion.

Conclusions: IC may rarely mimic a mass lesion worrisome for malignancy on colonoscopic examination. This "mass-forming" variant of IC shows a female predominance and a predilection for the proximal colon. Awareness of this phenomenon may prevent unnecessary resections in these patients.

683 Interobserver Agreement in the Phenotypic Classification of Barrett Dysplasia

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Background: The two most common types of Barrett dysplasia are intestinal and (gastric) foveolar. Intestinal dysplasia resembles colon adenomas. Foveolar dysplasia is cytologically characterized by round/oval nuclei, pale eosinophilic cytoplasm and apical mucin cap. Mixed dysplasia combines features of both. Another recognized pattern, non-adenomatous dysplasia, is a rare high grade variant characterized by crowded glands, cuboidal cells, high N:C ratio, non-stratified round nuclei, open chromatin and prominent nucleoli. Some early data suggests that these different types of dysplasia have different natural histories and biologic characteristics. Our aim was to evaluate the interobserver agreement in phenotypic classification of dysplasia in BE based on H&E stained slides.

Design: 40 consecutive endoscopic mucosal resections (EMR) were reviewed independently by 12 pathologists. Each pathologist provided a diagnosis of low grade dysplasia, high grade dysplasia, intramucosal carcinoma or submucosal invasion and classified the dysplasia type as intestinal, non-adenomatous, foveolar or mixed. The intraclass correlation (ICC) and Kendall's co-efficient of concordance (KC) were calculated for overall diagnosis and phenotypic classification. Additionally, Kappa (K) values were evaluated for each subtype of dysplasia to determine if observers were better at agreeing on any a particular type of dysplasia.

Results: The ICC and KC for overall grade of neoplasia was 0.604 (95% CI 0.500-0.719) and 0.688, respectively. However, for dysplasia type, the ICC and KC fell to 0.124 (95% CI 0.066-0.220) and 0.230. K values for intestinal, foveolar, non-adenomatous and mixed were 0.249, 0.176, 0.06 and 0.01, respectively. In 13 cases (32.5%), one or more pathologists (up to 3) returned a response of "Unsure" further highlighting the difficulty in recognizing dysplasia phenotypes on H&E.

Conclusions: Moderate agreement was achieved for dysplasia. There was poor agreement for dysplasia type classification with best agreement achieved for intestinal type dysplasia. This suggests that more objective measures, such as immunohistochemistry, should be evaluated to better the agreement of dysplasia type.

684 Method of Measurement of Invasion Depth and Scoring System To Predict LN Metastasis in Submucosal EGC

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Background: It is important to determine further surgical intervention after EMR/ESD, due to wider use of endoscopic resection as first treatment of EGC and controversies among investigators about the validity of known criteria. We have evaluated the risk factors of LN meta and validated methods of sm invasion depth in smEGCs and suggest new prediction scoring systems to indicate further surgery after EMR/ESD.

Design: We have reviewed 169 smEGCs and analyzed the risk factors affecting LN meta and made prediction score. Also we investigated patterns of mm visualized desmin immunostain and different methods of sm invasion measurement and suggestive most reliable method of sml invasion depth of measurement to predict LN meta of smEGCs.

Results: There are 15.9% (27/169) of LN meta in smEGC. Increased sm extent, depth of invasion, lymphovascular invasion and infiltrative growth pattern were associated with LN meta.

Predictive factors of lymph node metastasis in 169 sm EGCs

	B	OR (95% CI)	P
Lymphovascular tumor emboli (present vs absent)	2.128	8.396 (3.252-21.679)	<0.0001
Submucosal invasion width (≥0.75cm vs <0.75cm)	1.083	2.954 (1.041-8.381)	0.042

Note: B, coefficient; OR, odds ratio; CI, confidence interval. The independent factors for lymph node metastasis were analyzed by binary logistic regression analysis (backward, stepwise).

We have made predict score H=(2.128 x lymphovascular tumor emboli)+(1.083 x sm invasion width ≥0.75cm)+0.507xsm invasion depth≥1000um+(0.515x infiltrative growth pattern). H score yielded an area under the ROC curve of 0.809 in predicting LN meta of smEGC. Depth of invasion was higher in LN meta group than LN negative group by alternative method of measurement (distance from the lowest point of the imaginary line of mm), but statistically not significant by classic method (distance from the lowest point of the mm) (p=0.128), especially in discontinuous type of mm. The area under the ROC curve of alternative method was 0.652 compared to classic method.

Relationship between LN metastasis with depth of invasion in 169 smEGC

	LN metastasis(absent)	LN metastasis(present)	p value
Depth of invasion (imaginary)	1608.55±83.27	2142.59±205.10	0.012
Depth of invasion (imaginary)	1335.10±92.08	1684.25±198.60	0.128

Conclusions: we recommend that the use of prediction scoring system and alternative method of measurement of sml invasion depth can help decision making further surgical intervention after ESD in smEGCs.

685 Eosinophilic Gastritis in Children: A Clinicopathological Study

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Background: Eosinophilic gastritis(EG) in children is generally considered part of a more extensive allergic gastroenteritis. Evaluation of concurrent hypereosinophilia in different locations in the gastro-intestinal(GI) tract and response to therapy has not been systematically studied.

Design: Pathology files were searched for "eosinophilic gastritis" involving the gastric antrum and/or fundus for patients ≤18 years of age, from January 2005 to present. Medical charts were reviewed for medical history including atopy, symptoms at presentation, endoscopic findings and response to therapies. EG was defined histologically by marked, diffuse, eosinophilic infiltrates with ≥70 eosinophils per high power field (HPF). Pathology reports and slides were reviewed for concurrent eosinophilic esophagitis (EoE, >15 eosinophils HPF) and hypereosinophilia in the duodenum and lower GI tract. Post-treatment biopsies were reviewed.

Results: 31 patients (13 M, 18 F), ages 3 months to 16 years, were identified. Twelve (38%) were ≤ 3 years old. Of the 31 patients, 16 had food allergies, 18 had an atopic disorder, and 6 had a protein losing enteropathy (PLE). Main symptoms at presentation were abdominal pain, followed by vomiting and anemia. The most frequent endoscopic findings were focal ulcers, erythema, and nodular lesions. Histologically, 29 patients had marked antral eosinophilia but in 2 hypereosinophilia was limited to the fundus. Eleven had concomitant EoE. Four patients had duodenal eosinophilia with villous blunting of which 1 was diagnosed with celiac disease. Of 11 patients who also had lower GI tract biopsies, 4 had an increase in mucosal eosinophils. 20/31 patients had post treatment biopsies, of which 17/20 had complete clearance of gastric eosinophilia with dietary restriction therapy. However, 8 of the patients with resolved EG had persistent or denovo eosinophilia limited to the esophagus.

Conclusions: EG is a distinct clinical-pathological entity characterized by marked eosinophilia selectively in the gastric antrum or fundus. There is a strong association with food allergies and atopic disorders. Symptoms and endoscopic findings vary widely, highlighting the importance of obtaining biopsies for diagnosis. EG can be associated with EoE, and rarely with hypereosinophilia of the lower GI tract. EG largely responds to dietary restriction with resolution of symptoms and clearance of eosinophils in the stomach. Associated EoE may persist or present de-novo after treatment.

686 Pathological Correlates of Microsatellite Instability in Ulcerative Colitis-Associated Colorectal Carcinoma

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Background: Recognition of characteristics of colorectal cancer (CRC) that correlate with microsatellite instability (MSI) is of prognostic and therapeutic value and is a component of the revised Bethesda criteria for testing for Lynch syndrome. We investigated whether those histological features that correlate with MSI in the general population are useful in the setting of ulcerative colitis (UC)-associated CRC.

Design: A tissue microarray assembled from 47 CRCs resected from patients with UC between 2001-11 was used to evaluate the immunohistochemical expression of mismatch repair (MMR) proteins MLH1, PMS2, MSH6, and MSH2 as a surrogate for MSI. The corresponding original histologic slides were assessed by 2 pathologists for the following pathological variables: tumor infiltrating lymphocytosis (TIL, >2/hpf), Crohn's-like peritumoral infiltrate (CLPI), mucinous histology, dirty necrosis, grade, and anatomical location (right vs. left). Data were analyzed by Fischer's exact test.

Results: Loss of expression of MMR proteins occurred in 9/47 tumors (19%), including joint loss of MLH1 and PMS2 in 8/9 and loss of PMS2 only in 1/9. Of the pathological variables examined, TIL (P=0.0005), CLPI (P=0.02), grade G1 and G3 (P=0.04), and lack of dirty necrosis (P=0.03) were significantly more prevalent in MSI-CRCs compared to microsatellite stable CRCs. Mucinous histology and anatomical site did not correlate with MSI (P=0.47 and P=0.27, respectively).

Conclusions: The prevalence of MSI and corresponding histological features in UC-associated CRCs are similar to those of CRCs in the general population except for lack of correlation with anatomical site and mucinous histology. The latter parameters likely reflect overriding factors related to the pathogenesis of UC-associated CRCs. Loss of PMS2 in one of our UC-associated tumors may indicate concomitant Lynch syndrome, highlighting the importance of immunohistochemical screening for MMR deficiency even in the UC cancer population.

687 The Heterogeneity of HER2 Expression in Esophageal and Gastric Adenocarcinomas

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Background: The success of HER2-targeted therapy in the treatment of HER2 positive breast cancer has sparked interest in expanding the indications for this treatment in other solid tumors. Recent studies have shown that a subset of upper GI tract adenocarcinomas over-express the HER2 protein, and these patients will have increased overall survival when the HER2 targeted therapy, Trastuzumab, is added to chemotherapy. Correct identification of HER2 positive cancers in the upper GI tract has now become essential in order to stratify patients for therapy. The objective of this study is to further characterize the demographics of cases with HER2 expression, determine the extent of heterogeneity and its potential implications for HER2 testing strategies in these tumors.

Design: A retrospective review of gastric and esophageal adenocarcinoma biopsy and resection cases was prepared by searching the pathology archives for a 5 year period. Appropriate blocks were chosen for HER2 analysis. When available, 2 separate blocks containing tumor and lymph node metastases were chosen. Analysis of the IHC data (HercepTest, DAKO) and tumor demographics was performed. A subset analysis was performed on patients with IHC and corresponding FISH data.

Results: 176 patients were included in the study. 92 (52%) cases were adenocarcinomas arising in the esophagus, 80 (46%) arose in the stomach, and 4 (2%) cases were metastatic from either site. Overall, 21 (12%) of cases were positive for HER2 expression by IHC. HER2 positivity was more often seen in cases arising in the esophagus and in those with intestinal differentiation (p=0.02 and 0.21, respectively). Of the 103 cases with 2 or more blocks, discordant IHC results between blocks of 1 score or more were seen in 18 (17%) cases. Of those discordant cases, 14 (78%) patients would have had a change in HER2 status or needed additional HER2 FISH testing if only one block had been tested. In the subset population, 7 of 30 (23%) patients showed discordant results between the IHC and FISH testing. In 3 of the discordant cases, IHC staining on an additional block revealed concordant results with the FISH result.
Conclusions: HER2 expression is present in a subset of esophageal and gastric carcinomas, and significant heterogeneity was a frequent finding in these cases. Of the cases displaying heterogeneity, staining of multiple blocks for HER2 expression revealed a change in HER2 status in more than 75% of patients. These results indicate that IHC staining on a single block or on initial biopsy may not be sufficient for treatment stratification in upper GI adenocarcinoma patients.

688 Immunohistochemical Staining of Rectal Neuroendocrine Tumors Overlaps with Pancreatic Neuroendocrine Tumors

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Background: Neuroendocrine tumors (NETs) can present as liver metastases before discovery of the primary tumor. In our recent study with Islet 1 and PAX8, two markers of NETs of pancreatic origin, we observed aberrant immunoreactivity in primary rectal NETs with both Islet 1 and PAX8. Previous studies in the literature have reported that chromogranin A is infrequently expressed in rectal NETs. The purpose of this study was to further characterize the immunohistochemical staining patterns of primary rectal NETs with Islet 1, PAX8, CDX2, chromogranin A, and synaptophysin.
Design: A total of 36 primary rectal well-differentiated NETs from 32 patients were studied. Immunohistochemistry was performed with antibodies against Islet 1, PAX8, CDX2, chromogranin A, and synaptophysin. The extent of positive staining was assessed, and the intensity of staining was evaluated as weak, moderate, or strong. Tumors showing moderate to strong staining of at least 5% of cells or showing weak staining of at least 10% of cells were considered positive.
Results: Immunohistochemistry results separated according to staining intensity are summarized below:

Immunohistochemistry results for primary rectal NETs, n = 36

Staining intensity	Islet 1 (%)	PAX8 (%)	CDX2 (%)	Chromogranin A (%)	Synaptophysin
Moderate to strong	28 (78)	24 (67)	0	15 (42)	35 (97)
All (weak, moderate, strong)	30 (83)	28 (78)	0	15 (42)	36 (100)

The majority of tumors were immunoreactive with Islet 1 and PAX8 and showed moderate to strong staining. There was no difference between the rates of expression of Islet 1 (83%) and PAX8 (78%) in primary rectal NETs (p=0.77). Although results of staining for Islet 1 and PAX8 were concordant in the majority of cases, discordant staining was observed in 4 tumors. None of the tumors were positive for CDX2. Almost all tumors positive for chromogranin A and synaptophysin showed moderate to strong staining intensity. A significantly greater number of rectal NETs showed expression of synaptophysin (100%) compared to chromogranin A (42%) (p<0.0001).
Conclusions: Islet 1 and PAX8 are both highly expressed in primary rectal NETs, at rates similar to what we have seen previously for primary pancreatic NETs (Islet 1, 82%; PAX8, 88%). Synaptophysin expression is seen significantly more commonly than chromogranin A in primary rectal NETs.

689 HER2 Copy Number in the Assessment of HER2 Status in Gastric/GOJ Cancers: Does It Matter?

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Background: Following the results of the ToGA study, combination chemotherapy with trastuzumab was approved to treat HER2 positive advanced/metastatic gastric & GOJ carcinomas. HER2 positivity was defined as either an IHC 3+ score or HER2 amplification as determined by HER2/Chr17 ratio ≥2.0 rather than the HER2 copy number (CN) per cell. Sub group analysis showed that there was no survival benefit for the HER2 amplified but IHC negative group (score 0 or 1+) which was ~ 23% of the positive cohort. We aim to compare HER2 CN against HER2/Chr17 ratio in relation to the IHC score.
Design: HER2 status of gastric and GOJ cancers were assessed by IHC and silver in-situ hybridisation (SISH). IHC scoring (0-3+) was performed using "modified gastric" criteria. HER2 and Chr17 CNs were counted in at least in 20 cancer cells and the HER2:Chr17 ratio calculated as per protocol. These were compared to the IHC scores.
Results: There were 142 gastric & GOJ carcinomas.

IHC score vs HER2 copy numbers & ratio

IHC Score (No: of cases)	Mean CN	Range	Mean ratio	Range
0 (79)	2.6	1.3-4.7	1.2	0.7-2.0
1 (24)	2.7	1.7-5.1	1.3	0.9-2.6
2 (18)	4.5	1.8-15.0	1.8	0.9-5.3
3 (21)	20.1	3.6-32.0	10.2	1.3-16.0

CN=copy number

The HER2 CN & ratio for IHC score 3+ vs scores 2+, 1+ and 0 were significantly different (all p<0.001). The CN for IHC 2+ vs IHC 1+ and IHC 0 was significantly different (both p≤0.001) but the ratio was not. The CN and the ratio for scores 1 & 0 were not significantly different (p=0.6419). Using ToGA criteria 19% (27/142) were HER2 positive; 11%(3/27) did not show increase in CN; 7% (2/27) IHC negatives showed amplification by ratio, but the copy number was <6. An equivocal IHC score (2+) showed amplification in 4 of 18 (6%); 3 by both ratio & CN and one by ratio alone. An IHC score (3+) showed > 6 HER2 CNs and a ratio ≥2.0 in all but one case which showed no amplification by either CN or ratio (1/21).
Conclusions: Our findings show that the HER2 CN correlates better with the IHC score than the ratio. There was no survival benefit for IHC negative but amplified cases (~ 23%) in the ToGA trial. This subgroup was smaller (7%) in our cohort and neither showed a copy number >6. HER2 CN appears to be a better measurement than the ratio in determining the amplification status in IHC equivocal and negative cases.

690 Chronic Granulomatous Disease Involving Gastrointestinal Tract (Pathology Study of 87 Cases)

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Background: Chronic granulomatous disease (CGD) is an uncommon inherited immunodeficiency due to a disorder of phagocyte oxidase metabolism, occurring in about one out of 250,000 individuals. CGD patients develop recurrent and life threatening bacterial and fungal infections. Gastrointestinal (GI) involvement is a common problem for CGD patients who present with abdominal pain, diarrhea, constipation, obstruction and fistula. We evaluated a large number of GI biopsies on symptomatic CGD patients to characterize their morphologic features.
Design: We collected 313 GI biopsies (esophagus 23, stomach 71, duodenum 52, colon 165, and anus 2) from 87 CGD patients with clinical GI involvement. The patients were diagnosed at NIH Clinical Center from 1987 to 2011. All the biopsies were retrospectively evaluated for multiple parameters such as presence of pigmented macrophages (0~3), granuloma formation (0~4, poorly or well formed), inflammation (acute vs chronic), architecture distortion, intraepithelial lymphocytosis, ulceration, microscopic eosinophilic abscess and eosinophilia and atypia.
Results: We found that granuloma formation was present in 46% (76/167) of colon involving mucosa and submucosa, 23% (12/52) of duodenum, 6% (4/71) of stomach and 0% (0/23) of esophagus. Pigmented macrophages were present in 65% (109/167) of colon, and 13% (7/52) of duodenum. Inflammation was present in 58% (97/167) of colon with 20% (34/167) with acute cryptitis and/or crypt abscess and 38% (63/167) of chronic active/inactive colitis, 25% (13/52) of duodenum, 62% (44/71) of stomach, and 17% (4/23) of esophagus. Ulceration was found in 10% (17/167) of colon, 13% (7/52) of duodenum, and 5% (4/71) of stomach. Granulomas and abscesses were found in the 2 anal biopsies. Atypia/dysplasia was rarely encountered. Normal histology was found in 5% (4/87) of all selected patients.
Conclusions: Mild to severe pathologic changes of GI tract can be seen in the majority of CGD patients with GI symptoms. Colon is commonly affected. Granulomas, pigmented macrophages and inflammation are the most common pathologic changes.

691 Squamous Papillomas of the Esophagus: A Clinicopathologic Study of 171 Patients Revealing a Possible Association with Eosinophilic Esophagitis

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Background: Squamous papillomas, benign exophytic papillary proliferations of squamous epithelium and fibrovascular tissue, typically occur in the mid and distal esophagus. In adults, an association with reflux has been postulated. We have noticed cases containing increased numbers of intraepithelial eosinophils. Therefore, we reviewed our experience with squamous papillomas to determine whether there is an association with eosinophilic esophagitis.
Design: The surgical pathology archives at our institution from 2000-2009 were searched for cases of squamous papilloma. These were compared to a control cohort of patients with esophageal biopsy specimens matched for age, gender, and year of diagnosis. In each case, the patient age, gender, and presenting symptoms were recorded and the results of pathological review and comorbid conditions noted. Groups were compared using the chi-square test.
Results: The 171 patients (115 women) ranged in age from 18 to 86 years (mean = 52.8). Three patients had two papillomas. Based on biopsy specimens obtained separately from the papillomas, the histological inflammatory status of the esophagus was known in 93 patients. Seventy-eight showed inflammation including reflux-related changes (59 patients), histological eosinophilic esophagitis defined as >15 intraepithelial eosinophils per high magnification field (15 patients), and mucosal candidiasis (4 patients). Compared with the control cohort, squamous papillomas were more frequently associated with inflammation (p<0.0001), reflux-related histology (p=0.0002), and histological eosinophilic esophagitis (p<0.0001). No koilocytotic changes, squamous dysplasia, or carcinoma was encountered in either cohort. Repeat upper endoscopy with esophageal biopsy occurred in 18 patients; one patient had persistence/recurrence of esophageal papilloma at 3 months.
Conclusions: We conclude that, in adult patients, esophageal squamous papillomas show no histological evidence of HPV cytopathic effect and are not associated with squamous dysplasia or carcinoma. This study confirms a high rate of comorbid reflux but also reveals a possible association with histological eosinophilic esophagitis.

692 The Sydney System Twenty Years Later: Who Uses It and Does It Matter?

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Background: The original 1991 Sydney Gastritis Classification System recommended submitting two separate specimens each from antrum and corpus; the 1996 updated version added the *incisura angularis (IA)*. The extent to which the Sydney System

recommendations are followed remains unknown. This study was designed to determine the gastric sampling patterns in outpatient centers in the US and their diagnostic yield for *Helicobacter* infection and intestinal metaplasia (IM).

Design: We analyzed biopsy specimens submitted for the evaluation of gastritis from 400,738 endoscopic procedures performed between 1/1/08 and 6/30/11, using the Caris pathology database of biopsies received from outpatient endoscopy centers nationwide. Cases with an endoscopic impression of a discrete lesion were excluded. Based upon the requisition, biopsies were categorized as from: antrum, corpus, cardia, *IA*, or unspecified (NOS). The diagnostic yield for *H. pylori* infection (detected by IHC stain on all specimens) and IM were correlated with the number and sites of the specimens.

Results: There were 98,962 NOS biopsy sets; 264,604 specimens from the antrum; 69,737 from the corpus; 10,491 from the cardia; and 2,262 identified as *IA.* Specimens were from at least two sites (submitted in separate containers) in 33,072 procedures. Although neither version of the Sydney System was ever strictly followed, in 15,730 procedures (3.9%) there were at least two separate containers from antrum and corpus with at least two specimens each. The diagnostic yield for *H. pylori* and IM is summarized in Table 1.

Relative yield for different sets of gastric biopsies for the detection of Helicobacter and Intestinal Metaplasia

Biopsy set	Patients	H. pylori + (%)	OR (CI 95%)	IM (%)	OR (95% CI)
≤3 (A or C)	225,927	19,862 (8.8)	1	7,163 (3.2)	1
≥4 (A or C)	60,394	7,110 (11.8)	1.38 (1.35 - 1.42)	2,941 (4.9)	1.56 (1.50 - 1.63)
≥2A and ≥2C	15,730	2,324 (14.8)	1.80 (1.72 - 1.88)	945 (6.0)	1.95 (1.82 - 2.09)

A = Antrum; C = Corpus (2 specimens designated as antrum and 2 as corpus are compliant with the Sydney System.)

Conclusions: The Sydney System recommendations are rarely followed in the US. Compared to 3 or fewer biopsies, the diagnostic yield for both *H. pylori* and IM is nearly doubled by the submission of at least 2 separate antral and 2 corpus specimens. Cardia biopsies have the lowest yield. The detection of IM increases with the number of biopsies from any site (but cardia). The *IA*, originally added for its suspected early development of IM, yields only 3.5%. Sampling at least 2 antral and 2 corpus specimens is the best-yielding practice for the evaluation of gastritis.

693 Serrated Polyps in Patients with Inflammatory Bowel Disease

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Background: Serrated polyps (SPs), comprised of sessile serrated adenomas (SSAs) and traditional serrated adenomas (TSAs), are known precursors of colorectal cancer. It is also known that inflammatory bowel disease (IBD) increases the risk of colorectal cancer. SPs have been reported to occur in IBD, but because SPs outside of IBD have a different molecular pathway and outcome than adenomas (tubular and villous), it may also represent a different natural history in IBD. However, the prevalence of SPs in IBD patients is unknown and no guidelines exist for their management. This project aims to establish the frequency and anatomic distribution of SPs in IBD.

Design: All IBD patients with a gastrointestinal (GI) biopsy performed at the regional tertiary care hospital, from January 1, 2007 to December 31, 2008, were identified through a search of the centralized health region pathology database. Among these patients, all who had a biopsy diagnosed as adenoma, hyperplastic polyp (HP), SSA, or TSA were selected and their histologic sections reviewed by one GI pathologist who was blinded to the original diagnosis. We did not try to differentiate between dysplasia-associated lesions or masses and sporadic adenomas. Each patient's records were reviewed to classify the anatomic distribution of the lesions (proximal or distal to the splenic flexure).

Results: 663 IBD patients with a GI biopsy were identified. 78 patients had a diagnosis of at least one of adenoma, HP, SSA or TSA after the pathology review (Table 1). There was a change in diagnosis for several polyps including 4 SSAs previously diagnosed as HPs, 1 SSA previously a "serrated adenoma", 1 TSA previously an adenoma, and 1 TSA previously a "serrated adenoma". None of the SSAs had dysplasia.

Table 1: Polyp type, location, and frequency

	Proximal (% of total)	Distal (% of total)	Total	% frequency in IBD patients
Adenoma	19 (43.2%)	25 (56.8%)	44	6.64%
HP	13 (27.1%)	35 (72.9%)	48	7.24%
SSA	6 (100.0%)	0 (0.0%)	6	0.90%
TSA	1 (33.3%)	2 (66.7%)	3	0.45%

Conclusions: Our study is the first retrospective cohort study to identify the frequency of SPs in IBD patients. It also allows comparison between the frequency of SPs, HPs, and adenomas. As expected, all 6 SSAs were located proximally, and 2 of the 3 TSAs were distal. There were 5 times fewer SPs than either HPs or adenomas. One still cannot address the question of whether SPs develop independent of IBD, or if IBD represents an increased risk of SPs. While the frequency of SPs in our study is higher than in the general population, this is of uncertain significance due to our limited population. We intend to follow up on our detected SPs with BRAF mutation analysis.

694 Pyloric Gland Adenoma with Mismatch Repair Protein Loss and MSI-High Is a Precursor of Gastric Adenocarcinoma in Lynch Syndrome

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Background: Fundic gland polyposis is a gastric manifestation in patients with FAP. However, although gastric carcinoma is the second most common extra-colonic malignancy associated with Lynch syndrome, the detailed pathology or precursor lesions in the stomach are not described. In this study, we performed clinicopatholologic and molecular analyses using 13 gastric carcinomas from patients with Lynch syndrome.

Design: After computer search, 392 patients were identified to have both gastric and colonic adenocarcinomas. Additionally, 311 patients enrolled in familial cancer clinic suspected as Lynch syndrome were also retrieved. All the medical records of 703 patients in a single comprehensive cancer center from 1995 to 2011 were reviewed. Twenty patients met the Amsterdam II criteria and had been treated for gastric and colonic adenocarcinomas. Immunohistochemistry for mismatch repair (MMR) proteins, MSI tests, MLPA for hMLH1 and hMSH2 were performed to confirm Lynch syndrome.

Results: Thirteen patients were classified as Lynch syndrome and the average age of diagnosis of gastric carcinoma was 48 years. The location of tumor was antrum (n=8) followed by body (n=3) and cardia (n=2). Helicobacter pylori were demonstrated in 4 cases (30.8%) and background intestinal metaplasia and atrophy was identified in 11 cases (84.6%). The histology of gastric carcinoma included 10 tubular adenocarcinomas, 2 mucinous carcinomas, and a composite adenocarcinoma and endocrine carcinoma. In all cases, both gastric and colonic carcinomas were MSI-high and either hMLH1 or hMSH2 protein was lost in tumors.

Unexpectedly, pyloric gland adenoma (PGA) was identified in 4 cases around the carcinomas. PGAs mimicked fundic gland polyp except for the absence of oxyntic cells. Most tumor glands in PGAs were strongly positive for MUC6 and superficial layer was positive for MUC5AC, while MUC2 and CD10 were totally negative. In a PGA with germline hMLH1 mutation, hMLH1 protein expression was lost. Three PGAs with hMSH2 protein loss showed abnormalities in MLPA. The carcinomas around PGA were tubular adenocarcinoma of gastric mucin phenotype. In three cases, there was a direct transition from PGA to carcinoma and one PGA transformed to carcinoma over the follow up of 2 years.

Conclusions: We first identified that PGA may be a precursor lesion of gastric carcinoma in Lynch syndrome and accompanies MMR protein loss and MSI-high. Our findings suggest that MSI-phenotype is an early event and the MMR-deficient pathway also involves gastric carcinogenesis.

695 Diffuse Malignant Mesothelioma of the Peritoneum: An Immunohistochemical Study of 48 Cases

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Background: There are few studies describing the immunohistochemical profile of peritoneal mesotheliomas. p16 has been suggested to lose expression in poorly differentiated tumors, and D2-40 is a relatively recent mesothelial marker. In the current study, we review the immunohistochemical profile of 48 cases of peritoneal mesothelioma and study the utility of p16, D2-40 and CA125 expression in diagnosis.

Design: We retrospectively studied the cases of peritoneal mesothelioma accessioned as either cytoreductive surgery or biopsy at one institution from 2006 to 2011. Immunohistochemical staining was performed on representative paraffin embedded tumor blocks.

Results: There were 48 cases of peritoneal mesothelioma, 26 females (50 ± 14 years), 22 males (60 ± 12). 43 of these were classified as epithelioid and 5 were biphasic (with sarcomatoid areas). Of the 43 epithelioid ones, 28 were tubulopapillary, 9 showed solid growth (6 deciduoid and 3 myxoid), and 6 were well differentiated (4 multicystic, 1 papillary, 1 adenomatoid). All tumors but one were positive for calretinin, both nuclear and cytoplasmic staining, of which 97% were diffuse. 97% showed membranous positivity for EGFR in more than 3/4 of tumors cells. 94% were positive for CA125, mostly diffusely membranous staining. 86% were positive for p16, half of which were patchy positive (between 1/2 and 1/3 tumor cells), nuclear and cytoplasmic staining. 83% showed cytoplasmic staining with CK5/6, 1/3 of which were only focally positive. There was 67%, 57%, and 50% staining for HBME, EMA, and CK903, respectively. Only 47% of tumors stained with WT-1, and 33% with cytokeratin 7. 80% of tumors showed strong membranous positivity for D2-40. There was no correlation between differentiation and staining for any marker except D2-40, which was negative only for well differentiated tumors. The epithelial component in 4 of 5 sarcomatoid tumors showed strong (remaining one weak) and diffuse staining with calretinin in 100% of tumor cells; spindled areas displayed weak to moderate staining in 30-50% of cells. Strong staining with CK903 was seen in two cases, one deciduoid (membranous) and one sarcomatoid (cytoplasmic).

Conclusions: p16 is frequently positive in peritoneal mesothelioma, with no correlation between tumor differentiation and loss of expression. CA125 and EGFR are reliable markers. D2- 40 is nearly always positive in poorly differentiated tumors, which may pose diagnostic difficulties. The study confirms the high sensitivity for calretinin, and shows a high sensitivity for CA125 and EGFR for malignant mesothelioma.

696 Can We Distinguish Mycophenolate-Induced Colitis from Colonic Graft-Versus-Host Disease?

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Background: Mycophenolate mofetil (MMF) is an immunosupressive agent commonly used in solid organ transplant recipients. More recently it has been used to treat Graft-versus-host disease (GVHD). Previous studies have documented that MMF can cause GI tract damage that is very similar to GVHD. Our anecdotal experience was that MMF colitis often had dilated crypts containing esoinophils and debris, while GVHD did not. We undertook the following morphologic study to see if we could identify any histologic features that would allow us to separate these two entities.

Design: All colonic biopsies signed-out as either GVHD or MMF colitis between 2004 and 2010 were reviewed. The MMF cases entered into the study were all solid-organ transplant patients that had no evidence of GVHD (no bone marrow transplant patients). The GVHD cases were all bone marrow transplant patients that had no prior treatment with MMF. Twenty-eight cases of MMF colitis and 34 cases of GVHD had enough intact tissue in their biopsies for further study. All cases were reviewed blindly for the following features: amount and character of lamina propria and epithelial inflammation, Grade of GVHD, number of apoptotic bodies / 10 crypts, amount of crypt distortion and dilatation and number of Ki-67 positive cells/crypt.

Results: The number of apoptotic cells per 10 crypts was statistically higher in cases of colonic GVHD vs MMF colitis ($p<0.000002$) with a range of 6 - 60 for GVHD (mean 30.94) and 0 - 28 for MMF (mean 12.26). There were no statistically significant

differences with regards to Ki-67 labeling, crypt distortion or crypt dilation, nor any types or intensity of inflammatory cells. The only other significant finding was the presence of endocrine cell nests in the deep lamina propria of 8 severe cases of GVHD as compared to 0 cases of MMF colitis (despite having similar denuded areas)($p<0.006$).
Conclusions: This study confirms that MMF colitis is a great mimicker of colonic GVHD. While the number of apoptotic cells per 10 crypts was much higher in GVHD cases as a whole, there was considerable overlap, particularly in Grade 1 GVHD as compared to MMF colitis. In severe cases of GVHD, denuded areas often had endocrine cell nests at the base of the lamina propria that were not seen in cases of severe MMF colitis with similarly denuded areas. Dilated crypts containing eosinophils and necrotic debris were seen in cases of GVHD and MMF and were not helpful in distinguishing one entity from another.

697 Sporadic Fundic Gland Polyps with Low-Grade Dysplasia: A Large Case Series To Assess Clinicopathologic Behavior

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Background: Fundic gland polyps (FGPs) occur in two different clinical settings, sporadic and syndromic (familial adenomatous polyposis, or FAP). Sporadic FGPs are benign epithelial polyps associated with chronic use of proton-pump inhibitors (PPIs). Epithelial dysplasia (low- or high-grade) is extremely rare in FGPs, and, therefore progression data from large series are lacking. The aim of this study was to evaluate the clinical, morphologic and immunohistochemical features in a large series of sporadic FGPs with epithelial dysplasia.
Design: From a total of 28,000 FGPs diagnosed in our institution between January 2008 to July 2011, we extracted all cases with a reported diagnosis of low-grade dysplasia (LGD); we then reviewed the slides and selected cases that met the following criteria: hyperchromasia, loss of mucin, nuclear enlargement, and pseudostratification, limited to the surface or foveolar epithelium. Selected cases were stained for p53, Ki-67, and *B*-catenin.
Results: There were 48 FGPs (0.17%) with histologically confirmed low-grade dysplasia (LGD) and none with high-grade dysplasia; 13 were associated with FAP (age range 14 to 64 years, mean 46 years; 3 men and 10 women), and 35 were sporadic (age range 21 to 78 years, mean 52.1 years; 23 women and 12 men, 2:1 ratio). The indication for endoscopy in the sporadic FGP with LGD included heartburn, follow up of Barrett's, and non response to PPI (all of our study patients were on PPI). None of the cases had *Helicobacter pylori* infection. 15 sporadic and 5 syndromic FGPs with LGD and 5 sporadic FGPs without dysplasia were selected for immunohistochemical staining with p53, Ki-67, and *B*-catenin. None of the cases showed overexpression of p53 protein. Ki-67 showed a high proliferation index in the stem cell neck region and surface epithelium in the sporadic and syndromic FGPs with LGD. The deeper glands within the polyps were completely negative. The sporadic FGP without dysplasia showed absent surface nuclear staining. Nuclear accumulation of *B*-catenin was observed in 4 out of 5 (80%) syndromic FGPs with LGD and 2 out of 15 (13.3%) sporadic FGPs with LGD.
Conclusions: To date, this is the largest study evaluating the clinicopathologic findings of sporadic FGPs with LGD. Sporadic FGPs with LGD were more often seen in females and were associated with PPI use. The sporadic FGP with LGD had lower *B*-catenin nuclear staining. We found that syndromic and sporadic FGPs with LGD did not differ with respect to proliferative activity and p53 expression.

698 Gastric Intestinal Metaplasia with Dysplasia-Like Atypia: A Morphological and Biologic Evaluation

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Background: Gastric intestinal metaplasia (IM) can be hyperproliferative and display cytoarchitectural atypia. These changes fall short of qualifying for dysplasia and have been generally classified as indefinite for dysplasia. Few studies have evaluated the prevalence and the morphologic and biologic characteristics of this variant of gastric IM.
Design: Out of an institutional cohort of biopsies with chronic atrophic gastritis and/or dysplasia obtained from ethnic Chinese, we categorized the cases as either 1) simple IM (SIM) with uniform glands, mature goblet cells, basally oriented nuclei and surface maturation; 2) IM with hyperplasia (IMH) showing limited architectural abnormalities, reduced number of goblet cells, slightly elongated and hyperchromatic nuclei and surface maturation; 3) IM with dysplasia-like atypia (IM-DLA) showing crowded glands varying in size and shape with mucin depletion, hyperchromatic nuclei, moderate pseudostratification and surface maturation; or 4) gastric dysplasia (GED). The relationship between the morphologic subgroups and various clinicopathological features, mucin immunophenotypes (gastric-, gastrointestinal-, intestinal-, and small intestinal-type) and biologic characteristics (p53, Ki-67 and AMACR) was evaluated.
Results: The final cohort consisted of 554 cases including 424 SIM, 93 IMH, 16 IM-DLA, and 21 GED cases. Notably, IM-DLA had a prevalence (3.8%) similar to GED (5.0%). Both of these lesions were similar in body/fundus distribution (12.5%), common association with surface erosion (18.8%), acute inflammation (62.5%) and paucity of goblet cells (68.8%). IM-DLA and GED also shared biologic similarities but with a lower frequency of AMACR expression (25% vs. 62%), p53 expression (6.3% vs. 47.6%) and increased Ki-67 index on surface/pit and isthmic zones in IM-DLA. However, intestinal and gastric immunophenotypes and ectopic superficial MUC6 expression were found exclusively in GED ($p<0.001$). Alternatively, SIM and IMH (individually or combined) statistically differed from IM-DLA and GED with regard to the various characteristics evaluated.
Conclusions: Gastric IM can be divided into 2 broad categories, one combining SIM and IMH, and the other IM-DLA. These variants of IM are readily defined by cytoarchitectural and biologic characteristics. Based on the similarities between GED and IM-DLA, we postulate that IM-DLA represents an early step in the gastric carcinogenic sequence.

699 Infarct-Like Necrosis: A Distinct Form of Necrosis Seen in Colorectal Carcinoma Liver Metastases Treated with Perioperative Chemotherapy

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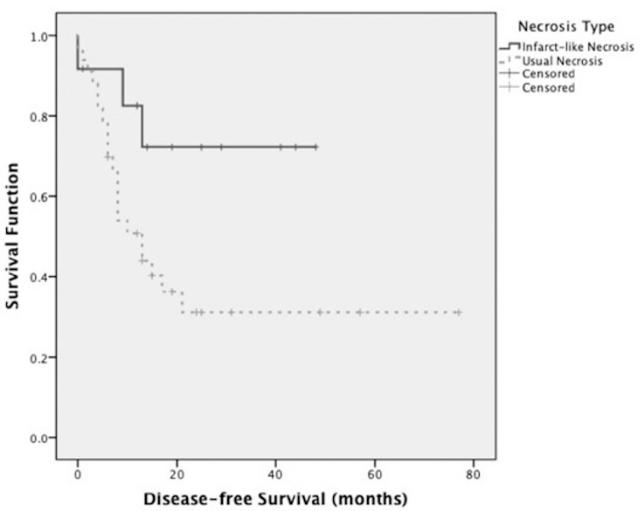
Background: The response of colorectal liver metastases (CRLM) to perioperative chemotherapy (PCx) can be assessed histologically. We classified necrosis in this setting into two types: infarct-like necrosis (ILN) representing therapeutic response to PCx, and usual necrosis (UN) signifying an absence of treatment effect. Tumor regression grading (TRG) is a previously described method that estimates response to PCx by measuring tumor replacement by fibrosis. We devised a modified TRG (mTRG) scoring system that incorporates ILN and compared its prognostic performance against TRG.
Design: A retrospective review was done of all partial hepatectomies performed for CRLM at our center between 2004-10. Cases were classified according to whether UN or ILN was more prominent. UN was defined as patchy necrosis containing nuclear debris and bordered by viable tumor cells; ILN was defined as confluent necrosis surrounded by hyaline fibrosis. UN and ILN were compared with respect to clinicopathologic features, including disease-free survival (DFS) and overall survival (OS). Each case was assigned two tumor regression scores: a TRG score using previously described criteria, as well as a mTRG score in which both ILN and fibrosis were deemed to represent treatment effect. DFS and OS using the two scoring systems were compared.
Results: Of the 109 cases reviewed, 46 received PCx. All 12 cases containing ILN occurred in those treated with PCx. Amongst patients receiving PCx, those with ILN had superior DFS than those with UN (Log-rank test, $P < 0.05$).



The mTRG score differed from the TRG score in almost all cases containing ILN (11/12). mTRG scores of 1-2 were associated with significantly better DFS and OS than mTRG scores of 3-5 (Log-rank test, $P < 0.05$). In contrast, use of TRG failed to demonstrate a significant difference in DFS and OS between histologic responders and non-responders to PCx.
Conclusions: ILN represents a therapeutic response to PCx and thus should be distinguished from UN. The prognostic utility of TRG is enhanced when ILN is considered a form of treatment effect.

700 miRNA Expression Pattern in Indeterminate Colitis

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Background: A diagnosis of idiopathic inflammatory bowel disease (IBD) is rendered by synthesizing clinical, radiographic, endoscopic, and histologic information. While most IBD cases can be specifically classified as either ulcerative colitis (UC) or Crohns disease (CD), 5-10% of patients have equivocal features, falling into the category of IBD indeterminate type or indeterminate colitis (IC). The molecular mechanism underlying IC is unknown. Herein, we explored the microRNA (miRNA) expression patterns in patients with IC.
Design: Frozen colon tissues from the distal part of the colectomy of 16 IC patients, 11 CD patients, 12 UC patients, and a control group of 11 patients with diverticular disease were identified. Total RNA was extracted and qRT-PCR was performed using five pairs of miRNA primers (miR-19b, miR-23b, miR-106a, miR-191, and miR-629). The level of each miRNA in the study groups was normalized to the mean value of the control. The general liner multiple regression analysis and the discriminant analysis were performed to evaluate the variance of miRNA expression and the capacity of five markers, as a whole, to differentiate the diseases of interest, respectively.
Results: There was significant difference in the expression of miR-19b, miR-106a and miR-629 between UC and CD groups ($P<0.05$). The expression level of all five

miRNAs was statistically different between IC and CD groups ($P<0.05$), respectively, but no significant difference existed between IC and UC groups. Discriminant analysis demonstrated that, as a whole, five miRNAs have a good capacity in distinguishing CD-like pattern from non-CD-like pattern. Nine of 14 CD patients (64.3%) showed CD-like miRNA pattern compared to 0% of UC patients. Among the 16 IC patients, definitive diagnosis of UC had been reached for four patients (25%) and all of them had UC-like miRNA pattern. Among the 12 patients whose diagnosis remained IC during follow-up, one showed CD-like pattern but the other 11 did not.

Conclusions: miRNA expression pattern in IC, as a whole, is similar to that of UC, not CD. Our study provides the molecular evidence to believe that most IC cases are UC. miRNA may be a useful diagnostic and prognostic marker to distinguish rare IC patients that will prove to have CD.

701 Sonic (Shh), Desert (Dhh) and Patched (PTCH1) Hedgehog Pathway Protein Expression Correlates with Aggressive Disease in Gastric/GE Junction Carcinomas (GCA)

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Background: Activation of hedgehog signaling pathway is implicated in embryonic development and the differentiation, proliferation and maintenance of multiple adult tissues. Altered expression of Shh, Dhh and PTCH1 has been linked to Barrett's metaplasia in the esophagus and neoplastic transformation in the stomach, but has not been related to clinical outcome in GCA.

Design: Formalin-fixed, paraffin-embedded tissue sections of 126 GCA were immunostained by automated methods (Ventana Medical Systems Inc., Tucson, AZ) using goat polyclonal Shh, Dhh and PTCH1 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA). Cytoplasmic immunoreactivity was scored based on staining intensity (weak, moderate, strong) and percentage of positive cells (focal <= 10%, regional 11-50%, diffuse >50%). Results were correlated with clinicopathologic variables.

Results: Immunoreactivity for Shh, Dhh and PTCH1 was predominately cytoplasmic. Shh overexpression was noted in 47% tumors and correlated with high grade [53% grade 3 vs 41% grade 2 vs 0% grade 1, p=0.042] and advanced stage [67% advanced vs 24% early, p=0.001]. Dhh overexpression was noted in 42% tumors and correlated with advanced stage [55% advanced vs 18% early, p=0.007], with a trend toward association with high grade [47% grade 3 vs 38% grade 2 vs 0% grade 1, p=0.089]. PTCH1 overexpression was noted in 84% tumors and correlated with high grade [83% grade 3 vs 90% grade 2 vs 40% grade 1, p=0.017]. On multivariate analysis, advanced stage [p=0.004] independently predicted shortened survival.

Conclusions: Protein expression of the Shh, Dhh, and PTCH1 members of the hedgehog pathway are associated with aggressive disease in GCA. Further study of hedgehog (Hh) signaling pathway in GCA appears warranted.

702 A Focused Peritumoral Evaluation for Lymph Nodes and a "Second Look" Protocol Improves Nodal Staging of Colon Cancer: A Prospective Study of 102 Colectomies

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Background: The number of colectomies for colon cancer (CC) in which a minimum of 12-15 lymph nodes (LNs) are examined has increased steadily over time. However, this has not led to a significant increase in the proportion of LN-positive CC. The region within a colectomy with greatest likelihood of finding positive LN is not addressed in the current guidelines. The goal of this study was to evaluate whether LN staging of CC can be improved by a focused LN search in the immediate vicinity of the tumor and a "second look" in cases which are LN-negative on initial examination.

Design: A novel protocol for examining LN in CC was used prospectively. LNs were submitted separately from the primary (PNB) and secondary nodal basin (SNB). PNB and SNB were defined arbitrarily as an area within 5 cm, and >5 cm away from the tumor edge, respectively. A second LN search was performed, by a different prosector, in all cases which were LN-negative on initial examination. 102 consecutive resections were analyzed during an 18 mo period in 2010-2011. 88 consecutive resections over an 18 mo period in 2008-2009 served as the control group for comparison.

Results: 42 CC were LN-positive and 60 LN-negative on initial examination. The number of LNs examined prospectively in cases with one LN search was 23 ± 15.3 (mean ± SD). Cases (initially LN-negative) in which a search was done twice had a LN count of 28 ± 11.9. This was significantly higher than LN counts (19 ± 7.5) in the control group ($p<0.0001$ and $p<0.007$, respectively).

The number of LNs in the PNB was 18 ± 8.3, and 22 ± 10.4 for cases with one and two searches, respectively. Positive LNs were present only in the PNB in 39/42 CC and in both the PNB and SNB in the remaining 3 cases.

A second LN search was done in 55/60 initially LN-negative CC. The mean number of additional LNs identified on a second search in the PNB and SNB, was 6.7 and 4.8, respectively. 5 of 55 (9%) initially LN-negative specimens had 1-4 positive LNs on a second search, upstaging CC from N0 to N1 (4 cases) and N2 (1 case). All positive LNs in the second search were in the PNB.

Conclusions: Positive LNs are most often found within the PNB and a focused search of this region yields 12-15 LNs in most resections. A second search in initially LN-negative cases leads to upstaging in 9% of resections. The number of LN examined from the PNB and a "second look" protocol can improve LN staging of CC and can be used as additional quality indicators in pathologic examination of CC.

703 Young Age and High Frequency of Multiplicity, Well Differentiation, Crohn's-Like Reaction, Tumor Heterogeneity, and Signet Ring Differentiation in Inflammatory Bowel Disease-Associated Colorectal Adenocarcinoma

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Background: Longstanding inflammatory bowel disease (IBD), either ulcerative colitis (UC) or Crohn's disease (CD), is associated with a high risk of developing IBD-associated colorectal adenocarcinoma (CAC). It is unclear if these patients should be screened for hereditary nonpolyposis colorectal cancer syndrome (HNPCC). We hypothesize that IBD-associated CAC is morphologically different from sporadic microsatellite stable CAC.

Design: We evaluated the demographic and morphological features of 108 IBD-associated CAC, including UC-associated (n=95) and CD-associated CACs (n =13), compared to 70 control cases of microsatellite stable sporadic CACs. Clinicopathologic features, including age, tumor multiplicity, tumor differentiation, tumor heterogeneity, the presence of signet ring differentiation, and CD-like reaction were compared between the two groups.

Results: The mean age of patients with IBD-associated CAC was 50 years, significantly younger compared to the mean age of 61.4 years in the control group ($p<0.05$). Two or more synchronous CACs were noted in 20.2% patients with IBD-associated CACs but only in 7.1% of the control group ($p <0.05$). Well differentiation of tumors, CD-like reaction, tumor heterogeneity, and the presence of signet ring differentiation were significantly more common in IBD-associated CACs compared to the control group ($p <0.05$).

Conclusions: IBD-associated CAC occurs 10 years earlier compared to that of non-IBD related sporadic microsatellite stable CAC and it is more commonly associated with synchronous tumors. Morphologically, IBD-associated CACs share the pathologic features seen in HNPCC, such as well differentiation, CD-like reaction, tumor heterogeneity, and signet ring cell differentiation. The young age and morphological similarity between IBD-associated CAC and HNPCC suggest that an age and morphology-based strategy prior to the screening test for HNPCC may be less effective in IBD-associated CAC than in the non-IBD population.

704 Identification of Novel Gene Mutations and Interactions That Determine Paneth Cell Granule Phenotype in Crohn's Disease

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Background: Previous studies have identified >90 susceptibility genes for Crohn's disease (CD). We have shown that combination of *ATG16L1* hypomorphism and persistent norovirus infection results in CD-like pathology in mouse models through altering Paneth cell (PC) granule phenotypes. However, little is known about the biologic implications of these abnormal granules and whether other susceptible genes have similar impact. The aims of this study are to investigate if abnormal PC phenotypes were associated with changes in ileal gene expression, and to correlate susceptibility genotype and PC phenotype in CD patients.

Design: Adult CD patients who underwent ileocolectomy between 2005 - 2010 with available genotype and tissue material were enrolled. H&E sections of uninvolved ileum were selected for lysozyme immunofluorescent stains and corresponding tissue blocks were used for microarray and RNA-Seq to assess gene transcription. PC were categorized based on previously described lysozyme stain patterns. Peripheral blood was collected and genotyped by Illumina HumanOmni1-Quad BeadChip. PC phenotypes were then correlated with genotype results.

Results: Seventy-seven patients who met the criteria were enrolled. Unsupervised hierarchal clustering of the microarray data set segregated the CD patients into 2 major clusters; ~6000 genes were differentially expressed between these clusters, including those involved in autophagy and viral sensing. Patients with an abnormal PC phenotype tended to associate with one cluster vs. the other. In addition, the presence of 1 or 2 NOD2 risk alleles was associated with an increased proportion of abnormal granules ($p<0.05$). *PTPN2* mutation alone showed significantly reduced normal granules and when presented with *NOD2* mutation, showed further increased abnormal granules ($p<0.05$). Mutations of *MUC19*, *TNFSF15* and *IBD-5* showed increased abnormal granules exclusively in the presence of *NOD2* mutation, whereas mutations of *IL-12B* and *C13orf31* showed increased abnormal granules exclusively in patients with wildtype *NOD2*.

Conclusions: Abnormal PC phenotype correlates with more global changes in gene expression in CD. *NOD2* is a major determinant in PC phenotype and shows similar but distinct changes from that associated with *ATG16L1* risk allele. *MUC19*, *PTPN2*, *TNFSF15*, and *IBD-5* mutations result in additive/synergistic effects in conjunction with *NOD2* mutation. Mutations of *IL-12B* and *C13orf31* show altered PC phenotype exclusively in *NOD2* wildtype background. These data provide valuable implication in interaction of these genes.

705 Paneth Cell in Colorectal Adenoma and Sessile Serrated Polyp: A Comparative Study

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Background: Few studies from Japan reported the prevalence and clinicopathologic features of Paneth cell-containing colorectal adenomas. Paneth cell differentiation has not been studied in sessile serrated polyp (SSP). This study aims to determine the prevalence of Paneth cell in colorectal adenoma and SSP, and histologic features associated with Paneth cell differentiation in these lesions.

Design: One-hundred seventy three consecutively diagnosed colorectal adenomas from 119 patients (61 males and 58 females) and 104 consecutively diagnosed SSPs from 92 patients (41 males and 51 females) were reviewed. The prevalence of Paneth cells

in adenomas and SSPs was determined and correlated with patient demographics and lesion characteristics. In addition, the prevalence of Paneth cell in the mucosa adjacent to SSPs was also assessed.
Results: SSP was likely to be right-sided than colorectal adenoma [76/104 (73.1%) vs. 95/173 (54.9%), p=0.003)]. Age and gender were not different between these two groups (61.0±9.2 vs 63.1±11.4 yrs, p=0.16; 44.5% patients being male in SSP group vs 51.3% in adenoma group, p=0.404). Thirty-one adenomas (17.9%) from 24 (20.2%) patients harbored Paneth cells. Patient age and adenoma size were not different between Paneth cell-containg adenomas and non-Paneth cell-containing adenomas (63.4±8.9 vs. 61.2±12.1 yrs, p=0.55; 0.62±0.41 cm vs. 0.66±0.61 cm, p=0.73). Paneth cell containing adenomas were more likely to occur in the proximal colon (77.4% versus 57.0%, p=0.04). There was a strong association between male gender and Paneth cell-containing adenomas, as 26 of 31 (83.9%) of these adenomas occurred in males compared to 75 of 142 (52.8%) non-Paneth cell-containing adenomas (p=0.002). One SSP (of 104, 0.9%) from 1 patient [of 92, 1.0%)] had Paneth cells [vs. 17.9% (31/173); p=0.000007) adenomas from 24 (of 119, 20.2%; p=0.00018) patients harbored Paneth cells]. Among the SSP group, adjacent mucosa was present in 96 cases and Paneth cell was identified in the adjacent mucosa in 13.5% (13 of 96) cases (vs. 0.9% of SSP harboring Paneth cell, p=0.0004).
Conclusions: Paneth cell differentiation is more common in coloretal adenoma than SSP. Paneth cell-containing adenomas are more common in males and are likely proximally located. Lower frequency of Paneth cell within SSP than that in the adjacent mucosa supports a clonal expansion of and active suppression of Paneth cell differentiation in SSP cells.

706 Profiling microRNA and mRNA in Esophageal Biopsies of Patients with Eosinophilic Esophagitis before and after Treatment

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Background: Eosinophilic esophagitis (EoE) is a chronic eosinophil-predominant allergic process. The role of microRNAs (miRNAs) in the development and control of inflammation is an active area of ongoing research, however, the miRNA profile of EoE biospies has yet to be elucidated. The purpose of this study was to define the miRNA and mRNA profile of a well characterized group of pediatric EoE patients.
Design: Formalin fixed paraffin embedded mucosal biopsies were obtained from five EoE patients. Each patient was biopsied before and after successful treatment with a combination of acid suppression and steroids. The pre-treatment biopsies had all of the classic histologic features of EoE whereas the post-treament biopsies were essentially normal with rare intraepithelial eosinophils. The epithelial layer of the mucosa was dissected and the miRNA and mRNA was isolated and profiled using TaqMan MicroRNA Arrays and Affymetrix chips, respectively. The top miRNA candidates were further confirmed by quantitative RT-PCR on a new set of both paired (8) and unpaired (10) biopsies.
Results: Thirty eight miRNAs was found to be differentially regulated following treatment with a p-value of < 0.05. Most of them (23) were up-regulated in the disease state and down-regulated after treatment. All six top up-regulated miRNAs (miRNA-145, miRNA-146a, miRNA-146b, miRNA 142, miRNA 214 and miRNA21) were further validated using a new set of biopsy specimens. In addition mRNA analysis was performed and 112 mRNAs were found to be dysregulated by a factor of 4 or more. These included a number of genes previously described as being differentially regulated in EoE such as CCL-26, POSTN, CPA3, FLG and SPRR3 as well as a number of genes as yet to be described in EoE.
Conclusions: This study is the first to determine miRNA and mRNA profiles of EoE patients before and after therapy. We identified certain mRNAs and miRNAs that have been previously described to play a role in immune or allergic responses as well as a number of novel new targets. Future studies will focus on investigation of the interaction between these differentially regulated miRNAs and mRNAs as well as to confirm the role of those novel dysregulated genes in the pathogenesis of the inflammatory response.

707 Tumor Budding Score Based on 10 High-Power Fields (HPFs) Is a Reliable and Reproducible Scoring System in Colorectal Cancer

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Background: One of the main challenges in colorectal cancer (CRC) research is to improve the prognostic stratification power of the TNM staging system, especially in Stage II CRC. Although recognized by the UICC/AJCC as an additional prognostic factor, tumor budding remains unreported in daily diagnostic work due to the absence of a standardized scoring method. The aim was therefore to assess the reproducibility of tumor buds measurement by evaluating 10-high-power fields (HPFs) at the invasive front and to confirm the prognostic value of tumor buds in our CRC setting.
Design: The study design was based on the REMARK guidelines published by the JNCI in 2005. Whole tissue sections of 215 CRCs with full clinico-pathological data as well as treatment and follow-up information were stained with cytokeratin AE1/AE3. Two pathologists scored the presence of buds across 10-HPFs at the invasive front and the measurements were correlated to patient and tumor characteristics.
Results: The 10-HPF scoring system was reproducible as underlined by an excellent inter-observer agreement (p<0.0001). Low-grade, moderate- and high-grade budding were defined as an average of <5 buds (n=78), 5-19 buds (n=108) and ≥20 buds (n=29), per 10-HPFs respectively. In univariate analysis, high-grade tumor budding was associated with higher TNM-stage (p=0.0003), vascular invasion (p<0.0001), higher tumor grade (p<0.0001), infiltrating tumor border configuration (p<0.0001) and reduced survival (p<0.0001). Multivariable analysis confirmed its independent prognostic (p=0.03) effect not only when adjusting for TNM-stage, L and V stage and tumor grade, but also when including adjuvant therapy (p=0.007). Additionally, tumor budding (HR=1.48) was a stronger prognostic parameter than tumor grade (HR=1.02).
Conclusions: Using the 10-HPF scoring system, tumor budding leads to an excellent inter-observer agreement and is a strong independent prognostic factor in CRC in accordance with the literature. Additionally, tumor budding seems to have a stronger prognostic power than tumor grade which makes it a valuable candidate parameter to better stratify CRC patients into prognostic subgroups.

708 Mitochondrial Mutagenesis and Inflammation in the Colorectal Adenoma-Carcinoma Sequence

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Background: Inflammation is known to play a role in colorectal carcinogenesis. We previously found that mitochondrial lipid peroxidation increases as the colorectal adenoma-carcinoma sequence advances suggesting that mitochondrial damage may contribute to neoplastic progression. This led us to investigate mitochondrial dysfunction & its relationship to inflammation in this sequence.
Design: The random mutation capture assay was used to detect mitochondrial DNA(mtDNA) point mutation frequency & mitochondrial:nuclear DNA ratio in fresh frozen tissue from 4 normal colonic biopsies, 6 adenomas with low-grade dysplasia(LGD), 4 adenomas with high-grade dysplasia(HGD) & 8 colorectal cancers(CRC) with matched normal tissue. T-lymphocytes(CD3) & macrophages(CD68) were assessed on formalin fixed paraffin embedded tissue sections from these cases, with IHC. MtDNA encoded cytochrome c oxidase subunit II(COII, part of electron transport chain complex IV), was also assessed with IHC. Data were analysed with Wilcoxon sign rank test, Mann-Whitney test & Spearman correlation.
Results: MtDNA mutation rate was lower in adenomas with LGD compared to HGD & CRC (all p values ≤ 0.02). A similar pattern was seen for mitochondrial:nuclear DNA ratio with the lowest ratio found in adenomas with LGD & higher ratios in adenomas with HGD & CRCs (all p values ≤ 0.05). There was significant positive correlation between mtDNA mutation frequency & COII loss in CRCs (r=0.8, p=0.03). Increased mutation frequency correlated with increased numbers of CD3-positive cells (r=0.9, p=0.05) in adenomas.
Conclusions: T cell infiltration increases in adenomas with higher mutation frequencies suggesting a link between inflammation & mitochondrial mutagenesis. As the adenoma-carcinoma sequence progresses to HGD & CRC, mutational frequency & mitochondrial:nuclear DNA ratio increase. Increasing COII deficiency correlates with increasing mtDNA mutation frequency, demonstrating an association between random mutations & inefficient oxidative phosphorylation in CRC. Adenomas with LGD had the lowest mutation frequency and lowest amount of mtDNA relative to nuclear DNA. We postulate that mutated mtDNA may be preferentially degraded to avoid accumulation of mutations which may impair cellular metabolism at this early stage in neoplasia. Degrading damaged mtDNA may assume a lower level of priority in HGD & CRC.

709 Reproducibility of Villous Component and High Grade Dysplasia in Colorectal Adenomas < 1 cm: Implications for Endoscopic Surveillance

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Background: In patients with 1 or 2 adenomas < 1 cm, the presence of high grade dysplasia (HGD) or villous component (VC) define an advanced adenoma (AA). Current consensus guidelines recommend that patients with AA undergo more intense post-polypectomy surveillance. In these clinical situations, the interobserver reliability in determining VC and HGD would play a major role in the credibility of these consensus guidelines. Therefore, the purpose of this study was to evaluate interobserver variability of VC and HGD in polyps < 1 cm before and after the development of consensus criteria among gastrointestinal (GI) pathologists.
Design: 5 expert GI pathologists evaluated 107 colorectal adenomas < 1 cm independently and classified them into tubular adenomas (TA), or adenomas with a villous component (A-VC) and into low grade dysplasia or HGD. After round 1; a consensus conference was held and consensus criteria for VC and HGD were developed by group review. The same set of 107 slides were re-reviewed independently by the same 5 GI pathologists. Interobserver variability utilizing kappa statistical analysis before and after the application of consensus criteria was assessed. A one-sided z-test was used to determine if kappa scores increased after the consensus conference.
Results: Interobserver agreement both before and after the consensus conference was poor for assessment of A-VC, HGD and AA (either A-VC or HGD).

Table 1: Kappa Indices for Interobserver Agreement

Feature	Kappa	P	95% CI	Interobserver agreement*
Pre-consensus diagnosis				
A-VC	0.21	<0.001	0.15 - 0.27	Poor
HGD	0.26	<0.001	0.20 - 0.32	Poor
AA	0.29	<0.001	0.23 - 0.35	Poor
Post-consensus diagnosis				
A-VC	0.37	<0.001	0.31 - 0.43	Poor
HGD	0.31	<0.001	0.25 - 0.37	Poor
AA	0.34	<0.001	0.28 - 0.40	Poor

* agreement beyond chance; poor: kappa<0.40 moderate: 0.40<kappa<0.75 excellent: kappa>0.75 ; Improvement in kappa (one-sided z-test): P=0.038 A-VC; P=0.11 HGD; P=0.14 AA

Conclusions: These data raise significant doubt regarding pathologists' ability to reliably discriminate advanced elements (A-VC or HGD) in adenomas < 1 cm, and hence calls in to question the validity of basing clinical decisions on this distinction.

710 Web-Based Teaching, a Contemporary Diagnostic Method of Educating Canadian Pathologists on Gastrointestinal Stromal Tumors (GIST)

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Background: Accurate diagnosis and risk stratification of GIST has become increasingly significant in view of rapidly changing treatment options including the emergence of adjuvant therapy. Following the NIH consensus in 2002, several risk stratification systems have been published, including the commonly used classification proposed by Miettinen et al (2006). Moreover the role of molecular testing for GIST is increasingly being considered as a prognostic factor in GIST. The rapidly evolving literature in this field has necessitated the development of a forum for dynamic, continuous education of pathologists to facilitate dissemination of up-to date information on the diagnosis of GIST.

Design: STEP (Stromal Tumours Evaluation For Pathologists) Program was developed at The Ottawa Hospital, Ottawa, ON as a tool to enhance education of pathologists regionally, provincially and nationally. Conceived as a one hour web-based interactive lecture using virtual microscopy, it includes an overview of GISTs, up-to-date diagnostic methods, differential diagnosis, molecular analysis and synoptic reporting. All participants provide independent assessment and evaluations upon completion of the course and have online access to the teaching set for six months post conference.

Results: In 2010, 5 webinars were completed, 4 in English and 1 in French, reaching 60 pathologists. Distribution of participants was: 17 (28%) Western Canada, 43 (72 %) Eastern Canada; 40 (66%) community hospitals and 20 (33%) academic centers. The overall satisfaction was rated as very good to excellent among 43 pathologists (72%). The program was rated with overall scores ranging from 4 (met expectations) to 5 (exceeded expectations) (mean 4.4).The mean scores for: meeting the learning needs, enhancing the learning process, relevance to the practice and digital pathology as an educational tool were 4.8, 4.6, 4.8 and 4.4 respectively.

Conclusions: Web based teaching using real-time, live digital slide conferencing and discussion, augmented by web-based teaching sets (accessible post conference) is a viable education tool with broad applicability, reaching both community and academic professionals over a large geographic distribution. In the rapidly changing fields of GIST diagnosis and management, this is a valuable and reproducible resource.

711 Histologic Subtypes and Pathologic Features of Epithelioid Malignant Peritoneal Mesotheliomas

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Background: There is currently no grading or staging system for malignant peritoneal mesothelioma (MPM). Most MPM are characterized into epithelioid, biphasic and sarcomatoid types based primarily on pleural mesothelioma classification schemes. While the histologic subtype of many tumors critically impacts patient care and prognosis, only recently has an association been made between the histologic subtypes of epithelioid pleural mesotheliomas and clinicopathologic features. We sought to explore histopathologic variables of epithelioid MPM that may have clinical significance.

Design: 24 cases of chemotherapy-naive epithelioid MPM were reviewed. Mean patient age was 54.6 yrs with 5:1 male:female ratio. Histologic subtypes and pathologic parameters were recorded. To assess for *CDKN2A*/p16 deletions, FISH was performed using a CEP9 probe and a *CDKN2A*/p16 locus-specific probe.

Results: The following histological subtypes (predominant pattern) were observed: **Papillary** (no secondary subset), n=3, 12.5%; **Tubular** (pure tubules or acini), n=4, 17.5%; **Tubulopapillary** (varying combinations of tubules and papillae), n=8, 33%; **Micropapillary** (papillary tufts lacking a central fibrovascular core), n=1, 4%; **Trabecular** (arranged in thin cords), n=0, 0%; **Solid** (sheets/nests of polygonal to round tumor cells), n=8, 33%; and **Pleomorphic** (anaplastic or with prominent giant cells), n=0, 0%. The pathologic parameters included: Depth of invasion (superficial < 0.5 mm, n=6, vs. deep > 0.5 mm, n=18); Mitotic count (<5/50 HPF, n=17, vs. > 5/50 HPF, n=7); Nuclear grade (low, n=9, vs. high, n=15); Lymphovascular invasion (LVI), n=7; Lymph node metastasis (LNM), n=4/11; Stromal desmoplasia, n=15; and Lymphocyte host response (LHR), none-mild (n=17), moderate-severe (n=7). Interestingly, LNM was present in solid and tubulopapillary subtypes with deep invasion, low mitotic index, stromal desmoplasia, mild to severe LHR and no p16 deletion. By contrast, papillary subtype showed superficial invasion, no stromal desmoplasia, none to mild LHR, no LNM and p16 deletion in 2/3 cases.

Conclusions: Our preliminary analysis reveals that specific histologic subtypes of epithelioid MPM appear to correlate with clinicopathologic parameters. These histologic subtypes and pathologic features may help in the development of a staging and grading system for MPM. Study of additional cases and correlation with outcome will strengthen these observations and are currently underway.

712 Utility of Sessile Serrated Adenoma as a Marker of Metachronous Colorectal Carcinoma

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Background: Identification of cancer precursors facilitates early detection of lesions with high risk of cancer. It is is well-established for conventional adenomas. The clinical significance of the sessile serrated adenoma (SSA) as a marker of metachronous colorectal carcinoma (MCRC) remains elusive, despite mounting evidence implicating the SSA as a significant player in the serrated carcinogenesis. The risk of MCRC (CRC detected more than 12 months after the index intervention) in non-dysplastic SSA is here evaluated.

Design: The base-line material comprised 219 SSAs, identified among 8.834 colorectal polyps originally coded as hyperplastic polyp (HP), as described previously [Mohammadi et al Pathol Res Pract 2011;207:410]. Out-come information within 17 months to 10 years of the index intervention was searched through pathology databases.

Results: During the follow-up period, 3 patients with SSA, all males, aged 45, 56, and 67 years, were diagnosed with MCRC, 1.5, 2, and 10 years, respectively. Further details on index polyp and MCRC are given in table 1.

Table 1.

Index polyp				MCRC			
	Mut. Status	Site	Size mm	Histologic type	Mut. Status	MLH1 protein	Site
1.	Braf-WT	Rectum	3	Glandular	NP	Retained	Rectum
2.	Braf-mut.	Asc.* Sigm.*	9	Glandular	Braf- and Kras WT	Retained	Rectum
3.	Braf-WT	Rectum	11	Mucinous	Kras-mut (12 wasp)	Retained	Left colon

Mut.: mutation; NP: not performed (insufficient material); Asc.: ascending colon; Sigm.: sigmoid colon; *two synchronous SSAs; WT: wild type.

Conclusions: The three examples of CRC identified were considered MCRC rather than lesions missed on the index endoscopy. Thus, index colonoscopy was performed under optimal conditions and the anatomic regions with CRC were all well visualized. Merely 1.4% of our study cases developed MCRC. This low figure was unexpected considering the following two facts: 1) the polyps were initially diagnosed as HP, implying that control surveillance was generally not offered, 2) the inconspicuous, sessile quality of SSA increases the risk of incomplete sampling. Apparently, such putative SSA-remnants were not prone to progress to MCRC, perhaps due to absence of dysplasia. Neither large size nor proximal site of the index polyp was consistent features of the MCRC cases. Of further note is the lack of convincing markers of a methylator pathway as might be anticipated if the index polyps played a role in the CRC development. Given the small sample size of this analysis, these observations need, however, to be addressed in additional large-scale studies prior to defining appropriate management strategies for this group of patients.

713 Glypican-3 Expression in Gastrointestinal and Pancreatic Carcinomas

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Background: Glypican-3 (GPC3), a cell membrane bound proteoglycan that can be overexpressed in certain malignancies, has been particularly linked to hepatocellular carcinoma (HCC). GPC3 is currently used as an immunohistochemical (IHC) marker of HCC but its expression in carcinomas of the gastrointestinal (GI) tract and pancreas, a common source of liver metastasis, has not been studied in detail. This study aims to evaluate GPC3 expression in carcinomas of the GI tract and pancreas.

Design: We examined IHC expression of GPC3 in 158 carcinomas including 52 adenocarcinomas (ADCA) of the pancreas and GI tract (8 esophagus, 8 stomach, 2 small bowel, 12 colon), 29 squamous cell carcinomas (SCC) of the GI tract (19 esophagus and 10 anus), 65 neuroendocrine carcinomas (NECA) of the pancreas and GI tract (2 esophagus, 8 stomach, 15 small bowel, 14 colon), and 12 pancreatic acinar cell carcinomas. Two control groups (32 HCC and 16 intrahepatic cholangiocarcinomas) were also stained with GPC3. All tumors were scored for cytoplasmic and membranous staining. A tumor was considered positive for GPC3 if >10% of neoplastic cells showed strong cytoplasmic and membranous immunoreactivity.

Results: 22 of 158 extrahepatic tumors (14%) were positive for GPC3. In the hepatic tumor group, none (0/16) of the cholangiocarcinomas and 24/32 (75%) of HCC were GPC3 positive. In extrahepatic tumors, GPC3 immunoreactivity was most frequent in pancreatic acinar cell carcinomas (58.5%), followed by SCC (27.5%), and ADCA of theGI tract (20%). All pancreatic ADCAs and NECAs were GPC3 negative. GPC3 expression correlated with poor differentiation in HCC but no such correlation was present in extrahepatic tumors. GPC3 positivity was more frequent in upper GI tract ADCA (28%) compared to lower GI tract ADCA (8.5%). In all tumors including HCC, GPC3 expression showed no significant correlation with tumor size. In all positive tumors, GPC3 immunoreactivity was characterized by strong cytoplasmic and membranous staining of 20-100% of the neoplastic cells. Diffuse positivity occupying 100% of tumor cells was only observed in HCC and pancreatic acinar cell carcinoma. SCCs typically demonstrated a predominant peripheral/basal distribution of GPC3 immunoreactivity.

Conclusions: GPC3 immunoreactivity occurs frequently in carcinomas of the GI tract and pancreas and is most often observed in pancreatic acinar cell carcinoma, SCC, and ADCA of the upper GI tract. As these tumors commonly metastasize to the liver, this can lead to a mistaken diagnosis of HCC; GPC3's lack of specificity should be recognized when evaluating tumors involving the liver to avoid potential diagnostic pitfalls.

714 HER2 Protein Overexpression in Gastric Adenocarcinoma: The Relationship between Its Histological and Immunohistochemical Profiles in a Japanese Population

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Background: Cases of gastric cancer with HER2 gene amplification and protein overexpression are treated by trastuzmab, and the frequency of these cases is reported to be 10-40% of gastric cancer. However, sufficient investigation has not been conducted regarding which histological types are found in cases of gastric cancer with HER2 expression. Therefore, we examined the frequency and the correlation between the histological types in HER2-expressing gastric cancers in Japanese patients.

Design: We reviewed a pathology database and collected all of the gastrectomy cases from April 2007 to June 2011. Then, a total of 132 consecutive cases were examined for their histological types and immunohistochemical HER2 expression. HER2 immunoreactivity was graded on a scale from 0 to 3 according to the ToGA study

criteria. Cases with HER2 expression of 2+ or more were considered to possess HER2 expression. We classified the histologic types based on Lauren's classification and the Japanese cancer classification.

Results: The number of cases in each histological type is shown in Table 1.

The number of HER2 expression cases in each histological type

HER2	pap	tub1/tub2	por1/por2	muc-w	muc-p	sig	NEC
3+	6	3/4	0/2	0	0	0	0
2+	1	1/3	0/1	0	0	0	0
1+	2	4/8	0/4	1	1	1	0
0	2	16/27	4/34	2	0	4	1
Total	11	24/42	4/41	3	1	5	1

The intestinal type of Lauren's classification includes pap, tub1 and tub2; the diffuse type includes por1, por2, muc-w, muc-p, sig and NEC. HER2 expression was significantly higher in the intestinal type of Lauren's classification (intestinal type, 18 out of 77cases, 23.5%; diffuse type, 3 out of 55 cases, 5.5%; overall, 21 out of 132 cases, 15.9%) and the papillary type in the Japanese classification (7 out of 11 cases; 63.6%). Significantly higher HER2 overexpression was noted in gastric cancers located at the distal stomach.

Conclusions: HER2 positivity was significantly higher in the intestinal type of Lauren's classification, and especially in the papillary type in the Japanese classification. It is interesting that the papillary type of gastric cancer is commonly observed in the proximal region of the stomach, and also that gastric cancer with the higher HER2 overexpression was frequently noted in the proximal portion of the stomach.

715 Liver Fatty Acid Binding Protein (L-Fabp): A Genetic Modifier of Murine Intestinal Polyposis

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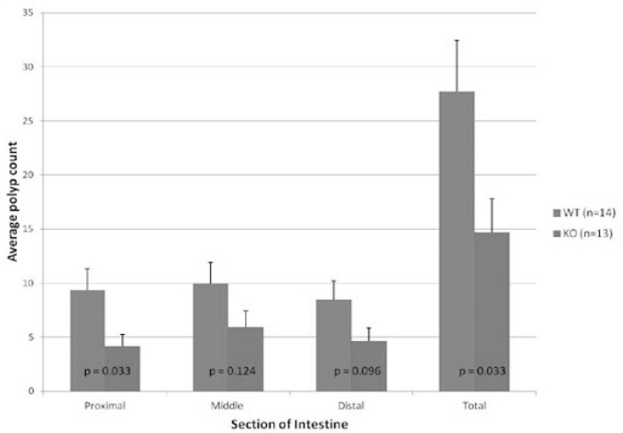
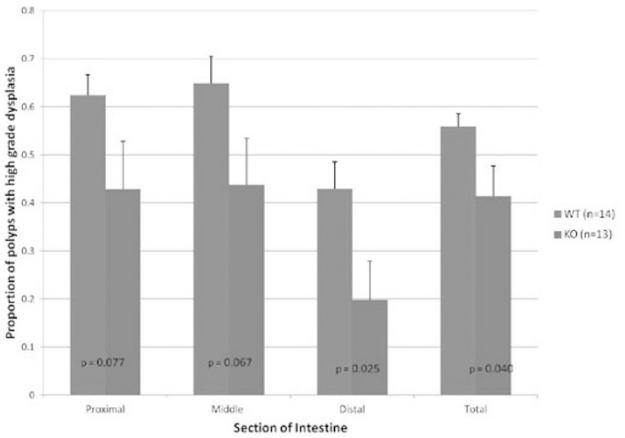
Background: Consumption of a high-fat diet is linked to an increased prevalence of colonic adenomas in the setting of obesity and insulin resistance. We examined the role of L-Fabp, a cytosolic transport protein that regulates trafficking of long chain fatty acids, bile acids, and cholesterol, as a genetic modifier(GM) of diet induced obesity. We predicted that L-Fabp is a potential GM linking obesity and dietary fat intake with adenoma susceptibility.

Design: *L-Fabp* $^{-/-}$ mice are protected against high fat diet-induced obesity. *Apc* $^{min/+}$ mice carry a heterozygous deletion in the APC gene and develop intestinal adenomas with loss of heterozygosity. We crossed *L-Fabp* $^{-/-}$ mice into the *Apc* $^{min/+}$ background to examine adenoma development and progression in *L-Fabp* $^{-/-}$, *Apc* $^{min/+}$ mice. Mice were fed a 10% fat diet, sacrificed at 100±4 days of age. Polyp number, regional distribution and histological characterization are undertaken to determine high grade dysplasia (HGD) for each genotype.

Results: The total number of polyps, and polyp area in proximal, mid and distal SI were significantly reduced in *L-Fabp* $^{-/-}$, *Apc* $^{min/+}$ compared to *Apc* $^{min/+}$ mice, with a wild type L-Fabp allele. The average total polyp count was 52.3 ± 5.5 in *Apc* $^{min/+}$ mice versus(vs) 35.5 ± 5.2 in *L-Fabp* $^{-/-}$, *Apc* $^{min/+}$ mice with protection against polyp development most significant in the proximal intestine.



The proportion of polyps with HGD was also reduced in *L-Fabp* $^{-/-}$, *Apc* $^{min/+}$ vs *Apc* $^{min/+}$mice (p=0.04). Protection against development of HGD was most significant in distal SI (p=0.025).



Conclusions: *L-Fabp* $^{-/-}$ *Apc* $^{min/+}$ mice are protected against intestinal polyposis, raising the possibility that alterations in the metabolism and/or trafficking of fatty acids, cholesterol or bile acids modulates adenoma growth.

716 Gastric Heterotopia in the Proximal Esophagus ("Inlet Patch"): Association with Adenocarcinomas Arising in Barrett Mucosa

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Background: The prevalence of inlet patches and their associations have been studied in tertiary care facilities; data generated from outpatient clinics are lacking. We designed this study to assess prevalence, demographics, and associated clinicopathologic features of inlet patches in patients who had esophagogastroduodenoscopy (EGD) in US outpatient settings.

Design: Using a large pathology database that includes biopsy specimens from US outpatient centers and relevant clinical and endoscopic information, we extracted pertinent data from patients who underwent EGD with biopsy between 1/2008 and 12/2010. Patients with inlet patch were identified by diagnostic codes and free-text searches. Controls were all patients who had EGD during the same period but no diagnosis of gastric heterotopia in the esophagus.

Results: Inlet patch was found in 870 (0.18%) of 487,229 unique patients (median age 56 years, 52.8% male) and was more common in males (p<0.0001). Dysphagia and odynophagia, globus, and upper respiratory symptoms were significantly more common in patients with inlet patch than in those without. The impression of a lesion in the upper esophagus was conveyed to the pathologist in 724 of the 870 patients (sensitivity 83.2%) with inlet patch. The inlet patch consisted of oxyntic mucosa lined by foveolar-type gastric epithelium; intestinal metaplasia was seen in 9 cases (1.0%) and dysplasia in none. *H. pylori* gastritis was less prevalent in patients with inlet patches than in controls (4.7% *vs.* 9.0%; p<0.001). Barrett mucosa (BM) was more common in men with inlet patches than in those without (15% *vs.* 11%; p<0.001). Of 31,401 patients with BM and no inlet patch, 654 (2.1%) had low-grade dysplasia; 342 (1.1%) had high-grade dysplasia; and 205 (0.7%) had adenocarcinoma. In comparison, of the 84 patients with both BM and an inlet patch, 1 (1.2%) had high-grade dysplasia, 1 (1.2%) had low-grade dysplasia, and 3 (3.6%) had adenocarcinoma (OR 5.64, 95% CI 1.77 - 18.0; p<0.001).

Conclusions: The prevalence of inlet patches in a tertiary care setting was lower than reported in prospective studies. Inlet patches were significantly associated with male gender, dysphagia, upper respiratory complaints, globus, Barrett mucosa, and adenocarcinomas arising in Barrett esophagus. Further studies will be needed to determine if patients with inlet patches and Barrett mucosa benefit from increased surveillance.

717 Ectopic Crypt Formation and Other Histological Parameters in Relation to BRAF and KRAS Mutation Status of Dysplastic Serrated and Non-Serrated Colorectal Polyps

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Background: Activating BRAF/KRAS mutations are determinants in the serrated pathway of carcinogenesis and are present in a variety of neoplastic polyp precursors. To date, no studies have related specific morphologic features of dysplastic serrated polyps (DSP) to oncogene status. Thus, we evaluated histological features of putative DSP and correlated them to BRAF/KRASmut status.

Design: 118 polyps, including 78 that exhibited both a serrated component and dysplasia and/or eosinophilic cell atypia and 34 conventional adenomas, the latter by size category, were sequentially accessioned from our dept. files. DNA was assayed for KRAS (codon 12&13) and BRAF (exon 15) mutations. The following histological parameters were assessed independently of the WHO diagnostic category of polyp: 1) Background (contiguous) hyperplastic polyp or SSA/P (HP/SSAP), 2) Presence or absence of ectopic crypt formation (ECF), 3) %Serration, 4) %Eosinophilic Cell Atypia, 5) %Villi and 6) High grade dysplasia/intramucosal adenocarcinoma (HGD). The reviewer was blinded to the BRAF/KRASmut status of the polyps.

Results: Among all polyps studied (118), ECF was present in 17/38 (44.7%) KRASmut, 13/50 (26%) BRAFmut and 4/30 (13.3%) KRAS/BRAFwt. ECF correlated significantly with villous component >25% (27/49 (55.1%) vs 7/69 (10.4%) [p<.0001]) but not with

the presence of serration (58/93 (62.4%) vs 10/25 (40%) [p=ns]). Among DSP only, background HP/SSAP was present in 23/ 50 (46%) that were BRAFmut compared to 3/38 (7.9%) KRASmut (p<.0001). Among DSP without background HP/SSAP, ECF did not correlate with oncogene mutation status (8/15 (53.3%) KRASmut vs 10/21(47.6%) BRAFmut). The frequency of HGD was 2/22(9.1%) and 2/19(10.5%) in KRASmut and BRAFmut DSP without HP/SSA, respectively, compared to 3/29(10.3%) BRAFmut DSP with contiguous HP/SSAP (p=ns).

Conclusions: The presence of ECF correlated with villous morphology but not serration and was not specific to either KRAS or BRAF mutations in DSP. Furthermore, ECF did not discriminate KRASmut DSP from BRAFmut DSP or KRASmut non-serrated adenoma. Contiguous HP/SSAP, on the other hand, was a robust indicator of BRAFmut status. The overall findings have implications for the classification of DSP and question the validity of ECF as the histological hallmark of TSA.

718 Do Molecular Features of Colorectal Cancers Change Abruptly at Splenic Flexure?

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Background: Colorectal cancer is typically classified into rectal, distal colon, and proximal colon cancer. Tumor molecular features in each of these locations have been shown to be different from those in the other sites. Considering a possible role of bowel contents (including microbiome) in carcinogenesis, we hypothesized tumor molecular features might gradually change along the bowel, rather than abruptly change at splenic flexure.

Design: We used 1443 colorectal cancers, and examined the frequencies of molecular features [CpG island methylator phenotype (CIMP), microsatellite instability (MSI), and *BRAF* and *KRAS* mutations] along the bowel segments. Linearity and non-linearity of molecular relations along the bowel were statistically tested by multivariate logistic regression models, adjusting for potential confounders.

Results: The frequencies of CIMP-high, MSI-high, and *BRAF* mutation gradually increased from rectum (<2.3%) to ascending colon (36-40%), followed by falls in the cecum (12-22%).

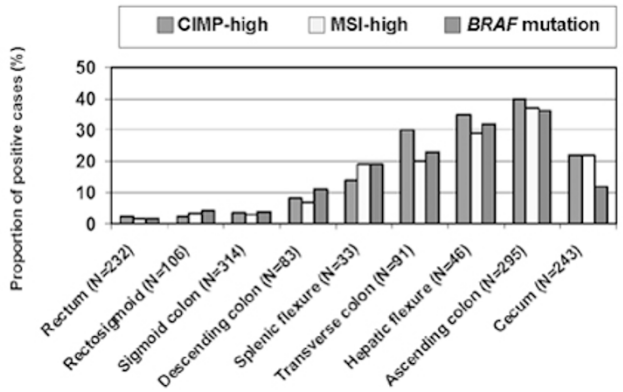

Figure 1

By linearity tests, these molecular relations were significantly linear along the bowel subsites from rectum to ascending colon (p<0.0001), and there was no evidence for non-linearity (p>0.12 by likelihood ratio tests). Notably, cecal cancers showed the highest frequency of *KRAS* mutations (52% in cecum vs. 27-35% in the other sites from rectum to ascending colon; p<0.0001).

Conclusions: The frequencies of CIMP-high, MSI-high, and *BRAF* mutation in colorectal cancer increased gradually along the bowel from rectum to ascending colon. Our novel data challenge the common conception of discrete molecular features of proximal vs. distal colorectal cancers, and have substantial impact on pathological, translational, and epidemiology research, which has typically been performed with dichotomous classification of proximal vs. distal tumors.

719 Large-Scale Genome-Wide mRNA Expression Profiling of 1003 Colorectal Cancers

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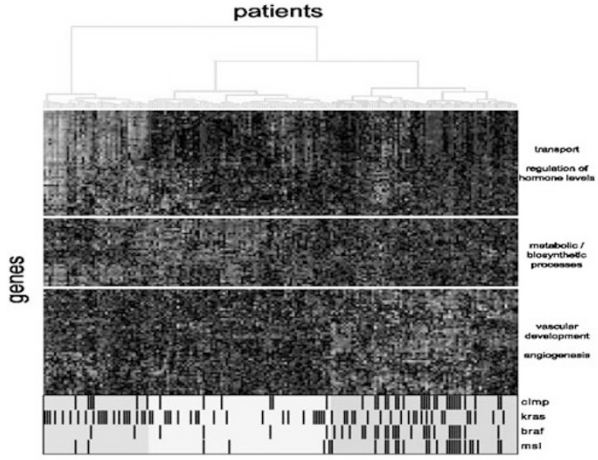
Background: Over 20 million archival tissue samples are stored annually in the U.S. alone, as formalin-fixed, paraffin-embedded (FFPE) tissue blocks. If we can unfold information on genome-wide expression signatures from FFPE tissue, such tissue samples can offer an enormous resource to link the molecular mechanisms of disease to its impact on patient outcome. In this study, our aim was to identify reproducible colorectal cancer subtype clusters and genome-wide mRNA expression signatures in 1003 colorectal cancers, using DASL (cDNA-mediated Annealing, Selection, Extension, and Ligation) microarray technology.

Design: We analyzed mRNA expression of over 24,000 genes in 1003 colorectal cancers using the DASL HumanRef8 v3 (24K probes) microarray. We also analyzed MSI, *KRAS, BRAF*, and *PIK3CA* mutations, and other tissue markers as well as clinical outcome of patients.

Results: We established extensive quality check pipeline. After thoroughly validating expression profiling data, we applied complete linkage hierarchical clustering to tumor samples using Pearson correlation and assessed associations with sample metadata. Each metadata variable was tested using an appropriate association test for its class (linear regression, F-test, or chi-square test), and p-values were adjusted for false discovery rate. Supervised clustering was conducted with a randomly held-out training set and a validation (test) set to confirm subtype clustering. We were able to identify colorectal cancer subtypes, which were associated with *KRAS* mutation, or with *BRAF* mutation, MSI and CpG island methylator phenotype (CIMP).



Each subtype cluster showed a unique signature of up- and down-regulation of functionally-relevant gene sets.

Conclusions: Genome-wide mRNA expression analysis on 1003 colorectal cancers has enabled an identification of colorectal cancer molecular subtypes and differentially expressed genes. We anticipate genome-wide expression studies being broadly applicable to future studies of molecular events in large cancer patient populations.

720 Concomitant Presence of PIK3CA Mutations in Both Exons 9 and 20 Predicts Aggressive Behavior of Colorectal Cancer

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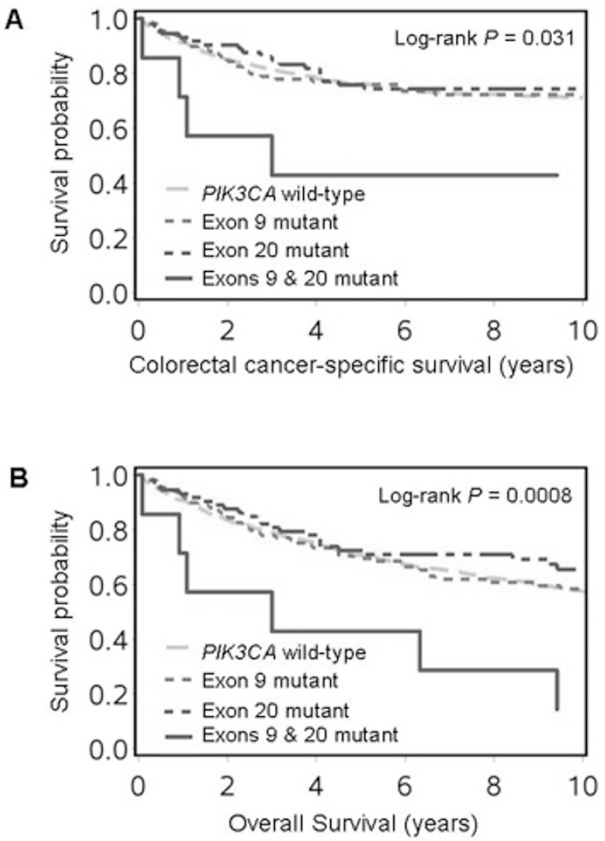
Background: *PIK3CA* mutation plays an important role in colorectal carcinogenesis. Experimental evidence suggests that *PIK3CA* exon 9 and exon 20 mutations trigger different biological effects, and that concomitant mutations in both exons 9 and 20 lead to enhanced tumor promoting effects. Thus, we hypothesized that *PIK3CA* exon 9 mutation and exon 20 mutation might have differential effects on colorectal cancer behavior, and that concomitant *PIK3CA* mutations in both exons 9 and 20 might confer aggressive tumor behavior.

Design: We sequenced *PIK3CA* by pyrosequencing on 1170 colorectal cancers. Cox proportional hazards model was performed to compute mortality hazard ratio (HR) by *PIK3CA* status, adjusting for clinical, stage and tumor variables including microsatellite instability, and *KRAS* and *BRAF* mutation status.

Results: *PIK3CA* mutation was detected in 189 (16% of 1170) cases; 109 in only exon 9, 73 in only exon 20 and 7 in both exons 9 and 20. Compared to *PIK3CA* wild-type cases, patients with concomitant *PIK3CA* mutations in both exons 9 and 20 experienced significantly higher colorectal cancer-specific mortality [univariate HR = 2.84; 95% confidence interval (CI), 1.05-7.69; multivariate HR = 3.51; 95% CI, 1.28-9.62), and higher overall mortality (univariate HR = 3.37; 95% CI, 1.58-7.15; multivariate HR = 2.68; 95% CI, 1.24-5.77).

Figure 1



In contrast, the presence of *PIK3CA* mutation in only either exon 9 or 20 was not significantly associated with patient survival.

Conclusions: The presence of concomitant *PIK3CA* mutations in both exons 9 and 20 in colorectal cancer is associated with poor prognosis. Our data support a more potent oncogenic effect of *PIK3CA* double mutants in both exons 9 and 20.

721 Silencing of P16 ^{Ink4a} in Colorectal Cancer Is Associated with *BRAF* Mutation and Independent of Microsatellite Instability

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Background: Sporadic colorectal cancer (CRC) with high-level microsatellite instability (MSI-H) is strongly associated with *BRAF* mutation and the CpG island methylation (CIMP) phenotype. However, the causal relationship between *BRAF* mutation and CIMP is not well understood. p16, a cell cycle inhibitor, is a specific marker of CIMP phenotype. Silencing of p16 is often caused by promoter methylation of CDKN2A gene in CRC. Recent study suggested that p16 is a marker of oncogene-induced senescence and is expressed in the precursor lesions of the serrated neoplasia pathway. We hypothesize that p16 silencing in CRC correlates with *BRAF* mutation independently of MSI-H status.

Design: Consecutive colectomy specimens for CRC were collected from 98 patients. *KRAS* mutations (codons 12/13), *BRAF* mutation (V600E), MSI status (NCI panel of 5 markers) and p16 immunohistochemical expression were evaluated on representative paraffin blocks. Additional staining for p16 was also done in normal colon, conventional adenomatous polyps (APs) and sessile serrated adenomas (SSAs). A case was considered positive for p16 if 5% or more cancer cells had nuclear and cytoplasmic staining.

Results: Based on the mutation status of *BRAF* and *KRAS*, 98 CRC cases were classified into 3 groups [Table 1]. Group 1(n=16, mean age 76) harbored *BRAF* mutation. Lack of p16 expression was seen in all but one (94%, n=15) cases, of which 10 were MSI-H and 5 were MSS (microsatellite stable). Group 2 (n=33, mean age 68) exhibited *KRAS* mutations. Five cases (15%) did not express p16. These 5 cases were either MSI-H (n=1) or MSS (n=4). Group 3 (n=49, mean age 64) contained wild types *BRAF* and *KRAS* genes. Ten cases (20%) lacked the expression of p16, of which nine were MSS and one was MSI-H.

Normal colonic mucosa (n=5) was negative for p16. Patchy p16 positivity was present in the lower crypts of SSA (n=6) and various areas in AP (n=8).

Table 1 Expression of p16 in CRC

	Group 1	Group 2	Group 3
Mean age (range)	76 (58-89)	68 (29-92)	64 (22-93)
BRAF	Mutated	Wild type	Wild type
KRAS	Wild type	Mutated	Wild type
Total case no.	16	33	49
p16 negative case no	15 (94%) ¶§	5 (15%) ¶	10 (20%)§

¶§ p value<0.001 (chi square test)

Conclusions: Silencing of p16, a specific CIMP marker, in CRCs is highly associated with *BRAF* mutation independently of microsatellite instability. These findings suggest that CIMP phenotype is a property of *BRAF* mutation rather than MSI status. Future studies of p16 and other markers related to *BRAF* or *KRAS* mutations in CRC may increase our understanding on the biological behaviors and cell cycle controls of CRC.

722 Sporadic Colonic Adenocarcinomas with a High Degree of Microsatellite Instability (MSI-H) Do Not Show Evidence of Wnt Signaling Abnormalities

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Background: Most sporadic colonic adenocarcinomas arise from adenomas *via* dysregulation of the Wnt signaling pathway, as evidenced by nuclear B-catenin immunostaining, and are microsatellite stable (MSS). Sessile serrated polyps are not dysplastic, but do show *BRAF* mutations and DNA methylation, and have been implicated in the serrated neoplastic pathway. Onset of MSI-H in serrated polyps coincides with progressive dysplasia and leads to sporadic cancers with *BRAF* mutations, MSI-H, and widespread promoter hypermethylation. Wnt signaling and MSI-H are generally considered to be mutually exclusive cancer progression models. However, several studies have described abnormal B-catenin staining in serrated polyps, suggesting a role for Wnt signaling in development of sporadic MSI-H cancers. We evaluated B-catenin staining in sporadic colon cancers to determine whether Wnt activation promotes carcinogenesis in MSI-H tumors.

Design: 43 colon cancer resection specimens were evaluated. All patients were >60 years old with tumors proximal to the splenic flexure. Immunostains for MLH1, PMS2, MSH2, MSH6, and B-catenin were performed. Aberrant B-catenin staining was defined by moderate-to-strong nuclear, and diminished membranous, staining in ≥30% of the tumor cells. Tumor DNA was extracted and assessed by PCR for MSI using 5 mononucleotide repeats (BAT-25, BAT-26, NR-21, NR-24, MONO-27). Pyrosequencing was carried out to assess for *BRAF* codon V600E mutations.

Results: 23 tumors showed complete loss of MLH1 and PMS2 staining with MSI-H, including 16 (70%) with *BRAF* mutations (M/F=6/17, mean age: 72 years). Only 1 (4%) MSI-H tumor displayed aberrant B-catenin staining and it was *BRAF* wild-type. Twenty cancers had preserved staining for DNA repair proteins, MSS, and wild-type *BRAF* (M/F: 11/9, mean age: 71 years). Of these, 75% showed abnormal B-catenin staining, which was significantly more than MSI-H tumors (p<0.0001).

Conclusions: MSS colonic carcinomas are usually *BRAF* wild-type and show nuclear localization of B-catenin with diminished cell membrane staining, reflecting aberrant Wnt signaling. Abnormal B-catenin staining is rare in sporadic MSI-H cancers and, in fact, we found no abnormal B-catenin expression among MSI-H cancers with *BRAF* mutations. Despite reports of nuclear B-catenin accumulation in serrated polyps, we conclude that Wnt signaling alterations are unlikely to be biologically important in sporadic MSI-H tumors.

723 "Basal Cell Carcinoma Where the Sun Doesn't Shine" – A Clinicopathologic Analysis of Basal Cell Carcinoma of the Anal Region and Its Distinction from Basaloid Squamous Cell Carcinoma

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Background: Basal cell carcinoma (BCC) of the anal region is rare and morphologically difficult to distinguish from basaloid squamous cell carcinoma (BSCC), particularly on biopsies. This distinction has therapeutic and prognostic implications. We reviewed morphologic features and determined the utility of Ber-EP4, bcl-2, p63, CK5/6, p16, and SOX-2 as diagnostic tools.

Design: A total of 9 BCCs and 15 BSCCs from the anal region between Jan 1993 – Jul 2011 were reviewed and analyzed for the following features: location, connection with surface epithelium, squamous differentiation, peripheral palisading, tumor retraction, mitosis/atypical mitosis, necrosis, cystic change, inflammatory response, and presence of an in-situ component. Cytoplasmic and membranous expression of Ber EP4, bcl-2, CK5/6, nuclear expression of p63, SOX-2 and nuclear and/or cytoplasmic expression of p16 were scored in a semi-quantitative manner: 1 + 1-10%, 2 + 11-50%, 3 + >50%. Statistical significance was determined using Chi^2 analysis and Student t-test.

Results: All BCCs were in the perianal region while all BSCCs were in the anal canal/anorectum. Patients with BCC were slightly older (median age 71 yrs vs. 62 yrs.; p=0.09). Both had a female preponderance (F:M = 4:1 BCC vs 2:1 BSCC). BCC subtypes included nodular (4), nodular/micronodular (2), nodular/infiltrative (1), nodular/superficial (1), and superficial (1). Retraction artifact was the only statistically significant histologic feature of BCC compared with BSCC (88% vs 26%; p=0.04). Peripheral palisading of BCC approached significance (88% vs 40%; p=0.07). Atypical mitoses were more common in BSCC (71% vs 11%; p=0.05). An in-situ component was exclusively present in BSCC and noted in 6/15 cases. BCC had 2-3+ immunoreactivity for Ber-EP4 (BCC 100% vs BSCC 40%; p=0.0007) and bcl-2 expression (BCC 100% vs BSCC 33%; p=0.005). 2-3+ immunoreactivity for p16 was only seen in BSCC (BCC 0% vs. BSCC 93%; p<0.0001). All BCCs showed patchy, cytoplasmic p16 expression at the periphery of the tumor nests. SOX-2 expression was exclusive to BSCC (BCC 0% vs. BSCC 93%; p<0.0001). There was no difference in p63 and CK5/6 expression.

Conclusions: Perianal location, retraction artifact, and lack of atypical mitoses are histologic features that help distinguish BCC from BSCC. An in-situ component, when present, supports the diagnosis of BSCC. Immunostains are extremely helpful as diffuse Ber-EP4 and bcl-2 expression is a feature of BCC and BSCC is typified by diffuse p16 and SOX-2 expression.

724 Expression of HPV L1 Capsid Protein in Anal Condyloma and Anal Squamous Intraepithelial Neoplasia (ASIN)

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Background: Morphologic evaluation and grading of anal dysplasia on biopsies remains problematic, especially in cases with limited sample. Although a handful of studies have evaluated the role of p16 as a surrogate biomarker in the diagnosis of anal squamous dysplasia, the expression of HPV L1, a capsid protein that confers viral entry ability, has never been analyzed in this regard. The aim of our study is 1) to systematically study the expression of HPV L1 capsid protein in anal condyloma and anal squamous dysplasia and 2) to correlate HPV L1 expression with p16 expression and grade of dysplasia (low grade/high-grade ASIN).

Design: A total of 80 anal lesions were retrieved and histologically reviewed. Expression of nuclear HPV L1 was studied using combined immunohistochemistry and in situ hybridization technique from Advanced Medical Science. p16 immunohistochemistry was performed with antibody from Ventana (clone E6H4). Two p16 immunostaining patterns were recorded: diffuse and full thickness staining and patchy/focal staining. HPV L1 and p16 expression was correlated with grade of dysplasia using Chi^2 analysis.

Results: Eighty anal lesions included 11 condyloma, 39 low-grade ASIN (AIN1) and 30 high-grade ASIN (AIN2-3). Expression of HPV L1 was seen in 31.3% (25/80) of all anal lesions, including 64% (7/11) condylomas, 46% (18/39) low-grade ASIN and 0% of high-grade ASIN lesions. The difference of HPV L1 expression between low-grade and high-grade ASIN lesions was statistically significant ($p<0.001$). p16 expression was identified in 86.3% (69/80) of all anal lesions. Of these, 38 lesions showed patchy staining pattern and 31 had diffuse staining pattern. Patchy p16 staining pattern was observed in 64% of condylomas and 80% of low-grade ASIN lesions, but not in high-grade ASIN lesions. In contrast, diffuse p16 staining pattern was found in 100% (30/30) of high-grade ASIN and only in 2.6% (1/39) of low-grade ASIN lesions. The difference of p16 patterns between low grade and high grade ASIN was statistically significant ($p<0.001$).

Conclusions: HPV L1 capsid protein is mainly expressed in anal condylomas and low-grade ASIN lesions and its expression is lost in high-grade ASIN. The two patterns of p16 immunostaining may have different biologic implications. Patchy p16 staining pattern is a feature of low-grade ASIN and some anal condylomas, while diffuse p16 staining pattern is a hallmark of high-grade ASIN. Our study indicates that application of p16 and HPV L1 immunohistochemistry can facilitate the accurate diagnosis and grading of anal squamous dysplasia.

725 Benign Fibroblastic Polyps (Mucosal Perineuriomas) Harbor *BRAF* Mutations, but Not in the Stromal Component: A Laser Capture Microdissection Study

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Background: Benign fibroblastic polyps (BFP) of the colon, also termed mucosal perineuriomas, are rare mucosal lesions characterized by a bland spindle cell proliferation within the lamina propria of a polyp with hyperplastic/serrated features. A high prevalence of *BRAF* V600E mutation has been reported in BFPs, which suggests that they may be peculiar variants of hyperplastic or sessile serrated polyps of the colon. We analyzed a series of BFPs for *BRAF* mutations in the entire polyp, and separately in microdissected polyp stroma, to determine whether the stromal component is part of the same clone.

Design: The pathology archives of three academic hospitals were searched for cases diagnosed as BFP and mucosal perineurioma. H&E slides were reviewed to confirm the diagnosis in each case. Polyps with insufficient stroma for microdissection and those without a definite serrated epithelial component were excluded from the study. DNA was extracted from formalin-fixed paraffin embedded tissue from each polyp and analyzed for *BRAF* V600E mutation. The stromal component from *BRAF* mutation-positive polyps was then microdissected by laser capture microdissection and tested separately for *BRAF* mutation.

Results: Fourteen cases were considered satisfactory for inclusion in the study based on the amount of lesional stroma in the polyps. The epithelial component of all polyps showed morphologic features of a hyperplastic polyp. The stromal component was positive for EMA (epithelial membrane antigen) by immunohistochemistry in 7/8 cases for which the data was available. Adequate DNA was extracted in 11/14 cases and a *BRAF* V600E mutation was detected in 8/11 (73%) polyps. Microdissected stromal DNA sufficient for *BRAF* mutation analysis was extracted in 5 of these 8 cases and showed wild-type *BRAF* in all five cases (100%) analyzed.

Conclusions: BFPs show a high prevalence of *BRAF* mutation which, in conjunction with morphology, supports their relationship to colonic serrated polyps. Furthermore, the stromal component appears to be consistently negative for *BRAF* mutation. "Stroma-rich serrated polyp" more accurately describes the biologic nature of these unique lesions.

726 Phylogeographic Origin of *Helicobacter pylori* Is Associated with Eosinophilic Infiltration of the Gastric Mucosa

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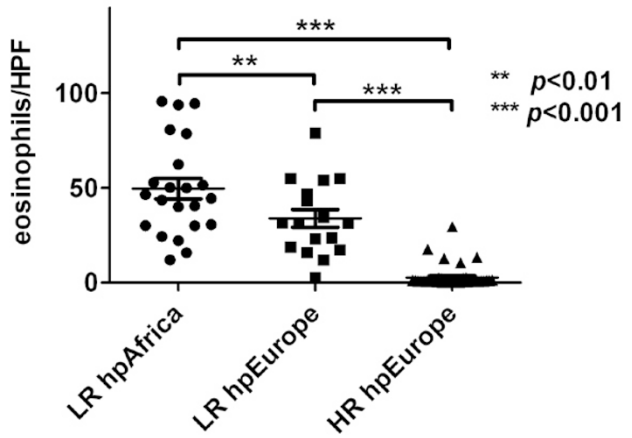
Background: Eosinophils participate in the immune response against *H. pylori* and are markers of an anti-inflammatory Th2-type response. We studied two Colombian populations with contrasting risks of gastric cancer and equally high *H. pylori* prevalence. The high-risk (HR) area is in the Andes Mountains, and the low-risk (LR) area on the Pacific coast. We reported that the phylogeographic origin of the *H. pylori* strains in these populations is associated with severity of the gastric histologic lesions. *H. pylori* strains of African origin were associated with less damage to the gastric mucosa than strains of European origin. We hypothesize that *H. pylori* strains of African ancestry may protect the gastric mucosa against cancer development by favoring a Th2 response associated with increased recruitment of eosinophils.

Design: Gastric biopsies from 80 Colombian males (40-59 years old) with *H. pylori*-associated gastritis were used in this study (39 from the LR area and 41 from the HR area). Eosinophils were counted in 5 high-power fields (HPF) in HE-stained sections from antral biopsies, in the areas with the highest eosinophil density. *H. pylori* was isolated from antral biopsies and the phylogeographic origin was determined by multilocus sequence typing.

Results: Mean values of eosinophils/HPF in the gastric mucosa were higher in the 22 subjects infected with *H. pylori* strains of African origin compared to the 58 subjects infected with strains of European origin (49.6 and 11.9 eosinophils/HPF, respectively; $p=0.0001$). Among subjects living in the LR area, those infected with African strains showed higher mean values of eosinophils/HPF compared to the subjects harboring strains of European origin.



Conclusions: Our results suggest that the recruitment of eosinophils to the gastric mucosa is partially dependent on the phylogeographic origin of the infecting *H. pylori* strains. However, other factors such as infection with helminths and host genetic background may also play an important role.

727 Scoring of Mesorectum: Confrontation between Surgeon's and Pathologist's Assessments

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Background: The adequacy of surgical resection of rectal cancer would be determined by a grading of mesorectum the so called total mesorectal excision (TME).

The aim of the study was first to investigate the TME score determined by the Surgeons and by the Pathologists and then to evaluate the factors that influence the completeness of mesorectal excision.

Design: Between January 2010 and February 2011, 100 patients (55male and .45 female) underwent surgery for rectal cancer (91 adenocarcinoma of the rectum and 9 squamous cell carcinoma) 80 % underwent standard laparoscopy, 2% underwent robotic assisted laparoscopy, and 18 % underwent abdomino-perineal resection. Sixty nine per cent have received neo-adjuvant chemo radiotherapy. Data on all patients entered prospectively onto a data base and Surgeon TME score and Pathologist TME score added to the data base.

The TME score was ranged from 1-3 with 3 being perfect specimen, 1 incomplete specimen and 2 intermediary specimen.

Results: Surgical and pathological TME scoring was concordant in 74 % of cases.

	Pathologist score		
Surgeon score	**1**	**2**	**3**
1	2	2	0
2	4	13	10
3	0	10	59

10 scored 2 by the surgeon were scored 3 by the pathologist, and on the opposite, 10 scored 2 by the pathologist were scored 3 by the surgeon.

The factors that predict the discordance were the abdomino perineal excision ($p=0.02$) and the gender female ($p=0.03$).

Incomplete mesorectal excision (scores 1and 2) were statistically influenced by the female gender ($p=0.001$), the localization in the low rectum ($p=0.001$), the abdomino-perineal resection ($p=0.005$) and a preoperative tumoral resection ($p=0.02$).

Conclusions: In 26% of cases there was a difference in TME scoring between the surgeon and the pathologist. The difference concerned scores 2 or 3 never 1 and 3. Consequently, and especially in abdomino-perineal excision a concomitant evaluation would be required.

728 Mutational Profiling in Ampullary Adenocarcinomas Using the 'SNaPSHOT' Platform

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Background: The ampulla of Vater is at the union of the pancreaticobiliary tree and the duodenum. Both pancreaticobiliary and intestinal epithelia may contribute to

the development of ampullary carcinoma. However, genetic alterations underlying ampullary carcinogenesis have not been well established. In addition, there are no effective adjuvant therapies available for patients with ampullary carcinoma. Molecular targeted therapies can potentially provide more effective and less toxic treatment options for these patients. To gain insights into the genetic basis of this tumor type, we analyzed 63 possible gene mutations in 7 cancer genes using multiplex mutational profiling.
Design: Twenty surgically resected ampullary carcinomas were included in the study and their histopathology reviewed. 5 unstained slides from each sample were prepared from formalin fixed paraffin embedded tissue. Microdissection was performed to achieve a neoplastic cellularity of >20%. DNA from the enriched neoplastic samples was screened for 63 mutations in 7 cancer genes (*AKT1, BRAF, KRAS, NRAS, PIK3CA, PTEN* and *SMAD4*) using the SNaPSHOT single base extension assay (Applied Biosystems).
Results: Eighteen of the 20 cases had intestinal type morphology. Among the intestinal type tumors, 9 (50%) displayed mutations in the genes analyzed. Seven of the 9 cases had a mutation solely in the *KRAS2* gene, including 6 at codon 12 and one at codon 61. One had a lone mutation in codon 545 of the *PIK3CA* gene. Only one case harbored 2 driver mutations with one in *KRAS2* at codon 13 and one in *SMAD4* at codon 361. Two cases included in the study had pancreaticobiliary histology. One of these possessed a *KRAS2* gene mutation at codon 12. Overall the *KRAS2* mutations were detected in 9 of 20 (45%) cases. *PIK3CA* and *SMAD4* mutations were present in a single specimen each.
Conclusions: *KRAS2* gene mutations are common in ampullary adenocarcinoma. Other genes associated with ampullary carcinoma include *SMAD4* and *PIK3CA*. We have utilized a reproducible and sensitive multigene assay for mutational screening in ampullary carcinoma. Accurate prognostication and therapeutic choice for these neoplasms may be driven by the results of these studies.

729 Lymphocytic Colitis: Effect of Therapy on Histologic Findings

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Background: Lymphocytic colitis (LC) is a distinct form of microscopic colitis characterized by a chronic, profuse, watery diarrhea that shows a endoscopically normal appearance; the salient histologic finding is intraepithelial lymphocytosis (IELs). The impact of therapy on the histology has not been thoroughly investigated.
Design: Inclusion criteria required a clinicopathologic diagnosis of LC with index and follow-up biopsies including symptom and medication history relevant to the treatment/diagnosis of LC. Histologic features that were studied included number of surface IELs / 100 colonocytes, presence of chronic changes, acute inflammation, extent of lamina propria expansion by chronic inflammation, degree of surface epithelial damage and site of biopsy (left v right colon).
Results: 228 patients with LC were found from 1/1998-1/2009. Of these, 47 had diagnostic and follow-up biopsies and 21 of these patients had sufficient clinical follow-up for study inclusion. Patients were predominantly female (F:M 18:3) with an average age of 52±15 years at diagnosis. Based on clinical data, patients were divided into two groups.
The first group (8pts) had complete symptomatic resolution. They had, on average, 54±14 IELs on diagnostic biopsy and 12±4 IELs on follow-up biopsy. Patients clinically improved without intervention(3pts), by following a gluten-free diet(1pt), and with discontinuation of rofecoxib(1pt); the remaining patients were treated with medications known to be efficacious in LC.
The second group (13pts) had persistent symptoms. They had, on average, 62±13 IELs on diagnostic biopsy. These patients were subdivided into two subgroups based on histologic follow-up. Subgroup A (4 patients; mean 66±11 IELs), despite persistent symptoms, did not have histologic findings consistent with LC on a follow-up biopsy (mean 14±3 IELs). Subgroup B (9 patients) had no significant change in the number of IELs from the diagnostic (61±13 IELs) to follow-up biopsy (58±10 IELs). All 13 patients in the second group were treated with anti-LC medications. The remaining histologic and clinical parameters, including medication type, did not correlate with symptomatic or histologic improvement.
Conclusions: Based on this preliminary study, complete histologic resolution correlates with symptomatic improvement in a substantial subset of patients with LC; a minority of patients show a discordance between the histologic appearance and presence of diarrhea. The type of anti-diarrheal medication does not seem to affect the histology of follow-up biopsies.

730 CDX2 Expression and Previously Undescribed CDX1 Expression in Primary Gastrointestinal and Metastatic Carcinoid Tumors: An Immunohistochemical Study of 43 Cases

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Background: CDX1 and CDX2 are homeobox genes which encode for transcription factors essential for intestinal organogenesis. CDX2 has been demonstrated in gastrointestinal (GI) carcinoid tumors whereas CDX1 has never been tested. The aim of this study was to evaluate the expression of CDX1 in GI carcinoid tumors, the relative site-specificity of CDX1 and CDX2, and to determine their patterns of expression in both paired and unpaired analyses of primary carcinoid tumors and metastases.
Design: Immunohistochemical study of CDX1 and CDX2 transcription factors was performed on tissue microarrays from 32 archival tumor samples and on recuts from 11 archival paraffin embedded tissue sections of primary gastrointestinal (GI) carcinoids and extra-gastrointestinal (EGI) tract metastases from the years 1990-2011. Specimens were categorized as either primary tumors or metastases and also by anatomic location as foregut, midgut, hindgut, or EGI. The Fisher Exact test was employed to determine the statistical significance of differences in the proportion of positive immunoperoxidase staining between members of each category.
Results: The proportion of specimens staining positively was higher in metastases compared to primary tumor for both CDX1 (55% vs. 41%) and CDX2 (76% vs. 69%). Neither of these differences achieved statistical significance. The percentage of positively staining specimens differed for each marker depending on the anatomic location.

1

Site of tumors	CDX1 (%)	CDX2 (%)
Foregut	25	50
Midgut	26	90
Hindgut	57	11
EGI	71	89

Differences in staining were statistically significant for CDX1 midgut vs. EGI (p=0.018) and CDX2 midgut vs. hindgut (p=0.0001) as well as hindgut vs. EGI (p=0.0002). CDX1 expression was the highest in hindgut, and CDX2 expression was the highest in midgut compared to other GI locations.
Conclusions: Carcinoid tumors originating in different sites have similar histologic features. Immunoperoxidase markers with differential expression may prove useful in distinguishing the origin of metastatic carcinoids. In our study, both CDX1 and CDX2 exhibited differential expression based on anatomic location and metastatic status of tumor. Our results, like others, demonstrate that CDX2 expression persists in metastases with similar findings in CDX1 expression. Retention of these markers in neoplastic endocrine cells, in contrast to the downregulation in colon cancer, has unknown significance. The potential use of CDX1 as a more sensitive marker for hindgut tumors needs more studies.

731 Conversion of Goblet to Non-Goblet Columnar Metaplasia of the Esophagus. A Clinical/Pathologic and Molecular Study of 8 Cases

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Background: In patients with Barrett's esophagus (BE), it is presumed that only columnar epithelium with goblet cells is at risk for neoplastic progression. We have noted, that a small proportion of patients with BE convert to non-goblet columnar epithelium. The aim of this study was to evaluate the clinical, pathologic and flow cytometric abnormalities of BE patients who have converted from goblet to non-goblet columnar epithelium. The results have implications with regard to the ACG definition of BE.
Design: During a 7 year period (2001-2008) in a prospective BE surveillance cohort in which all patients had 4 quadrant biopsies every 2 cm of esophagus, 8 BE patients out of 333 (2.4%) who converted from goblet to non-goblet cell columnar epithelium of the esophagus as confirmed in their last 2 surveillance endoscopies, were identified. After exclusion of 137 patients who had cancer or EMR during surveillance, the final (N=8) cases and controls (N=186) were evaluated for clinical and pathologic features, including flow cytometric characteristics.
Results: BE converters (mean follow-up = 59.6 months) showed a similar male/female ratio (5/3), but were significantly older (mean age: 67 years) compared to the 186 non-converters (mean follow-up = 48.7 months) (M/F:149/37, mean age: 63.7 years). Converters showed a significantly shorter mean length of esophageal columnar epithelium at baseline (1.6 cm, range: 1-6) and a significantly shorter length at last endoscopy (mean 1.3 cm, range 1-4) compared to non-converters (5.4 (1-17) and 5.0 (1-16), respectively, $p<0.01$ for both comparisons). The 8 converters showed a similar proportion of cases with tetraploidy (30%), aneuploidy (40%), or any flow abnormality (50%) compared to non-converters (23%, 16% and 31%, respectively, $p> 0.05$ for all comparisons). Immunohistochemical analysis demonstrated that the metaplastic epithelium of converters maintained positivity for intestinal differentiation markers. The prevalence rate of dysplasia/cancer in the two patient groups were statistically similar.
Conclusions: Conversion of goblet (BE) to non-goblet columnar epithelium of the esophagus is rare, but is more common in patients with shorter segments of columnar epithelium. Conversion of BE to non-goblet epithelium is not associated with loss of flow cytometric abnormalities nor loss of expression of intestinal differentiation markers, suggesting that these patients may still be at risk for cancer and should remain in endoscopic surveillance.

732 MACC1, a Potential Diagnostic Marker for Early Stage Colorectal Cancer

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Background: Metastasis-Associated in Colon Cancer 1 (MACC1), a newly identified gene, may act as a key regulator of the HGF-MET pathway, and is associated with both malignant and metastasis of colorectal carcinoma (CRC). To explore potential markers for early detecting malignancy and predicting metastasis of CRC, we evaluate the expression of MACC1 and MET in adenoma, adenoma with high grade dysplasia, malignant polyps and adenocarcinoma with metastasis.
Design: A total of 48 cases were analyzed, including 13 tubular adenomas, 11 adenomas with high grade dysplasia, 19 malignant polyps (15 intramucosal, 4 invasive adenocarcinoma), and 5 adenocarcinomas with distant metastasis. MACC1 and MET expression were investigated by immunohistochemical method. Cytoplasmic staining for each protein was semiquantified as negative (0), positive with low level expression (1) or positive with high level expression (2). The expression of MACC1 and MET in different groups was evaluated and statistically analyzed.
Results: Positive MACC1 staining was seen in 15% of adenoma, 63% of adenoma with high grade dysplasia, 89% of malignant polyps (13 intramucosal and 4 T1 invasive carcinoma) and 100% of invasive carcinoma with metastasis ($P<0.01$). Furthermore, high level MACC1 expression was observed in all (5/5) of carcinoma with metastasis

and 16% (3/19) of malignant polyps, but not in adenoma or adenoma with high grade dysplasia (P<0.01). Although no significant different expression of MET was found among experimental groups, the correlation between MACC1 and MET expression was seen in carcinoma with metastasis. 80% of metastatic carcinoma showed high expression of both MACC1 and MET. On 10 to 53 months follow up, 7 of 19 of patients with malignant polyps had subsequent segment resection, none of the 19 patients with malignent polyps develops distant metastasis.

Conclusions: Our study suggests that MACC1 may be an important indicator for malignant transition from adenoma to high grade dysplasia, and invasive adenocarcinoma. The expression of MACC1 may serve as a marker for early diagnosis of colorectal cancer. High expression of both MACC1 and MET in colorectal adenocarcinoma with metastasis confirmed the essential roles of these two proteins in the process of cancer metastasis. Met expression does not have diagnostic value in early colorectal malignancy.

733 An Interobserver Study on IgG4 Related Disease

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Background: IgG4 related disease (IgG4-RD) is a multisystem multiorgan inflammatory disease that responds to immunosuppressive therapy. The common organs involved include the pancreas, biliary tract, lung, lymph nodes, and kidney. In part, the diagnosis is dependent on histopathological features: dense lymphoplasmacytic infiltrate, obliterative phlebitis and storiform type of fibrosis. However, there are organ based variations in the histopathological appearance. A quantitative analysis of a tissue IgG4 stain is widely accepted and indispensable test for the diagnosis of IgG4-RD; however, there are no widely accepted cut points. The purpose of this study was to prospectively evaluate interobserver agreement on the histopathological diagnosis of IgG4-RD.

Design: Sixteen pathologists participated in this study. A virtual library of 31 biopsies and resection specimens was constructed. Readers reviewed the slides online and filled out a questionnaire for histopathologic findings and diagnosis. The study group included 20 cases of IgG4-RD and 11 mimics of this process. The specimens in this data set included: liver (6), kidney (4), salivary gland (4), lymph node (4), aorta (3), lung (3), orbit (4), and retroperitoneal fibrosis (3). The readers were provided an H&E slide, an immunohistochemical stain for IgG4 and an IgG4 to IgG ratio.

Results: The overall kappa coefficient was 0.775 (substantial/good agreement). The kappa value for pathologists that sign out a single subspecialty was lower than those who evaluate ≥ 2 specialties (p=NS). However, there was a wide variation in the cut points for IgG4 used by the pathologists for the diagnosis of IgG4-RD. The two commonest histological features used by the pathologist included a dense lymphoplasmacytic infiltrate and a storiform type fibrosis.

Conclusions: We demonstrate substantial κ agreement in the diagnosis of IgG4-RD among pathologists with an interest in IgG4-RD.

734 Polyp Landscape in Serrated Polyposis Syndrome

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Background: Serrated polyposis syndrome (SPS), also known as hyperplastic polyposis, is a syndrome of unknown genetic basis defined by the occurrence of multiple serrated polyps in the large bowel and associated with an increased risk of colorectal cancer (CRC). There is a variety of presentations of SPS, which may encompass a continuum of phenotypes modified by environmental and genetic factors. To explore the phenotype of SPS, we recorded the histologic and molecular characteristics of multiple colorectal polyps in 100 patients with SPS.

Design: Patients were selected from a cohort of individuals recruited between 2000 and 2010 from genetics clinics in Australia, New Zealand, Canada and the USA. Pathology review of polyps was undertaken by 3 specialist gastrointestinal pathologists. Polyps were classified into conventional adenoma or serrated polyp with various subtypes following the criteria from the WHO. Mutations in *BRAF* and *KRAS* and mismatch repair protein expression were determined in a subset of polyps.

Results: A total of 100 patients were selected for the study, comprising 58 females and 41 males. The total polyp count per patient ranged from 6 to 150 (median: 30). The vast majority of patients (89%) had polyposis affecting the entire large intestine. From this cohort, 406 polyps were reviewed. Most of the polyps (83%) were serrated polyps: microvesicular HP (n=156), goblet cell HP (n=25), SSA/P (n=110), SSA/P with cytological dysplasia (n=28) and TSA (n=18). *BRAF* mutation was mainly detected in SSA/P with dysplasia (95%), SSA/P (84.5%), microvesicular HP (76.2%), and TSA (53.8%) while *KRAS* mutation was present mainly in goblet cell HP (50%) and in tubulovillous adenoma (45.4%). Four of 6 SSA/Ps with high grade dysplasia showed loss of MLH1/PMS2 expression. CRC was diagnosed in 39 patients who were more often found to have a conventional adenoma compared to patients without CRC (p=0.003).

Conclusions: SPS referred to genetics clinics is a pancolonic disease with a wide range of polyp counts, mostly represented by microvesicular HP, SSA/P and occasional TSA with a high rate of *BRAF* mutation. The occurrence of CRC was associated with the presence of conventional adenoma.

735 Double Immunostain with SATB2/Cytokeratin 20 Is Useful in the Differentiation of Appendiceal from Ovarian Mucinous Neoplasms

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Background: SATB2 is a DNA-binding protein involved in transcriptional regulation and chromatin remodeling. SATB2, CDX2, cytokeratin 20 (CK20), and Villin are all expressed in most colon cancers. Mucinous carcinomas in the peritoneal/pelvic cavities may be of gynecologic or gastrointestinal origin. We investigated the utility of SATB2 in distinguishing appendiceal mucinous neoplasms (AMN) from ovarian mucinous neoplasms (OMN), using double immunostains (DS).

Design: AMN and OMN (borderline tumors and carcinomas) from 2000 to 2011 were obtained from our archives, and tissue microarrays of 40 AMN and 18 OMN were made. DS were performed pairing nuclear stains CDX-2 and SATB2 with cytoplasmic stains CK20 and Villin, and stains were scored as positive (> 5% cells staining) or negative.

Results:

Expression of CDX-2, SATB2, CK20, and Villin in Appendiceal and Ovarian Mucinous Neoplasms

	CDX-2	SATB2	CK20	Villin
Appendix	37 (92.5%)	32 (80%)	39 (97.5%)	37 (92.5%)
Ovary	8 (44.4%)	3 (16.7%)	6 (33.3%)	14 (77.8%)

CK20 was the most sensitive stain for AMN. Villin was the most sensitive for OMN, but also stained many AMN. SATB2 was expressed in the fewest cases at both sites, staining 80% of AMN and 16.7% of OMN.

Double Staining of Appendiceal and Ovarian Mucinous Neoplasms

	CDX-2/CK20	CDX-2/Villin	SATB2/CK20	SATB2/Villin
Appendix both positive	36 (90%)	34 (85%)	31 (77.5%)	30 (75%)
Appendix either positive	40 (100%)	40 (100%)	40 (100%)	40 (100%)
Appendix both negative	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Ovary both positive	5 (27.8%)	8 (44.4%)	0 (0%)	1 (5.6%)
Ovary either positive	9 (50%)	14 (77.8%)	9 (50%)	16 (88.9%)
Ovary both negative	9 (50%)	4 (22.2%)	9 (50%)	2 (11.1%)

All AMN expressed at least one of the stains in each DS, and many AMN stained for both. DS with Villin were least useful, as Villin was commonly expressed at both sites. DS for SATB2/CK20 showed the greatest potential clinical utility. When both SATB2 and CK20 were positive, OMN was unlikely (0%) compared to AMN (77.5%). Conversely, when both were negative, OMN (50%) was much more likely than AMN (0%). Results with CDX2 DS were similar to SATB2 DS, but more OMN expressed CDX2, decreasing specificity for appendix. In cases with one positive and one negative stain, the site was not as clear, but favored appendix, especially if SATB2 was the positive stain.

Conclusions: The use of 2, rather than 1 immunostain, improves the distinction of AMN from OMN. The SATB2/CK20 DS is useful in distinguishing AMN from OMN in the peritoneal/pelvic cavity, particularly if material is limited. In challenging cases, the addition of CDX2 may be helpful.

736 Bone Marrow Micrometastases in Esophagogastric Cancer – 10-Year Follow-Up Confirms Prognostic Significance

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Background: A majority of esophagogastric cancer (EGC) patients with potentially curative R0 resections succumb to metastatic (haematogenous spread) disease. Current pathologic staging guidelines do not include assessment systemic micrometastatic disease. We present 10-year outcome data of EGC patients with rib marrow examined for micrometastases correlated with treatment and conventional pathologic tumor staging.

Design: With multi-institutional research ethics board approval 77 patients with localized oesophagogastric tumors having radical en-bloc oesophagectomy were followed until death or for 10 years, with >50% patients receiving neoadjuvant (5-fluorouracil/cisplatin-based) chemoradiotherapy (CRT) and the remainder being treated with surgery alone (SO). Posterior rib segments were excised at thoracotomy prior to tumor manipulation. Marrow flushed from the rib segment was enriched for mononuclear cells and stained using a monoclonal anti-cytokeratin 18 antibody. Standard demographic and pathologic parameters were recorded and patients were followed for a mean 10.04 years. Disease recurrences and all deaths in the follow-up period were recorded.

Results: No patients were lost to follow up and 46 EGC-related and 10 non-EGC related deaths occurred. The identifiable prognostic determinants were lymph node positivity, the presence of bone marrow micrometastases and neoadjuvant chemotherapy. Lymph node positivity was a significant predictor of poorer disease specific survival (DSS) in both CRT and SO patients. Multivariate Cox model analysis of risk showed bone marrow micrometastasis positivity differentiated low and high risk groups among SO but not CRT patients. Taking these groups as a whole and allowing for lymph node status micrometastasis positivity increases risk of death in all SO patients but also in lymph node negative CRT patients. The overall contribution of micrometastasis positivity to DSS is significant using likelihood ratio tests (p = 0.022).

Conclusions: Bone marrow micrometastases provide contributory prognostic data in lymph node negative EGC patients and consideration should be given to routine examination for same. Systemic micrometastic disease (bone marrow micrometastases) continues to provide a window on the metastatic process and may be used to refine pathologic staging of EGC to improve outcome prediction.

737 Squamous Morules in Colon Polyps: Endocrine Differentiation in an Under-Recognized Mimic of Invasive Carcinoma

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Background: Squamous morules encountered in colorectal adenomas are groupings of monotonous cells showing both squamous and endocrine differentiation, the latter not well-recognized. On our consult service, adenomas with squamous morules are often submitted with a concern for invasive carcinoma, or not recognized/reported. To further characterize this important carcinoma mimic, we studied the clinicopatholigic features of squamous morules.

Design: The study group consisted of prospectively identified colon polyps with squamous morules.

Results: We prospectively identified 10 colon polyps with squamous morules from 10 patients (5 men) ranging in age from 28-82 years (mean, 60 years). Eight were consultation cases; 2 were prospectively identified in our material. Indications for colonoscopy were known in 7 patients: screening colonoscopy (3), GI bleed (3), and diarrhea (1). The polyps involved the right (4), transverse (1), and left colon (4), and the site was not specified in one case. The average polyp size was 19 mm, and most were tubulovillous adenomas (7). Associated findings included prolapse change (10), fibrosis (4), high grade dysplasia (3), and serrated features (3).

The individual cells of squamous morules were cuboidal with abundant eosinophilic, dense cytoplasm. The nuclei were montonous and round with "salt-and-pepper" chromatin. Pleomorphism, hyperchromasia, and mitotic activity were absent. Squamous morules often had a quasi-infiltrating architecture (10), occasional lumen formation (7), a connection to adjoining adenomatous epithelium (8), and a fibrotic background (4). The majority of squamous morules were multifocal (7), extending over an average length of 0.4 cm. The morules displayed synpatophyin reactivity, variable chromogranin reactivity, and a negligible Ki-67 index.

In the 8 cases with a submitted diagnosis or impression, the squamous morules were interpreted as invasive carcinoma (2), not reported (3), or appropriately recognized as squamous morules (3). The two cases misinterpreted as invasive carcinoma showed quasi-infiltrative architecture, fibrotic background reminiscent of desmoplasia, and associated high grade dysplasia.

Conclusions: We report squamous morules with endocrine differentiation as an important mimic of invasive carcinoma. Squamous morules were found in polyps sharing the following core features: large size, tubulovillous features, prominent prolapse changes, fibrotic background, and, occasionally, high grade dysplasia and serrated features. Awareness of the unique morphology of squamous morules is important to avoid misdiagnosing carcinoma.

738 Biliary Strictures: Methods for Improving Diagnostic Accuracy

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Background: Biliary strictures may be caused by inflammatory or neoplastic processes. Differences in management emphasize the need to establish the underlying causes. Biliary brushing is the initial intervention to assess the etiology of strictures, and often concurrent biopsies and FISH studies are also performed. Prior studies have established that brushings have a high specificity but low sensitivity. The aim of this study was to determine the etiology for the low cytological yield and to assess which sampling method(s) (cytology, biopsy and/or FISH) improve the diagnostic yield of pancreatobiliary tumors.

Design: A total of 48 patients with biliary strictures including 29 cases of histology-proven pancreatobiliary adenocarcinomas (PBCa) and 19 'control' cases (benign strictures on resection and/or 17-40 months of clinical follow-up) were retrieved. Brush cytology specimens, FISH analysis (UroVysion probes for chromosomes 3, 7, and 17, and locus 9p21) and bile duct biopsies were re-reviewed by 2 pathologists. Cytology specimens were evaluated for overall cellularity and presence of the following features: large atypical cells with foamy cytoplasm (LACF), drunken honeycomb (DH) and loosely cohesive clusters of round cells (LCCRC). Biopsy specimens were examined for presence or absence of stromal invasion (SI).

Results:

Brushing vs. FISH vs. biopsy

	PBCa* (n=29)	Control* (n=19)	Sensitivity	Specificity	PPV	NPV
Brushing#	13/29 (44.8)	1/19 (5.3)	0.448	0.947	0.929	0.529
FISH	21/29 (72.4)	2/19 (10.5)	0.7	0.895	0.913	0.654
Forceps biopsy	16/21 (76.2)	0/7 (0)	0.786	0.938	0.971	0.625
FISH and brush or biopsy	19/29 (65.5)	1/19 (5.3)	0.655	0.947	0.95	0.643

* positive/total (%); # positive defined as suspicious or positive for adenocarcinoma on cytology

Scant cellularity was present in 55.2% of PBCas and 63.2% of control cases and resulted in 7 of 8 cases of non-diagnostic or false negative cytology results. LACF, DH and LCCRC were identified in 90.5%, 66.7% and 57.1% of PBCas vs. 12.5%, 12.5% and 0% of controls, respectively) (p values 0.0002, 0.007 and 0.001, respectively). The presence of SI aided in diagnosis of carcinoma in biopsies (11 of 16 positive).

Conclusions: Our study suggests that higher specimen cellularity and the presence of LACF, DH, and LCCRC increase the sensitivity in cytology specimens. In addition, sampling of stromal tissue may facilitate the diagnosis of invasive PBCa. Concurrent biopsies and FISH studies are helpful in increasing the diagnostic yield of pancreatobiliary tumors.

739 Inflammatory Bowel Disease Associated with Primary Sclerosing Cholangitis Has Different Phenotypes: PSC-IBD *vs.* IBD-PSC

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Background: Inflammatory bowel disease (IBD) associated with primary sclerosing cholangitis (PSC) is reported to be mild and prone to right-side predominance with rectal sparing. While IBD onset often follows the diagnosis of PSC the opposite may occur; no data are available on possible differences. Thus, we examined colonic disease distribution and severity based on primary disease presentation (colonic versus hepatic).

Design: We identified patients with PSC and IBD seen at the University Health Network-Toronto between 1995 and 2011. Colonic biopsy specimens were independently reviewed in a blinded fashion. When available, each colonic region was assessed on a six-grade scale (0 to 5) for architectural distortion, Paneth cell metaplasia, and degree of inflammation. Clinical, endoscopic, and follow up data were collected. Patients were divided into two groups based on site of first disease presentation: group 1, colitis first (IBD-PSC) and group 2, sclerosing cholangitis first (PSC-IBD). We excluded patients in whom information on disease presentation was not available (n = 12). We then compared both groups for disease distribution and severity.

Results: We examined 58 patients (54 with ulcerative colitis and 4 with Crohn's disease). Colitis preceded liver disease in 28 patients (IBD-PSC). Colitis followed liver disease in 30 (PSC-IBD). Colitis, in both groups, was mild with focal deep plasmacytosis and occasional mild cryptitis - more severe active cryptitis was not identified. Individual biopsies from both right and left regions of the colon were available in 42 patients only. IBD preceding PSC had a tendency to have a 'pan-colitis' distribution, see table – this group included all patients with Crohn's disease. IBD following PSC was more likely right-sided (sparing the descending and sigmoid colon and rectum ($p = 0.012$)).

Distribution	IBD-PSC (n=22)	PSC-IBD (n=20)
Right sided (only)	5	13
Left sided (only)	1	0
Pan-colitis	16	7

Conclusions: The histological picture of both groups (IBD-PSC and PSC-IBD) is distinctively mild, but the distribution tends to be pancolitis in the IBD-PSC group and right sided ulcerative colitis in the PSC-IBD group. This suggests that patients with what appears to be right sided ulcerative colitis might be a separate phenotype associated with PSC and should lead to consideration of evaluation of liver function tests if not already carried out.

740 BAF250a (*ARID1A*) Loss Is Frequent in High-Grade Gastric Adenocarcinomas and Is Associated with Mismatch Repair Protein Loss

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Background: BAF250a, the protein encoded by the tumor suppressor gene AT-rich interactive domian1A gene (*ARID1A*), a subunit of the SWI/SNF chromatin remodeling complex, is considered to be an essential part of early tumorigenesis. We reported frequent mutations of *ARID1A* in clear cell and endometrioid ovarian carcinomas and loss of the corresponding protein BAF250a by immunohistochemistry (IHC). These finding have generated interest in assessing the mutation and loss of *ARID1A* in other malignancies. Our group recently reported that BAF250a is also frequently lost in endometrial cancers with loss observed in 29% (29/101) of grade 1 or 2 and 39% (44/113) of grade 3 endometrioid carcinomas of the endometrium. In preliminary studies gastric cancer has also displayed an increased frequency of BAF250a loss and we now report a series of gastric carcinomas with associated clinical follow-up.

Design: The study cohort consisted of 222 gastric carcinomas (mean age 63.5 y; m:f 163:59) from patients referred for treatment at the BCCA. A tissue microarray (duplicate 0.6 mm cores) was immunostained for BAF250a (Anti-ARID1A, HPA005456, Sigma), MLH1 (ES05, Leica), MSH2 (25d12, Leica), MSH6 (44/MSH6, BD Biosc.) and PMS2 (A16-4, BD Biosc.). Immunoreactivity was considered positive in tissue cores that contained any positive tumor cell nuclear staining, regardless of intensity. Negatively scored tissue cores were ones that showed completely absent tumor cell nuclear staining, as well as positive non-neoplastic cell nuclear staining. The significant association of BAF250a with biomarkers and survival were determined by Fisher's exact test and Kaplan Meier plot and log rank tests respectively.

Results: Overall, loss of BAF250a expression as measured by IHC was observed in 32.9 % (n=49) of gastric adenocarcinomas. This was preferentially seen in higher-grade tumors (G3: 52%, G2: 43%) than in low-grade lesions (G1: 4.2%) and was significantly associated with absent expression of at least one mismatch repair (MMR) protein ($p<0.0001$). Whereas loss of MMR protein expression was correlated with better overall survival ($p<0.001$), BAF250a was not.

Conclusions: Loss of BAF250a expression, and thus mutation of *ARID1A*, is relatively common in high-grade gastric adenocarcinomas and significantly correlates with loss of expression of mismatch repair proteins offering a potential therapeutic approach for these cancers.

741 Mycophenolic Acid (Cellcept and Myofortic) Associated Eosinophilic Enterocolitis in Adult Transplant Recipients

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Background: Mycophenolic acid (Cellcept and Myofortic) is an immunosuppressive drug used in prevention of graft rejection in solid organ transplant recipients. Common gastrointestinal side effects include diarrhea, nausea, vomiting and epigastric pain. The spectrum of histologic changes seen in GI biopsies of patients receiving Mycophenolic acid have been categorized as inflammatory bowel disease-like, graft versus host disease-like, ischemia-like, and self-limited colitis-like. We report eosinophilic enterocolitis as an additional pattern of injury in GI biopsies in symptomatic patients taking Mycophenolic acid.

Design: Five solid organ transplant recipients who were taking Mycophenolic acid and underwent concurrent small intestinal and colon biopsies were identified on a pathology information system search. Of the five patients, 3 were heart transplant recipients, 1 liver and 1 kidney. All patients were symptomatic at biopsy time, and on follow-up, available for all 5 patients, symptoms improved when Mycophenolic acid dose was decreased or withdrawn (1 patient). All patients with IBD, PSC, history of parasitic infection, previously diagnosed GVHD, eosinophilic esophagitis, eosinophilic gastroenterocolitis, celiac sprue, and known food hypersensitivity/allergy were excluded. From these 5 patients, a total of 1 duodenal and 5 colonic biopsies were examined.

Results: Pathologic findings included increased eosinophilic infiltration of the lamina propria, crypt epithelium, and submucosa. The duodenal biopsy showed preserved villous architecture, normal crypt architecture, focally increased lamina propria eosinophilic infiltrates up to 40/HPF, and cryptitis characterized by single cell infiltration of the crypt epithelium by eosinophils. The colon biopsies were taken from all parts of the colon and uniformly showed increased lamina propria eosinophils, ranging from

50-80/HPF in areas of highest density. The biopsies all showed crypt architectural distortion, lamina propria edema, reactive lymphoid hyperplasia, cryptitis similar to that seen in the duodenal biopsy, and occasional eosinophilic crypt abscesses. Two of the colon biopsies additionally showed sheets of eosinophils infiltrating the submucosa. None of the biopsies showed crypt epithelial apoptosis suggestive of GVHD.
Conclusions: Eosinophilic enterocolitis should be included in the spectrum of histologic changes seen in GI biopsies of adult transplant recipients taking Mycophenolic acid.

742 Interaction of Cancer Stem-Like Cells and Growth Factor Receptors in the Evolution of Colorectal Cancers during Aging

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Background: The incidence of colorectal carcinoma increases exponentially with age. The exact reason for this is yet unknown. We have previously reported that the age related rise in adenomatous polyps in normal appearing mucosa of humans is associated with increased expression of cancer stem cell (CSC) markers. Additionally we have demonstrated that this is accompanied by an increased expression of growth factor receptors such as epidermal growth factor receptor (EGFR) and insulin like growth factor receptor (IGFR), suggesting a relationship between the CSCs and EGFR. We hypothesize that Growth factor receptor over-expression may be involved in the upregulation of CSCs during development of colon cancer.
Design: We investigated the co-expression of phosphorylated EGFR and phoshorylated IGFR in the same cells as the stem cell marker CD166 in 4 study groups: (1) colonic adenocarcinomas in patients <60 years; (2) colonic adenocarcinomas in patients >60 years; (3) adenomatous polyps and (4) normal colonic mucosa of patients with polyps (<60 vs >60). We used double color immunofluorescence staining pairing a growth factor receptor marker with CD166. The co-expression score was determined by counting the percentage of co-expressing cells within a crypt and counting 20 crypts and averaging per crypt. Data was statistically analyzed using Chi-Square tests.
Results: We found a significant increase in co-expression of pEGFR/CD166 and pIGFR/CD166 in normal mucosa of aging individuals, when stratified by 60 years of age (p=0.01 for pIGFR/CD166, p=0.001 for pEGFR; <60 vs. >60 years of age). We also investigated the co-expression of pIGFR/CD166 and pEGFR/CD166 in colon adenomas (n=20). In all adenomas the level of co-expression was higher than both groups of normal colonic mucosa samples. In addition the co-expression of pEGFR and pIGFR with CD166 was higher in colonic adenocarcinomas of >60 compared to <60. (p=0.001 for both pEGFR and pIGFR).
Conclusions: These findings suggest that cancer stem cells show increased expression of growth factor receptors during cancer development and aging. This will give a survival and proliferative advantage and allow for accumulation of new mutations to promote the cancer phenotype. The mechanism for the interaction between the two systems is unknown but microRNAs are the most likely culprits.

743 Prognostic Role of Combined MSI and BRAF Mutation Status in Colorectal Cancer: Toward Routine Clinical Use

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Background: Microsatellite instability (MSI) analysis and *BRAF* sequencing are becoming routine clinical tests for colorectal cancer (CRC) prognostication and familial risk assessment. Although MSI-high [MSI-H] has been associated with good prognosis and *BRAF* mutation with poor prognosis, it remains uncertain whether MSI-H (or *BRAF* mutation) influences clinical outcome independently of *BRAF* (or MSI) status. To clarify the prognostic roles of MSI and *BRAF* status, a large sample is required for adequate statistical power, because *BRAF* mutations and MSI-H exist in a minority of CRCs.
Design: The relationship between MSI and *BRAF* status and patient survival was analyzed in 1343 CRCs. The Cox proportional hazards model was used to compute mortality hazard ratios (HRs) according to combined MSI/*BRAF* status, adjusting for potential confounders, including disease stage.
Results: MSI-H was detected in 15% and *BRAF* mutation in 14% of CRCs, respectively. The percentages for the combined MSI-*BRAF* subtypes were: MSI-H *BRAF*-mutated [mut], 8%; MSI-H *BRAF*-wildtype [wt], 7%; MSS (non-MSI-H) *BRAF*-mut, 6%; and MSS *BRAF*-wt, 79%. Using MSS *BRAF*-mut as the reference group, the multivariate HRs (95% confidence interval [CI]) for CRC-specific mortality were: MSI-H *BRAF*-wt, 0.19 (0.09, 0.41); MSI-H *BRAF*-mut, 0.41 (0.22, 0.77); and MSS *BRAF*-wt, 0.56 (0.39, 0.80). HRs (95% CI) for overall mortality were: MSI-H *BRAF*-wt, 0.47 (0.29, 0.74); MSI-H *BRAF*-mut, 0.74 (0.48, 1.14); and MSS *BRAF*-wt, 0.68 (0.49, 0.93). Kaplan-Meier analysis showed that survival was lowest for the MSS *BRAF*-mut patients, and highest for the MSI-H *BRAF*-wt patients ($p < .0001$ for CRC-specific and $p = .0003$ for overall mortality.

Figure 1. CRC-specific survival by MSI–*BRAF* subtype

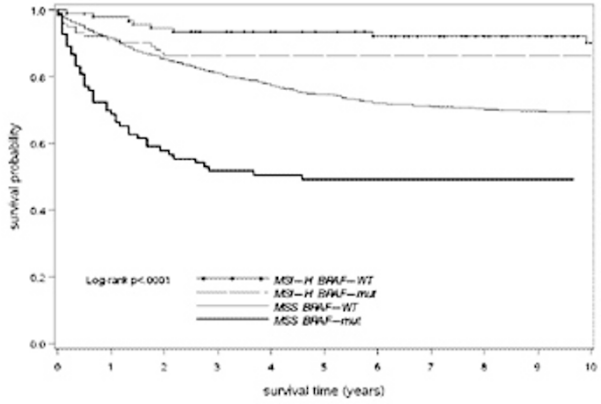
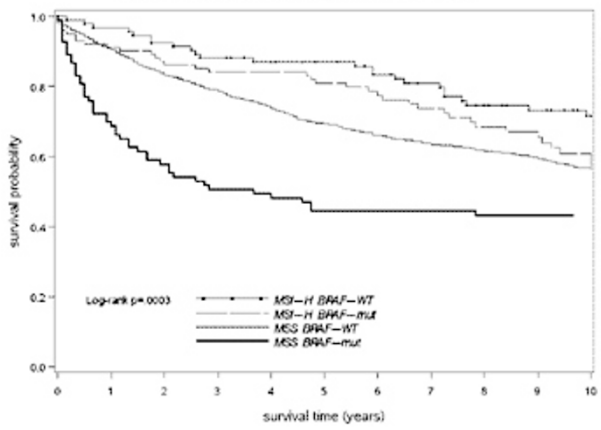


Figure 2. Overall survival by MSI–*BRAF* subtype



No significant interaction was present between MSI and *BRAF* (p=0.52), indicating that the prognostic role of MSI-H (or *BRAF* mutation) was independent of *BRAF* (or MSI) status.
Conclusions: By combined MSI/*BRAF* subtyping, MSI-H *BRAF*-wt patients have the best prognosis, whereas MSS *BRAF*-mut patients have the worst. Thus, the routine clinical use of both MSI and *BRAF* testing to subclassify patients will aid prognostication, resulting in better-informed clinical decision making and improved patient care.

744 Dachshund Homolog 1 Is Associated with Colorectal Carcinogenesis

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Background: The human dachshund homolog 1 (DACH1) gene is located in chromosome 13 and encodes a protein composed of two highly conserved domains, dachshund domain 1 and 2, engaging in multiple context-dependent complexes that activate or repress transcription, and regulate cell proliferation and differentiation. DACH1 expression is reduced in a variety of human tumors. Reduced DACH1 expression predicts poor outcomes of breast and endometrial cancer patients. The expression levels and the role of DACH1 in colorectal cancer are largely unknown. Our aims were to characterize DACH1 expression in normal, hyperplastic, dysplastic, and carcinomatous colon tissues; and to determine any relation of DACH1 expression to colon carcinogenesis.
Design: A colorectal progression tissue microarray (TMA) was designed and constructed for evaluation of 173 normal, 130 hyperplastic, 60 dysplastic and 222 adenocarcinoma cores obtained from 131 cases of colorectal carcinoma patients and normal control tissues. Standard immunohistochemistry using anti-human DACH1 was performed. The immunostaining intensity was analyzed as: 0, no staining; 1, weak staining; 2, intermediate staining; 3, strong staining. A composite index was calculated based on the combination of intensity and percentage for each core (range 0-300). Statistical analysis using repeated measures ANOVA by SAS was performed.
Results: DACH1 staining of colon is predominantly cytoplasmic, nuclear, or both nuclear and cytoplasmic. There was absent or very weak staining in normal colonic mucosa, and few positive cells were seen at the base of crypt at the proliferating zone. The staining indices in normal, hyperplasia, dysplasia and cancers were 26, 56, 124 and 161, respectively. The differences among groups were significant (p<0.0001).
Conclusions: Our results show that there is a progression of DACH1 immunoexpression in normal colon, hyperplasia, dysplasia, and colorectal carcinoma, suggesting that DACH1 plays a role in colorectal carcinogenesis. This study uncovers a novel role for DACH1 as an oncogene in colorectal carcinogenesis, unlike that of the tumor suppressor role of DACH1 that has been reported in hormone-responsive breast and endometrial cancers. Further studies using a larger cohort to investigate the correlation

between DACH1 expression in various tumor grades, stages, and metastases status, are necessary to stratify prognosis, and to determine whether DACH1 is indeed an oncogene in colorectal carcinogenesis.

745 Gastric Chief Cell Adenomas: Proposal for a New Entity

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Background: Chief cell predominant adenocarcinoma (CCP-AD) is a rare type of gastric carcinoma characterized by phenotypic and immunophenotypic differentiation toward chief cells. These tumors demonstrate low proliferative rates, lack of p53 overexpression, and less aggressive behavior as compared to usual gastric carcinomas. CCP-AD has only recently been described and its morphologic precursor is unknown.

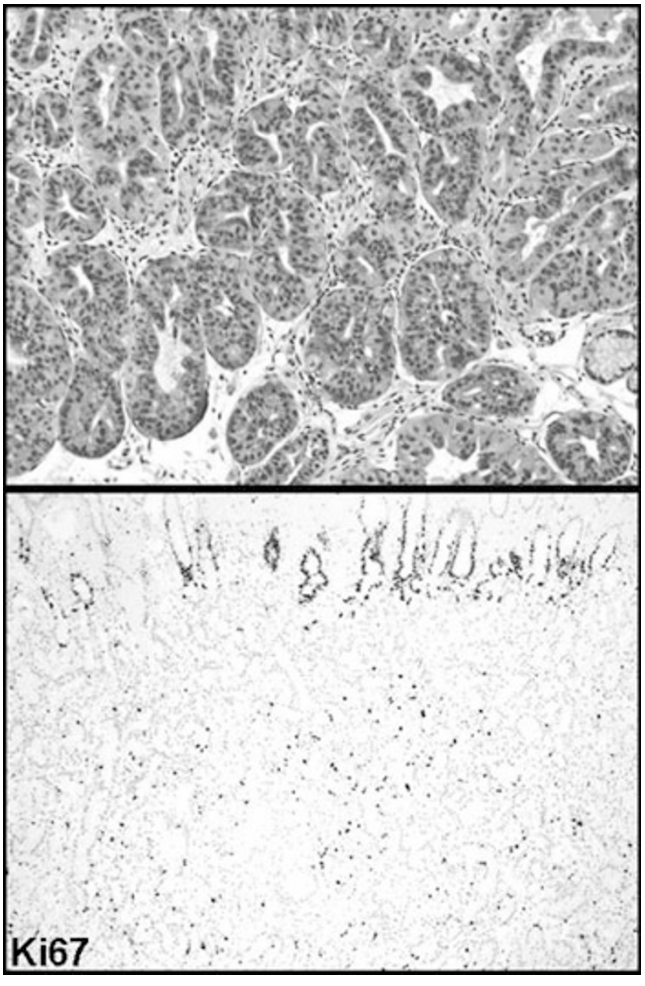
Design: We studied 16 polyps from 10 patients who underwent gastric biopsy (n=7) or resection (n=3). Cases were collected prospectively because of their unusual histology (cytologic dysplasia and architectural atypia of chief cell predominant deep fundic glands). Age, gender, polyp size, morphology, multiplicity, concomitant adenocarcinoma, other gastric polyps, and results of immunohistochemistry for Ki67 and p53 were recorded.

Results: Lesions were centered in the basal aspect of oxyntic mucosa and comprised primarily of chief cells with moderately enlarged nuclei. Involved glands were mildly dilated with budding/anastomosing architecture (n=8), cribriforming (n=1), multilayered cells (n=14), luminal apoptotic debris (n=2), and inspissated basophilic secretions (n=2). In 6 cases, glands were admixed with splayed muscularis mucosae and 2 extended into superficial submucosa. Four lesions also had foveolar dysplasia and one of these abutted a submucosally-invasive CCP-AD. Ki67 immunostaining demonstrated abnormal extension of the proliferative zone down into the deep glandular compartment, although the proliferative rate was low (≤5%). p53 was negative.

Chief Cell Adenomas

Age	Sex	No. polyps	Size	Other lesions
49	M	3	Largest 0.5 cm	FGPs x7
50	F	1	0.3 cm	No
36	M	1	1.7 cm	Chief cell predominant adenocarcinoma
66	M	1	0.2 cm	FGPs x2
77	M	1	0.2 cm	FGP
73	M	1	0.8 cm	FGPs x3
80	M	3	Largest 0.7 cm	No
71	M	5	Largest 0.45 cm	Foveolar adenoma and dysplastic FGP
73	M	1	0.1 cm	No
69	F	1	0.3 cm	No

FGP, fundic gland polyp



Conclusions: We describe a novel gastric polyp and propose the name "chief cell adenoma" based on the presence of cytologic dysplasia of chief cells, architectural atypia, and abnormal Ki67 labeling of deep fundic glands. The occurence of foveolar dysplasia in a subset of these lesions, and one patient with associated CCP-AD, suggest that chief cell adenomas might represent a precursor of CCP-AD.

746 Stem Cell Phenotype in Cirrhosis and Hepatocellular Carcinoma

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Background: Recent data support the existence of cancer stem cells (CSCs) in some and perhaps, all cancers. While cancers are a heterogeneous mix of cells at various stages of differentiation, undifferentiated tumor-specific stem cells acquire the transforming mutations necessary for tumourigenesis. In the present study, we determined whether stem cells play a significant role in the initiation and/or progression of hepatocellular carcinoma (HCC).

Design: We have investigated the role of CSCs in the genesis of HCC by analyzing CD133, a specific stem cell marker, in cirrhosis and HCC arising in cirrhotic and non-cirrhotic liver with comparison to normal liver. Paraffin-embedded archival material was used to identify stem cells and to examine protein and mRNA expression. Quantitative immunostaining was done for CD133 and Hep-par1. Real time RT-PCR was performed to evaluate CD133, Oct4, and Nanog (all stem cell markers) mRNA expression. All of these analyses were also performed on fibrolamellar carcinoma.

Results: Normal liver demonstrated rare presence of CD133+ cells and low mRNA levels of stem cell markers. In cirrhosis, CD133+ cells and mRNA expression of stem cell markers was significantly (p=0.04) higher. These CD133+ cells were localized primarily to neoplastic hepatocytes. Similarly, in HCC with cirrhosis, CD133+ cells were significantly increased (p=0.001) in the neoplastic hepatocytes and were localized in the periphery of the tumour nodules. Several such cells co-expressed Hep-par1. On the contrary, HCC without cirrhosis did not show significant increase in both stem cell number and mRNA expression of CD133 or other stem cell markers. Fibrolamellar carcinoma showed increase in these cells similar to HCC with cirrhosis. Interestingly, no significant changes in the stem cell number or mRNA levels were observed in HCC without cirrhosis and normal liver. Other stem cell markers such as Nanog and Oct 4 showed similar trend.

Conclusions: Our findings indicate presence of CD133-expressing stem/precursor cells in hepatocellular carcinoma with cirrhosis, fibrolamellar carcinoma and cirrhosis. Based on this data, CD133+ stem cells are significantly increased in cirrhosis with or without HCC, suggesting a role of these stem cells in the regenerative/reparative process. This implies a bystander role of these stem cells. In fibrolamellar carcinoma, likely the presence of fibrosis within the tumour caused increased expression of CD133. The tumourigenic capacity of these CD133+ stem cells in the immunodeficient mice will potentially delineate the underlying pathogenesis.

747 Biopsies from the GEJ Area Composed of Pure Oxyntic Glands Is Not Necessarily Indicative of the Proximal Stomach

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Background: Barrett's esophagus (BE) has been proposed to develop via conversion of mucosa comprised of oxyntic glands to one comprised of mucous glands prior to goblet cell metaplasia. Based on this theory, some authorities have suggested that biopsies from the GEJ area composed of pure oxyntic glands always represent mucosa from the proximal stomach. The aim of this study was to evaluate the prevalence and significance of mucosa composed of pure oxyntic glands when detected in biopsies of the GEJ region in a large study of GERD patients either with (N=214) or without (N=295) columnar-lined esophagus (CLE).

Design: Biopsies of the GEJ region from 509 GERD patients (M/F ratio: 281/228, mean age: 51 years), all of whom were endoscoped and interviewed prospectively as part of a community clinic-based study of GERD patients in Washington state, were evaluated in a blinded fashion for the presence or absence of pure oxyntic-type mucosa and goblet cells, without knowledge of the clinical characteristics or anatomic location (esophagus or stomach) of the biopsy. Patient endoscopic features, presence or absence of CLE and clinical risk factors for BE, such as gender, race, waist/hip ratio, smoking history, severity of GERD symptoms, and body mass index (BMI) were compared between patients with vs without pure oxyntic-type mucosa and, of the former, with or without goblet cells.

Results: Biopsies composed of pure oxyntic glands occurred in 126/509 (24.8%) patients overall. Pure oxyntic-type mucosa was significantly more common in patients without CLE (30.5%) compared to patients with CLE (18.9%, p=0.003), and no significant difference was observed in short (21.8%) vs long segment (15.8%) type. Goblet cells occurred in 11/126 (8.7%) cases, and no significant differences were observed in CLE (12.5%) vs non-CLE (7%). No measured risk factors for BE or demographic characteristics of the patients were significantly related to the presence of pure oxyntic-type mucosa, or to those cases with or without goblet cells with one exception; males (80%) were more likely to have pure oxyntic-type mucosa vs females (68%, p=0.004).

Conclusions: Pure oxyntic-type mucosa may occur in a substantial proportion of esophageal biopsies in patients with CLE; thus, mucosa with this phenotype does not necessarily represent tissue from the proximal stomach. Contrary to the prevailing theory of BE pathogenesis, in some cases, goblet cells may be derived directly from pure oxyntic-type mucosa, without an intervening stage of mucous gland metaplasia.

748 Morphologic Subtypes of Dysplastic Colonic Polyps with Emphasis on Those with Clear Cell Change

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Background: Rarely, conventional adenomas (Ad) may show areas of serrated change. Conversely, sessile serrated adenoma/polyp (SSA/P) and traditional serrated adenoma (TSA) may develop dysplasia reminiscent of Ad. Finally, previous authorities have identified clear cell change in Ad, occasionally in a serrated pattern, that has unknown biological significance. The aim of this study was to reclassify a consecutive series of polyps originally diagnosed as Ad in 1995, based on contemporary WHO 2010 polyp criteria, and to evaluate the clinicopathologic and immunohistochemical features of the unusual group of Ad with clear cell change.

Design: 205 consecutive Ad (from 153 patients) diagnosed in 1995 were re-evaluated for the type of polyp [Ad, TSA with dysplasia (TSA-D), SSA/P with dysplasia (SSA/P-D)] based, in part, on evaluation of the background non-dysplastic component, and also for the presence and degree of clear cell (mucinous) change in the polyp (grade 1=<10%, 2=10-90%, 3=>90%). The clinicopathologic features and association with carcinoma upon long term follow up were evaluated. In addition, a subgroup of 35 polyps (7 Ad, 24 Ad with clear cell change, 3 TSA-D, 1 SSA/P-D) were evaluated for DAS1, MUC1, 2, 5AC, 6, CDX, and P53.

Results: Of the 205 consecutive polyps, 2 were reclassified as SSA/P-D (1%), 11 were reclassified as TSA-D (5.4%) and 52 (25.3%) showed at least focal clear cell change [grade 1=24 (11.7%), 2=23 (11.2%), 3=5 (2.4%)]. Ad with any mucinous component showed a similar gender distribution, mean age, anatomic distribution, and shape, to Ad without clear cell change, but were significantly larger in size (0.83 cm vs 0.55 cm, p=0.0005). Ad with clear cell change showed significant decreased DAS1 and MUC6, and significantly increased MUC5, in areas of clear cell change versus areas with no clear cell change. All other immunomarkers were similar. Overall, 7 patients (4.6%) had or developed cancer, 1 who had only clear cell Ad (2%), none with only SSA/PD (0%), none with only TSA (0%), 5 with Ad (3.6%), and 1 with a clear cell Ad, a TSA, and an Ad.

Conclusions: Based on the current WHO polyp classification, about 6% of polyps actually represent dysplastic serrated polyps (mostly TSA-D and a few SSA/PD). Up to 25% of Ad show clear cell change which shows a different immunophenotype from epithelium without clear cell change, but otherwise similar clinicopathologic features and association with cancer. Cancer outcome was similar in all groups, but further studies with larger numbers of patients are needed.

749 Histologic Features, Particularly Eosinophilic Inflammation, Can Discriminate Mycophenolate-Induced from GVHD-Induced Colitis

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Background: Mycophenolate Motif (MMF) is a T cell inhibitor frequently used for the treatment of acute allograft rejection. MMF may cause colitis that clinically and histologically resembles Graft Versus Host Disease (GVHD). Some bone marrow (BM) transplant patients also receive MMF, and, are at risk for both forms of colitis. The aim of this study was to evaluate a wide range of histologic features that may help differentiate MMF from GVHD-induced colitis, and to validate any significant features on a test set of BM transplant patients who also received MMF.

Design: Routinely processed colonic biopsies from 17 patients (mean age: 48 years, M/F ratio: 6/11) and 40 cases of GVHD (mean age: 57 years, M/F ratio: 26/14) (grade 1:10 cases, 2:10 cases, 3:10 cases, 4:10 cases) were evaluated histologically for a wide range of inflammatory, epithelial, and architectural changes in a blinded manner, in both an absolute and semiquantitative manner, by evaluation of 10 high power fields (HPF). Statistically significant features were then tested on a group of 20 BM transplant patients who also received MMF (N=20) and developed a diarrheal illness.

Results: Patients with MMF-induced colitis showed significantly fewer lymphocytes/HPF (24.0 vs 40.0, p=0.03), crypt atrophy (p<0.0001), and significantly fewer apoptotic cells per crypt (1.1 versus 10.6, p=0.005), compared to GVHD. In contrast, both the presence and quantity of neutrophils (p=0.01 for both) and, in particular, eosinophils (EOS) (17/17 vs 28/40, median=40.0 vs 2.0, p=0.0002) were far more common in MMF colitis. Regarding EOS, ≥15 EOS/10 HPF showed 75% specificity and 100% sensitivity for MMF. Many of these features, were also significant when MMF colitis was compared to each individual grade of GVHD separately. Evaluation of the test (validation) set of cases in a blinded manner, showed that the quantity of lymphocytes, and the presence or absence, and quantity, of neutrophils and EOS, and the degree of crypt distortion, showed high sensitivity and specificity for distinguishing MMF from GVHD. In particular, EOS ≥ 15 showed 100% sensitivity and 67% specificity.

Conclusions: Evaluation of histologic features, such as the presence and quantity of lymphocytes, neutrophils, and, in particular, EOS, atrophy and apoptosis, can help distinguish MMF from GVHD-induced colitis in a reliable manner. The presence of EOS >15/10 HPF is a highly sensitive and reasonably specific indicator of MMF induced colitis in BM transplant patients who have received this drug in addition to usual immunosuppressive therapy.

750 Hamartoma-Like Schwann Cell Proliferation and Architectural Distortion of the Appendix: A Study of 46 Appendiceal Diverticular Disease and Serrated Epithelial Lesions

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Background: S-100 positive Schwann cell proliferation in the appendiceal mucosa has been known as intramucosal neuroma. The recently described entity "mucosal Schwann cell hamartoma" (MSCH) is a benign mucosal Schwann cell proliferation in colonic polyps and holds morphologic resemblance to intramucosal neuroma of the appendix. We identified benign spindle cell proliferations of the appendiceal lamina propria encountered in conditions of mucosal expansion including diverticular disease and serrated polyps, and studied the prevalence and distribution of MSCH-like lesions of the appendix.

Design: 46 appendectomy cases of diverticular disease (23) and serrated lesions (23) to include sessile serrated polyp, hyperplastic polyp, and epithelial hyperplastic change were retrieved from the surgical pathology files from 2000 to 2011. H&E slides were reviewed and distribution of the spindle cells in the lamina propria in relation to epithelial alteration or crypt architectural distortion, if present, was studied. Representative tissue sections from paraffin-embedded blocks were subject to immunoperoxidase staining for S-100, EMA and Neurofilament protein (NFP).

Results: 13 of 46 cases (28%) showed localized or diffuse proliferation of S-100 positive Schwann cells in the lamina propria associated with prominent crypt distortions. The mean age of the patients was 46 (22-77) years and included 7 females and 6 males. No patient had a history of a syndromic disorder. 10 mucosal schwann cell proliferations were from appendiceal diverticular cases. In 2 cases the secondary epithelial alterations had been interpreted as serrated adenoma (1) and epithelial hyperplastic change (1). One had been diagnosed as neural hyperplasia (1). No diffuse Schwann cell proliferation was seen within the serrated polyps. One case showed focal NFP staining mixed with Schwann cell proliferation. EMA was negative in the spindle cells of interest.

Conclusions: In the appendix with diverticular disease, MSCH-like prominent mucosal Schwann cell proliferation is common, being encountered in 43% (10 of 23) of the cases studied. The Schwann cell proliferation is usually associated with crypt architectural distortion. Awareness of this common finding of MSCH-like proliferation in the appendix is required to avoid misinterpreting secondary epithelial alterations as preneoplastic conditions. On the contrary, Schwann cells are inconspicuous within true serrated polyps of the appendix. Perineuriomas are uncommon in the appendix.

751 The Prognostic Value of MUC16 (CA125) on the Cell Surface of Gastrointestinal Cancers

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Background: Mucin core protein 16 (MUC16, CA125) is a cell surface glycoprotein that plays a role in promoting cancer cell growth and survival in ovarian cancer. We examined MUC16 expression in a large number of gastrointestinal carcinomas and precursor lesions to determine whether MUC16 up-regulation is correlated with patient outcome in gastrointestinal neoplasia.

Design: Tissue microarrays were constructed using surgical resection tissues from gastrointestinal adenocarcinoma patients. Tissue cores included: Pancreatic (115 normal, 11 precursors, 200 pancreatic ductal adenocarcinomas), Esophageal (86 normal, 104 precursors, 95 esophageal adenocarcinomas, 35 lymph node metastases); Gastric (176 normal, 43 precursors, 119 gastric adenocarcinomas, 62 lymph node metastases); Colorectal (34 normal, 17 precursors, 39 colorectal adenocarcinomas). Membranous MUC16 was scored and samples were categorized into four tiers: negative, focal, moderate, or diffuse staining. Six pancreatic and two esophageal adenocarcinoma cell lines were analyzed for MUC16 presence using Western Blot.

Results: MUC16 was detected in 81.5%, 69.9%, 41.2%, and 64.1% of the pancreatic ductal, esophageal, gastric, and colorectal adenocarcinomas, respectively. MUC16 was seen in a subset of non-invasive precursor lesions. Multivariate analysis indicated that moderate/diffuse MUC16 in pancreatic ductal adenocarcinomas is strongly associated with poor survival (P<0.001), independent of other prognosis predictors. A similar trend was observed for esophageal (P=0.160) and gastric adenocarcinomas (P=0.080). Focal MUC16 in colorectal adenocarcinomas was significantly correlated (P=0.044) with a better patient outcome, when compared to MUC16 negative cases. MUC16 was found in three pancreatic ductal and one esophageal adenocarcinoma cell lines.

Conclusions: We conclude that MUC16 expression occurs in the majority of the gastrointestinal adenocarcinomas and a subset of non-invasive precursor lesions. MUC16 expression is an independent predictor of poor outcome in pancreatic ductal adenocarcinomas, and potentially in gastric, and esophageal adenocarcinomas. As MUC16 expression appears to parallel aggressive tumor behavior, MUC16 may function as a prognostic marker and therapeutic target.

752 Acute Cellular Rejection in Small Bowel Transplantation: The Nebraska Experience

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Background: Acute cellular rejection (ACR) is a significant complication of small bowel transplantation. Since the 2003 Eighth International Small Intestinal Transplantation Symposium, a standardized grading system has existed for evaluation. Other authors have noted significant rates of biopsies graded as indeterminate and mild while we have observed lower rates for both grades. The aim of this study was to document the frequency of the grades of ACR and to determine if the grade correlated with length of duration of acute rejection.

Design: All small bowel transplant mucosal biopsies performed at our instituition from Janurary 2004 to September 2011(2082 biopsies from 235 patients) were retrospectively analyzed. Specimens were assigned as: no evidence of ACR (grade 0), indeterminate for ACR, mild ACR (grade 1), moderate ACR (grade 2), severe ACR (grade 3), treated/on-going rejection, or no crypts present to evaluate ACR. The length of each ACR episode was determined by the time from index biopsy to the first of two consecutive grade 0 biopsies. Other findings such as PTLD, CMV enteritis, and adenovirus enteritis were also documented.

Results: The distribution of ACR was as follows: grade 0 (84.6%), indeterminate for ACR (5.5%), grade 1 (4.6%), grade 2 (1.7 %), grade 3 (1.6%), treated/on-going rejection (1.5%), no crypts present to evaluate ACR (1.3%). The average length of each rejection episode for a given grade was as follows: grade 1 (14.6 days +/- 11.8 days), grade 2 (53.3

days +/- 90.1 days), grade 3 (43.4 days +/- 49.6 days). The average length of rejection was statistically significant between episodes called mild versus moderate ($p<0.05$). However, there was no statistically significant difference in length of rejection between episodes called moderate versus severe ($p=0.54$).

Conclusions: Acute Cellular Rejection is an uncommon but significant complication of small bowel transplantation. These findings demonstrate a low frequency of biopsies graded as indeterminate at our institution. Although there was a statistically significant difference in rejection time between mild and moderate ACR, no such difference existed between moderate and severe ACR in this analysis. Finally, these findings demonstrate that the current grading scheme may correlate with length of acute cellular rejection.

753 Serrated Polyps of the Extracolonic Gastrointestinal Tract: Histologic Findings and Genetic Alterations

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Background: Serrated polyps are now recognized as precursors to a subset of colorectal carcinomas (CRC) and have been classified as hyperplastic (HP), sessile serrated adenoma (SSA), traditional serrated adenoma (TSA) and mixed adenomatous/serrated polyp (MP). HP and SSA frequently harbor BRAF mutations, while dysplastic lesions (TSA and MP) more commonly harbor KRAS mutations. CRC arising through the serrated neoplasia pathway often demonstrates high-level microsatellite instability (MSI-H) and CpG island methylator phenotype (CIMP). In contrast to serrated polyps of the colon, extracolonic serrated polyps are virtually undescribed and their genetic alterations are unknown.

Design: Fifteen serrated polyps from small bowel (n=9), stomach (n=4), and ampulla (n=2) were classified in accordance to serrated lesions of the colon as HP (n=2), SSA (n=1), TSA (n=6) and MP (n=6). Purified DNA from these lesions was subjected to pyrosequencing for BRAF (exons 11, 15) and KRAS (codons 12, 13, 61) mutations, immunohistochemistry and/or PCR for MSI status, and methylation-specific PCR for CIMP.

Results: Eleven (73%) of 15 serrated polyps were associated with invasive carcinoma, including 5 with mucinous features and 1 with signet ring cells. Oncogenic KRAS mutation was the most common abnormality, present in 8 of 14 cases (57%). MSI and CIMP were less common (Table 1). Only one polyp (duodenal HP) harbored a BRAF mutation (Fig 1).

TABLE 1. Extracolonic serrated polyps

Location	Histology	KRAS	BRAF	MSI	CIMP
Ileum	TSA	G13D	Neg	Stable	Low
Jejunum	TSA	G12D	Neg	Stable	Neg
Ileum	MP	Neg	Neg	--	Neg
Jejunum	MP	Q61E	Neg	Stable	Low
Duodenum	MP	--	--	MSI-L	--
Duodenum	TSA	Neg	Neg	Stable	--
Duodenum	SSA	G12D	Neg	Stable	Low
Duodenum	MP	G12D	Neg	--	--
Duodenum	HP	Neg	V600E	Stable	--
Stomach	TSA with fundic gland polyp	Q61H	Neg	Stable	Neg
Stomach	HP	Neg	Neg	Stable	Low
Stomach	MP	G12D	Neg	MSI-H	High
Stomach	MP	Neg	Neg	--	--
Ampulla	TSA	G12A	Neg	Stable	Neg
Ampulla	TSA	Neg	Neg	Stable	Low

--, insufficient DNA

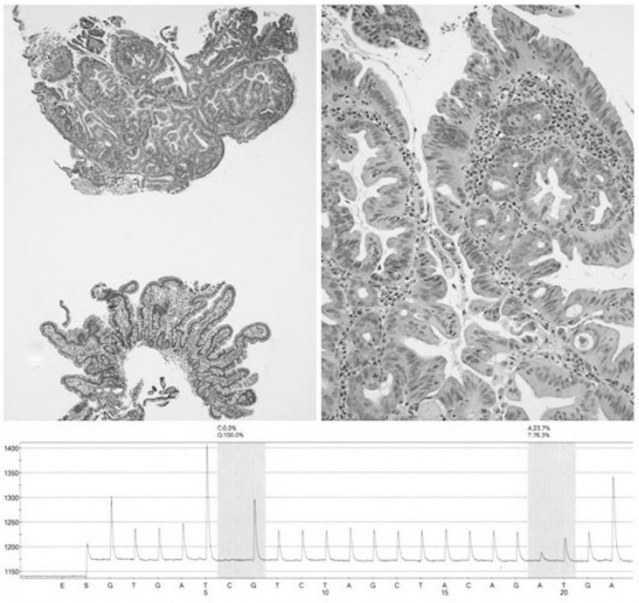

Figure 1. Duodenal hyperplastic polyp with BRAF V600E mutation

Conclusions: In contrast to serrated polyps of the colon, serrated polyps in extracolonic locations have higher rates of dysplasia and more frequently contain invasive carcinoma. In our analysis, KRAS mutations were common in dysplastic serrated lesions and BRAF mutation was restricted to a nondysplastic duodenal HP.

754 Reproducibility of the Diagnosis of Malignant Colorectal Polyps

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Background: Due both to introduction of screening of colorectal cancer and improvement of endoscopically techniques, the resection of malignant polyps (Tis and T1) is increasing. On a limited specimen, the pathologist has the critical task of determining whether there is a significant risk of recurrence or metastasis which may result in complementary surgical resection of the colorectum. To date, however, the level of agreement among pathologists in diagnosing malignant polyps has not been well established.

Design: Using a web-based virtual microscopy, we examined the observer variation in the diagnosis of 100 malignant colorectal polyps (Tis and T1) endoscopically resected by 13 gastrointestinal pathologists. Different pathological parameters were assessed as tumor differentiation, angiolymphatic vessel invasion, tumor budding, Haggitt's or Kudo's levels, measurement of submucosal invasion and base resection margin status. Results were analyzed by kappa (k) statistics and for percentage agreement.

Results: k analysis indicated that the strength of agreement was substantial for resection margin (k=0.61), moderate for pTis/pT1 classification (k=0.54), tumor budding (k=0.44), and slight for tumoral differentiation (k=0.14). Interobserver agreement was fair and moderate for Haggitt's and Kudo's classifications respectively. A better agreement was observed when measurement of the width or depth of submucosal invasion was performed (k=0.58 and k=0.54). According to the presence or not of pejorative criteria, there was a moderate agreement among pathologists on patient management strategies (k=0.54). A better agreement was observed among pathologists working in most active endoscopic centers and for specimens well orientated.

Conclusions: Gastrointestinal pathologists demonstrate moderate agreement for differentiate intramucosal carcinomas (pTis) from invasive adenocarcinomas (pT1) in endoscopically colonic resected specimens. The high interobserver variability concerning the tumoral differentiation parameter is clinically irrelevant. Measurement of the extent of invasion in submucosa appears more reproducible than the Haggitt's and Kudo's classifications.

755 Immunohistochemical Expression of IMP3 in Superficially Invasive pT1 Esophageal Adenocarcinoma (EAC) Is Associated with Lymphovascular Invasion (LVI) and Can Help in the Risk Stratification of These Patients

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Background: A problem in the management of patients with Barrett's esophagus-related pT1 EAC is to distinguish those who should be treated conservatively either by EMR(Endoscopic mucosal resection) and/or RFA (Radio-frequency ablation) from those who require gastro-esophagectomy. Recently, LVI has emerged as on of the best predictors of regional lymph node metastasis (LNM) and recurrence-free survival (RFS) in pT1 EAC (*AJSP 2011; 1045:1052*). However, LVI may be underestimated, both because of interobserver variability and incomplete sampling. The aim of our study was to determine whether IMP3 expression by immunohistochemistry (IHC) in pT1 EAC is associated with LVI.

Design: Depth of invasion, assessed in five sublevels (m2, m3, sm1, sm2, and sm3), LVI, and expression of IMP3 were studied in 30 patients who underwent gastro-esophagectomy for pT1 EAC (2001-2010) at Hartford Hospital, and correlated with LNM and RFS. IMP3 was considered positive when expressed in >50% of the malignant cells with an intensity of stain of 2-3+.

Results: The results are summarized in Table1. 10 of 18 (55.5%) cases with IMP3 expression demonstrated LVI and 2/10 (20%) showed LNM and DOD. In contrast, none of the 12 IMP3 negative cases showed LVI ($p<0.004$; 2-tailed Fisher exact test) or had LNM/DOD.

Table 1: Clinical, histological and IMP3 labeling

	LVI+/IMP3+	LVI-/IMP3+	LVI-/IMP3-	LVI+/IMP3-	Total
m2	0	2	6	0	8
m3	0	2	3	0	5
sm1	0	1	0	0	1
sm2	5	1	1	0	7
sm3	5	2	2	0	9
Total(%)	10(33.4%)	8(26.6%)	12(40%)	0(0%)	30(100%)
LNM(%)	2/10(20%)	0/8(0%)	0/12(0%)	0/0(0%)	2/30(6.6%)
DOD(%)	2/10(20%)	0/8(0%)	0/12(0%)	0/0(0%)	2/30(6.6%)

m: mucosal, sm: submucosal, DOD: died of disease

Conclusions: In pT1 EAC, 1) IMP3 expression is associated with an increased likelihood of LVI. 2) Absence of IMP3 expression is associated with a significantly reduced risk of LVI (Negative Predictive Value: 100%). 3) Assessment of IMP3 in pT1 EAC may be useful in the risk stratification and potentially on the type of treatment of these patients.

756 Use of Elastic Stain in Identification of Venous Invasion in Polyps with Early Invasive Adenocarcinoma (pT1)

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Background: Venous invasion (VI) has been reported to be one of the significant predictors for distant metastases in colorectal carcinomas. Unfortunately the assessment of VI on routine hematoxylin eosin (H&E) stained slides is prone to subjectivity. To our knowledge no previous study has looked for VI by elastic stain on polyps with early invasive adenocarcinomas. The aim of our study was to evaluate VI in these lesions using elastic stain and correlate its presence with distant metastases and the clinical outcome.

Design: 15 cases of early invasive carcinoma (pT1) on polypectomy specimens were selected and reviewed by two pathologists for venous invasion. Based on previously described histological features suspicious for VI on H&E sections, such as protruding tongue sign (round-oval smooth-bordered protrusion of tumor into pericolic fat with visible adjacent artery) and unaccompanied artery sign (thick walled artery is visible adjacent to an oval shaped focus of tumor with no normal vein) (J Clin Pathol 2009; 62: 1021-1025), a representative tissue block was selected for Verhoff's Elastic stain. The follow up data for metastatic disease was obtained from medical records.

Results: H&E stain identified VI only in 3 cases (3/15=20%) while elastic stain identified VI in 7/15 cases(47 %). The median follow-up was 3 years. During this period 2/15 cases developed distant metastases. One of these two cases negative on H&E stain, showed VI on elastic stain. The other case did not show VI either on elastic or H&E stain.

VI on H&E and Elastic stain (n=15)

	Positive VI	Negative VI
H&E stain	3	12
Elastic stain	7	8

Conclusions: 1. Routine use of elastic stain significantly enhances the detection of VI in polyps with early invasive adenocarcinoma (from 20% to 47%).2. The presence of distant metastasis in one of our cases with VI identified only on elastic stain suggests that consideration should be given to the use of elastic stain in cases demonstrating the protruding tongue sign or an unaccompanied artery sign.3. However due to the limited number of cases in our series, we cannot establish if the presence of VI in these malignant polyps has a negative impact on outcome. 4. Consequently additional study with a large number of patients should be done to identify the significance of VI as a risk factor for distant metastases.

757 Immunohistochemical Differences between Pancreatic Adenocarcinoma and Intraductal Papillary Mucinous Neoplasms: Insights into Progression of a Precursor Lesion

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Background: Alteration of p16, p53, PTEN, and Bcl2 occur frequently in pancreatic adenocarcinoma (PCa). Recent studies have demonstrated p16, p53, Bcl2, and Ki67 as prognostic immunohistochemical (IHC) biomarkers of PCa. Intraductal papillary mucinous neoplasms (IPMN) represent precursor lesions that can progress to PCa. The molecular alterations that mediate progression from IPMN to PCa, and the differences between these lesions in terms of IHC, remain poorly understood. We have previously reported the PTEN, p53, and p16 IHC profile of 36 IPMN cases. In this study, we characterize the differences between these IPMN cases and 19 PCa cases, in terms of p16, p53, PTEN, Bcl2, and Ki67 IHC.

Design: We analyzed 36 cases of IPMN and 19 cases of PCa. DNA was extracted from paraffin-embedded tissue. Tissue microarrays consisting of 3 cores/case were constructed. Expression of p16, p53, PTEN, Bcl2, and Ki67 was assessed by IHC. PTEN expression within lesional epithelium was scored as normal (2+), decreased (1+), or negative (0). Bcl2 expression was scored as increased (2+) or normal (1+) compared to non-neoplastic epithelium. Staining for p53 (0,1+,2+,3+) and p16 (0,1+,2+) was scored according to intensity. Ki67 positivity was scored as a percentage.

Results: We observed significant differences in terms of p53 and PTEN IHC between PCa (p53=1.02/3; PTEN=0.36/2) and IPMN (p53=0.48/3; PTEN=1.47/2) ($p<0.05$). No significant differences were observed between PCa and IPMN in terms of p16 (IPMN=0.65/2; PCa=0.45/2), Bcl2 (IPMN=1.37/2; PCa=1.36/2), or Ki67 (IPMN=14%; PCa=15%). Differences in p53, PTEN, and p16 staining were significant when low-grade (LG) and intermediate-grade (IG) IPMN (p53=0.33/3; PTEN=1.70/2; p16=0.89/2) were compared to high-grade (HG) IPMN and PCa (p53=0.83/3; PTEN=0.80/2; p16=0.43/2) ($p<0.05$).

Conclusions: The observed differences between IPMN and PCa in terms of p53 and PTEN staining suggest that PTEN loss and decreased p53 function contribute to the progression from IPMN to PCa. The observed differences between LG and IG IPMN versus HG IPMN and PCa indicate roles for p16, p53, and PTEN in the evolution of HG IPMN, prior to the development of cancer. We did not observe differences in Bcl2 IHC, perhaps because alterations of Bcl2 occur early in the development of IPMN, or cannot be detected by IHC. These findings provide insight into molecular changes that mediate the progression of IPMN into PCa.

758 Clinicopathologic and Molecular Characterization of PIK3CA Mutations in Colorectal Neoplasms

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Background: PIK3CA mutations have been described in 10-20% of colorectal carcinomas. Only a small number of studies have examined their significance, often with conflicting results.

Design: We analyzed frozen tissue from 613 colorectal neoplasms including 34 adenomas for hotspots mutations in PIK3CA (exons 4, 7, 9 and 20), KRAS, and BRAF using a MALDI-TOF mass-spectrometry based genotyping assay. TP53 mutations were also detected using Sanger sequencing. Microsatellite instability testing was performed in 352 cases and clinicopathologic data was collected in all cases. Mutant allele frequency (MAF) was determined in 2 cases using deep sequencing.

Results: PIK3CA mutations were detected in 69 (11.3%) cases including 5 (14.7%) adenomas and the majority of mutations were in exon 9 (n=44, 63.8%). Right-sided tumors were more frequently mutated compared to left-sided and rectal tumors (16.7% vs 9.6% and 9.7%, p=0.03), but there was no association between PIK3CA mutations and sex, age or stage at presentation. PIK3CA mutations were more common in microsatellite unstable (MSI-H) tumors (29% vs. 9.7%, $p<0.001$), and were observed both in sporadic and hereditary non-polyposis colorectal cancer-associated cases. Compared to PIK3CA wild-type tumors, PIK3CA-mutated tumors were more likely to harbor KRAS mutations (16.1% vs. 7.7%, p=0.002). There was no significant association between PIK3CA and TP53 or BRAF mutations, although none of the tumors with PIK3CA exon 20 mutations harbored a BRAF mutation. In 2 KRAS/PIK3CA mutant cases tested the PIK3CA MAF was lower compared to the KRAS MAF, suggesting the PIK3CA mutation occurred later than the KRAS mutation. There was no significant difference in disease specific survival between PIK3CA mutant and wild type tumors even after MSI-H tumors were excluded from analysis.

Conclusions: PIK3CA mutations are present in adenomas, suggesting that they occur relatively early in colorectal carcinogenesis. They show a strong association with KRAS mutations and are more frequent in right-sided and MSI-H tumors. Contrary to a prior study, we did not observe an association with adverse prognosis.

759 Heat Shock Protein (HSP)-90 Is Overexpressed in Gallbladder Carcinoma (GBC)

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Background: GBC is one of the most common malignancies in Chile with the highest mortality rate in the world. It is the principal cause of oncologic death in women. Usually it presents with advanced stage with no effective treatment. Heat shock proteins (HSPs) are molecular chaperones that are known to have tumoral activity. Of these, HSP90 had been shown to interact with different molecules related to differentiation, proliferation and oncogenesis in cancer cells. Two molecules have shown to inhibit the action of HSP90 (geldanamycin (GA) and 17-allylamino-17-des-methoxygeldanamycin (17-AAG)) and hence a potential target for adjuvant therapy. HSP90 expression and antitumoral activity of GA and 17-AAG have not been studied in GBC.

Design: Forty-seven GBC cases were selected for this study. Immunohistochemical studies in paraffin-embedded tissue were done using a monoclonal HSP90 antibody (clone JPB24, dilution 1:200, Novocastra, Newcastle upon Tyne, UK). A positive case was interpreted if at least 50% of the tumor showed cytoplasmic staining. Cell lysates (15mg) were prepared from gallbladder cancer cell line (GB-d1), human epithelial gallbladder cells (HGBEC) and normal human embryonic kidney cells (HEK293) for Western Blot (WB) analysis using anti-HSP90 (1:250). For viability assay, GB-d1 cells were seeded in 96-well plates at a density of 5000 cells per well. After an overnight attachment period, cells were exposed to various concentrations of GA and 17-AAG for 24 h, 48 h and 72 h. Control cells received DMSO only. The percentage of viable cells was assessed with a colorimetric MTS cell proliferation assay (Promega, Madison, WI).

Results: HSP90 protein expression was seen in 45 cases by immunohistochemical analysis (45/47). Average expression was 78%. The staining intensity ranged from weak to strong with 35 cases (74%) showing moderate intensity. HSP90 expression was higher in GB-d1 cell line compared to normal gallbladder cells. The MTS cell proliferation assay demonstrated that both GA and 17-AAG similarly elicited cytostatic and cytotoxic effects on GB-d1 cells. After 48 h treatment at concentrations >10 uM, the GB-d1 cell viability was reduced by GA and 17-AAG. Treatment with 0.1% of DMSO as equimolar solvent control does not affect the GB-d1 cell viability.

Conclusions: GBC is a heterogeneous group of neoplasms with overexpression of proteins related to proliferation and survival. Our results showed that HSP90 is highly expressed in GBC making it a good candidate for targeted therapy.

760 Molecular Characteristics of HPV Positive anal Carcinoma

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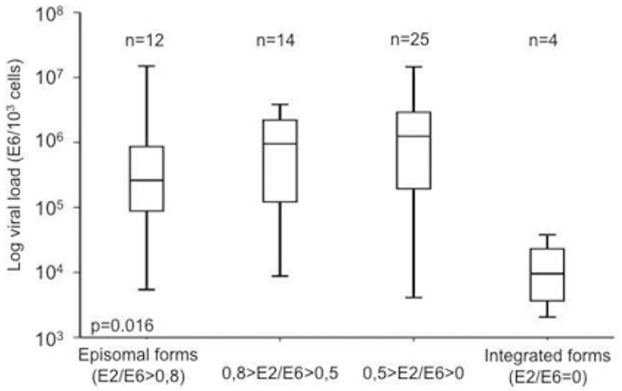
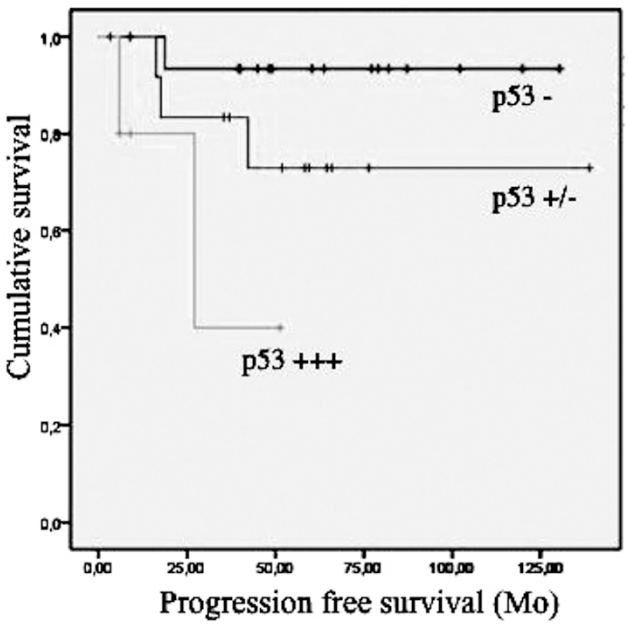
Background: Incidence of invasive anal carcinoma is increasing. Most anal invasive carcinoma is caused by high-risk human papillomaviruses (HPV) and HPV16 is the most prevalent type.

Design: HPV genotype distribution was determined in 86 diagnostic biopsies of anal cancers, collected between 1997 and 2007. Viral load and physical state of the viral genome were evaluated in HPV16 positive tumours with type-specific real-time PCR targeting E6, E2 and albumin genes. Expression of p16, Ki-67, p53 and p21 was also analyzed by immunohistochemistry. Biologic data were correlated with OS and PFS of patients to highlight theranostic factors.

Results: 80% of cases were squamous cell carcinoma and 97.5% of samples were positive for alpha-HPV DNA. HPV16 was the most prevalent genotype (90%), followed by HPV39 and HPV33 (3.75% each). Fully integrated HPV16 DNA estimated by the E2/E6 was observed in less than 10% of cases associated with a low viral load compared with cases containing either pure episomes or mixes of integrated and episomal DNA in which the HPV16 median load was at least higher of 2 log.



The majority of HPV16+ tumours expressed p16 (90%) and showed a high proliferation index (82%). In contrast a minority of tumours presented an overexpression of p53 (14%) and its target p21 presented a different expression pattern. An interim statistical study showed that p16 expression and absence of p53 expression seemed to be positive prognostic markers in term of progression free survival and overall survival.



Conclusions: The high prevalence of HPV16 is confirmed in this French series of anal carcinoma with a high viral load except in cases harbouring pure integrated HPV16 DNA. The subsequent deregulated p53 expression might predict response to radiochemotherapy.

761 *IDH1* and *IDH2* Mutations Detected by Pyrosequencing in Cholangiocarcinoma

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Background: Somatic mutations in isocitrate dehydrogenase 1 and 2 genes (*IDH1* and *IDH2*) are common in gliomas and can help stratify brain cancer patients into histologic and molecular subtypes. However, these mutations are considered rare in other solid tumors. The aim of this study was to determine the frequency of *IDH1* and *IDH2* mutations in cholangiocarcinoma (CCA).

Design: DNA was extracted from 94 paraffin-embedded CCA resection specimens (67 intrahepatic and 27 extrahepatic). Pyrosequencing was performed using a Qiagen PyroMark Q24 system (Valencia, CA), assessing for mutations in codons 132 of *IDH1* and codons 140 and 172 of *IDH2*. Identified mutations were confirmed by Sanger sequencing. The association between the occurrence of mutations in *IDH1* and *IDH2* and clinicopathologic results were examined using the Chi-square and Fisher's exact tests, as appropriate. Kaplan-Meier curves were plotted and survival was compared using the logrank value. All reported P-values were two-sided and P-values less than 0.05 were considered statistically significant.

Results: Mutations were detected in 21 (22%) of the 94 evaluated specimens including 14 *IDH1* and 7 *IDH2* mutations. *IDH1* mutations included R132C (n=9), R132S (n=2), R132G (n=2), and R132L (n=1). The 7 *IDH2* mutations included R172K (n=5), R172M (n=1) and R172G (n=1). Mutations were more frequently observed in intrahepatic CCA compared to extrahepatic CCA (28% vs. 7%, respectively; P=0.030). There were no significant differences in the frequency of *IDH1/2* mutations when compared to age, gender, lymph node metastasis, or associated PSC status. Patients with an *IDH1/2* gene mutation appeared to have better overall survival a year following surgical resection when compared to patients without an *IDH1/2* mutation (95% vs. 83%, respectively), however, the overall median survival was not significantly different (P=0.338).

Conclusions: The results of this study show for the first time that *IDH1* and *IDH2* genes are mutated in CCA. The results of this study are encouraging because it identifies a potential biomarker for earlier detection of CCA and a new target for genotype-directed therapeutic trials. Additional studies with larger cohorts are needed to determine whether patients with *IDH1/2* mutations are clinically distinct from CCA patients without an *IDH1/2* mutation.

762 Concordance and Interobserver Agreement of HercepTest™ and 4B5 Immunohistochemical Staining in Gastric Carcinoma

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Background: Reliable and reproducible detection of HER2 overexpression in gastric carcinoma is essential for determining patient eligibility for trastuzumab (Herceptin™) therapy. HercepTest™ and 4B5 immunohistochemistry (IHC) were directly compared to determine the performance characteristics of each assay for detection of HER2 status in gastric carcinoma.

Design: Twenty-six formalin-fixed, paraffin-embedded gastric adenocarcinomas were evaluated for HER2 status by IHC (HercepTest™ and 4B5) and fluorescence *in situ* hybridization (FISH) (PathVysion®). IHC slides were reviewed by two pathologists. Interobserver agreement and methodology concordance were determined for HercepTest and 4B5, using FISH as the gold standard.

Results: Agreement between HercepTest and 4B5 results was 81% (21/26). There was a noticeable tendency for higher scoring with 4B5 as compared to HercepTest™.

Table

	HercepTest 0	HercepTest 1+	HercepTest 2+	HercepTest 3+
4B5 0	7	0	0	0
4B5 1+	6	1	0	0
4B5 2+	2	3	2	0
4B5 3+	1	1	1	2

Interobserver agreement among 2 IHC viewers for HercepTest™ or 4B5 was 77% (20/26) and 73% (19/26), respectively. All 4B5 discrepant cases were correctly classified, as confirmed by FISH, for clinical decision. Correct clinical classification was achieved in 5 of 6 discrepant HercepTest™ cases. Concordance with HER2 FISH was 89% (23/26) for HercepTest™ and 96% (25/26) for 4B5.

Conclusions: Evaluation of HER2 overexpression in gastric carcinoma by 4B5 shows higher concordance with HER2 FISH compared to HercepTest™. There was no significant difference in interobserver variability between 4B5 and HercepTest™.

763 Ileal 'Carcinoid' Tumours – Small Size Belies Deadly Intent: High Rate of Nodal Metastasis in Tumours ≤1cm

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Background: Neuroendocrine ('carcinoid') tumours account for 2% of tumours of the GI tract, most occurring in the small intestine. A size of 2cm is generally regarded as a cut off point for risk of lymph node metastasis in intestinal neuroendocrine tumours in the absence of other high risk features. However, metastatic disease has been reported in 12% in tumours of the jejunum and ileum measuring 1cm or less.

Design: Archives from 2 institutions were searched for ileal neuroendocrine tumours measuring 1cm or less. Gross, histologic, demographic, clinical and follow-up data were recorded.

Results: Twenty-one ileal neuroendocrine tumours were identified measuring ≤10mm (mean 6.5 mm (range 1-10mm), male:female ratio 11:10, mean age 64y (range 36-90y)). At least 6 cases were incidental findings either at colonoscopy or at surgery for other causes. Seven (33%) were multiple and 19 (90%) met the WHO criteria for well differentiated neuroendocrine carcinoma. Regional lymph nodes were examined in 14 cases (67%) and 10 of these cases (71%) showed lymph node metastasis (mean number of nodes examined 8.79 (range 1-25), mean number of positive nodes 2.36 (range 1-11)). Mean primary tumour size in cases with nodal metastasis was 7.3mm (range 3-10mm) and 4 (29%) were multifocal. Seven (33%) had distant metastasis at diagnosis (6 liver, 1 mesentery). Follow up data were available for 17 patients (81%) with a mean follow up period of 39 months (range 1-135 months). Four patients (19%) were dead from disease, 8 (38%) were dead from other causes, 3 (14%) were alive without disease at last follow up and 2 (10%) were alive with disease.

Conclusions: In this series of ileal neuroendocrine tumors ≤1cm in size, the rate of lymph node metastasis was 48% overall and 71% for cases with regional lymph node resections. In addition, 33% showed distant metastasis at the time of diagnosis (possible referral bias). It is apparent that tumours as small as 3mm and those confined to the submucosa can give rise to lymph node metastasis. These data emphasise the need for serious consideration of local resection with regional lymphadenectomy, even for sub-centimetre ileal neuroendocrine tumours.

764 Poor Agreement for Detection of Goblet Cells in Esophageal and GEJ Biopsies

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Background: The American Gastroenterological Association (AGA) criteria for Barrett's esophagus (BE) includes endoscopic recognition of columnar mucosa and histologic confirmation of goblet cells in mucosal biopsies. Goblet cells are often few

in number, and may be difficult to differentiate from pseudogoblet cells. This study was performed to determine the interobserver variability for detection of goblet cells, and other cell types, in biopsies from the distal esophagus and GEJ.
Design: Digitally scanned mucosal biopsies (Aperio system) from 34 patients, obtained from either the distal esophagus (N=16, 5 long segment BE, 11 short segment BE) or GEJ (N=18) were evaluated by 7 GI pathologists for a variety of histologic features, such as goblet cells, multilayered epithelium (ME), pseudogoblet cells, and type of glands, and then the pathologists were asked whether they believed the biopsies were diagnostic of BE. Overall, 18 cases with few, or only rare, goblet cells diagnosed by the original signout pathologist, who did not participate further in the interobserver study, were selected for study. The data were analyzed by Kappa statistics.
Results: Overall interobserver agreement for detection of goblet cells was poor (Kappa = 0.35), and agreement was worse for esophageal versus GEJ biopsies. All 7 reviewing pathologists agreed on the presence of goblet cells in only 12 cases. Kappa values for detection of ME and pseudogoblet cells were very poor (0.29 and 0.10, respectively). The highest levels of agreement were obtained for detection of gland type (Kappa = 0.54). However, even regarding gland type, all 7 pathologists agreed on the presence of mucous glands (vs. oxyntic vs. mixed mucous/oxyntic) in only 29% of cases. Agreement regarding whether the observers believed the biopsy fulfilled the histologic criteria for BE was extremely poor (Kappa=0.15).
Conclusions: Based on the results of this study, the current AGA criteria for BE is problematic, since interobserver agreement for detection of goblet cells when the latter are few in number is poor.

765 Histological Findings in Acutely Symptomatic Ulcerative Colitis Patients with Superimposed *Clostridium difficile* Infection

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Background: In patients with acutely symptomatic ulcerative colitis (UC), pathologists may be asked to differentiate between a UC flare versus superimposed infection. Ulcerative colitis patients are prone to *Clostridium difficile* infection and UC patients tend to have worse outcomes when *C. difficile* positive. However, no recent studies have examined whether it is possible to detect histological evidence of *C. difficile* related colitis in the setting of acute UC. If stool testing has not been performed, histological suspicion may prompt earlier testing and treatment.
Design: The microbiology database at St. Michael's Hospital was searched for UC patients tested for *C. difficile* by stool enzyme immunoassay (2004-2011). 10 Patients with a diagnosis of ulcerative colitis with colonic biopsies taken during a flare that coincided with a positive *C. difficile* (*C. difficile* +) test were identified. 32 controls were found who were biopsied during a UC flare but tested negative for *C. difficile* (*C. difficile* -) Slides from these cases were reviewed by a gastrointestinal pathologist who was blinded to patient cohort. Features examined included evidence of pseudomembranes (defined as "mushroom" shaped fibrinopurulent exudate at the mucosal surface), degree of active and chronic colitis, and presence of lamina propria hemorrhage and neutrophils. Endoscopy reports were examined for comments regarding macroscopic pseudomembranes. Statistical analysis was performed with two-tailed Fisher's exact test.
Results: In *C. difficile* + patients, 4 of 10 (40%) had microscopic pseudomembranes. In controls, 4 of 32 (13%) demonstrated pseudomembranes. This difference approached but fell short of significance (p=0.076). Only one of the *C. difficile* + patients and none of the controls had endoscopically evident pseudomembranes. There were no differences in the severity of colitis on biopsy between *C. difficile* + and negative patients, with most having moderate acute and chronic colitis. The presence of hemorrhage or neutrophils in the lamina propria did not correlate with *C. difficile* positivity.
Conclusions: There are no specific features which separate UC patients with and without *C. difficile* infection. However, the presence of microscopic pseudomembranes is suggestive of super-imposed *C. difficile* infection in UC patients. While it is not particularly sensitive or specific, commenting on this histological feature may prompt earlier microbiologic definitive testing and treatment.

766 Immunohistochemical Profiles of Small Intestinal Adenocarcinomas

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Background: Small intestinal adenocarcinomas (SIAC) are rare tumors associated with poor prognosis. Immunohistochemical studies have revealed variable staining profiles. Our study examined a series of markers with potential diagnostic or therapeutic significance.
Design: 22 non-ampullary SIAC were identified. A history of Crohn's, celiac, ulcerative colitis, Peutz-Jeghers, and Lynch syndrome was present in 12 patients. Sections were immunohistochemically stained for CK7, CK20, HER2, P53, HepPar1, CD10, AMACR, MLH1, and MSH2. CK7, CK20, CD10, and HepPar1 were graded as weak, moderate and strong staining. HER2 was interpreted by the gastric adenocarcinoma criteria. The other stains were interpreted as positive or negative, with at least moderate tumor staining required for positivity.
Results: The majority of SIAC were CK20 positive: 15 showed moderate to strong staining and 4 had focal or weak positivity. In contrast to a previous study, CK7 positivity was less common and seen in only 12 tumors, including 2 that were focal and weak. HER2 showed 3+ in one tumor and 2+/equivocal positivity in 4 tumors; in situ hybridization studies are pending. P53 was positive in 13 tumors. Normal small bowel consistently stained for CD10 and HepPar1, while expression was present but weaker in 6 and 8 tumors respectively. Contrary to a previous report, AMACR was up-regulated in 11 tumors, with adjacent normal tissue showing faint to non-existent staining. In 18 tumors tested with MLH1 and MSH2, 4 had loss of staining consistent with microsatellite instability. History of chronic or hereditary gastrointestinal diseases, location (proximal vs. distal), and tumor differentiation were not associated with any unique staining pattern.
Conclusions: SIAC show variable CK7 and CK20 profiles, but most the majority show a degree of CK20 expression. HER2 over-expression was less common, but if amplified, it has therapeutic implications. P53 mutation likely has a role in tumorigenesis of these adenocarcinomas. CD10 and HepPar1 are present in a proportion of tumours: this may aid in differentiation from metastatic carcinomas. AMACR up-regulation appears to be more common than in previous reports. Microsatellite instability is present in a minority of small bowel adenocarcinomas.

767 Detection of Duodenal Mucosal Invasion by Pancreatic Ductal Adenocarcinoma: A Novel Immunopanel with Prognostic and Therapeutic Implications

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Background: Identification of duodenal mucosal invasion by pancreatic ductal adenocarcinoma (DMI-PDAC) is a diagnostic challenge on biopsy specimens. Despite their aggressiveness, well-differentiated PDACs are morphologically indistinct from normal or reactive duodenal/ampullary epithelium. We investigated a series of pancreaticoduodenectomy specimens with an immunopanel to identify DMI-PDAC, including markers of poor prognosis and targets of promising novel immunotherapies. This panel may increase the sensitivity of detection, thus accelerating the curative resection timeline and increasing surgical eligibility, while offering theranostic information.
Design: Seventeen consecutive pancreaticoduodenectomy specimens with DMI-PDAC and one control case of PDAC without invasion were included. Immunohistochemical labeling for MUC1 (VU4H5), MUC1 (MA5), MUC2, MUC4 (8G7), MUC5AC, MUC6, PAM4, DPC4, CDX2, and mesothelin were performed on formalin-fixed, paraffin-embedded sections of duodenal-ampullary-pancreatic junctions. Staining patterns of DMI-PDAC were independently recorded by three pathologists (SCW, GP, CS).
Results: Normal/reactive duodenal and ampullary mucosa were MUC1+/MUC2+/MUC4-/MUC5AC-/MUC6-/MA5-/PAM4-/DPC4+/CDX2+/Meso- and MUC1+/MUC2+/MUC4-/MUC5AC-/MUC6+/MA5+/PAM4-/DPC4+/CDX2-/Meso-, respectively. In general, PDACs stained MUC1+/MUC2-/MUC4+/MUC5AC+/MUC6-/MA5+/PAM4+/DPC4-/CDX2-/Meso+. In DMI-PDACs, MUC1 (VU4H5) labeling was strong and uniform regardless of differentiation (18/18, 100%). DMI-PDACs were also labeled with MUC5AC (17/18, 94.4%), mesothelin (15/18, 83.3%), and PAM4 (13/18, 72.2%). MUC2, DPC4, and CDX2 were not expressed in 94.4% (17/18), 55.6% (10/18), and 72.2% (13/18) of the PDAC, respectively. A tumor subset expressing DPC4 and/or CDX2 was differentiated from duodenal or ampullary mucosa with mesothelin. DMI-PDACs also labeled with MUC6 (7/18, 38.8%) and showed increased MUC4 and MUC1 (MA5) labeling with de-differentiation (13/18, 72.2% and 16/18, 88.9%).
Conclusions: An abbreviated panel of MUC1 (VU4H5), MUC4, MUC5AC, mesothelin, PAM4, CDX2 and DPC4 consistently highlights DMI-PDAC. Although MUC1 and MUC5AC are sensitive markers of DMI-PDAC, expression of mesothelin and PAM4 and loss of DPC4 and CDX2 expression are more specific for DMI-PDAC. MUC4 and MUC1 expression are known to associate with higher grade and poor prognosis. Cases expressing PAM4, mesothelin, or MUC1 may be eligible for promising novel immunotherapies.

768 Increased IgG4+ Cells in Duodenal Biopsies Are Not Specific for Autoimmune Pancreatitis

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Background: Endoscopic ampullary biopsies showing increased IgG4+ plasma cells have been recently reported as an alternative to pancreatic biopsy in diagnosing autoimmune pancreatitis. In some institutions, pathologists are being asked to perform IgG4 immunostaining on duodenal biopsies without clear clinical indications or clear guidelines on how to interpret the significance of finding an increase in IgG4+ cells. The purpose of this study was to assess whether increased IgG4+ cells can be seen in duodenal biopsies outside the context of autoimmune pancreatitis.
Design: Duodenal biopsies from 28 patients undergoing endoscopy for suspected celiac disease (16), abdominal pain (6), recurrent pancreatitis (1), inflammatory bowel disease (IBD) surveillance (3), or post-*Helicobacter pylori* treatment (2) were stained immunohistochemically for IgG4.
Results: All duodenal biopsies that were histologically normal (n=5) or showed increased intraepithelial lymphocytes (IELs) without villous blunting (n=8) were negative for IgG4. Scattered IgG4+ cells (up to 10 per hpf) were found in 5 cases of 10 serologically confirmed celiac disease patients, with higher numbers of IgG4+ cells in biopsies showing significant villous blunting. In addition, there was heavy IgG4 positivity (10 to >30 per hpf) in 5 patients who had normal pancreata on ultrasound; 1 patient with recurrent pancreatitis and ulcerative colitis, 1 patient with primary sclerosing cholangitis (PSC) and ulcerative colitis, and 3 patients with confirmed celiac disease. Of these patients, the duodenal biopsies from the recurrent pancreatitis patient showed gastric heterotopia; her serum IgG4 was normal. In the PSC patient, the biopsies showed villous blunting, increased IELs, ulcer, and foveolar metaplasia. Biopsies of the celiac disease patients also showed severe villous blunting and increased IELs.
Conclusions: The finding of increased IgG4+ cells in duodenal biopsies is not specific for autoimmune pancreatitis. Even with a large number of IgG4+ cells, appropriate clinical findings and radiologic correlation are necessary to make a diagnosis of autoimmune pancreatitis. In our study, scattered IgG4+ cells were found particularly in duodenal biopsies with villous blunting and increased IELs, with 5 cases showing

dense IgG4+ infiltrates. The significance of IgG4 positivity in celiac disease and in IBD is unclear. Nevertheless, the sole finding of increased IgG4+ plasma cells in biopsies is not specific for IgG4-related sclerosing disease, as seen in this study and reports of other conditions such as gastritis in pernicious anemia and in rheumatoid arthritis.

769 High Grade Neuroendocrine Carcinoma of the Anorectum: A Clinicopathologic Study of 7 Cases of a Rare Entity

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Background: High grade neuroendocrine carcinomas (HGNEC) of the gastrointestinal tract are uncommon and may be classified as small cell or large cell type. Given their therapeutic and prognostic implications, it is imperative to establish an accurate diagnosis by histology and immunohistochemistry in order to rule out tumors that may mimic HGNEC. The aim of this study was to evaluate the clinical and pathologic features and outcome of 7 cases of anorectal HGNEC.

Design: Seven cases were obtained by searching the pathology files at two major medical institutions, indicating the rarity of this tumor. Clinical records were assessed for treatment and outcome. We examined many features such as the type of HGNEC (small vs large cell) and the presence or absence of anal intraepithelial neoplasia (AIN) and/or adenomatous changes, among others. All cases were evaluated for synaptophysin, chromogranin, CDX2, p63, and cytokeratin 5/6 expression.

Results: Four cases were large cell NEC and 3 were small cell carcinoma. Two cases revealed a second type of carcinomatous component (1 adenocarcinoma and 1 squamous cell carcinoma), one case had an adenomatous component with high grade dysplasia; no cases demonstrated evidence of AIN. Immunohistochemically, the large/small cell components of the tumors stained for synaptophysin (6/7), chromogranin (5/7), CDX 2 (1/7, focal weak), and CK 5/6 (1/7, focal weak). All cases were negative for p63. All patients were treated with adjuvant chemoradiation with platinum-based chemotherapy for metastatic disease. Four patients died of disease within 2 years of treatment, 2 patients were lost to follow-up, and one patient is alive ten years following diagnosis and currently stable on chemotherapy.

Conclusions: HGNECs of the anorectum are rare, as only 7 cases were identified in the pathology files at two major medical institutions. Correct diagnosis of these tumors is critical given their therapeutic and prognostic implications. Our study suggests that HGNECs of the anorectum may be associated with non-neuroendocrine carcinomatous components. In our experience, such tumors are treated similarly to pure HGNECs.

770 Interobserver Reproducibility and Feasibility of Polymerase Chain Reaction (PCR)-Based Assay in Distinguishing Ischemic Colitis from *Clostridium Difficile* Colitis in Endoscopic Mucosal Biopsies

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Background: Ischemic colitis and C. *difficile* colitis may be difficult to distinguish on histology alone. More recently, PCR-based assays on stool samples have shown higher sensitivities than the widely used *C. difficile* toxin enzyme immunoassay (EIA), which has a sensitivity of only 48%. This study examines the utility of *C difficile* PCR-assay in endoscopic mucosal biopsies. In addition, we also evaluate the interobserver reproducibility of distinguishing ischemic colitis from C. *difficile* colitis based on previously proposed histologic features. (Dignan CR AJSP 1997).

Design: Biopsies with an ischemic-pattern of injury (N=40) were culled from our database between Jan 2005-Dec 2010 and evaluated for: pseudomembranes, lamina propria hyalinization, withered crypts, thrombi, mucosal hemorrhage, and necrosis. Endoscopic findings, *C. difficile* EIA result, treatment, and vascular work-up were documented. Biopsies were blindly reviewed by two GI pathologists and categorized as either *C. difficile* or ischemic colitis and interobserver reproducibility was assessed using kappa statistics. Following microdissection and DNA extraction, PCR assay was performed using the BD Geneohm (Franklin Lakes, NJ) *C. difficile* assay. Considering clinical work-up, including *C. difficile* EIA results as the gold standard, the histologic diagnosis and PCR assay results were compared and statistically analyzed.

Results: The cohort comprised of 21 females and 19 males; there was no significant difference in age between patients with ischemic colitis (N= 20) and *C difficile* colitis (N=18; mean age 56 yrs vs 63 yrs). In 2/40 cases, the etiology was unclear. There was good agreement between histologic and clinical diagnosis (kappa=0.7). The interobserver agreement between the two GI pathologists was excellent (kappa = 0.84). A total of 34 cases (19 ischemia, 15 C. *difficile*) were analyzed by PCR. None of the ischemic colitis cases were positive by PCR (100% specificity), but only 3/15 *C. difficile*-confirmed cases were PCR positive (20% sensitivity). One of these 3 cases, however, was *C. difficile* EIA negative.

Conclusions: Interobserver reproducibility for histologically distinguishing ischemic colitis from *C difficile* colitis was excellent in this study. The *C. difficile* PCR test, as applied to endoscopic mucosal biopsies, has excellent specificity but limited sensitivity. Nevertheless, it may be helpful in identifying *C. difficile* infection in patients with histologic evidence of *C. difficile* enterocolitis, but who had a negative EIA test.

771 Protein Tyrosine Phosphatase T/Paxillin – An Unappreciated Pathway of Colon Carcinogenesis

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Background: Protein tyrosine phosphatase T (PTPRT) is the most frequently mutated tyrosine phosphatase in cancer - having been identified in a number of differing human cancer types. Paxillin is an adaptor protein involved in cell adhesion, migration, proliferation and apoptosis. We recently discovered that paxillin is a substrate of PTPRT, that PTPRT directly dephosphorylates a previously unappreciated paxillin phosphotyrosine site Y88 (pY88). We noted that the PTPRT system complex acts as a tumor suppressor and that over expression of pY88 occurs in colon cancers (CCs). This study investigates the extent of pY88 expression, site of activity of the PTPTRT/paxillin pathway in CC and a possible therapeutic option for chemotherapeutic modulation via Src kinase since pY88 is a known phosphorylation site for this enzyme.

Design: Immunohistochemistry was performed on a range of CCs and matching controls with anti-pY88 antibody. Cell fractionation studies of CC cell lines to independently identify the site of pY88 cellular localization was performed. Finally, CC cell cultures with variable pY88 expression were exposed to Src tyrosine kinase inhibition using a specific inhibitor 'Dasatinib' and the effects observed.

Results: 74% of Stage IV, 53% of Stage III, and 37% of Stage I & II CCs show strong pY88 paxillin IHC staining. No association with tumor grade was found. Immunohistochemistry and cell fractionation analyses consistently showed that pY88 accumulates in cancer nuclei. CC cell lines harboring high levels of pY88 paxillin are hyper-sensitive to Src kinase inhibitor 'Dasatinib' in tissue culture.

Conclusions: Our data suggest that paxillin Y88 phosphorylation may play a significant role in colon cancer progression and demonstrate its preferred localization to the nucleus. This is consistent with its newly discovered carcinogenic functions. Interestingly, as postulated from the known association of pY88 with the Src kinase pathway, over expression of paxillin pY88 may be a marker of colon cancer susceptibility to a Src kinase inhibitor chemotherapeutic effect.

772 Gastric Adenocarcinomas Display Unique Human and Viral RNA Signatures Related to Epstein-Barr Virus Infection

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Background: Gastric carcinoma was diagnosed in an estimated 21,250 United States patients last year, with 10,340 attributable deaths. Disease pathogenesis is complex and likely represents the end point of interactions between environmental and host factors, including Epstein-Barr virus (EBV) and *Helicobacter pylori* infection, genetic susceptibility, and nitrate ingestion. Most patients present with advanced disease and then die of disease, indicating the need for earlier detection and better management strategies. RNA-based expression profiling shows promise for early detection, monitoring tumor burden, and predicting response to therapy.

Design: To promote assay development and validation, we used the Nanostring nCounter system to measure 96 RNAs, including non-coding and viral RNAs, in 116 paraffin-embedded gastric adenocarcinoma tissues that had been macrodissected to enrich for malignant cells.

Results: Expression patterns revealed four distinct molecular subtypes of gastric cancer. Molecular Group 4 (n=13) expressed both lytic and latent EBV genes and was downregulated for many factors previously reported to be gastric cancer-specific. Unlike EBV negative cancers, the EBV positive cancers lacked chromogranin A (CHGA) and they expressed CXCL1 and TRAF1, suggesting NFKB signaling downstream of viral LMP1. The majority of gastric cancers clustered into Molecular Group 1 (n=52) and overexpressed many genes previously reported to be associated with gastric carcinoma, including REG4 and CDH17, while Molecular Group 2 (n=24) lacked these two RNAs. Intermediate levels of lytic viral RNA were seen in groups 2 and 3 but these 2 groups differed from each other in levels of GPR183 (EBI2), an EBV-associated G-protein, and in the pro-apoptotic factor BCL2L11 (BIM). Given that the list of profiled genes was enriched for those that distinguish benign from malignant gastric tissue, it is not surprising that benign mucosa displayed an expression profile unlike that of the predominant group 1 cancers.

Conclusions: Overall, this study shows that molecular subtyping is feasible on paraffin-embedded biopsy or resection tissue and that gastric cancer is heterogeneous from a molecular standpoint. These findings may promote the development of screening tests for the major cancer subtypes and may help control cancer by tailoring therapy to the unique biochemical pathways that characterize a given patient's tumor.

773 Low-Grade Appendiceal Mucinous Neoplasms Show Activation of the Mitogen Activated Protein (MAP) Kinase Pathway

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Background: Low-grade appendiceal mucinous neoplasms are classified as mucinous cystadenomas (MCAD) when confined to the appendiceal muscularis mucosae and well-differentiated mucinous adenocarcinomas (MACA) when they invade the appendiceal wall or spread to the peritoneum (pseudomyxoma peritonei). The molecular features of these uncommon tumors have not been extensively studied. We evaluated 86 cases, including 60 MCADs and 26 MACAs, for mutations affecting RAS/RAF/MEK/ERK signaling.

Design: DNA was extracted from 60 MCADs and paired samples of appendiceal and peritoneal tumors from 26 patients with MACAs using a 96-well plate format. Template DNA (10-20 ng) and PCR master mixes were dispensed into 384 well plates for PCR cycling. Subsequent primer extension products were analyzed using the Sequenom platform, which is more sensitive than direct sequencing and amenable to multiplex assessment of multiple candidate mutations. Amplified products were dispensed onto the Sequenom Spectro-chip and assessed for *KRAS*, *BRAF*, *PIK3CA*, *AKT1*, and *NRAS* mutations using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS).

Results: Oncogenic *KRAS* point mutations were detected in 31 (52%) MCADs involving codons 12 (n=29) and 13 (n=2). Three (5%) MCADs contained hotspot mutations in *PIK3CA* (1 E542 and 2 E545 mutations). Eighteen (69%) MACAs had *KRAS* mutations affecting codons 12 (n=16) and 13 (n=2) that were identical in appendiceal and peritoneal

components in 9 cases. Five MACAs showed mutations in the appendiceal tumor, but not the peritoneum, reflecting the paucicellular nature of peritoneal disease. Four others had new mutations in the peritoneal tumor compared to the appendix. Three (12%) MACAs showed activating *PIK3CA* mutations limited to peritoneal deposits (1 E542K and 2 M1043I mutations). All appendiceal mucinous neoplasms were *BRAF*, *AKT*, and *NRAS* wild-type.

Conclusions: A high proportion (58%) of low-grade appendiceal mucinous neoplasms harbor activating *KRAS* mutations. These changes are present even when the tumor is a mucinous cystadenoma confined to the appendix. Activating *PIK3CA* mutations are infrequent (7%), but may be more common in advanced tumors. Mutations affecting *BRAF*, *AKT*, and *NRAS* do not play a major role in appendiceal mucinous neoplasia. These findings may be important to developing treatment strategies in the future.

774 Clinicopathological Review of 62 Cases of Primary Duodenal Adenocarcinoma

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Background: Primary duodenal adenocarcinoma (PDuA) is uncommon, and the clinicopathologic features and prognostic factors in patients with PDuA are unclear.

Design: We reviewed sixty-two cases of PDuA who underwent surgical resection for PDuA at our institution. The archival H & E stained slides were reviewed. Patient clinical and follow-up information was extracted from patient medical records or review of the U.S. Social Security Index. Statistical analysis was performed using Statistical Package for Social Sciences software with two-sided significance level of 0.05 for all statistical analyses.

Results: Our patient population consisted of 31 males and 31 females with age ranging from 35 to 88 years (median: 59 years). 48 patients underwent pancreaticoduodenectomy, 13 patients underwent segmental duodenal resection and one underwent transduodenal polypectomy. The tumor size ranged from 0.3 to 7.4 cm (median: 3.5 cm). Adenoma was present in 37% (23/62) of the cases. Well, moderate and poorly differentiated adenocarcinomas were present in 3, 42 and 17 patients, respectively. Ten cases (16%) showed mucinous differentiation. 4, 12, 27 and 19 patients had T1, T2, T3, and T4 tumor respectively with lymph node metastasis identified in 31/59 (53%) patients who underwent lymphadenectomy. Stage I, II, II and IV disease was present in 4 (6.5%), 23 (37%), 31 (50%), and 4 (6.5%) of patients respectively. The follow-up information was available in 59 patients (range: 6 to 222 months, median: 111 months). During follow-up, metastasis to liver, lung, retroperitoneum, skin and local recurrence were detected in 4, 3, 4, 1 and 7 patients respectively. The median survival was 111 ± 39 months. Lymph node metastasis and AJCC stage correlated significantly with patient survival ($P<0.05$). No significant correlation between survival and other pathological parameters was observed.

Conclusions: Our study show that patients with PDuA who underwent surgical resection have a good prognosis.

775 SOX9 and CDX2 Expression Predicts the Development of Barrett's Esophagus

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Background: The diagnosis of Barrett's Esophagus (BE) requires both endoscopic evidence of columnar mucosa (CM) and the presence of goblet cells (GC) by histology. The identification of GC can depend on the number of biopsy samples obtained and the location where the biopsies were taken. Several cellular signaling pathways responsible for modulation of intestinal differentiation have been shown to be involved in the development of metaplastic mucosa in BE, including sonic hedgehog (SHH), bone morphogenic protein 4 (BMP4), SOX9, and CDX2. In this study, we investigate whether the expression of these molecules in GC-negative CM from esophageal biopsies predicts the identification of GC in followup biopsies.

Design: Pathology databases from the Universities of Chicago and Washington were searched from 1990 to 2008 for esophageal biopsies containing CM in patients with endoscopic evidence of BE. Of these, 11 cases that were negative for GC on initial biopsies were found to have GC (without dysplasia) on followup biopsies (BE group; average age 54.9, GC detected at an average of 59.0 months after initial biopsy). Additionally, 25 cases that were initially negative for GC and remained negative on followup biopsies served as controls (average age 51.2, followed for an average of 49.4 months). Immunohistochemistry for CDX2, SOX9, BMP4, SHH, and p63 was performed on the initial biopsies and graded independently by at least 2 pathologists in a masked manner.

Results: CDX2 was positive in the CM of 6/11 cases in the BE group, and in 0/25 controls, resulting in a sensitivity of 55% and specificity of 100% (positive predictive value 100%, negative predictive value 83%). Focal and weak immunoreactivity for SOX9 was seen in most examples of CM; however, strong diffuse immunoreactivity for SOX9 was detected in 7/10 in the BE group and in 0/11 controls, giving rise to a sensitivity of 70% and specificity of 100% (positive predictive value 100%, negative predictive value 79%). p63 highlighted a focal basal-cell-like layer beneath the columnar mucosa in a few cases, but no difference existed between the two groups. SHH and BMP4 also showed no significant difference between the BE and control groups.

Conclusions: SOX9 and CDX2 are highly specific predictive markers for the identification of GC in followup biopsies of patients with GC-negative CM upon initial biopsy. These markers may be useful in identifying a subset of patients with CM who have a higher risk of developing BE and need closer followup.

776 Loss of Expression of DAXX and ATRX in Low-Grade Neuroendocrine Tumors (Carcinoid Tumors)

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Background: DAXX, a protein associated with FAS-mediated apoptosis, and ATRX, SWI/SNF-2 family chromatin remodeler, form a complex that has been associated with deposition of histone H3.3 at heterochromatin region of the genome. Recently, DAXX and ATRX genes were found to be frequently altered in pancreatic endocrine tumors. Tumors with these mutations were associated with a better overall survival. Loss of immunolabeling for DAXX and ATRX correlate with mutation of the respective genes. It is largely unknown whether the altered expression of DAXX and/or ATRX protein are present in neuroendocrine tumors other than pancreatic origin.

Design: Tissue microarrays consisting of 73 patients with pancreatic endocrine tumors; 53 patients with small bowel, stomach or colorectal neuroendocrine tumors; and 16 patients with pulmonary neuroendocrine tumors. These were low to intermediate-grade neuroendocrine tumors. Immunohistochemical studies were performed on paraffin-fixed tissue microarray sections. Nuclear staining was scored as negative (lack of staining) or positive. These staining results were correlated with clinicopathological data, including patients overall survival, by Chi-square and Kaplan-Meier analyses.

Results: Loss of expression of DAXX was present in 33/61 (54%) pancreatic endocrine tumors, 8/42 (19%) gastrointestinal neuroendocrine tumors, and 8/15 (53%) pulmonary neuroendocrine tumors. There was significant difference in loss of DAXX staining result among pancreatic neuroendocrine tumors and neuroendocrine tumor of other sites ($p=0.001$). In contrast, loss of expression of ATRX was found in 17/63 (27%) pancreatic endocrine tumors, 12/42 (27%) gastrointestinal neuroendocrine tumors, and 5/16 (31%) pulmonary neuroendocrine tumors. There was no significant difference in loss of ATRX staining results among pancreatic neuroendocrine tumors and neuroendocrine tumor from any other sites. Loss of either protein was not associated with patients overall survival, including each subsite.

Conclusions: Loss of nuclear expression DAXX and ATRX was frequent observed in pancreatic neuroendocrine tumor and neuroendocrine tumors from other sites. However, loss of either protein was not associated with patients overall survival.

777 In Situ Validation of an Intestinal Stem Cell Signature in Colorectal Cancer

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Background: Wnt/Tcf, Lgr5, Ascl2 and/or Bmi1 signaling are believed to define the mouse intestinal stem cell niche(s) from which adenomas arise. However, there is insufficient evidence that these observations are relevant to human colorectal adenocarcinomas. The aim of this study was to determine the relevance of these putative intestinal stem cell markers to human colorectal cancer using in situ hybridization.

Design: Nineteen putative intestinal stem cell markers, including Ascl2 and Lgr5, were identified from published data and an evaluation of a human colorectal gene expression database. Associations between these genes were assessed by isotopic in situ hybridization in 57 colorectal adenocarcinomas. Multiplex fluorescent in situ hybridization was also performed for Ascl2/Lgr5/Olfm4.

Results: Ascl2 and Lgr5 were expressed in 85 and 76% of cancers respectively, and expression of Ascl2 and Lgr5 was positively correlated ($p=0.003$). Expression of Bmi1 was observed in 48% of cancers, but expression was very weak in 92% of cases with expression. Both Ascl2 and Lgr5 were positively correlated with expression of Axin2, Cdca7, Dkc1, EphB2, EphB3, Ets2, Rnf43, Sox9 and Znrf3, but neither were correlated with expression of Cdk6, Gpx2, Olfm4 or Tnfrsf19.

Conclusions: These data suggest that 76-85% of colorectal cancers show a Lgr5/Ascl2 intestinal stem cell signature and support the hypothesis that a large proportion derive from crypt stem cells. In addition, the results demonstrate that not all stem cell markers in mouse small intestine are relevant to human colorectal cancer. Finally, the predominantly uniform expression patterns of Ascl2, Lgr5 and EphB2 suggest that intestinal stem cell genes may not mark tumour-initiating cells.

778 Simple Algorithm for the Prediction of Low-Level Microsatellite Instability (MSI-L) in Colorectal Cancer

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Background: Colorectal cancers with low-level microsatellite instability (MSI-L) are thought to represent a subgroup of highly aggressive tumors leading to unfavorable outcome. Whereas MSI-high (MSI-H) cancers can be distinguished from microsatellite stable (MSS) cases using immunohistochemistry for mismatch repair markers MLH1, MSH2, MSH6 and PMS2, the same is not true for MSI-L since both MSI-L and MSS will result in a positive immunohistochemical pattern. The aim of this study was to establish a discriminating protein profile for MSI-L colorectal cancers.

Design: MSI was assessed by analysing BAT25, BAT26, D2S123, D5S346, and D17S250. MSS and MSI-L were defined as instability in 0 and 1 marker, respectively. A tissue microarray was stained and analysed for 41 different proteins selected from a larger pool of markers to represent major signalling pathways (WNT, RAS/MAPK, TGF-beta, and AKT), stem cell markers, cellular processes such as cell cycle, proliferation, apoptosis, angiogenesis, metastasis and markers of differentiation and mucin expression. Simple and multiple logistic regression and receiver operating characteristic (ROC) curve analysis were performed and area under the curve (AUC) was evaluated.

Results: From 172 cases included in this study, 98 (57%) were MSS and 20 (11%) were MSI-L. Only loss of nuclear beta-catenin (OR (95%CI): 0.21 (0.1-0.9); p=0.027), Cdx2 (OR (95%CI): 0.23 (0.1-0.63); p=0.005), CK20 (OR (95%CI): 0.16 (0.03-0.7); p=0.018) and membranous E-cadherin (OR (95C%): 0.2 (0.1-0.55; p=0.002) could

significantly predict MSI-L from MSS. More importantly, in multivariate analysis, beta-catenin, CK20 and Cdx2 were independent predictors of MSI-L and together strongly discriminating (AUC=0.82). These features could be combined into a single score and visualized as an algorithm for the prediction of MSI-L. The parameter estimates from logistic regression analysis were used as weights for each marker then a total score and probability of MSI-L were obtained.

Conclusions: These results suggest that in contrast to MSS, MSI-L cancers may not frequently arise through deregulation of WNT pathway signaling. In addition, a simple combined score of beta-catenin, CK20 and Cdx2 may be useful as a predictive algorithm for the MSI-L status.

Genitourinary

779 *KRAS* Mutation Is Present in a Small Subset of Primary Urinary Bladder Adenocarcinomas

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Background: The realization that the presence of *KRAS* mutations is a near absolute contraindication to the use of EGFR-targeted therapies in colonic adenocarcinoma and non-small cell lung carcinoma has significantly altered the clinical management of individual patients with these types of cancer. The aim of this study is to determine whether *KRAS* mutations occur in primary bladder adenocarcinoma and/or urothelial carcinoma with glandular differentiation.

Design: Twenty cases of primary bladder adenocarcinoma were identified. Clinical histories and hematoxylin and eosin slides of each case were reviewed to confirm primary bladder origin. An additional 5 cases of urothelial carcinoma with significant degree of glandular differentiation were included. DNA was extracted from formalin-fixed paraffin embedded tissue, amplified with Shifted Assay Termination (STA) technology. The STA reaction recognizes wild type or mutant target sequences and selectively extends detection primers with 1 to 20 labeled nucleotides. This extension is repeated 20 times to enrich the mutation signal. The enriched mutation signals are then detected by capillary electrophoresis fragment analysis. The PCR amplification products were analyzed on an ABI-3130XL analyzer with GeneMapper® software (Applied Biosystems® Inc., Foster City, CA). Analysis included 6 different mutations on codon 12 (G12C, G12V, G12S, G12D, G12R, G12A) and codon 13 (G13C, G13V, G13S, G13D, G13R, G13A) along with negative and positive controls. 55 cases of colonic adenocarcinomas were also analyzed.

Results: Two of twenty (10%) cases of primary urinary bladder adenocarcinoma were found to contain a *KRAS* mutation. Both cases exhibited G13D mutations on codon 13. None of the 5 cases of urothelial carcinoma with glandular differentiation displayed *KRAS* mutation. Eighteen (33%) of 55 cases of colonic adenocarcinoma contained a KRAS mutation.

Conclusions: *KRAS* mutations are present in a small subset of primary urinary bladder adenocarcinomas, suggesting a possible role in tumorigenesis. KRAS mutations do not provide a means of distinguishing between primary urinary bladder adenocarcinoma and primary colonic adenocarcinoma.

780 Gleason Pattern 5 Is Frequently Underdiagnosed on Prostate Needle Core Biopsy

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Background: The current study assesses underdiagnosing Gleason pattern 5 on biopsy and discuss the potential consequences for patient management.

Design: We retrieved 300 consecutive prostate biopsy cases from our consultation files from 2009-2010 in which we identified Gleason pattern 5. All cases were diagnosed by one expert GU pathologist, and were sent in as a final diagnosis, where the outside pathologist was not requesting consultation as a result of difficulty with the diagnosis. Cases typically were sent for consultation at the request of the patient or the outside treating physician.

Results: Table compares Gleason grades rendered upon expert review (EXP) compared to those at the outside institutions (OS).

	OS 3+3=6	OS3+4=7	OS4+3=7	OS3+5=8	OS5+3=8	OS4+4=8
EXP3+5=8	2 (6.5%)	15 (48.4%)	1 (3.2%)	10 (32.2%)	0	1 (3.2%)
EXP5+3=8	0	0	4 (44.5%)	0	3 (33.3%	1 (11.1%)
EXP4+5=9	1 (0.8%)	11 (8.8%)	21 (16.8%)	3 (2.4%)	1 (0.8%)	51 (40.8%)
EXP5+4=9	0	1 (1.4%)	5 (7.0%)	2 (2.8%)	1 (1.4%)	24 (33.3%)
EXP5+5=10	0	0	0	1 (1.6%)	1 (1.6%)	8 (12.7%)

	OS4+5=9	OS5+4=9	OS5+5=10	Total
EXP3+5=8	2 (6.5%)	0	0	31 (100%)
EXP5+3=8	1 (11.1%)	0	0	9 (100%)
EXP4+5=9	33 (26.4%)	4 (3.2%)	0	125 (100%)
EXP5+4=9	23 (31.9%)	15 (20.8%)	1 (1.4%)	72 (100%)
EXP5+5=10	19 (30.1%)	8 (12.7%)	26 (41.3%)	63 (100%)

Of the 300 cases, 203 cases (68%) were under-graded, 93 cases (31%) were graded the same, and 4 cases (1%) were overgraded relative to the grade assigned by the expert pathologist. In 146 (48.7%) of the cases, Gleason Pattern 5 was not identified by the outside pathologists. Of the 146 cases, the outside Gleason score was ≤7 in 61 (20.3%) and 4+4=8 in 85 (28.4%) of the cases. Even when the tumor was diagnosed at our institution as Gleason score 5+5=10), only 26 (41.3%) were diagnosed as the same by the outside pathologists.

Conclusions: Gleason pattern 5 is commonly underdiagnosed by outside pathologists compared to an expert GU pathologist. One explanation is a hesitancy of pathologists to render the worst grade pattern possible and its adverse implications on prognosis and treatment. Another explanation is that some cases may have consisted of mostly pattern 4, less pattern 3 (or vice versa), and tertiary pattern 5. According to the ISUP modified grade, Gleason score on biopsy is derived by adding the most prevalent and highest grade patterns as opposed to the most and 2nd most common patterns. Not using the ISUP modified grading system could have accounted for at most 36/146 (24.7%) of the cases where Gleason pattern 5 was not recorded by the outside pathologists. Recognition of undergrading pattern 5 is the first step to improving its grading in the future by diverse educational means.

781 De-Differentiated Tubulocystic Carcinoma of the Kidney: A Series of 3 Cases with FISH Analysis

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Background: Tubulocystic renal cell carcinoma (RCC) is relatively rare and was first described as a low grade variant of collecting duct carcinoma. This variant was not recognized in the 2004 WHO classification and only received its current name in 2009 in a series of 39 cases from 7 large institutions. It is low grade with only 2/17 cases with follow-up in this prior series developing metastases. Its relationship to collecting duct carcinoma is controversial and recent studies have linked it with papillary RCC. Only 1 case from 2011 describes a sarcomatoid tubulocystic RCC.

Design: 3 consult cases of de-differentiated tubulocystic RCC were identified. FISH was performed on 2 cases with available material.

Results: Two lesions measuring 9.5 cm. and 3.8 cm. were described as partly solid and cystic. One case was grossly a 14.0 cm cyst with a granular lining. Microscopically, all had classic areas of circumscribed tubulocystic RCC occupying 30%, 80%, and 90% of the tumor. 2 cases had small components of papillary RCC and 1 case a central large cystic component. In 2 cases, a proliferation of small tubules infiltrated away from the main mass with typical features of collecting duct carcinoma. In the 3rd case, a focus of sarcomatoid carcinoma was seen adjacent to the tubulocystic RCC. In 2 cases, tumor invaded peri-renal tissue. The 3rd case was organ confined with vascular invasion. 1 patient died 9 months post-operatively with metastases to the abdominal wall and femur. The 2nd case developed metastases to retroperitoneal nodes 3 years post-operatively. The 3rd patient was lost to follow-up.

Tumor Pattern	Chromosome 7	Chromosome 17	Chromosome Y
Case 1. Tubulocystic	Disomy	Trisomy	Loss
Case 1. De-differentiated	Disomy	Trisomy	Disomy
Case 2. Tubulocystic	Disomy	Trisomy	Disomy
Case 2. De-differentiated	Disomy	Trisomy	Disomy

Conclusions: This is the first series and only the 2nd report of de-differentiated tubulocystic RCC. FISH results showed some features that are seen with papillary RCC in both the tubulocystic and dedifferentiated components and with one exception showed identical cytogenetic findings between the 2 components. Morphologically, in 2 cases the de-differentiated areas were also indistinguishable from collecting duct carcinoma suggesting a relationship between the 2 entities. De-differentiated tubulocystic RCC increases the risk of aggressive behavior above that of usual tubulocystic RCC.

782 Virtual Karyotype of Renal Carcinoid Tumors by SNP Microarrays

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Background: Renal carcinoid tumors (RCT) are rare neoplasms. Unlike carcinoid tumors at other anatomic site, scarce data are available on the genetic changes in RCT. We sought to determine if there were characteristic chromosomal copy number changes in RCT using SNP arrays on archival formalin-fixed paraffin embedded (FFPE) tissue blocks.

Design: We obtained demographic, clinicopathologic information and archival FFPE tissue blocks from 5 patients with RCT from multiple institutions. DNA from the FFPE tissue blocks was analyzed with 250K Nsp SNP microarrays (Affymetrix, Santa Clara, CA). Virtual karyotypes were obtained detailing the genomic imbalances and loss of heterozygosity (LOH).

Results: The average age was 56 years (Male:Female 2:3). Tumors invaded perinephric adipose tissue (n= 5), lymph nodes (n=3) or presented with metastatic disease (n=2, both liver). Three renal carcinoid tumors showed no chromosomal abnormalities (NCA). One tumor showed focal deletions in 3q [-3q(q21.31-q26.32]. One tumor, an atypical carcinoid, showed multiple chromosomal abnormalities including loss of 3p and other chromosomal losses [-1(p31.1-p31.1), -1(p22.3-p11.2), -3(p26.3-q11.2), -10(q21.2-q26.3), -10(q21.1-q21.1), -11(q14.3-q23.3), -13, -16(q13-q24.3)].

Conclusions: Most renal carcinoid tumors showed no chromosomal abnormalities by SNP microarrays (3/5 tumors). Chromosomal abnormalities of chromosome 3 (3q or 3q loss) were present in the 2/5 tumors and one tumor (atypical carcinoid) showed complex abnormalities (loss 3p, additional chromosome loses). Loss of 3p is of interest as this is characteristic of clear cell (conventional) renal cell carcinoma. VHL mutation analysis in this tumor is underway. With the exception of more complex chromosomal changes being present in an atypical carcinoid, in the remaining tumors there was no difference between NCA tumors and those with no chromosomal abnormalities. A larger study of these rare tumors may identify recurrent abnormalities that may associated with clinical behavior.