Gastrointestinal Pathology

565 Unusual Immunohistochemistry Staining Patterns Encountered in Cancers Screened for Lynch Syndrome

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Background: Mismatch repair deficiency is associated with Lynch syndrome in a subset of colorectal and endometrial cancers, and universal screening is now recommended for these tumors. Immunohistochemistry for four mismatch repair proteins (MLH1, MSH2, MSH6, and PMS2) is the most frequently used screening method. Although interpretation is usually straightforward for these stains, unusual patterns are encountered occasionally. Unusual staining patterns can lead to uncertainty about the possible underlying gene defects.

Design: Retrospective review of 3,564 cases of cancer tested by immunohistochemistry indicated that 727 (20%) were abnormal. The usual abnormal pattern was defined as loss of both MLH1 and PMS2, loss of PMS2 alone, loss of both MSH2 and MSH6, or loss of MSH6 alone in the entire tumor tested. Of the abnormal cases, a significant fraction (93, almost 13%) showed unusual staining patterns. Unusual cases were stratified into categories based on the staining pattern and histopathologic features.

Results: The most common categories of unusual staining are indicated in Table 1. The patterns indicate different underlying mechanisms, both intrinsic to the biology of the tumor (genetic heterogeneity or multiple gene defects) and extrinsic to the tumor (chemoradiation and tissue preservation artifacts).

Staining pattern	Colorectal Cancer (n = 57)	Endometrial Cancer (n = 31)	Other Cancers (n = 5)	Total
True heterogeneity (partial staining of the tumor; absent staining in whole regions of the tumor with retained internal control staining).	22	20	2	44
Non-standard loss of staining indicating a defect in more than one gene.	12*	3	1	16
Unusual staining in the setting of prior chemoradiation.	5	0	0	5
Artifacts of tissue preservation and staining, preventing interpretation of mismatch repair status in the tumor.	12	6	2	20
Unknown / other mechanisms.	7	2	0	9

(*) 1 case showed two unusual staining patterns.

Conclusions: Multiple mechanisms can contribute to unusual staining patterns. These unusual patterns are encountered infrequently overall but represent a significant proportion of abnormal cases (13% in our series). Better understanding of the mechanisms causing unusual immunohistochemistry staining patterns will enable improved classification of tumors with mismatch repair deficiencies.

566 Prognostic Implication of Optimized Detection of CMV By Immunohistochemistry in CMV PCR-Positive Inflammatory Bowel Disease Patients

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Background: The significance of superimposed CMV infection in patients with IBD has been an issue of debate. Independent studies have reported a significantly higher incidence of colon CMV infection in patients with steroid-refractory IBD as compared to those with non-refractory disease, suggesting a contributory role of this virus in the disease process. Despite the relative high incidence, CMV was identified only in a small proportion (20 – 40%) of the steroid resistant patients. The reported lack of colon CMV infection in the majority of these patients raises the question whether CMV infection is truly not associated with the patients or the negative result is due to the low sensitivity of the examination.

Design: To address this question, we identified 26 IBD patients whose intestinal biopsies or resection specimens were CMV PCR positive, and performed CMV IHC on all the tissue blocks that were initially found to be CMV negative per H&E.

Results: Re-examination of 153 tissue blocks derived from 37 separate colonoscopy and colectomy specimens from 26 IBD patients (representing 28 separate clinical CMV infections) identified additional CMV inclusions in patients whom were diagnosed CMV negative by H&E or IHC performed only on selected blocks in the initial examination. However, despite the enhanced sensitivity, IHC on all the blocks only detected CMV in about half the patients. While the reason for the negative results in the remaining patients remains to be completely understood, it appears at least partially due to the small number of biopsies taken. In the procedures where 6 or more segments of the intestine were sampled, the CMV positive rate was as high as 83%, whereas in the patients who had only 1 – 5 areas biopsied, the positive rate was only 29% ($p < 0.01$). The majority of IHC^{pos} patients underwent colectomy or died, contrasting most of the IHC^{neg} patients who did well with conservative management, suggesting IHC positivity is a better predictor of prognosis than PCR positivity alone.

Conclusions: Our results demonstrate that while performing IHC on all blocks increases the sensitivity, adequate sampling is essential for optimal CMV detection. As identification of CMV by histology/IHC rather than PCR has clinical implications, our data support the notion that optimized IHC identification of CMV may serve as a gold standard for the diagnosis of CMV colitis.

567 Retrospective Study of Clinicopathologic Features and Patient Outcome of Primary Adenosquamous Carcinoma of the Esophagus

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Background: Primary adenosquamous carcinoma (ASC) of the esophagus is an infrequent tumor with limited information on its biology and clinical behavior.

Design: In a retrospective search of the institutional database (2001-2014) of primary esophageal ASC, we identified 73 patients whose pathology was reviewed by a GI Pathologist. Forty-three of 73 patients with ASC diagnosed on pretreatment biopsies were included in the study. Pretreatment clinical staging was confirmed by endoscopic ultrasound and/or CT/PET CT scans. The presence of Barrett's esophagus was assessed either on esophagogastroduodenoscopy or pathology specimens. Adenosquamous carcinoma was diagnosed based on presence of separate or admixed glandular and squamous component identified on H&E with or without mucicarmine or immunohistochemistry for keratin 5/6, p63, p40 and CDX-2. All patients with advanced locoregional disease underwent chemoradiation with or without surgery. All stage IV patients underwent chemotherapy with or without palliative localized radiation. Esophagectomy specimens after preoperative chemoradiation were reviewed for presence of Barrett's esophagus, amount of residual tumor and pathologic stage.

Results: The patient population included 36 men and 7 women with an age range of 49-88 years. Tumor location was mid or distal esophagus in 25 (58%) and gastroesophageal junction in 18 (42%). Barrett's esophagus was found in 13 (30%) patients. Two thirds (n=29) of patients presented with stage IV (n=28) or locally advanced unresectable disease (n=1). Six patients with locoregional disease demonstrated disease progression after preoperative chemoradiation. Five patients with locoregional disease had extensive residual tumor in surgical resection specimens. Three patients with locoregional disease demonstrated radiologic response to preoperative chemoradiation and were not operated due to high risk of operative morbidity. Only 3 of 41 (7%) patients survived more than 3 years.

Conclusions: Primary esophageal adenosquamous carcinoma has higher stage at presentation and poor response to preoperative chemoradiation.

568 Prevalence and Clinical Significance of Microsatellite Instability in Colorectal Cancers With Retained DNA Mismatch Repair Proteins By Immunohistochemistry

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Background: DNA mismatch repair (MMR) protein immunohistochemistry (IHC) is now used more often than microsatellite instability (MSI) testing by PCR to screen colorectal carcinomas (CRC) for Lynch Syndrome (LS). The rate of discordance reported in the literature between these two screening tests varies widely. The aim of our study was to determine the discordance rate between MMR IHC and MSI in a large series of CRC and to analyze its clinical significance.

Design: MMR IHC to screen for LS was performed in 2116 CRC between 1991-2014. MSI testing was also performed in 947/2116 cases, most commonly due to young age (<50 yrs) at CRC diagnosis. MMR IHC status was assessed using a four antibody panel (MLH1, MSH2, MSH6, and PMS2) and scored as MMR deficient only when complete loss of nuclear staining was seen in the tumor cells. Tumors were classified as MSI-high if >30% of tested loci showed instability.

Results: Mean age of the study group was 54 years and M:F ratio was 1:1.1. MMR IHC was intact in 839/947 (89%) patients in whom both MMR IHC and MSI testing was performed. Only 6/839 cases (0.7%) with intact MMR IHC were MSI-H, and represented 6% of the 106 MSI-H cases. Germline testing in two of these 6 patients (due to young age, 35 and 46yrs, respectively, and strong family history of CRC) confirmed LS due to *MLH1* point mutations in both cases. The results of genetic testing were not available in the remaining four patients but one had a family history suggestive of LS. Conversely, 8/841 (0.95%) patients with either microsatellite stable (MSS) tumors (n=738) or tumors with low level MSI (MSI-L) (n=103) showed abnormal results by MMR IHC . 3/8 discordant cases were MSI-L and two of these three were LS patients (both *MSH6* mutations) . The third MSI-L patient had loss of MLH1/PMS2 on IHC but no genetic testing was available and family history was not suggestive of LS. Of the 5/8 discordant MSS cases (3 with MSH2/MSH6 loss, 2 with only MSH6 loss), 3 were negative for LS by genetic testing, and 2 did not have genetic testing and did not have a family history suggestive of LS.

Conclusions: Discordance between intact MMR IHC and MSI testing is rare (0.7%) in CRC, and there was no clear trend favoring either method. Performing MSI testing in patients with intact MMR IHC when they present at a young age (<50 yrs) or have a positive family history helps in identification of additional LS patients.

569 Prospective Evaluation of Histologic Changes in Acute Reflux Esophagitis in Humans: Support for a Cytokine-Mediated, Lymphocytic Pathogenesis

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Background: Recent animal studies suggest that reflux esophagitis (RE) is not due to direct destruction of squamous cells by acid, but rather due to induction of a cytokine-mediated, lymphocytic infiltration. Since clinicians rarely see patients with new-onset GERD, the evolution of histologic changes in human RE has never been evaluated prospectively. Our aim was to evaluate the evolution of RE in patients with acute GERD induced by discontinuing PPI therapy.

Design: 9 patients [M/F ratio: 7/2, mean age; 67 yrs] with previously documented severe RE (endoscopic LA grade C) who were healed with PPI therapy had a baseline endoscopy performed, after which PPIs were stopped, and then it was repeated at 1 and 2 weeks thereafter. At each endoscopy, multiple biopsies were obtained from non-ulcerated esophageal mucosa. Esophageal pH monitoring was performed at baseline and at 2 weeks. Biopsies were evaluated for multiple histologic features including the presence and degree of basal and papillary hyperplasia, spongiosis, surface injury and microerosions, and for the type and degree of inflammation (lymphocytes, eosinophils, neutrophils graded on a scale of 0-3), both in the lamina propria-(LP) and epithelium.

Results: All patients developed endoscopic RE by 2 weeks (2 LA grade A, 3 LA grade B, 4 LA grade C). Mean esophageal acid exposure (total % time pH<4) increased from 6.6% at baseline to 28.4% at 2 weeks. At week 1, a significant ($P < 0.05$) increase was observed in LP (mean grade 0.3 vs 1.2) and intraepithelial lymphocytes (0.4 vs 1.1), reactive basal and papillary hyperplasia (0.3 vs 1.7), and intercellular peripapillary spongiosis (0.3 vs 1.6). Other features, such as perivascular lymphoid aggregates were also increased. Except in areas adjacent to erosions, neutrophils were not present in the LP or epithelium and eosinophils were only present in 2 patients, and in both, they were very few in number [mean < 0.5 per HPF]. At week 2, LP and intraepithelial lymphocytes increased further, and reactive epithelial changes worsened, but no other features were increased.

Conclusions: Patients with healed RE can develop severe RE within 1 week of stopping PPIs. The development and progression of epithelial and inflammation changes observed supports the new hypothesis that reflux causes esophagitis to develop via a cytokine-mediated, lymphocytic mechanism, and suggests that eosinophils are not an important component in the early phases of disease.

570 MOC-31 Expression of Background Cirrhotic Nodules in Liver Biopsies of Hepatic Lesions – A Potential Pitfall in Small Biopsy/Cytology Specimen

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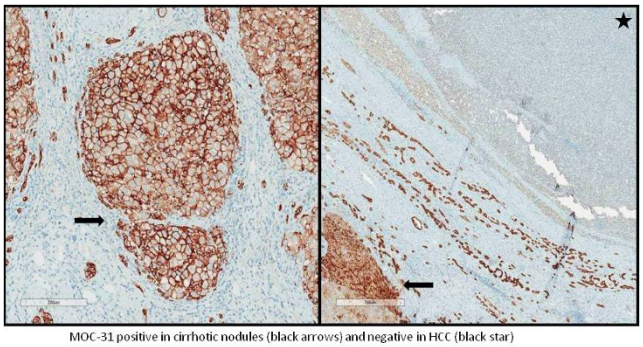
Background: Due to advances in imaging diagnostics of Hepatocellular Carcinoma (HCC), the use of liver biopsies for diagnosing hepatic malignancies is decreasing, but the difficulty in their interpretation is increasing, as nodules that are not typical of HCC are usually targeted for biopsy. Differentiating HCC from cholangiocarcinoma (CC), combined HCC-CC or metastases, which are managed differently, sometimes may be difficult. IHC can be used to document hepatocytic (HepPar1, arginase) differentiation and glandular differentiation (MOC31). However, we have recently observed extensive membranous MOC31 staining of hepatocytes within regenerative/cirrhotic nodules surrounding HCC nodules, which prompted us to review the usefulness of MOC31 in regards to small biopsies (including cytology specimen) sometimes showing only focal involvement by malignancy or extensive fragmentation, as to avoid potential diagnostic pitfalls.

Design: We utilized our pathology database to identify liver specimens in which MOC-31 was used to rule out CC, CC component or metastases. 25 cases were identified over a 5-year search (2009-2014). We reviewed these cases for expression of MOC31 in hepatocytes of cirrhotic/non-cirrhotic background liver as well as in pure HCC lesions. Well-defined membranous staining of regenerative or neoplastic hepatocytes was recorded.

Results: 7/25 cases had cirrhosis and all but one showed focal or diffuse strong membranous staining for MOC-31 in the cirrhotic nodules (6/7; 86%). 18/25 of the cases showed no cirrhosis; 14 exhibiting no MOC31 staining and 4 showing focal weak staining. Pure HCC showed no MOC31 staining in all cases.



MOC-31 positive in cirrhotic nodules (black arrows) and negative in HCC (black star)

Conclusions: Our findings demonstrate strong MOC31 staining in non-neoplastic cirrhotic nodules present in the background of a lesion/tumor. While in larger specimens (wedge resections/hepatectomies) where tissue is abundant and morphologic features allow easier discrimination of benign cirrhotic nodules from neoplastic tissue, when confronted with smaller/fragmented specimen, aberrant expression of MOC-31 (especially when utilized in a limited panel) within cirrhotic/regenerative nodules may lead to misinterpretation of hepatocellular tissue as being glandular/epithelial differentiation.

571 Tracing Immunohistochemical and Molecular Markers of Serrated Carcinoma Along the Serrated and Conventional Polyp Pathways of Colorectal Carcinogenesis

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Background: In contrast to conventional carcinoma (CC), the sequence of precursor polyps leading to serrated adenocarcinoma (SAC) is not clearly defined. A plausible way to recognize possible precursor lesions of SAC is by tracing those typical SAC immunohistochemical (IHC) and molecular markers in a series of colorectal polyps representing the major histological spectrum of serrated polyps (SP) and conventional polyps (CP). In order to give the proper weight to each marker a multivariate logistic regression model was constructed.

Design: In total, 303 polyps were included: 121 SP (33 hyperplastic polyps (HP), 37 sessile serrated adenoma (SSA), 51 traditional serrated adenomas (TSA)), 143 CP (72 tubular adenoma (TA), 34 tubulovillous adenoma (TVA), 37 villous adenoma (VA)) and 39 biphenotypic serrated-conventional polyps (SCP). The IHC markers used were identified from previous studies dealing with SC and CC expression profiling. A representative subset of 106 of these polyps was selected for molecular marker analysis based on oncogene mutation and microsatellite instability status.

Results: Serrated and conventional polyps differ in terms of most of the SC IHC markers, FSCN1 showing the highest difference in positivity (66.9% vs. 42.7%; p=0.0001). Despite sharing serrated morphology, SSA and TSA are very different regarding location, IHC expression of EPHB2 and PTCH and BRAF mutation. Logistic regression analysis revealed that SSA is the polyp type sharing more IHC and molecular markers with SC (70% and 82%, respectively). VA and TA also show important differences according to polyp size and FSCN1 expression and also as their similarity to SC markers (64.9% vs. 38.9%; p=0.010).

Conclusions: This study reports that certain polyps display more typical IHC and molecular markers of carcinoma with serrated morphology than others, SSA being the most plausible precursor of this type of cancer. In addition, our results show that VA, being a conventional polyp, shows a considerable grade of similarity with SAC on IHC grounds supporting the notion that polyp pathways overlap, thus adding complexity to the linear conception of these pathological sequences. These results may help to define colorectal carcinogenic route markers with possible prognostic value.

572 Brunner Gland Duct Adenoma Mimicking Pancreatic Intraductal Papillary Mucinous Neoplasm

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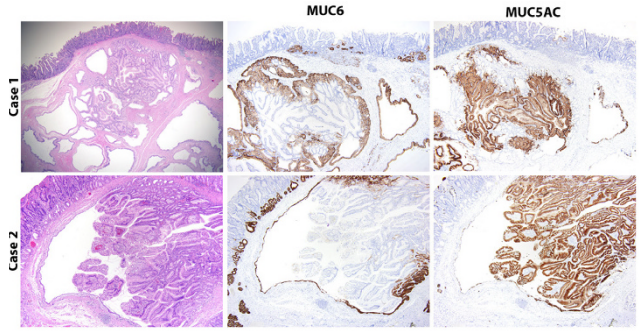
Background: Recently we encountered several examples of an unusual duodenal polypoid lesion consisting of a submucosal mucinous columnar cell proliferation within dilated Brunner gland ducts. Focally the proliferation developed a papillary configuration reminiscent of a pancreatic branch-duct type IPMN.

Design: Five cases were identified from the surgical pathology files of two institutions. Representative sections of each lesion were stained immunohistochemically for mucin markers MUC1, MUC2, MUC5AC, and MUC6. Sections containing normal Brunner glands (including examples with mild duct dilatation) and duodenal adenomyomas were stained as controls. The morphologic features and mucin phenotype were analyzed.

Results: There were 1 female and 4 male patients, ranging from 41 to 74 years of age. All cases were recognized endoscopically as protruding nodules or polyps located in the duodenal bulb or the second portion of the duodenum. The endoscopists were certain that the lesions were too proximal to represent either the ampulla of Vater or the minor papilla. The size ranged from 0.8-1.2 cm. All lesions were grossly well-circumscribed, and one had a microcystic cut surface. Histologically, the lesions arose from submucosal Brunner glands, with one extending to the mucosal surface. Three cases exhibited prominent intraductal mucinous proliferation in the form of micropapillae. One case showed focal mild dysplasia. Immunohistochemically, while surrounding Brunner glands and ductal epithelium demonstrated reactivity for MUC6, the abnormal proliferation showed diffuse strong MUC5AC reactivity and no reactivity for MUC6.



Conclusions: To our knowledge, this is the first detailed description of a novel Brunner gland lesion that morphologically resembles a pancreatic branch-duct IPMN. This lesion is morphologically quite distinct from the more commonly encountered Brunner gland adenomas, hyperplasia and hamartomas. By way of morphologic and immunohistochemical analogy to similar pancreatic tumors, we suspect this represents a neoplastic lesion, and thus propose the name Brunner gland duct adenoma (BGDA). The natural history of these lesions is currently unknown.

573 Universal IHC Testing of Colorectal Carcinomas for Microsatellite Instability: The Role of Histology, Impact on Clinical Care and Financial Implications

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Background: Universal IHC testing for microsatellite instability (MSI) has been advocated for colorectal carcinomas (CRCs) due to the inability of current screening guidelines to detect all MSI tumors. However, morphologic criteria have been underutilized in MSI screening strategies, and their use in the Revised Bethesda Guidelines (RBG) is limited to patients under age 60. We reviewed our CRC cases with MSI IHC testing to assess the number and type of MSI cases that would have been missed had our proposed MSEye criteria (RBG clinical criteria plus histologic criteria without age restrictions) been used. Charges generated by different screening strategies were also compared.

Design: Cases of CRC tested for mismatch repair (MMR) protein status by IHC between 1/2009 and 7/2014 were identified. Two GI pathologists independently reviewed all slides with tumor in a masked manner and recorded the presence of tumor infiltrating lymphocytes, Crohn's-like reaction, medullary pattern, mucinous/signet-ring differentiation, and other features. Each case was then evaluated by the MSEye criteria and by an online morphology-based MSI predictor (http://sitemaker.umich.edu/gruber.lab/files/msi_pre.htm). Follow-up data was collected for all patients with MSI tumors, and charges for MSI-related testing and counseling were calculated.

Results: A total of 115 CRC cases with slides available for review were identified, of which 31 (27%) had loss of >= 1 MMR protein by IHC. Using the MSEye criteria, 65 cases would have been tested by IHC and 5 cases with MSI by universal IHC would have been missed; the online predictor also missed 5 MSI cases. None of the 7 Lynch syndrome cases were missed using the MSEye criteria or the online predictor. MSI results affected subsequent therapy recommendations for only 10/31 (32%) MSI patients. Screening of cases based on MSEye criteria would have resulted in an estimated $164,710 reduction in charges compared to universal testing.

Conclusions: Most cases of CRC with MSI, including all confirmed cases of Lynch syndrome, would have been selected for testing using the MSEye criteria. Additionally, MSI status only influenced therapy recommendations in 1/3rd of patients. It is therefore reasonable to use histology-based screening strategies, in combination with RBG clinical criteria, to select CRC cases for MSI IHC testing, especially considering the significant reduction in charges when compared to universal testing.

574 DNA Methylation Array Shows Overlapping Methylation Profiles in Hyperplastic Polyps (HP) and Sessile Serrated Adenoma/Polyps (SSA/P) of the Colorectum

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Background: Development of evidence based surveillance guidelines for patients with serrated polyps of the colorectum is hampered by the lack of reproducible morphological distinction between HP and SSA/P. We tested the hypothesis that HP and SSA/P are distinctive lesions and therefore must cluster separately when analyzed by an epigenome-wide methylation array to determine their DNA methylation status.

Design: Patients with serrated polyps in the New Hampshire Colonoscopy Registry were reconsented for use of tissue for research. Polyps were selected to ensure an equal distribution between HP and SSA/P, polyp location (right vs. left), size distribution and presence of sufficient tissue for DNA methylation array analysis. Polyps with >25% admixed normal colonic mucosa and serrated polyps with dysplasia were excluded. DNA methylation array was performed using the Illumina Infinium Human Methylation 450k beadchip (Illumina, San Diego, CA) which simultaneously profiles >485,000 CpG sites covering all CpG islands. Polyps were also analyzed for *BRAF* and *KRAS* mutation. We used principal component analysis to assess most prevalent sources of variability across the entire array of methylation values. The association between the top principal components and clinical and demographic variables was then examined using linear regression.

Results: The methylation array analysis included 35 HPs (21 left; 14 right) and 42 SSA/Ps (20 left; 22 right). The majority of samples were positive for *BRAF* V600E mutation (80% HPs; 88% SSA/Ps tested). The principal component analysis showed that DNA methylation was most strongly related to smoking status, gender and polyp size. A locus specific analysis using methylation status as the dependent variable and morphology (SSA/P vs HP) as the predictor, with adjustment for age, gender, polyp size, and anatomic site, showed very few loci with low *P* values and none reached the Bonferroni threshold for a statistically significant difference. Hierarchical clustering analysis of the 50 loci with the lowest p values failed to show separate clustering by morphologic type.

Conclusions: HPs and SSA/Ps do not cluster separately in methylation array analysis supporting the hypothesis that they arise through similar epigenetic pathways. Our findings provide a pathogenetic basis for developing clinical surveillance guidelines based on the number and size of serrated polyps rather than histological distinction between HP and SSA/P.

575 Histopathologic Features of IBD in Patients With PSC Do Not Predict Need for Orthotopic Liver Transplantation

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Background: Primary sclerosing cholangitis (PSC) is a chronic cholestatic disease of the biliary tree associated with inflammatory bowel disease (IBD) in 70-80% of patients (pts). Ulcerative colitis (UC) is most often associated with PSC, though a minority of pts have Crohn disease (CD). There is evidence that gut-liver crosstalk may contribute to the pathogenesis of PSC-IBD. It has been reported that more severe PSC is associated with lower IBD disease severity in PSC-IBD pts. These reports suggest that histopathologic findings in colonic and ileal specimens may be useful in predicting the risk of orthotopic liver transplant (OLT) in PSC-IBD pts.

Design: The aim of this study was to determine if histopathologic features of IBD in PSC-IBD pts correlate with the need for OLT.

Colonic biopsies (bx) and resection specimens from PSC-IBD pts between 1999 and 2013 were identified. Histologic and clinical features including disease severity and distribution, age, IBD duration at the time of bx, medical treatment, colectomy, and need for OLT were obtained. CD20, CD3 and IgG4 immunostains were performed, enumerated and averaged over 10 high power fields (400x).

Results: 26 PSC-UC pts and 6 PSC-CD pts were identified. IBD duration, average age, and gender were not significantly different between PSC-UC and PSC-CD pts (p=0.3635, p=0.764, and p=0.4056, respectively). Follow-up duration was 4.8 years in PSC-CD pts and 8.2 years in PSC-UC pts (p=0.1317). Confirming previous studies, a high proportion of PSC-UC pts had ileal involvement (31%), right colon only involvement (29%), and pancolitis (67%). Rectal involvement (67%) was also less frequently seen compared to pts with UC without IBD.

In PSC-UC pts, OLT was not associated with colectomy, medical treatment, disease severity or distribution, mucosal CD3, CD20, or IgG4 counts. But, PSC-UC pts that required OLT had more severe inflammation of the left colon compared to PSC-UC pts who did not require OLT (p=0.0568). And, no PSC-CD pts required OLT, compared to 10 of 26 pts (38.5%) with PSC-UC (p=0.06).

Conclusions: None of the histopathologic variables examined showed a significant association with OLT, suggesting histopathologic features of IBD may not be independent predictors of the need for OLT in PSC-IBD pts. Still, the lack of OLT in PSC-CD pts and the increase in disease activity in the left colon of PSC-UC pts requiring OLT are interesting findings, although not statistically significant. Larger studies with longer follow-up are necessary to further characterize the link between OLT and IBD type as well as histologic features.

576 The 12-Gene Colon Cancer Assay: Experience With 12,776 Stage 2 Patients

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Background: Genomic assays have the potential to provide information beyond the traditional methods used for assessing risk of recurrence. The 12-gene Colon Cancer Assay (Onco*type* DX) is clinically validated and predicts recurrence risk after surgical resection in patients (pts) with stage 2 colon cancer (CC).[1-3] We report the Genomic Health Clinical Laboratory experience with stage 2 CC since the product became commercially available. The assay incorporates the expression of 12 genes (7 cancer related [3 cell-cycle genes, 3 stromal genes, and the early response gene, *GADD45B]* and 5 reference genes) and gives a Recurrence Score result (a numeric score between 0 and 100) that is a quantitative estimate of the risk of recurrence based on individual tumor biology in MMR-proficient tumors.

Design: 12,776 samples from stage 2 CC pts submitted 4/2010 to 8/2014 passed pathology review and RT-PCR quality measures. Descriptive statistics for the clinical characteristics of pts, Recurrence Score results and distributions were calculated. Low, intermediate, and high risk groups are defined as: <30, 30-40, and ≥41, respectively.

Results: Of 12,776 samples, 11,696 (91.6%) were adenocarcinoma and 1,062 (8.3%) were mucinous. Median age was 64; with equal numbers of men (6,401, 50%) and women (6,375, 50%). Samples were received from 36 countries. The median Recurrence Score result was 25 (range 0-75); 8,684 (68%) low, 2,926 (23%) intermediate, and 1,166 (9%) high. Mucinous carcinoma had a significantly higher median Recurrence Score result (34) than adenocarcinoma (24; Wilcoxon test p<0.001). Of adenocarcinoma pts, 8,330 (71%) had low scores; 2,547 (22%) intermediate, and 819 (7%) had high. Mucinous pts were evenly distributed in the three risk groups with 32% low, 35% intermediate and 32% high.

Conclusions: In the first 4 years of experience, there were more than 12,000 samples submitted from stage 2 CC pts for the 12-gene colon assay. While the vast majority of samples were adenocarcinoma, mucinous subtype was found to have a higher median score (34 vs 24) and more high scores (32% vs. 7%). Overall there was a wide range of Recurrence Score results (0-75) indicating that risk of recurrence is continuous and not simply high or low. Traditional methods for assessing risk do not reveal the full picture and the 12-gene colon cancer Recurrence Score result provides a quantitative and more individualized risk assessment for stage 2 CC pts beyond T-stage and MMR status. This will greatly improve the ability to personalize care and treatment decisions in pts with stage 2 CC.

[1]Gray, et al, 2011; [2]Venook, et al, 2013, [3]Yothers, et al, 2013.

577 Tumor Cell Detection in Peritoneal Lavage Fluid of Gastric Cancer Patients: RT-PCR and Cytology

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Background: Cytological status of peritoneal lavage fluid is an important prognostic factor in locally advanced gastric cancer. It is a positive indicator of the recurrence risk and of the decrease in the survival ratio and influences the handling of patients.
According with literature although highly specific, conventional peritoneal lavage fluid cytology has a low sensitivity in predicting peritoneal recurrence. It rises to 22 to 75% when using immunocytochemistry (ICC).
We considered using molecular techniques (RT-PCR) as an option to rise sensitivity in determining the extension of the neoplasm.
Design: A total of 106 peritoneal lavage fluid samples from gastric cancer patients were obtained between January 2011 and July 2014. Half the material was processed for cytological study and the other half by Real Time RT-PCR with two epithelial markers, CEA and CK20. The material for cytology was centrifuged and when possible, we did a cell block; in cases where the cell block could not be done we reserved two cytospin for ICC study. The ICC study was done when histological type of gastric adenocarcinoma was diffuse or poorly differentiated and the primary tumor expressed CK20 and/or CEA . The follow up of the last 25 patients is less than one year. In 9 cases we don't know the patient's current status.
Results: 20 cases (19%) were positive by cytology, and 2 cases (2%) were reported as presence of scattered atypical cells. Using RT-PCR, significative CEA and/or CK20 expression was detected in 34 (32%) patients. There were 87 cases negative by cytology, of them 16 were positive by RT-PCR analysis (18%). Only a case with positive cytology was RT-PCR negative and a relevant % of tumor cells didn't express either CEA or CK20. The other 18 cases with positive cytology were positive by RT-PCR.
Of 97 patients we followed, 24 died due to disease progression: 11(46%) had positive cytology and 17 (71%) positive RT-PCR. Among 73 patients alive or dead by other causes, there were 11 (11%) with positive cytology and 16 with RT-PCR positive (22%).
Conclusions: - Detection of CEA or CK20 by RT-PCR in peritoneal lavage increases sensitivity than cytology alone in the detection of extension of gastric cancer.
- There is an association between positive peritoneal lavage and worse outcome, thus the results of cytology and RT-PCR analysis are important for therapeutic decisions.

578 Correlation of p53 Staining With Mutational Load Analysis in Barrett Esophagus Patients

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Background: Patients with Barrett esophagus (BE) undergo routine biopsies to monitor extent of disease and disease progression. Risk stratification of patients who progress from intestinal metaplasia (IM), low grade dysplasia (LGD) or indefinite for dysplasia (IND) to high grade dysplasia (HGD) or esophageal adenocarcinoma (EAC) has routinely been based on histologic classification. However, the pathologic interpretation is subjective with considerable interobserver variability. A more objective risk stratification prior to progression could enable targeted monitoring and earlier intervention. In our previous research, progression of BE was shown to be associated with mutational load. P53 is a tumor suppressor gene located at 17p13 that has been implicated as one of the 10 loci that undergoes mutation in EAC. Use of immunohistochemical (IHC) staining to detect truncated mutations (no staining) or point mutations (stabilized protein and increased staining) has proven useful in various malignancies. The purpose of this study was to compare p53 with mutational load as an ancillary test and for risk stratification in patients with BE.
Design: Cases included 9 known progressors (P) (n=20) and 25 known non-progressors (NP) (n=30). P were those patients that had an initial biopsy with IM, IND, and/or LGD who later developed HGD or EAC. NP were those patients that remained stable at IM, IND or LGD during their surveillance time. H & E slides were blindly reviewed and the area of the most aggressive histology was correlated with p53 staining. Staining was scored by percentage of nuclear staining (0: 0-5%; 1: 5-25%; 2: 25-50%; 3: 50-75%; 4: 75-100%) and by staining intensity (1+: mild; 2+: moderate; 3+: strong). These results were correlated to previous mutational load analysis.
Results: Nuclear staining of p53 was diffuse (3-4) and strong (3+) in 80% (17/20) of samples from P group and within this group loss of heterozygosity (LOH) or microsatellite instability (MSI) for 17p/p53 was present in 80% (17/20). NP group showed no staining in 30% (9/30); 5-25% staining in 36.6% (11/30); 25-50% staining in 30% (9/30) and 50-75% staining in 6.6% (1/30). Mutational load was low or non-existent in the NP group.
Conclusions: 1. P53 IHC staining correlated well with BE patients who had disease progression and harbored LOH and/or MSI.
2. P53 IHC staining may be useful in histologic evaluation of patients monitored for BE and serve as an ancillary tool for risk stratification of progressors.

579 Impact of the Amplified RAS Mutational Analysis in Colombian Patients With Colorectal Cancer: Study of 1204 Cases

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Background: New 2014 guidelines from The National Comprehensive Cancer Network (NCCN) related with the management of colorectal cancer (CRC) have been modified and to define predictive response on treatment with anti-EGFR antibodies it is now recommended to analyze the mutational status of KRAS codons 12,13,61,117 and 146 and NRAS codons 12,13,61,117 and 146. About 40% of RAS mutations are point mutations and occur in codons 12 and 13 of the KRAS gene, however, mutations in other codons and the NRAS gene are less frequent. The aim of this study was to evaluate the impact related on the new 2014 recommendations on the mutational status of KRAS and NRAS genes in Colombian patients with CRC.
Design: A retrospective evaluation of samples diagnosed as CRC in our pathology department was conducted. KRAS mutational status of codons 12 and 13 during the period January 2009-December 2013 was assessed. Cases diagnosed in January to September 2014, were evaluated for the mutational status of exons 2,3,4 of KRAS and NRAS genes. In both cases the mutation analysis was evaluated from paraffin embedded tissues and by direct sequencing.
Results: 844 patients with CRC were studied in the period 2009-2013 (50% females and 50% males) average age of 56 years (16-87 years). Mutations analysis in codons 12 and 13 of the KRAS indicated that 36.4% of the cases were mutated (37.5% women and 35.3% men). From the 317 mutated cases, 74.3% at codon 12 and the remaining 25.7% at codon 13. The second group of cases included 360 patients diagnosed during the first 8 months of this year, 49.7% females and 50.3% males, average age 57 years (16-89 years). Mutational analysis including codons 12, 13, 59, 61, 117 and 146 of the KRAS genes presented a mutation frequency of 44.4% (48.6% of women and 40.6% in men). From the group of wild type cases by KRAS gene, 6 cases presented mutations at codon 12 and 4 cases at codon 61 of the NRAS gene, meaning that the percentage of patients with mutations in both KRAS and NRAS genes corresponded to 47.2% of the cases.
Conclusions: Change in NCCN recommendations involving extended mutational analysis at codons 59,61,117,146 and 159 of the KRAS gene and codons 12,13,59,61,117,146 and 159 of the NRAS gene, allowed us to identify about 10% additional mutated cases compared to previous evidence that included only codons 12 and 13 of the KRAS gene.

580 Olfactomedin-4 Is an Independent Prognostic Marker in Stage II Colorectal Carcinoma

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Background: Olfactomedin-4 (OLFM4) is an anti-apoptotic glycoprotein. Proteomic work from our group has shown that OLFM4 is a key protein that is differentially expressed between normal colonic mucosa and colorectal cancer (CRC). Here, we investigate OLFM4 as a new prognostic marker.
Design: We investigated the OLFM4 expression by IHC (rabbit polycolonal Ab) in a well characterized stage II cohort of 57 CRC patients (median follow-up, >60 months). OLFM4 expression was grouped into high (2-3+) and low (0-1+) based on the most prevalent ('modal') intensity observed in the tumor. OLFM4 expression was correlated with tumor pathological features including TNM staging as well as clinical outcomes such as overall survival and progression-free survival. Survival curves were generated using the Kaplan-Meier method, with significance evaluated using the Mantel-Cox log-rank test. Risk ratios were calculated using the Cox proportional hazard model in both univariate and multivariate analyses. The values of $p<0.05$ were considered statistically significant.
Results: High OLFM4 expression was observed in 29.8% of stage II CRC. Colorectal cancer patients with high OLFM4 expression had reduced recurrence free survival of 33.3% at 5 years as compared to 70.0% with normal OLFM4 expression ($p=0.012$). In a multivariate analysis, there was no association of OLFM4 with age, gender, tumor location, histologic grade, tumor size, pT, number of lymph nodes resected, and chemotherapy given.
Conclusions: High OLFM4 expression is an independent prognostic marker for poor progression-free survival in stage II CRC. Our results highlight subgroups of CRC patients whose tumors are driven by OLFM4, and development of a proteomics-based biomarker panel can act as an adjunct to decide treatment strategies in stage II patients.

581 Loss of DNA Mismatch Repair Genes Appears Extremely Rare in Stomach Adenocarcinoma: A Single Institution Cohort Study

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Background: Gastric cancer is the fourth leading cause of cancer-related death on a global scale. Most gastric adenocarcinomas occur sporadically, but 8-10% demonstrate a familial component. Among gastric adenocarcinomas associated with a familial component, half are attributed to hereditary germ-line mutations. Hereditary non-polyposis syndrome (Lynch syndrome) is characterized by germ-line mutations in DNA mismatch repair genes that lead to microsatellite instability. The DNA mismatch-repair gene germ-line mutations include *MLH1, PMS2, MSH2,* and *MSH6*. Studies show varying prevalence of gastric adenocarcinoma in Lynch syndrome, 0.8% to 30% with *MLH1* and *MSH2* germ-line mutations being the most common. Since stomach cancer is more common seen in younger patients and with family history in US, screening Lynch syndrome using immunohistochemistry approach appears reasonable.
Design: Screening Lynch syndrome using immunohistochemistry approach was performed for sixty cases of gastric adenocarcinoma including gastric cardia, body,

and antrum from 2010-2013. The patients' ages ranged from 41 years to 91 years, and the average age was 69 years. Among the 60 cases of gastric adenocarcinomas, there were 27 females and 33 males (M:F = 1.22:1). The expression of the mismatch repair proteins: MLH1, PMS2, MSH2, MSH6 was determined by immunohistochemistry on 5 um sections of paraffin embedded tissue. Tumor samples classified as showing loss of MLH1 by immunohistochemistry will be further tested to determine if the results is a consequence of epigenetic methylation of MLH1 due to BRAF mutation or Lynch Syndrome due to germ-line mutation of MLH1 using mutant BRAF immunohistochemistry and qPCR and sequencing.

Results: Sixty cases of gastric adenocarcinoma of the stomach were of intestinal and signet-ring type. Using proper positive and negative controls, all sixty cases showed positive expression of the mismatch repair proteins, MLH1, PMS2, MSH2, and MSH6. No MLH1 epigenetic methylation or Braf mutation were seen.

Conclusions: Our single institutional large cohort study indicates that Lynch syndrome is not present or is extremely rare in our cohort of stomach adenocarcinoma. As such, this study demonstrates that mismatch repair protein screening for Lynch associated gastric adenocarcinoma is low yield and should not be used as it is in cases of colonic adenocarcinoma.

582 Reevaluation of p53 Immunohistochemistry in Barrett's Esophagus-Associated Neoplasia Using a Molecular-Based Approach

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Background: Dysplasia assessment, the "gold standard" for risk stratification in Barrett's esophagus (BE), is fraught with significant interobserver variability, especially for diagnoses of low-grade dysplasia (LGD) and indefinite for dysplasia (IND). Numerous biomarkers have been studied in hopes of improving accuracy. Among the best studied is p53 immunohistochemistry (IHC). Most previous studies have equated p53 "overexpression" with a "positive" result. Unfortunately, both wild-type *TP53* and missense-mutant *TP53* may be "overexpressed." *TP53* truncating mutations/deletions may result in *complete absence* of p53 staining; in our experience this "null-pattern" staining is often misinterpreted as "negative." We performed a "molecular-based" p53 assessment on a large cohort of BE specimens across the dysplasia spectrum to answer the following: 1. what is the frequency of "null-pattern" staining, 2. what fraction of IND cases can be upgraded to LGD based on abnormal p53 IHC, and 3. what is the rate of p53 abnormality in LGD (reported rate 9-89%)?

Design: 167 BE samples were reclassified by a GI pathologist as follows: no dysplasia (ND) 46, IND 56, LGD 9, high-grade dysplasia (HGD) 21, intramucosal carcinoma (IMC) 12, at least submucosally invasive carcinoma (SMI) 23. p53 IHC (clone DO-7) was assessed as wild type (weak to moderate staining), missense (clonal areas of diffuse, strong staining), or null (clonal areas of completely absent staining).

Results: "Null-pattern" staining accounted for 18% of abnormal p53 IHC results. 27% of IND and 67% of LGD specimens had an abnormal p53 IHC result. Detailed data are presented in the Table.

p53 Patterns as a Function of Diagnostic Category						
	Diagnostic Category					
p53 Pattern	ND (n=46)	IND (n=56)	LGD (n=9)	HGD (n=21)	IMC (n=12)	SMI (n=23)
Wild Type	45 (98%)	41 (73%)	3 (33%)	1 (5%)	0	1 (4%)
Missense	1 (2%)	13 (23%)	4 (44%)	16 (76%)	9 (75%)	17 (74%)
Null	0	2 (4%)	1 (11%)	1 (5%)	2 (17%)	5 (22%)
Combo	0	0	1 (11%)	3 (14%)	1 (8%)	0

Combo represents presence of 2 patterns, at least 1 of which is missense or null

Conclusions: "Null-pattern" staining accounts for a significant minority of abnormal p53 IHC results. Around a quarter of IND specimens can be upgraded to LGD based on abnormal p53 IHC staining, and most LGDs are p53-abnormal. We advocate a "molecular-based" interpretation of p53 IHC, which we find especially useful when considering a differential of IND versus LGD.

583 Molecular Characterization of Acquired Resistance To Anti-EGFR Monoclonal Antibodies in Colorectal Cancer Patients

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Background: Drug resistance invariably develops in colorectal cancer patients treated with anti-EGFR antibodies, cetuximab and panitumumab. To increase our understanding on mechanisms of acquired resistance to anti-EGFR treatment, we evaluated molecular changes before and after receiving anti-EGFR treatment in paired tumor samples from CRC patients.

Design: A total of 37 colorectal cancer patients were included in the study. We systematically re-biopsed cancer lesions and obtained blood samples from patients before treatment and at the time of tumor progression following anti-EGFR based therapy. Mutations in *EGFR*, *KRAS*, *NRAS*, *BRAF* and *PIK3CA* genes were evaluated by next-generation pyrosequencing. *KRAS*, *EGFR* and *HER2* gene amplification was evaluated by FISH.

Results: Molecular analysis of samples at the time of progression showed the emergence of novel alterations in 81% of patients. *RAS* mutations were the most frequent event (40% of patients) and mostly affected exons 3 and 4, followed by mutations in *PIK3CA* (19%), *BRAF* (11%), *EGFR*S492R (8%) and novel mutations in *EGFR* ectodomain (5%). FISH analysis showed *EGFR* and *HER2* gene amplification in 57% and 18% of patients, respectively, whereas *KRAS* gene amplification was not detected in any of the cases. Of note, in 9 patients the mutations were already detected by next-generation sequencing at diagnosis as well as in 8 patients *EGFR* and *HER2* amplification. In some cases different molecular mechanisms of resistance overlapped and evolved during the course of the disease.

Conclusions: Multiple mechanisms are responsible for acquired resistance to cetuximab-based therapy in mCRC. These results provide a solid basis for a rational development of treatment strategies to overcome or prevent acquisition of resistance to anti-EGFR therapies in colorectal cancer patients that can be individualized based on molecular studies at disease progression.

584 A Clinicopathological and Molecular Analysis of 107 Sessile Serrated Adenomas With Dysplasia

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Background: The sessile serrated adenoma (SSA) is a common colorectal polyp, accounting for up to 14.7% of all polyps. Cases with dysplasia (SSAD) or carcinoma (SSAC) are much rarer, representing around 0.4% of polyps. SSAs progress to carcinoma along either a mismatch repair deficient (MMRD) or a mismatch repair proficient (MMRP) pathway, depending on the methylation status of the *MLH1* gene. The clinicopathological and molecular significance of these disparate pathways has not yet been determined.

Design: 107 histologically advanced SSAs were retrieved retrospectively from the files of a gastrointestinal pathology practice. For inclusion cases required unequivocal SSA with abrupt transition to dysplasia or carcinoma in the one tissue fragment. Clinicopathological data, *BRAF* and *KRAS* mutation testing and immunohistochemistry for MLH1, β-catenin, p16, p53, CK7 and CK20 was performed on all cases.

Results: The clinicopathological data and mutation status of the cases is shown in table 1.

SSAD subtype	Mean age	Female	Mean size	Proximal		BRAF mutant	KRAS mutant
Mismatch repair deficient	77	56/77	13.3mm	69/76	25/74	73/77	1/77
Mismatch repair proficient	71	10/30	9.7mm	20/28	16/13	27/30	0/30
P-value	0.0028	0.0003	0.0751	0.0239	0.0723	0.03976	1.000

The immunohistochemical features are shown in table 2.

SSAD subtype	P53 positive	P16 loss	Nuclear β-catenin positive	CK7 positive	CK20 negative
Mismatch repair deficient	3/77	31/77	42/77	27/77	25/77
Mismatch repair proficient	9/30	9/30	16/30	4/30	3/30
P-value	0.0005	0.3788	1.000	0.0325	0.0259

Conclusions: MMRD and MMRP advanced SSAs show different clinicopathological and molecular features. MMRD advanced SSAs are more likely to occur in the proximal colon of older women when compared to MMRP cases. They are less likely to have *TP53* mutation and are more likely to show aberrant expression of CK7 and CK20. Both groups tend to occur at a relatively small size and lesions <10mm are common. These findings are important for understanding the biology of these polyps and may also have implications for colonoscopic surveillance intervals.

585 Upper Gastrointestinal Polyps in McCune Albright Syndrome

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Background: McCune Albright Syndrome (MAS), a rare sporadic disorder, results from postzygotic activating mutations in the GNAS gene, which encodes the alpha subunit of the stimulatory G protein. Subsequent decrease in GTPase activity leads to constitutive activation and persistent elevation of cyclic adenosine monophosphate. Somatic manifestations include at least two of the following three features: 1) polyostotic fibrous dysplasia, 2) café-au-lait skin pigmentation, and 3) autonomous endocrine hyperfunction (gonadotropin-independent precocious puberty, acromegaly, hyperthyroidism, etc). With the exception of a recent report of four patients with hamartomatous upper gastrointestinal polyps, there are almost no published data regarding gastrointestinal lesions in patients with MAS.

Design: We queried our institution-wide database for patients with established MAS who underwent upper gastrointestinal endoscopy and reviewed the following for each case: demographics, clinical notes, endoscopy procedure notes, and biopsy material.

Results: Three patients with MAS underwent upper endoscopy between November2013 and August2014. The first was a 55-year-old male undergoing endoscopic ultrasound for evaluation of pancreatic cystic lesions (likely representing a mixed-type intraductular pancreatic mucinous neoplasm [IPMN]) who manifested multiple gastric lesions,

including a circumferential cardiac polypoid gastric adenoma without high-grade dysplasia, fundic gland polyps and foveolar hyperplasia in the body, and multifocal gastric heterotopia in the duodenum. Immunohistochemistry performed on the gastric adenoma demonstrated prominent MUC5AC (gastric foveolar differentiation) and MUC6 (pyloric gland type differentiation) labeling, with focal CDX2 and MUC2 (intestinal differentiation) expression. The second patient was a 20-year-old male with a history of acute recurrent pancreatitis who underwent upper endoscopy for evaluation of reflux, which revealed a fundic gland polyp in the distal gastric body and reactive/chemical gastropathy in the proximal body and antrum. The third patient was a 46-year-old female undergoing endoscopic ultrasound for evaluation of multiple pancreatic cysts (likely branch-duct IPMNs), who had a gastric hyperplastic polyp at the gastroesophageal junction and multifocal gastric heterotopia in the duodenal bulb.
Conclusions: Our findings confirm the presence of various upper gastrointestinal mass lesions in patients with MAS. Given the rarity of this syndrome, further characterization of the incidence and histologic features of these lesions requires routine gastrointestinal endoscopy in affected patients.

586 miRNA Expression Profiles of Gastrointestinal Neuroendocrine Tumors for Different Sites and Stages

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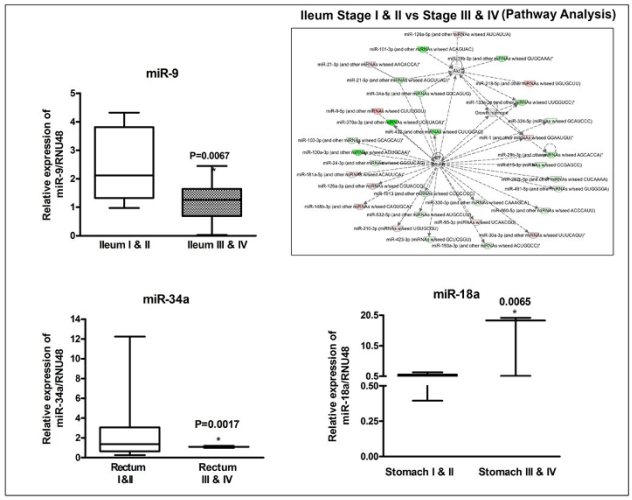
Background: The natural history of gastrointestinal neuroendocrine tumors (NET) may not correlate with histologic grade. We explored differences in miRNA expression in NET originating at different sites. In addition, stage III and IV (group1:Gp1) tumors were compared to stage I and II (group2:Gp2) tumors.
Design: NET of stomach (Gp1:n=3, Gp2:n=8), ileocecum (Gp1:n=17, Gp2:n=5), colon (Gp1:n=3), rectum (Gp1:n=3, Gp2:n=15) and appendix (Gp2:n=14). The tumors were categorized into group 1 and 2 as per the AJCC 7. Total RNA was isolated from formalin fixed paraffin embedded (FFPE) tissue using RNeasy kit (Qiagen). Microarray expression profiling was performed on purified pooled samples by LC sciences utilizing miRBase version 16. Real time qRT-PCR using SYBR green master mix was performed on individual samples for validation. Relative expression of miRNA was analyzed using Ct method and was normalized by RNU48 expression. Ingenuity software pathway analysis was used to assess molecular networks and target genes.
Results: Gp1 ileal NET tumors showed lower expression of miR-9 and higher expression of miR-301a and miR-367c. Gp1 rectum NET showed lower expression of miR-34a in comparison to Gp2. Gp1 NET of stomach showed lower expression of miR-215 and higher expression of miR-18a and miR-20a. Stage matched Gp1 colon tumors revealed higher expression of miR-205 and lower expression of miR-863 in comparison to rectum NET. Stage matched Gp1 ileal tumors revealed higher expression of miR-29b and lower expression of miR-499 in comparison to colonic NET. Pathway analysis revealed involvement of Akt pathway in gp1 NET arising from ileum and stomach.



Conclusions: NET from different sites have distinct miRNA profiles which implies diagnostic significance. Similarly, miRNA profiles from Stage III and IV NET from different sites have distinct profiles, implying impact on prognosis and risk stratification. Akt pathway involvement in higher stages of ileal and stomach NET may have therapeutic potential. Many of these differences may be evaluated by in situ methods on FFPE sections in future for differential diagnosis and prognostic purpose.

587 Yap1, a Hippo-Pathway Transcriptional Coactivator, Is Overexpressed in Microsatellite-Stable BRAF Mutant Colorectal Adenocarcinoma

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Background: Yes-associated protein 1 (Yap1) is a downstream transcriptional coactivator of the Hippo signaling pathway. Inactivation of the Hippo pathway results in translocation of Yap1 to the nucleus where it drives gene expression through its interaction with various transcription factors, resulting in upregulation of cell processes that promote tumorigenesis. Nuclear and cytoplasmic Yap1 expression has been reported to be both higher and more frequent in colorectal adenocarcinoma (CRC) than in normal colonic tissue, and recent studies have shown that Yap1 upregulation is involved in the bypass of *KRAS* inhibition in *KRAS*-mutant pancreatic and colonic adenocarcinomas. Given the frequency of Yap1 overexpression in CRC and the association between Yap1 expression and mutations in MAP-kinase pathway mediators, we set out to establish the relationship between Yap1 expression and mutations commonly found in CRC.
Design: We performed IHC for Yap1 (clone: CST#4912, 1:40 [Cell Signaling Technology, MA, USA]) on tissue microarrays (TMAs) consisting of 165 cases of CRC. Mutational and microsatellite instability status was known in all cases, as determined by PCR and immunohistochemistry for mismatch repair proteins, respectively. Yap1 staining was scored through stratification of cytoplasmic and nuclear staining into negative, low, and high, as described in prior studies.
Results: Cytoplasmic and nuclear Yap1 staining was absent in 6% and 7%, low in 78% and 21%, and high in 16% and 73% of cases, respectively. High nuclear Yap1 expression was seen more frequently in *BRAF* mutant than non-*BRAF* mutant cases (p=0.049) and in microsatellite-stable (MSS) *BRAF* mutant cases compared to all others (p=0.036) and compared to microsatellite-unstable *BRAF* mutants (p=0.17). There was no correlation between cytoplasmic or nuclear Yap1 expression and tumor treatment status, overall microsatellite instability status, or any other mutation, including *KRAS*, *NRAS*, and *p53*, among others.
Conclusions: Yap1 is overexpressed in *BRAF* mutant colorectal adenocarcinoma, particularly in the subset of cases that is microsatellite stable. Prior studies have demonstrated that MSS *BRAF* mutant CRCs are clinically aggressive tumors that are associated with a poor prognosis. Our findings suggest a potential role of Yap1 overexpression in these tumors. Additional studies are needed to further examine the role of Yap1 in colonic carcinogenesis and in MSS *BRAF* mutant tumors in particular.

588 Should We Evaluate Microscopic Surgical Resection Margins in Primary Colonic Adenocarcinoma?

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Background: In the pathologic evaluation of primary colon adenocarcinoma, documentation of the margin status is critical. However, the vast majority of surgical resection margins (mesenteric and mucosal) are not microscopically involved by carcinoma, particularly when they are grossly uninvolved. We investigate how often microscopic margins are positive and whether the subsequent cost incurred by the laboratory is justified.
Design: All Emory University cases documenting primary colon adenocarcinoma with microscopically positive margins between January 1, 2011 and April 18, 2014 were reviewed for demographic information, margin status, histologic type, pathologic grade and stage, presence of lymphovascular or perineural invasion, and clinical outcome. The data were analyzed to determine if gross positivity predicted microscopic positivity. A cost analysis was also performed.
Results: A total of 4842 colectomies with adenocarcinoma were identified. Six (0.1%) of the specimens had a positive mesenteric margin. None had involved mucosal margins. There were two right hemicolectomies, two left colecetomies, one sigmoid resection, and one total abdominal colectomy. The mean patient age at the time of surgery was 70 (range: 53-85) years. Five patients were African American and one was caucasian. Five patients had grossly identifiable tumor at the margin. The one patient with a grossly negative margin had extensive metastatic disease and the positive shave margin was described in a comment as "0-1 mm from the margin." All six cases were low grade adenocarcinoma. Lymph nodes were involved in 67%, lymphovascular invasion was present in 50%, and perineural invasion was seen in one case (16.6%). Four patients (67%) were alive with no evidence of disease and are undergoing surveillance. Two had recurrence and are undergoing treatment.
The cost to the laboratory to submit margin sections was approximately $5.10 per case (averaging two blocks), which is $24,694 over the course of this study.
Conclusions: A minute percentage of colectomies had positive margins, and those that did were either grossly positive or had extensive multifocal metastatic disease. The technical cost incurred by the laboratory to submit sections of margins from colectomy specimens is significant. This cost underestimates the professional investment in evaluating the margin slides. While it appears that in most cases the microscopic margin status is predicted by the gross, it is widely accepted to submit microscopic margins for all cases; therefore, re-evaluating this practice should be considered.

589 Gastrointestinal Tract Pathology in PTEN Hamartoma Tumour Syndrome: A Review of 43 Cases

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Background: PTEN hamartoma tumour syndrome (PHTS) results from germ line mutation of PTEN gene (10q23.3). Most cases correspond to Cowden syndrome and the terms are essentially interchangeable. Inheritance is autosomal dominant and malignancies can develop in multiple organs. Gastrointestinal tract manifestations are protean and include hamartomatous polyps, ganglioneuromas and lymphoid polyps.
Design: All patients with documented PTEN mutation or definite clinical diagnosis of Cowden syndrome were identified from the authors' institutions and all the gastrointestinal biopsies were reviewed. Abnormal findings were documented in each site in the gastrointestinal tract. Hamatomatous polyps were further characterised as to the presence of adipose tissue, ganglioneuromatous elements and lymphoid tissue.
Results: 43 patients were identified. M:F~1:1, Age range 5-85, mean 42 yrs. Abnormalities at each site were: Esophagus – glycogenic acanthosis (7/43 cases); Stomach – hamartomatous polyps (5/43 cases); Duodenum – hamartomatous polyps

(3/43 cases), ganglioneuroma (1/43 cases); Ileum – nodular lymphoid hyperplasia (3/43 cases); and Colon – hamartomatous polyps (17/43 cases), adenomatous (10/43 cases), inflammatory (4/43 cases), ganglioneuromas (6/43 cases), and lymphoid follicles (4/43 cases). Adipose tissue (30% of cases), ganglioneuromatous elements (23% of cases), and lymphoid follicles (28% of cases) were components of the hamartomatous polyps.
Conclusions: Gastrointestinal tract manifestations of PTHS are diverse. Pathologists should be aware of the various morphologies in order to suggest a potential syndromic association when this has not been previously considered.

590 Role of Serial Sectioning in the Classification of Serrated Colon Polyps

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Background: Colonic sessile serrated adenomas (SSAs), unlike microvesicular hyperplastic polyps (HPs), are considered presumptive premalignant lesions. Recent consensus guidelines have proposed a lower diagnostic threshold for SSA from earlier WHO criteria to include serrated polyps (SPs) with even a single SSA-type crypt (Rex DK, et al. Am J Gastroenterol. 2012;107:1315-29), while another study has proposed criteria for SSA that are even less stringent, i.e., provisional SSAs (pSSAs) (Bettington et al., Am J Surg Pathol. 2014;38:158-166). We investigated whether serial sectioning of SPs fulfilling minimal SSA criteria might result in sufficient differences to produce discordant results depending which section was examined.
Design: Of 148 SPs encountered consecutively in our routine practice, each initially comprising 6-9 serial sections per slide, we identified 30 (17 left-sided, 13 right-sided) fulfilling the minimal SSA citeria of Rex et al. (Group A, N=6); of Bettington et al. (i.e., pSSA3; Group B, N=15); or neither, but which contained 1 or 2 crypts with equivocal SSA-like features (Group C, N=9). The polyps were each further processed into 27-30 additional serial sections. Each section was evaluated jointly by 2 pathologists and reclassified as [1] SSA per WHO or Rex et al., [2] pSSA per Bettington et al., or [3] microvesicular hyperplastic polyp (HP).
Results: Refer to table below.

Initial classificationand criteria	N	Location (no. right sided) (%)	Initial classification retained in all serial sections	Biopsies reclassified in serial sections (polyp types)	Reclassified polyps by location
Group A:1 or 2 non-adjacent SSA-type crypts	6	4 (60%)	5	1 (HP)	1 HP left-sided
Group B:≥3 crypts with poorly-developed SSA features	15	5 (33.3%)	1	14 (4 HPs, 3 SSAs per Rex et al., 7 SSAs per WHO)	3 of 4 HPs left-sided; 6 of 10 SSAs left-sided
Group C:1 or 2 crypts with poorly developed SSA features	9	4 (44%)	6	3(2 SSA per Rex et al., 1 SSA per WHO)	2 SSAs right-sided; 1 SSA left-sided

Conclusions: The current minimal criteria for SSAs are subject to discordant classification when subjected to serial sectioning, indicating a baseline error rate if insufficient sections are examined. Although the clinical implications are uncertain, this source of potential variation should be recognized in designing pathologic studies.

591 Gastrointestinal Tract Adenocarcinomas Display Intratumoral Heterogeneity Detectable By 47 Gene Next Generation Sequencing Panel

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Background: Gastrointestinal tract adenocarcinoma(GITA) displays two types of intratumoral heterogeneity(ITH); cytogenetic and evolving subclones. Next generation sequencing (NGS) gene panels are often performed on advanced stage GITA. The purpose of this study is to identify ITH by allelic imbalances(AI) and subclones(SC) in GITA via an NGS gene panel and to investigate any histological associations.
Design: H&E sections were noted for the number of growth patterns, necrosis, grade (low vs. high), and nuclear pleomorphism(NP) (mild-moderate vs. severe). The percent tumor, specimen (biopsy vs. resection/excision), and tumor state (metastasis vs. primary) was noted. DNA was extracted from FFPE samples(Qiagen), enriched for 47 genes (TruSeq cancer panel), and sequenced on a MiSeq (Illumina, CA). Variant calls were made by an in-house bioinformatic pipeline. AI and SC were called based on allele frequencies and percent tumor. Statistics were performed in SAS JMP (Cary, NC), using ANOVA, Chi-squared, or fisher's exact test. Alpha was set at 0.05.
Results: A total of 75 cases were studied with 166 variants detected for a mean of 2.2 (stdev 1.2) per case and an average read depth of 6759. AI was detected in 49/166(30%) variants in 36/75(48%) cases. TP53, KRAS, and APC were the most frequently mutated genes showing AI. SC was detected in 11/75(15%) cases with PIK3CA(3/11), FBXW7(2/11), and TP53(2/11) being the most frequent genes defining SC. On univariate analysis (table 1), SC was only found in resection/excisions (p=0.0056) and cases with tumor percentage 26% or greater(p=0.038). No associations were found between AI or SC and sampled tumor state, number of growth patterns, NP, grade, and necrosis.
Conclusions: ITH is detectable in GITA by a 47 gene NGS panel and was not associated with any histological features. Importantly, evolving subclones which harbored actionable mutations were only found in resection/excisions and cases with greater than 25% tumor. These findings may be useful for quality assurance, interpretation of complex allele frequencies, and treatment decisions.

Table 1. Univariate analysis of GI tract adenocarcinoma intratumoral heterogeneity by histologic and specimen characteristics (n=75)

	Pathogenic variants per case		Allelic imbalance detected			Evolving subclone detected		
			No	Yes		No	Yes	
	mean (stdev)	p-value	(n=39)	(n=36)	p-value	(n=64)	(n=11)	p-value
Specimen type		**0.0123**			0.4914			**0.0056**
Biopsy (n=28)	1.8 (0.88)		16	12		28	0	
Resection/excision (n=47)	2.5 (1.2)		23	24		36	11	
Sampled tumor state		0.313			0.9406			0.2263
Metastasis (n=33)	2.1 (1.1)		17	16		30	3	
Primary (n=42)	2.4 (1.2)		22	20		34	8	
Percent tumor		0.5379			0.5819			**0.0381**
11-25% (n=17)	2.2 (1.1)		7	10		17	0	
26-50% (n= 37)	2.4 (1.1)		20	17		32	5	
>50% (n=21)	2.0 (1.2)		12	9		15	6	
Histologic growth patterns		0.8031			0.4354			0.993
One (n= 34)	2.2 (1.3)		16	18		29	5	
Multiple (n=41)	2.2 (1.0)		23	18		35	6	
Nuclear pleomorphism		0.437			0.326			0.5139
Mild-moderate (n=48)	2.3 (1.2)		27	21		40	8	
Severe-bizzare (n=27)	2.1 (1.1)		12	15		24	3	
Tumor necrosis		0.2751			0.4634			0.2875
Yes (n=24)	2.0 (1.1)		11	13		22	2	
No (n=51)	2.3 (1.2)		28	23		42	9	
Tumor grade		0.2059			0.6083			0.131
Low(G1, G2) (n=46)	2.3 (1.1)		25	21		37	9	
High (G3, G4)(n=29)	2.0 (1.3)		14	15		27	2	

Bold= Statistically significant

592 LIN28 Was Expressed in Gastric Hepatoid Adenocarcinoma: An Immunohistochemical Study of 33 Cases With Comparison To SALL4, AFP, Glypican-3, Hepatocyte and Polyclonal-CEA

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Background: Primary gastric hepatoid adenocarcinoma (PGHA) is uncommon and sometimes it poses diagnostic difficulty especially in metastatic sites. Previous studies have identified several diagnostic immunohistochemical makers for PGHA including AFP, Glypican 3 (GPC3) and SALL4. In this study we investigated the expression of a novel marker, LIN28 in 33 PGHAs to explore its potential diagnostic utility. We also compared LIN28 to SALL4, AFP, GPC3, hepatocyte and polyclona-CEA (p-CEA).
Design: Thirty-three PGHAs were included for this study. For each case, one paraffin block containing tumor was retrieved to generate 5um unstained slides for immunohistochemical staining with antibodies to the following proteins: LIN28, SALL4, AFP, GPC3, hepatocyte and p-CEA. Only nuclear staining was considered positive for SALL4. Only canalicular pattern was considered positive for p-CEA. For other markers, the staining was cytoplasmic. The staining was semi-quantatively scored as 0 (no tumor cells stained), 1+ (1-25%), 2+ (26-50%), 3+ (51-75%), and 4+ (>75%).
Results: The staining results were summaried in the following table.

Marker	0	1+	2+	3+	4+
LIN28	17 (52%)	9(27%)	0	3(9%)	4(12%)
SALL4	6 (18%)	12(36%)	4(12%)	5(15%)	6(18%)
AFP	8(24%)	10(30%)	4(12%)	1(3%)	10(30%)
Glypican-3	11(33%)	5(15%)	2(6%)	4(12%)	11(33%)
Hepatocyte	15(45%)	9(27%)	6(18%)	3(9%)	0
p-CEA	16(48%)	12(36%)	3(9%)	1(3%)	1(3%)

Positive staining of LIN28, SALL4, AFP, GP3, hepatocyte and p-CEA was seen in 16/33 (48%), 27/33 (82%), 25/33 (76%), 22/33 (67%),18/33 (55%) and 17/33 (52%), respectively. LIN28 and SALL4 demonstrated the following combinations: SALL4+/LIN28+ in 11/33 (33%, either AFP or GPC3 + in 11/11), SALL4+/LIN28- in 15/33(45%, either AFP or GPC3 + in 12/15), SALL4-/LIN28+ in 6/33 (18%, either AFP or GPC3 + in 5/6), and SALL4-/LIN28- in 1/35 (3%, AFP + in 1/1).
Conclusions: LIN28 was expressed in approximately half (48%) of PGHAs and was not sensitive as other markers. Among the 6 markers, SALL4 is the most sensitive one and the diagnostic sensitivity can be further enhanced in combination with LIN28. However, the maximal diagnostic sensitivity was only achieved with a panel including LIN28, SALL4, AFP and GPC3.

593 M2-Polarized Tumor Associated Macrophages (TAMs) Predicts Poor Prognosis of Esophageal Adenocarcinoma Without Neoadjuvant Therapy

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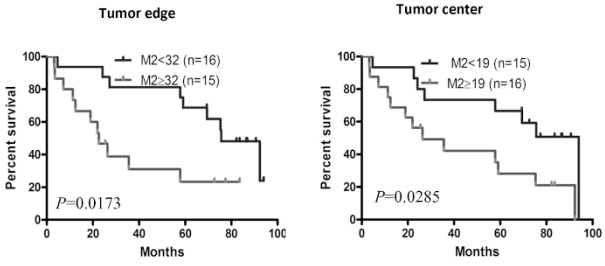
Background: Despite the advances in early detection and implementation of neoadjuvant therapy, the prognosis of esophageal adenocarcinoma (EAC) is still poor. Accurately prediction of lymph node status in preoperative stage is critical for optimal management of EAC. We studied the distribution of subtype tumor associated macrophages (TAMs) in EAC and explored their potential roles in prediction of prognosis.

Design: 53 EAC resection specimens (33 without neoadjuvant therapy, 20 with neoadjuvant chemoradiation) were immunostained with CD68, CD40 and CD163. Average numbers of M1 (CD68+/CD40+) or M2 (CD68+/CD163+) macrophages were counted at five hot spots at 400x high power field in tumor center and tumor edge, and correlated with clinicopathological factors. Student *t*-test was also employed for data analysis. The association between subtype of TAMs and overall survival was analyzed using Kaplan-Meier method.

Results: In EAC without neoadjuvant therapy, the median number of M2 TAMs was 32 per high power field at tumor edge and 19 in tumor center. A high M2 macrophage count was associated with poor patient overall survival, independent of the locations of macrophages in tumor.



Interestingly, the M2/M1 ratio that represented the balance between M2 and M1 macrophages was significantly higher in nodal positive EAC than that in nodal negative group, and positively correlated with patient survival. M1, M2 count, or the ratio of M2/M1 was not associated with patient's age, gender, tumor differentiation or stage. Neoadjuvant therapy diminished the correlation between M2 macrophages and patient survival.

Conclusions: M2-polarized TAMs in EAC predicts poor prognosis prior to chemoradiation treatment. The M2/M1 macrophage ratio may serve as a sensitive marker to predict lymph node metastasis and poor prognosis in EAC without neoadjuvant therapy.

594 Gastrointestinal Graft Versus Host Disease in Autologous Versus Allogeneic Transplant Patients: Earlier Onset and More Apoptosis

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Background: Graft versus host disease (GVHD) is a complication of autologous stem cell transplantation (AuSCT) that arises in the gastrointestinal (GI) tract, skin, and liver. Previous reports of GI GVHD in the setting of AuSCT have noted histology indentical to allogeneic GI GVHD, but have not formally characterized it, or compared to allogeneic stem cell transplant (AlloTPX) controls. Prior studies have also reported an early onset of GVHD in AuSCT, despite the commonly held belief that GVHD cannot be diagnosed confidently <21 days post-transplant. The aim of this study was to histologically and clinically characterize GI GVHD in AuSCT patients and compare to a group of AlloTPX controls.

Design: Colon biopsies taken to evaluate GVHD in AuSCT and AlloTPX patients from 2008-2014 were evaluated blindly by a GI pathologist for the maximum number of apoptotic bodies per 10 contiguous crypts and were graded according to the 1974 Lerner criteria. 124 biopsies from 86 patients were evaluated. Clinicopathologic information was collected through chart review.

Results: The AuSCT group (mean age 59, M:F; 7:9) included 13 patients with multiple myeloma (MM), and 3 with non-Hodgkin's lymphoma (NHL). 7 patients had features of grade 1 GVHD, 3 grade 2, 4 grade 3, and 2 grade 4. 70 AlloTPX patients made up the control group (mean age 52.5, M:F; 32:38). AuSCT patients were significantly more likely to develop GVHD symptoms <21 days post-transplant (53% vs 7.3%, p<0.001). The median time to GI symptoms post-transplant was 20 days for AuSCT vs. 91 days for AlloTPX. All AuSCT patients who developed symptoms <21 days post-transplant (9/17) required steroids and symptomatically resolved following treatment. AuSCT patients exhibited more average apoptotic bodies per 10 contiguous crypts, though the difference was not statistically significant (7.1 vs 4.9, p=0.14). Further analysis indicated that the trend was driven by a significant difference between AuSCT and AlloTPX patients with HLA matched-related donors (MRD) (mean: 7.1 vs 3.5, p=0.04).

Conclusions: GI GVHD in the setting of AuSCT exhibits a full histologic spectrum, with examples ranging from rare crypt apoptosis (Grade 1) to mucosal ulceration (Grade 4). GI GVHD in AuSCT patients occurs earlier than in AlloTPX and with more numerous apoptotic bodies compared to MRD AlloTPX patients. Despite a significant number (53%) of AuSCT patients developing GVHD symptoms <21 post-transplant, all resolved with steroid treatment, challenging the idea that GVHD cannot be confidently diagnosed in this time period.

595 Objective Measurement of HER2 and Downstream Signaling Targets in Gastric Cancer

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Background: HER2 overexpression/amplification characterizes a subtype of gastric cancer (GC) associated with potential of benefit from anti-HER2 targeted therapies. The prognostic role of HER2 overexpression in GC remains unclear. HER2 testing in GC is performed using chromogenic immunohistochemistry (IHC) and in situ hybridization methods. Evaluation of HER2 IHC is subject to diverse methodological and interpretative challenges. Here, we measured HER2 protein in GC using objective methods and correlated its levels with downstream intracellular signal pathways activation and clinico-pathological features.

Design: We measured HER2 protein using quantitative immunofluorescence(QIF) with 2 domain-specific antibodies (e.g. intracellular [ICD] and extracellular domain [ECD]) in a retrospective collection of 302 GCs represented in 2-fold redundancy in tissue microarray format. The levels of HER2 were stratified using a permutation Jointpoint regression analysis. In addition, the levels of the HER2 target S6 protein and its major phosphorylated forms (pS6 s235-6 and pS6 s240-4) were measured in consecutive tissue sections to interrogate the signaling pathway activation status. The associations between HER2 levels, S6 pathway activation, clinico-pathological variables and survival were studied.

Results: Elevated HER2 protein using both ICD and ECD assays was significantly associated with intestinal histology, earlier clinical stage (stages I-II) and high levels of total and phosphorylated S6 protein. Some discordance was seen between ECD and ICD, but only cases with lower levels of HER2. Phosphorylation of S6 was elevated in 40-60% of the population compared to HER2 which was elevated in less than half of those with high phospho-S6. Elevated HER2 protein was not independently associated with survival in GC. However, high pS6 was associated with increased overall survival in cases with HER2 protein overexpression (log-rank *P*=0.005, HR: 4.72, 95% C.I.: 1.95-36.89).

Conclusions: In GC, HER2 expression is associated with intestinal phenotype, earlier clinical stage at diagnosis and elevated total S6 and pS6 protein levels. Although HER2 alone is not prognostic in GC, co-expression of phosphorylated S6 protein predicts better outcome, suggesting pathway activation may be broader than seen when using high HER2 alone to define pathway activation. Taken together, our results indicate that objective measurement of HER2 and downstream signaling targets may identify a wider range of responders to anti-HER2 pathway therapies than reflected by simple measurement of HER2.

596 Examining the Utility of PET/CT in Predicting HER2 Overexpression in Gastric Adenocarcinoma

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Background: 18 fluorodeoxyglucose (FDG) positron emission tomography-computed tomography (PET/CT) is useful for pre-operative staging of gastric carcinoma and for following disease progression. The amount of 18-FDG uptake reflects a tissue's level of metabolic activity, reported semi-quantitatively as the maximum standard uptake value (SUVm). HER2 overexpressing (HER2+) gastric and gastroesophageal junction adenocarcinomas (GC and GEJC, respectively) represent a clinically more aggressive form of disease which may correlate to the tumor's metabolic activity. SUVm could be a preoperative parameter used to predict HER2 status of GC/GEJC. We analyzed GC/GEJC cases with HER2+ and normal expression levels of HER2 (HER2-) at our institution and compared their SUVm.

Design: A retrospective search of our pathology reports over 3 years (2011-2014) revealed 26 total HER2+ GC and GEJC biopsies and resections, all confirmed by FISH. We identified 61 random cases of HER2- (by immunohistochemistry) GC/GEJC distributed evenly over the same time period. The following variables were studied: patient age, gender and tumor size, histologic diagnosis, differentiation, presence of lymph node (LN) metastasis and pre-treatment tumor SUVm.

Results: Our study group consisted of 87 total patients (62 male, mean age 68 years) with GC/GEJC. Patients with HER2+ and HER2- GC/GEJC showed no difference in age (70 and 67 years, P=0.2, respectively) or gender (77% and 70% men, P=0.6). There was no difference in SUVm between HER2+ and HER2- cases (7.5 and 8.4, P=0.6). No difference was seen between HER2+ and HER2- cases in tumor histology (76% and 58% intestinal type, P=0.2), tumor differentiation (56% and 72% poorly differentiated, P=0.3), or presence of LN metastasis (33% and 40%, P=0.3). Tumor size was different between HER2+ and HER2- cases (1.8 cm and 3.3 cm, P=0.01).

Conclusions: Our findings reveal that gastric and gastroesophageal junction tumors with differential HER2 expression by FISH show no difference in several clinicopathologic factors, in agreement with others' conclusions. There is a tendency, although not significant in our cohort, for HER2+ cases to demonstrate intestinal phenotype. To our knowledge, this is the first study which compared the radiologic SUVm from patients with HER2+ and HER2- gastric and gastroesophageal junction carcinoma matched by age and gender. We found no difference in SUVm level between the two groups, thus we provide no evidence for the predictive role of SUVm with relation to HER2 status of gastric and gastroesophageal junction carcinomas.

597 Identification of Serrated Polyposis in a Large Patient Cohort Using WHO Diagnostic Criteria

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Background: Serrated polyposis syndrome (SPS) is characterized by multiple colonic serrated polyps (SP) and increased risk of microsatellite instability (MSI) colorectal cancer (CRC). In 2010, the WHO established criteria for SPS: 1) ≥5 SP proximal to sigmoid with ≥2 being ≥1 cm; 2) any SP proximal to sigmoid in a patient with a 1st-degree relative with SPS or 3) ≥20 SP. SPS incidence is unknown and a confirmatory test is unavailable. We examined the utility of the WHO criteria in identifying patients with SPS in a large cohort.

Design: 1114 consecutive patients with at least 1 SP (hyperplastic polyp, HP; sessile serrated polyp, SSP; and traditional serrated adenoma, TSA) were identified via retrospective review of the pathology database over 6 months in 2014. Each patient's complete pathology record was screened for SPS WHO criteria. Clinicopathologic characteristics of patients meeting criteria were evaluated.

Results: We identified 18 patients (11 female, mean age 65 years) who met SPS WHO criteria. A mean of 3.1 colonoscopies was needed to meet criteria; 3 met criteria at the 1st colonoscopy. 9 patients met criterion 1 (Group A), 6 met criterion 3 (Group B), 3 met both and none met criterion 2. The mean number and size of SP differed significantly between Group A and B (9.6 and 24.5; p=0.00 and 0.77 cm and 0.41 cm; p=0.00, respectively). In addition, the anatomic distribution and histologic SP types varied significantly between group A and B, with 48% and 11% right sided (p=0.00) and 36% and 16% SSP (p=0.00), respectively. Conventional adenoma was seen in 89% Group A and 67% Group B patients. 3 patients had SSP with advanced neoplasia (AN), including 1 with high grade dysplasia and 2 with CRC (1 CRC was negative for MSI by PCR).

Conclusions: We identified 18 patients meeting SPS criteria among 1114 patients with at least one SP (1.6% incidence). SP histology, size and anatomic distribution varied between patients meeting criterion 1 or 3, confirming others' findings of 2 SPS phenotypes and validating WHO criteria for identifying both. We did not identify patients meeting criterion 2, a limitation of our study design, in part due to lack of awareness of SPS among clinicians who do not obtain complete family histories. 17% of SPS patients had SSP-AN, confirming an increased risk of carcinoma in patients with SPS. Most patients met SPS criteria after 2 or more colonoscopies. Until a confirmatory test becomes available, we recommend pathologists routinely reviewing prior pathology and medical records of patients with a diagnosis of any SP to screen for SPS.

598 Role of Foregut Transcription Factor Sox2 in the Pathogenesis of Barrett's Esophagus (BE)

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Background: BE is defined by columnar metaplasia of the esophagus with goblet cells and *Cdx2* is believed to play an important role in its pathogenesis. Anecdotal evidence suggests that BE develops in a background of cardia type mucosa but the role of foregut transcription factors in the pathogenesis of BE has not been evaluated systematically. The aim of our study was to analyze *Sox2* expression, a key transcription factor of foregut differentiation, in esophageal biopsies from patients with columnar metaplasia of the distal esophagus without goblet cells (COLME), short segment BE (SSBE) and long segment BE (LSBE).

Design: Inclusion criteria were endoscopic and histologic evidence of columnar mucosa in the distal esophagus with or without goblet cells. Endoscopy findings were reviewed in COLME patients to ensure endoscopic evidence of columnar mucosa in the distal esophagus. Immunohistochemical staining for Sox2, Cdx2 and p63 was performed in all cases and scored separately in COLME (n=23), SSBE (n=25), LSBE (n=24), and in foci of multilayered epithelium (ME; n=19), when present. Three sets of biopsies (proximal, mid, distal) from all LSBE patients were evaluated for any gradient in Sox2 or Cdx2 staining.

Results: The mean age of the study group was 59.8 yrs (range 21-83 yrs) and the M:F ratio was 1.4. Sox2 was strongly expressed in columnar metaplastic epithelium without goblet cells and in foci of ME (100%; See Table). p63 was positive only in the basal layers of ME, but Sox2 was present in both p63 positive basal and p63 negative superficial columnar cells. There was a significant reduction in Sox2 expression in BE compared to COLME (p=0.0001) but similar prevalence was noted in short and long segment BE (52% vs. 33%, p=0.39). No gradient of Sox2 or Cdx2 expression was seen in proximal, mid, and distal biopsies from LSBE patients.

Cdx2 was completely absent in COLME (0), showed a few positive nuclei in ME, and a significant progressive increase from SSBE to LSBE (36% vs. 75%, p=0.02). Finally, p63 was expressed only in basal layers of ME, but was completely absent in columnar epithelium with and without goblet cells.

Conclusions: Our findings implicate foregut transcription factor Sox2 in the initial stages of squamous to columnar transformation and in the pathogenesis of BE.

	COLME (n=23)	ME (n=19)	SSBE (n=25)	LSBE (n=24)
Sox2	23/23	19/19	23/25 (strong in 13/25;52%)	22/24 (strong in 9/24; 33%)
Cdx2	0/23	3/19 (rare cells)	22/25 (strong in 9/25;36%)	24/24 (strong in 17/24;75%)
p63	0/23	15/19 (squamous only)	0/25	0/24

599 Utility of CD123 for Diagnosing Low Grade Graft-Versus-Host Disease in the Stomach

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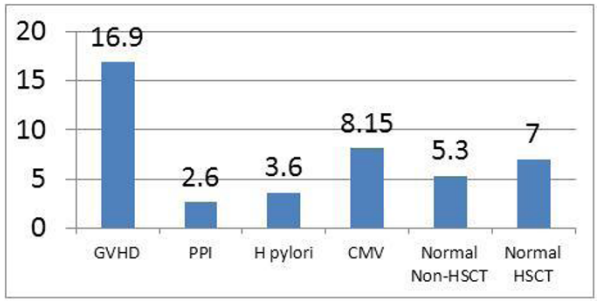
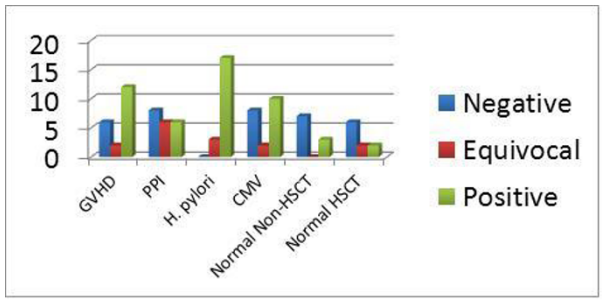
Background: Graft-versus-host disease (GVHD) is one of the most common complications following hematopoietic stem cell transplantation (HSCT), and the gastrointestinal tract is a major target for disease. The histologic hallmark of GVHD is the presence of epithelial apoptotic bodies; however, this finding is non-specific, as it can be seen with infection, inflammatory conditions, and drug-related injury. No reliable markers exist to differentiate GVHD from other histologic mimics. The aim of this study was to examine the utility of CD123 for diagnosing low-grade GVHD in gastric biopsies.

Design: A 10-year retrospective review of patients diagnosed with GVHD at our institution was performed. Gastric antral and oxyntic biopsies were reviewed from 6 patient groups (n=20 each): (1) histologically normal stomach, (2) *H. pylori* positive, (3) cytomegalovirus (CMV) positive, (4) on proton-pump inhibitor (PPI) therapy, (5) HSCT patients with Grade 1 or 2 GVHD on biopsy, and (6) HSCT patients with histologically normal stomach. Baseline apoptotic counts and CD123 immunohistochemistry (IHC) were performed on all groups.

Results: The mean apoptotic count for the GVHD versus non-GVHD patients was 16.9 versus 4.9 (p < 0.0001) (Figure 1). CD123 had a sensitivity and specificity of 60% and 53%, respectively, for detecting low-grade GVHD in the stomach (Figure 2).





Conclusions: While expression of CD123 is not limited to patients with GVHD, and can be seen most notably with *H. pylori* infection, it may be a useful adjunct in conjunction with mean apoptotic counts for diagnosing low-grade GVHD in the stomach.

600 Somatostatin Receptor 2A Expression in Liver Metastases From Small Intestine Neuroendocrine Tumors

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Background: Somatostatin receptors (SSTRs) are G-protein-coupled receptors that inhibit adenylate cyclase and cAMP production upon ligand binding. Five subtypes, including SSTR1, SSTR2A, SSTR3, SSTR4, and SSTR5, have been characterized, all of which have been detected in gastroenteropancreatic neuroendocrine neoplasms. SSTR2A shows high affinity for somatostatin analogs (SSAs). SSAs are the standard diagnostic and treatment tools in small intestine neuroendocrine tumors (NETs) expressing SSTR2A. While most well-differentiated NETs strongly express SSTR2A, high-grade neuroendocrine carcinomas frequently display reduced SSTR2A expression. In this study, we would like to compare SSTR2A expression in primary small intestine NETs and their liver metastases and to explore possible correlation between SSTR2A expression and Ki67 proliferative rate in the liver metastases.

Design: There were 20 patients (9 males and 11 females) with ³2 liver metastasis, of which 9 had a primary tumor available for immunohistochemical studies. A representative formalin-fixed paraffin-embedded tumor section from liver tumors and available primary tumors was used for immunohistochemical labeling for SSTR2A. SSTR2A expression in liver tumors was divided into two groups based on labeling intensity (score 0-3) and percentage of expressing tumor cells: group 1 with negative/weak (intensity X percentage <100) and group 2 with moderate/strong (100-300) expression. Ki67 proliferative rate was available for all lesions included in the study.

Results: We had 20 patients with 129 total liver tumors. 10 of 20 (50%) patients had liver tumors in both groups, whereas 3 (15%) had only group 1 liver tumors and 7 only group 2 liver tumors. Intratumoral heterogeneity of SSTR2A expression was also observed. 9 primary tumors showed moderate/strong SSTR2A expression in 6 cases and negative/weak expression in 3 patients. These primary tumors with moderate/strong SSTR2A expression gave rise to both groups of liver metastases, whereas liver metastases from those with negative/weak expression were mainly group 1 tumors. The

SSTR2A expressions did not correlate with Ki67 proliferative index (r=-0.07). 11 of 18 (61%) liver lesions with a Ki67 proliferative index >20% and 35 of 51 (69%) with a Ki67 rate £2% had moderate/strong SSTR2 expression (p>0.05).
Conclusions: Liver metastases from small intestine NETs have heterogeneous SSTR2A expression. SSTR2A expression status of primary tumors does not predict expression in liver metastases. There is no significant correlation between SSTR2A expression and Ki67 proliferative index.

601 Longitudinal Monitoring of ALOX15 Expression in Pediatric Patients Undergoing Treatment for Eosinophilic Esophagitis and Its Correlation With STAT6 Expression

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Background: It has been previously shown that ALOX15 immunohistochemistry (IHC) correlates well with the number of eosinophils per high power field (eos/HPF) in patients with eosinophilic esophagitis (EoE) and is positive in 95% of initial biopsies. STAT6 is a transcription factor that binds to the promoter region of the ALOX15 gene and can be blocked by omeprazole (PPI). Monitoring ALOX15 expression during therapy and its correlation with STAT6 expression might provide insight into their pathogenic role and/or potential to respond to therapy.
Design: The study included 212 biopsies from 31 pediatric EoE patients with positive ALOX15 IHC in the initial biopsy. Moderate and strong cytoplasmic staining of ALXO15 and suprabasal nuclear staining of STAT6 in more than 10% of squamous cells was considered positive. The IHC results were correlated with histologic and endoscopic findings, and resolution of symptoms.
Results: Each patient had a minimum of 1 followup biopsy after initial diagnosis with an average of 3 biopsies per patient (range: 1-8). 26/31 patients were treated with steroids and PPI, 3 patients were treated with steroids and H2 blocker and 2 patients with PPI only. Both patients treated with PPI only failed to respond. The results are summarized in Table 1.

	Responded to therapy (n=16)	Failed therapy (n=15)
ALOX15	0/16 (0%)	15/15 (100%)
STAT6	0/16 (0%)	8/15 (53%)
Average number of EOS/HPF PROXIMAL/DISTAL	4/4	64/105

Every biopsy with £15 eos/HPF was ALOX15 negative and every biopsy with ³15 eos/HPF was ALOX15 positive. The average time to symptomatic and histologic resolution was 9 months and the average followup in patients who failed therapy was 19 months.
Conclusions: ALOX15 and STAT6 expression correlate well with response/failure to therapy. Positive IHC for ALOX15 and its transcription factor STAT6 in a subset of patients who fail therapy suggests that PPI not always blocks STAT6 in the clinical environment. While detection of ALOX15 and STAT6 by IHC on esophageal biopsies does not provide additional diagnostic information to the histologic findings alone, its close association with disease status makes it a potential candidate for disease monitoring by non-invasive mechanisms and/or for targets of molecular therapy.

602 Semiquantitative Analysis of Histologic Spectrum of Intestinal Fibrostenosis in Crohn's Disease By Using a Novel Scoring Scheme

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Background: The simplistically and ambiguously termed 'fibrostenosis' of bowel is a hallmark of severe Crohn's disease and a major contributor to medical treatment failure. Noninvasive assessment for and novel therapy targeting at this phenotype are under investigation, which requires a better understanding and categorization of the underlying histologic basis.
Design: In 26 patients (M 15, F 11, 22-66 yo) of stricturing Crohn's ileitis or colitis that required surgical resection, a representative transverse section of the fibrostenotic bowel from each case was reviewed for a detailed analysis. For each layer of bowel wall [mucosa including muscularis mucosae (MU), submucosa (SM), muscularis propria (MP), subserosal adventitia (SS)], various histologic abnormalities were evaluated individually, including active and chronic inflammation, fibrosis, smooth muscle proliferation and hypertrophy, neural hypertrophy, adipocyte proliferation. A semiquantitative grading scheme was also proposed to determine the weight (score 0 to 3) of each histopathologic element.
Results: Segmental fibrostenosis involved ileum in 17 and colon in 9 cases, 3-30 cm in length, 2.0-5.8 cm in circumference, and 0.4-2.0 cm in wall thickness. The most significant histologic changes are chronic inflammation in MU/SM/SS, and muscular hypertrophy in MP. Muscular proliferation/hypertrophy is more prevailing than fibrosis in all layers (MP > MU > SM), with muscularization of SM. Fibrosis is most prominent in SM, followed by SS, but only minimal in MU and negligible in MP. Neural hypertrophy is common in MP and SM. Adipocyte proliferation is also present in SM and MP but less prominent. Overall, the weight of histopathologic elements is in the order of chronic inflammation > muscle hyperplasia > active inflammation > fibrosis > neural hypertrophy. The volume expansion is most significant in muscular layer (MP > SM > SS > MU).
Conclusions: In Crohn's 'fibrostenosis', the most prominent histologic change secondary to inflammation is smooth muscle hyperplasia. Fibrosis is less significant. The predominant volume-expanding compartment is also muscular layer. The '*inflammation-smooth muscle hyperplasia axis*' may be more important than fibrosis in the development of Crohn's stricture and in the consideration of novel therapeutic target. Our scoring scheme would be a useful tool for studying the prevalence of different histopathologic components.

603 Improving HER2 Assessment in Gastroesophageal Junction (GEJ) and Gastric Adenocarcinoma (GADC): Lessons From Concurrent Immunohistochemistry (IHC) and Fluorescence In Situ Hybridization (FISH) – Study of 117 Specimens

Zongming Chen, Hong Yin, Jinhong Li, Shaobo Zhu, Fan Lin. Geisinger Medical Center, Danville, PA.
Background: Since the ToGA trial demonstrated a survival benefit with trastuzumab therapy in GEJ or GADC patients with HER2 overexpression, assessment of HER2 status in these tumors has been routinely performed. Over 100 cases were tested, and useful lessons were learned to further improve test accuracy.
Design: A total of 117 invasive GEJ and GADC specimens were tested for HER2 status by both IHC (4B5, Ventena) and FISH (Dako). Results were interpreted according to the modified criteria by Hofmann et al (*Histopathology*. 2008;52(7):797-805). Data from the two methods were compared (Table). Potential causes for discordance were analyzed in detail.
Results: HER2-positive rates were 21% and 23% by IHC and FISH, respectively. Four of 69 IHC-negative cases were FISH positive. Upon retrospective review, consensus opinion reclassified 2 cases from 1+ to 2+. Thus, a false-negative rate was 2% (2/117). Three of 24 IHC 3+ cases were FISH negative, but a true false-positive error was a case of a signet ring cell carcinoma demonstrating reproducible cytoplasmic positivity mimicking a membranous staining pattern. The other 2 included a case with an IHC-positive focus not present on the FISH slide and a case with polysomy 17; both were regarded as true-positive events. Thus, the false-positive rate was 0.8% (1/117). The results are summarized in the Table.

IHC Results	FISH		Concordance Rate (%)
	Amplified	Not amplified	
Negative (0 and 1+)	4 (1+ in 3; 0 in 1)	65	65/69 (94)
Equivocal (2+)	2	22	N/A
Positive (3+)	21	3	21/24 (88)

Conclusions: Based on the current criteria, a false-negative result is the more frequent error in HER2 assessment. Performing FISH in cases with a borderline 2+ IHC result may improve result accuracy. Although false-positive results are rare, signet ring cell carcinoma with aberrant HER2 expression in cytoplasm may represent a potential pitfall.

604 "Indefinite for Dysplasia" in Barrett"s Esophagus: Active Inflammation and Abnormal Flow Cytometric DNA Content Are Significant Predictors of Early Detection of Dysplasia or Adenocarcinoma

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Background: Dysplasia arising from Barrett's esophagus (BE) precedes esophageal adenocarcinoma (EAC). However, some cases are difficult to diagnose as dysplastic, especially in the setting of inflammation, ulceration, or technical issues; these may be designated "indefinite for dysplasia (IND)." At present, histologic identification of dysplasia is the best marker of an increased risk of malignancy, although flow cytometric analysis of DNA content has been shown to be valuable for detecting patients at high risk for developing EAC. Few reports in the literature have specifically evaluated the natural history or outcome of IND. In fact, neither the American College of Gastroenterology nor American Gastroenterological Association specifies guidelines for the management of IND.
Design: We analyzed a series of 96 IND patients initially diagnosed between 2005 and 2013 to determine the outcome of IND and to identify factors (including histologic features and flow cytometric data) to predict subsequent detection of dysplasia or EAC.
Results: Our data show that 25% of IND cases were found to have low-grade dysplasia (LGD), high-grade dysplasia (HGD), or EAC within 1 year, with 37% and 47% detected within 2 and 3 years, respectively. The 1-, 2- and 3-year detection rates of HGD or EAC were 10%, 13%, and 20%, respectively. Active (neutrophilic) inflammation in the area of IND and abnormal DNA flow cytometric results were significant risk factors for detection of dysplasia or EAC (hazard ratio (HR) = 3.4, p = 0.0005; and HR = 5.7, p = 0.003, respectively). When active inflammation and DNA flow cytometric results were considered together, the resulting HR for the combined markers was 18.8 ($p < 0.0001$). The sensitivity and specificity of the combined markers for predicting detection within 3 years was 100% (95% confidence interval (CI) = [91%, 100%]) and 60% (95% CI = [31%, 83%]), respectively, resulting in 100% negative predictive value (95% CI = [61%, 100%]) and 89% positive predictive value (95% CI = [76%, 96%]).
Conclusions: IND in BE is a diagnosis that may cause difficulty in planning further patient management. Our results show that in the context of IND, histology with the support of DNA flow cytometry can identify a subset of patients who may merit more frequent endoscopic surveillance for early detection of dysplasia or EAC.

605 Outcome of "Indefinite for Dysplasia" in Inflammatory Bowel Disease: Correlation With Flow Cytometry and Other Risk Factors of Colorectal Cancer

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Background: Dysplasia that develops in the background of inflammatory bowel disease (IBD) precedes colorectal cancer (CRC). The category of "indefinite for dysplasia (IND)" is used often in equivocal cases, but its clinical significance remains unclear. Additional biomarkers in the setting of IND are needed to better define risk of progression to high-grade dysplasia (HGD) or CRC, as well as to differentiate between non-dysplastic and truly dysplastic epithelium. Flow cytometric analysis of DNA content (aneuploidy) has shown some promise in stratifying patients into low or high risk for CRC, but there are few reports that specifically evaluate its potential in the setting of IND.

Design: We analyzed a series of 84 IBD cases with IND diagnosed between 2003 and 2013 to determine the outcome of IND (mean follow-up of 28 months). Among the 84 IND cases, 53 patients had concurrent flow cytometric analysis of tissue taken from the same endoscopic location. Electronic medical records and our pathology information system were further reviewed to correlate outcomes with the following factors: type of lesion identified (flat vs. polypoid), primary sclerosing cholangitis (PSC), active inflammation, and flow cytometric data.

Results: Thirteen percent of IND cases yielded low-grade dysplasia (LGD) after a mean follow-up of 28 months, whereas only 2% of IND cases developed advanced neoplasia (HGD or CRC). There was no statistical difference between ulcerative colitis and Crohn's disease groups ($p = 0.10$ from log-rank test). The risk of LGD, HGD, or CRC was not significantly associated with the type of lesion ($p = 0.94$), PSC ($p = 0.94$), or active inflammation ($p = 0.41$) in our cohort of IND patients. However, the finding of aneuploidy at baseline IND was predictive of LGD and HGD ($p = 0.037$). Patients with IND and aneuploidy had 1-year, 2-year, and 3-year progression rates to neoplasia of 12%, 17%, and 30%, respectively. In contrast, IND patients with normal baseline flow cytometric results had 1-year, 2-year, and 3-year progression rates to neoplasia of 0%, 5%, and 5%, respectively.

Conclusions: Flow cytometry may be a useful adjunct in determining patient surveillance and management in the setting of IBD with IND. IND patients with abnormal flow cytometry may warrant more intensive follow-up, but conversely, IND in the setting of normal flow cytometry may require less frequent surveillance colonoscopy.

606 MCM4 and MCM7, Significantly Correlated With Ki67 Expression and Prograssion in Esophageal Adenocarcinoma, and Precancerous Lesions

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Background: Minichromosome maintenance (MCM) proteins play important roles in DNA replication. The deregulation of these proteins has been shown to contribute to tumorigenesis and cell proliferation. The present study investigated the correlation between MCM4, MCM7, and a conventional proliferative marker, Ki67, expression levels in esophageal carcinoma and precancerous lesions to determine the predictive value of these markers for the progression of esophageal diseases.

Design: Esophageal tissue microarrays consisting of 82 squamous epithelium (SE), 60 columnar cell metaplasia (CM), 33 Barrett's esophagus (BE), 38 low-grade dysplasia (LGD), 14 high-grade dysplasia (HGD), 108 esophageal adenocarcinoma (EAC), and 24 esophageal squamous cell carcinoma (ESCC) were immunohistochemically stained. Nuclear staining of each marker was considered positive. The percentage (0-100%) positively stained cells were recorded. The association of markers with clinicopathological charateristics was analyzed. A *p*-value of <0.05 is considered significant.

Results: The mean percentage of expression for MCM4, MCM7 and Ki67 significantly increased from SE (5.5%, 7.3% and 5.9%, respectively), CM (11.2, 13.5% and 3.4%), BE (27.7%, 35.3% and 8.3%), LGD (42.6%, 52.2% and 12.9%), HGD (63.2%, 77.7% and 29.6%), to EAC (61.3%, 75.5% and 24.5%) and ESCC (74.1, 85.4% and 36.3%). The distributions of mean percentage of expression between the markers significantly correlated across each examined esophageal lesion. The percentage of Ki67 expression was found to be significantly associated lymph node metastasis.

Conclusions: Our study demonstrated both MCM4 and MCM7 expression correlated with Ki67 in esophageal lesions. MCM4 and MCM7 may serve as proliferative markers for evaluating esophageal precancerous progression and for differentiating diagnosis of various precancerous lesions in difficult pathologic cases.

607 Tumor Budding as a Prognostic Marker in Ampullary Adenocarcinoma

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Background: Individual outcomes from ampullary adenocarcinomas (AACs) are difficult to predict and can pose a significant challenge in management and prognosis. An established prognostic marker in colorectal carcinoma, tumor budding has been suggested as a useful predictor of outcome for AACs.

Design: 60 cases of resected AACs were identified from a cancer database in a tertiary centre. All tumor slides were assessed for budding. Budding was defined as the presence of ≥ 5 isolated single cancer cells or clusters composed of ≤ 5 cancer cells per 20X field (Ueno et al. [1]). Budding was classified as 'high' if there were ≥ 3 & 'low' if there were < 3 budding foci. Cases were re-evaluated by a second pathologist using a double-head microscope. Data were statistically analysed & correlated with clinicopathological features & patient outcome.

Results: See table.

Comparison of Low & High Budding Groups				
Characteristics		Low(n=22)	High(n=38)	p value
Age(y)	Mean(±sd)	63.5 (12.5)	66.9 (8.5)	nsa
Follow up(months)	Median(range)	15.5 (5-113)	13.0 (1-132)	nsb
		(%)	(%)	(X2)c
Gross subtype	Ductal	19	81	0.02
	NOSd	23.1	76.9	
	Duodenal	44.4	55.6	
	IAPNe assoc.	64.7	35.3	
Histologic subtype	Pancreaticobiliary	28.6	71.4	0.047
	Intestinal	54.5	45.5	
Differentiation	Well	72.7	27.3	0.006
	Moderate	36.4	63.6	
	Poor	12.5	87.5	
LVI	Yes	22.2	77.8	0.005
	No	58.3	41.7	
PNI	Yes	16	84	0.005
	No	51.4	48.6	
Nodes	N0	50	50	0.021
	N1	21.4	78.6	
Stage	1	83.3	16.7	0.016
	2	50	50	
	3	23.8	76.2	
	4	20	80	
Recurrence/Cancer death	2.8148(CI 0.9-8.8)			0.074f
a=Independent t-test; b=Mann Whitney U test; c=Chi-square test; d=Not otherwise specified; e=Intra-ampullary papillary tubular neoplasm; f=Odds ratio				

Conclusions: Although our cohort has a relatively short median follow up, budding was found to have significant association with other recognized predictors of poor prognosis including poor differentiation, node involvement, advanced stage, lymphovascular/perineural invasion (LVI/PNI) and showed significant trend for poor outcome. A correlation with sub-type was also identified. Therefore, in keeping with recent publications[2], tumor budding should be routinely assessed in AAC & may help stratify patients for adjuvant chemotherapy.

1)Ueno H. Histopath.2002;40:127-132. 2)Adsay NV. Am J S Path.2010;34:1417-24.

608 MicroRNA Expression Profiling Distinguishes LS From Sporadic Microsatellite Unstable Colorectal Carcinomas

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Background: Colorectal cancer (CRC) patients with Lynch syndrome (LS) are characterized by microsatellite instability (MSI) due to germline mutations in mismatch repair genes, while BRAF mutated MSI tumors are more likely to be sporadic (Sp), due to methylation and silencing of *MLH1*. MicroRNAs (miRs), noncoding RNAs, are a fairly new class of genes that play a regulatory role in expression of protein coding genes by inhibiting or degrading mRNA. They seem to be fundamental in many biological and pathological processes. We studied MSI CRC to decipher new tumorigenic pathways by comparing miR expression in CRC from patients with LS to Sp MSI CRC.

Design: 24 MSI CRC (loss of immunoexpression of MLH1 and PMS2) were selected from the tissue archives including 12 with confirmed LS and no BRAF mutation, and 12 with BRAF mutation and therefore presumed Sp methylation. Representative areas from tumors were marked and cored. RNA was analyzed using Nanostring technology. RT-PCR with Taqman probes was used for validation of deregulated miRs.

Results: NanoString analysis of the miR profile identified 10 deregulated genes in LS CRC compared to Sp MSI CRC. There were only 3 genes downregulated, whereas 7 were upregulated, and the level of deregulation was greater in downregulated vs. upregulated genes (2 to 3x less vs. 0.5 to 1.5x more). Among the downregulated genes, miR494 was noteworthy with 3.74 less expression in LS vs. Sp MSI CRC. Among the upregulated genes, miR222, was increased in LS 1.18x compared to Sp MSI CRC. RT-PCR confirmed the deregulation of 7 out of 10 miRs identified by NanoString including miR494 and miR222 as down and upregulated, respectively.

Conclusions: The comparative miR gene profiling of LS vs. Sp MSI CRC identified a surprisingly specific signature with 10 miRs deregulated, of which 7 were upregulated and 3 were downregulated. The most downregulated miR, miR494, has important cancer related targets such as BACH1. In addition, miR222 is a known oncogene that targets key proteins, including ETS1. BRAF related miR, miR9, was not identified as deregulated

as expected, which suggests an intricate complex of molecular interactions that regulate tumorigenesis in MSI CRC. The specific miR signature that LS CRC exhibit compared to the Sp MSI CRCs could be used for diagnostic, or more importantly, therapeutic purposes as both miR494 and miR222 are potential therapeutic target candidates. Additional studies are necessary to investigate these novel findings.

609 The Incidence of Epidermoid Metaplasia in 1048 Consecutive Esophageal Tissue Samples and 58 Cases of Esophageal Squamous Neoplasia

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Background: Esophageal epidermoid metaplasia is defined by a prominent granular layer with overyling hyperorthokeratosis, resembling the epidermis of skin. The typical endoscopic correlate is a white plaque. An association between epidermoid metaplasia and esophageal squamous neoplasms (dysplasia and squamous cell carcinoma) has been proposed in small studies. However, the strength of this association and the incidence of epidermoid metaplasia in patients with and without squamous neoplasms remains unclear. This study reviews a large number of archived esophageal biopsies and resections to test the hypothesis that epidermoid metaplasia is associated with squamous neoplasia and is a potential a precursor lesion.

Design: Slides from all archived esophageal biopsies and resections for any indication from a 15 month period in 2013-2014 at one institution were reviewed retrospectively for epidermoid metaplasia. All archived esophageal tissues from patients with a diagnosis of esophageal squamous cell carcinoma or dysplasia from 2005-2014 were reviewed.

Results: Review of 1048 consecutive esophageal biopsies and resections for any indication identified two cases (0.19%) of epidermoid metaplasia. The incidence of epidermoid metaplasia was significantly higher ($p<0.001$) in a series of 58 consecutive patients with squamous cell neoplasms (4 with dysplasia, 54 with squamous cell carcinoma), of which two (3.5%) had concurrent epidermoid metaplasia. One case was the only verrucous carcinoma in the series and the other was a relatively superficial (pT1) and predominantly exophytic well differentiated squamous cell carcinoma. No patients had epidermoid metaplasia in an esophageal biopsy prior to the diagnosis with squamous neoplasia. Review of two additional verrucous carcinomas from a separate institution revealed one additional case with associated epidermoid metaplasia.

Conclusions: The relatively increased incidence of epidermoid metaplasia observed in patients with squamous neoplasms provides additional support for the proposed association with squamous neoplasia. This association may be stronger with verrucous carcinomas, based on our limited sample of this rare tumor type. It remains unclear if epidermoid metaplasia is a precursor to verrucous carcinoma or other squamous neoplasms. However, epidermoid metaplasia is so rarely identified in esophageal biopsies that it seems justifiable to follow these patients with periodic endoscopies.

610 Detection of the Loss of Hes1 Expression Differentiate Sessile Serrated Adenoma/polyp From Hyperplastic Polyp

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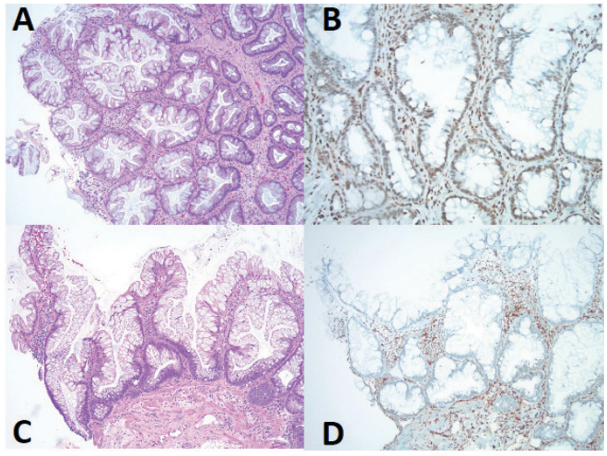
Background: The differential diagnosis between sessile serrated adenoma/polyp (SSA/p) and hyperplastic polyp is mainly based on the architectural criteria, and not uncommonly the classification of serrated polyps could be challenging. Hes1 is a transcriptional factor that is the downstream target of Notch signaling pathway controlling enteric cell differentiation. In this study, we would examine the expression pattern of Hes1 in serrated polyps and determine the role of Notch pathway in SSA/p development and whether the Hes1 could be used as a diagnostic marker for SSA/p.

Design: A consensus of 47 SSAs and 31 HPs polyps were selected in the study, which were independently confirmed by 2 GI pathologists. Among them, 31 SSAs and 9 HPs were located in right colon, while 16 SSAs and 22 HPs were located in left colon. Immunohistochemistry (IHC) of Hes-1 (Abcam, MA) using an automated immunostainer (Ventana) was performed.

Results: Hes1 staining is present in the nuclei of normal colonic mucosa throughout of the colon. Strong nuclear staining of Hes1 is present in >90% of glands of HPs in both right and left colon of 31/31 (100%) cases. Complete loss or very weak nuclear Hes1 expression is observed in dilated crypts of SSAs located in 13/16 (81%) cases in left colon and 30/31 (97%) cases in right colon. For the diagnosis of SSA/p, the overall sensitivity and specificity would be 91% and 100% respectively.

Conclusions: SSA/p and HP may have some morphological similarity, but they have different molecular pathogenesis pathway. Most SSA/ps have dysregulated Notch signal transduction pathway with the presentation of the loss of Hes1, while all HPs have normal Notch signaling pathway. The loss of Hes1 could be used as a sensitive and specific marker to differential SSA/p from hyperplastic polyp, which helps to make the correct diagnosis in morphological challenging cases.



A. HP, H&E; B. HP, Hes-1 IHC; C. SSA, H&E; D. SSA, Hes-1 IHC.

Group	Loss of Hes1	Hes1 Expression	Total (n=)
HP	0 (0%)	31 (100%)	31
SSA/p	43 (91%)	4 (9%)	47
Total	43	35	78

611 Quantitative Multiplexed Biomarker and Morphology Analysis To Aid Diagnosis of Dysplasia in Barrett's Esophagus

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Background: The histologic diagnosis of dysplasia in Barrett's esophagus (BE) is limited by intra- and inter-observer variability. Immunohistochemical detection of biomarkers such as Ki-67, p53 and AMACR has been used to aid diagnosis, however, interpretation of diagnostic markers by light microscopy is challenging. This study aimed to determine whether a quantitative, multiplexed biomarker-morphology imaging approach could objectively identify aberrations in biomarker expression and nuclear morphology in metaplastic cells that are correlated with grade of dysplasia.

Design: We evaluated TissueCypher, a platform for fluorescence whole slide digital image reading and multiplexed biomarker/morphology measurements, to quantify biomarkers and nuclear morphology in BE biopsies. BE cases with subspecialist confirmed diagnoses of no dysplasia (ND, n=132 patients), low grade dysplasia (LGD, n=28 patients) and high grade dysplasia (HGD, n= 20 patients) *(Fig1A-C)* were retrieved from four institutions. Formalin-fixed, paraffin-embedded BE biopsy sections were fluorescently immunolabeled for Ki-67 and CK-20 plus Hoechst labeling of nuclei. Whole slide fluorescence digital images of the biopsy sections *(Fig1D-F)* were analyzed by TissueCypher to measure biomarker and morphology features within the appropriate subcellular and tissue compartments.

Results: In the ND-LGD-HGD sequence there was an increasing proportion of CK-20+ cells proliferating (Ki-67+) (Fig1D-G). Ki-67+ CK-20+ cells showed higher Ki-67 intensity, larger nuclear area and equivalent diameter and loss of nuclear solidity in HGD and LGD versus ND (Fig1H-I).

Conclusions: A quantitative, multiplexed biomarker-morphology imaging approach detects significant differences between BE with ND, LGD and HGD and may provide an adjunctive tool to conventional pathological analysis for the objective assessment of BE.

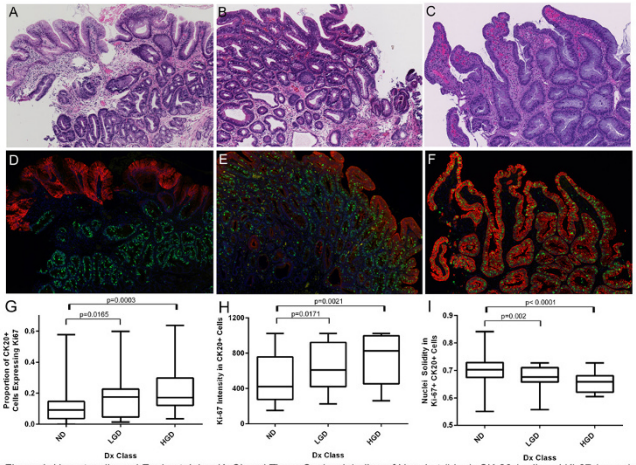

Figure 1. Hematoxylin and Eosin staining (A-C) and TissueCypher labeling of Hoechst (blue), CK-20 (red) and Ki-67 (green) (D-F) in sections of Barrett's biopsies with no dysplasia (ND), LGD, HGD, respectively. Box and whisker plots showing quantitative image analysis features in Barrett's biopsies with ND, LGD, HGD; G: Proportion of CK-20+ cells expressing Ki-67, H: Ki-67 intensity in CK-20+ cells and I: Nuclei solidity (indicator of morphology aberration) in CK-20+ Ki-67+ cells. P values shown on panels G-I are from two-tailed Mann-Whitney tests.

612 Possible Interplay Between Tumor Budding, Hypoxia-Related Markers Glut1 and Glut3 and Angiogenesis in the Tumor Microenvironment of Colorectal Cancer

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Background: In colorectal cancer (CRC), tumor buds are thought to represent tumor cells with a migratory phenotype, enabling dissemination via angioinvasion. Indeed, high-grade tumor budding has consistently been linked to vessel invasion. However, the mechanisms and interplay between buds and vessels are unclear. Hypoxia-related genes and angiogenesis have also been proposed to play a role in facilitating cancer progression. The aim of this study was to investigate the role of hypoxia-related markers Glut1 and Glut3 in CRC within the context of budding and vasculature in the tumor microenvironment (TME).

Design: 117 CRC cases were included in this study. To examine the TME, a next-generation tissue microarray was constructed. 2 punches each of tumor in areas with highest budding/lymph vessel index (selected on pancytokeratin/D240 double immunostain), areas of highest budding/blood vessel index (selected on pancytokeratin/caldesmon double immunostain) and tumor center were taken. Immunohistochemistry for Glut1 and Glut3 was performed and tumor budding using the '10 HPF' method was scored. Blood (BVD) and lymph vessel density (LVD) in the corresponding TME areas were assessed.

Results: Glut1 and Glut3 expression in buds was significantly increased compared to the tumor center and front ($p<0.001$). Glut1 expression in the tumor center predicted high-grade budding ($p=0.004$), in the tumor front was associated with higher grade ($p=0.027$) and in buds with higher TNM stage ($p=0.007$). Glut3 expression in the tumor center was associated with higher grade ($p=0.015$) and in the tumor front with higher grade ($p=0.034$), higher pT stage (p.0.032), higher TNM stage ($p=0.0449$) and venous invasion ($p=0.01$). There was a strong trend between Glut1 expression in the invasive front and higher BVD ($p=0.054$) and a trend between Glut1 expression in buds and higher LVD ($p=0.077$). Also, a higher number of buds was strongly correlated with increased BVD ($\rho=0.36$, $p<0.001$).

Conclusions: Glut1 and Glut3 expression in CRC, especially at the tumor front and in buds, is associated with adverse clinicopathological features, in line with previous studies. Additionally, tumor budding is strongly correlated with an increase in blood vessel density in the TME. Our results underline the complexity of interplay between budding, angiogenesis and vessel invasion. Future studies may involve a multi-marker panel focusing on all of these components.

613 The Role of the Tumor Suppressor PTEN in Colorectal Cancer Is Highly Dependent on the Tumor Area

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Background: Expression of the tumor suppressor gene PTEN is reportedly lost in 20-35% of colorectal cancers (CRCs). Loss has also been demonstrated in cancers harboring KRAS and PI3K mutations, yet the prognostic and predictive relevance of PTEN remains unclear. PTEN immunohistochemistry is included on the latest CAP biomarker checklist, although at present there is no standardized scoring method. Therefore, the aim of this study was to analyze expression of PTEN in different tumor areas which may influence future scoring systems.

Design: PTEN immunohistochemistry was performed on a mixed-stage cohort of 198 CRCs. Cytoplasmic and nuclear PTEN (CPTEN and NPTEN) expression was assessed (% positivity) across 10 high power fields on whole tissue slides in tumor center, front, peritumoral buds (PTBs), intratumoral buds (ITBs) and mucosa. Assessment of tumor budding using the '10 HPF' method, KRAS and BRAF mutational analysis and immunohistochemistry of mismatch repair (MMR) proteins was performed.

Results: CPTEN expression decreased stepwise from mucosa to tumor center and front (average positivity 65%, 34% and 28% respectively, $p<0.001$), with lowest expression in buds (average 4.61% in PTBs and 7.23% in ITBs ($p<0.001$)). Average NPTEN expression in mucosa was 6.86%, 0.15% in the main tumor body and never observed in buds. Lower CPTEN in the tumor center, front and in ITBs was associated with KRAS mutations ($p<0.001$ for all areas). Lower CPTEN in the tumor center was more frequent in MMR-deficient tumors ($p= 0.03$) and had a negative effect on survival, maintained after adjusting for pT, pN and pM stage on multivariate analysis ($p=0.032$, OR=1.44, 95%CI=0.492-0.968). Although low CPTEN in the tumor front was associated with more advanced pT and pN classification ($p= 0.008$ and $p =0.036$), no effect on survival was observed.

Conclusions: This appears to be the first study to perform geographic analysis of PTEN immunohistochemistry, revealing considerable differences among tumor compartments. Associations between low PTEN expression and KRAS mutations are in line with studies demonstrating co-occurrences of these events. More importantly, the prognostic significance of PTEN may be restricted to cytoplasmic expression in the tumor center. The potential role of PTEN as a prognostic and predictive biomarker calls for accurate and standardized scoring systems reflecting the heterogeneity of its expression.

614 Gastric Adenocarcinoma and Proximal Polyposis Syndrome (GAPPS): Not Just Fundic Gland Polyps

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Background: Gastric Adenocarcinoma and Proximal Polyposis of the Stomach (GAPPS) was reported in 2011 as a new autosomal dominant gastric polyposis syndrome. It is characterised by exclusive involvement of the gastric body and fundus by multiple polyps. The histopathology is reported as fundic gland polyposis +/- areas of dysplasia, occasional hyperplastic, adenomatous and mixed polyps and adenocarcinoma. This study aims at systematic description of lesions in a cohort of GAPPS family members.

Design: Gastric biopsies and gastrectomies of 15 patients belonging to a GAPPS family were collected. There were 26 biopsies (4 had multiple biopsies) and 2 gastrectomies. Slides were reviewed with further stains when appropriate. A literature search identified previously described microscopic features.

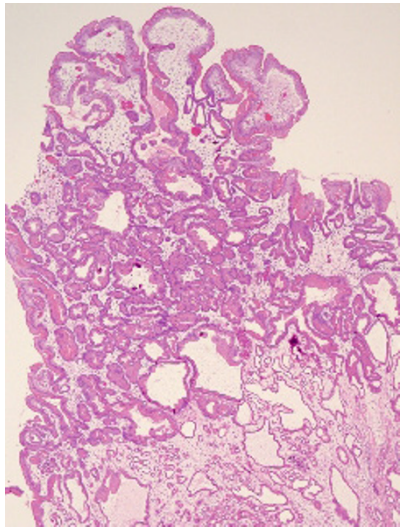
Results:

Table 1: Endoscopic and microscopic features of 15 patients with 26 biopsies and 2 gastrectomies.

	Biopsy patientsN=15			GastrectomyN=2*
Endoscopy	Normal	Polyposis	Mass	Polyposis
No. of patients	5	9	1	2
FGPs	0	6	0	2
Hyper-proliferative aberrant pits	0	8	0	2
Polypoid foveolar hyperplasia	0	1	0	0
Dysplasia- Intestinal - Gastric - Hybrid	NA	3^021	1^001	2^020
Adenocarcinoma	NA	0	1	0
Helicbacter gastritis	2	1	0	0

* Both gastrectomy patients had a previous biopsy
^The dysplasia was in 6 different patients

Polyposis of the gastric body and fundus were seen in 9 of 15 patients.
Six patients had dysplasia (gastric or hybrid phenotype) identified in the setting of gastric foveolar adenoma x2, hyper-proliferative aberrant pits x2 and polypoid foveolar hyperplasia x1, adenocarcinoma x1.



Active chronic gastritis with *Helicobacter* (HP) was seen in one patient with polyposis.

Conclusions: The spectrum of gastric pathology associated with GAPPS is wider than previously reported and include newly identified hyper-proliferative aberrant pits, polypoid foveolar hyperplasia, predominantly gastric and/or hybrid dysplasia through to WHO tubular type adenocarcinoma.

615 Prevalence and Natural History of Non-Alcoholic Fatty Liver Disease (NAFLD) in Patients Presenting With Autoimmune Hepatitis (AIH)

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Background: Given the increasing burden of NAFLD in the general population, a similar rise might be expected in AIH patients. We sought to determine the prevalence of NAFLD in patients presenting with AIH and to document the incidence of fatal and non-fatal outcomes in these patients as compared to patients presenting with AIH only.
Design: We identified all AIH cases at our institution in the last 35 yrs. with documentation of age, gender, comorbidities(DM, hyperlipidemia, obesity, HTN), alcohol abuse, lab data(auto-Abs,Igs,HBV/HCV serologies) and outcome data. All cases of concurrent AIH and NAFLD were identified, and NAFLD cases were further subdivided into simple steatosis and NASH. Patients were placed into one of three cohorts (AIH only, AIH & simple steatosis, AIH & NASH). The following outcome and clinical data among the three cohorts were analyzed: incidence of death (all cause), incidence of death (liver-related), incidence of liver-related adverse outcomes and prevalence of cirrhosis at the time of index biopsy. Adverse outcome was defined as the histologic confirmation of cirrhosis or HCC, or the clinical development of portal hypertension, ascites or hepatic encephalopathy.
Results: Of 123 database patients, 73 (59%) met criteria for AIH (age 11-78;mean 50; 62% female). Of these, 15% had simple steatosis and 16% had NASH. Presence of NASH was significantly associated with DM (p=0.027), hyperlipidemia (p=0.008) and HTN (p=0.037). Presence of simple steatosis was significantly associated with obesity (p=0.020) and HTN (p=0.008). 50% of NASH patients had cirrhosis at time of index biopsy as compared to 18% of AIH only patients (p=0.032). Patients with AIH & NASH had a relative risk of 7.65 (95% CI: 1.43-40.83) for incidence of liver-related death and a relative risk of 2.55 (95% CI: 0.92-7.09) for incidence of liver-related adverse outcomes, as compared to the AIH only cohort. There was no significant difference in outcome measures when comparing AIH patients to patients with AIH and simple steatosis.
Conclusions: Patients with AIH who were exposed to metabolic damage in the form of NASH were more likely to present with cirrhosis and were more likely to develop adverse clinical outcome with decreased survival as compared to AIH patients who did not have NASH. These findings suggest that simultaneous exposure to these two chronic liver diseases confers a clinically significant increased level of risk which may warrant closer follow-up and surveillance.

616 Selective Staining of Gastric Biopsies for H. pylori Does Not Affect Detection Rates or Turnaround Time and Improves Cost Compared To Reflexive Staining

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Background: Recently, the Gastrointestinal Pathology Society recommended against reflexive ancillary stains to detect *H. Pylori* in gastric biopsies and instead encouraged selective staining of biopsies with appropriate inflammatory patterns. The current study evaluates the impact of this recommendation on *H. Pylori* detection rates, turnaround time (TAT), and cost savings when a laboratory changes from a policy of reflexive to selective staining.
Design: *H. pylori* detection rates, TAT, and cost of performing stains were evaluated in all cases in the year leading up to the policy change (all reflexive Geimsa stains) and in all cases diagnosed in the 8 months following the policy change. During the latter time frame, pathologists were instructed to order an ancillary stain if they diagnosed chronic active or chronic inactive gastritis and *H. Pylori* was not seen on H&E stains. As there are no universally accepted minimal criteria for chronic inactive gastritis, an arbitrary but low threshold of 5 near-touching mononuclear cells was determined sufficient to trigger an ancillary stain. Each pathologist interpreted this threshold on his/her own with no further direction from the group's gastrointestinal pathologist.
Results: 1,497 patients comprised the reflexive stain (RS) cohort of which 228 (15.2%) were *H. Pylori* positive. 996 patients comprised the selective stain (SS) cohort of which 155 (15.6%) were *H. Pylori* positive. Of the positive SS patients, 87 (56%) required H&E *only* for detection and 68 (44%) required ancillary stains (66 IHC, 2 Geimsa). In the SS cohort, ancillary stains were performed on 32% of total cases (317 IHC, 6 Geimsa) and the positive rate for all stained cases was 21% (68/323). There was no significant difference in *H. Pylori* detection rates between the RS and SS cohorts (OR=1.03, 95% CI=0.82-1.28, p=0.82). TATs were similarly equivalent with a mean of 68.30 hours for the RS cohort and 68.34 hours for the SS cohort (p=0.98), both of which included a resident preview day. We calculated an average laboratory cost savings of $12.66 per patient, which is projected to save our department nearly $19,000 in 2014.
Conclusions: Our results support a policy of selective staining for *H. Pylori* as opposed to reflexive staining and go on to show that laboratories that change their policy can expect to generate cost savings without compromising detection rates or TAT.

617 Lymphocytic Esophagitis: An Inflammatory Pattern Strongly Associated With Immune-Mediated Conditions

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Background: Lymphocytic esophagitis (LE) is a poorly understood inflammatory condition characterized by increased intraepithelial lymphocytes with minimal granulocytosis. It has been reported in association with gastroesophageal reflux disease (GERD), allergies, infections, achalasia, Crohn disease, and some medications, although its role in the pathogenesis of these disorders is not clear. The purpose of this study was to evaluate the clinicopathologic and immunophenotypic features of LE.
Design: We identified 30 esophageal biopsy cases diagnosed as LE or intraepithelial lymphocytosis and recorded relevant clinical information. Cases were assessed for the number, distribution, and location of intraepithelial inflammatory cells, basal zone hyperplasia, spongiosis, dyskeratosis, sloughing and infection. Ten cases were further evaluated with CD3, CD4, CD8, CD20, CD1a and S100 immunostains. We also performed CD1a and S100 stains on 20 controls (10 GERD cases and 10 normal biopsies).
Results: Most patients were older women (mean: 47 years, range: 12-85, M:F=1:2) who presented with GERD-like symptoms, including dysphagia (n=10), heartburn (n=5), or pain (n=4). Twenty-one (70%) patients had at least one immune-mediated disease, 16 of whom required immunosuppressive therapy. Eleven patients had Crohn disease and several others had asthma, connective tissue disorders, hypothyroidism, achalasia, psoriasis, eczema, Sjögren syndrome, celiac disease, ulcerative colitis and lymphocytic colitis. None of the patients carried a diagnosis of lichen planus. Characteristic histologic features included striking mucosal infiltration by CD8+ cytotoxic T cells (mean: 41/HPF, range: 10-81) with spongiosis (93%), basal zone hyperplasia (80%), and dyskeratosis (57%), but none of these features was specifically associated with any clinical condition. Langerhans cells were mostly concentrated in the basal zone and peripapillary epithelium, but were not increased in LE (mean: 20 range: 7-43) compared to GERD (mean: 14/HPF, range: 5-24) and normal controls (mean: 15/HPF, range: 9-25).
Conclusions: In addition to intraepithelial lymphocytes, LE often shows scattered dyskeratotic cells in the mid to upper epithelium, spongiosis, and expansion of the basal zone, reminiscent of lichen planus. This pattern of injury in adults often reflects underlying immune-mediated disorders, particularly Crohn disease, similar to reported associations among pediatric patients.

618 Utilization of the NIH Consensus Guidelines Improves the Diagnostic Accuracy of Gastrointestinal Graft Versus Host Disease: A Five-Year Retrospective Study

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Background: Graft versus host disease (GVHD) of the gastrointestinal tract is a common complication of hematopoietic stem cell transplant (HCT) associated with significant morbidity and mortality. Clinical impression, endoscopy, and histology are vital tools in diagnosing GVHD and excluding other diseases. In 2006, the NIH published consensus guidelines to aid in the histologic diagnosis of GVHD.
Design: A retrospective search from 1/1/05 to 1/1/11 of our EMR identified 1,024 adult patients (pts) who underwent HCT (397 allogeneic, 586 autologous, 35 both, and 6 syngeneic). Endoscopies with histology were available for 169 allogeneic pts, resulting in 251 endoscopic events. A chart review was performed. The clinical diagnosis at the time of biopsy was considered the gold standard. In 2010, the NIH guidelines were instigated at our institution; thus, the original pathology reports were interpreted into one of the NIH categories (not-, possible-, consistent with-, or unequivocal GVHD). Biopsies were jointly reviewed by 2 pathologists, blind to the clinical findings, and an NIH consensus diagnosis was independently made for each site (excluding esophagus).
Results: Of the 251 endoscopies, 211 had a clinical diagnosis of GVHD. A diagnosis of consistent with or unequivocal GVHD was made for 117 (47%) and 190 (76%) of the original and reread pathology diagnoses, respectively. Within the original pathology, there were 3 (1%) false positives (FP) and 97 (39%) false negatives versus 21 (8%) and 42 (17%) within the reread group, respectively. For the 21 FP rereads, pts were clinically diagnosed with infection (14), drug toxicity (4), GERD (2), dietary intolerances (2), and/or other (4).

Table 1. The original and reread pathology diagnoses and statistics

	Original pathology	Reread pathology
Not GVHD	84 (33%)	42 (17%)
Possible GVHD	50 (20%)	19 (8%)
Consistent with GVHD	76 (30%)	112 (45%)
Unequivocal GVHD	41 (16%)	78 (31%)
Sensitivity	54%	80%
Specificity	93%	48%
PPV	97%	89%
NPV	28%	31%

Conclusions: Utilization of the NIH consensus guidelines markedly increases histologic sensitivity. Additionally, it aids in creating uniformity and diagnostic clarity. Our decreased specificity is likely due to the lack of clinical information, laboratory results, and endoscopic impressions at the time of reread and a lower threshold for diagnosing GVHD. Correlation with these factors is critical in avoiding potential FPs.

619 Circulating Tumor Cells and Circulating Tumoral DNA in Diagnosis of Pancreatic Tumor

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Background: Pancreatic fine needle aspiration (FNA) is mandatory for diagnosis of pancreatic adenocarcinoma. Bleeding, intestinal perforation and spread of tumor cells may complicate pancreatic FNA. The aim of this work was to evaluate non-invasive diagnostic methods, circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA), in patients with pancreatic adenocarcinoma.

Design: CTC research by Screencell® technique was performed prior to pancreatic FNA in patients with CT scan suspected pancreatic tumor. Collected CTC were evaluated according to morphological criteria. Free circulating DNA was obtained by extraction from blood plasma: ctDNA was revealed by the presence of *KRAS* exon 2 mutation (previously identified in the matched tumor FNA sample) using digital PCR (Life Technologies ®).
Results: Sixty-three patients were enrolled in the study; 52 had histological proven pancreatic adenocarcinoma and 11 had benign pancreatic lesion. Thirty-one out of the 52 patients with pancreatic adenocarcinoma presented 5 to 350 CTC (sensitivity 59.6%). Two patients with benign pancreatic lesions disclosed circulating epithelial cells, with CTC criteria (specificity 81.8%). *KRAS* mutation was present in 71% (22/31) of pancreatic adenocarcinoma with available tissue for mutation screening. Free circulating DNA concentrations ranged between 34 and 213 ng/ml. ctDNA was detected in 4 of the 9 patients tested, with a copy number between 0.67 and 5.58% of free circulating DNA.
Conclusions: Screencell® is an easy and reliable technique and can highlight numerous CTC in patients with pancreatic adenocarcinoma. Two patients without pancreatic neoplasia presented circulating cells classified as tumor cells on morphological criteria. Although circulating epithelial cells can be detected in patients with benign pancreatic lesions (Rhim et al, Gastroenterology, 2014), the challenge for pathologists is to differentiate them from malignant CTC. Furthermore, digital PCR by Life technologies® is an easy and sensitive technique to detect ctDNA in patients with pancreatic adenocarcinoma. Together, these techniques could represent an attractive alternative for non-invasive diagnosis of pancreatic tumors.

620 CDX2 Negative Colorectal Carcinomas Are Associated With ARID1A Loss, Microsatellite Instability and Poor Prognosis

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Background: Loss of ARID1A expression occurs in a small subset of colorectal carcinoma (CRC), and is reported to be associated with mismatch repair deficiency, *BRAF*V600E mutation, and clinicopathologic features associated with somatic hypermethylation. Loss of CDX2 and/or CK20 expression has also been shown to correlate with *BRAF* mutant, MLH1 deficient, CIMP-high CRC, and the loss of CDX2 and CK20 has been reported as an independent poor prognostic factor among MSI-H CRC. The aim of this study was to determine the relationship between CDX2 and ARID1A expression and microsatellite instability and to determine the prognostic significance of CDX2 and ARID1A deficient CRC.
Design: 546 CRC resections with known microsatellite instability status were reviewed for a predetermined set of histologic features, including tumor size, location, histological type, pT and N stage, LVI and PNI. Patient demographic and survival data was available for all cases. A tissue microarray (TMA) was prepared with two cores per tumor and stained by immunohistochemistry for ARID1A, CDX-2, and p53. CDX2 staining was scored as deficient (complete loss), intermediate, or diffuse moderate/strong. ARID1A staining was scored as retained or lost, with loss defined as complete absence of nuclear staining in tumor cells with intact staining background stromal cells.
Results: The patient cohort included 319 men and 227 women (mean age: 63 years). 483 tumors were microsatellite stable, 22 MSI-low and 41 MSI-high. Loss of CDX2 expression was seen in 39/546 (7.1%) of cases (5% of MSS, 0% of MSI-L, and 17.1% of MSI-H) and correlated with large tumor size, location in the right colon, poor differentiation on histology, higher T stage, presence of PNI and LVI, presence of lymph node metastases, MSI-H status, and loss of ARID1A expression. Loss of CDX2 was associated with a poor prognosis, regardless of microsatellite status (68.8 months for CRC with complete loss of CDX2 vs. 89.2 and 96.9 months for cases with intermediate or diffusely retained CDX2 expression, respectively). Loss of ARID1A staining in CRC was inversely associated with p53 mutant staining pattern, and was not an independent predictor of prognosis.
Conclusions: CDX2 expression is lost in 7% of CRC, and correlates with MSI-H status and loss of ARID1A expression. While MSI-H colorectal carcinomas are typically associated with a better prognosis, the loss of the tumor suppressor CDX-2 defines a subset with poor prognosis.

621 Risk of Inflammatory Bowel Disease (IBD)-Associated Colorectal Neoplasia in IBD-Primary Sclerosing Cholangitis (PSC) Appears Greater in Patients Diagnosed With IBD Before PSC

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Background: Patients with inflammatory bowel disease (IBD) are at increased risk of developing colorectal cancer (CRC). Risk is modulated by colitis extent, inflammatory activity, presence of dysplasia, and family history of CRC. According to the 2010 American Gastroenterological Association (AGA) medical position statement on IBD, patients should undergo colonoscopy at 8 years from disease onset to define the extent of disease. Patients with extensive disease (involving ³1/3 of the colon) are entered into surveillance within 1-2 years, with colonoscopy q 1-3 years. Patients with IBD and primary sclerosing cholangitis (PSC) are considered at especially high CRC risk. The AGA recommends colonoscopy at the time of PSC diagnosis (dx), with patients found to have IBD entered into immediate, intensive (q 1-year) surveillance. A recent study has challenged this practice, suggesting that CRC risk in IBD-PSC patients is a function of which disease comes first, with only those patients presenting with IBD first at increased risk.
Design: We identified patients diagnosed with PSC from 1991-2013 based on a query of our anatomic pathology information system. The following clinical information was extracted from the electronic medical record: age at IBD dx, age at PSC dx, duration of IBD follow up, IBD type, presence of IBD-associated neoplasia [CRC, flat dysplasia, or dysplasia-associated lesion or mass (DALM)].
Results: We identified 67 PSC patients with available clinical follow up, 44 (66%) of whom had IBD (37 UC, 7 Crohn's). 28% of 25 patients diagnosed with IBD prior to PSC developed IBD-related neoplasia, including 4 cancers, while none of the patients diagnosed with PSC prior to or concurrently with IBD have developed neoplasia. Detailed clinicopathologic information is presented in the Table.

Disease presentation	Mean Age IBD Dx	Mean Age PSC Dx	Mean Colitis Follow Up (Years)	Neoplasia in UC	Neoplasia in Crohn's	Total Neoplasia
IBD prior to PSC (n=25)	25	41	29	6 of 22 (27%)	1 of 3 (33%)	7 of 25 (28%)
PSC prior to IBD (n=6)	38	30	13	0 of 4 (0%)	0 of 2 (0%)	0 of 6 (0%)
PSC and IBD concurrently (n=13)	22	22	12	0 of 11 (0%)	0 of 2 (0%)	0 of 13 (0%)
PSC only (n=23)	NA	46	NA	NA	NA	0 (0%)

Conclusions: Patients with IBD diagnosed prior to PSC are at increased risk of developing CRC. The risk in patients diagnosed with PSC prior to or concurrently with IBD appears much less so. Although this may, to some extent, represent a function of more limited colitis follow up in this group (12 vs 29 years), it challenges the paradigm of placing all patient with PSC and IBD into immediate, intensive colonoscopic surveillance.

1. 622 Intensity of NFκB Expression Correlates With the Degree of Dysplasia in Barrett's Esophagus

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Background: The activation of nuclear factor kappa B (NFκB) transcription factor family is considered to have a key role in the tumorigenesis of many tumors. Few studies showed that in Barrett's esophagus (BE), NFκB is a major inflammatory pathway associated with cancer progression at the molecular level. However, there is limited data concerning the use of immunohistochemistry (IHC) to assess NFκB expression in BE. In this study, we used IHC to assess NFκB expression and correlate with the degree of dysplasia.
Design: A total of 43 cases of Barrett's esophagus, 9 cases of esophageal adenocarcinoma, and 4 control tissues from normal gastroesophageal junction mucosa were included in this study. IHC for 2 NFκB molecules, RELA and NFκB -1, were performed in all cases. The expression of both NFκB molecules in each case was graded as negative, weakly positive, or strongly positive. Statistical correlation was calculated using t-test and analysis of variance (ANOVA).
Results: In the 43 cases of Barrett's esophagus: 17 cases showed no dysplasia, 16 cases showed low grade dysplasia, and 10 cases showed high grade dysplasia. In cases of BE with no dysplasia: RELA was expressed weakly in 35% of the cases (n=6) and NFκB-1 was expressed weakly in 65% of the cases (n=11). In BE with low-grade dysplasia, RELA was weakly expressed in 50% of the cases (n=8), and NFκB-1 was weakly expressed in 75% of the cases (n=12). None of the BE with no dysplasia or BE with low-grade dysplasia showed strong expression of either molecule. Cases of BE with high-grade dysplasia showed strong expression of RELA in 80% (n=8) and strong expression of NFκB-1 in 100% of cases (n=10). Cases of adenocarcinoma showed strong expression of both RELA and NFκB-1 in 100% of cases (n=9). None of the control cases showed any expression of either RELA or NFκB-1. Strong expression of either NFκB molecule was strongly correlated to BE with high grade dysplasia and adenocarcinoma (p=0.0003).
Conclusions: IHC for NFκB, using antibodies against RELA and NFκB-1, is expressed in most cases of BE. Our study shows that the intensity of the staining correlates with the degree of dysplasia. NFκB is strongly expressed in cases of BE with high-grade dysplasia and adenocarcinoma; however, it is only weakly expressed or not expressed in cases of BE with low-grade dysplasia and BE with no dysplasia. Thus, IHC for NFκB may be useful in confirming cases of BE-HG and providing a better screening for early cancer risk.

623 Comprehensive Genomic Profiling (CGP) of 122 Appendiceal Tumors Identifies Genomic Alterations (GA) Associated With Neuroendocrine and Signet Ring Differentiation

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Background: Malignant vermiform appendix neoplasms account for <1% of intestinal malignancies and have no clear standard for management after initial resection. Higher grade and signet ring cells predict a worse prognosis in the mucinous adenocarcinoma (MA) subtype. In contrast, the rare goblet cell carcinoid (GCC) subtype, characterized by both glandular and neuroendocrine differentiation, tends to have a better prognosis. We compared the CGP of 122 Stage 4 primary appendiceal tumors to better understand the differing biologic behaviors of the subtypes and to provide rationale for systemic therapeutic options.
Design: DNA was extracted from 40 microns of FFPE sections from 122 appendiceal tumors. CGP was performed on hybridization-captured, adaptor ligation based libraries to a mean coverage depth of 681X for 3,769 exons of 236 cancer-related genes plus 47 introns from 19 genes frequently rearranged in cancer. Cases were categorized by a genomic alteration-blind histologic review into MA (n=71), MA with signet ring cells (MA-SC; n=34), and GCC (n=17). Patient samples were analyzed and reported for all classes of GA, which include base substitutions, small insertions/deletions, rearrangements, and copy number alterations.
Results: GCC and MA-SC occurred more commonly in women (71%F;76% F) at a slightly younger age (51.3y; 53.9y) compared to MA (47% F; 55.7y). 111 of 122 (91%) appendiceal tumors had at least one or more clinically relevant GA (CRGA). In MA without signet cells or neuroendocrine differentiation, *KRAS* was the most common

alteration (80%) regardless of grade. *GNAS* was associated with low grade MA (G1 71% vs. G3 24%), while *TP53*, *APC,* and *SMAD4* was associated with high grade MA. In contrast, *RAS* mutation was less common in MA-SC (38%) and rare in GCC (11.8%) despite comparable rates of *SMAD4* (17.6%; 17.6%) alteration. Additional low frequency potentially targetable CRGA included *ATM* (8.1%), *BRAF (2.5%), BRCA2 (1.6%), CCND1 (3.3%), ERBB2 (3.3%), FBXW7 (4.1%), IDH1 (2.5%), KDR (1.6%), MCL1(6.6%), NRAS (4.1%)*, and *PIK3CA (9%).*

Conclusions: Appendiceal cancer features a high frequency of CRGA across the different histologic subtypes. GCC and the MA-SC have a distinct genomic profile from poorly differentiated MA. Novel targets were identified at a high frequency in GCC and MA with and without signet ring cells providing rationale for CGP-directed therapeutic decision-making.

624 Annexin A10 Is Useful in Screening for Lynch Syndrome By Decreasing the Need for MLH1 Promoter Hypermethylation: A Consecutive Analysis of 575 Colorectal Adenocarcinomas

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Background: Universal screening of colorectal carcinomas (CRCs) for Lynch syndrome (LS) is becoming common practice. Once a CRC is found to demonstrate loss of MLH1 expression, the tumor is assessed for a *BRAF* V600E mutation. If the tumor is *BRAF* wild-type (WT), *MLH1* promoter methylation is performed, but this test is time consuming and only performed at a few centers. We recently demonstrated that immunohistochemistry for annexin A10 (ANXA10), a marker of a sessile serrated polyp, can be useful in distinguishing sporadic from LS-associated microsatellite-high (MSI-H) CRC. Recent studies have also demonstrated that MSI-H colorectal adenocarcinomas are associated with loss of expression of the tumor suppressor *ARID1A*. In this study, we investigated the utility of ANXA10 and ARID1A immunohistochemistry in identifying sporadic MSI-H CRC in a consecutive series of cases.

Design: 575 consecutive CRC cases were screened for LS at our institution from July 2013 to June 2014. Seventy (70) cases demonstrated microsatellite instability (MSI-H). Sixty (60) of these cases demonstrated loss of MLH1. Immunohistochemical staining for ANXA10 and ARID1A were performed on 53/60 (88%) cases. The cases were evaluated for any ANXA10 nuclear expression and complete loss of ARID1A nuclear expression. These results were compared with *BRAF* V600E mutation and *MLH1* promoter hypermethylation analysis.

Results: *BRAF* mutations were present in 37/53 (70%) CRCs with loss of MLH1. ANXA10 was expressed in 16/37 (43%) *BRAF* mut CRCs. The remaining 16 *BRAF* WT CRCs were all subjected to *MLH1* promoter methylation analysis. Three (6%) were not methylated at the *MLH1* promoter consistent with LS. These 3 CRCs were negative for ANXA10. Six of 13 (46%) *BRAF* WT, *MLH1* hypermethylated CRCs expressed ANXA10. Loss of ARID1A expression was identified in only 3 cases of *BRAF* mut CRC.

CRC with MLH1 loss (n=53)	n(%)
BRAF mutated	37(70)
ANXA10 positive	22 (42)
BRAF mutated and/or ANXA10 positive	43 (81)
ANXA10 positive , BRAF WT, MLH1 promoter hypermethylation	6 (11)
ANXA10 negative , BRAF WT, MLH1 promoter hypermethylation	7 (13)
ANXA10 negative, BRAF WT, no MLH1 promoter hypermethylation	3 (6)

Conclusions: While *BRAF* mutational analysis is extremely useful in identifying sporadic tumors, a substantial number of sporadic CRCs are *BRAF* WT and require testing for *MLH1* promoter hypermethylation. ANXA10 immunohistochemistry can obviate the need for *MLH1* promoter hypermethylation analysis in ~40% of cases.

625 BRAF Mutations in Sessile Serrated Polyps Associated With Inflammatory Bowel Disease and Comparison With Sporadic Counterparts

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Background: Despite our understanding of the cancer risk of sporadic sessile serrated polyps (SSP), little is known about the clinical significance of SSP in patients with inflammatory bowel disease (IBD). Our preliminary findings presented at USCAP 2014 and anecdotal information from other groups suggest that sporadic and IBD-associated SSP may be similar. However, there is no published data in the English literature regarding their molecular profile and how it compares to their sporadic counterparts. In the sporadic setting, SSP frequently harbor *BRAF* mutations and methylation of CpG islands. Assessing if both lesions, sporadic and those associated to IIBD, follow the same or different molecular pathway will further help understanding the biology of these lesions.

Design: We identified a group of serrated polyps arising in patients with IBD that includes: sessile serrated polyps without dysplasia (SSP), and serrated polyps with low grade cytologic dysplasia (SSP-D). A control group of sporadic SSP was included. Demographic and clinical information was obtained from review of medical records. H&E stained slides were reviewed to confirm the diagnosis. *BRAF* mutation analysis was performed in both groups.

Results: The study group included 30 serrated polyps from 22 patients with diagnosis of IBD (27 SSP and 3 SSP-D). The control group included 20 SSP from 20 patients without diagnosis of IBD (sporadic).Summarized data is shown in Table 1.

	Sporadic SSP (control group)	IBD-associated SSP (study group)
Number of polyps	20 (20 pts)	30 (22 pts)
Age (years)	41-77 (mean: 62)	34-77 (mean: 49)
Female:male	12: 8	12: 10
Diagnosis	20 SSP	27 SSP, 3 SSP-D
Polyp location	Right:13 Transverse:3 Left:4	Right:19Transverse:3Left:4 Rectum:3Colon (not specified): 1
Polyp size (cm)	0.6-2.6 (mean: 1.16)	0.2 -3 (mean: 1.25)
BRAF mutation positive	14/20 (70%)	26/30 (86%)

In both groups a slight predominance in females was noted and most lesions occurred in the right colon. Even though a slightly higher *BRAF* mutation rate was noted in the IBD associated SSP/SSP-D in comparison to the sporadic SSP, this was not statistically significant (p=0.17).

Conclusions: IBD-associated SSPs have a high frequency of *BRAF* mutation, similar to its sporadic counterpart. Further molecular evaluation, including CpG methylation could potentially help understand the molecular pathways implicated in the development of these lesions and further support the application of management guidelines currently used in sporadic SSP.

626 Calretinin and Limited Histologic Sectioning in the Pre-Operative Diagnosis of Hirschsprung Disease (HD)

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Background: The gold standard for the pre-operative diagnosis of HD is confirmation of absence of ganglion cells in distal rectal submucosal plexuses, with many pathologists preferring to exhaust the tissue block in this evaluation. Recently, HD has been associated with loss of calretinin-positive intrinsic nerves in the rectal lamina propria and muscularis mucosae. Since 2010, we have added calretinin immunohistochemistry to five hematoxylin and eosin (H&E) stained slides with about five sections on each slide in the initial work-up of possible HD cases.

Design: Cases from January 2010 to August 2014 after implementation of the new protocol were reviewed; 212 rectal suction biopsies from 95 patients (age range 2 days-18 years) were identified. We compared the histologic findings from the initial five H&E levels and calretinin immunostain to final histologic and clinical diagnosis.

Results: Of biopsies with adequate submucosa, 52 biopsies were from patients with HD and 145 biopsies were not. Of the HD patients, the five initial levels + calretinin i.e., lack of ganglion cells + no calretinin nerve twigs, correctly classified HD in 51/52 biopsies; one (1) biopsy lacked ganglion cells, but demonstrated equivocal calretinin staining. Two (2) challenging biopsies from a HD patient showed equivocal ganglion cells and absent hypertrophic nerves, but intrinsic nerves were negative for calretinin. Of the non-HD patients, the five initial levels + calretinin correctly excluded HD in 145/145 biopsies; 3/145 biopsies lacked ganglion cells, but demonstrated calretinin-positive intrinsic nerves. Of biopsies lacking adequate submucosa, two (2) had HD on follow-up and 13 did not. In this group, the five initial levels + calretinin correctly classified HD in 2/2 biopsies and excluded HD in 11/13 biopsies.

Conclusions: Our results show that the calretinin immunochemistry in addition to five initial H&E levels correctly classified 196/197 (99.5%) of biopsies with adequate submucosa and 13/15 (86.7%) of biopsies lacking adequate submucosa. This protocol is quite effective in rapidly triaging possible HD cases, and the initial findings warrant a discussion with the patient's treating physician prior to examining hundreds of additional H&E levels.

627 How Effective Is Preoperative Assessment Prior To Sleeve Gastrectomy Surgery?

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Background: Few studies have observed the stomach histopathologies of patients undergoing bariatric gastrectomy, which could create guidelines to identify the optimal pre-operative screening tool for patients undergoing bariatric gastrectomy - in fact, in a cost-conscious environment pathologic evaluation of these specimens may be regarded by some as unnecessary. Currently, different centers employ different practices, including upper GI contrast studies and upper GI endoscopies. Our current study seeks to determine the histopathologic abnormalities identified in specimens from bariatric gastric surgery and the role of gastric biopsies prior to bariatric gastrectomy.

Design: This study analyzed 204 partial gastrectomies performed for weight reduction performed between 2010 and 2014 and determined what pathologic findings were present. Pathologic diagnoses were grouped into abnormal with potential clinical implication, abnormal without clinical implication and no significant abnormality. The prior endoscopic and biopsy results, when available, were examined for the patients with significant pathologic findings to determine if this information was known prior to surgery.

Results: Nine patients (4.5% of the total cases) were found to have either a leiomyoma, intestinal metaplasia, neuroendocrine tumor, autoimmune atrophic gastritis, or Helicobacter pylori (HP) gastritis were considered to have potential clinical implications.

Pathologic Findings	No. of Cases	Percentage
Abnormal Findings with potential Clinical Implication	9	4.5
Histologic Changes without Clinical Implication	70	35.2
No Significant Abnormality	125	61.3

These patients were females between the ages of 31 and 63 (84.3% of the total population were female); 6/9 had no significant findings on endoscopy prior to surgery, two patients had no records of pre-operative endoscopy, while one demonstrated erythema on endosopy leading to biopsy prior to surgery. This biopsy had mild chronic active gastritis which was negative for HP by immunohistochemistry with the subsequent specimen showing HP associated chronic gastritis.

Conclusions: We found that 4.5% of bariatric gastrectomies had histopathologic findings which may have clinical implications, ranging from HP gastritis to neuroendocrine tumor. In only one case was a pre-operative biopsy obtained. Our findings indicate that bariatric gastric specimens can show abnormalities with potential clinical implications and these findings may not be identified with clinical workup prior to surgery.

628 Identification of a Novel Single Nucleotide Polymorphism in the Region of the CSFR-1 Gene By Next-Generation Sequencing in Medullary and MSI-Associated Colorectal Carcinomas

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Background: While they commonly present at more advanced stages, both medullary colorectal adenocarcinomas and the closely related microsatellite instability (MSI) associated adenocarcinomas have a much better prognosis. In the present study we performed next-generation sequencing (NGS) on a set of medullary and MSI associated colorectal carcinomas to identify any common single nucleotide polymorphisms (SNP).

Design: The Powerpath database at our institution was queried to identify all colorectal adenocarcinomas diagnosed between 2006 and 2014. Pathology reports were reviewed and special immunostains were run to identify poorly differentiated colorectal adenocarcinomas with medullary features and MSI-high colorectal adenocarcinomas. These tumors were compared against MSI-negative non-medullary adenocarcinomas found through the Powerpath database and used as a control. Formalin fixed paraffin embedded tissue from these cases was sequenced using the Ion AmpliSeq Cancer Hotspot panel V2 (Life Technologies) in the Ion Torrent sequencer. Results were reviewed using Variant Caller (Life Technologies) and the Integrative Genomics Viewer application (Broad Institute). Statistical analysis was performed using Microsoft Excel and STATA 11.0.

Results: A total of 20 cases were sequenced and analyzed. Total read counts ranged from 109,532 to 883,399 with a mean read length of 107 bp. There was a coverage depth of 98% aligned bases. A set of two contiguous SNPs consisting of a substitution of G for T followed by A for G on in the 3' UTR of Exon 22 in the CSF1R gene was consistently identified in all medullary and MSI-high colorectal adenocarcinomas, while it was only present in one third of non-MSI, non-medullary colorectal adenocarcinomas.

Conclusions: CSF1R codes for the colony stimulating factor receptor, which regulates the function and differentiation of macrophages. Animal and human models have suggested a link between the CSF1R gene, macrophage stimulation, and immune-associated antitumor effect in colorectal cancer. With this in mind, a consistently identified SNP in the CSF1R gene may be of clinical significance and is a possible genetic basis for this phenomenon. Further studies are warranted to determine the effects of this variant in gene expression.

629 Differentiated Anal Intraepithelial Neoplasia: An Under-Recognized Variant That Can Be Reliably Distinguished By P16/P53 Expression

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Background: Most anal intraepithelial neoplasia (AIN) is HPV-related, progressing through well characterized stages of conventional, Bowenoid-type dysplasia. However, a subset of AIN, known as differentiated (simplex) dysplasia, is often challenging to diagnose, marked by atypical basal cells with surface maturation. Differentiated dysplasia is widely recognized in the female genital tract, but has not been well studied in the anus. The goal of this study was to better characterize the histologic and immunophenotypic features differentiated AIN.

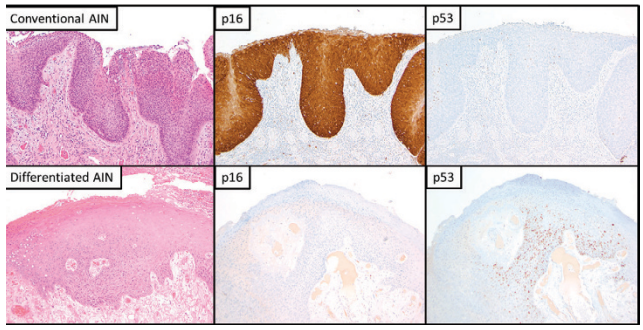
Design: 27 cases of high-grade AIN from 27 individual patients were evaluated by three gastrointestinal pathologists. 18 cases were conventional AIN and 9 were differentiated AIN. Formalin-fixed, paraffin-embedded tissue sections were stained with antibodies for p16 and p53. p16 positivity was defined as diffuse block staining. p53 positivity was defined as increased basal staining. Follow-up information was available for 24 patients with mean follow-up time of 3 years.

Results: 8 of 9 (88.9%) cases of differentiated AIN were p53 positive and p16 negative (p53+/p16-). In contrast, all 18 cases of conventional AIN were p53 negative and p16 positive.

Table 1: p16 and p53 Expression in AIN

	P16+/P53-	P16-/P53+
Differentiated AIN (n=9)	11.1%	88.9%
Conventional AIN (n=18)	100%	0%
P value	<0.05	<0.05

Histologically, differentiated AIN features atypical cells in the basal layer with surface maturation or abrupt keratinization. Acanthosis, spongiosis and parakeratosis are common. High-grade, conventional AIN is characterized by the absence of maturation with atypical cells in an expanded basal zone occupying the majority of the epithelial thickness. Review of the clinical data found no statistically significant differences between these AIN subtypes in parameters such as age, gender or clinical outcome.



Conclusions: p16/p53 expression patterns reliably distinguish between differentiated and conventional AIN. The p16 results support assertions that differentiated dysplasia in the anus, as in the female genital tract, is not HPV-related. Further study is needed to characterize the clinical and pathologic features of differentiated AIN.

630 Comprehensive Genomic Profiling of Advanced Stage Colorectal Carcinoma By Next Generation Sequencing Reveals Frequent Alterations in Histone Modifying Genes MLL3 and MLL2

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Background: Histone modifying enzymes affect gene expression and DNA repair, and are thought to function as tumor suppressors. Genomic alterations in histone modifying genes have been implicated in the tumorigenesis of various cancers but their profile in colorectal cancer (CRC) is less well-characterized. We analyzed genomic alterations in histone modifying genes by next generation sequencing (NGS) of clinical CRC samples.

Design: Hybridization capture of 3,769 exons from 236 cancer-related genes and 47 introns of 19 genes commonly rearranged in cancer was applied to ≥ 50ng of DNA extracted from 25 FFPE samples of advanced stage, treatment resistant CRC and sequenced to high, uniform coverage by Foundation Medicine (Cambridge, MA). Genomic alterations (base substitutions, small indels, rearrangements, copy number alterations) were determined and then reported for these patient samples. *MLL3* mutations were analyzed in an additional cohort of 56 CRC patients, including 29 microsatellite unstable (MSI) tumors by a multiplex mass spectrometry Sequenom assay developed in our laboratory.

Results: A total of 33 genomic alterations were identified by NGS in 6 histone modifying genes *MLL3, MLL2, ATR, PRKDC, CREBBP, and NSD1* in a cohort of 25 advanced stage CRCs. The most frequent genomic alterations were detected in histone-H3 modifying genes *MLL3* (36% of cases), *MLL2* (24%), and *NSD1* (4%). In 3 cases (12%) both *MLL2* and *MLL3* were mutated. Another set of genes that modify histone-H2A, namely *ATR* and *PRKDC*, were mutated in 3 cases each (12%). *CREBBP*, a gene that encodes histone H3 lysine acetyltransferase, displayed a splice site variant in one case (4%). Mutations in more than one gene (up to 4) per case were identified in 4 cases. Multiple mutations (up to 4) in individual genes were identified in 6 cases. Two MSI cases (8%) had recurrent c.8390delA frameshift mutations in the *MLL3* gene. Further multiplex mass spectrometry analysis of this frameshift mutation performed on an additional cohort of 56 CRC patients revealed mutations in 13 patients (23%). These *MLL3* frameshift mutations were strongly associated with MSI tumors, (P<0.0003).

Conclusions: In this study genomic alterations in histone modifying genes, particularly *MLL2* and *MLL3*, were identified in up to 36% of advanced stage colorectal adenocarcinomas. Frameshift mutation c.8390delA in *MLL3* was particularly associated with MSI tumors. Alterations in the tumor suppressor activity of these genes may play a role in colorectal carcinogenesis.

631 Prospective BRAF V600E Mutation Testing of dMMR Colorectal Cancer: Detailed Correlation With Patholological and Clinical Features

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Background: Lynch syndrome (HNPCC) accounts for 1-3% of all Colorectal (CRC) cases. They can be identified by deficient mismatch repair proteins (dMMR) status as detected by immunohistochemistry (IHC). The presence of a BRAF V6000E mutation in a dMMR patient is strongly associated with sporadic non hereditary CRC. Since 2012, we have performmed BRAF molecular testing on all newly diagnosed dMMR CRC cases.

Design: 266 dMMR cases (12%) with IHC loss of MMR proteins were identified from 2165 patients tested. BRAF molecular testing was performed on 80 cases that showed loss of MLH1 or PMS2. IHC with a BRAF V600E specific antibody was performed on a subset of cases (n=50). Full clinical and pathological data reported with a standard proforma was available for each case. Statistical analysis for Fisher's exact test p values and odds ratio was performed.

Results: 48/80 dMMR were BRAF V600E mutation positive (60%) and 32/80 dMMR cases were BRAF V600E mutation negative (40%). There was 100% concordance in BRAF status between molecular and IHC testing.

	% BRAF Positive (n=48)	% BRAF Negative (n=32)	p Value	OR
Age 70+	68.7	43.7	0.037	2.8
FHX CRC	22.2	57.7	0.007	0.2
Poor Differentation	53.3	28.1	0.036	2.92
Female	75	56.2	ns	2.33
Right Sided CRC	91.7	90.6	ns	1.13
Multiple CRCs	10.4	12.5	ns	0.81
Tumour Size over 7 cnm	35.5	37.5	ns	0.91
Tumour Perforation	15.5	15.6	ns	0.99
Stage 1 or 2	72.7	75.9	ns	0.84
Stage 3	27.3	20.7	ns	1.43
Stage 4	0	3.4	ns	0.21
Node = N0	73.3	77.5	ns	0.8
T4	44.4	38.7	ns	1.27
Lymphovascular Invasion	40	50	ns	0.67
Extramural Invasion	22.2	16.7	ns	1.43
Neural Invasion	8.7	9.7	ns	0.89
Over 50% Mucinous	8.9	15.6	ns	0.53
Infiltrative Margin	28.9	30	ns	0.94
Tumour Budding	20.9	14.3	ns	1.58

Conclusions: BRAF V600E negative dMMR colorectal cases were significantly associated with younger age, positive family history and well to moderate tumour differentiation. Tumour budding was present in a small percentage of both groups. Both BRAF positive and negative cases were associated with early stage (N0) disease. The introduction of BRAF testing in our institution has reduced by 60% the number of dMMR cases that require additional testing for Lynch syndrome. Hypermethylation status of MLH1 will further assist in the clinical characterisation of the BRAF negative group.

632 Immunohistochemical Subtyping of Gastric Adenocarcinoma

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Background: The Cancer Genome Atlas Research Network recently classified gastric adenocarcinoma into four subtypes via molecular analysis: Epstein-Barr virus-positive tumors; microsatellite-unstable tumors; genomically stable tumors; and tumors with chromosomal instability (CIN), of which 71% harbor *TP53* mutation. Immunohistochemical (IHC) analysis might be useful in similarly categorizing individual tumors, and Her2 expression might be related to subtype.

Design: We identified 71 cases of gastric or gastroesophageal adenocarcinoma in our files with available clinicopathologic patient data and tissue blocks. We performed IHC staining for MLH1 and P53 and EBER in-situ hybridization on sections from all cases. We then stratified them into four groups based on positive EBER expression, loss of MLH1 expression, overexpression or loss of p53 in the absence of the previous two findings, and unremarkable staining for all three markers. We then compared the groups by patient age, sex, follow-up, histologic pattern (intestinal or diffuse types), stage, and (when available) IHC and fluorescence in-situ hybridization (FISH) Her2 data.

Results: Cases were categorized as follows: Group 1 (EBER-positive), 6 cases (8%); Group 2 (MLH1-deficient), 15 cases (21%); Group 3 (aberrant p53 staining, EBER-negative, retained MLH1), 24 cases (34%); and Group 4 (unremarkable staining pattern), 26 cases (37%). This distribution was nearly identical to that found by the Research Network, after accounting for the *TP53* mutation rate in the CIN group. Average patient age was 68 years, and 72% of patients were male; these did not significantly differ by group. Average follow-up was 22.5 months (median, 9 months), with 66% of patients dying of disease or unknown cause; Group 1 averaged 55 months of follow-up (median, 67 months; p=0.008). Diffuse-type features were seen in 45% of cases; in Group 1, all six were intestinal type (p=0.03). The four groups appeared to be comparable in stage, though 62% of cases were biopsied but not resected, precluding pathologic staging. Her2 overexpression was most common in Group 4. No Group 2 cases overexpressed Her2 by IHC analysis or FISH. In Group 3, only 1/21 cases was IHC-positive, but 3/10 were FISH-positive.

Conclusions: Staining for EBER, MLH1, and P53 offers an efficient, reasonably accurate alternative for molecular subtyping of gastric adenocarcinoma, though not all cases with CIN can be identified. These findings have potential prognostic and therapeutic implications. Her2 expression is likely to be absent in microsatellite-unstable tumors and may be IHC-negative but FISH-positive in tumors with CIN.

633 Role of EBV Infection in Refractory Ulcerative Colitis (UC)

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Background: EBV infection has been related to Inflammatory Bowel Disease (IBD) but the potential relationship with disease course and severity is not clear.

Design: To analyze the role of EBV infection on disease course and severity three groups of patients were studied: Group I(n=27) diagnosed with UC that underwent colectomy because of refractory disease. In this group, all previous endoscopic biopsies were reviewed and investigated for EBV. All cases were also studied for CMV. Group II(n=14) patients with stable UC, and group III(n=19) refractory UC not needing surgical treatment. Clinical data including Mayo Score, treatment and evolution of disease were also recorded. EBV *in situ* hybridization (EBER) was performed in all surgical specimens. In positive cases, a complete EBV study (LMP1, EBNA2 and ZEBRA) was also performed.

Results: EBV was observed in 15(56%) patients undergoing colectomy in the ulcers associated to inflammatory infiltrate. The pattern of EBV-related infiltrates were: polymorphic in 8(53%), EBV+ mucocutaneous ulcer in 2(13%), plasma cell hyperplasia in 1(7%) and unspecified in 4(27%). EBV+ cells infiltrating the muscular layer were observed in 60% of the cases and EBV follicular colonization in 2 samples.

Most cases (86%) showed latency pattern I (EBER+, LMP1- and EBNA2-). Replication was observed in 5/15(33%). Five of 15(33%) EBV+ cases had concomitant CMV infection. All EBV+ UC had a clinically severe acute exacerbation (Mayo score 8-9) compared to EBV- UC (54%) p=0.005. In prior endoscopic biopsies EBV+ cells were already found in 10/12(83%). Regarding treatment, 3 EBV+ patients(23%) were receiving thiopurines at the moment of the colectomy vs 11(77%) of the EBV– group (p=0.007). No differences were found among patients treated with cyclosporine or antiTNF. We also study 23 samples of 14 patients with IBD who responded to treatment and no cases were positive for EBV or CMV. Among 19 cortico-refractory patients who do not underwent surgery, only 2(10.5%) cases were EBV positive whereas 10(75%) patients that need colectomy for the management of the disease were positive for EBV (p=0.0001).

Conclusions: The presence of EBV in patients with cortico-refractory UC is associated with a higher requirement of colectomy. The analysis of EBV in biopsies and surgical specimens of UC refractory patients may help to select high-risk patients, candidates to alternative therapy approaches.

634 Co-Expression of MUC5AC and TFF1 Distinguishes Sessile Serrated Adenomas/Polyps From Hyperplastic Polyps

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Background: Sessile serrated adenomas/polyps (SSA/Ps) have been extensively studied as possible precursor lesions to colorectal cancer. However, it is difficult to distinguish SSA/Ps from hyperplastic polyps (HPs) in many cases, especially smaller lesions that are not right sided.

In work previously published by members of our group, RNA sequencing of SSA/Ps from serrated polyposis syndrome patients was compared with uninvolved tissue adjacent to these polyps and normal biopsies from patients undergoing screening colonoscopy. MUC5AC and TFF1 were overexpressed in SSA/Ps, both of which encode proteins seen in gastric mucosa, but not typically in colon.

Design: 12 SSA/Ps and 5 HPs were confirmed by 2 gastrointestinal pathologists. Two samples of normal colon were also examined. Formalin-fixed, paraffin-embedded tissue sections were stained with DAPI and immunofluorescent antibodies for MUC5AC and TFF1. Expression was scored based on percentage of serrated crypt cells staining (0: none; 1: 1-25%; 2: 26-50%; 3: 51-75%; 4: >76%) and intensity of staining (0-4). Merged images were used to analyze co-expression.

Results: Normal colon samples had no expression of MUC5AC while one had mild TFF1 staining. HPs showed patchy, weak staining of MUC5AC and variable TFF1 expression. SSA/Ps exhibited strong, diffuse staining for both. The differences in average expression scores of SSPs and HPs for MUC5AC, TFF1 and MUC5AC/TFF1 co-expression were statistically significant (two-tailed t-test).

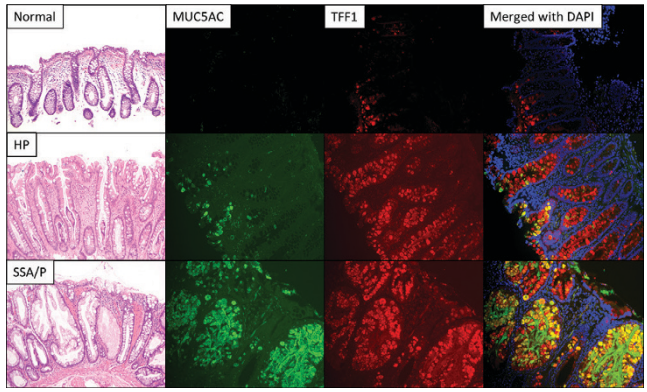

Table 1: MUC5AC, TFF1 and MUC5AC/TFF1 Co-expression in SSA/Ps and HPs

	MUC5AC	TFF1	MUC5AC/ TFF1 co-expression			
	Percentage	Intensity	Percentage	Intensity	Percentage	Intensity
SSA/Ps (n=12)	3.17	3.5	3.25	3.33	3.08	3.41
HPs (n=5)	1.4	1.6	1.8	2.0	0.8	1.0
P value	0.00688	0.00369	0.0363	0.0498	0.00129	0.000241

Conclusions: Strong and diffuse co-expression of MUC5AC and TFF1 is seen in SSA/Ps, consistent with previous RNA sequencing data. Further study is needed, but these findings may help distinguish SSA/Ps from HPs in diagnostic practice.

635 Basaloid Squamous Cell Carcinoma of the Anus Revisited

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Background: Basaloid squamous cell carcinoma (SCC) of the anus, previously called cloacogenic carcinoma, is a subtype of SCC with peculiar morphologic features. The WHO has recommended against subtyping of anal SCC because of diagnostic difficulty, lack of diagnostic reproducibility and morphologic heterogeneity within tumors. However there is a scarcity of data on the morphologic variation within basaloid SCC of the anus, the clinicopathologic features of basaloid SCC and the evaluation of novel therapeutic biomarkers including *FGFR1* and *FGFR3* which have been implicated in SCC at other sites.

Design: We retrieved cases of basaloid SCC from 1994 to 2013. The slides were reviewed for diagnostic confirmation and detailed histologic characterization. Patient demographics, tumor stage and clinical follow up were obtained. CK5/6, p40, p16, SOX2, KIT, DOG1 and FGFR1 immunohistochemistry were performed. Fluorescence in situ hybridization (FISH) for *FGFR3* rearrangement was done in a subset of cases.

Results: We identified 27 cases of basaloid SCC with a F:M of 4:1 and a median age of 60 (range 42-92). There were 19 resections, 6 biopsies and 2 liver metastases. Morphologically, basaloid SCC could be categorized into 4 groups: transitional cell carcinoma-like (n=10), basaloid with peripheral palisade (n=13), adenoid cystic carcinoma-like (n=3) and mucinous microcystic (n=1). In 19 cases, the histologic patterns were pure and in the remainder, a mixture of patterns was seen. CK5/6, p40 and p16 were positive in all cases. SOX2 was positive in 17/21 cases and DOG1 and KIT were negative in all cases. FGFR1 overexpression was absent in 6/6 and *FGFR3* was not rearranged in 4/4 cases. Most patients (62%) presented with T2 lesions and nodal metastases were present in 7/15. Clinical follow up was available on 60% of cases; 9 patients (53%) developed local recurrence or metastasis after a median 24 months and 5 (29%) died of disease after a median 30 months.

Conclusions: Basaloid SCC of the anus is characterized by 4 major histologic patterns; most often in pure form. Tumors may not express SOX2 but consistently express CK5/6 and p40. Basaloid SCC of the anus is p16-positive and unlike SCC from other sites is negative for FGFR1 overexpression and *FGFR3* rearrangement. Most patients present with localized disease but recurrence and metastasis are not uncommon.

636 Everything But the Kitchen SINK: Spectrum of Upper Gastrointestinal Submucosal Lesions and Diagnostic Pitfalls Using a Novel Biopsy Technique

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Background: Submucosal lesions of the gastrointestinal tract are difficult to biopsy and diagnosis based on clinical features and imaging may be inaccurate. Single-incision needle-knife (SINK) biopsy is a novel technique used to endoscopically sample submucosal lesions. The aim of this study was to review the diagnostic yield of this technique, accuracy in diagnosis and pitfalls and challenges encountered in diagnosis.

Design: 19 submucosal lesions biopsied using the SINK technique were identified; 13 stomach, 2 distal esophagus/gastroesophageal junction (GEJ), 2 duodenum and 1 mid esophagus. The clinical database was reviewed for lesion characteristics, suspected diagnosis, previous biopsy/FNA attempts and surgical resection, if applicable. H&E and immunohistochemistry slides were reviewed.

Results: Sufficient sampling for a specific pathologic diagnosis was obtained in 16 (84%) cases. Of the 10 lesions with prior non-diagnostic biopsies or FNA, a specific diagnosis could be made in 7 (70%) of cases. The pathologic diagnosis matched clinical suspicion in 10/19 (53%) cases, however 4 cases of suspected GISTs were found to be leiomyoma, glomus tumour, inflammatory fibroid polyp and schwannoma. A suspected leiomyoma at the GEJ was found to be a GIST. Non-diagnostic cases were due to inability to distinguish leiomyoma from normal smooth muscle, crushed pancreatic rest tissue misinterpreted as oxyntic mucosa and non-specific immunostaining of a spindle cell lesion. One GIST classified as grade 1 on biopsy was upgraded on resection.

Conclusions: SINK biopsy technique provides adequate tissue for immunohistochemistry and a specific histologic diagnosis in most cases. Diagnoses not suspected clinically may be made which may avoid unnecessary surgery, but it is important to recognize diagnostic pitfalls and limitations.

637 HER2 Expression in Dysplastic Lesions of the Gastroesophageal Junction and Stomach

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Background: HER2 amplification is seen in approximately 15-20% of adenocarcinomas (AC) arising from the gastroesophageal junction (GEJ) and stomach, more frequently in intestinal type AC which are presumed to arise from dysplastic lesions. In breast lesions, non-invasive ductal carcinoma in situ is often HER2 amplified while adjacent tumours may not show amplification. Few studies have investigated pre-invasive lesions of the gastrointestinal tract for HER2 amplification.

Design: 150 cases of in-house and consultation cases previously tested for HER2 amplification of AC were evaluated for the presence of dysplastic lesions. These were graded as low grade (LGD) or high grade (HGD) dysplasia. Immunohistochemistry (IHC) for HER2 (4B5, Ventana) was used to grade HER2 staining in these dysplastic areas; staining was scored as 0-3 according to gastric HER2 scoring criteria.

Results: Of the 150 cases reviewed, 29 (19.3%) cases had LGD (3 cases), HGD (15 cases) or a combination of grades (11 cases) present. 16 cases were from the stomach and 13 from the GEJ. 14 cases were from resections (surgical or endoscopic mucosal resections) and 15 were biopsies. For LGD cases, the majority did not show significant HER2 staining.[table 1] Several showed 2+/equivocal staining, in only one case associated with an amplified AC. Only one case of LGD showed 3+ staining, associated with both HGD and invasive AC with HER2 amplification. For HGD, while the majority were negative for HER2 staining, a greater proportion showed HER2 staining compared to LGD. Several cases with 2+/equivocal well as strong 3+ staining were seen, all associated with invasive AC with HER2 amplification.

Conclusions: Pre-malignant lesions of the GEJ/stomach do show HER2 amplification, which increases from LGD to HGD. When present, this most often correlates with the adjacent invasive carcinoma.

HER2 IHC Scoring	LGD (% of total)	HER2 result in associated AC	HGD (% of total)	HER2 result inassociated AC
0-1	10 (71.4%)	1 HER2 amplified AC 9 HER2 negative AC	12 (54.5%)	4 HER2 amplified AC 8 HER2 negative AC
2	3 (21.4%)	1 HER2 amplified AC 2 HER2 negative AC	4 (18.2%)	4 HER2 amplified AC
3	1 (7.2%)	1 HER2 amplified AC	6 (27.3%)	6 HER2 amplified AC
Total cases	14		22	

638 The Knowledge of Residual Mesorectal Metastatic Lymph Nodes and Tumor Deposits Distribution in Rectal Cancer After Radiochemotherapy Can Permit Changes in the Surgical Approaches? A Prospective Study in 116 Patients

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Background: In rectal cancer, radiochemotherapy (RCT) with total mesorectal excision (TME) reduces the local recurrence rate, partly due to the excision of mesorectal metastatic lymph nodes (N +) and/or mesorectal tumor deposits (MTD). But TME with low anastomosis alters patient's quality of life related to impaired sphincter function with a high risk of permanent stoma. A better understanding of mesorectal N + and /or MTD topographies related to the tumor location could reduce the extent of TME in order to improve patients quality of life, or even could allow a simple local excision (LE).

Design: A meticulous topographic analysis of mesorectal N + and MTD was performed prospectively in surgical specimens of patients with rectal cancer after RCT.

Results: 116 patients were operated for low (n = 69), medium (n = 46) and high (n = 1) cancer rectum. The stages were ypT0 /is (n = 22), T1 (n = 9), T2 (n = 33), T3 (n = 50) and T4 (n = 2). It was noted in the 1551 lymph nodes analyzed, 95 N + and 39 MTD in 44 (38%) patients. The N+/MTD were located in 41% (n = 55) in peritumoral, 58% (n = 78) above the proximal edge of the tumor.

In high/ middle rectum subgroup (n = 47), no N + was noted on the 95 lymph nodes located below the distal tumor edge. However, one MTD (2%) was observed 2cm away the distal tumor edge.

For stages ypT0-T2 subgroup (n = 64) wherein LE could be discussed, 12 patients (19%) had mesoretal N + and/or MTD located more than 1 cm above the proximal tumor edge associated to vascular pedicle involvement in 7 cases.

Conclusions: Our results suggest that LE in the ypT0-2 subgroup would generate a too high risk of local recurrence rate due to an incomplete tumor resection because of persistence of N + and/or MTD away from the proximal tumor edge in 19% of patients. On the other side, for the upper / middle cancer rectum after RCT, a distal reduction of the TME would be licit in 98% of patients. However, despite a very meticulous pathologic work-up and the obtainment of the same conclusions than Sprenger and al study realized in 81 patients, both studies are limited by their sample sized. Only, a multicenter study would allow general statement on distal metastatic spread of rectal cancer after RCT and possible consequences for surgical approaches.

639 Enumeration of Th1/Th2 Polarization of CD4+ T Cell Subsets in Colonic Mucosal Lamina Propria Helps To Distinguish Between Ulcerative Colitis and Crohn's Disease

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Background: Distinction between the two forms of IBD, ulcerative colitis (UC) and Crohn's disease (CD), is often challenging both clinically and histopathologically due to overlapping features. Aberrant mucosal adaptive immunity contributes to the

pathogenesis of IBD, and it is fundamentally different between UC (Th2-like) and CD (Th1 pathway). It is of clinical interest whether analysis of certain distinct $CD4^+$ (Th) cell subsets in bowel mucosa can discriminate between UC and CD.
Design: Fresh endoscopic biopsies of inflamed colonic mucosa were used to isolate lamina propria mononuclear cells. Multicolor flow cytometry was used to detect $CD4^+$ T cell subsets separated by key cytokines and master transcription factors (IFNγ/T-bet for Th1, IL13/Gata3 for Th2, IL17/RoRgt for Th17, FoxP3 for Treg). 20 CD and 20 UC patients, whose diagnosis was made based on current criteria and concurred by 3 IBD expert physicians, were enrolled into training cohort, and other 12 with established diagnosis and 10 newly diagnosed patients into test cohort. Using training cohort data and linear discriminant analysis, discriminant equation and scores were obtained and receiver-operating characteristic (ROC) curves were constructed by calculating the areas under curves (AUCs). The discriminant equation with the highest AUCs was then applied to test cohort.
Results: In training cohort, CD cases showed increased $IFN\gamma^+$ and $T\text{-}bet^+$ Th1 populations ($P<0.001$) and decreased $IL13^+$ and $Gata3^+$ Th2 populations as compared with UC cases ($P=0.026$ and 0.001). No difference in Th17 or Treg populations existed between the two groups. Among the four subsets, $IFN\gamma^+$ subset had the highest AUCs (95% CI) of 0.828. A combination of more than one subset showed further higher AUCs than any single one. The combo of all four subsets yielded the highest AUCs of 0.945, and its sensitivity, specificity and accuracy for the distinction were 80.0%, 95.0% and 90.0%, respectively, at a cutoff value of 0.5. In test cohort, the sensitivity, specificity and accuracy of the combo of four were 94.5%, 66.7% and 90.1%. Only 2/22 patients were classified in contrast to clinical diagnosis.
Conclusions: Flow cytometric analysis of $CD4^+$ T cell subsets in lamina propria, focusing on Th1/ Th2 polarization, may be a promising clinically useful test to distinguish between UC and CD in difficult cases.

640 Revisiting Colonic Polyposis in Resection Specimen: Increased Recognition of Serrated Polyposis Syndrome

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Background: The causes of colonic polyposis include a variety of well characterized inherited conditions, such as familial adenomatous polyposis syndrome (FAP), hamartomatous polyposis or MYH-associated polyposis (MAP). Recently, serrated polyposis syndrome (SPS) has emerged as a cause of colonic polyposis associated with increased risk of colorectal carcinoma (CRC). The aim of our study was to examine the prevalence and clinicopathologic features of SPS in resection specimens.
Design: A retrospective search of the pathology database (2004-2014) was performed to identify all patients with a diagnosis of polyposis who also underwent colonic resection. The parameters noted included: age, gender, resection type and surgical indication, pre-operative polyposis diagnosis if any, and polyp histology, size, number and distribution. Preoperative colonoscopies and pathology records were also reviewed. World Health Organization (WHO) 2010 criteria were utilized for establishing a diagnosis of SPS.
Results: We identified 22 patients (9 men, 13 women, average age of 56 years, range 19-86) who underwent colon resection and had a diagnosis of polyposis. The spectrum of polyposis syndromes included FAP (5/22), MAP (2/22), IBD (2/22) and 6/22 patients suspected of having FAP or MAP. 5/22 patients met WHO criteria for SPS. The remaining 2 patients had multiple serrated polyps and the possibility of SPS was raised, however, strict WHO criteria were not met (insufficient polyp size). Polyposis syndromes due to FAP, MAP, and IBD were all established pre-operatively, and most of these patients (8/9, 88%) underwent total colectomy. In the 5 SPS patients, 2 were diagnosed preoperatively. Polyp histology in SPS patients consisted of exclusively serrated polyps (SP) in 3 and a mixture of SP and conventional adenomas in the other 2. The number of serrated polyps ranged >20 to >100. Indications for resection in SPS patients included CRC (2), intramucosal carcinoma (1) and dysplasia arising in IBD (1). Total colectomy was performed in 3 SPS patients, including 1 colectomy specifically performed for SPS (without CRC). In 2 SPS patients harboring CRC, both showed MSI-H, BRAF V600E mutation phenotype.
Conclusions: SPS was seen in 23% of colectomies with polyposis. In most instances, SPS was detected after examination of specimens in which SPS was not suspected preoperatively. Our data show that SPS is an underrecognized entity in resection specimens. Recognition of SPS requires awareness of the syndrome and diligent gross pathological examination.

641 Traditional Serrated Adenoma Frequently Coexists With Other Polyps

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Background: Traditional serrated adenomas (TSA) are preneoplastic lesions leading to colorectal cancer. Typical examples are easily recognizable but heterogeneous morphologic features can pose a diagnostic challenge.
The purpose of this study is to explore the occurrence of an admixed polyp – hyperplastic (HP), sessile serrated adenoma (SSA), conventional adenoma (CAd) with TSAs and also identification of histologic variants in a large series of TSAs.
Design: 147 TSAs were retrieved from the archives of the Pathology departments at UHN, Canada and Oxford, UK over a 2-year period, reviewed and re-evaluated for the presence of other component(s) (HP, SSA and CAd) admixed with the TSA. The incidence of goblet cell-rich variant (GCR more than 50% of polyp containing goblet cells) was also sought.
Results: - 79/147 (%54) cases showed another coexistent polyp type:
- HP in 24/79 (30%), 23 in left (LT), 1 in right (RT) colon
- SSP in 12/79 (15%), 5 in RT, 7 in LT colon
- CAd in 33/79(41%), 27 of the 33 in LT colon
- > 2 polyp types in 10 (12%): 4 SSA/HP, 4 HP/Cad, 1 SSA/HP, 1 SSA/CAd
- 68/147 (46%) cases showed pure histologic features of TSA;
- 57/68 in LT, 11/68 in RT colon
- 7 /68 showed conventional dysplasia, 6 in LT, 1 in RT colon
- 23/147 (15%) were considered as GCR-TSA;
- 12/23 (52%) with an admixed component, 16/23 (70%) in LT colon
- 13/23 (56%) GCR-TSA showed no/very few ectopic crypt foci.
Conclusions: In our series;
- More than half of the TSAs showed another polyp type admixed
- In RT-sided TSAs, SSP component is more common and in LT sided TSAs, HP is the common coexisting polyp
- Conventional dysplasia is more common in LT-sided pure TSAs compared to RT sided ones
- GCR-TSAs contain less ECFs and are more common in LT side
-These "mixed" polyps suggest a morphologic spectrum with final progression to conventional dysplasia.

642 Tumor-Infiltrating Lymphocytes in Colorectal Adenocarcinomas: Tracking the Immune Response Through Different Tumor Stages

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Background: The presence of tumor-infiltrating lymphocytes (TIL) has been associated with a favorable prognosis for colorectal adenocarcinomas. However the exact contribution of each TIL subtype is still a matter of controversy. Even less is known about the relative TIL composition with regard to different stages of disease (pT1-pT4).
Design: B-cells (CD19+ and CD20+, resp.), T-cells (CD3+, CD4+, and CD8+, resp.), activated T-cells (CD25+), Treg (FoxP3+) and tissue macrophages (histiocytes, CD68+) were stained by immunohistochemistry and counted in 131 colorectal adenocarcinomas of different stages in three representative fields of view each. Only TIL along the invasive margin of the tumor were counted. TIL within the stroma or those organized in follicular structures were excluded. MSI status of each tumor was analyzed, using the MSI Analysis System (Promega).
Results: The immune response in colorectal tumors was dominated by T cells in all tumor stages. Specifically, CD4+ cells were more abundant than CD8+ cells throughout all TNM stages except for pT2. In pT2, CD8+ T cells were significantly more abundant in relation to all other tumor stages ($p<0,02$). The high CD8+ count in pT2 correlated with increased numbers of activated CD25+ T cells and histiocytes. 67% of all patients with pT4 tumors had a CD4/CD8 ratio of >2, significantly more than in all other stages ($p<0,005$). Throughout different tumor stages we observed a clear correlation of CD8+ T-cell counts with numbers of histiocytes, and even more pronounced a correlation of activated T lymphocytes and histiocyte numbers. Infiltrating B cells were significantly present only in pT1 ($p<0,05$). A final analysis will provide additional details on Treg counts and the MSI status in correlation with distinct tumor stages.
Conclusions: This work characterizes the immune response in colorectal cancer against the background of different tumor stages. The strongest CD8+ cytotoxic T cell response was found in pT2, pointing towards a functional immune response in early stage disease. As disease progresses to higher stages, the cytotoxic CD8+ T cell response retracts, correlating with a decreased number of activated lymphocytes and histiocytes, (and a simultaneous increase in the CD4/CD8 T cell ratio). Together, our data indicate a quantitative retraction and a qualitative loss of efficacy of the immune response in advanced tumor stages, potentially contributing to worse prognosis.

643 Serologic Testing and Duodenal Pathology in Diagnosis of Autoimmune Enteropathy in Adults

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Background: Autoimmnue enteropathy (AIE) is an uncommon condition causing protractible diarrhea in pediatric and adult patients (pts). The diagnosis (dx) is frequently delayed since clinical, endoscopic and histologic features frequently overlap with other entities.
Design: 69 patients with a clinical concern for AIE were identified. 43 were tested for anti-enterocyte antibodies (AEA): 11 positive (+), 8 equivocal (E) and 24 negative (-). Specimens from pts with + AEA but a clinical dx of other diseases were excluded. Total of 134 biopsies (33 + serology, 134 E and (-) serology) from 36 pts (20 female, 16 male, age 18 - 90 years old) were reviewed in a blinded fashion. In efforts to determine distinguishing characteristics of AIE, histologic findings including increase in eosinophils (> 20 per high power field), and serologic data were compared.
Results: Out of 8 AIE pts with + AEA, specimens from 7 pts were available. Pts with E serology had clinical dx of Gluten sensitive Enteropathy (GSE) (3), Crohn disease (CD) (1), drug induced colitis (1), microscopic colitis (MC) (2), irritable bowel syndrome (IBS) (1). IBS (7), CD (5) and MC (4) were the most common dxs in the AEA (-) group. Most striking pathology was noted within duodenum (11 +, 13 E, 22 (-)). Although more commonly seen in AIE specimens, increase in mixed lamina propria (LP) inflammation (91% vs 69%), villous blunting (81.8% vs 61.5%), and increase in intraepithelial lymphocytes (IEL) (63% vs 53.8%) were shared between AIE bxs and bxs with equivocal AEA. In contrast increase LP eosinophils (eos) (63% vs 16%), deep crypt lymphocytosis (54% vs 15%), and activity (63.6% vs 30.8%) were much more common in the AIE bxs. Notably, loss of Paneth and/or goblet cells and architectural distortion

were only present in the AIE bxs in comparison to serology equivocal specimens. Bxs with (-) serology also show many of the above reported features shared by serology + and equivocal cases but with less frequency.
Conclusions: Even though several histologic features were shared amongst AIE, GSE, MC, CD and IBS pts; deep crypt lymphocytosis, increase in LP eos and activity were much more commonly noted in AIE bxs. In addition loss of Paneth and/or goblet cells and architectural distortion were only seen in AIE. Even though the diagnosis remains challenging, these features might be helpful in diagnosing AIE in pts with equivocal serology.

644 BRAF Mutation Frequency Is Fewer in Asian Colorectal Carcinomas (CRCs) Patients and Immunohistochemical Detection of BRAF V600E Mutant Protein Using the VE1 Antibody in Colorectal Carcinoma Is Highly Concordant With Molecular Testing

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Background: The BRAF V600E mutation occurs in 15-17% of White patients colorectal carcinomas (CRCs) and has important genetic, prognostic, and therapeutic implications. A monoclonal antibody (VE1) targeting the BRAF V600E mutant protein has become available with variable efficacy in literature reports. The BRAF mutation in CRCs of Taiwanese was investigated by the immunohistochemical expression of BRAF V600E protein using the VE1 antibody and by PCR in order to assess BRAF V600E mutation incidence and also the sensitivity and specificity of VE1 immunohistochemistry.
Design: Four hundred and thirty-nine patients with CRC receiving colectomy at the Taipei Veterans General Hospital in 2012 were identified by a retrospective search of pathology records. All slides were reviewed by two pathologists and BRAF V600E mutation detection by PCR and immunohistochemistry using VE1 antibody are performed. The intensity of staining was scored as negative (0), weak (1+), moderate (2+), strong (3+). Associations between BRAF V600E mutation and patient's age, sex, tumor location and stage were also evaluated.
Results: BRAF V600E mutation was identified in 24 cases by PCR. All these cases showed positive BRAF V600E mutation demonstrated cytoplasmic positivity with the VE1 antibody with most tumors (22/24, 92%) demonstrating moderate to strong staining. Of the 415 BRAF V600E–negative cases, 408/415 CRCs (98%) were negative with the VE1 antibody while 7 CRCs (2%) demonstrated weak cytoplasmic staining. The sensitivity and specificity of VE1 were 100% and 98%, respectively. A right-side predominance in tumor location is noted (Right vs. Left, 11.7% vs. 2.9%, p=0.0002) in BRAF mutation while there is no significant difference in different tumor stages (I+II/III+IV, 3.9% vs. 7.1%, P=0.14) and patients' age groups (<=60 y/o vs. >60 y/o, 5.4% vs. 5.5%, p=0.97) and sex (Male/Female, 6.1% vs. 4.3%, p=0.39).
Conclusions: Our results indicate that BRAF mutation is more frequent in right-side CRCs but the overall frequency is fewer in Asian patients than White patients (5.5% vs. 15-17%) and VE1 immunohistochemistry is a useful surrogate for the detection of the BRAF V600E mutation in CRC, although weak staining must be evaluated by BRAF PCR analysis to exclude a false positive result.

645 Who Are You Calling Indeterminate? Classification of Colon Biopsies With 6 or Fewer Apoptotic Bodies Predicts Decision To Treat in the Evaluation of Gastrointestinal Graft Versus Host Disease

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Background: Gastrointestinal Graft Versus Host Disease (GI GHVD) is a complication of stem cell transplantation (SCT) with significant morbidity. Currently, crypt apoptosis is the chief histologic feature for diagnosis of mild GI GVHD. Given the broad differential diagnosis of rare apoptosis, a recent study proposed that biopsies with ≤6 apoptotic bodies per 10 contiguous crypts (1-6ap) be considered "indeterminate for GVHD" (iGVHD). Of note, 3 symptomatic patients with 1-6ap had improved without treatment. The aim of this study was to assess colon biopsies with 1-6ap to determine if diagnosis was predictive of the decision to treat and outcome.
Design: Colon biopsies taken to evaluate for GVHD from 2008-2014 were evaluated blindly by a GI pathologist for the maximum number of apoptotic bodies per 10 contiguous crypts. 129 biopsies from 89 patients were evaluated. Clinicopathologic information was collected from chart review. CMV positive cases were excluded.
Results: 16 patients (18%) were not treated for GVHD, and were diagnosed as follows: 1 with grade 1 GVHD(G1), 3 iGVHD, and 12 negative for GVHD (Neg). The mean apoptotic count was 1.2 (range 0-8). Five patients showed no apoptosis, one of whom was asymptomatic. One patient was asymptomatic with 2ap. The 10 remaining symptomatic patients with 1-6ap counts had resolution of symptoms without treatment. Compared to a diagnosis of G1, patients were 28 times less likely to be treated if diagnosed Neg (12/21, 57%), and 16 times less likely if diagnosed iGVHD (3/9, 33%), despite similar rates of 1-6ap (Table 1).

Table 1: Diagnosis, Management, and Apoptotic Counts

Diagnosis	Not Treated, N(%)	Symptoms Resolved, N(%)	Ap/10Crypt, mean(range)	1-6Ap/10Crypt, N(%)
Neg	12/21(57)	15/21(71)	1.1(0-4)	13/21(62)
iGVHD	3/9(33)	8/9(89)	2.5(1-8)	8/9(89)
G1	1/44(2)	34/44(77)	5.0(2-22)	38/44(86)
G2-4	0/28(0)	21/28(75)	9.3(2-27)	12/28(43)

Legend: Ap=apoptotic bodies; Neg=Negative for GVHD; iGVHD=indeterminate for GVHD; G1,2-4=Grades 1, 2-4 GVHD

Conclusions: In symptomatic SCT patients with biopsies showing 1-6ap, the diagnoses of Neg and iGVHD were predictive of a higher rate of conservative management and a similar rate of symptom resolution. Our findings support the diagnosis of iGVHD, and we wonder how many of our G1 patients with 1-6ap (38/44, 85%) might have been spared increased immunosuppression. Prospective studies of iGVHD biopsies might answer this question.

646 Overexpression of DNAJC12 Serves as a Poor Prognosticator in Patients With Rectal Cancers After Neoadjuvant Chemoradiotherapy

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Background: Neoadjuvant concurrent chemoradiotherapy (CCRT) followed by surgery has been regarded as a standard treatment for locally advanced rectal cancer. It is of great value to search for potential biomarkers in predicting tumor response and survival after neoadjuvant CCRT. Protein folding plays an important role in the functional conformation of proteins, and genes associated with protein folding have been found to have certain prognostic significance in a subset of cancers.
Design: Through data mining from a public transcriptomic dataset of rectal cancer (GSE35452) with the focus on genes associated with protein folding, *DNAJC12* (encoding a member of the HSP40/DnaJ protein family) was identified as the most significant gene correlated with the response to CCRT. We further evaluated the expression of DNAJC12 by immunohistochemistry in the pre-treatment tumor specimens from 172 patients with rectal cancers. The association of DNAJC12 expression with various clinicopathological factors, tumor regression grade, overall survival (OS), disease-free survival (DFS), and local recurrence-free survival (LRFS) was statistically analyzed.
Results: High expression of DNAJC12 was significantly associated with advanced pre-treatment tumor status (P<0.001), advanced pre-treatment nodal status (P<0.001), advanced post-treatment tumor status (P<0.001), advanced post-treatment nodal status (P<0.001), increased vascular invasion (P=0.015), increased perineural invasion (P=0.023), and lower tumor regression grade (P=0.009). More importantly, high expression of DNAJC12 was found to be a poor prognosticator for OS (P=0.0012), DFS (P<0.0001), and LRFS (P=0.0001). In multivariate analysis, DNAJC12 overexpression still emerged as an independent prognosticator for worse OS (hazard ratio [HR]=2.119; P=0.040), DFS (HR=3.878; P<0.001), and LRFS (HR=3.605; P=0.016).
Conclusions: DNAJC12 overexpression served as a negative predictive factor for the response to neoadjuvant CCRT and was significantly associated with shorter survival in patients with rectal cancers receiving neoadjuvant CCRT following surgery.

647 AKT1 E17K in Colorectal Carcinoma Is Associated With BRAF V600E But Not MSI-H status: A Clinicopathologic Comparison To PIK3CA Helical and Kinase Domain Mutants

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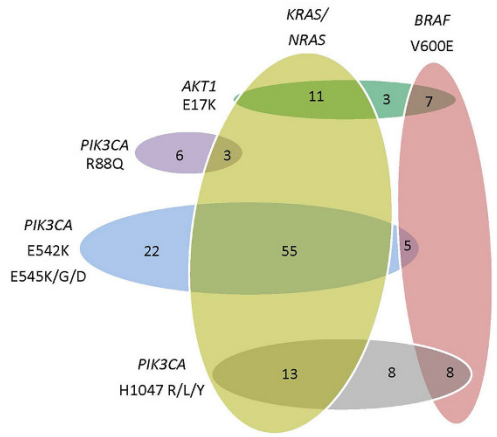
Background: The PI3K/AKT/mTOR pathway is activated through multiple mechanisms in colorectal carcinoma (CRC). We aimed to characterize the clinicopathologic and molecular features of AKT1 E17K-mutated CRC with comparison to PIK3CA-mutated CRC.
Design: All institutional CRC submitted for clinical molecular testing were screened for mutations in AKT1 (E17K) and PIK3CA involving codons 542, 545 and 1047 in exons 2, 10 and 21, respectively. Results were correlated with clinical and pathologic characteristics.
Results: Of 2631 CRC, there were 18 AKT1 E17K and 120 PIK3CA mutants: 9 R88Q, 82 E542K and E545K/G/D, and 29 H1047R/L/Y. Eight-six percent and 70% of AKT1 and PIK3CA mutants, respectively, had RAS or BRAF mutation. In comparison to PIK3CA mutants, AKT1 E17K was associated with mucinous morphology (p=0.0006) and concurrent BRAF V600E mutation (p=0.005). Among PIK3CA-mutants, exon 21 mutations were associated with BRAF V600E mutation (p=0.002), MSI-H status (p=0.0002) and poor differentiation (p=0.007), while exon 10 mutations were associated with KRAS/NRAS mutations (p=0.035) (figure 1). Three of 4 AKT1 mutants with primary and metastasis results available had concordant AKT1 mutation status in both lesions. Both patients with AKT1 E17K alone exhibited primary resistance to cetuximab, whereas 7 of 8 patients with PIK3CA mutation alone experienced tumor shrinkage or stability with anti-EGFR therapy. Conclusion: AKT1 E17K mutation in advanced CRC is associated with mucinous morphology, PIK3CA wild type status, and concurrent RAS/ RAF mutations with similar pattern to PIK3CA exon 21 mutants.
Conclusions: AKT1 E17K mutation in advanced CRC is associated with mucinous morphology, PIK3CA wild type status, and concurrent RAS/ RAF mutations with similar pattern to PIK3CA exon 21 mutants. AKT1 E17K mutations may contribute to primary resistance to cetuximab and serve as an actionable alteration.

Figure 1. Distribution of PI3K Pathway Mutations in Colorectal Carcinoma.



648 ERBB2 (HER2) Gene Amplification and Protein Overexpression in Ampullary Carcinoma

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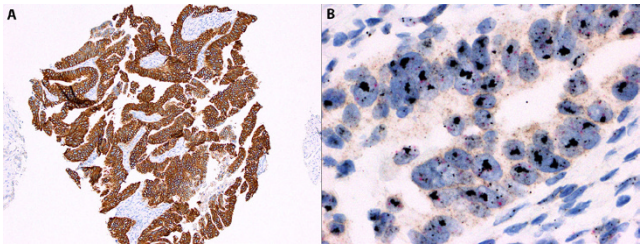
Background: Ampullary carcinoma (AC) is a rare malignancy with high mortality, poor response to chemotherapy, and a relative lack of known targetable alterations. Our recent work on a smaller cohort has shown that ERBB2 (HER2) amplification may be a frequent event in AC. We aimed to characterize HER2 overexpression and gene amplification in a large cohort.

Design: Tissue microarrays were constructed from the resection specimens of 101 cases, using 3 cores per AC. Immunohistochemistry (IHC) (4B5, Ventana) using the scoring criteria for gastric carcinoma, were used to assess HER2 overexpression. Chromogenic in situ hybridization (CISH) (Ventana) was performed to assess concordant gene amplification. Clinicopathologic features were collected from electronic medical records.

Results: HER2 was amplified in 17 (17%) of 101 AC (table 1). IHC in amplified cases was 3+ for 9 cases and 2+ in 8 cases, which 8 additional cases of 2+ staining were not amplified (50% of 2+ cases). No cases with 0 or 1+ staining were amplified, demonstrating high sensitivity of IHC. All cases positive (3+ membranous staining) by IHC (Figure 1A) were positive by CISH (Figure 1B). HER2 amplified and non-amplified AC shared similar clinicopathologic profiles, including median age (60 years vs. 66 years) male predominance (M:F = 12:5 vs. 45:39), and differentiation (intestinal: mixed: pancreatic = 5:4:8 vs. 36:16:32).

Conclusions: Data from our large cohort indicate that ERBB2 (HER2) gene amplification and protein overexpression occur in 17% of AC, without predilection for differentiation, age, or sex. IHC screening for HER2 has high sensitivity for this potentially actionable alteration.



	IHC 0	IHC 1+	IHC 2+	IHC 3+
CISH amplified	0	0	8	9
CISH not amplified	60	16	8	0

649 p53 But not Cytokeratin 7 Expression Predicts Colorectal Neoplasia Risk in Patients With Inflammatory Bowel Disease and Mucosal Changes Indefinite for Dysplasia

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Background: We previously showed a significant colorectal neoplasia risk in inflammatory bowel disease (IBD) patients with mucosal changes indefinite for dysplasia (IND) and a potential diagnostic use of p53 and cytokeratin 7 immunohistochemistry in IBD-associated neoplasia. This study aims to determine the predictive value of these two markers in neoplasia risk in IBD-IND population.

Design: We retrieved 44 cases of colonic biopsy with IND from 44 IBD patients [mean age 46.6 (±15.1), male 57%] who had subsequent follow-up [median 101 months (range: 6-247)] after the IND diagnosis. We analyzed the expression of p53 and cytokeratin 7 in the colonic biopsies by immunohistochemistry and correlated their expression and clinicodemographics with colorectal neoplasia outcome.

Results: Among these 44 patients, 11 (25%) progressed to neoplasia [low-grade dysplasia (6), high-grade dysplasia (2), and cancer (3)] at a median follow-up of 34.7 months (range: 19-145). Univariate analysis confirmed that age and p53 expression were associated with neoplasia progression.

Table 1. Univariate analysis of risk factors associated with neoplasia progression in IBD-IND

Characteristics	Hazard Ratio	95% Confidence Interval	p value
Age, every 1-yr increase	1.07	1.02-1.12	0.003
Gender (male vs female)	1.85	0.49-6.97	0.37
Extensive colitis (yes vs no)	1.37	0.41-4.56	0.61
Primary sclerosing cholangitis (yes vs no)	2.18	0.57-8.28	0.25
Duration of colitis, every 1-yr increase	0.93	0.84-1.04	0.2
p53 weak nuclear staining, every 1% increase	1.02	0.99-1.04	0.19
p53 strong nuclear staining, every 1% increase	1.05	1.01-1.09	0.009
p53 strong cytoplasmic staining, every 1% increase	1.04	1.01-1.08	0.025
Composite p53 score, every 1% increase	1.01	1.003-1.02	0.007
Cytokeratin 7 staining, every 1% increase	0.99	0.97-1.01	0.39

Conclusions: IBD Patients with IND are at significant risk for colorectal dysplasia and cancer; age and p53 expression are associated with their neoplasia progression. Further large confirmatory studies are warranted.

650 Tactile Corpuscle-Like Bodies in the Gastrointestinal Mucosa: A Case Series

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Background: Tactile corpuscle-like bodies (TCLB) are microscopic, neural-derived bodies that simulate the superficial touch receptors of the peripheral nervous system. TCLB were originally described as a presumably neoplastic component of neurofibromas and have subsequently been described in melanocytic nevi, peripheral nerve sheath tumors, and ovarian mature cystic teratoma. Only rarely have TCLB been described in the gastrointestinal (GI) tract, with the presumption that they represent incidental reactive neural proliferations. We aimed to compile cases of TCLB involving the GI tract in non-syndromic patients to further characterize this finding.

Design: Cases of TCLB involving GI mucosa were compiled from in-house and consult material from 3 institutions (The Johns Hopkins Hospital, The Ohio State University, and the University of Pittsburgh Medical Center). Demographic and clinical information were obtained via the respective institutional records.

Results: Nine cases of TCLB involving the GI tract were identified (male = 6, female = 3, ages 39-79, mean 56). Sites of involvement were esophagus/GE junction (n=6), stomach (n=1), sigmoid colon (n=1), and gastric heterotopia of the cricopharynx (n=1). Endoscopy was abnormal in 6 of 7 cases (including changes consistent with Barrett esophagus [BE] and polypoid/nodular mucosa) and normal in 1 of 7 cases where this information was available. The foci of TCLB ranged from <0.1 mm to 1.5 mm. Other abnormal histopathologic findings were identified in the mucosa in 6 of 9 cases (including diffuse neurofibroma-like proliferation, BE, chronic gastritis, ECL-cell hyperplasia, and gastric intestinal metaplasia). Immunohistochemical labeling of TCLB in these cases was as follows: S-100 (6/6), SOX-10 (2/2), Nestin (1/1), Collagen-4 (1/1), synaptophysin (1/1; speckled pattern), EMA (0/3), CD68 (0/3), CD117 (0/2), AE1/AE3 (0/3), SMA (0/1), DOG-1 (0/1), CD34 (0/3), MSA (0/1), NSE (0/1), NFP (0/1), GFAP (0/1). No morbidity/mortality related to TCLB was reported.

Conclusions: TCLB are rare findings in the GI tract. To our knowledge, this is the largest series of TCLB in the GI tract. Most of the TCLB in this series were found in association with histologic evidence of mucosal injury, implicating a repairative process. The immunohistochemical profile of TCLB is most in keeping with Schwannian origin. Recognition of this rare finding in the GI tract is important to prevent misdiagnosis and potential overtreatment.

651 Discrepancy Between HER2 Immunohistochemistry and Fluorescence In Situ Hybridization in Esophageal and Gastric Adenocarcinoma

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Background: Esophageal/gastric cancer (EGC) is the second leading cause of cancer-related death worldwide. Because of the aggressive and systemic nature of the disease, many patients receive chemotherapy. Response rate to chemotherapy is only 20 – 40 %. It has been shown that trastuzumab, a HER-targeted agent, combined with chemotherapy improves the response rate and the prognosis on HER2 positive adenocarcinoma. Therefore, accurate diagnosis of HER2 status is essential in order to correctly treat patients. The current recommendation for HER2 testing is immunohistochemistry (IHC) on all cases with reflex fluorescence in situ hybridization (FISH) testing on equivocal (IHC 2+) cases. Recently, we have observed frequent discrepancies between HER2 IHC and FISH studies in EGC. In this study, we explored the prevalence of discrepancies between HER2 IHC and FISH studies.

Design: There were 253 EGC specimens sent for HER2 studies, 205 of them had both IHC and FISH tests performed in the past four years at our institution. Results of IHC and FISH were compared in all cases with both FISH and IHC results as well as within subgroups (biopsies vs. resection, esophageal/gastroesophageal junction (GEJ) vs. gastric, in house vs. referral, and pretreatment vs. no treatment).
Results: 145 specimens were from esophageal/GEJ cancer, and 105 from gastric cancer. The prevalence of HER2 over-expression/amplification (either by IHC or FISH) in esophageal/GEJ and gastric cancer is 32% (47/145), and 14% (15/105) respectively. Among the 205 cases with both HER2 IHC and FISH studies, 17 (8%) cases were positive in FISH only, 18 (9%) positive in FISH and equivocal in IHC, 4 (2%) positive in IHC only, and 17 (8%) positive in both FISH and IHC. Most FISH-positive and IHC-negative cases were seen in referral biopsy (38%, 8/21) and resection cases (36%, 8/22). There was only one in-house biopsy with positive FISH but negative IHC (11%, 1/9). There was no significant difference in discrepancy between esophageal/GEJ (33%, 13/39) vs. gastric (31%, 4/13) or pretreatment (33%, 4/12) vs. no treatment (33%, 3/9) specimens.
Conclusions: Significant discrepancy is observed between IHC and FISH results in EGC, which is mainly caused by false negative IHC. The false negative IHC may result from preanalytical variables, including poor preservation and/or fixation of specimens, since most cases with the discrepancy were resection specimens and referral biopsies. Given the frequent false negative IHC, future guidelines on EGC HER2 testing should consider greater utilization of FISH testing.

652 Epithelial Mesenchymal Transition in Micropapillary Colorectal Carcinoma

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Background: Micropapillary colorectal carcinomas (CRCs) behave more aggressively and are associated with a poorer prognosis compared to typical CRCs. Based on our observation, the interface between micropapillary carcinoma and adjacent stroma often does not show clear transition from tumor epithelial cells to mesenchymal stromal cells, suggestive of epithelial mesenchymal transition (EMT). EMT is characterized by loss of epithelial markers, acquisition of mesenchymal markers, and activation of signalling pathways such as transforming growth factor β (TGFβ) signaling. EMT in cancer cells has been associated with increased metastatic potential. In this study, we investigated whether micropapillary CRC is highly associated with EMT.
Design: 29 CRCs with at least focal micropapillary features were included in the study. Immunohistochemical studies were performed for epithelial markers (AE1/AE3 and E-cadherin), mesenchymal markers (vimentin and smooth muscle actin (SMA)), and TGFβ signaling (SMAD4).
Results: Focal loss or decrease in cytokeratin AE1/AE3 expression, especially membranous expression, was observed in 18 of 29 (62%) tumors. In addition, focal loss of E-cadherin membranous labeling was seen in 21 of 27 tumors (78%). These reduced expressions were mainly present in the transitional area between tumor cells and stroma, single cancer cells or cancer cells in small clusters. Focal vimentin expression in tumor cells was also identified in 13 of 29 tumors (45%). 22 of 29 (76%) carcinomas displayed prominent myxoid tumor stroma associated with the micropapillary component. All 22 of these cases showed single cancer cells and small clusters of 2-3 cancer cells, whereas only 1 of 7 cases (14%) without prominent myxoid stroma showed single cancer cells and small clusters of cancer cells ($p<0.01$). In addition, the stroma around the micropapillary component in the 27 of 29 cases (93%) showed dense and strong SMA staining, as seen in cells undergoing EMT. 24 of 29 tumors (83%) showed nuclear expression of SMAD4, indicating the activation of TGFβ signaling.
Conclusions: In this study, we showed that micropapillary CRCs are highly associated with features of EMT, such as loss of epithelial markers, gain of mesenchymal markers, and TGFβ signaling activation. EMT therefore may be a reason that micropapillary CRCs have a higher metastatic rate than typical CRCs.

653 CA9 – A Novel Biomarker for Cholangiocarcinoma and Pancreatic Ductal Carcinoma

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Background: Cholangiocarcinomas (CC) and pancreatic ductal adenocarcinomas (PDAC) are aggressive malignancies and often present with metastasis before primary diagnosis. Unfortunately, there is no reliable IHC marker to distinguish CC and PDAC from other adenocarcinoma. By analyzing "human protein atlas" (http://www.proteinatlas.org) data, we found CA9 could be a biomarker for CC and PDAC. In this study, we want to investigate if CA9 can be a diagnostic marker for CC and PDAC.
Design: After IRB approval, we collected 25 cases of CC and 20 cases of PDAC. Tissue microarray (TMA) was constructed from those cases with non-neoplastic adjacent tissue as control. TMA contain all non-neoplastic tissue and ~200 common carcinomas were also used in this study. TMA slides and conventional whole tissue slides were subjected for IHC stain of CA9. Only membrane staining was considered positive. Stains were interpreted as negative (<5%), focal (5-20%), patchy (20-50%), diffuse (>50%); weak or strong intensity.
Results: For all benign tissue, only gastric mucosa, bile duct and pancreatic duct (weak) are positive. All other tissues are negative. 25/25 of CC and 20/20 PDAC are positive for CA9. Most of them are diffusely and strongly positive, only 2/25 of CC and 1/20 PDAC are patchy positive. In 200 common carcinomas, only clear cell renal cell carcinomas (ccRCC) are diffusely and strongly positive for CA9. 18 other carcinomas are focally or patchy positive. Interestingly, CA9 staining is much stronger in PDAC than the staining in benign pancreatic duct.
Conclusions: CA9 is strongly and diffusely positive in CC and PDCA. The only other common carcinoma has similar CA9 staining patten is ccRCC which is relatively easy to be distinguished from CC and PDAC. The carcinogenic role of CA9 in CC and PDCA warrant for further investigation and may lead to therapy target for CC and PDCA.

654 Histological Features of Colon Allograft in Intestinal Transplant Patients

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Background: Inclusion of colon as a component of intestinal transplant (IT) has been associated with increased transit time and water absorption with improvement in stool formation and fecal continence. Although initially thought to predispose to graft loss, additional larger studies showed no difference in graft outcome. Subsequently, inclusion of colon has become a more common procedure in intestinal transplantation, raising the need to characterize the histology of colon allograft (CA) biopsies.
Design: Thirty-two CA biopsies derived from 6 IT patients were studied. All 32 biopsy sets also included small intestine allograft (SIA) and 7 had native colon (NC) samples. We studied the histological features of IT biopsies and compared the features of rejection in CA and SIA using the current small intestinal transplant acute cellular rejection grading system. This included apoptotic body (AB) count, with assessment of apoptotic crypt dropout, erosions and inflammatory infiltrate (indeterminate: 3-5 ABs, mild: ≥6 ABs, moderate: confluent crypt apoptosis or focal crypt dropout, severe: extensive crypt dropout and erosion/ulceration).
Results: Fifteen SIAs showed features of rejection (3 -mild, 10 -moderate, 2 -severe) and 14 corresponding CAs (1 -mild, 5 -moderate, 8 -severe). Only 1 SIA, with features of mild rejection, had negative corresponding CA. The affected CAs showed ulcerations more frequently (8/14 of CAs compared to 2/15 of the corresponding SIAs). The CAs matching the 3 SIAs with mild increase in apoptosis classified as indeterminate for rejection, showed features of moderate (1) and severe (2) rejection. Fourteen SIAs were negative for rejection and 11 corresponding CAs were also negative, with the remaining 3 showing features of moderate (1) and severe (2) rejection. NC, sampled in 6 negative biopsies and 1 with moderate CA rejection, showed no increase in apoptosis and no pathological features. In CA mucosa with extensive crypt dropout and ulcers, residual neuroendocrine cell clusters were seen, confirmed by chromogranin immunostain.
Conclusions: CA biopsies displayed a spectrum of pathological changes similar to SIAs, including patchy increased AB count, apoptotic crypt dropout and, more frequently, ulcers. Neuroendocrine cell clusters were seen in CA ulcers and further studies may be helpful to determine if it is a feature more common to rejection rather than other ulcer etiology (i.e., infection, ischemia, drugs). In the biopsy set studied, CAs were more affected than the corresponding SIAs, however, the clinical significance of these findings remains unclear.

655 ARID1A Expression in Colonic Adenocarcinoma: An Exploration of Its Prognostic Significance

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Background: *ARID1A*, a chromatin remodeling gene, has been found to be mutated in a number of tumors including colorectal carcinoma (CRC). Loss of ARID1A has been associated with outcome in tumors such as ovarian, endometrial and gastric adenocarcinomas. While most studies suggest an association of ARID1A loss with an adverse outcome, conflicting results also exist. Major limitations of some outcome studies include small sample size and heterogeneous patient population. Our study aimed at analyzing the prognostic significance of ARID1A in a homogeneous group of early stage colonic cancer patients, a patient population where prognostic markers are particularly relevant.
Design: The study population consisted of 578 patients with stage I or II colonic adenocarcinoma who had undergone surgery with curative intent, without neoadjuvant or adjuvant therapy, and with a median follow up of 82 months. ARID1A expression was analyzed by immunohistochemistry (IHC) using tissue microarrays, and was correlated with various clinicopathological characteristics including mismatch repair (MMR) protein expression. Statistics were performed using SPSS v21.
Results: ARID1A loss was observed in 56 of 541 analyzable tumors (10%). Loss of ARID1A was more frequent in females ($p<0.001$) and in tumors with loss MMR protein expression ($p<0.0001$). Correlative analysis revealed an association of ARID1A loss with poor differentiation (PD, $p<0.001$) and lymphovascular invasion (LVI, $p=0.007$); however, no significant association was detected between ARID1A loss and age, tumor location, perineural invasion, T stage, or pre-op CEA. The median overall survival (OS) of the entire group was 115 months (95% CI, 99-132). While age ($p<0.001$; OR=1.06; CI: 1.04-1.08), LVI ($p=0.02$; OR=1.8; CI: 1.1-3), histologic subtype ($p<0.001$; OR=1.9; CI: 1.4-2.6), and tumor stage ($p<0.001$; OR=2.5; CI: 1.6-4.1) correlated with OS on multivariate analysis, the status of ARID1A showed no association with survival.
Conclusions: This study represents the first to systematically evaluate the prognostic value of ARID1A in early stage colonic adenocarcinomas. Our finding of 10% of the tumors losing ARID1A is similar to the reported frequencies in CRCs in general. While ARID1A loss showed an association with PD and LVI, it also correlated with MMR deficiency, and ultimately did not impact on the overall survival in our patient population. Such data are clinically relevant as efforts are ongoing in identifying markers that can detect the small but significant subset of early stage CRCs that will have an adverse outcome.

656 Digital Whole Slide Imaging and Two Photon Microscopy for Liver Fibrosis Scoring and Quantification

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Background: The progression of fibrosis in inflammatory or infectious diseases of the liver guides treatment decisions. The histologic assessment of fibrosis is subjective and varies based on the experience of the assessors and the system used. Whereas the Batts-Ludwig, Ishak or Metavir scoring systems are useful tools for viral hepatitis, their utility is limited in steatohepatitis. Moreover, the clinician has to be familiar with the scoring system for correct interpretation. Therefore, a more objective and uniform method is needed to optimize patient care. We sought to study fibrosis using digital imaging analysis and two photon excitation microscopy combined with second harmonic generation (2PE/SHG) in comparison with clinically used scoring system in hopes of decreasing subjectivity.

Design: 49 non-fragmented liver core needle biopsies with either hepatitis C or steatohepatitis, sampled between 2012 and 2014, were selected from our archives. The Masson's trichrome stains were evaluated using a 0-4 scoring system (0 – no fibrosis, 1 –portal expansion or pericellular fibrosis, 2 – periportal fibrosis, 3 – bridging, and 4 – cirrhosis) by two experienced pathologists and a pathology resident. Kappa statistics was performed. Masson's trichrome slides from 38 cases were digitally scanned with an AT2 scanner (Leica, Vista, CA) and assessed via a color deconvolution algorithm (ScanScope V9). Data was analyzed using ANOVA with Tukey's HSD. 5 of these cases underwent 2PE/SHG imaging (Genesis®200, Histoindex Pte Ltd) to obtain aggregated fiber percentage (AFP), total number of fibers (TNF), and number of fiber cross-links (FCL).

Results: The overall Fleiss kappa value between three assessors was 0.59. ANOVA test showed a significant difference between percent fibrosis in the groups (p-value of 0.019) analyzed via digital imaging. Plots demonstrated positive correlation between average scores and percent fibrosis (r^2=0.47, P=0.003), AFP (r^2=0.85, P=0.06), TNF (r^2=0.84, P=0.07) and FCL (r^2=0.96, P=0.007).

Conclusions: Our results demonstrate only fair interobserver agreement between the scores of the three assessing pathologists, highlighting the need for more objective fibrosis scoring. Preliminary data show a positive correlation between the scores and percent fibrosis obtained on digital imaging and AFP, TNF and FCL obtained on 2PE/SHG suggesting the promise of this novel technology. Additional cases are being analyzed and will be presented at the meeting.

657 Agreement for the Histological Diagnosis of Aberrant Crypt Foci – A Study of 687 Rectal Samples

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Background: Aberrant Crypt Foci (ACF) have come up in the last years as potencial precursors of colorrectal carcinoma (CRC) as they have been involved in the ACF-adenoma-carcinoma sequence. Nonetheless the reported prevalence of dysplasia in these lesions is widely variable, probably due to multiple factors (technical, histological classification). Our aim was to investigate intra- and inter- observer agreement for their histological diagnosis.

Design: Rectal ACF were detected and biopsied using high definition methylene blue chromoendoscopy on healthy subjects (n=100), individuals with adenomas (n= 50) and with CCR (n=50). A total of 687 rectal ACF were included. Frozen serial sections of ACF were stained with H&E. Two independent pathologists (A, B) blinded to each other and to endoscopic assessment, classified the samples into inadequate, normal mucosa, hyperplasic, serrated and dysplastic. Each pathologist examined twice the samples in a 6 months period. Finally, a consensus diagnosis was reached. Weighed k-statistics was calculated inter- and intra-explorer and defined as fair: 0,21-0,40; moderate: 0,41-0,60; good: 0,61-0,80 and very good: 0,81-1,00.

Results: At final consensus 35 (5,1%) samples were considered inadequate for histological diagnosis, 93 (13,5 %) normal mucosa, 399 (58,1 %) hyperplasic ACF, 59 (8,6 %) serrated ACF and 101 (14,7 %) dysplastic ACF. Weighted κ for intraobserver variation was 0.59 (A) and 0.71 (B) (p[thinsp]£[thinsp]0.001). The best κ for interobserver variation was 0,25 (p[thinsp]£[thinsp]0.001).

Conclusions: The intra- and inter-observer agreement for ACF histological diagnosis is moderate and fair, respectively. This raises the need for consensus standardised diagnosis histology criteria for ACF.

658 Role of Pancreatic Core Biopsy in the Diagnosis of Autoimmune Pancreatitis

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Background: Autoimmune Pancreatitis (AIP) has been recognized as a chronic fibro-inflammatory disease that can mimic pancreatic cancer in both clinical presentation and radiographies. Diagnosis of AIP can be challenging and pancreatic core biopsy may offer some help. However, the lymphoplasmacytic infiltration and IgG4-positive cells can also be seen in pancreatic cancer. Therefore, it is critical to interpret core biopsy in the appropriate clinical context.

Design: We retrospectively analyzed 20 cases that were clinically suspicious of AIP vs malignancy. The information of clinical history, laboratory tests, radiographic images, biopsies, steroid trial and clinical follow-up was collected. The pancreatic core biopsies were reviewed and immunostained with IgG4 antibodies. In addition, 6 resected AIP and three pancreatic cancer cases with AIP-like inflammation were compared.

Results: Among 20 cases suspicious for AIP, 9 cases were confirmed to be AIP; 6 cases were nonspecific pancreatitis; 4 cases were pancreatic cancer; one was uncertain due to lack of follow-up. On imaging studies, 7 of 20 showed mass-like lesions, of which 4/7 confirmed to be cancer and 3/7 turned out to be AIP. Classic AIP-like radiographies were seen in 10 of 20 cases, of which 7/10 were AIP, and 3/10 were nonspecific pancreatitis. Six additional AIP had Whipple resection after unsuccessful cytology biopsy. In total15 AIP cases, only two had elevated serum IgG4 and two had post-surgically elevated IgG4. Pancreatic core biopsies were performed in 19 cases. 7/19 had minimal tissue for diagnosis, 3/19 had normal tissue, 2/19 had carcinoma, and 7/19 showed AIP. The histological features in the 7 AIP cases were lymphoplasmacytic infiltration with eosinophils and cellular fibrosis. Neutrophil in small ducts was seen in one case. IgG4 immunostains showed 3/7 with increased IgG4-positive plasma cells (> 50/HPF), and 4/7 with few IgG4-positive cells (0- 10/HPF). IgG4-positive plasma cells were also seen in 1/ 3 pancreatic cancers (>10/HPF). 8/9 AIP patients had steroid trial, and all of them had good response on subsequent follow-up.

Conclusions: Distinguish between AIP and cancer can be very difficult. Pancreatic biopsy shows lymphoplasmacytic infiltrate with fibrosis but negative for IgG4-positive plasma cells, steroid trial should be attempted. However, the AIP-like histology and increased IgG4- positive cells on biopsy are not specific to AIP. Therefore, clinico-histological correlations are essential for the correct diagnosis of AIP.

659 Ki-67 "Hot Spot" Digital Analysis Is Useful in the Distinction of Hepatic Adenomas and Well-Differentiated Hepatocellular Carcinomas

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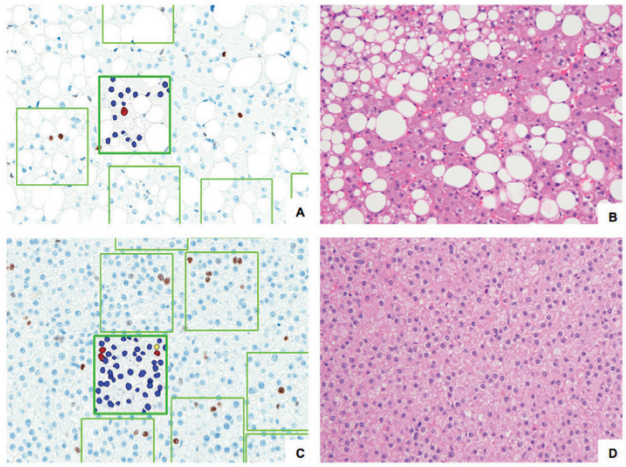
Background: The diagnostic evaluation of well-differentiated (WD) hepatocellular neoplasms is challenging. Ki-67 proliferative index is currently performed routinely in various tumors and digital analysis represents the most accurate method for this assessment. We aimed to evaluate the utility of Ki-67 by digital analysis in the context of WD hepatocellular neoplasms.

Design: Resection cases of typical hepatic adenomas (HAs), atypical HAs, and WD hepatocellular carcinomas (HCCs) were selected from our files.

Group	Criteria
Adenoma	Typical morphology, intact reticulin
Atypical Adenoma	Atypical morphology, not fullfilling criteria for HCC. Intact reticulin. Atypical clinical features: postmenopausal female, male gender, anabolic steroids, glycogen storage disease
Hepatocellular Carcinoma	Well-differentiated (Grade I)

Ki-67 proliferative index by IHC was evaluated by digital analysis, using a standardized neuroendocrine tumor "hot spot" protocol.



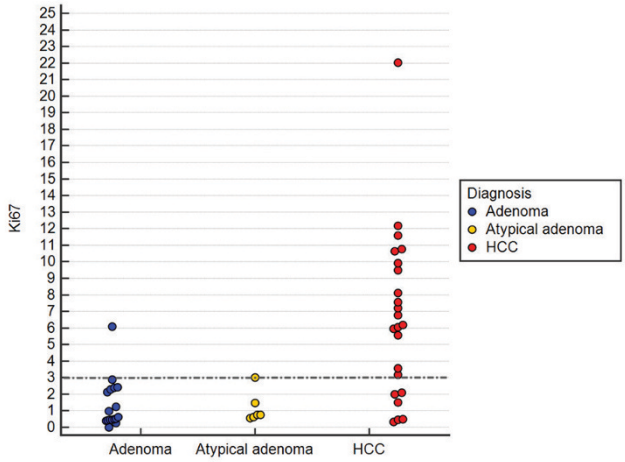
Hepatic adenoma (A and B) and well-differentiated hepatocellular carcinoma (C and D). Photograph from scanned Ki-67 IHC slide with digital analysis using a "hot spot" protocol (A and C, 200X magnification). Hematoxylin-eosin stain (B and D, 200X magnification).

Results: 17 cases of HAs, 6 atypical HAs, and 23 WD HCCs were included. Clinicopathologic features are summarized in table 2.

	HAs n=17	Atypical HAsn=6	HCCsn=23
Age*	39 (24-57)	36 (9-74)	65 (23-83)
Male:Female	0:17	3:3	16:7
BMI*	35 (21-44)	24 (17-37)	29 (19-46)
OCP*	10 (59%)	0	2 (9%)
Anabolic steroids*	0	1 (17%)	0
Glycogen storage disease	0	3 (50%)	0
# of nodules*	2 (1-33)	3 (1-15)	1 (1-2)
Largest nodule, (CM)*	5.8 (0.4-17)	2.7 (0.9-8.8)	6.2 (2.3-14.5)

* median (range)

Nearly all HAs and atypical HAs showed a proliferative rate ≤3%, while 74% of WD HCCs showed higher proliferation.



Conclusions: Ki-67 is a potentially useful adjunct marker in the evaluation of WD hepatocellular neoplasms, as tumors with >3% proliferative rate most likely represent WD HCC.

660 Stromal Expression of miR-21 Is an Independent Predictor of Early Tumor Relapse in Advanced Colon Cancer But Not Rectal Cancer

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Background: miR-21 is an oncogenic microRNA that regulates the expression of multiple cancer-related target genes. Metastasis-associated protein1 expression and loss of E-cadherin expression are correlated with cancer progression and metastasis in many cancer types. In advanced colorectal cancer, the clinical significance of miR-21 expression remains unclear. We aimed to investigate the impact of miR-21 expression in advanced colorectal cancer and its correlation with protein markers associated with colorectal cancer progression.

Design: From 2004 to 2007, 277 consecutive patients with T3-4a colorectal cancer treated with R0 surgical resection were included. Patients with neoadjuvant therapy and distant metastasis at presentation were excluded. The expression of miR-21 was investigated by in situ hybridization. Immunohistochemistry was used to detect E-cadherin and metastasis-associated protein1 expression.

Results: High stromal expression of miR-21 was found in 76 of 277 (27.4%) colorectal cancer samples and was correlated with low E-cadherin expression (P=0.019) and high metastasis-associated protein1 expression (P=0.004). Colorectal cancer patients with high miR-21 expression had significantly shorter recurrence-free survival than those with low miR-21 expression. When analyzing colon and rectal cancer separately, high expression of miR-21 was an independent prognostic factor of unfavorable recurrence-free survival in colon cancer patients (P=0.038, HR = 2.45; 95% CI = 1.05-5.72) but not in rectal cancer patients. In a sub-classification analysis, high miR-21 expression was associated with shorter recurrence-free survival in the stage II colon cancer (P=0.007) but not in the stage III subgroup.

Conclusions: Stromal miR-21 expression is associated with the expression of E-cadherin and metastasis-associated protein1 in colorectal cancer. Colon cancer patients with high levels of miR-21 are at higher risk for tumor recurrence and should be considered for more intensive treatment.

661 Correlation Between the Elastic Modulus of Colorectal Cancer Tissue and Clinicopathological and Histological Findings

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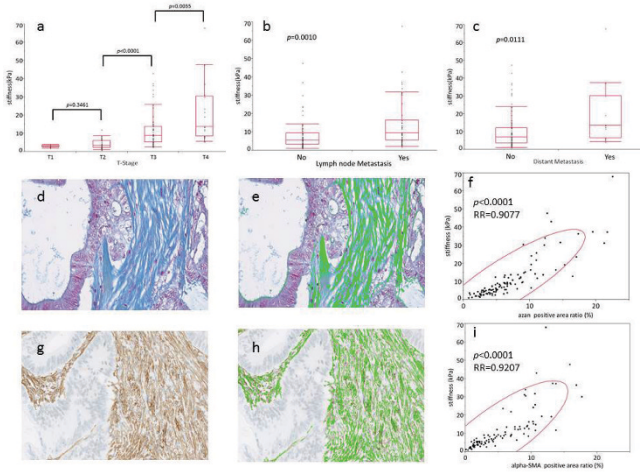
Background: Generally, cancer tissue is palpable as a hard mass. In the other hand, it is not clear the nature of elasticity in cancer tissue. We recently establish the quantitative assessment method of consistency in human tissue. The elastic modulus (EM) of colorectal cancer tissue measured by a tactile sensor may provide us crucial pre- and post- operative information.

Design: Using a Venustron system, a tactile sensor (AXIOM Axim, Fukushima, Japan), we measured the EM of 106 surgically resected colorectal cancer tissues. The data of the EM werecompared with the clinicopathological findings and the azan-Mallory (azan) positivearea ratio and the alpha-smooth muscle actin (α-SAM) positive area ratio,calculated by morphometric software (WinROOF, Mitani Corporation, Tokyo, Japan).

Results: The EM in cancer tissue was significantly higherthan that in normal tissue (7.51 kPa VS 0.936 kPa, p<0.0001). Higher EM intumor was associated with pathological T-, N- and M-Stage (figure1 a, b, c). TheEM strongly correlated with azan positive area ratio (figure1 d, e, f, RR=0.9077)and α-SAM positive area ratio (figure1 g, h, i, RR=0.9207).



Conclusions: The Assessment of the EM of the resected colorectalcancer tissue measured by tactile sensor was correlated with TNM classificationand the azan positive and α-SAM positive stroma. Assessment of consistency canbe available to estimate more accurate preoperative clinical stage estimation.

662 HER2 Expression in Dysplasia-Carcinoma Sequence of Barrett Esophagus and Its Role in the Different Pathways of Carcinogenesis in Barrett Esophagus

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Background: HER2, a proto-oncogene, is amplified in a proportion of esophageal adenocarcinoma, however, there is limited data on HER2 expression in early Barrett-associated adenocarcinoma, and its expression in the metaplasia-dysplasia-carcinoma sequence.

Design: The cohort consisted of 23 endoscopic mucosal resections (EMR) with dysplasia and intramucosal carcinoma (IMC) arising in Barrett esophagus (BE). Immunophenotype was evaluated by expression of gastric (MUC5AC,MUC6) and intestinal (MUC2,CD10) markers, together with semi-quantitative scoring of CDX2 expression (CDX2 score = intensity (0-3) x percent staining (0-4)). HER2 immunohistochemistry was assessed using ToGA criteria. The immunophenotype of dysplasia and adjacent IMC were compared to determine phenotypic concordance during neoplastic progression.

Results: Of the 23 cases analyzed, 20 cases demonstrated phenotypic stability with consistent gastric (n=16), intestinal (n=3) and hybrid (n=1) phenotype along the carcinogenic sequence. Overall, HER2 positivity (score 3+) was observed in 18.2% (4/22) dysplasia (high grade only) and 26.0% (6/23) IMC, with HER2 positivity noted in both IMC and adjacent dysplasia (high grade) in 3 cases. HER2 positivity was observed only in lesions with gastric phenotype. The mean CDX-2 score was 6.89 and 5.76 in HER2 negative dysplasia and IMC, respectively, whereas mean CDX2 scores of 3.5 and 3.33 were observed in HER2 positive dysplasia and IMC, respectively. HER2 positivity was only observed in neoplastic BE and never in non-neoplastic BE.

Conclusions: HER2 positivity appears to be strongly associated with the pathway of BE carcinogenesis presenting with a gastric phenotype, particularly late in the dysplasia-carcinoma sequence. HER2 positivity demonstrates an inverse relationship with CDX2 expression, suggesting reciprocal regulation and consistent with its role as a potential oncogene in BE carcinogenesis.

663 Inverted Pattern of MUC1 Expression at the Invasive Front of Malignant Colorectal Polyps Is a High-Risk Factor of Lymph Node and Distant Metastasis

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Background: Previous studies have demonstrated that colorectal carcinomas (CRCs) with micropapillary pattern, and those showing high counts of tumor budding (TB), defined as clusters of less than five cancer cells, and poorly-differentiated clusters (PDCs), defined as five or more cells with no gland formation, have a high probability to develop metastatic disease. In these settings MUC1 (usually expressed at the apical cell membrane of the neoplastic glands of CRCs) shows an inverted pattern of expression. It has been speculated that the inverted pattern of MUC1 expression reflects the loss of epithelial properties and possibly indicates epithelial mesenchymal transition in colorectal carcinoma, similar to the phenomenon of tumor budding. The aim of our study was to asses the pattern of MUC1 expression at the invasive front of malignant polyps and its correlation with clinical outcome.

Design: We investigated 29 polypectomies with submucosal invasive adenocarcinoma which subsequently underwent colon resection. One unstained slide from each case was immunolabeled for MUC1 (clone MRQ-17; Cell Marque). All cases were blindly reviewed by two pathologists to analyze the pattern of MUC1 expression in malignant glands, PDC and TB along the entire advancing front of invasion. Inverted pattern of MUC1 was recorded when reactivity at the cell membrane facing the stroma was observed.

Results: Inverted pattern of MUC-1 expression was present in PDC and/or TB of 7/29 (24%) malignant polyps, but in none of the invasive malignant glands; 4/7(57%) of these had metastasis (2 in regional lymph nodes, 1 in mesentry, and 1 in pleural fluid). Only 4/22 (18%) cases without MUC-1 inverted pattern developed metastasis while remainder 18/22 (81.8%) didn't develop metastasis. The sensitivity, specificity, positive predictive value, and negative predictive value were 50%, 85.71%, 57% and 81.82%.

Conclusions: Inverted pattern of MUC-1 expression occurs only in PDC and/or TB of malignant polyps and it is a highly specific (85.7%) finding that may account for the higher metastatic potential of these lesions. Furthermore, its absence has a high negative predictive value (81.82%) that may be helpful in the risk stratification of these lesions.

664 Peroxiredoxin5 Affects Prognosis via Increase of EMT in Gastric Cancer

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Background: Peroxiredoxins (PRXs) are novel peroxidases containing high antioxidant efficacy and are associated with tumor biology in several kinds of cancer. EMT is a process by which polarized epithelial cells lose their polarity, adhesion and tight junction. In cancer progression, it is well known that proper Reactive oxygen species (ROS) production can promote migration and invasion, which are typical EMT properties, but excessive ROS production can inhibit EMT of cancer cells. However, expression of PRXs, its impact on disease prognosis, patient's survival, and the relationship of between EMT and PRXs as antioxidant is rarely studied in human gastric cancer.

Design: First of all, the nine gastric cancer cell lines were investigated expression of PRXs using RT-PCR, Western blotting. To determine EMT properties, we examined expression of E-cadherin and Vimentin using RT-PCR, and Western blotting in 2 gastric cancer cell lines. We studied the expression of all six isofom PRXs by immunohistochemical staining in tissue microarrays containing 210 gastric cancer tissues, and investigated the correlation between expression of PRXs and the clinicopathological parameters.

Results: In all nine gastric cancer cell lines, PRXs transcripts and proteins were detectable by RT-PCR and Western blotting. As a result, the expression of PRX5 was high expressed in SNU-668, but not SNU-216. In addition, SNU-668 was increased the expression of Vimentin but, decreased E-cadherin. Interestingly, although the expression of PRX1-4 was not associated with clinicopathological parameters, PRX5 was significantly associated with larger tumor size (PRX5; $P = 0.000$), depth of tumor (T status, PRX5; $P = 0.000$), lymph node involvement (N status, PRX5; $P = 0.003$,), and higher TNM stage (PRX5; $P = 0.000$). Also they tended to be correlated with a reduced 5-year survival rate (PRX5; 36.8% vs. 58.7%, $P = 0.007$). In multivariable analysis, PRX5 was only isoform that associated with patient's survival (high PRX5; $P = 0.01$).

Conclusions: We found that expression of PRXs is increased in gastric cancer, suggesting the induction of PRXs as response to increased production of reactive oxygen species in carcinoma tissue. Especially, PRX5 contributes to intensify EMT. Therefore, upregulated PRX5 can be considered prognosis marker of gastric cancer. Thus the expression of PRX5 plays an important role during gastric cancer progression and patient's survival.

665 Deamination Effects in Formalin-Fixed Paraffin-Embedded Tissue Samples in the Era of Next Generation Sequencing

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Background: Deamination of nucleotides in DNA causes C:G>T:A changes in formalin-fixed paraffin-embedded (FFPE) tissue samples and causes false positives in next generation sequencing (NGS) data. Deamination becomes more pronounced as the paraffin blocks age. Uracil DNA glycosylase (UDG) has been shown to help eliminate the process. In fresh paraffin blocks, deamination is common in CpG sequences. However, the effects of UDG in different conditions such as prolonged fixation time, differing formalin pHs and ischemia have not been studied.

Design: Both tumor and normal tissue were obtained from two patients with advanced gastric adenocarcinoma. The tissue was fixed for 1 to 5 days in formalin with a pH of either 5, 7, or 9. One sample was subjected to 24-hours of ischemia prior to fixation. We also included four other FFPE tumor blocks greater than 10 years old. In all samples, we performed semiconductor-based NGS to evaluate nucleotide changes, and we used UDG to test for deamination-related artifact.

Results: There was a robust increase in both variant calls and C:G>T:A changes after 3 days of fixation. These changes occurred regardless of the formalin pH used, however, more variant calls were seen in the pH5. Tumor and ischemic normal tissue showed increased variant calls which appeared to be independent of the fixation time. The number of CG>TG changes accounted for 9 to 50% of the total calls. The coverage of variant calls showed significant variation as the fixation time increased. Treatment with UDG eliminated all C:G>T:A changes with the exception of the CG>TG sequence in normal tissues fixed for 3 and 4 days in pH7. In the aged blocks, the proportion of C:G>T:A changes eliminated by UDG varied from 65.7 to 85.6%. The C:G>T:A changes with coverage of less than 300 were substantially decreased in these blocks when treated with UDG.

Conclusions: False positive variant calls due to deamination increase after 3 days of fixation and with prolonged cold ischemia time. Most of these changes can be eliminated with the treatment of UDG. When performing NGS on recently embedded blocks, it is important to notice that poorly fixed samples subjected to the above conditions may be at risk of being deaminated. UDG treatment can help to eliminate this confounding artifact.

666 High-Throughput Sequencing and Copy Number Variation Detection in Gastric Adenocarcinoma for Personalized Cancer Therapy

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Background: In the era of targeted therapy, mutation profiling of cancer is a crucial aspect of therapeutic decisions. To characterize cancer at a molecular level, the use of formalin-fixed paraffin-embedded tissue is important.

Design: We tested the Ion AmpliSeq Cancer Hotspot Panel v2 and nCounter Copy Number Variation Assay in 89 formalin-fixed paraffin-embedded gastric cancer samples to determine whether they are applicable in archival clinical samples for personalized targeted therapies. We validated the results with Sanger sequencing, real-time quantitative PCR, fluorescent in situ hybridization and immunohistochemistry.

Results: Frequently detected somatic mutations included *TP53* (30.3%), *APC* (10.1%), *KDR* (7.9%), *PIK3CA* (5.6%), *KRAS* (4.5%), *SMARCB1* (4.5%), *SMO* (4.5%), *STK11* (4.5%), *CDKN2A* (3.4%), *SMAD4* (3.4%), *CDH1* (2.2%) and *RET* (2.2%). Amplifications of *HER2*, *CCNE1*, *MYC*, *KRAS* and *EGFR* genes were observed in 8 (8.9%), 4 (4.5%), 2 (2.2%), 1 (1.1%) and 1 (1.1%) cases, respectively. In the cases with amplification, fluorescent in situ hybridization for *HER2* verified gene amplification and immunohistochemistry for HER2, EGFR and CCNE1 verified the overexpression of proteins in tumor cells.

Conclusions: In conclusion, we successfully performed semiconductor-based sequencing and nCounter copy number variation analyses in formalin-fixed paraffin-embedded gastric cancer samples. High-throughput screening in archival clinical samples enables faster, more accurate and cost-effective detection of hotspot mutations or amplification in genes.

667 The Impact of Routine Elastin Staining on Venous Invasion Detection in Colorectal Cancer

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Background: Venous invasion (VI) is an independent prognostic indicator in colorectal cancer (CRC). Data from our own centre and at a provincial level indicate that VI is under-reported. Elastin staining has been shown to enhance the detection of VI and affords superior prediction of survival compared to standard staining alone. The aims of this quality improvement study were (1) to determine the impact of the introduction of routine elastin staining on VI detection rates and (2) to determine its impact according to the sub-specialist interest of the reporting pathologist.

Design: In April 2012, routine elastin staining (≥ 5 tumor containing blocks/case) on all CRC resection specimens was implemented in our centre. Pathology reports one year prior to (n=145) and one year after this date (n=138) were reviewed for the presence or absence of VI, as well as other mandatory elements of the College of American Pathologists (CAP) protocol. Comparative analyses were adjusted for baseline differences in TNM stage, administration of neoadjuvant therapy and tumor location.

Results: Following the implementation of routine elastin staining, there was an overall increase in the VI detection rate from 20.0% to 42.0% (adjusted Odds Ratio, 3.54 [95% confidence interval, 1.89-6.63]; P<0.001). Significant increases in VI detection rates were observed among both gastrointestinal (GI) and non-gastrointestinal (non-GI) pathologists, although there were no significant differences in VI detection rates between GI and non-GI pathologists in either the pre-implementation (23.9% vs. 16.7%: adjusted OR, 0.54 [95% CI, 0.10-2.99]; P=0.48) or post-implementation periods (40.5% vs. 44.1%: adjusted OR, 1.49 [95% CI, 0.66-3.37]; P=0.34). There was a corresponding reduction in the overall rate of small vessel/lymphatic invasion (SVLI) from 23.4% to 13.8% (adjusted OR, 0.27 [95% CI, 0.13-0.57]; P=0.001). No significant changes in the rates of perineural invasion or discontinuous tumor deposits were observed.

Conclusions: The implementation of routine elastin staining was associated with a more than two-fold increase in the detection of VI, irrespective of the sub-specialist interest of the reporting pathologist. The reduced reporting of SVLI (which is of less prognostic value) probably reflects increased detection of VI over SVLI when elastin stains are used.

668 Widespread Variability in Assessment and Reporting of Colorectal Cancer Specimens Among North American Pathologists: Results of a Canada-US Survey

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Background: Challenges exist in standardizing reporting of colorectal cancer (CRC) resection specimens, related either to new concepts, or issues with interpretation of reporting guidelines. This study aimed to determine practice patterns of North American pathologists, with reference to their country of practice and their sub-specialist interest, in order to identify differences in assessment and reporting.

Design: A population-based online survey was developed and distributed to all members of the Canadian Association of Pathologists and a randomly selected cohort from the College of American Pathologists' directory.

Results: Completed surveys were received from 389 pathologists (144 Canadian: (48 GI, 96 non-GI) and 245 US: (84 GI, 161 non-GI)). There was substantial variation in reporting between and within groups; selected examples are reported here. Statistically significant differences were present between GI and non-GI pathologists with respect to defining T4a status ($p<0.02$), assessment of radial margins in colon cancer ($p<0.02$), use of lymph node revealing solution ($p<0.0001$), defining tumor deposits ($p<0.02$), reporting VI ($p=0.002$), routine use of elastin stains ($p<0.01$), reporting of tumor budding ($p<0.0001$) and ordering of MMR IHC ($p<0.0001$). Canadian pathologists were more likely than US pathologists to gross rectal cancer specimens according to the method of Quirke et al (65.4% vs. 19.0%, $p<0.0001$), to routinely grade the quality of total mesorectal excisions (89.2% vs. 55.2%, $p<0.0001$), to report venous invasion (VI) separately from small vessel invasion (60.8% vs. 39.4%, $p=0.0002$) and to use elastin stains routinely in VI diagnosis (29.4% vs. 2.4%, $p=0.0001$), but were less likely to perform MMR IHC on patients aged <60 years (25.2% vs. 42.0%, $p<0.005$).

Conclusions: Reporting practices among pathologists vary according to country and sub-specialist interest. Of note are several differences that could have an impact on staging and survival, and may limit the comparability of outcomes data between centres. Many issues surrounding interpretation of definitions could be resolved through knowledge transfer programs with future guidelines focusing on the standardization of specimen assessment.

669 Anal Lesions in IBD Are Associated With High Risk HPV Subtypes

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Background: A high prevalence of anal human papillomavirus (HPV) and anal dysplasia has been reported recently in patients with inflammatory bowel disease (IBD), (Cranston, R, Gastroenterology 2013;144: S-41) the basis for which is uncertain, but conjectured to involve altered immune status. We conducted a retrospective pathological and molecular study of anal neoplastic lesions in our patient population with IBD.

Design: Anal lesions identified in surgical and endoscopic specimens from 18 patients with clinically proven IBD (8M, 10F; 11 ulcerative colitis, 7 Crohn disease) were identified retrospectively and their pathology was reviewed. The tissues were evaluated for p16 overexpression by immunohistochemistry and for HPV by global and type-specific molecular PCR (1 consult case had been referred with positive p16 and HPV16 in situ hybridization slides). Lesions with p16 block-positive staining (p16+) and negative PCR for HPV underwent additional immunostaining for p53.

Results: The 18 cases comprised 2 (11%) low grade anal intraepithelial neoplasms (ASIN-L), 9 (50%) high grade anal intraepithelial neoplasms (ASIN-H), 6 (33%) invasive squamous cell carcinomas (SCCs), and 1 (5.5%) small cell carcinoma arising from ASIN-H. The results are summarized in the table. The HPV+/p16+ group comprised 2 SCCs (HPV16), 7 ASIN-H (HPV16), and 1 small cell carcinoma (HPV18). Two cases of SCC were HPV-/p16-. One case of SCC that was HPV16-positive but p16-negative was considered HPV-associated but not HPV-driven. The one SCC that was HPV-/p16+ showed "null" p53 immunostaining, suggesting mutation. One ASIN-L and 3 ASIN-H that were HPV-/p16+ showed wild-type p53 immunostaining and probably resulted from sampling error due to limited lesional tissue.

	HPV+	HPV-	Total
p16+	102 SCCs and 6 ASIN-H: HPV16+ PCR1 ASIN-H: HPV16+ ISH1 Small cell carcinoma arising from ASIN-H: HPV18+ PCR	51 SCC3 ASIN-H1 ASIN-L	15
p16-	11 SCC: HPV16+ PCR	22 SCCs	3
Total	11	7	18

Conclusions: High-risk HPV infection occurs in a broad spectrum of anal neoplasia in patients with IBD. Screening and surveillance for anal lesions should be considered in this population.

670 Serrated Colorectal Polyps in Inflammatory Bowel Disease

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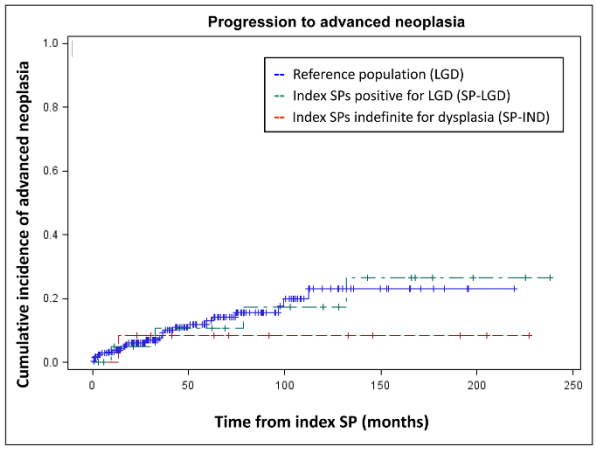
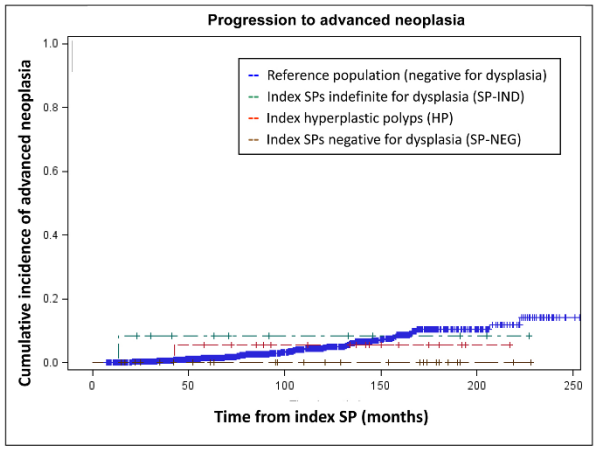
Background: Traditional serrated adenomas (TSAs) and sessile serrated adenomas/polyps (SSA/Ps), collectively serrated polyps (SPs), are presumptive precursors of at least 20% of sporadic colorectal carcinomas, however, their significance in inflammatory bowel disease (IBD) is unclear. We retrospectively characterized SPs in IBD with respect to morphology, sex, anatomical location, KRAS and BRAF mutational status and risk of neoplastic progression.

Design: 78 SPs resected endoscopically from 6,602 IBD patients undergoing surveillance endoscopy were evaluated. Actuarial rates of progression to high-grade dysplasia or carcinoma (advanced neoplasia, AN) among patients without prior or synchronous neoplasia were compared to reference IBD cohorts without SPs including a subset with conventional hyperplastic polyps (HPs).

Results: Most SPs without dysplasia (SP-NEG) occurred in the proximal colon in females and contained *BRAF* mutations. Most SPs with low-grade dysplasia (SP-LGD) and those graded indefinite for dysplasia (SP-IND) occurred in the distal colon in males and contained *KRAS* mutations. Morphologically, SP-NEG resembled sporadic SSA/Ps and SP-LGD resembled sporadic TSAs, whereas SP-IND comprised a mixed group. Rates of prevalent neoplasia associated with SP-NEG, SP-IND and SP-LGD were 11%, 39% and 76%, respectively (P=.0018). Ten-year actuarial progression rates to AN were SP-NEG (0%), SP-IND (8%) and SP-LGD (17%). The corresponding rates among patients with HPs and those in the reference patient cohort with no dysplasia or conventional LGD at baseline were 5%, 6% and 23% respectively.





Conclusions: In IBD patients with SPs, dysplasia grade correlates with morphology, sex, anatomical location, *BRAF* and *KRAS* mutation status, prevalent conventional neoplasia and risk of progression to AN. IBD patients with SPs resembling sporadic SSA/Ps are least likely to develop non-serrated dysplasia or carcinoma, whereas those with SP-LGD SP-IND may be at comparable risk as IBD patients with conventional LGD.

671 A TWIST To the Tale: Effect of TWIST1 and TWIST2 Promoter Methylation and Protein Expression in Tumor Stroma on the Tumor Budding Phenotype in Colorectal Cancer

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Background: Tumor budding may be the result of epithelial mesenchymal transition (EMT). This hypothesis is supported by loss of E-cadherin and up-regulation of E-cadherin repressors like TWIST1 and TWIST2 in colorectal carcinomas (CRC) with high grade tumor budding. Here we investigate a possible epigenetic link between TWIST proteins and the tumor budding phenotype.

Design: TWIST1 and TWIST2 promoter methylation and protein expression was investigated in tumor stroma and correlated with tumor budding and expression of EMT markers in two independent, well characterized patient cohorts (n_1=185; n_2=115). Laser capture microdissection (LCM) of tumor epithelium and stroma from low- and high-grade budding cancers was performed. Results were cross-validated with six human CRC cell lines. Patient cohort 2 (n=115) was used to assess prognostic effects.

Results: In both patient cohorts, TWIST1 and TWIST2 expression was highly restricted to stromal cells. In cohort 1, loss of stromal TWIST expression significantly corresponded with promoter hypermethylation. Based on LCM of tumor stroma an inverse correlation between tumor budding, TWIST1 and TWIST2 expression in the stroma and promoter methylation could be confirmed. All cell lines had marked methylation (TWIST1:55%-94%; TWIST2:87%-97%) and no protein expression of TWIST1 or TWIST2. TWIST hypomethylation in the stroma and high-grade budding correlated with EMT marker expression in tumor tissue (increased nuclear β-catenin, p=0.0061; ZEB2, p=0.008; loss of CDH1, p<0.0001 and CDX2, p=0.0002). On cohort 2, TWIST1 stromal cell staining predicted adverse clinicopathological features including advanced pT (p=0.0044), nodal metastasis (p=0.0301), lymphatic invasion (p=0.0373), perineural invasion (p=0.0109) and poor survival (p=0.0226).

Conclusions: Our results provide a link between TWIST promoter methylation in the tumor stroma and the tumor budding phenotype. Further studies are needed to investigate the mechanistic link between stromal cells and tumor budding in colorectal cancers.

672 Tumor Budding in Colorectal Cancer: A Multicenter Inter-Observer Study on Behalf of the Swiss Association of Gastrointestinal Pathology (SAGIP)

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Background: Tumor budding in colorectal cancer is recognized as an additional prognostic factor by the UICC. Diagnostic implementation has been delayed by disagreement on the optimal scoring method. Here, the Swiss Association of Gastrointestinal Pathology performs a multi-center inter-observer study on tumor budding to compare H&E with pancytokeratin (panCK) stains in the 10 high-power fields (10HPF) and hotspot method.

Design: Two serial sections of 50 TNM stage II-IV tumors were stained for H&E or panCK (AE1/AE3). Slides (n=100) were digitized and independently assessed at six centers in Switzerland and Austria using the 10HPF and hotspot method. Inter-observer assessment was calculated using the Pearson correlation (r) and intra-class correlation coefficients (ICC).

Results: In panCK stains, up to 3.7 fold more tumor buds were detected compared to H&E (p<0.0001). For H&E stained slides using the 10HPF method, correlation coefficients between centers ranged variably from r=0.46 to r=0.91, with an overall average r=0.64, ICC=0.58. The hotspot H&E method performed markedly worse with an overall r=0.55, ICC=0.49. In contrast, strong correlations were found using the 10HPF method in the assessment of panCK stained slides with values of 0.73£r£0.95 and an average overall r=0.85, ICC=0.83. Based on panCK stains, the hotspot method performed similarly well with correlation coefficients from 0.74 to 0.93 and an overall correlation coefficient of r=0.83, ICC=0.80.

Conclusions: Based on previous literature and findings herein, we recommend 1) use of panCK staining for the assessment of tumor budding 2) the 10HPF method for resection specimens and 3) the hotspot method for limited material (preoperative biopsy or pT1).

673 Construction of an Objective Pathological Diagnostic System of Blood and Lymphatic Vessel Invasion

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Background: Although blood and lymphatic vessel invasion (BLI) are strong prognostic factor, observation of BLI is known for its weakness of high inter-observer variability. The goal in this study is to create an objective pathological diagnostic system of BLI assessment.

Design: H&E slides with or without histochemical/immunohistochemical staining were assessed by Japanese pathologists and concordance of BLI assessment was checked. Next, histological findings associated with BLI having good concordance were reviewed. Based on these results, framework for developing diagnostic criterion was developed, using Delphi method. Constructed criterion was validated among 18 pathologists from 7 countries.

Results: Concordance was low for BLI diagnosis and was not any better when additional staining was provided. Although we failed to find H&E findings with good agreement, we found elastica and D2-40 findings with good concordance. Based on this observation, we developed a framework for pathological diagnostic criterion. Although this criterion improve concordance of in Japanese pathologists, that decreased consistency in pathologists from other countries. The decreased level of consistency was different between US/Canada and European pathologists.

Conclusions: A framework for pathological diagnostic criterion was developed by reviewing concordance and using Delphi method. Different effects of diagnostic criterion on pathologists from different countries can be associated with varied interpretations. International criterion should be constructed by participants from around the world.

674 Calretinin and D2-40 Staining in Mimics of Peritoneal Mesothelioma

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Background: Mesothelioma can mimic a variety of other neoplasms, and because it is a rare disease, a panel of markers is recommended to establish the diagnosis. The differential diagnosis of peritoneal mesothelioma includes widely metastatic gastric or pancreatic cancer. While it is well known that D2-40 is a positive marker for mesothelioma, it is not known whether a subset of gastric or pancreatic cancers might also stain.

Design: A total of 52 consecutive cases of surgically resected adenocarcinoma (26 each of gastric and pancreatic) from 2010 to 2013 were selected. Paraffin blocks were chosen based on amount of viable tumor present and sections were stained for calretinin and D2-40. Distribution of staining was recorded as 0 (completely negative result), 1 (1-25%), 2 (26-50%), 3 (51-75%), or 4 (76-100%). Similar scoring was applied to 52 consecutive peritoneal mesotheliomas for which D2-40 and calretinin stains were available for review. The slides were scored independently by two pathologists; discrepancies were resolved through consensus review.

Results: The staining results are summarized in the table below. Calretinin was found to have 100% sensitivity. Specificity was 79% when any staining (nuclear or cytoplasmic) is counted as positive, but 100% when only nuclear staining is considered positive. D2-40 was found to have 96% sensitivity and 94% specificity. A significant difference between adenocarcinoma and mesothelioma for both calretinin and D2-40 was identified (p <0.01).

	% positive calretinin	% positive D2-40	Mean score calretinin	Mean score D2-40
Mesothelioma (n=52)	100	96	3.89	3.66
Pancreatic adenocarcinoma (n=26)	31	4	0.42	0.12
Gastric adenocarcinoma (n=26)	12	8	0.27	0.08

Conclusions: While calretinin is a well-established marker for differentiating peritoneal mesothelioma from gastric and pancreatic adenocarcinoma, D2-40 is a newer marker with little data available evaluating this marker in gastric and pancreatic adenocarcinomas. Both calretinin and D2-40 are useful immunohistohemical stains for separating these malignancies. However, it is important to be aware that a subset of both gastric and pancreatic adenocarcinomas will show cytoplasmic staining with calretinin. Nuclear staining with calretinin is more specific for mesothelioma. Stains for D2-40 should be interpreted with caution due to staining of nerves, desmoplastic tumor stroma, smooth muscle, germinal centers, and lymphatics.

675 HER2 Testing in Advanced Gastric Cancer: Stringent Criteria for Amplification

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Background: The definition of "HER2+" G/GEJ cancer varies globally. We aim to establish a robust definition using our experience of centralised testing.

Design: The HER2 Testing Advisory Board (GaTHER) oversaw testing at 5 reference laboratories. IHC and ISH were performed in parallel. Data on cancer site, specimen type, IHC score, mean HER2 gene copies (CN), HER2:CEP17 ratio (R) and heterogeneity were collected. HER2 status was determined using a stringent variation of the GaTHER definition (GaTHERs; IHC2+ or 3+ and CN>6 and R>2) and compared with other published criteria.

Results: Of 891 samples tested 791 were included in the analysis; 10% were metastatic tumours. HER2+ rate was 15.7%, 15.3%, 14.2% and 13.4% as per FDA, CAP/EMA/ Asia-Pacific, GaTHERs and Gomez-Martin* criteria, respectively. Heterogeneity was noted in 34%.

	CN >6 & R >2, n (%)	ISH HER2 CN		ISH HER2:CEP17 ratio	
		mean (SD)	median (range)	mean (SD)	median (range)
IHC0, n=327	0 (0.0%)	2.18 (0.66)	2.0 (1.0-5.4)	1.13 (0.22)	1.1 (0.6-2.0)
IHC1, n=215	0 (0.0%)	2.20 (0.66)	2.0 (1.2-5.0)	1.12 (0.23)	1.1 (0.7-2.2)
IHC2, n=144	14 (9.7%)	3.24 (2.56)	2.6 (1.2-22.2)	1.48 (1.09)	1.2 (0.8-10.1)
IHC3, n=105	98 (93.3%)	15.79 (6.70)	15.1 (1.5-30)	8.39 (4.45)	7.3 (1-25)
GaTHERs "Negative", n=678	0 (0.0%)	2.28 (0.79)	2.1 (1-9.5)	1.14 (0.27)	1.1 (0.6-2.8)
GaTHERs "Positive", n=112	112 (100%)	15.78 (6.24)	14.8 (6.1-30) p<0.0001a	8.29 (4.27)	7.1 (2.6-25) p<0.0001a

[a]Wilcoxon-rank sum comparing + and - cases.

Conclusions: Variability of reported HER2+ G/GEJ cancer is partly due to lack of universal definition. IHC and ISH (by both CN and R) combined should be used to assess HER2 status. Discrepancies including true IHC3+ and nonamplified cases need personalised clinicopathological approach.

*Gomez-Martin et al JCO 2013:31:4445-52

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676 Patterns of Gastric Inflammation in Early Helicobacter Pylori Infection in Healthy Volunteers

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Background: The knowledge on pathological features of gastric inflammation due to *Helicobacter pylori* (HP) infection is mostly derived from chronically infected, symptomatic patients. The aim of this study is to describe the less well documented features of gastric inflammation and its relationship to bacterial presence and the host response following acute infection, based on a unique group of subjects who were voluntarily infected with HP.

Design: Thirty six HP sero-negative individuals with normal endoscopic and biopsy findings were enrolled into the trial. Thirty subjects were infected with 5 different *H. pylori* strains (6 each per strain). Six subjects were given placebo. Endoscopy was performed at 2 and 12 weeks post challenge and biopsy (antrum and corpus), serology and culture results were independently assessed in all 36 subjects. Inflammatory pattern and presence of HP organisms by histology were assessed blindly (MPK).

Results: Table 1: Summary of pathology findings and HP status at 2 and 12 weeks post challenge.

ACG=active chronic gastritis, LG=lymphocytic gastritis

	2 weeks		12 weeks	
	challenged (n=30)	placebo (n=6)	challenged (n=30)	placebo (n=6)
Activity	29	0	27	0
Inflammatory pattern				
ACG only	26	0	20	0
LG+ACG	3	0	8	0
No inflmmation	1	6	2	6
Presence of HP organisms				
Histology	18	0	15	0
Culture	26	0	19	0

LG pattern (n=8) compared to the ACG pattern (n=20) at 12 weeks correlated with transient infection, sero-conversion and few bacteria related to only 2 of 5 *H. pylori* strains. There was a trend to have higher numbers of eosinophils with the LG pattern compared to ACG pattern. Generally lymphocytes dominated the inflammation at 2 weeks with higher numbers of plasma cells at 12 weeks.

Conclusions: Active gastric inflammation persisted after clearance of HP organisms. Absence of bacteria in biopsies or by culture does not exclude "*H. pylori*-related gastritis". Plasma cells are not the most prevalent chronic inflammatory cell in early infection unlike the HP gastritis pattern seen in clinical practice. Our findings suggest that self-limiting HP infection may be responsible for a significant proportion of so called "non HP gastritis". Lymphocytic gastritis pattern appears to be related to specific HP strains.

677 CK7 Expression in Anal Squamous Cell Carcinoma Is Independently Associated With an Aggressive Disease Course

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Background: Recent studies have implicated the presence of "junctional-type" cells at the squamocolumnar junctions of the esophagus, cervix and anus. Additional work has found that a junctional phenotype in cervical dysplasia, characterized by CK7 expression, portends an aggressive disease course. This study examines the potential prognostic role of CK7 in anal squamous cancer.

Design: 30 cases of invasive anal squamous cell carcinoma (ASCC) were confirmed by three gastrointestinal pathologists. 27 of these cases have associated high-grade anal intraepithelial neoplasia (AIN), including 18 with conventional-type dysplasia and 9 with differentiated-type dysplasia. Sections from formalin-fixed, paraffin-embedded tissue were stained with antibody for CK7. CK7 positivity was defined as cytoplasmic expression in greater than 10% of malignant cells. The medical records were reviewed, identifying well documented follow-up information available on 24 patients with mean follow-up time of approximately 3 years.

Results: Positive CK7 expression was seen in 9 of 30 ASCC cases overall, and in 8 of 24 cases with available clinical follow-up information. In 4 (50%) of these CK7-positve cases, death due to ASCC was noted. In contrast, only 1 of 16 (6.25%) patients with CK7-negative tumors succumbed to disease. This difference was statistically significant. Other parameters, including age, gender, recurrence rate and associated AIN type, showed no statistically significant differences.

Table 1: CK7 Expression in ASCC

	CK7-Positive ASCC	CK7-Negative ASCC	P value
Total cases (n=30)	9	21	
Age (mean, years)	53.2	52.7	0.449691
M:F	2:7	12:9	0.118441
Initial T-stage (T1/T2/T3/T4)	3/2/1/2	6/5/5/1	
Low-stage at diagnosis (T1 or 2)	62.5%	64.70%	1
High-stage at diagnosis (T3 or 4)	37.5%	35.3%	1
Associated conventional AIN	42.1%	57.9%	0.195271
Associated differentiated AIN	11.1%	88.9%	0.195271
Recurrence rate	62.5%	66.7%	1
Death due to ASCC	50%	6.25%	0.0267668
Length of follow-up (average, years)	2.21	2.85	0.413631

Conclusions: As in cervical dysplasia, CK7 expression in ASCC predicts an aggressive disease course. Further study is needed to fully evaluate the role of CK7 as a prognostic biomarker in ASCC.

678 Intestinal Ischemic Necrosis Confers an Increased Risk of Death in Patients With Small Intestinal Neuroendocrine Tumors

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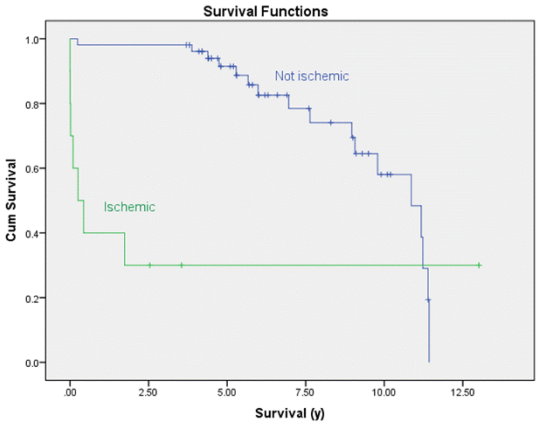
Background: A subset of patients with small intestinal neuroendocrine tumors (SINETs) show intestinal ischemic necrosis (IIN) at their initial resection. We sought to compare the pathologic features of SINETs with IIN to those without IIN and to assess the impact of IIN on patient survival.

Design: H&E slides of the initial resections of 10 SINETs with IIN and of 52 SINETs without IIN were collected from our surgical pathology archives over a 14-year period. Pathologic features were assessed including pT, pN, pM, tumor size, tumor grade (assessed by mitotic count), perineural invasion, angiolymphatic invasion, presence of multiple tumors, proximal and distal margin status, presence of mesenteric deposits, length of resected small bowel, and synchronous carcinoma. Survival information was analyzed by the Kaplan Meier method with statistical differences tested by the Cox proportional hazards model.

Results: The median age of patients with SINETs with IIN was 83.0 years, while that of patients with SINETs without IIN was 65.5 years (p=0.001). At one year post-resection, only 40% (4/10) of patients with IIN were alive, while 98% (51/52) of those without IIN were alive (p<0.001). Only IIN and age were significant adverse prognostic factors in univariate analysis. By multivariate analysis, IIN was associated with a 3.5-fold increased risk of death (p=0.01), while age was associated with a 1.1-fold increased risk of death (p=0.004).



Conclusions: Patients with SINETs complicated by IIN have a significantly higher risk of death than those without IIN. Although patients with IIN tend to be older, this effect is independent of age and other pathologic features. It is therefore important for pathologists to report the finding of IIN in resections of SINETs considering the significant association between IIN and decreased survival.

679 MicroRNA Expression Profiling for Prediction of Resistance To Neoadjuvant Radiochemotherapy in Squamous Cell Carcinoma of the Esophagus

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Background: microRNAs (miRNA) have been shown to play an important role in biology of malignant tumours, including sensitivity to chemotherapy and radiation. Neoadjuvant radiochemotherapy (RCTX) followed by surgery is a standard treatment strategy for locally advanced esophageal squamous cell carcinoma (ESCC). However, a subset of patients does not respond to RCTX. In the present study we evaluated whether miRNA expression profiling on pretherapeutic biopsies can predict resistance to RCTX in ESCC.

Design: 54 patients with locally advanced ESCC (cT3-4, cN1-3, M0-1) underwent preoperative radiochemotherapy with cisplatin, 5-fluorouracil and 30-45 Gy, followed by resection esophagectomy. Tumor response was evaluated by determination of histopathological tumour regression. MiRNA profiling was done in pre-therapeutic formalin-fixed and paraffin embedded (FFPE) biopsies using the Agilent Human Microarray platform (Release 16.0), encompassing 1205 human miRNAs on 31 samples. Differential miRNA expression was identified in responders (n=15) and non-responders (n=16) by applying appropriate biostatistics and validated by real-time quantitative PCR (qRT-PCR) in the larger collective of 54 patients.

Results: The miRNA expression profiles of pre-therapeutic ESCC biopsies within and between non-responders (n=16) and responders (n=15) were highly similar (average correlation coefficients r=0.96, 0.94 and 0.95), indicating a generally homogenous miRNA profile in (locally advanced) ESCC. However, 12 miRNAs were identified to be differentially expressed in non-responders (p≤ 0,025). In particular, non-responders showed an upregulation of six miRNAs (miR-1323, miR-3678-3p, miR-194*, miR-3152, miR-665, miR-3659) from 22 to at least 2- fold and a downregulation of four miRNAs (miR-126*, miR-484, miR-330-3p, miR-3653) from 10 to at least 2-fold. Additionally performed qRT-PCR analysis for specific miRNAs confirmed these microarray findings for miR-194*, miR-665, miR-3459.

Conclusions: Our results indicate that miRNAs are involved in therapy response to radiation and chemotherapy, and suggest that miRNA profiles could be used to predict response to RCTX in ESCC. The potential of miRNA regulation as potential therapeutic target warrants further investigations.

680 Effect of Medications on Distribution of Inflammation in Pediatric Ulcerative Colitis Patients Undergoing Colectomy

Margaret Lawless, Bonnie Cole, Michele Schaffer, Ghassan Wahbeh. University of Washington, Seattle, WA; Seattle Children's Hospital, Seattle, WA.

Background: Ulcerative colitis (UC) is associated with significant morbidity in children. In the pediatric population, UC most frequently involves the entire colon, less so in adults. There have been reports suggesting that anti-TNF therapy can result in preferential healing of the distal colon. The potential for medications to impact disease distribution is an important consideration during endoscopic evaluation and surgical management, particularly if patchy colonic involvement raises the question of Crohn's disease. We sought to examine whether exposure to anti-TNF therapy and other drugs before colectomy impacts the distribution of the inflammation within the removed colon.

Design: We performed a retrospective review of 27 colectomy specimens from a pediatric cohort between 2007-2013. Inflammation in the distal, transverse, and proximal colon segments was evaluated independently by two pathologists blinded to the patientsʼ treatment history. A modified Riley score was used to grade the degree of acute inflammation on a scale of 0-7. The patient's history was reviewed for administration of anti-TNF antibodies (infliximab, adalimumab), immunomodulators (6-mercaptopurine, azathioprine, methotrexate), prednisone, mesalamine, and antibiotics. Linear mixed-effects models were used to evaluate possible association between inflammation at various sites with medication use.

Results: There was no statistically significant differential effect of any medications on the distribution of inflammation. However, the direction of the differential effect of inflammation varied amongst medication types, with anti-TNF therapies and immunomodulators having lower scores in the distal colon compared to transverse and proximal colon, and mesalamine having higher scores in the distal colon compared to more proximal colon segments.

Conclusions: Overall, there was a trend towards lower inflammation in the distal colon when compared with the transverse colon in patients treated with anti-TNF therapies and immunomodulators. However, the differential effect of any particular medical therapy on acute inflammation scores was not statistically significant in this cohort. Larger studies may be necessary to determine whether these medications alter the pattern of inflammation in a subset of pediatric ulcerative colitis patients. Additionally, such differential healing may be more focused in the rectum which is not included a colectomy specimen in UC surgery.

681 Long-Term Outcomes in Appendiceal Goblet Cell Carcinoids Favor a 2- Instead of a 3-Tiered Grading System

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Background: Goblet cell carcinoid (GCC) is a rare appendiceal malignancy with both neuroendocrine and glandular features. The use of a 3-tiered histologic classification scheme that stratifies patients into the following prognostic groups has been suggested: typical GCC - group A; signet ring cell adenocarcinoma ex-GCC - group B; and poorly-differentiated adenocarcinoma ex-GCC - group C. However, the distinction between goblet and signet ring cells, and hence the distinction between groups A and B, is subjective and challenging. This study examined a large cohort of cases to determine whether it is appropriate to use a binary histological grading system for GCC.

Design: A continuous population-based cohort of GCC (n=87), diagnosed between 1980 and 2014, was identified from the British Columbia Cancer Registry and the Vancouver Lower Mainland pathology archive. Clinical data were obtained from a prospective database and available health records. Histology was classified into groups A, B, or C according to previously published criteria. Overall and recurrence-free survival (OS, RFS) were examined using Kaplan Meier analysis.

Results: A total of 87 cases were included and the median follow-up period was 43 months. The mean age was 55 years and 47% were male. The majority of cases were Stage II (63%) or Stage IV (23%) at diagnosis with only a minority being Stage I (2%) or Stage III (12%). By histologic classification, 60% were group A, 33% were group B, and 8% were group C. The strongest predictor of overall survival was the presence of metastatic disease (median OS 15.4 vs. 42.8 months, p=0.009). In the non-metastatic cohort, 5-year OS and RFS were similar between groups A and B (97.7% vs. 100.0%; 86.0% vs. 82.5%, respectively), while group C patients had worse prognosis (0% 5-year OS and RFS, p<0.01 for each). Among patients with metastatic disease, the presence of ³2 mitotic figures per 10 HPF was associated with poor outcome (2-year OS 10.0% vs. 60.0%, p<0.05).

Conclusions: In patients with GCC, the presence of metastatic disease is the strongest predictor of outcome. In non-metastatic cases, outcome was similar between groups A and B, whereas group C had a significantly worse outcome. A simplified 2-tiered grading system that stratifies GCC into low-grade (groups A and B) and high-grade (group C) tumors may be preferable over a 3-tiered scheme.

682 Adenocarcinoma Arising in Atrophic Autoimmune Pangastritis: Confirmation of Its Neoplastic Potential

Sun Mi Lee, Melissa Taggart, Dipen Maru, Susan Abraham. University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Atrophic autoimmune pangastritis (AAP) is a rare but distinctive form of atrophic gastritis that differs from both environmental and classical autoimmune gastritis. It is characterized by intense mucosal inflammation, persisting even in severely atrophic mucosa; diffuse involvement of antrum and body; lack of neuroendocrine hyperplasia; and lack of association with *Helicobacter pylori*. In the original publication (*Am J Surg Pathol* 2006;30:1412-9) low grade epithelial dysplasia was noted in one of 8 patients with AAP, suggesting that the condition could have neoplastic potential.

Design: We present 7 patients with gastric adenocarcinoma (AdCa) arising in AAP. Cases were collected prospectively (2) and retrospectively (5) by a computerized search through Surgical Pathology reports of gastrectomy specimens that were noted to have severe gastritis and/or severe glandular atrophy. Potential cases were reviewed by two pathologists and were included in the study only if both antrum and body demonstrated the aforementioned histologic features. Exclusion criteria included *H. pylori* infection (or history of treated infection) and receipt of neoadjuvant therapy. AdCa in the study cases was classified as intestinal or diffuse and was evaluated for degree of differentiation, location, and multiplicity. Background gastric mucosa was scored on a 0-3+ scale for degree of glandular atrophy, intestinal metaplasia, mononuclear infiltrates, and neutrophilic inflammation.

Results: Study patients comprised 5 (71%) males and 2 (29%) females aged 19-82 yr (mean 59 yr); one patient had common variable immunodeficiency and enterocolitis. Most AdCa's were of intestinal type (86%), poorly differentiated (71%), and multiple (57%, including 2 carcinomas in each of 3 patients and 3 carcinomas in another), with a slight predilection for gastric body/fundus (58%). All AdCa's arose in a background of moderate to severe (2-3+) glandular atrophy and moderate to severe (2-3+) mononuclear inflammation in a pangastric distribution. Intestinal metaplasia was moderate to extensive (2-3+) in most (71%) patients and demonstrated patchy loss of goblet cells in one. Neutrophilic infiltrates were typically of mild (1+) intensity (71%), but one patient had multiple erosions and another had a history of bleeding gastric ulcers.

Conclusions: Most AdCa's in AAP are intestinal-type and most arise in a background of multifocal intestinal metaplasia. These findings confirm that AAP has the potential for neoplastic transformation, and suggest that patients with AAP require endoscopic follow-up to exclude development of dysplasia and carcinoma.

683 Histologic Grading of Tumor Response in Posttherapy Pancreaticoduodenectomies for Patients With Pancreatic Ductal Adenocarcinoma

Sun Mi Lee, Huamin Wang, Asif Rashid, Anirban Maitra, Li Liu, Manonmani Sundar, Hua Wang, Matthew Katz, Gauri Varadhachary, Jeffrey E Lee, Robert A Wolff, Jason B Fleming, Peter Pisters. University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Neoadjuvant therapy has been increasingly used to treat patients with potentially resectable pancreatic ductal adenocarcinoma (PDC). Although the CAP grading system for tumor response in posttherapy specimens has been used, its clinical significance hasn't been validated. We have proposed a modified 3-tier CAP grading system based on our previous study, which showed that only patients with either complete pathologic response or microscopic residual disease in posttherapy pancreaticoduodenectomies(PD) had better prognosis. In this study, we sought to validate our modified CAP grading system for tumor response using a new cohort of patients.

Design: The study population consists of 167 consecutive patients with PDC who completed neoadjuvant therapy and PD between 2008 and 2012 (mean age: 65 years, 83 women and 84 men). Histologic tumor response (HTR) was graded using the 4-tier CAP grading system (Grade 0, no viable tumor; grade 1, ≤5% viable tumor cells; grade 2, 5-50% viable tumor cells; and grade 3, > 50% viable tumor cells). The results are correlated with clinicopathologic parameters and patient survival.

Results: CAP grade 0, 1, 2 and 3 responses were present in 3 (1.8%), 18 (10.8%), 95 (56.9%) and 51 (30.5%) patients respectively. CAP grade 0 or 1 HTR was associated with lower frequency of lymph node metastasis (P=0.004) and recurrence (P=0.01) and lower ypT (P<0.001) and AJCC stage (P<0.001) than those with grade 2 or grade 3 HTR. Patients with CAP grade 0 or 1 HTR had better disease-free survival (DFS, *p*=0.004) and overall survival (OS, *P*= 0.02) than those with either grade 2 or grade 3 HTR. All 3 patients with grade 0 HTR were alive with no evidence of disease at last followup. However, there was no difference in either DFS or OS between the group with grade 2 HTR and those with grade 3 HTR (P>0.05). In multivariate analysis, CAP grade 0 or 1 HTR was an independent prognostic factor for better DFS (P=0.025), but not OS (P=0.12).

Conclusions: Our study demonstrated that HTR is a key prognostic factor in patients with PDC who completed neoadjuvant therapy and PD. We propose to modify the CAP grading system for tumor response to a 3-tier grading system (Grade 0, no viable tumor; grade 1, ≤5% viable tumor cells; and grade 2, >5% viable tumor cells).

684 Indian Ink Tattooing for Tumor Visualization in Laparoscopic Colectomy Facilitates Lymph Node Harvest

Wen-Yih Liang, Pao-Shu Wu. Veterans General Hospital-Taipei, Taipei, Taiwan; National Yang-Ming University School of Medicine, Taipei, Taiwan.

Background: Status of lymph node metastasis is a very important prognostic factor in colorectal cancer. Indian ink (carbon dye) tattooing is a commonly used method preoperatively in laparoscopic colectomy to mark and visualize the lesion site. The aim of this study is to assess the effect of Indian ink tattooing on lymph node harvest and whether it can ensure adequate lymph node staging and provide a higher sensitivity in predicting metastasis and/or recurrence of the disease.

Design: A total 193 cases (39 cases with Indian ink tattooing as the study group and 154 cases without using any dye tattooing as the control group) of colorectal adenocarcinoma receiving colectomy in Taipei Veterans General Hospital in 1998 were analyzed retrospectively. The number of lymph node harvest, nodal status, disease free and metastasis/recurrence between the two groups were analyzed.

Results: The number of lymph node harvest was significantly higher in the study group with mean lymph node number of 26.2 vs. 16.6 (p<0.0001) and sufficient staging rate (number of lymph node harvest >=12) of 97% vs. 81% (p<0.05). The sampled lymph node number in stage I/II (N0) and stage III/IV (N1-2) also showed significant difference (24.12.0 vs. 15.60.8, p<0.0001 in N0 status; and 30.42.6 vs. 17.60.8 in N1-2 status, p<0.0001, respectively). The study group also has a higher disease-free survival rate (84.8% vs. 63.7% in all cases, p=0.0197; 91.7% vs. 76.8 in N0 status, p=0.1958; and 66.7% vs. 43.4% in N1-2 status, p=0.1958).

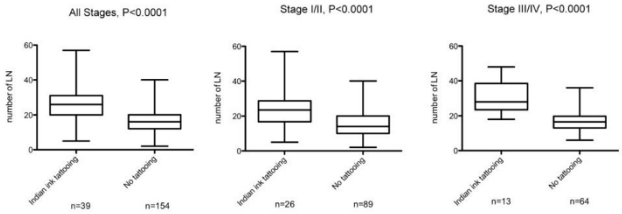

Figure 1. Number of harvest lymph nodes in Indian ink tattooing and conventional method.

Conclusions: Indian ink tattooing before laparoscopic colectomy is useful to mark the primary tumor site and significantly increased the number of lymph node harvest which can ensure adequate lymph node staging in colectomy and also provides a higher disease free survival rate compared to conventional lymph node sampling method.

685 pT3 Tumors in Colorectal Cancers With Elastic Lamina Invasion Should Be Upstaged To pT4a

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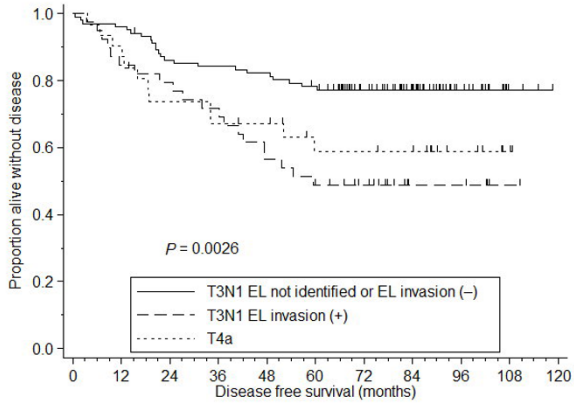
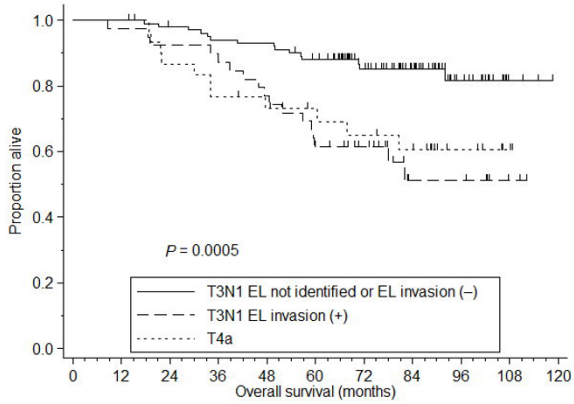
Background: Peritoneal involvement is an important adverse prognostic factor in colorectal cancer (CRC) and determines a shift in pTNM stage. Peritoneal involvement can be difficult to identify both macroscopically and microscopically. We had evaluated a cohort of pT3N0Mx CRCs for elastic lamina invasion (ELI) using an elastic stain in our previous study and found a significant prognostic different between two groups and there is no significant difference between pT3N0Mx cases with ELI and pT4aN0M0 in disease free survival and overall survival. The aims of this study were to further evaluate if the difference is also present in pT3N1Mx CRCs.

Design: One hundred and forty-one patients with T3N1Mx and 31 patients with T4aN1Mx CRC treated at the Taipei Veterans General Hospital between 2003 and 2007 were identified by a retrospective search of pathology records. Elastic stain was performed on the most deeply involved slide. Associations between peritoneal ELI and and outcomes (recurrence, metastasis, and survival) were evaluated.

Results: The elastic lamina was identified in only 62 cases (44%). Of those, 39 cases (27.6%) displayed ELI. This finding was associated with significantly worse (p<0.001) disease free survival (5 year DFS=48.7%) and significantly worse (p<0.001) overall survival (5 year OS=61.4%) compared to patients with no ELI (5 year DFS=73.9%, OS=95.7%) and those for whom no elastic lamina was identified (5 year DFS=79.5%, OS=85.7%). There is no significant difference between pT3N1Mx cases and pT4aN1Mx in disease free survival and overall survival. (p=0.4746 and 0.6464).





Conclusions: The findings in our current and previous studies suggest that application of an elastic stain is a significant prognostic marker which should be considered for routine use in pT3 CRCs either in the node negative or positive setting. Upstaging of pT3 tumors with elastic lamina invasion should be considered in future iterations of the AJCC/UICC TMN staging system for CRC.

686 miR-31 Expression in Ulcerative Colitis-Associated Dysplasia and Sporadic Adenoma: A MicroRNA Study

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Background: In patients with ulcerative colitis (UC), polypoid dysplastic lesions are morphologically and immunologically similar to sporadic adenomas. Our previous studies have shown that microRNAs, especially miR-31, are specifically expressed in UC.

Design: This study examines whether miR-31 can differentiate UC-associated dysplasia from sporadic adenoma. miR-31 enrichment by qRT-PCR was performed on formalin-fixed, paraffin-embedded colonic mucosa tissue from patients with a diagnosis of UC (n=36), noninflammatory bowel disease patients with sporadic adenoma (n=40), and patients with diverticular disease (n=29) as the "normal" control.

Results: The expression of miR-31 was significantly increased from cohorts of UC compared to the control ($P<0.05$). There was also a significantly different expression of miR-31 between the sporadic adenoma group and the control ($P<0.05$). However, there was no significant difference between the cohorts of UC and the sporadic adenoma ($P>0.05$).

Conclusions: Our study confirms that miR-31 is specifically expressed in UC. However, miR-31 expression is also elevated in some sporadic adenomas precluding it as a differential marker to distinguish UC-associated dysplasia from sporadic adenomas in formalin-fixed, paraffin-embedded colonic tissue.

687 Margin Status of Posttherapy Pancreaticoduodenectomy and Prognosis in Patients With Pancreatic Ductal Adenocarcinoma

Li Liu, Matthew Katz, Sun Mi Lee, Laurice Fischer, Manonmani Sundar, Hua Wang, Gauri Varadhachary, Robert Wolff, Jeffrey Lee, Peter Pisters, Anirban Maitra, Jason Fleming, Jeannelyn Estrella, Asif Rashid, Huamin Wang. University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Margin-negative (R0) resection is crucial for the prognosis in patients with pancreatic ductal adenocarcinoma (PDC). However, the definition of a negative retroperitoneal margin (RPM) varies. The American Joint Committee on Cancer (AJCC) defines a positive RPM when tumor cells are present at the margin, while the European protocol is based on the 1 mm clearance. The purpose of this study is to compare the prognostic significance of RPM in posttherapy pancreaticoduodenectomies (PD) using these two criteria.

Design: Our study group consists of 411 consecutive patients with PDC who completed neoadjuvant therapy and PD at our institution from 1999 to 2012 (mean age: 64 years; 183 females and 228 males). A standardized protocol was used to process all PD specimens. The entire RPM was submitted perpendicularly in all cases and distance to tumor was measured microscopically. The results were correlated with clinicopathologic parameters and survival using SPSS Statistics.

Results: Using the AJCC criteria, 32 (7.8%) had positive margin and 379 (92.2%) had negative margin. Among the margin negative group: RPM is £1mm in 66 cases and >1 mm in 313 cases. There is no difference in either disease-free survival (DFS) or overall survival (OS) between those with positive RPM and those with RPM £1 mm (P>0.50). However, those with RPM >1 mm had better DFS and OS than those with positive RPM (P<0.001) and those with RPM £1 mm (P<0.001). RPM>1 mm correlated with lower ypT (P<0.001) and AJCC (P<0.001) stage, and fewer lymph node metastasis (P=0.002) and recurrence (P<0.001). By multivariate analysis, RPM>1 mm, lymph node metastasis and tumor grade were independent prognostic factors for both DFS and OS.

Conclusions: Our study demonstrates that patients with RPM >1 mm had better prognosis than those with positive RPM and those with RPM £1 mm in PDC patients who received neoadjuvant therapy and PD. There is no difference in prognosis between those with positive RPM and those with RPM ≤ 1 mm. Therefore, our data provide strong support for RPM >1 mm to achieve an R0 resection in posttherapy PD specimens.

688 An Integrated Risk Stratification System Incorporating Paneth Cell Phenotype and Clinical Parameter Predicts Outcome in Post-Operative Crohn Disease

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Background: We have previously shown that Crohn Disease (CD) patients with significant Paneth cell defects (bad Paneth cell phenotype) correlates with CD susceptible genes (*ATG16L1*, *NOD2*), and is associated with immune-activation gene expression and prognosis after surgery. While the results were promising, the study was performed with enrichment in certain genotypes, and hence the predictive power of Paneth cell phenotype in a 'real life' setting remained untested. Moreover, the interplay of Paneth cell phenotype and other potential confounding factors in predicting the disease course have not been evaluated in detail. We hypothesized that an integrated risk stratification system incorporating Paneth cell phenotype and clinical factors may further classify CD into clinically distinct subgroups.

Design: We retrospectively analyzed all consecutive CD resection cases in our institution for 3 years (2011-2013). Demographics, age at diagnosis and surgery, clinical phenotype (Paris classification), smoking and treatment history were collected. Paneth cell was classified by lysozyme immunofluorescence, and bad Paneth cell phenotype was defined as ³20% of all Paneth cells with defect in granule number/distribution. The primary end point was time to endoscopic recurrence. Multivariate analysis was performed for analysis.

Results: A total of 121 patients were enrolled. The majority of patients (89%) were Caucasians, and 53% were males. The average age at surgery was 38 years old (y/o; range, 10-82y/o), and the average at diagnosis was 21 y/o (range, 9 – 59y/o). The majority (66%) of the patients had ileocolonic disease, and only 4 cases were of colonic involvement only. Most (91%) patients had fibrostenosing disease. Forty-nine patients (41%) were smokers. Only 23 patients did not receive postoperative prophylaxis. By multivariate analysis, we found that postsurgical prophylaxis, smoking, and Paneth cell phenotype each independently predicts prognosis (P=0.0359, 0.0223, and 0.0232, respectively), whereas age, gender, ethnicity, and clinical phenotype did not ($P>0.05$ for all). Among patients who received prophylaxis, stratification by smoking and Paneth cell phenotype showed that smokers with bad Paneth cell phenotype had the shortest time to recurrence (P=0.0081).

Conclusions: Paneth cell phenotype and smoking history synergizes to predict prognosis after surgery in CD patients. Employment of a simple integrated stratification system incorporating key clinical and pathologic parameters can allow for more accurate prognosis prediction in CD.

689 BATF2 Deficiency Promotes Progression in Human Colorectal Cancer Via Activation of HGF/MET Signaling: A Potential Rationale for Combining MET Inhibitors With IFNs

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Background: BATF2, a novel IFN-stimulated gene, inhibits tumor cell proliferation, invasion, and migration. The objectives of this study were to determine how BATF2 expression is associated with colorectal cancer (CRC) progression and patient outcome, to investigate how BATF2 overexpression inhibits HGF/MET signaling, and to elucidate the rationale for combining MET inhibitors with interferons (IFNs).

Design: BATF2 expression in CRC tissues was assessed to determine the association of BATF2 expression with CRC patient prognosis. BATF2 overexpression in CRC cells allowed for the assessment of its effects on tumor cell proliferation, invasion, and migration. The *in vitro* functional effects of BATF2 on HGF/MET signaling were examined. Tumor xenograft models were used to validate the effects of BATF2 on CRC xenograft growth and assess the efficacy of the combination of MET inhibitors with IFNs in CRC.

Results: In CRC tissues, BATF2 was found to be significantly downregulated, and its expression negatively correlated with MET expression. Decreased BATF2 expression was associated with progression and shorter patient survival in CRC. BATF2 overexpression promoted apoptosis and inhibited proliferation, migration, and invasion in CRC cells, as well as dramatically blunted tumor xenograft growth. Additionally, MET inhibitors in combination with IFN-β produced synergistic cytotoxicity both *in vitro* and *in vivo*.

Conclusions: Together, these novel findings suggest that BATF2, a suppressor gene, is a potent negative regulator of HGF/MET signaling, and these results provide a rationale for combining MET inhibitors with IFNs in preclinical trials.

690 Esophageal "Squamous Papilloma" – An Inflammatory Process Highly Associated With Eosinophilic Esophagitis and Gastroesophageal Reflux Disease

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Background: Esophageal squamous papilloma (SP) is a rare epithelial lesion most commonly found incidentally in the lower esophagus during endoscopy. Most SPs are small, exophytic growths composed of fibrovascular cores covered by mature squamous epithelium while a smaller number exhibit endophytic growth. An association of HPV, inflammatory or neoplastic processes with SP development is unclear and association between SP development and other esophageal disorders such as eosinophilic esophagitis (EoE) or gastroesophageal reflux disease (GERD) has not been established. Therefore, the etiology of SP remains elusive. We identified 97 SP cases, evaluated histologically diagnosed EoE, assessed HPV and reviewed clinical and endoscopic findings for correlation with SP development.

Design: SP was identified in 97 patients (59 female, 38 male, age 29-82) between 2002 and 2014. 66 had exophytic and 31 had inverted growth. Endoscopy and chart review for each patient was performed to identify previous esophageal findings. EoE was confirmed in previously diagnosed patients by identification of at least 3 high power fields with >15 intraepithelial eosinophils. Immunohistochemistry (IHC) for Ki-67, p53, and p16 and in-situ hybridization for HPV were performed on the most recent cases. Expression patterns of each marker were compared to those of normal control esophagus.

Results: Median SP size at endoscopy was 0.3 mm (range 1-5 mm). 50% of patients with SP had concurrent or previously diagnosed EoE and/or GERD. Concurrent EoE was identified in 17 cases (12 exophytic, 4 inverted) while 31 cases (21 exophytic, 10 inverted) had a clinical history of GERD. Each SP exhibited a typical morphology with occasional intraepithelial lymphocytes or eosinophils and no dysplasia. Ki-67 expression was limited to the proliferative zone, similar to control esophageal tissue. HPV, p16 and p53 were each negative in all cases examined.

Conclusions: We present the first SP large single institution cohort study and demonstrate that EoE and GERD are highly associated with SP development. Although recent studies suggest HPV may be associated with SP in the distal esophagus, we demonstrate that HPV is not associated with SP development and those associated

with EoE or GERD are more likely to occur more proximally. Our results indicate that inflammatory processes like EoE and GERD may play a significant role in SP development and that the underlying pathogenesis needs to be further elucidated.

691 Unique Histopathologic and Immunophenotypic Characterization of Achalasia in Heller Myotomy Specimens: A Single Institution Large Cohort

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Background: Achalasia is a primary esophageal motility disease affecting 1 to 3 per 100,000 people annually, which presents with progressive dysphagia, regurgitation, chest pain and weight loss. Studies suggest that achalasia is the result of aberrant immune response resulting in neuronal inflammation, loss of ganglion cells in the myenteric plexus and subsequent lower esophageal muscle relaxation. However, the exact morphologic alteration of this disease is not yet established. Heller myotomy is an important surgical approach to treat achalasia. Therefore, we evaluated 54 biopsy specimens from Heller myotomy cases and systematically analyzed the histology as well as the immunohistochemical profile of neuronal inflammation.

Design: Full thickness esophageal muscularis propria biopsies obtained during laparoscopic Heller myotomy were identified from 54 patients (mean age 45, range 15-84) from our institutional pathology database. Blinded biopsies were graded for presence or absence of muscular atrophy, inflammation and fibrosis. Neuron loss was analyzed and graded based on ganglion cell number similar (score=0), decreased (score=1) or absent (score=2) in comparison to normal controls. Immunohistochemistry for CD3, CD4, CD8, CD20 and FOXP3 were performed on all cases with inflammation.

Results: From this large cohort, 78% of cases showedneuronal loss, 11% demonstrated intramuscular and submucosal fibrosis and only 1% displayed muscle atrophy. Predominantly perineural inflammation was present in 17% of cases and consisted entirely of lymphocytes. Immunohistochemical analysis demonstrated that most inflammatory cells were CD3 and CD8 positive T cells (80-90%) with fewer CD4 positive T cells (10-20%). No regulatory T cells (Treg; FOXP3 positive T cells) were identified. Only 1 case displayed predominantly eosinophils and all cases were negative for CD20 positive B lymphocytes.

Conclusions: We present a single institution large cohort study evaluating morphology and inflammation in achalasia and demonstrate that increased neuronal loss is a dominant morphologic alteration. Although some studies suggest myopathy as a cause for achalasia we demonstrate that muscle damage or fibrosis is not, or may be a rare, cause for disease pathogenesis. Our results further demonstrate that CD8 positive T lymphocytes, with no Treg lymphocytes, are the predominant perineural inflammatory component and suggest that neuronal loss may be secondary to cytotoxic T cell mediated inflammation.

692 Histologic Changes in GI Biopsies From Patients With Idelalisib-Induced Enterocolitis

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Background: Idelalisib is a highly specific small-molecule phosphoinositide-3-kinase delta (PI3KΔ) inhibitor that was recently approved by the FDA for the treatment of chronic lymphocytic leukemia (CLL). The known side effects of idelalisib include severe diarrhea and colitis. Here we report the first description of the histologic findings in idelalisib-induced enterocolitis in CLL patients.

Design: Colon (and small bowel, when available) biopsies from 10 patients receiving idelalisib for CLL treatment were obtained over a 4 year period (2011-2014) at our institution. The histologic features of the 10 cases were documented, and immunohistochemical stains were performed on one case to characterize the lymphocytic infiltrate.

Results: All 10 patients were receiving idelalisib and underwent colonoscopy for a workup of diarrhea. None of the patients received a bone marrow transplant. Histologically, in the colon biopsies, all 10 cases showed some degree of apoptosis within crypts, with 5 cases showing severe apoptosis involving the majority of the crypts. Immunohistochemical stains for CMV were performed in 7/10 cases and were negative. No viral inclusions were seen in any case. 8/10 cases showed acute cryptitis, and 7 of these cases showed architectural distortion. A lymphocytic infiltrate within the lamina propria in addition to increased intraepithelial lymphocytes within crypts was seen in 5 cases; immunohistochemical stains performed on one of these cases showed the lymphocytes to be mostly T cells with a predominance of CD8+ T cells, with the majority expressing the alpha/beta T cell receptor. The possibility of graft-versus-host-disease (GVHD) was raised in error for one patient; in the others, diagnoses of infectious enterocolitis versus inflammatory bowel disease were strongly considered. Duodenal biopsies were available for three patients, and apoptosis was present to some degree in all biopsies. For two of the patients, numerous intraepithelial lymphocytes were noted in addition to severe villous blunting; the possibility of celiac disease was raised for both cases.

Conclusions: Histologic changes are frequently observed in patients presenting with diarrhea who are receiving idelalisib for CLL treatment. Awareness of the histologic features, including apoptosis, acute cryptitis, increased intraepithelial lymphocytes, and architectural distortion is important to distinguish idelalisib-induced enterocolitis from potential mimics including GVHD, inflammatory bowel disease, celiac disease, and CMV/infectious enterocolitis.

693 A Reappraisal of the Gastric Mucosal Pathology in Portal Hypertensive Patients

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Background: With the exception of portal hypertensive gastropathy (PHG), the detailed endoscopic and pathologic features of gastric mucosa of portal hypertension (pHTN) patients are largely unknown. We characterized the endoscopic and pathologic findings of gastric biopsies from pHTN patients and compared with those obtained from non-pHTN patients.

Design: The study group consisted of 550 patients with pHTN, the majority of whom (438/550, 80%) underwent upper endoscopy for esophageal varices surveillance in the setting of cirrhosis. The control group consisted of 281 individuals without pHTN; the main indication of endoscopy in the control group was nonspecific gastrointestinal symptoms (127/281, 45%). Two-sided Fisher's exact test was used for statistical analysis.

Results: Endoscopic findings seen exclusively in patients with pHTN included PHG (153/550, 28%; $P < 0.0001$), gastric varices (12/550, 2%; $P = 0.0108$) and congestion (9/550, 2%; $P = 0.0326$). Nodular / papular mucosa on endoscopy was also significantly more common than those in the control group (32/550 vs 4/281, $P = 0.0021$). In contrast, endoscopic findings of erythema (97/550 vs 105/281; $P = 0.0001$) and polyp (69/550 vs 53/281; $P = 0.0172$) were significantly less than those in the control group.

Histologically, while PHG and gastric antral vascular ectasia (GAVE) were diagnosed exclusively in the study group, they were infrequent findings (12%, and 1%, respectively). The most common histologic findings in pHTN patients were acute and/or chronic gastritis that was not associated with *Helicobacter pylori* and nonspecific reactive epithelial changes (41% and 29% respectively; $P < 0.0001$ compared to the histologic findings of PHG and GAVE). Compared to those in the control group, histologic findings of reactive epithelial changes (29% vs. 51%, $P < 0.0001$), proton pump inhibitor-related changes (15% vs. 30%, $P < 0.0001$), and malignancy (1% vs. 3%, $P = 0.0138$) were significantly less common in patients with pHTN. In contrast, hyperplastic polyps were more common in patients with pHTN (6% vs 3%, $P = 0.0314$).

Conclusions: Our results show that the classical endoscopic and pathologic features of PHG only represent a small fraction of pHTN cases. In contrast, there is a spectrum of endoscopic and pathologic findings in gastric biopsies in pHTN patients. The predominant pathologic features in pHTN patients include non-*Helicobacter pylori* gastritis and nonspecific reactive epithelial changes, both of which may be caused by an impairment of gastric microcirculation in pHTN patients.

694 Collagenous Gastritis: Do Children Present Differently From Adult Onset Patients?

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Background: Collagenous gastritis (CG) is a rare condition characterized by surface epithelial damage, subepithelial collagen deposition and a lamina propria inflammatory infiltrate. Previous studies have proposed two clinicopathologic subtypes: (1) children (£18 years old) presenting with severe anemia, nodular gastric mucosa and isolated gastric disease and (2) adults with chronic watery diarrhea that is associated with diffuse collagenous involvement of the GI tract. However, notable exceptions exist and there is a broad variability in clinical presentation, etiology, treatment response and disease course. In order to better define the clinicopathologic features of CG, we have collected 31 cases and describe their clinical, endoscopic, pathologic and follow-up findings.

Design: The study group consisted of 10 children and 21 adults with CG from 6 academic institutions over a 20-year period. Patient demographic information, presenting symptoms, medication use, endoscopic findings, histopathologic features (including subepithelial collagen thickness) and follow-up data were analyzed.

Results: Both children and adults presented with similar clinical symptoms including anemia (50% and 32%, respectively), epigastric/abdominal pain (50%, 47%) and diarrhea (40%, 58%). Endoscopically, a nodular gastric mucosa was frequently identified in both cohorts (75%, 44%). Extra-gastric collagenous involvement was also seen with comparable frequency (40%, 45%). Of note, one child presented with common variable immunodeficiency (CVID) and another with celiac disease. Concomitant autoimmune metaplastic atrophic gastritis (AMAG) was identified in 10% of adults. Olmesartan and Venlafaxine usage in adults was identified in 31% and 10%, respectively. Histologically, there were no differences in mean collagenous layer thickness, location of gastric involvement and eosinophilic infiltrate ($p > 0.05$). Follow-up information was available for 25 of 31 (81%) patients. Despite medical management in most cases, persistence of symptoms or CG on subsequent biopsies was seen in 100% of children and 75% of adults.

Conclusions: Contrary to prior reports, no clinicopathologic differences were identified among pediatric and adult patients with CG. While CG remains an enigmatic and heterogeneous condition, immune abnormalities, such as CVID, celiac disease and AMAG, or medication effects, such as Olmesartan and Venlafaxine, may be possible disease triggers.

695 Gastrointestinal Graft Versus Host Disease After Stem Cell Transplantation: A Comparison of the Diagnostic Yield of Biopsy Sites

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Background: Discordance in diagnosing gastrointestinal (GI) graft versus host disease (GVHD) between biopsies from the upper (U) and lower (L) GI tract is common. The biopsy site(s) that is more likely to yield positive results remains unclear. In this study, we sought to study the diagnostic yields of matched biopsies from both the UGI and LGI tract.

Design: The study group consisted of 80 GI GVHD cases from 78 post allogeneic hematopoietic stem cell transplantation patients (mean age: 48 years old; range: 2 – 69) from two institutions over the past 10 years. All patients had undergone simultaneous UGI and LGI biopsies; all were confirmed to have GVHD by clinical response to GVHD management. Chi-square test and one-way ANOVA were used for statistical analysis.

Results: Fifty-nine (74%) of 80 cases showed features of GVHD in both UGI and LGI tract, whereas UGI-only (up to proximal small bowel) GVHD was found in 6%, and LGI (including terminal ileum)-only in 20%. The prevalence of GVHD was the lowest in esophagus (35%) and significantly increased in the stomach (54%) and duodenum (83%; $P < 0.0001$). Prevalence was the highest in terminal ileum (95%) while a similar prevalence ($P = 0.5438$) was also detected in the colorectum including the right (75%), transverse (80%), left (85%) and sigmoid colon (95%), and rectum (89%). Overall, the prevalence of GVHD in LGI biopsies was statistically higher than that of UGI biopsies ($P < 0.0001$).

The histologic grade of GVHD among LGI biopsies was also significantly higher than that of UGI biopsies ($P < 0.0001$). The histologic grade was the lowest in esophagus (mean histologic grade = 0.7) and significantly increased ($P < 0.0001$) in stomach (0.9) and duodenum (1.6). The histologic grade among LGI biopsies increased from terminal ileum (mean histologic grade = 1.8) to the left (2.2) and sigmoid colon (2.5) and rectum (2.6), although this was not statistically significant ($P = 0.0602$). Overall, the histologic grades of LGI biopsies were higher than, the same as, and lower than, those of the UGI biopsies in 51%, 34% and 15% of cases, respectively.

Conclusions: Our results suggest that LGI biopsies have higher diagnostic yield for GVHD and also reveal more severe disease than UGI biopsies. Biopsies of the rectum, sigmoid colon and terminal ileum have similar high yield in detecting GI GVHD. Biologically, our results may suggest foregut derived organs such as the esophagus and stomach may be less susceptible to immune-mediated injury than organs derived from midgut or hindgut.

696 RAS Mutations Vary Between Lesions in Synchronous Primary Colorectal Cancer: Testing Only One Lesion Is Not Sufficient To Guide Anti-EGFR Treatment Decisions

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Background: Mutations in *KRAS* and *NRAS* are negative predictors of the response to anti-EGR therapies in the treatment of metastatic colorectal cancer. There are few reports on RAS testing in synchronous primary colorectal cancer (SP-CRC) and a lack of recommendations on which tissue should be tested for the mutation in this disease. In this study, we analyzed the *RAS* status of both lesions in SP-CRC patients and in their metastases.

Design: Study participants comprised patients of both genders and of all ages with a diagnosis of SP-CRC. DNA was obtained from one area of each patient's formalin-fixed paraffin-embedded tissue lesion, and *KRAS* and *NRAS* mutations in codons 12, 13, 61, 117 and 146 were analyzed by pyrosequencing.

Results: RAS status was heterogeneous in 6 (75%) of 8 SP-CRC patients between primary lesions. Five showed heterogeneity regarding *RAS* mutational status [1 lesion with wild-type (WT) *RAS* status and 1 mutated], and from these, four presented with metastasis: 3 cases (75%) had WT metastatic tissue, and 1 case (25%) had mutated metastatic tissue [table 1]One patient showed divergence regarding *RAS* mutation type.

Conclusions: *KRAS* and *NRAS* mutations vary significantly between SP-CRC lesions, and the status of the metastasis is unpredictable. Testing for *RAS* mutations in only 1 of the primary lesions in SP-CRC can misguide the clinical decision and hinder the interpretation of the prediction potential with regard to anti-EGFR treatment. Based on that, in a routine molecular pathology laboratory, a more appropriate approach in metastatic SP-CRC is to test the metastatic tissue or both primary lesions for providing more accurate mutation scenery and consequently supporting more assertive clinical decisions.

697 Lynch Syndrome Screening: Discordance in MMR and Germline Test Results

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Background: Increased screening for Lynch syndrome (LS) has identified patients with tumors that have deficiency in mismatch repair (MMR), but no germline mutations in genes encoding MMR proteins. This "Lynch-like syndrome" accounts for 2.5 to 3.9% of all patients tested and poses a dilemma for genetic counseling. To determine possible sources of error in MMR testing, discordant MMR and germline mutation results from a single institution that utilizes universal screening of colorectal cancer (CRC), endometrial cancer (EC) and ovarian cancer (OC) were reviewed.

Design: All tumors were studied for MLH1, PMS2, MSH2, and MSH6 mismatch repair protein deficiency (dMMR) by immunohistochemistry (IHC). Weak or partial tumor nuclear expression and/or weak or absent expression in adjacent stromal and/or lymphocyte nuclei was scored as equivocal. IHC was repeated on all cases with equivocal expression. Tumors with MLH1 deficiency underwent methylation analysis (EC, OC) or *BRAF* V600E mutation analysis (CRC). Targeted germline mutation analysis was performed for *MLH1*, *PMS2*, *MSH2*, *MSH6*, and *EPCAM*, when indicated.

Results: 21 cancers had discordant IHC and germline mutation results: 14 CRC, 5 EC, 1 OC, and 1 small bowel. The most common discordant results were in tumors with deficiency in MLH1/PMS2 (7, including 1 germline *APC* mutation and 1 hypermethylated but *BRAF* wild type), followed by deficiency in MSH2/MSH6 (6). One of 2 tumors deficient in MSH6 (MSH2 intact) harbored a germline mutation in *MSH2* while another tumor with equivocal expression for MSH6 (MSH2 intact) also had a germline mutation in *MSH2*. A germline mutation in *PMS2* was also identified in 1 of 2 tumors with equivocal PMS2 expression. Microsatellite instability (MSI) analysis in 5 of the remaining tumors without germline mutation showed 1 with MSI-L and 4 with MSI-H.

Conclusions: Potential explanations for discordant MMR and germline mutation results include germline mutations not detected by current screening methods, bi-allelic tumor DNA mutations in MMR genes, somatic mosaicism for a MMR gene mutation, and false positive screening test results. In absence of corroborating evidence for an underlying, undetected germline or somatic mutation (i.e., MSI-H, strong personal or family history, etc.), it appears there may be a small, but significant rate of false positive IHC screening results for patients with LS-associated tumors. Accurate interpretation of MMR IHC is critical as tumors with equivocal expression for PMS2 or MSH6 may harbor germline mutations. MSH6 is particularly problematic, as equivocal expression or isolated deficiency may rarely occur with germline *MSH2* mutation.

698 Human Papilloma Virus in Rectal Adenocarcinoma

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Background: Several studies have investigated the prevalance of human papilloma virus (HPV) in colon adenocarcinomas, often with contradictory results: using PCR HPV was identified between 0 and 97 percent of cases. We utilize a sensitive in hybridization (ISH) assay to examine for the presence of HPV in a cohort of rectal adenocarcinomas.

Design: 24 rectal adenocarcinomas and 22 adenomas resected between 2012 – 2013 were identified. ISH for HPV mRNA (E6 and E7 genes) was performed [QuantiGene® ViewRNA technology (Affymetrix, Santa Clara, CA)]. Immunohistochemistry for P53, P16 and mismatch repair proteins MLH1, MSH2, MSH6, PMS2 were performed. The ISH probes included a cocktail for high-risk HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 66). Demographic data was extracted from electronic medical records. 98.8 % of cases were subject to chemo radiation prior to resection.

Results: 6/24 (25%) were HPV +, 18/24 (75%) were HPV -. There was a male preponderance in the HPV + cohort (p=0.55)

HPV cohort	Mean age	Gender M:F	Precursor adenoma	Lymph node positive	P53 deletion	P 16
HPV +	52	5:1	0	83% (5/6)	33%(2/6)	100%(6/6)
HPV-	66	3:2	45% (8/18)	16% (3/18)	33%(6/18)	65%(11/17)
P value	0.085	0.55	0.025	0.005	NS	0.069

The HPV + cohort was younger than the HPV - cohort (P= 0.085). HPV + tumors were more likely to show nodal metastasis (p=0.005), and higher T stage (p=0.046). The HPV + cohort lacked a precursor lesion, while adenomas were identified in 8 (45%) of the HPV - cases (p=0.025). There were no histological features that distinguished the HPV + group from the HPV - group. 33% cases in both groups were P53 positive. P16 was more often positive in the HPV + cohort (P=0.069). Mismatch repair proteins were intact in 6 (100%) HPV + and 17 (99.5%) of HPV - cases. All colonic adenomas were negative for HPV.

Conclusions: HPV is detected in 25% of rectal adenocarcinomas. The younger age, p16 reactivity, male status, lack of precursor adenomas, advanced T stage and increased lymph node involvement suggests that the virus may identify a unique subset of rectal carcinomas. The HPV positive adenocarcinomas may show aggressive clinical behavior. Alternatively, these tumors are less responsive to conventional chemoradiation. A larger cohort is currently being investigated to further explore the biology of HPV + colon carcinomas.

[table1]

699 LINE-1 Overexpression Is a Hallmark of Gastrointestinal Cancers and Pre-Neoplastic Diseases

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Background: Long interspersed nuclear element-1 (L1), a retrotransposon, accounts for approximately 17% of the human genome. In a prior study, L1 encoded protein was identified in a broad range of human cancers but was not expressed in non-neoplastic tissue. In this study we explore a RNA based in-situ hybridization(ISH) assay for L1 in pre-neoplastic and neoplastic gastrointestinal (GI) tumors.

Design: ISH using probes aligned to the open reading frame 1 of L1 was performed on colonic adenomas (n=18), colon carcinomas (n=112), pancreatic ductal adenocarcinomas (PDAC) (n=94) and esophageal carcinomas (ECA) (n=21). To explore the diagnostic utility of the assay in dysplastic lesions, we evaluated samples of Barrett's esophagus (BE) negative for dysplasia (n=24), low grade dysplasia (LGD) (n=7) and high grade dysplasia (HGD) (n=17). Non-tumor samples (n=91) included esophagus (n=21), colon (n=26) and chronic pancreatitis (n=44). ISH was performed (QuantiGene® ViewRNA technology (Affymetrix, Santa Clara, CA) and scored as follows: 1 = equal staining intensity in stromal and tumor cells (Negative), 2 = increase in staining intensity of tumor cells with respect to stroma (Positive). We also quantitated the stain using BZ-

II analyzer software of BZ-9000 (Keyence - Biorevo). The difference in number of transcripts per cell between tumor and stroma represents the number of excess L1 RNA transcripts per tumor cell. Immunohistochemistry for MLH1, PMS2, MSH6 and MSH2 was performed on colon cancer cases.
Results: Positive L1 staining was identified in 82/112 (73%) of colonic carcinomas, 19/21 (90%) ECA and 91/94 (97%) PDAC. Negative staining in the colon carcinoma cohort correlated directlywith the presence of microsatellite instability (P=0.0001). All the non-tumor samples showed negative L1 staining. The colonic adenomas were uniformly positive for L1. All 18 non dysplastic BE biopsies stained negative; in comparison 6/7 (86%) LGD and 16/17 (94%) HGD cases were L1 positive. The L1 transcript per tumor cell was significantly higher in BE dysplastic cases (dysplasia vs. no dysplasia P=0.03); there was no difference between LGD and HGD (P=0.2).
Conclusions: L1 RNA expression is increased widely in GI malignancies. A positive L1 stain support a diagnosis malignancy and likely represents global DNA hypomethylation. Increased L1 RNA expression is seen in pre-neoplastic lesions of the GI tract. Increased L1 in BE associated dysplasia represents a novel biomarker for the diagnosis of dysplasia.

700 Inflammation in the Terminal Ileum in Ulcerative Colitis (UC) in Clinical Remission Is Not Due To Backwash Ileitis

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Background: In UC patients with intractable disease who have had a total colectomy, terminal ileitis is presumed to be due to backwash of colonic contents ("backwash ileitis"), but this has never been proven. In contrast, little is known regarding the prevalence and cause of ileitis in UC patients in clinical remission. The aim of this prospective study was to determine the prevalence rate and clinicopathologic associations of ileitis in UC patients enrolled in a surveillance program.
Design: The study cohort consisted of 72 UC patients and 90 healthy controls undergoing surveillance and screening colonoscopy, respectively. The endoscopic appearance and histology of the terminal ileum (both groups) and colon (UC group only) were evaluated in a standardized fashion. Biopsies were evaluated for the presence and degree of colitis and ileitis (inactive, mild, moderate or severe). Clinical and serologic data obtained was extensive and included clinical (Mayo score; 0-6) and endoscopic severity (Rutgeert's score; 0-3), medications (including probiotics) and NSAIDs, alcohol intake, type of bowel prep, smoking history, serum ANCA, ASCA, Prometheus, type of symptoms, and extra-intestinal manifestations (including PSC). The findings in the ileum were correlated with the clinical and serologic parameters.
Results: A significantly higher proportion of UC patients [Mean Mayo Score; 0.8, Rutgeert's Score; inactive (44%), mild (40%), moderate (15%), severe (0%)] had chronic active (N=1) or active (N=15) ileitis (total N=16) vs controls [N=4(4%), p=0.003 vs UC]. There were no significant differences between the UC group and controls regarding any clinical or serologic features, including medication use (such as NSAIDs), alcohol use, type of bowel prep, smoking and alcohol intake. In a comparison of UC patients with, versus those without, ileitis, no association was noted with any of the clinical or serologic parameters, including medication use, type of bowel prep, length and severity of colitis, including the presence or degree of cecal inflammation. In fact, only 4 of the 16 UC patients (25%) with ileitis had active inflammation in the cecum and in all 4 it was mild. None of the patients has developed Crohn's disease (CD).
Conclusions: Based on our data, ileitis may represent a primary manifestation of UC in patients in clinical remission. UC patients with ileitis should not have their diagnosis changed to CD due to the presence of ileitis in biopsies.

701 Pseudomyxoma Peritonei of Appendiceal Origin: Molecular Abnormality Characterization

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Background: Pseudomyxoma peritonei (PP) is a secondary tumor of the peritoneum, resulting from rupture of a mucinous lesion in the appendix called "Low grade Appendiceal Mucinous Neoplasm" (LAMN). This rare tumor with difficult nosologic classification (closer to a borderline than an authentic malignant tumor) has poor prognosis without maximally invasive chemo-surgical treatment but with significant postoperative complications. The aim of this project was to characterize the molecular features of PP from a homogeneous series of 14 cases.
Design: Molecular characterization of PP was performed on normal and tumor DNA of 14 patients. We used a targeted approach by primer-extension reaction (SNaPshot®), Quantitative Multiplex PCR of Short Fluorescent Fragment (QMPSF), and search for microsatellite instability (phenotype RER for Replication ERror) and whole genome by complete exome analysis (Next Generation Sequencing NGS).
Results: According to literature data, the majority of PP in our series (57%) carried *KRAS* mutations, starting with their in situ stage in the appendix. The novelty is the presence of *NRAS* mutation in one PP (7%). There was no MCL1 or JUN gene coamplification (litterature data) detected by QMPSF but isolated *MCL1* amplification, gene involved in the control of apoptosis. No microsatellite instability was detected in any tumors in our cohort. In addition, the complete exome sequencing of one sample (never performed until now) revealed GNAS gene mutation (already described in literature on these tumors) and mutation in *RYR3* gene (never reported to date for these tumors). *RYR3* appears to be a perfect candidate relevant to its oncogenic implications which are already well known (in mammary carcinoma among other things) and its role in the control of intracellular calcium level. Mutational *RYR3* hotspots will thus be studied by primer-extension reaction on all PP of the series and the results will be presented.
Conclusions: Characterization of molecular abnormalities of PP is necessary to better understand the oncogenesis of this tumor by detecting new genes of interest for the development of targeted therapies and ultimately perhaps to avoid maximally invasive treatment for these patients.

702 Prognostic Stratification of Nodal Negative Colon Cancer By a New Morphology Based Risk Score

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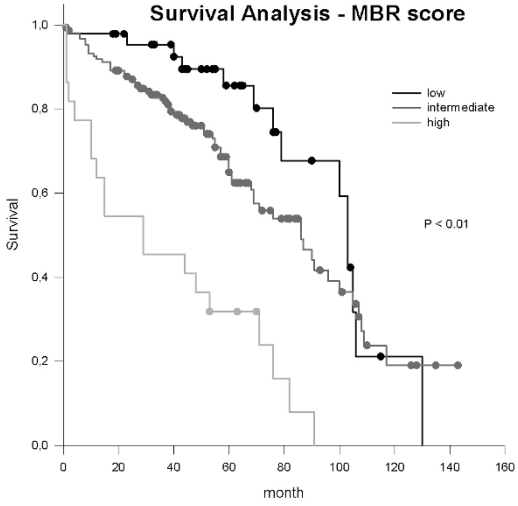
Background: Lymph node staging is still of crucial importance in colon cancer. The occurrence of lymph node metastases are associated with worse outcome compared to nodal negative cases. However, it is also well known that about 20% of nodal negative cases show an aggressive course. Oncologist, therefore, ask for additional prognostic factors to identify these patients with increased risk. Despite huge efforts none of many suggested molecular markers found general acceptance to serve as stratification tool in nodal negative cancer. We hypothesized that a simple morphology based risk (MBR) score could be an appropriate alternative. Besides conventional factors the paucity of medium and large sized lymph nodes (LN) was included to enhance the prognostic accuracy.
Design: A retrospective study was performed enrolling 218 nodal negative colon cancer cases collected in the time between 2002 and 2005. The MBR score included the factors pT stage (1/2 vs.3/4), invasion type (expansive vs. infiltrative) tumor budding (none/low grade vs. high grade) vascular invasion (L/V0 vs. L/V1) and paucity of medium and large sized LNs. The latter factor is defined as < 2 LNs of > 5 mm in diameter. Zero or one point was given for each factor depending on its quality (Score range: 0 – 5 points). Based on the score the cases were categorized into one of three groups: low MBR (0-1 point), intermediate MBR (2 points) and high MBR (3-5 points).
Results: 47 (22%), 149 (68%) and 22 (10%) cases were categorized into the MBR low, intermediate and high group, respectively. The corresponding median overall survival times differed highly significantly (P < 0.01) between the three groups with 103, 86 and 29 month, respectively.



Conclusions: The MBR score is easy to assess and could be shown as prognostic in stage I/II colon cancer. The cases of the MBR high group show an aggressive course. These patients could benefit from adjuvant chemotherapy.

703 CD44v9 Is Expressed in Human Duodenal Carcinoma and CD44v9-Positive Cancer Cells Exhibit Stem Cell-Like Features

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Background: Cancer stem cells are associated with cancer therapy resistance. CD44 in particular has been identified as a marker for stem cells. The splicing variant of CD44 is associated with the prognosis and cancer stage in colon cancer. However, little has been reported on CD44 variant 9(v9), one of the CD44 splicing variants in duodenal carcinoma. Here we report the clinicopathological characteristics of CD44v9-positive tumor cells in duodenal carcinoma.
Design: We evaluated clinicopathological features of 34 duodenal carcinomas and assessed CD44v9 expression by immunohistochemistry. Double staining with CD44v9 and Ki67, and CD44v9 and cleaved caspase 3 were performed. In immunofluorescence analysis, high density and low density areas of CD44v9-positive cells were selected as CD44v9-positive and -negative areas, respectively. Three CD44v9-positive areas and three CD44v9-negative areas were chosen and evaluated for positive Ki67 and cleaved caspase 3 expression.
Results: All cases had CD44v9-positive tumor cells except one case. The degree of CD44v9-positive cells varied. We did not observe any correlation between histological type and CD44v9-positive tumor cells. A positive correlation, although weak, was found between the degree of CD44v9-positive tumor cells and inflammatory cell infiltration (r=0.3997, $P < 0.0234$). The percentage of cells that showed Ki67 positive staining was significantly lower in CD44v9-positive areas (29.51%; 14.94–46.99) compared with CD44v9-negative areas (54.49%; 43.25–66.8) (P < 0.0001). The percentage

of cells that showed cleaved caspase 3 positive expression was significantly lower in CD44v9-positive areas (1.35%; 0–2.61) compared with CD44v9-negative areas (11.09%; 4.25–19.77) ($P < 0.0001$).

Conclusions: The degree of CD44v9 positive cells might be affected by inflammatory cell infiltration in duodenal carcinoma. This resistance is one of the characteristics of stem cells. CD44v9-positive cells are more likely to have lower Ki67 expression, and the mitotic ability of CD44v9 positive cells may be low. CD44v9-positive cells are also more likely to have lower cleaved caspase 3 expression, and CD44v9-positive cells may have the property that apoptosis is hard to have. These characteristics suggest the possibility that CD44v9-positive cells may be stem cells in duodenal carcinoma.

704 Clinicopathologic Comparison of Colorectal Carcinoma in Patients With Suspected Lynch Syndrome without Germline Mutation (Lynch-Like Syndrome) versus Patients with Germline Mutation (Lynch Syndrome)

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Background: Microsatellite instability (MSI) and mismatch repair (MMR) protein immunohistochemical analysis of colorectal carcinoma (CRC) identifies patients suspected of having Lynch syndrome (LS). Some patients with suspected LS have no germline mutation of MMR genes and are classified as Lynch-like syndrome (LLS). The aim of this study is to compare the features of CRC in patients with LS and LLS.

Design: 3213 patients with CRC were analyzed and if ³2/6 markers showed instability by PCR and/or the tumor displayed abnormal MMR protein expression, the tumor was classified as MSI-H. Patients with CRC with loss of MSH2/MSH6, isolated loss of MSH6 or PMS2, or loss of MLH1/PMS2 expression with concurrent wild-type *BRAF* and negative *MLH1* promoter hypermethylation were referred for germline sequencing and rearrangement analysis for suspected LS. All CRCs were analyzed for grade, location, tumor infiltrating lymphocytes (TILs), Crohn's-like reaction, mucinous / signet ring / medullary histology, and precursor lesions.

Results: 337/3213 CRC (10.4%) demonstrated MSI-H and 114 patients (3.5%) were classified as suspected LS. 48 of the suspected LS patients underwent germline genetic mutation analysis. In 33 patients (69%) a deleterious germline mutation in a MMR gene was identified with germline mutations in *MSH2* (19), *MLH1* (5), *MSH6* (7), and *PMS2* (9). 15 patients (31%) were classified as LLS with no germline mutation identified and harbored CRC with loss of MLH1/PMS2 (6), loss of MSH2/MSH6 (7), and isolated loss of PMS2 (3) expression. No LLS patients had CRC with isolated loss of MSH6 compared with 18% of LS patients (p=0.08). Patients with LLS more frequently had right-sided CRC compared with LS patients (93% vs. 45%, p=0.002). Patients with LS more frequently had synchronous CRC compared with LLS patients (21% vs. 7%) (p=0.10). There was no significant difference in age, gender, stage, tumor histology, and precursor lesions between CRC in LS and LLS patients.

Conclusions: 31% of patients with suspected LS will lack a germline MMR gene mutation. Compared with LS, patients with LLS are more likely to have right-sided CRC and less likely to have synchronous CRC. Importantly, our results indicate that patients with CRC demonstrating isolated loss of MSH6 expression or left-sided tumor location are more likely to have LS confirmed by germline mutation analysis.

705 'BowelScreen': The Polyp Experience in a Screening Centre in the Irish FIT Programme

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Background: The Irish National Cancer Screening Service (NCSS) commenced colorectal cancer screening ('BowelScreen') in 2013 & it is the first National Programme based on faecal immunohistochemical testing (FIT).

This prospective observational study aims to report the initial experience of the pathology of polyps, lesions and tumours detected endoscopically at 'BowelScreen' at a single institution by analyzing prospectively collected data using standardized proforma reporting.

Design: 'BowelScreen' patients were highlighted using a unique 'BowelScreen' identifier on the laboratory requisition forms.

Data was prospectively extracted from the standardized histopathological reports.

Microsoft Excel was used to record data and to collate results.

Results: Between 20th February 2013 and 31st August 2014, 1061 histology samples were received on 356 patients, with an average weekly workload of 4 cases, with an average of 3 samples per case.

During this 18 month period, 869 polyps and 23 cancers were confirmed histologically. Most polyps (64.2%) were diminutive (<5mm), and the majority were adenomas (74.8%). The high grade dysplasia rate was 1.7%. The adenoma architecture was villous in 0.3%, tubulovillous in 9.7% and tubular in 90% of adenomas. Epithelial misplacement was present in 5.7% of adenomas but did not pose diagnostic difficulty in small polyps. 15.4% of adenomas were > or = 10mm (intermediate or high risk for surveillance, as per European Guidelines). 32 patients (9%) had > or = 5 adenomas (high risk for surveillance).

Sessile serrated polyps accounted for 5.5 % of polyps. Only 1 traditional serrated adenoma was detected.

Margins for dysplasia could only be assessed in 68.4% cases & were involved in 1.3%.

Conclusions: Comprehensive data analysis of pathological data has the ability to inform both clinical and policy decision-making in a National FIT programme.

706 Lymph Node Ratios as a Predictor of Outcome in Stage III Colorectal Carcinomas

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Background: Nodal disease is an adverse feature in colorectal carcinoma (CRC). Further refinement of this pathological feature may add prognostic information and inform clinical decision making. We evaluated whether lymph node ration (LNR, ratio of positive lymph nodes to overall lymph node count) added prognostic information in CRC.

Design: All CRC surgical specimen reports from 1996-2012 in St Vincent's Healthcare Group were identified and analysed. Nodal counts including total node numbers, positive node numbers and negative node numbers were counted in the total cohort. LNRs were calculated only in stage III tumours. Five & 10 year survival data, overall and cancer specific was calculated. Statistical analysis included Chi Squared testing, Kaplan-Meier curves and Cox regression analyses using SSPS 18.0.

Results: 1909 CRC surgical cases were identified. 433 were clinical stage III. The mean total node count was 13.6 (range 0-66). For stage III CRCs , the mean negative node count was 11.8 (range 0-65) and lymph node ratio was 0.245 (range 0.2-1). High LNR was significantly associated with perineural invasion, tumour grade and peritoneal involvement. High LNR in stage III (14.8% of cases) was associated with poorer survival on both univariate and multivariate analysis (p<0.0001). High LNR was associated with decreased survival using Cox Proportional Hazards (HR 2.7, (5% CI: 1.393-5.237), however when LNR, positive node numbers and negative node numbers are compared using multivariate analysis in the entire cohort, only positive node numbers remains an independent predictor of survival.

Conclusions: Lymph Node Ratio predict survival in stage III CRCs but is not superior to the number of positive nodes which remains the strongest predictor in this cohort of patients.

707 Evaluation of Fibrosis Patterns and Glutamine Synthetase Staining in Congestive Liver Disease

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Background: Liver pathology in congestive liver disease (CLD) includes sinusoidal dilation, congestive necrosis, and vascular injury. The development of fibrosis in CLD can be heterogeneous and difficult to assess on biopsies due to sampling. Glutamine Synthetase (GS), which normally stains pericentral zone 3 hepatocytes, has been reported to be altered in CLD. However, GS correlation with histologic features has not been well described. The goal of this study is to evaluate liver biopsies in CLD for GS staining alteration, fibrosis, nodular regenerative hyperplasia (NRH) features and assesses correlations between GS staining and features of CLD including fibrosis.

Design: Fibrosis was scored as follows 0: none, 1: mild/zone 3, 2: septal and 3: advanced, including cirrhosis. Vascular injury was evaluated based on % of portal tracts with loss of portal veins, % loss of hepatic veins (0: no loss, 1: loss < 1/3, 2: loss 1/3-2/3 and 3: loss > 2/3), sinusoidal dilation (0: none, 1: Zone 3, 2: Zone 2 and 3: Pan-zonal) and congestive necrosis (1: present or 0: absent). NRH features and fibrosis heterogeneity were also recorded. GS staining was assessed by scoring zone 3 staining (0: No staining, 1: reduced, 2: Normal).

Results:

Table 1.

Fibrosis and NRH	# Cases (%)Total 40 cases
Any fibrosis	34 (85%)
Advanced fibrosis	12 (30%)
Advanced fibrosis and NRH	2 (17%)
Subtle NRH	18 (45%)
Obvious NRH	6 (15%)

Heterogeneity of advanced fibrosis in CLD liver biopsies (9 cases, 22%) was seen between biopsy cores and within cores.

Table 2.

GS immunohistochemistry	# Cases (%)Total 40 cases
GS decreased or absent	30 (75%)
Absent GS	14 (40%)
GS Loss associated with increased sinusoidal dilation	r = -0.415, P = 0.008
Sinusoidal Dilation association with Vascular Injury Score	r = 0.494, P = 0.001

Reduced GS staining did not correlate with the degree of fibrosis (P = 0.33).

Conclusions: Assessment of fibrosis severity from liver core biopsy in CLD may not be reliable due to heterogeneity, and may require multiple biopsies and correlation with clinical findings.

NRH pattern and advanced fibrosis can coexist and are not mutually exclusive; each caused by the vascular alterations in CLD.

GS staining pattern corresponds to the severity of the vascular injury, but may not provide additional information in the assessment of the severity of fibrosis.

708 TWIST2 Protein Overexpression Is Associated With Aggressive Disease in Colorectal Adenocarcinomas (CRCs)

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Background: TWIST2 (twist basic helix-loop-helix transcription factor 2)is one of the basic helix-loop-helix (bHLH) transcription factors that have been implicated in cell lineage determination and differentiation. TWIST2 expression functions as an inhibitor of osteoblast maturation and has been found to be upregulated in certain types of cancer. The prognostic significance of TWIST2 expression in CRC has not been extensively studied.

Design: Formalin-fixed paraffin-embedded tissue sections from 106 CRC were immunostained by a manual method (DAKO EnVision+ Dual Link System-HRP) using mouse monoclonal DERMO1 (TWIST2, Abnova, Taipei, Taiwan). Cytoplasmic and/or nuclear immunoreactivity was scored based on intensity and percentage of positive tumor cells in both the tumor and adjacent benign epithelium (when present) in each case. Results were correlated with histologic and prognostic variables.

Results: TWIST2 immunoreactivity was predominately cytoplasmic and variably expressed. Intense, diffuse cytoplasmic overexpression was noted in 30/106 (28%) CRC and correlated with histologic grade (40% grade 3; 26% grade 2, and 0% grade 1, p=0.019); advanced (stage III, IV) vs early (stage I, II) tumor stage (38% adv vs 20% early, p=0.036); positive lymph node status (38% LN+ vs 14% LN-, p=0.007); and overall survival (37% expired vs 11% alive, p=0.005). On multivariate analysis, positive lymph node status (p<0.0001) independently predicted shortened overall survival.

Conclusions: Cytoplasmic overexpression of TWIST2 correlated with high histologic grade and advanced tumor stage in CRC as well as with positive lymph node status and overall survival. Further study of the prognostic significance of TWIST2 overexpression and its potential to serve as a target of therapy for CRC appears warranted.

709 High Incidence of Kras and GNAS Mutations Could Represent a New Insight of Biology of Pseudomyxoma Peritonei

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Background: PMP refers to peritoneal involvement by appendiceal mucinous tumors. WHO distinguishes high grade and low grade PMP only based on morphological criteria. Post-surgical PMP survival rate is 86% in low grade and 50% in high grade PMP. Elucidation of the molecular mechanisms driving mucinous tumors of appendiceal origin to PMP is a priority to identify new prognostic and predictive biomarkers. To date, only KRAS has been supposed to have a role in mucinous differentiation pathway in colorectal cancer. Given that the histological architecture in which tumoral cells may be deceptively bland and sparse within an abundant mucinous background, we aimed to perform a biological characterization of pseudomyxoma peritonei, using a high-sensibility gene sequencing technique.

Design: From our monoinstitutional series of 206 patients with PMP homogeneously treated with Cytoreductive Surgery with Peritonectomy Procedures and Hyperthermic Intra Peritoneal Chemotherapy, 45 subjects signed informed consent for this biological study. KRAS gene status was assessed in all cases by direct sequencing of exons 2, 3 and 4. The wild type exon 2 cases were subsequently analyzed through a specific mutant enriched PCR technique that is more sensitive in detecting codon 12 /13 KRAS mutations. To widen the spectrum of possible alterations of genes involved in PMP evolution, 13 cases (9 mutated and 4 wt for KRAS gene, respectively) of PMP were analyzed by NGS (Iontorrent technology, PMG) by using the Hot-spot cancer panel.

Results: According to WHO classification, 29 were low and 16 high grade tumors. *KRAS* mutations were detected in 38 cases (84%) and were constituted mainly by codon 12 mutations (90%), principally causing the pG12D substitution, and were not correlated with grading. Sequencing of exon 4 lead to identification of A146V mutation in one case. NGS analysis confirmed *KRAS* status in all cases and revealed also pR844H GNAS mutation in almost half of cases (6/13=46%). Moreover, we observed TP53 (23%), PIK3CA (8%) and APC (8%) mutations.

Conclusions: PMP carcinogenesis is a multifactorial and complex process, linked to high frequency of *KRAS* mutations. Further studies are necessary to explain the role of GNAS mutations. Molecular study in PMP should be suggested in order to select mutations useful to drive a PMP tailor made treatment.

710 Genotype Driven Anti-Cancer Therapies for Colon Cancer

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Background: Colon adenocarcinoma patients who have exhausted standard-of-care are likely to benefit from next-generation sequencing analysis. Large NGS targeted panels provide more therapeutic options with off-label and investigational drugs specifically designed to target the patient's molecular profile (genotype). In this study, we provide data in support of such an expanded and targeted gene panel.

Design: DNA from ten colon adenocarcinoma FFPE samples was sequenced on Illumina HiSeq 2500 or MiSeq sequencers using a CLIA-certified 358-gene targeted sequencing assay. FASTQ files generated from Illumina's CASAVA software were submitted to a Clinical Genome Analytics (CGA) data analysis pipeline to perform automated read quality assessment, alignment, and variant calling for SNPs and in/dels. Clinical annotations for actionable variants and therapeutic interventions were curated through extensive literature searches and a comprehensive clinical report was written for each sample. Actionable variants were defined as: 1) gene variant associated with an approved drug in colon cancer, 2) gene variant associated with an approved drug in another tumor type, 3) gene variant associated with a drug that is in an active colon or solid tumor clinical trial, and/or 4) gene variant confers resistance to a drug.

Results: An average of 2.7 actionable variants were identified per sample with KRAS mutations in exons 12 and 13 being the most common. The average number of therapeutic options was 1.5, 10.7, and 15.4 for FDA approved drugs in colon cancer, FDA approved drugs in other tumor types, and investigational drugs in clinical trials, respectively. At the time of clinical reporting, an average of 70.3 clinical trials were actively recruiting either for the patient's specific molecular profile and/or utilizing therapies targeting the patient's molecular profile. Common FDA approved therapeutic options included bevacizumab and regorafenib, while options for off-label drugs were everolimus, vandetanib, cabozantinib, ponatinib, trametinib, sorafenib, sunitinib, dabrafenib, and temsirolimus.

Conclusions: Expanding small-targeted gene panels to larger next-generation sequencing panels increases the therapeutic options for the oncologist. Colon cancer patient biopsies subjected to a 358-genel panel had an average of 10.7 and 15.4 off-label and investigational therapies available, respectively.

711 Is the Routine Microscopic Examination of Proximal and Distal Resection Margins in Colorectal Cancer Surgery Justified?

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Background: Colorectal cancer (CRC) is the second most common cause of cancer death in the United States. Complete surgical excision of all detectable tumor is verified by the pathologist's examination and documentation of the tumor-free status of all surgical margins. Microscopic examination of the proximal and distal resection margins is part of the routine pathologic evaluation of colorectal surgical specimens removed for adenocarcinoma. Anastomotic "donuts" are also frequently received and microscopically examined.

Design: All cases of primary colorectal adenocarcinoma resected between 2004 and 2013 at our institution were reviewed. Pathology reports were used to record the distance of the tumor from the proximal and distal resection margins prior to formalin fixation, the microscopic presence or absence of carcinoma at the proximal and distal margins, whether or not the specimen was received with anastomotic "donuts," and the presence or absence of carcinoma in the anastomotic "donuts." The status of the circumferential (radial) margin, defined as any adventitial soft tissue unencased or incompletely encased by peritoneum closest to the deepest penetration of tumor, was also documented.

Results: Out of a total of 594 cases, definitive direct involvement of a proximal or distal margin of resection by adenocarcinoma was found in only three cases. All three cases also showed tumor at the margin grossly. Only one possible case of intramural tumor spread by a mass grossly located 1.7 cm away from the microscopically-involved margin was found in a patient who also had extensive metastatic disease. The shortest distance measured between the tumor and a negative proximal or distal resection margin was 0.4 cm in an abdominoperineal resection specimen. All 242 anastomotic "donuts" were free of carcinoma.

Conclusions: Our study suggests that the proximal and distal margins of colorectal cancer specimens need not be examined microscopically if the tumor is 5 cm or more away from the margins. Also, in cases in which anastomotic "donuts" are included with the case, these may be microscopically examined in place of the bowel specimen margins as they represent the true margins of resection. Anastomotic "donuts" need not be examined at all if the tumor is 5 cm or more away from the proximal or distal margin.

712 Targeted Genomic Profiling of Small Intestinal Adenomas Reveals Frequent APC Point Mutation Association

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Background: The molecular alterations of duodenal adenomas in sporadic versus familial adenomatous polyposis (FAP) cases are incompletely understood.

Design: A retrospective cohort of 30 formalin fixed, paraffin embedded duodenal adenoma cases, including 20 sporadic and 10 known FAP cases, were subjected to targeted next generation sequencing (NGS) using the Oncomine Cancer Research Panel, comprising recurrent alterations in 126 oncogenes and tumor suppressors identified by pan-cancer analysis.

Results: Targeted NGS averaged 2,396,502 mapped reads per sample, with high (mean >950X) coverage over targeted bases. An average of 204 variants were called per sample. Across the 30 cases, 62 mutations were prioritized as possible driving variants. Twenty-five of thirty cases (83%) were positive for *APC* mutations, including 18 of 20 (90%) sporadic and 7 of 10 (70%) FAP cases. Interestingly, 6 of 20 (30%) sporadic and 3 of 10 (30%) FAP cases had a single C to T mutation in the mutation cluster region of *APC* (C4294T; R1432X), validated by Sanger sequencing. Ten of thirty cases (33%) were positive for *KRAS* mutations, including 8 of 20 (40%) sporadic and 2 of 10 (20%) FAP cases. Seven of these *KRAS* mutations occurred at glycine 12 or 13. Of the thirty cases, three (10%) had *ERBB3* mutations, two (6.6%) had *RAF1* mutations, two (6.6%) had *TP53* mutations, and one case each harbored a *ATM, FBXW7, ERBB2, MSH1, MAP2K1, FGFR3, BAP1,* or *CDH1* mutation.

Conclusions: There were no statistically significant differences by Fisher's exact test between molecular alterations of sporadic and FAP-associated duodenal adenomas in our cohort. Previous reports have shown *KRAS* is mutated in 40-60% and *APC* is mutated in 7-13% of small intestinal adenocarcinomas. However, we observed *KRAS* mutations in 33% and *APC* mutations in 83% of our adenoma cohort, with 30% of our cases containing the *APC* (C4294T; R1432X) point mutation. This study suggests that whereas sporadic and FAP-associated duodenal adenomas have similar scope and frequency of mutation, duodenal adenomas and adenocarcinomas have distinct *APC* mutation rates, or previous studies underestimate *APC[/talic] mutation rates in duodenal adenocarcinoma.*

713 CD200 Is Expressed in Gastrointestinal Neuroendocrine Tumors and Correlates With Grade

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Background: CD200 is a membrane-bound glycoprotein normally expressed by a variety of cell types, including neurons and follicular dendritic cells. Recently, we demonstrated that diverse types of neuroendocrine neoplasms (NEN) also express CD200. The purpose of this study was to evaluate CD200 expression in NENs of gastrointestinal (GI) origin, and to correlate its expression with clinical and pathologic features, including WHO grade (determined by Ki67 and mitotic count), mitosis-specific marker Phosphohistone-H3 (PHH3), the presence of necrosis, and survival.

Design: 113 resected NENs of pancreas (47), small bowel (51), colon (4), and appendix (11) were assessed for mitotic count (MC), necrosis, and immunohistochemical staining for Ki67, PHH3, and CD200. Grade (G1-3) was based on MC & Ki67, in accordance with the 2010 WHO classification. Grade based on PHH3 was determined by counting PHH3+ cells in 50 HPF, and stratified using the same criteria for MC. CD200 staining was scored as positive or negative. Clinical data were obtained from chart review and from the National Death Index. Fisher's exact test was used to examine the significance between CD200 expression and tumor grade. The mean square contingency coefficient between CD200, MC, and Ki67 was also calculated to determine the strength of the association.

Results: 93% (105/113) of the NENs showed diffuse positivity for CD200. The higher grade NENs (G2-3) were more likely to be CD200 negative ($p<0.001$) and the strength of this association was high ($\Phi = -0.4$). This association between higher grade and CD200 negativity was regardless of grading method used (Ki67 index, MC, PHH3 count, $p<0.001$ for each measure). Of the 3 markers, CD200 expression was most strongly associated with Ki67 index. There was also a trend towards an association between the CD200 negativity and the presence of necrosis, but this did not reach statistical significance ($p=0.056$). At a median follow-up of 53 months, the median survival of the population had not been reached. However, 62% of the patients with CD200 negative NENs were deceased compared to 12% of patients with CD200+ NENs.

Conclusions: CD200 is positive in the majority of GI NENs, and negative staining is strongly associated with higher tumor grade. In this study, patients have an overall excellent prognosis after resection (evidenced by the median survival not being reached). However, of the patients with CD200-negative NENs, 62% have died, indicating that CD200 may also be helpful in evaluating prognosis.

714 Novel Oncogene and Tumor Suppressor Mutations in KIT and PDGFRA Wild Type Gastrointestinal Stromal Tumors Revealed By Next Generation Sequencing

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Background: 10- 15% of GISTs are negative for KIT and PDGFRA, and most of these cases are SDH deficient. Recent literature has provided data on additional molecular alterations such as KRAS in KIT mutant GISTs. We aimed to assess the frequency and spectrum of somatic mutations in common oncogenes as well as copy number variations in our institutional GISTs negative for KIT and PDGFRA mutations.

Design: GISTs wild type for KIT/ PDGFRA between 2009 and 2013 were tested via next generation sequencing for somatic mutations in 341 genes. SDHB immunohistochemistry to evaluate for SDH deficiency was also performed.

Results: Of 267 GISTs tested for KIT and PDGFRA mutations, 15 were wild type, of which 8 cases had material available for further testing. All 8 cases lost SDHB expression and had various molecular alterations involving ARID1A, TP53, and other cancer-related genes (Table 1). One case had a KRAS G12V (c.35G>T) mutation in both the primary gastric tumor and a post-imatinib recurrence. The tumor had anaplastic features and was resistant to multiple tyrosine kinase inhibitors, ultimately resulting in cancer-related mortality within 2 years of diagnosis.

Conclusions: KRAS mutations occur in rare GISTs with wild type KIT and PDGFRA and may confer primary resistance to tyrosine kinase inhibitors. Mutations in tumor suppressor genes are frequent and distributed across a variety of genes.

Clinicopathologic Characteristics and Somatic Mutations in SDH-Deficient Wild Type Gastric GISTs

Age	Sex	Size (cm)	Mitotic Rate (50 HPF)	Morphology	Lymph Node Status	Somatic Alterations
67	M	4.7	99	epithelioid	0/0	*KRAS* p. G12V *ATRX* p. G75X *ATRX* p. E1509_1510delinsX *DICER1* p. L805Q *MSH2* p. T568fs *NF1* p.Q4234fs *PMS1* p. W198L *IGF1* amplification *MDM2* amplification
35	M	8	71	epithelioid	0/2	*ARID1A* p. S499fs with loss of ARID1A immunohistochemical expression
32	F	2	12	spindle	0/0	none
43	F	3.5	1	epithelioid		*SDHA* p. R352X *DAXX* p. T568I *BMPR1A* splice site (c. 675+1G>T)
59	F	4	4	mixed	0/0	**BRD4* p.1113del **CREBBP* p.686Q **FLT1* p. L927S **HNF1A* p. N62S **SDHB* p. D204fs **TP53* p. A138V **TP53* p. R306fs **TP53* p. 191_192del
28	F	4		epithelioid		*TP53* p. R342fs *TP53* p. 191-192del
61	F	7.5	1	epithelioid		**SDHA* p. R210X **RAD52* p. G118D **BRCA2* p. T598A **BRCA2* p. T2097M **AXL* p. T328M
32	F	10	2	epithelioid	0/0	**NOTCH2* p. R2105W **MSH2* p. M813W **KDR* p. N205S **SDHA* p. C311F

*Sequenced against pooled normal due to unavailability of matched normal DNA.

715 Reappraisal of Serosal Invasion in Patients With pT3 Colorectal Cancer By Elastic Stain: Clinicopathological Study of 129 Surgical Cases with Special Reference To Peritoneal Elastic Lamina Invasion

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Background: Peritoneal elastic lamina invasion (PELI) has been reported to be an important adverse prognostic factor in pT3 colorectal cancer (CRC). However, the data supporting this contention are limited. To clarify the associations between PELI pT3 CRC and prognostic significance, 129 consecutive surgical cases of pT3 CRC were examined.

Design: One hundred and twenty nine consecutive in-house surgical cases of pT3 CRC between 1993 and 2011 were reviewed. Thirty consecutive surgical cases of pT4a CRC resected during the same period were also examined for comparison. Case selections were restricted to pT3 CRCs with the sections containing the deepest adenocarcinoma invasion partially or entirely covered with the peritoneum. Elastic staining was performed on one section containing the deepest tumor invasion partially or entirely covered with the peritoneum. The associations between the presence of PELI and clinicopathological factors including prognosis of the patients were examined.

Results: PELI were identified in 28.7 % (37/129) of the pT3 CRCs. PELI was associated with primary site ($p=0.006$), lymph node metastasis ($p<0.001$), lymphovascular invasion ($p<0.001$), recurrence ($p=0.007$), and patient's age ($p=0.002$). The proportion of patients with a 4-year-recurrence-free period in those with negative PELI, positive PELI and pT4a CRC was 90.3 %, 66.7 % and 28.9 %, respectively ($p<0.0001$).

Conclusions: Elastic staining is useful to evaluate the serosal invasion of CRC. Positive PELI is a significant predictive factor for lymph node metastasis and recurrence-free survival in patients with pT3 CRC, indicating that PELI can be useful for stratifying patients with pT3 CRC.

716 GNAS Mutation as an Alternative Mechanism of Activation of the Wnt/β-Catenin Signaling Pathway in Gastric Adenocarcinoma of the Fundic Gland Type

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Background: Gastric adenocarcinoma of the fundic gland type (GAFG) is a rare variant of gastric tumor. We have recently reported the frequent accumulation of β-catenin in GAFGs and showed that approximately half of the cases studied harbored at least one mutation in *CTNNB1*/*AXINs*/*APC*, leading to the constitutive activation of the Wnt/β-catenin pathway. However, the mechanisms of Wnt signaling activation in the remaining cases are unknown. Accumulating evidence showed that the activating mutation in *GNAS* promotes tumorigenesis via the activation of the Wnt/β-catenin pathway or the ERK1/2 MAPK pathway.

Design: Tissue sample were collected from GAFGs (n=26) . For comparison, sample (n=32) from patients with sporadic fundic gland polyps (FGPs) were selected. We analyzed the mutasions in *GNAS* (exon 8, 9), *KRAS* (exon 2) to elucidate the association of *GNAS* mutations with Wnt/β-catenin pathway activation in GAFGs and FGPs. We employed immunohistochemistry (IHC) to examine nuclear β-catenin expression (nuclear β-catenin labeling index was classified as follows: 5% or less, negative; more than 5%, positive) and PCR followed by direct sequencing to see the genetic alterations in *CTNNB1* (exon 3), *APC* (exon 15), *AXIN1* (exon 5), and *AXIN2* (exon 1 and 5), *GNAS* and *KRAS*.

Results: Immunohistochemistry revealed nuclear β-catenin expression in 22 out of 26 GAFGs, and ten out of 26 cases (38.5%) harbored at least one mutation in *CTNNB1*/

AXINs/APC. Activating mutations in *GNAS* were found in five out of 26 GAFGs (19.2%), all of which harbored R201C mutations. Activating mutations in *KRAS* were found in two of 26 GAFGs (7.7%), and both of these also contained *GNAS* activating mutations. Four out of five cases with *GNAS* mutation showed nuclear β-catenin expression (P=0.01). Furthermore, three of these four cases did not harbor mutations in *CTNNB1*, *AXINs*, or *APC*, suggerting that mutations in the Wnt component genes and those in *GNAS* occur almost exclusively.
Conclusions: The present study demonstrates that a small subset of GAFG harbors activating *GNAS* and *KRAS* mutations. Furthermore, *GNAS* mutation may act as an alternative mechanism of Wnt/β-catenin signaling activation that contributes to tumorigenesis.

717 Loss of Function Alterations in Intestinal Stem Cell Regulator SOX9 Are Common in Colorectal Adenocarcinoma

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Background: Sox9 is a key regulator of differentiation in the intestinal stem cell niche and is a known Wnt signaling antagonist. It has been reported to play both an oncogenic and tumor suppressor role in colorectal cancer (CRC). However, the spectrum and significance of *SOX9* genetic alterations in CRC is unclear. The aim of this study was to document the types of *SOX9* alterations seen in CRC to delineate a possible role in the pathogenesis of these tumors.
Design: DNA was extracted from FFPE or fresh frozen tissue samples of CRC from 196 patients. Samples were analyzed using the OncoPanel platform, a hybrid-capture-based NGS assay that interrogates 275 cancer genes for mutations and copy number variation. Sequencing was performed using an Illumina HiSeq 2500 sequencer and data was analyzed via a customized pipeline that incorporates a variety of open source and internally developed tools. The potential functional consequence of *SOX9* mutations was assessed using a combination of open source in silico prediction tools.
Results: The study group included 141 colonic and 55 rectal adenocarcinomas. Overall, evidence of Sox9 loss of function was seen in 16% (30/186) of MMR-proficient CRC cases. In this group, *SOX9* mutations were detected in 18 cases (10 frameshift mutations, 4 non-sense mutations and 4 single nucleotide variations (SNVs) computationally predicted to impair Sox9 function) while single copy loss of the *SOX9* locus was detected in 14 cases. Interestingly, two cases harbored compound single copy loss of a *SOX9* allele and a likely impairing SNV. *SOX9* mutations were detected in 30% of the 11 MMR-deficient cases. *SOX9*-mutant MMR-proficient tumors were significantly more likely to harbor activating *KRAS* mutations (odds ratio 3.3, p-value 0.02 by Fisher's Exact Test), while mutations in additional key CRC genes were not strongly associated with *SOX9* alterations. Concurrent mutations in CRC-associated genes included *APC* (17), *BRAF* (1), *KRAS* (13), *NRAS* (2), *PIK3CA* (2), *SMAD4* (5) and *TP53* (12). *SOX9* loss of function alterations were present in 15% of right-sided colon cancer, 15% of left-sided colon cancer and 27% of rectal cancers.
Conclusions: *SOX9* is commonly altered in CRC via multiple genetic mechanisms that likely lead to impaired protein function. The resulting stem cell dysregulation and potential impact on Wnt signaling may play a role in the pathogenesis of CRC. The broad anatomic distribution of CRC with *SOX9* alterations suggests that these changes occur across both serrated and conventional molecular subtypes of CRC.

718 Clinicopathologic Analysis of Colorectal Carcinoma With High-Grade Neuroendocrine Differentiation: Identification of a Unique Subtype with Mixed Large Cell Neuroendocrine Carcinoma/Signet Ring Cell Adenocarcinoma with Frequent BRAF Mutation and Poor Overall Survival

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Background: Colorectal carcinoma (CRC) with neuroendocrine differentiation (CRC-NE) may present as pure neuroendocrine carcinoma or as a component of a combined carcinoma (mixed adenoneuroendocrine carcinoma, MANEC). The aim of this study was to perform a clinicopathologic and molecular analysis of CRC-NE.
Design: 140 CRC including 34 CRC-NE and 106 conventional CRC were analyzed for tumor histology; *KRAS* and *BRAF* mutation, and microsatellite instability (MSI); and overall survival. High-grade neuroendocrine differentiation was defined as small cell and/or large cell histology with immunoreactivity with at least 1 neuroendocrine marker (synaptophysin or chromogranin) and with mitotic count >20/10 HPF and/or Ki67 index >20%.
Results: Compared with conventional CRC, CRC-NE was more frequently located in the right colon (76% vs. 46%, p=0.003), more often presented with metastasis (59% vs. 18%, p<0.0001) and often had perineural (68%), lymphatic (97%), and venous (62%) invasion and high tumor budding (71%) (p<0.001). *BRAF* mutation was more common in CRC-NE compared with conventional CRC (53% vs. 12%, p<0.0001). Factors associated with poor overall survival included neuroendocrine differentiation (p<0.0001), stage (p<0.0001), *BRAF* mutation without MSI-H (p=0.02), and signet ring histology (p=0.03). In the multivariable analysis, neuroendocrine differentiation and stage were independent predictors of overall survival (p<0.01). CRC-NE was further divided into large cell neuroendocrine carcinoma (LCNEC, n=8), MANEC composed of LCNEC and conventional adenocarcinoma (MANEC-conventional type, n=10), and MANEC composed of LCNEC and signet ring cell carcinoma (MANEC-signet ring type, n=16). MANEC-signet ring type more often had a *BRAF* mutation compared with LCNEC and MANEC-conventional type (73% vs. 14% vs. 50%, p=0.034). *KRAS* mutation was identified in 0% of MANEC-signet ring type, 25% of LCNEC, and 43% of MANEC-conventional type (p=0.032). LCNEC, MANEC-conventional type, and MANEC-signet ring all had a worse overall survival compared to conventional CRC (log-rank p<0.0001).
Conclusions: CRC-NE more often involves the right colon, exhibits frequent *BRAF* mutation and is an independent predictor of poor survival. We identified a unique subtype composed of LCNEC and signet ring cell carcinoma (MANEC-signet ring type) with frequent (73%) *BRAF* mutation and poor survival.

719 Tumor Budding in Intestinal Type Gastric Adenocarcinoma Is a Predictor of Nodal Metastasis and Recurrence

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Background: Gastric adenocarcinoma accounts for 10% of all cancers worldwide and has a poor prognosis. Histologically, they can be classified as intestinal or diffuse. In general, intestinal type cancers are more common and have been reported to have a better prognosis compared to diffuse type. However, this remains controversial. Recent studies have shown the presence and amount of tumor budding in intestinal adenocarcinomas of the colon and esophagus to be predictive of nodal metastasis and recurrence. The aim of this study is to determine if tumor budding in intestinal type gastric adenocarcinomas correlates with any prognostic features.
Design: 104 gastric resections from patients treated with primary surgical excision between 1999 and 2013 were identified. Pathologic variables including histologic type (intestinal vs. diffuse), tumor grade (1, 2, or 3), T-stage, and presence or absence of lymph node metastasis were evaluated. Tumor bud scores were assigned to all intestinal type cancers using methods previously described (Wang, *Am J Surg Path*, 2009) for colorectal adenocarcinoma. Tumor bud scores of <1 were designated as low and scores of ≥ 1 were designated as high.
Results: Mean patient age was 67.5 years (range 40 to 91) with a M:F ratio of 1.5:1. Tumor characteristics were as follows: 49 intestinal (48%); 38 diffuse (37%) and 16 mixed (15%). Most tumors were poorly differentiated (69%). Overall, diffuse type tumors had a higher recurrence rate (39%) than those with intestinal histology (24%) (p<0.001), suggesting a more aggressive behavior.
Of the 49 cases with intestinal histology, 4 were grade 1 (8%), 27 were grade 2 (55%), and 18 were grade 3 (37%). T stages were as follows: 14 T1 (29%); 11 T2 (22%); 14 T3 (29%); 10 T4 (20%). Thirty-two (65%) of the intestinal tumors had high tumor bud scores. Overall, cases with high tumor bud scores were associated with higher T-stage, N-stage, and grade (p-value <0.001). These cases also had a higher likelihood of recurrence compared to low (p-value <0.001). In addition, intestinal type tumors with high tumor bud scores had a similar recurrence rate (31%) compared to diffuse-type tumors (39%).
Conclusions: In our cohort, cases with high tumor bud scores in intestinal type cancers have higher T-stage, N-stage, grade, and likelihood of recurrence. Therefore, assessment of tumor budding may identify a group of patients requiring closer clinical follow-up.

720 Blood-Based Gene Expression Biomarkers Are Useful To Predict Disease Type and Activity in IBD

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Background: Biomarkers that are able to separate disease types, and predict levels of inflammation, are essential in IBD patients given the poor utility of currently available clinical biomarkers. The aim of this study was to prospectively identify panels of genes that can help distinguish Crohn's Disease (CD) from Ulcerative Colitis (UC) and separate levels of activity in these disorders, using mRNA gene expression profiling.
Design: 109 IBD patients [CD; 53, UC;56] were grouped into disease categories and severity by histologic grading of standardized segmental colonic mucosal biopsies. Whole blood was collected from each patient by PAXgene Blood RNA collection tubes [PreAnalytiX] and gene expression analysis using mRNA was conducted by microarray hybridization. All biopsies were graded as either inactive, mild (<50% of mucosa shows cryptitis/crypt abscesses), moderate (>50%), or severe (ulceration). Subsequently, patients were graded overall as inactive, mild or moderate-severe (M-S) based on a summation of the biopsy findings. 10 samples from each activity group with the best match of age, sex, race, duration and extent of disease were selected. Logistic regression was performed on multiple combinations of common probe sets, and data was evaluated in terms of discrimination by computing the area under the ROC curve. To improve specificity, samples from 120 other healthy patients and individuals with various non-IBD diseases served as controls.
Results: Panels of 6 individual genes successfully discriminated type and stages of disease activity with a high degree of sensitivity and specificity. The gene panel discriminated stages of activity; CD mild activity [ROC-AUC 0.89]; CD M-S activity [ROC-AUC 0.98]; UC mild activity [ROC-AUC 0.92]; and UC M-S activity [ROC-AUC 0.99]. Validation by real time RT-PCR confirmed the Affymetrix microarray data. The sensitivity and specificity of the CD mild panel was 88% and 76-100% respectively, which showed 100% specificity against each of the UC groups. The CD M-S panel separated samples according to severity by isolating the M-S activity samples from all other samples; sensitivity of 100% and specificity 80-100%. The UC M-S panel had sensitivity of 90% and specificity of 78-100% (100% specificity vs UC mild, 89% vs UC active).
Conclusions: Specific whole blood gene panels reliably distinguish CD from UC and help separate activity of disease with a high degree of sensitivity and specificity.

721 Biopsy Site Changes in Colonic Adenomas Mimic Invasive Adenocarcinoma

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Background: Biopsy of colonic adenomas prior to their removal may introduce mucosal elements into the submucosa. Displaced adenomatous epithelium can closely mimic invasive adenocarcinoma and, in our experience, is often more challenging to recognize than typical "pseudoinvasion" in pedunculated polyps. In this study, we evaluated the histologic features of endoscopically manipulated adenomas compared to those of adenomas with pseudoinvasion and invasive carcinoma.

Design: We identified 44 consecutive adenomas that were previously biopsied. Of these, 20 (45%) contained displaced adenomatous epithelium in the submucosa and formed the study group. We identified 22 controls, including 12 pedunculated adenomas with misplaced epithelium and 10 malignant polyps (*i.e.* adenomas with adenocarcinoma in submucosa). Submucosal epithelial elements in all groups were evaluated for architectural, cytologic, and stromal features, and subjected to Ki67 and p53 immunostains.

Results: Most study polyps were large (mean: 30 mm, range: 5-50 mm) and, unlike adenomas with misplaced epithelium, most occurred in the right colon (59%). Displaced epithelium commonly (40%) had an infiltrative appearance and was composed of singly dispersed dysplastic cells in fibrin or granulation tissue in 4 cases. Lamina propria surrounded displaced epithelium in 16 (80%) cases and 9 (45%) contained extruded mucin. Biopsy site changes consisting of erosions, granulation tissue, hemorrhage and/or hemosiderin, and fibrosis were seen in 25%, 45%, 20%, 20% of cases, respectively. In contrast, misplaced epithelium of adenoma controls always had a lobular contour with a rim of lamina propria, 2 (17%) contained mucin pools, 10 (83%) showed hemorrhage and/or hemosiderin, and none displayed single epithelial cells in the submucosa. All invasive carcinomas contained overtly malignant epithelium surrounded by desmoplastic stroma. Study and control adenomas showed a similar, or decreased, proportion of Ki67 and p53-positive epithelial cells in the submucosa compared to the surface adenoma, whereas invasive carcinomas showed increased staining for both markers (70% and 80%, respectively) compared to the adjacent adenoma.

Conclusions: Endoscopic manipulation of colonic adenomas often displaces dysplastic epithelium into the submucosa and this epithelium may have an infiltrative appearance that simulates malignancy. Pathologists should be aware of this potential pitfall when considering a diagnosis of invasive adenocarcinoma in previously sampled polyps.

722 Immunohistochemical and Molecular Findings in Sessile Serrated Polyps With Dysplasia

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Background: The serrated neoplastic pathway implicates sessile serrated polyps as precursors to sporadic colon cancers with MSI-H. Unlike conventional adenomas, these polyps lack Wnt signaling abnormalities, but harbor *BRAF* mutations. Cumulative DNA methylation in sessile serrated polyps presumably silences *MLH1* and heralds dysplasia onset. However, molecular changes in sessile serrated polyps with dysplasia are ill-defined. We performed this study to better evaluate the features of these polyps.

Design: We assessed sessile serrated polyps with (n=40) and without (n=27) dysplasia. The former showed discrete areas of non-dysplastic serrated epithelium and dysplasia, which was noted to be conventional (*i.e.* tubular adenoma-like), serrated, or mixed. All polyps were immunostained for annexin A10 (a phospholipid binding protein touted as a marker of the serrated neoplastic pathway), MLH1, and β-catenin. Pyrosequencing for *BRAF* V600E mutational status was performed on sessile serrated polyps and foci of dysplasia.

Results: Most sessile serrated polyps contained mutant *BRAF* (89%) and showed diffuse nuclear and cytoplasmic annexin A10 staining (87%), but none showed loss of MLH1 nuclear staining or nuclear localization of β-catenin. Most dysplastic foci had *BRAF* mutations (75%), 33% showed MLH1 loss, and 18% displayed nuclear β-catenin staining. Three (75%) high-grade dysplastic foci showed MLH1 loss. Only 32% of dysplastic foci were annexin A10 positive compared to 82% of adjacent non-dysplastic areas. Dysplastic foci appeared serrated (63%), conventional (15%), or mixed (22%). Serrated dysplasia showed more frequent *BRAF* mutations (84%), abnormal β-catenin staining (16%), and annexin A10 positivity (55%) compared to conventional dysplasia [50% (p=0.1), 0% (p=0.4), and 0% (p=0.03), respectively]. MLH1 loss was similar among serrated (36%) and conventional (33%) dysplasia (p=0.6).

Conclusions: Sessile serrated polyps with dysplasia often show *BRAF* mutations regardless of morphology, supporting their role in the serrated neoplastic pathway, but other features of the pathway are inconsistently present. Annexin A10 is a sensitive marker for sessile serrated polyps, but expression decreases with progression to dysplasia. Although MLH1 loss may occur late in carcinogenesis and aberrant β-catenin staining may reflect transient Wnt signaling activation, it is also possible that sessile serrated polyps with dysplasia progress *via* complex mechanisms not limited to the serrated neoplastic pathway.

723 microRNA Sequencing of Pancreatic and Intestinal Neuroendocrine Tumors Reveals Organ-Specific Profiles and Potential Molecular Subgroups

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Background: Neuroendocrine tumors (NETs) of digestive organs share many pathologic features, but differ in their clinical behavior. We have previously reported that pancreatic and ileal NETs have distinct miRNA expression profiles by small RNA sequencing. The purpose of this study is to compare these miRNA expression profiles to those of appendiceal and rectal NETs, as well as NETs from other organs.

Design: Total RNA was extracted from formalin-fixed paraffin-embedded tissues of well-differentiated pancreatic (N=12), ileal (N=15), appendiceal (N=10) and rectal (N=4) NETs. These samples was subjected to small RNA sequencing. Unsupervised clustering was performed using normalized data and mean miRNA expression levels were statistically compared. Lung NETs and tumors from thyroid, parathyroid and adrenal were also profiled and compared.

Results: Unsupervised hierarchical clustering separated pancreatic NETs from all intestinal NETs. This separation can be mostly attributed to the low expression of a set of miRNAs in pancreatic NETs, notably miR-615-3p, miR-196a/196b, miR-653, and miR-137 ($p< 0.00001$ and >40 fold differences for all comparisons). In contrast, relatively few miRNAs were overexpressed in pancreatic NETs, most notably miR-216b (P=0.0008).

Among the intestinal NETs, ileal and rectal NETs were clearly separated. Most (8/10) appendiceal NETs clustered with rectal NETs, but 2 clustered with ileal NETs. 67 miRNAs were differentially expressed between the ileal and rectal-appendiceal groups, with miR-653 and miR-196a again among the most significantly different miRNAs (P=0.0006 and 0.004, respectively). Pancreatic NETs formed two subgroups that were separated by differential expression of the miR-216a/216b/217 cluster. Review of clinicopathological findings did not reveal features that would account for the two appendiceal or the two pancreatic subgroups.

Analysis above pointed to miR-653, miR-615, miR-196a/196b, and miR-216a/216b/217 cluster as important miRNAs in various pancreatic and intestinal NETs. Examination of these miRNAs in NETs of other organs showed miR-653 and miR-216a/216b/217 to be almost exclusively expressed in intestinal and pancreatic NETs, whereas miR-615 and miR-196a/196b were also expressed in pheochromocytoma, but not in NETs of other organs.

Conclusions: miRNA sequencing revealed a set of novel miRNAs that are unique to and differentially expressed by the NETs of digestive organs. Experiments to validate and explore potential biological roles of these miRNAs are ongoing.

724 Classification, Immuno-Profile and Clinicopathological Predictors of Mortality in Ampullary Carcinomas

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Background: Histopathological classification of Ampullary Carcinomas (AC) is essential due to variation of prognostic and therapeutic modalities in Pancreaticobiliary (PBT) and Intestinal type (IT) of carcinomas arising from anatomic complex region of ampulla of Vater.

Design: We analyzed 60 AC cases from two large medical centers. On FFPE whole tissues immunohistochemistry was performed for CK7, CK20, CDX2, MUC2 and MUC5ac by Leica antibodies and Leica Bond-Max automated system (Leica). IHC expression equal to or greater than 5% among tumor cells is considered positive. Area under the receiver operating characteristic (ROC) curve was employed to assess the classification of AC with above biomarkers using SAS 9.3 (SAS Inc., Cary, NC).

Results: Of the 60 patients with AC, 30 (50%) were males, 51 (85%) were Caucasian and 4 (6.7%) were African Americans with a mean age of 63.7±11.7 years. The concordance between H&E and IHC diagnosis was 94%. The combination of CK7, CK20 & MUC2 had the greatest ability to discriminate PBT from IT (C-Statistic 0.968). CK20 and MUC2 were positively correlated with lymphovascular invasion and with increasing histology grade, respectively. CK-7 and MUC5ac were highly correlated with primary tumor, number of positive nodes and overall tumor stage. 68.7% and 42.1% (P= 0.062) patients with PBT and IT survived with a median follow up of 13 (range 7, 42.5) and 29 months (range 1, 71) (P=0.572), respectively. Clinical predictors of mortality are:

Spearman Correlation Coefficients for Mortality with Clinical Characteristics (p<0.10) and Univariate Cox Proportional Hazards Models for Predictors of Mortality (p<0.10)

	Correlation Coefficient	P-Value	Hazard Ratio (95% CI)	P-Value
Insulin Dependent	0.29	0.026	10.90 (2.90, 40.98)	<0.001
Anemia	-0.25	0.054	0.45 (0.18, 1.14)	0.091
Surgical Complication: Resp Failure	0.23	0.081	28.71 (4.68, 176.03)	<0.001
Histopathology:				
Tumor Size (mm)	0.43	<0.001	1.03 (1.01, 1.05)	0.005
Positive Node Status	0.30	0.020	3.17 (1.31, 7.68)	0.011
Peri-pancreatic invasion Present	0.31	0.015	3.37 (1.39, 8.21)	0.007
Lymph Vascular Invasion Present	0.25	0.078	1.61 (0.66, 3.89)	0.293
Staging:				
Regional Lymph Nodes N1	0.25	0.058	2.91 (1.20, 7.07)	0.018
Residual Tumor	0.32	0.074	9.79 (1.77, 54.22)	0.009
Recurrence	0.76	<0.001	13.09 (3.78, 45.40)	<0.001

Conclusions: The combination of CK7, CK20 & MUC2 IHC antibodies are best suited to classify AC into PBT and IT. The expression of CK7, CK20, CDX2, MUC2 and MUC5ac, however, does not correlate with patient mortality. Primary tumor size, peripancreatic tissue invasion and recurrence are the most significant predictors of mortality.

725 Protein Kinase C-θ Expression Correlates With KIT Overexpression and Can Be Used as a Prognostic Factor in Gastrointestinal Stromal Tumors

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Background: Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. Although KIT and protein kinase C-θ (PKC-θ) overexpression are well known, the role of PKC-θ in GISTs has not been elucidated. On the contrary, wild type KIT recycling via protein kinase C-Δ (PKC-Δ) activation has been reported in a subset of colon cancers. Therefore, to elucidate the relationship between PKC-θ and KIT expression, and their roles in GIST tumorigenesis, we compared PKC-θ, PKC-Δ, and KIT expression in 50 GISTs.

Design: GIST tissue samples from 50 patients were used in this study. Clinicopathologic factors and the mutation status of KIT and platelet-derived growth factor receptor α (PDGFRA) were compared with the expression levels of KIT, PKC-θ, and PKC-Δ by western blotting and immunohistochemistry. Overall survival and disease-free survival were analyzed by using Kaplan-Meier method. The median follow-up period after surgery was 44 months.

Results: PKC-θ was expressed in 30 out of 50 cases and PKC-Δ was expressed in 34 out of 50 cases by immunohistochemistry. PKC-θ and PKC-Δ were significantly expressed in high grade GISTs based on the modified NIH consensus criteria (P = .005 and .038, respectively); and PKC-θ was more frequently and strongly expressed in cases showing recurrence or metastasis (P = .008). When we compared the expression levels of PKC-θ and PKC-Δ with that of KIT by western blotting, only PKC-θ expression correlated with KIT expression (Pearson's correlation coefficient = .682). In survival analyses, patients with PKC-θ expression showed shorter disease-free survival than those without PKC-θ expression (P = .003).

Conclusions: PKC-θ is expressed in a subset of GISTs, especially in the high grade tumors and cases showing recurrence or metastasis; and the level of PKC-θ expression correlates with that of KIT overexpression. Our findings indicate that PKC-θ overexpression can play a role in GIST tumorigenesis and progression.

726 Risk of Lymph Node Metastasis in Early Gastric Cancer With Diffuse and Mixed Type Histology

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Background: With improvement in endoscopic techniques, endoscopic submucosal dissection (ESD) have been developed and currently widely accepted treatment modalities for early gastric cancer (EGC), even in diffuse type within expanded criteria based on large datasets indicating an almost absent risk of lymph node (LN) metastasis. However, there are several safety and efficacy issues regarding endoscopic resection of diffuse type EGCs. Therefore, it is very important to review lymph node metastasis and make consensus about indication of ESD of diffuse type EGC based on probability of lymph node metastasis.

Design: We reviewed clinicopathologic features of 525 diffuse and mixed type EGCs related with LN metastasis.

Results: In our datasets of 525 diffuse and mixed type EGCs, overall, there are 12.9% (71/550) of LN metastasis in EGC (mucosal EGC: 5.2% (15/288), submucosal EGC: 21.4% (56/262)). Increased size, gross type (elevated), depth of invasion, perinueral and lymphovascular invasion were associated with LN metastasis.[table1] [table2] Significantly LN metastasis (2/92, 2.2%) was noted with tumors falling within established expanded ESD criteria, i.e., less than 2cm mucosal cancer without ulceration, lymphovascular tumor emboli. Regarding histologic components of EGC, increased size of total diffuse component, poorly component was associated with LN metastasiscompared to size of signet ring cell component or intestinal component.

Conclusions: Taken together, we recommend that more centers worldwide survey their experience with regard to LN metastasis in cases of diffuse or mixed type EGCs to possibly refine the criteria of ESD as therapeutic modalities of EGC and careful identification of poorly component in ESD specimen is needed to select cases with further surgical intervention in diffuse or mixed type EGC.

727 Serrated Polyps in Lynch Syndrome Patients

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Background: Lynch syndrome (LS) is characterized by an increased risk of microsatellite unstable (MSI-H) colorectal cancer (CRC), usually believed to arise from conventional adenoma, due to a germline defect in a mismatch repair gene. Sessile serrated polyps (SSP) have emerged in recent years as precursor lesions of sporadic MSI-H CRC and are associated with BRAF V600E mutation. The role of SSP and whether they contribute to carcinogenesis in LS patients is not well established. We examined the clinicopathologic features of LS patients diagnosed with serrated polyps (SP), including SSP, hyperplastic polyps (HP) and traditional serrated adenomas (TSA).

Design: The pathology database was retrospectively searched to identify all LS patients between 2009-2014. The parameters recorded for each patient included age, gender, mode of LS diagnosis, history and molecular features of CRC (if present) and any history of extra-intestinal carcinoma. Patient endoscopic features, polyp location and histology, and history of chronic GI disease were also noted. Each patient was also evaluated for the possibility of sessile serrated polyposis syndrome (SPS) using the WHO 2010 criteria.

Results: We identified 34 patients (23 women, 11 men, average age 52.5, range: 24 to 80 years) with a diagnosis of LS. LS diagnosis was made by gene sequencing (44.2%), Amsterdam criteria (23.5%) or IHC/PCR of CRC (32.3%). 19 patients had a diagnosis of CRC, 18 had extra-intestinal cancers, 9 patients had both and 6 patients had no history of carcinoma. The three most common extra-intestinal carcinomas included endometrial adenocarcinoma, mammary ductal carcinoma, and urothelial carcinoma. 73.5% of LS patients had a diagnosis of SP, including 60% HP, 28% SSP, and 12 % TSA. Majority (59%) patients had 2 or more SP while 41% had one SP. None of the LS patients met WHO criteria for SPS and none had SP associated low grade dysplasia or advanced neoplasia. None of the CRC in our patient LS cohort exhibited molecular features of sporadic MSI-H cancer.

Conclusions: We found patients with LS commonly had a diagnosis of at least one SP, including 64.7% of LS patients with SSP, a result significantly higher than previous reports. Interestingly, we did not find evidence of sporadic MSI-H CRC in our patient cohort, suggesting that germline mutations in mismatch repair genes do not contribute to the serrated pathway of carcinogenesis in LS patients. Furthermore, these data also support the established hypothesis that MSI-H CRC in LS patients arises from conventional adenoma.

728 Next Generation Sequencing (NGS) Identifies Mutational Distinction Between Synchronous and Metachronous Distant Metastases of Colorectal Carcinoma (CRC)

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Background: The pathophysiology of --non-hereditary CRC is a multistep process of "driver" mutation accumulation that ultimately results in invasive neoplasia. Unfortunately, identification of consistent genetic alterations that distinguish tumors that metastasize from those that stay localized remains elusive. Herein, a pilot study was undertaken to further characterize the genetic aberrations involved in CRC progression through sequencing of paired primary and metastatic tumors.

Design: Thirty-two samples from eleven patients with metastatic CRC were selected from institutional archives. Seven patients had synchronous (at the time of presentation) distant metastases and 4 patients developed metachronous distant metastases. A representative formalin-fixed paraffin-embedded block was selected from each primary tumor (PT), distant metastasis (DM), and regional lymph node metastasis (LNM) when present. Samples were evaluated using the AmpliSeq cancer panel v2 with aberrations filtered to include only non-synonymous amino acid changes in 2855 "hotspots" frequently mutated in 50 cancer genes.

Results: Samples included 11 PT, 9 LNM and 12 DM. Across all patients, 25 different hotspot mutations were identified involving *TP53*, *APC*, *BRAF*, *PTEN*, *PIK3CA*, *KRAS*, *SMAD4*, *FBXW7*, and *KDR*. Mutations per case ranged from 1-4. Recurrent aberrations between different patients, each occurring in 2 individuals, were *APC* c.4037C>A, *KRAS* C.35G>A and *TP53* c.262_272del. Six cases (55%) had the same mutations in all tumors, 3 cases (27%) had no overlap between their primary and metastases, and 2 cases (18%) had some overlap. Seven of 9 cases (78%) with a synchronous LNM overlapped in some or all mutations with the PT. Of the 7 cases with an available synchronous DM, 4 (57%) had overlap in mutations with the PT. In contrast, all 4 cases (100%) with an available metachronous DM overlapped with the PT in some or all mutations.

Conclusions: Pathogenetic differences may occur between tumors that metastasize synchronously versus metachronously to distant sites. About half of synchronous DM demonstrated completely divergent mutations from the PT. This is in contrast to metachronous DM in which all had the same or overlapping mutations as the PT. These results may suggest that DM arising near the time a PT is established manifest higher genetic instability that underlies their early aggressiveness; the indolent nature of later metastases may reflect a lower level of mutagenesis resulting in genetics that more closely resemble the PT.

729 Endoplasmic Reticulum Stress, the Unfolded Protein Response and Development of Colon Adenocarcinoma

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Background: When misfolded proteins accumulate in the endoplasmic reticulum (ER) during hypoxia or nutrient deprivation, the cell is said to experience ER stress. This triggers an unfolded protein response (UPR) to restore the balance between misfolded proteins and ER chaperones such as BiP. UPR signalling is required for the growth of many solid cancers and higher levels of ER stress have been observed in more aggressive tumors. In chronic ER stress, factors including GADD34 have been shown to mediate cell death. It has recently been shown in mesothelioma that more aggressive sarcomatoid tumors lose expression of GADD34. Colorectal adenocarcinoma arises due to progressive changes within pre-malignant lesions. Our aim was to test the hypothesis that the expression of markers of the UPR (BiP, CHOP and GADD34) correlates with the progression of those lesions to invasive adenocarcinoma.

Design: The expression of BiP, CHOP and GADD34 was estimated by immunohistochemical staining of a tissue microarray generated from formalin-fixed paraffin-embedded cores of colon epithelium, comprising normal tissue (n=117), low-grade adenoma (n=86), high-grade adenoma (n=62), invasive colon adenocarcinoma (n=175), and lymph node metastasis of colon adenocarcinoma (n=6). We used A549 cell lines treated with the ER stress-inducing drug tunicamycin as positive controls.

Results: We observed that as colonic epithelium shows increasing evidence of pre-malignant and then frankly malignant change, BiP expression significantly increases ($p < 0.001$), whereas CHOP and GADD34 expression is progressively attenuated ($p < 0.001$).
Conclusions: We identified a positive relationship between BiP expression and colon carcinogenesis, and a negative correlation for CHOP and GADD34 expression. These findings are consistent with a model in which ER stress accompanies malignant transformation and where loss of proteins that mediate the toxicity of ER stress, such as GADD34, may facilitate tumorigenesis. This raises the exciting possibility that restoration of GADD34 function might antagonize the malignant process.

730 Recognizing Precursor Lesions for Autoimmune Metaplastic Atrophic Gastritis

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Background: Autoimmune metaplastic atrophic gastritis (AMAG) is a significant risk factor for both anemia and gastric neoplasia. Still, the histologic features of AMAG are often overlooked, especially in the early stages of the disease. The purpose of our study was to catalogue the progression of changes that occur in patients who ultimately develop AMAG with the goal of recognizing those who may benefit from close clinical follow-up.
Design: An initial search was performed to identify a patient cohort with the histologic diagnosis of AMAG. After identification, each patient's history of gastric biopsies was reviewed and correlated with demographic and laboratory data.
Results: From January 2012 to August 2014, the diagnosis of AMAG was rendered on material from 184 patients, with 65 (35%) of these patients being represented by outside consult material. Our final cohort, then, was limited to the 119 patients who were seen in-house during this period. Ninety-three patients were women, and the average age for the initial diagnosis of AMAG was 59 years. Prior gastric body biopsies had been performed on 54 patients in the cohort (45%), and the majority of specimens had shown AMAG. Sixteen of the previous biopsies, however, carried a diagnosis other than AMAG: 11 inactive chronic gastritis, 2 acute *H. pylori* gastritis, and 1 each of eosinophilic gastritis, iron pill gastritis, and parietal cell pseudohypertrophy. These 16 biopsies were taken from 11 patients from 1 to 13 years before a definitive histologic diagnosis of AMAG. Upon review of the oxyntic mucosal histology in this subset of cases, the most common findings were intestinal or pyloric metaplasia (9/16), heavy chronic inflammation deep in the lamina propria (9/16), prominent lamina propria eosinophils (8/16), inflammatory destruction of oxyntic glands (8/16), and parietal cell pseudohypertrophy (4/16). At least 2 of these histologic features were present in 13/16 of the biopsies. Importantly, 3 of these patients were found to have positive anti-parietal cell antibodies between the early gastric biopsy and the later definitive histologic diagnosis of AMAG.
Conclusions: Although subtle, histologic features of developing AMAG may be identified in routine gastric body biopsies. When metaplasia, prominent eosinophils, and/or oxyntic destruction are seen, a note suggesting laboratory testing and/or close clinical follow-up in this subset of patients may be warranted.

731 Reflex Universal Testing for Microsatellite Instability for Colorectal Carcinoma – Experience in a Large Academic Center

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Background: Recent declines in colorectal carcinoma (CRC) incidence rates have been attributed to early detection, improved treatment and understanding of the molecular basis of CRC, including defects in mismatch repair (MMR). MMR testing is used in screening for Lynch Syndrome and predicting prognosis and response to chemotherapeutic agents. The aim of this study is to compare the yield of selective MSI MMR testing following the NCI and Bethesda guidelines to that in the era of universal testing.
Design: A retrospective search of our pathology database for CRCs identified at colonoscopy or resected surgically from 2010 to 2014 was undertaken. Our institution implemented universal testing of CRCs for MMR by immunohistochemistry in November 2013 (University of Miami/Jackson Memorial Hospital). The results of selective and universal MMR testing in CRCs during 2 different periods (2010 to 2013 and 2013 to 2014) were reviewed. BRAF V600E mutation results in CRCs with MMR protein deficiency were also reviewed.
Results: 468 CRCs were diagnosed from October 2010 to October 2013. During this period, 108 CRCs were tested for MMR. Out of those, 15 (14%) CRCs displayed MMR deficiency, representing 3% of the overall cases. Ten of these were BRAF V600E negative. The BRAF mutation analysis was not performed in the remaining 5 MMR deficient cases. A total of 109 CRCs were diagnosed from November 2013 to June 2014. During this period, 82 CRCs were tested for MMR. Out of those, 8 (10%) showed MMR deficiency, representing 7% of the overall cases. All 8 were tested for the BRAF V600E mutation, and 4 (50%) were BRAF V600E positive, consistent with sporadic CRC.
Conclusions: Implementation of universal MMR screening in CRC increased the identification of patients with MMR deficiency from 3% to 7% when compared to selective testing following the NCI and Bethesda guidelines. Even though the sample of universal testing is smaller, the percentage of patients harboring molecular changes requiring genetic counseling for possible Lynch Syndrome cases increased from 2% (10/468) to 3% (4/109) when universal testing was applied. Our institution will look to improve the efficiency of MMR testing, as our universal testing protocol failed to screen 25% eligible patients, exemplifying the challenges of implementing universal testing in large hospital systems.

732 Ineffective Esophageal Motility (IEM) Is Associated With CD4 T-Cell Predominance and Lymphocytic Esophagitis (LE)

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Background: IEM, a primary motility disorder, is defined only in functional terms with no established histologic criteria. It is found in 20-50% of patients with gastroesophageal reflux disease (GERD), however it is etiologically distinct from GERD. Histologic recognition of IEM may benefit the management of patients with upper GI symptoms. The goal of this study was to characterize possible histologic abnormalities in IEM.
Design: Forty-four patients were diagnosed with IEM at our institution from 2003 -2014. Esophageal biopsies were available for 27 patients. The control group consisted of 20 patients with GERD complaints and histologic reflux esophagitis (RE) with increased IEL (REIL) and no evidence of dysphagia or demonstrated dysmotility. Motility status was normal in 2 cases and unknown in the rest of the control group. LE was defined as either focally or diffusely increased peripapillary IEL with no or few granulocytes. Normal cutoffs for IEL were as follows: 42 - mid esophagus, 48 - distal esophagus, 62 - gastroesophageal junction (Putra et al. Mod Pathol 2014, 27 Suppl2, 198A). IEL were counted in the most affected x400 field of view. CD4 and CD8 T-cells were analyzed by immunohistochemistry. The CD4:CD8 ratio > 1 and ≤ 1 indicated the predominance of CD4 and CD8 T cells, respectively. Data is presented as mean ± SD.
Results: Increased IEL were observed in 44% (12/27) patients with IEM. Of these, 67% (8/12) patients had LE pattern (0.9 ± 1.7 eosinophils/ total biopsy) and 33% (4/12) patients had RE pattern (14 ± 13 eosinophils/ HPF; see Table for histologic features). IEL were CD4 T-cell predominant in 83% (10/12) of IEM patients, in contrast to 25% (5/20) of REIL patients (p=0.003). CD4:CD8 ratio for IEM and REIL groups was 2.55 ± 1.79 and 0.98 ± 1.22, respectively (p=0.009).

	IEM (n=12)	REIL (n=20)	P-value
Age	58 ± 10	55 ± 16	
M:F	1:1.4	1.2:1	
IEL count	89 ± 59 (range: 47-271)	121 ± 43 (range: 59-237)	0.088
Focally increased IEL	67% (8/12)	25% (5/20)	0.030
Peripapillary localization of IEL	67% (8/12)	20% (4/20)	0.021
Prominent spongiosis	75% (9/12)	65% (13/20)	0.703

Conclusions: IEM is associated with increased CD4 predominant IEL. This immunophenotype and the pattern of LE may be helpful in suggesting IEM over REIL.

733 Inflammatory Bowel Disease in Type 2 Autoimmune Pancreatitis: Clinical and Histologic Features

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Background: The term autoimmune pancreatitis (AIP) is applied to two apparently distinct groups of patients: those with the pancreatic manifestation of IgG4-related disease (AIP1) and those with a pancreas-specific condition (AIP2). Compared to IgG4-related pancreatitis, AIP2 patients are younger and have a more equal sex distribution. It has been proposed that there is an association between AIP2 and inflammatory bowel disease (IBD). We reviewed our experience with AIP2 in order to better characterize the IBD that develops in these patients.
Design: We reviewed our clinical data base of patients with AIP2 to identify those who also carried a diagnosis of IBD, then reviewed all available gastrointestinal tissue from those patients. Because some patients with AIP2 have increased tissue IgG4 in pancreas, we did immunohistochemistry for IgG4 on representative gastrointestinal sections. We reviewed the medical record to collect the clinical features of IBD in affected patients.
Results: Of the 46 patients with AIP2, 14 (34%) were also diagnosed with IBD (12 UC, 1 Crohn's disease and 1 type unclassified). Six of the 14 patients had IBD before their diagnosis of AIP2; both diagnoses were made simultaneously in 4 patients, and IBD was diagnosed after AIP2 in 4. The histologic activity of IBD was mild in 6 patients, moderate in 2 and severe in 6. Seven of the 12 patients with UC had left-sided colitis, while 5 had pancolitis. One patient had PSC. Four UC patients eventually required colectomy for refractory disease. Our review did not reveal any unique or characteristic histologic features. Two patients did have a diffuse increase in mucosal IgG4 (maximum density 15 and 35/high power field). The patient with the highest IgG4 count also had PSC, but that diagnosis was made on imaging only, so no tissue was available for review.
Conclusions: One-third of our patients with AIP2 have IBD, supporting the impression that there is an association between the two conditions. We did not identify clinical or histologic features to suggest that AIP2 patients have a unique form of IBD or an atypical clinical course.

734 Phenotype-Genotype Correlation in Serrated Precursor Lesions of the Colon based on Recent Classifications

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Background: Consensus in the diagnosis of serrated polyps has now been achieved with guidelines based on well-defined morphological criteria (e.g. WHO-classification 2010). However, phenotype-genotype correlations of serrated lesions were previously defined in an era when terminology was still unclear. Based on this, studies on cytologically

atypical lesions like traditional serrated adenoma (TSA) or sessile serrated adenoma with dysplasia (SSA-D) are apparently rare due to their low frequency in daily routine practice.

Design: In a multicentre study involving four centres, we collected 519 polyps, consisting of 111 classical adenomas (CAD) with different degrees of villosity, 115 hyperplastic polyps (HYP) of different subtypes, 180 SSAs, 45 SSA-D and 68 TSA. Available clinical parameters included gender, age, localization of the lesion and size. Molecular analysis was performed for BRAF and KRAS mutational status. Additionally, MLH1 and p16 promoter CpG methylation status was achieved with Bisulfite-Pyrosequencing.

Results: In CADs KRAS mutation seems to be a key mutator event (24.3%), increasing with the villosity of the lesion. In HYP and regular SSA BRAF mutations are frequently seen (72.2 % and 84.4%, respectively). P16 hypermethylation occurs earlier in SSA progression than MLH1 hypermethylation. Regarding TSA a mixed situation of high amounts of BRAF mutated and KRAS mutated polyps exist.

Conclusions: In conclusion, the new consensus classifications sharpen the entities on a molecular level in comparison to data from former literature. Especially SSA has become a very distinct lesion. The parallel pattern of KRAS and BRAF mutated TSAs possibly needs a subgrouping. Additionally, the review of exceptional molecular events on the morphological level reveals several pitfalls in the diagnosis of truly dysplastic serrated lesions.

735 GNAS Mutations Common To Intestinal Adenomas and Pancreatic IPMNs Are Not a Feature of Intra-Ampullary Papillary Tubular Neoplasms (IAPNs): Further Evidence for a Distinct Category of IAPN

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Background: *GNAS* mutation is common in intestinal type adenomas as well as pancreatic intraductal papillary mucinous neoplasms (IPMNs), especially in intestinal subtype, to the extent that it is now used in diagnosing IPMN in pancreatic secretions and cyst fluid. Intra-ampullary counterparts of "duodenal adenomas" and IPMN-like tumors ("noninvasive papillary neoplasms" per WHO) have recently been proposed to be classified as a distinct group, intraampullary papillary tubular neoplasm (IAPN).[1]

Design: From a cohort of 90 IAPNs, defined as preinvasive (adenomatous) neoplasm forming a tumor >1cm occurring predominantly (>75%) within the ampulla, 15 representative IAPNs, enriched with cases of intestinal pattern, were selected. Tissue from noninvasive IAPN components was microdissected and subjected to *GNAS* pyrosequencing.

Results: Tumor Characteristics: There were 10M, 4F, age 43-81 yrs (mean,60). Tumor size ranged from 15-65mm (mean,32), 12 had invasive carcinoma (2-50mm; mean 13) and 5 had LN mets. In terms of cell lineage in the preinvasive component, 10 had predominant intestinal features (3 pure, 7 mixed). 6 morphologically intestinal cases (1 pure and 5 mixed) also had immunohistochemical (IHC) confirmation as intestinal by MUC/CDX2/CK profile criteria recently established by Ang *et al*[2]. 5 cases had more pancreatobiliary pattern in the preinvasive component, of which 3 had IHC and 2 proved to have intestinal lineage by Ang criteria. ***GNAS* Results:** Of the 15 cases tested, 1 yielded no product, and in 3 there was very low DNA concentration (<0.45ng/ul). Of the 11 cases with sufficient DNA for extraction (lowest concentration, 2.24, median 10.56ng/ul) all showed wild-type *GNAS* without evidence of targeted mutations; 9 of these were of intestinal phenotype, with confirmatory IHC in 4.

Conclusions: Unlike intestinal adenomas and pancreatic IPMNs (of intestinal type) *GNAS* mutations are not common in IAPNs, even in those with intestinal phenotype. This goes along with the distinct and hybrid nature of these ampullary tumors, despite their morphologic resemblance to other tumors. It also supports the notion that IAPNs are not merely intraampullary version of intestinal adenomas or IPMNs. Further molecular studies are warranted to comparatively characterize IAPN and its kinship to other seemingly similar tumors.

Reference:
1. Ohike et al. AmJSurgPathol.2010;34:1731-48.
2. Ang et al. AmJSurgPathol.2014;38:1371-9.

736 Colorectal Medullary Carcinoma Exhibits Overlapping Histologic and Molecular Features With Poorly Differentiated Adenocarcinoma

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Background: Colorectal medullary carcinoma (MC) typically displays a solid growth pattern with abundant tumor infiltrating lymphocytes (TILs). Most MCs show mismatch repair deficiency (MMRd) and pursue a relatively indolent course. As a result, MC is now regarded as a biologically favorable subtype despite lacking glandular differentiation. However, defining MC on morphology can be difficult and application of the WHO criteria (i.e. sheets of malignant cells with vesicular nuclei, prominent nucleoli and abundant eosinophilic cytoplasm exhibiting prominent infiltration by intraepithelial lymphocytes) is not always straightforward, especial if the MMR status is unknown. We performed this study to determine whether the WHO morphological criteria reliably distinguish MC from other poorly differentiated colorectal carcinomas (CRCs).

Design: Our study included 89 CRCs with solid growth pattern in ≥50% of the tumor but without neuroendocrine differentiation (NED). Cases were selected from a patient population fulfilling Bethesda guidelines for possible Lynch syndrome. Tumors were evaluated for increased TILs (>=10 TILs/HPF) and classified into: typical MC (fulfilling WHO criteria); non-medullary poorly differentiated carcinoma (PDC, based on high grade cytology and pleomorphism); or indeterminate cases (IC, uniform cytology but not fulfilling WHO criteria for MC). The morphological subtypes were then correlated with other clinicopathological features and TNM stage.

Results: Twenty-seven (30%) cases fulfilled WHO criteria for MC; 41 (46%) were PDC; and 21 (24%) were IC. The latter resembled MC but had other discordant features: a cord-like growth pattern, nodules of cribriform glands with small lumina, small glands admixed with solid areas, an irregular tumor configuration, stippled chromatin reminiscent of NED, or squamoid cytology. A substantial number of ICs showed increased TILs and MMRd (Table 1).

	MC (n=27)	Indeterminate (n=21)	PDC (n=41)	p value
TIL-high	27 (100%)	16 (76%)	7 (17%)	
MMRd	26 (96%)	14 (67%)	9 (22%)	
TNM stage I or II	15/25 (60%)	10/21 (48%)	7/32 (22%)	0.021

Conclusions: A substantial number of poorly differentiated CRCs do not fulfill morphologic criteria for MC, yet show a similar "low grade" clinicopathological profile (MMRd, TIL-high, and low stage). Inclusion of MMRd in the definition of MC may be the most reliable way to distinguish these biologically low grade tumors from other poorly differentiated CRCs that may pursue a more aggressive clinical course.

737 Hypomethylation of Long Interspersed Element-1 Is a Prognostic Factor in Stage III or High-Risk Stage II Colorectal Cancers Treated With Adjuvant FOLFOX

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Background: Hypomethylation of Long intersperse element-1 (L1) is considered a surrogate marker for a decrease in methylcytosine content in tumor cells. Tumoral L1 hypomethylation correlates worse clinical outcome in patients with gastric cancer or esophageal cancer. However, it remains unclear whether low L1 methylation is a prognostic marker in colorectal cancers (CRCs). We aimed to elucidate whether tumoral L1 hypomethylation may have a prognostic role in CRCs treated with adjuvant FOLFOX.

Design: We analyzed 427 resected stage III or high-risk stage II CRC for their statuses in L1 methylation, CpG island methylator phenotype, microsatellite instability, and KRAS/BRAF mutation. L1 methylation at four CpG sites was assayed by pyrosequencing and average methylation levels at CpG 2 and 3 were obtained for each CRC case.

Results: L1 hypomethylation was closely associated with nodal metastasis but did not show any association with age of onset, gender, tumor subsite, tumor differentiation, mucinous histology, lymphatic emboli, venous invasion, perineural invasion, T stage, and *KRAS*/*BRAF* mutation. Multivariate analysis revealed that L1 hypomethylation as well as mucinous histology, T stage, N stage, lymphatic emboli and *KRAS* mutation was an independent prognostic parameter heralding poor prognosis.

Characteristic	Variable	Adjusted HR (95% CI)	P-value
L1 methylation (CpG site 2 and 3)			
	Q1	2.000 (1.115-3.585)	0.002
	Q2-4	1	
Mucinous histology			
	Non-mucinous	1	0.014
	Mucinous	3.906 (1.322-11.540)	
T stage			
	T1-3	1	0.043
	T4	1.969 (1.021-3.798)	
N stage			
	N0,1	1	
	N2	2.122 (1.184-3.802)	0.011
KRAS			
	Wild	1	
	Mutant	3.304 (1.888-5.781)	
Lymphatic emboli			
	Absent	1	
	Present	3.086 (1.602-5.944)	0.001

Conclusions: Tumoral L1 hypomethylation correlated independently with poor prognosis in patients with resectable CRC treated with adjuvant FOLFOX.

738 Duodenal Giardiasis Miss Rates: Lessons From a Large Tertiary Hospital

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Background: Giardiasis in the most common parasitic infection in the United States and is the leading cause of infectious malabsorption in children. Most cases are detected with stool antigen tests, but many cases are discovered on biopsy. The aim is to study the sensitivity of giardiasis detection in tissue biopsy, and pertinent histologic clues to timely diagnosis.

Design: A computerized search identified 507 unique patients with Giardia positive stool antigen tests from January 2004 to June 2014. From these patients, 44 duodenal biopsies from 40 patients were retrieved. Pertinent clinicopathologic data were collected.

Results: Upon retrospective review, Giardia was histologically identified in 8 of 44 biopsies (18%). Three cases were not prospectively identified (38% miss rate). For these discordant cases, the background mucosa was histologically unremarkable, had focal active inflammation, or showed severe villous blunting and extensive active inflammation. Two of the three missed cases showed numerous organisms. There was a lag time of 8, 10, and 35 months between the date of the missed biopsies and the date of positive stool antigens.

Conclusions: In summary, we report that histologic evaluation has a sensitivity of 18% percent in patients with a Giardia positive stool antigen study. We detected a 38% miss rate, which resulted in a treatment delay of up to 35 months. This study emphasizes that giardiasis remains challenging to detect, even at a large tertiary care center with subspecialty sign-out. While historically giardiasis is thought to occur in a histologically unremarkable background, we show that 2 of the 3 discordant cases had active inflammation and or villous blunting. Accordingly, this study highlights the importance of routinely examining the small bowel lumen for Giardia organisms to ensure timely treatment.

739 The H. pylori Gastritis Pattern Without Identifiable Organisms: Correlation With Non-Invasive Laboratory Testing

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Background: *Helicobacter pylori* (HP) infection is usually accompanied by a distinct histologic pattern in gastric biopsies. When this inflammatory pattern is observed, HP organisms are usually present. However, many biopsies show a histologic pattern highly suggestive of infection but without identifiable organisms, even with the use of special stains. At our institution, we typically suggest to the clinician that ancillary testing may be helpful to confirm the diagnosis in these cases. Two commonly employed non-invasive laboratory tests are IgG serology and stool antigen testing. The goal of this study is to investigate how ancillary tests are used in these cases and to determine what portion of the patients likely had an underlying HP infection despite the lack of organisms on tissue biopsy.

Design: We conducted a search our institutional pathology database from January 2004 to June 2014 using a specific text string that is included in the diagnostic text when we suspect HP in a gastric biopsy but no organisms are found. We then determined which of these patients received an order for HP stool antigen or IgG ELISA within six months of the biopsy date.

Results: We identified 752 biopsies from 737 unique patients that matched our search criteria. Within a six month window of the biopsy date, ancillary tests (IgG or stool antigen) were ordered in 238 of 737 patients (33%). There was either a positive IgG or positive stool antigen test result in 128 of 238 patients (53%) that had laboratory testing. Considering each test independently, IgG was ordered for 170 patients (23%). It was positive in 115 patients (67%), negative in 41 patients (24%), and the order was cancelled in 14 patients (8%). Stool antigen testing was ordered on 125 patients (17%). It was positive in 23 patients (18%), negative in 60 patients (48%) while the order was cancelled in 40 patients (32%). Matched IgG and stool antigen results were available in 56 patients. 43 were positive only by IgG while 10 were positive for both IgG and stool antigen. In this subset, all patients with positive stool antigen tests were positive by IgG.

Conclusions: Our data show ancillary testing is a valuable adjunct to tissue biopsy for the evaluation of HP when the tissue biopsy shows a HP gastritis pattern histologically, but without organisms. When ancillary testing was ordered, 53% of patients had a positive IgG or stool antigen test. These data underscore the importance of alerting clinicians of our histologic suspicion and suggesting that non-invasive testing may be helpful in these cases.

740 Comparative Genetic Analysis of Invasion-Resistant (Complex Non-Mucinous Pyloric) and Invasion-Prone Types of Intracholecytstic Papillary-Tubular Neoplasms of the Gallbladder

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Background: Intracholecytstic papillary-tubular neoplasms, ICPN, represent a unifying entity for WHO's "adenomas" and "intracystic papillary neoplasms" of gallbladder and have 2 distinct subsets: 1) complex-pyloric non-mucinous type (CP), which are virtually never associated with invasion (though most are typically diagnosed as "tubular/tubulopapillary adenocarcinoma" due to cytoarchitectural complexity), and 2) Non-CP types (pyloric-mucinous, gastric-foveolar, intestinal, and biliary) which are commonly (> 70%) associated with invasion.

Design: Whole transcriptome (RNA-Seq) and whole exome (WES) sequencing were performed on FFPE tissues of 7 ICPNs (4 CP, and 3 non-CP) and 3 matched controls (uninvolved gallbladder).

Results: Unpaired hierarchical clustering revealed distinct expression profiles for each of the 2 subtypes. CP type showed alterations in transcripts from the Notch and Wnt/ß-catentin (CTNNB1) signaling pathways, including elevation in transcripts for JAG2, CTNNB1, DKK1, CST1, FGF20, and NKD1. Immunohistochemical confirmation was obtained showing strong nuclear beta-catenin labeling in scattered clusters in all 4 CP, but not in non-CP cases. In contrast, transcripts altered in non-CP ICPNs included several established drivers of tumor progression including PIM1, MCL1, COLCA1, and PLXNB3. Both CP and non-CP ICPNs harbored mutations in a number of tumor suppressor and/or oncogenes, including ATM, APC2, FGFR3, MLL2, and MLL3, though no signature lesion in either group could be identified.

Conclusions: ICPNs harbor mutations in a number of tumor suppressor and/or oncogenes, going along with their pre-malignant nature. CP type ICPNs, which are invasion resistant, appear to be driven by the Notch and Wnt/CTNNB1 signaling pathways, bearing mutations in APC2 and MLL2 (two known regulators of ß-catenin signaling) and demonstrate aberrant nuclear CTNNB1 protein expression. In contrast, the non-CP group, which is highly invasion prone, shows deregulation of a number of other transcripts pivotal for tumorigenesis. Further analysis of differential molecular alterations in these biologically distinct pathways will likely shed new light to the mechanisms of invasive cancer formation in the gallbladder.

741 KRAS, NRAS and BRAF Mutation Profiling in a Series of 90 Metastasic Colorectal Cancer Spanish Patients – An Estimation of Cost Savings

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Background: Metastasic colorectal cancer (mCRC) patients harbouring a mutation in codon 12 or 13 of the *KRAS* does not benefit from therapy with antibodies targeting epidermal growth factor receptor (*EGFR*). Recently, several studies have shown that an extended study of the *RAS* family genes (*KRAS* exons 3 and 4; and *NRAS* exons 2, 3 and 4) could help to detect those patients that although having a wild-type *KRAS* exon 2 will not respond to anti-EGFR treatment. The aim of this study was to assess the *KRAS, NRAS* and *BRAF* mutational profiling in a series of spanish patients with mCRC and to extrapolate these results to the economic implications of a better triage.

Design: 90 mCRC were included in a prospective study from December 2013 to August 2014. DNA was extracted from formalin-fixed paraffin embedded sections. Mutations in exon 2 (codons 12 and 13) of *KRAS* and *BRAF* V600E mutation were analized with KRAS-BRAFStripAssay℗ (Viennalab). Further mutations in *KRAS* exon 3 and 4 and exons 2, 3 and 4 of *NRAS* were tested when no mutation in *KRAS* exon 2 was found. The extended study was performed as well in those cases with a G13D mutation (a proposed predictive marker of response to anti-EGFR). *KRAS* exon 3 and 4 (codons 59, 61, 117 and 146) were analized using RAS extension Pyro℗ Kit (Qiagen). *NRAS* mutations in exon 2 (codons 12 and 13), 3 (codon 59 and 61) and 4 (codons 117 and 147) were analized using the Therascreen℗ NRAS Pyro℗ kit and RAS extension Pyro℗ Kit (Qiagen). In our Hospital the median cost per month of a treatment with anti-EGFR is 2.000€ and the median progression-free survival is 8 months.

Results: Thirty-six (40%) cases had a mutation in *KRAS* exon 2. Fifty-three (58,9%) were native and were tested with the extended study together with 5 cases that carried a G13D mutation. None of the G13D cases had another mutation. Nine (17%) of the 53 *KRAS* exon 2 native cases had a mutation in another exon of *KRAS* or *NRAS*. Eight (8,9%) cases had V600E mutation in *BRAF*, no other mutation was identified. In 1 (1,1%) case a valid result was not achieved.

Conclusions: In this series the implementation of the *RAS* extended study allows the identification of a further 17% of the *KRAS* exon 2 native patients that will not respond to anti-EGFR therapy; supposing a saving of 144.000€. The global percentage of patients with a mutation in a *RAS* family gene arises to 57%. The authors N.R. (corresponding author) and R.R.L. contributed equally.

742 Mesenchymal Expression of CD90 and Nuclear Expression of FOXM1 Correlate With Disease Progression in Hepatocellular Carcinoma

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Background: Cancer stem cells (CSC) serve as a proliferation reservoir and contribute to the development of hepatocellular carcinoma (HCC). CD90, a CSC marker, plays an important role in cell-cell and cell-matrix interactions. Previous studies have looked at hepatocytic but not mesenchymal expression of CD90. FOXM1 (Forkhead box M1) is a proliferation-associated transcription factor that is absent to minimal in normal liver cells and is up-regulated in HCC. Digital analysis of both nuclear and cytoplasmic expressions of FOXM1 has not been done. This study evaluated the expressions of mesenchymal CD90 and nuclear and cytoplasmic FOXM1 through non-dysplastic cirrhosis (NDC)-liver cell dysplasia (LCD)-HCC progression.

Design: Explanted liver from 101 subjects with end-stage liver disease and HCC were collected at the University of Illinois Hospital. Eight subjects without significant liver disease were collected as controls. Tissue microarrays were generated containing representative tissues with NDC, LCD, and HCC. Immunohistochemistry (IHC) was performed for CD90 and FOXM1. Tumor was graded from 1 to 3 based on modified Edmondson–Steiner grading system. Mesenchymal CD90 IHC was graded from 0 to 3 based on intensity of staining. FOXM1 IHC was analyzed by Vectra® automated

multispectral imaging system to delineate mean nuclear and mean cytoplasmic staining intensity. Friedman's test and non-parametric Spearman's correlation were used for stastistical analysis.
Results: CD90 expression is significantly upregulated in HCC mesenchyme and significantly increases in NDC-LCD-HCC progression ($p<0.0001$) and correlates positively with tumor grade ($r=0.37$, $p=0.0002$). Nuclear FOXM1 expression significantly increases in mean intensity ($p=0.043$) in NDC-LCD-HCC progression while cytoplasmic FOXM1 expression does not significantly increase in NDC-LCD-HCC progression ($p=0.075$). This may correspond to the translocation of FOXM1 from the cytoplasm to the nucleus to exert its transcriptional role in HCC progression.
Conclusions: These results suggest that mesenchymal CD90 and FOXM1 nuclear expressions play important roles in the progression of HCC. Targeting CD90 and FOXM1 may provide diagnostic, prognostic, and therapeutic strategies in HCC management.

743 Programmed Cell Death Ligand 1 Expression Is Associated With BRAF Mutation, Microsatellite Instability, Medullary Morphology, and Helps To Characterize Tumor Behavior in Colorectal Carcinoma

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Background: Programmed Cell Death Ligand 1 (PD-L1) is a key regulatory molecule that suppresses the cytotoxic immune response in a variety of physiologic and pathologic pathways. Recently, PD-L1 overexpression has been correlated with a lessened immune response and consequent worse prognosis in a variety of cancers. However, the role of PD-L1 in colorectal carcinoma (CRC) is less clear.
Design: We performed immunohistochemistry for PD-L1 (clone: E1L3N #13684, 1:200, Cell Signaling Technology, MA) on tissue microarrays (TMAs) consisting of 336 tissue cores from 164 CRC cases, both primary (n=124) and metastases (n=40), including pre (n=109), and post (n=55) chemotherapy. Mutational and microsatellite instability (MSI) status was known in most cases, as determined by a multiplex PCR assay (SNaPshot) and immunohistochemistry, respectively. Stringent grading based on membranous staining only was used. The same TMAs were stained with anti-CD8 (clone: IgG2b 4B11, RTU, Leica, IL) to semiquantitatively evaluate the extent of cytotoxic tumor infiltrating lymphocytes (TILs). Correlation of PD-L1 expression and TILs with clinicopathologic data was performed.
Results: Fifteen cases (9%) showed positive membranous staining for PD-L1 (PD-L1-P), these cases were more likely to be older ($P<0.01$) and female ($P<0.02$) than PD-L1 negative cases (PD-L1-N). PD-L1 expression correlated with abundant TILs ($P<0.01$), the presence of *BRAF V600E* mutation ($P<0.01$), and MSI-H compared to PD-L1-N cases ($P<0.001$). The PD-L1-P cases all demonstrated a medullary phenotype, which was seen in only a minority of PD-L1-N cases ($P<0.001$). Compared to PD-L1-N tumors, fewer of the PD-L1-P tumors went on to develop metastases ($p<0.01$). Considering only cases with medullary histology, PD-L1-P cases trended towards a higher level of CD8+ TILs ($P=0.067$), however, other clinicopathologic features were similar.
Conclusions: Our finding that PD-L1 expression is associated with abundant TILs and less aggressive tumor progression is somewhat counterintuitive but this finding is similar to that found in some other CRC cohorts. This study supports these findings and suggests a mechanism for this unexpected result may involve the presence of the *BRAF* mutation, MSI, and a medullary phenotype, explaining a potential difference between CRC and other cancers, where PD-L1 expression portends a poor prognosis.

744 Comprehensive Genomic Profiling of Extrahepatic Cholangiocarcinoma Reveals a Long Tail of Therapeutic Targets

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Background: Extrahepatic cholangiocarcinoma (EHCCA), also known as bile duct carcinoma, is distinct in clincopathologic findings from intrahepatic cholangiocarcinoma (IHCCA) and gallbladder carcinomas (GBCA) is a progressive form of cancer with a poor prognosis. We queried whether EHCCA featured clinically relevant genomic alterations (CRGA) that could lead to targeted therapies for patients with clinically advanced disease.
Design: DNA was extracted from 40µ FFPE sections from 57 clinically advanced EHCCA. Comprehensive genomic profiling (CGP) was performed on hybridization-captured, adaptor ligation based libraries to a mean coverage depth of 719X for 3,230 exons of 182 cancer-related genes plus 37 introns from 14 genes frequently rearranged in cancer. The results were evaluated for all classes of genomic alterations (GA). CRGA were defined as GA linked to drugs on the market or under evaluation in mechanism driven clinical trials.
Results: There were 36 male and 21 female patients with a median age of 58.9 years. Two (4%) EHCCA were grade 1, 42 (74%) grade 2 and 13 (23%) grade 3. There was 1 (2%) stage II, 14 (25%) stage III and 42 (74%) stage IV tumors at the time of CGP. A total of 248 alterations were identified (mean 4.4; range 0-13) in 84 genes. Fifty one (89%) of EHCCA patients featured at least 1 clinically relevant alteration including *KRAS* (42%); *CDKN2A* (28%); *ERBB2* (11%); *PTEN* (11%); *ATM*; *MCL1*; *NF1* and *PIK3CA* (all 7%) and *AKT2*; *BRAF*; *BRCA2*; *CCND1*; *CDK4; CDK6*; *FBXW7*; *FGFR1*; *FGFR3; NRAS*; *PTCH1*; *RAF1* and *STK11* (each altered in a single patient). In contrast with previously studied IHCCA, no *IDH1/2* mutations or *FGFR* gene fusions were identified. The 11% *ERBB2* alteration frequency in EHCCA was similar to our previously studied GBCA at 14% and both were significantly greater than the complete absence of *ERBB2* alteration seen in IHCCA ($p<0.0001$). The clinical benefit in patients with EHCCA has been observed with targeted MTOR and HER2 inhibitors will be presented.
Conclusions: EHCCA is a distinct form of biliary tract cancer that features a genomic landscape more characteristic of GBCA than IHCCA. With CGP a variety of CRGA for patients with relapsed and metastatic EHCCA can be identified. The resulting long tail of potential therapy targets generated from the CGP analysis mandates comprehensive diagnostic approaches to maximize targeted treatment options for patients with clinically advanced disease to potentially improve disease outcomes for this aggressive form of malignancy.

745 Frequency and Characteristics of Serrated Lesions of the Appendix in Serrated Polyposis Patients

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Background: Patients with serrated polyposis develop multiple serrated polyps throughout the large bowel. The majority of serrated polyps show activation of the mitogen-activated protein kinase pathway through mutation in the *BRAF* or *KRAS* gene. The proportion of mutation in each gene depends on the subtype of serrated polyp and their location in the large bowel. The majority (up to 80%) of sessile serrated adenomas (SSA) harbor a *BRAF* mutation and no *KRAS* mutation. In serrated lesions of the appendix, *KRAS* mutation has been reported to be the most common alteration, including in SSA. The frequency and the characteristics of appendiceal serrated lesions in serrated polyposis patients have not been well reported.
Design: Patients were selected from our database between 2008 and 2014 when fulfilling the following criteria: total or right colectomy specimen with sampling of the appendix and a diagnosis of serrated polyposis made prior or at time of surgery. Slides from the appendix were reviewed to classify the lesions according to their closest resemblance to subtypes of serrated polyp in the rest of the large bowel using the current histological criteria. DNA extraction was performed from the serrated lesions for *BRAF* and *KRAS* mutation testing.
Results: A total of 34 colectomy specimens were retrieved and a serrated lesion of the appendix was identified in 23 (68%), which was classified as hyperplastic polyp (HP) for 4, SSA for 16 including 2 with dysplasia, and traditional serrated adenoma (TSA) for 3. The frequency of mutations for each polyp subtype is shown here:

Polyp Subtype	Number	KRAS Mutation	BRAF Mutation
Hyperplastic polyp	4	0	0
Sessile serrated adenoma	16	9 (56%)	5 (31%)
Traditional serrated adenoma	3	2 (66%)	0
Total	21	11 (52%)	5 (24%)

Conclusions: Serrated lesions of the appendix are frequently found in serrated polyposis patients and are most commonly of SSA-type morphology. *KRAS* and *BRAF* mutations were found in 52% and 26%, respectively. All 5 *BRAF* mutations were in SSA-type lesions. The high rate of *KRAS* mutation in SSA-type lesions (56%) suggests that the histologic molecular correlation reported for subtypes of serrated polyp in the large bowel may not be applicable in the appendix for serrated polyposis patients.

746 Retained Colorectal Carcinoma MLH1 Expression in Lynch Syndrome Patients Carrying a Germline MLH1 Mutation

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Background: Immunohistochemistry for mismatch repair (MMR) proteins MLH1, PMS2, MSH2 and MSH6 is used to screen for Lynch syndrome in patients with colorectal carcinoma. The pattern of loss of expression is usually indicative of the underlying genetic defect. Loss of PMS2 with normal MLH1 expression in tumor cells suggests a germline mutation in *PMS2*; however no deleterious mutation is found in a significant proportion of these cases and no diagnosis of Lynch syndrome can be made. We hypothesized that a germline mutation in *MLH1* may explain some of these cases with solitary loss of PMS2 expression.
Design: Patients with colorectal carcinoma were selected from the Colon Cancer Family Registry based on the presence of a microsatellite instability phenotype, solitary loss of PMS2 immunohistochemical expression and absence of germline *PMS2* mutation by long-range PCR and MLPA (multiplex ligation-dependant PCR amplification). Germline *MLH1* mutation testing was performed by Sanger sequencing and MLPA.
Results: There were 76 colorectal carcinomas showing a solitary loss of PMS2 expression by immunohistochemistry. A germline mutation in *PMS2* was identified in 59 cases (78%). From the 17 cases with no mutation detected, blood-derived DNA for *MLH1* testing was available for 12 patients. A deleterious *MLH1* mutation was identified in 4 patients (c.113A>G p.Asn38Ser; c.230G>A p.Cys77Tyr; c.199G>A p.Gly67Arg; c.350C>T p.Thr117Met) and an unclassified variant was identified in 3 patients (c.299G>C p.Arg100Pro; c.187G>C p.Asp63His; c.1607C>T p.Pro536Leu). All *MLH1* variants were missense suggesting that the MLH1 protein may retain its antigenicity, accounting for the immunohistochemical results.
Conclusions: A missense mutation in *MLH1* may explain more than half of colorectal carcinoma cases with solitary loss of PMS2 expression for which no *PMS2* mutation has been found, establishing the diagnosis of Lynch syndrome. Patients with colorectal carcinoma showing loss of PMS2 and normal MLH1 expression by immunohistochemistry should be screened for *MLH1* mutation if no *PMS2* mutation has been identified.

747 Discordant Mismatch Repair Protein Immunoreactivity in Lynch Syndrome-Associated Neoplasms: A Recommendation for Screening Synchronous/Metachronous Lesions

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Background: Approximately 2-5% of patients newly diagnosed with colorectal and/or endometrial cancer will have Lynch syndrome (LS). These patients are important to identify due to their genetic predisposition to synchronous/metachronous LS-associated neoplasms (LSAN). Mismatch repair protein (MMRP) immunoreactivity is widely available to screen for LS. We evaluated LS patients with >1 LSAN and assessed the MMRP staining pattern to determine whether screening of all neoplasms is necessary.

Design: From 2009-2014, 13 LS patients with available tissue from 29 synchronous and/or metachronous primary LSAN were identified (men=6, women=7; age range=16-84). Neoplasms involved large (17) and small intestine (3), endometrium (1), and skin (sebaceous tumors, 8). All were stained for MSH2, MSH6, MLH1, and PMS2, and immunoreactivity was scored as intact or lost. For patients with ³3 cutaneous neoplasms, only the 2 most recent were evaluated.

Results: Nine of 13 patients (69%) with 20 synchronous/metachronous neoplasms showed concordant MMRP results (germline mutations; 6 *MSH2*, 2 *MLH1*, and 1 *MSH6*), and 4 patients (31%) with 9 tumors showed discordant MMRP results.

Immunoreactivity in Discordant Lynch Syndrome-Associated Neoplasms

Patient	Age	Tumor 1	Tumor 2	Tumor 3	Germline Mutation
1	61	Cecum; Loss of MSH2	Hepatic flexure colon; Loss of MSH2	Transverse colon; Loss of MSH2 & MSH6	MSH2
2	49	Proximal left colon; MMRP intact	Distal left colon; Loss of MLH1 & PMS2		No sequencing; Tumor BRAF mutation negative; Suspicious for LS
3	50	Left upper arm skin; Loss of MLH1 & PMS2	Right upper arm skin; MMRP intact		MLH1
4	51	Sigmoid colon; MMRP intact	Endometrium; Loss of PMS2		PMS2

In patients with discordant expression, patient 1 showed loss of MSH2 in 2 neoplasms with loss of MSH2 and MSH6 in the third, patients 2 and 3 showed loss of MLH1 and PMS2 in 1 neoplasm with intact MMRP in the synchronous neoplasm, and patient 4 showed loss of PMS2 in 1 neoplasm with intact MMRP in the synchronous neoplasm.

Conclusions: Of 13 LS patients with synchronous/metachronous LSAN, 4 (31%) showed discordant MMRP results. LS diagnosis could have been missed if only the neoplasm exhibiting intact MMRP expression was screened. Accordingly, our findings support the recommendation to test all primary LSAN, especially in those patients with clinical and pathologic features concerning for LS.

748 Typhlitis: Diagnostic Clues To an Elusive Entity Associated With a High Mortality

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Background: Typhlitis is an invasive microbial infection of the bowel with a historically high mortality rate. Basic information about its incidence, etiology, and outcome are incomplete due to its rarity.

Design: The study group consisted of 3 biopsies, 15 resections, and 3 autopsies from 21 patients at 7 institutions over a 19-year period.

Results: All patients were immunocompromised, most with a known malignancy (n=19). Of the 17 patients with available history, 16 received chemotherapy within the preceding month. The majority had both clinical and pathologic diagnosis of typhlitis (n=14), with 7 discordant diagnoses [clinical only [n=4; pathology=appendicitis (n=2), CMV (n=1), and lymphoma (n=1)]; pathology only (n=3; clinical= colitis, infection vs GVHD, or leukemia, one each]. Common clinical presentations included neutropenia (n=11/11), fever (n=8/15), and gastrointestinal symptoms (n=17/17). Sites of involvement included the right colon (n=11/13), appendix (n=5/11), terminal ileum (n=5/13), and descending and sigmoid colon (n=2/13). Imaging studies showed either thickened small bowel (n=2), thickened right colon (n=3), or thickened stomach and intestines (n=2). Most patients had positive microbial cultures (n=10/12; bacteria=8; *Candida*=2). Histologic examination showed invasive bacteria (n=8/13), fungus (n=4/13), or both (n=1/13). Perforation was rare (n=3/17). Treatment included a combination of surgery and supportive care (n=13/17), or supportive only (n=4/17). Most patients with a pathologic diagnosis of typhlitis were deceased (n=12/17) at time of follow-up (follow-up=average 6 years).

Conclusions: In summary, we found a 71% risk of mortality in patients with typhlitis, emphasizing the importance of disease awareness. Helpful clinical clues include a history of immunosuppression, fever, neutropenia, ileocecal thickening, gastrointestinal symptoms, and positive microbial cultures. Histologic features included infiltrating organisms (bacteria > fungus) in a background of depleted lamina propria inflammatory cells. We report 35% of diagnoses were discordant. As such, we propose a modified diagnostic criterion that requires histologic identification of invasive micro-organisms in an inflammatory depleted background.

749 LINE-1 RNA In Situ Hybridization: A Novel Application for Assessing Global Methylation Status

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Background: Long interspersed nuclear element-1 (L1) is a retrotransposon that accounts for approximately 17% of the human genome. Loss of methylation of L1 is believed to account for much of the genomic hypomethylation observed in human cancer, and serves as a surrogate marker for global methylation status. Epigenetic alterations such as global DNA hypomethylation are common events in colon cancer. L1 methylation status has thus far been assessed via whole genome bisulfate sequencing (WGBS), a time and labor-intensive method. We propose a novel application of chromogenic in situ hybridization (ISH) to assess L1 mRNA expression. Further, we explore its utility in colon cancer as a marker of L1 methylation.

Design: Whole genome bisulfate sequencing (Illumina) was performed on two colon cancer cell lines (HCT116 and SW620) and LINE-1 genomic DNA methylation status was evaluated with and without treatment with 5-azacitidine (DNA hypomethylating agent). Using the same treatment parameters, ISH (QuantiGene® ViewRNA, Affymetrix) using an RNA probe aligned to the open reading frame 1 of L1 was performed on both cell lines and quantitated using the Keyence fluorescent scope system (Biorevo). L1 ISH was performed on tissue microarrays (TMAs) representing 161 colonic adenocarcinomas and 18 samples of normal colonic mucosa.

Results: HCT116 and SW620 cells treated with 5-azacitidine demonstrated hypomethylation of L1 genomic DNA as measured by WGBS and expressed more LINE-1 mRNA than untreated cells via ISH ($p < 0.0001$). In colorectal TMAs, decreased L1 RNA was associated with MSI+ (p=0.0001), BRAF mutation (p=0.007), and later age of onset (p=0.01). A trend was observed with right-sided carcinomas and no correlation was found between the intensity of L1 reactivity and TNM status or sex.

Conclusions: Both colon cancer cell lines demonstrated an increase in L1 mRNA expression in the presence of 5-azacitidine via ISH. Increased L1 expression, corresponding to L1 hypomethylation, was confirmed by the standard WGBS method, suggesting that ISH might serve as an alternate method for assessing L1 methylation status. Furthermore, by applying this method to colorectal carcinomas, we identified a direct relationship between L1 expression with MSI, the presence of BRAF mutation, and age of onset. We propose that L1 in situ hybridization may provide a more robust assessment of L1 methylation status and serve as a surrogate marker for global genome methylation.

750 Clinicopathologic Features and Outcomes of Rectal Adenocarcinoma in Young Patients

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Background: Recent population-based studies suggest an increasing incidence in rectal cancer in patients 40 years and younger, a trend also observed at our institution. While the pathology of colorectal cancer in young patients has previously been examined, there are few reports focusing specifically on rectal adenocarcinoma in the 40 years and younger age group. Our aim was to compare the clinicopathologic features and outcomes between young (≤40 years of age) and older patients with rectal adenocarcinoma.

Design: Patients with rectal adenocarcinoma resected between1990-2014 were identified in a single-institution database of colorectal cancer resections. Patients were divided into those aged ≤40 years and those 41 years or older. Clinicopathologic parameters and outcomes were analyzed using standard bivariate methods and Cox regression. Slides from resection specimens were reviewed to record high-risk features.

Results: Special subtypes such as signet ring cell carcinoma and mucinous carcinoma occurred at similar frequencies in both age groups. High levels of microsatellite instability were noted in 4 (38%) cases in the young patients, compared to 5 (13%) in older patients. Younger patients were more likely to present with Stage III (37%) and Stage IV disease (19%) than older patients (Stage III, 28%; Stage IV, 10%). However, survival analysis disclosed no significant differences in overall or disease-specific survival between younger and older patients in univariate analysis.

Table 1.

Age (years)	Total	Neo-adjuvant therapy	LVI* (p=0.023)	TDs† (p=0.006)	PNI (NS)	DOD
10-40	42	26 (62%)	7/12 (58%)	12/35 (34%)	6/33 (18%)	15/40 (38%)
41-95	365	165 (45%)	77/184 (42%)	48/306 (16%)	28/317 (9%)	89/345 (26%)

* in patients who did not receive neoadjuvant therapy; †includes patients who received and did not receive neoadjuvant treatment.

LVI= lymphovascular invasion; PNI=perineural invasion; TDs=tumor deposits; DOD= died of disease; NS= not significant.

Conclusions: Rectal adenocarcinomas in young patients are more often associated with high risk features (lymphovascular invasion and tumor deposits) compared to those in older patients, suggesting more aggressive behavior, but are otherwise morphologically similar. Despite more advanced disease at diagnosis, disease-specific survival is similar to older patients, perhaps due to fewer co-morbitities in younger patients.

751 Expression of CD24, a Stem Cell Marker, in Pancreatic and Small Intestinal Neuroendocrine Tumors

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Background: Well differentiated neuroendocrine tumors (NETs) of the gastroenteropancreatic system compromise approximately two-thirds of total NETs and the pathogenesis of foregut NETs is distinct from that of midgut NETs. CD24 has been has been recognized as both a normal and a malignant stem cell biomarker; despite extensive studies its expression in intestinal and pancreatic NETs remains unknown. In this study, we wanted to determine CD24 expression in pancreatic and small intestinal NETs.

Design: In this study, expression of CD24 by immunohistochemical labeling was observed in normal duodenal and ileal mucosa, benign pancreatic sections, primary duodenal NETs, and primary and metastatic ileal and pancreatic NETs.

Results: We observed scattered CD24 positive cells in the duodenal and ileal crypts, most of which showed strong subnuclear labeling pattern. Same pattern of the expression was observed in 95% of primary ileal NETs, but only in 15% duodenal NETs ($p<0.01$). In addition, metastatic ileal NETs retained CD24 expression. Pancreatic islets did not express CD24, and only rare cells had subnuclear labeling of CD24 in the pancreatic ducts. Unlike ileal NETs, only 5% pancreatic NETs expressed CD24 in the subnuclear compartment ($p<0.01$). In particular all 5 of these NETs shared unique morphologic features, including dense fibrosis, small nests/tubules, and an infiltrative growth pattern.

		MIDGUT NETs		PANCREATIC NETs	
CD 24+	DUODENAL NETs (n=26)	Primary (n=43)	Liver Metastatsis (n=18)	Primary (n=92)	Liver Metastasis (n=18)
	4 (15%)	41 (95%)	17 (94%)	5 (5%)	0 (0%)

Table 1. Expression of CD24 in Duodenal, Midgut and Pancreatic Neuroendocrine Tumors (NETs)

Conclusions: Immunohistochemical studies for CD24 expression might have potential clinical implications. Ninety five percent of midgut NETs expressed CD24, whereas only 5% of pancreatic and 15% of duodenal NETs had CD24 expression. Importantly, metastatic midgut NETs retained subnuclear CD24 expression. We propose CD24 has a novel marker for identifying primary midgut neurendocrine tumors as well as a subset of primary pancreatic neuroendocrine tumors with a unique histomorphologic pattern of disease. The distinction is important, as CD24 has potential as small intestinal cancer stem cell marker; CD24 reactive NETs could be prospective candidates for both anti-cancer stem cell and anti-angiogenic therapeutic regimens.

752 FISH Assessment of ALK Rearrangements in Neuroendocrine Tumors – High Frequency of ALK Deletions

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Background: Anaplastic lymphoma receptor tyrosine kinase (*ALK*, 2p23) rearrangement is a potent predictor for benefit on treatments with *ALK* inhibitors. Neuroendocrine tumors (NETs) are rare neoplasms with an increasing incidence. Oncogenic pathways of NETs are still poorly understood, and no standards treatments are available for these tumors. The aim of this study was to analyze *ALK* rearrangements and copy number alterations (CNAs) by fluorescence *in situ* hybridization (FISH) in a NETs subset.

Design: A total of 152 NETs were retrospectively collected from our institution between 2004 and 2014. Regarding clinical data, media age was 68 and 62.5% were in clinical stage I-II. Tumors were located in gastrointestinal tract (80), lung (30), pancreas (25) and other sites (17). Paraffin samples were screened for *ALK* status using a break-apart FISH probe (Abbott Molecular). *ALK+* samples were tested for *ALK-EML4* fusion (Zytovision) and *ALK* immunohistochemistry (IHC) (Dako). Cut off values for CNAs were set from ten non-tumor tissues as 35% for deletions and 10% for gains. Statistical analysis was performed to evaluate the association between *ALK* gene alterations and clinicopathological features.

Results: A total of 109 out of 152 NETs were evaluated in this study (43 cases had insufficient material and/or gave inconclusive FISH results). Two cases presented *ALK* rearrangement (1.9%). One case exhibited *ALK-EML4* fusion and the other had unknown *ALK*-partner, both having *ALK* positive staining by IHC. Regarding CNAs, 41.5% had deletions (median 1.58 copies/nuclei; range 1-2) and 24.5% had gains (median 2.48 copies/nuclei; range 2-6). The percentage of nuclei with *ALK* deletions was 44.4% (range 35 to 70%) and ALK gains 39.3% (range: 10 to 82%). The low number of positive cases precluded the finding of statistical associations.

Conclusions: Although rare, *ALK* rearrangements are present in NETs. This reveals a potential new indication for *ALK* inhibitors but further investigation is needed. At time of assessment by FISH, *ALK* deletions should be taken into account as a frequent event in NETs.

753 Different Mutation Patterns Characterize Dysplasia of Alternative Epithelial Lineages in Gastric Hyperplastic Polyps

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Background: Gastric hyperplastic polyps (GHPs) are the most common type of polyps occurring in the stomach. Although GHPs are broadly interpreted as benign lesions, they may progress to dysplasia (0.2 to 10%) and adenocarcinoma (0.6%–3%). In this study, we aimed to identify genomic mutations that characterize and may drive malignant transformation in GHPs by using next-generation sequencing.

Design: Eight GHPs (4 with dysplasia and or intramucosal adenocarcinoma and 4 without) were identified from our pathology archives. Only large polyps (>1 cm) with primarily gastric differentiation were included in this study. Adenomatous polyps (intestinal-type) were excluded. The polyps were further characterized by immunohistochemistry for MUC5A, MUC6, CDX-2, TP53, and Ki-67. DNA was extracted from 10 microdissected FFPE sections cut at 5 micron and sequenced for the detection of somatic mutations. DNA was measured by Qubit fluorimetric quantitation. Multiplex sequencing was done with the TrueSeq Amplicon Cancer Panel in theMiSeq platform. Sequencing data files were analyzed with MiSeq Reporter v1.3+ software (Illumina). Variant annotation and visualization were performed using NextGENe (SoftGenetics) software.

Results: No pathogenic mutations were detected in GHPs without dysplasia. *TP53* mutation was the most common genetic alteration in GHPs with at least dysplasia (3 of 4 cases). GHPs with pyloric type dysplasia were associated with *PIK3CA* mutations (2 of 2 cases), and foveolar dysplasia carried *TP53* mutations.

Conclusions: *TP53* mutations are a common alteration in the malignant transformation of GHPs. GHPs with dysplasia may show hybrid gastric and intestinal differentiation. Pyloric type dysplasia was associated with *PIK3CA* gene mutations whereas foveolar dysplasia carried *TP53* mutations. The identification of carcinoma-associated mutations in large GHP provides additional evidence of their neoplastic nature and emphasizes the need for their complete resection and follow-up.

754 Targeted Next-Generation Sequencing Reveals Cancer-Related Mutations in Duodenal Hyperplastic Polyps

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Background: Duodenal hyperplastic polyps (DHP) frequently show morphologic features that resemble colorectal microvesicular hyperplastic polyps, including similar mucin expression profiles. Frequently, gastric foveolar and/or pyloric-type differentiation can be seen as a component of DHP. Currently, little is known about the molecular background through which duodenal hyperplastic polyps develop with rare reports of duodenal DHP harboring dysplasia and/or carcinoma. This study aimed to investigate the genetic alterations associated with the development of DHP.

Design: Search of our surgical pathology archives identified DHPs resected between 2000 and 2013. Adenomatous polyps and intra-ampullary lesions were excluded. DNA was extracted from 10 FFPE sections cut at 15 micron and sequenced for the detection of somatic mutations using the TrueSeq Amplicon Cancer Panel and next generation sequencing (NGS) in the MiSeq platform. DNA was measured by Qubit fluorimetric quantitation. Multiplex sequencing was done with the TrueSeq Amplicon Cancer Panel in the MiSeq platform. Sequencing data files were analyzed with MiSeq Reporter v1.3+ software (Illumina). Variant annotation and visualization were performed using NextGENe (SoftGenetics) software. of progression or recurrence of DHP.

Results: Three DHPs were selected (mean age=55.3 ± 20.8 years; M:F=1:2). The polyps ranged from 1.5 to 2 cm in size and were located in the second part of the duodenum. Two polyps showed focal surface erosion, inflammation and areas of marked cytological atypia, but no definite dysplasia. Targeted sequencing revealed carcinoma-associated mutations in 2 out of 3 polyps. The most significant mutations were in polyp 1 with >20% mutant allele in *SMAD4, PIK3CA, PTEN, CDH1*. Both polyps 1 and 2 carried *VHL* mutation. By immunohistochemistry, these 2 polyps displayed weak p53 labeling and focal increase in Ki67 staining, in a pattern most commonly interpreted as reactive.

Conclusions: This is the first study to identify cancer-related mutations in benign duodenal hyperplastic polyps, confirming the neoplastic nature and demonstrating the malignant potential of these lesions. The mutational targets in duodenal hyperplastic polyps differ from their colonic counterparts. Our results highlight the importance of complete resection and clinical follow-up of benign-appearing DHPs. More studies are needed to evaluate the risk.

755 Characterization and Clinical Value of Tumor Infiltrating Lymphocytes (TILs) in Gastric Cancer

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Background: Increased tumor infiltrating lymphocytes (TILs) have been associated with better outcome in diverse human neoplasms, including gastric cancer (GC). TILs are usually determined with single-marker staining of different (serial) tissue sections and using subjective semi-quantitative methods. Here, we characterized the lymphocyte infiltrates in GCs using objective measurements.

Design: Using multiplexed quantitative fluorescence (QIF), we simultaneously measured the levels of CD3 (T cells), CD8 (cytotoxic T cells), CD20 (B-lymphocytes), cytokeratin (tumor cells) and DAPI (all nuclei) in a retrospective collection of 302 GCs represented in tissue microarray format. The level of TILs was scored using the AQUA method. Measurements were performed in two cores obtained from different tumor areas to assess heterogeneity of the markers.

Results: To first assess uniformity of TIL subtype expression, association between measurements obtained from 2 different tumor areas (e.g. inter-core regression coefficient [R^2]) was prominently lower for CD20 (R^2=0.001) than for CD3 (R^2=0.34) and CD8 (R^2=0.42).None of the markers showed association with age and anatomic tumor location. GCs with diffuse histology showed higher levels of CD20 than intestinal neoplasms (P=0.04). All markers showed a trend for lower levels in stage IV tumors. Using the median score as cutpoint, elevated CD8 but not CD3 or CD20 signal was significantly associated with longer overall survival (log-rank P=0.004).
Conclusions: CD20 positiveB-lymphocytes are more heterogeneous than T cells in GC and are associated with diffuse tumor histology. Increased levels of CD8, but not CD3, predict better outcome in GC.

756 Objective Measurement of PD-L1 Protein in Gastric Carcinomas

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Background: Blockade of the PD-1/PD-L1 immune (co-)inhibitory pathway induces lasting clinical responses in patients with diverse solid malignancies. Tumor PD-L1 protein expression measured using chromogenic immunohistochemistry has been associated with clinical benefit, but the sensitivity and specificity of the test are limited. A fraction of gastric carcinomas (GC) show increased PD-L1 expression and early phase clinical trials indicate that ~30% of patients with advanced GC benefit from anti-PD-1 therapy.
Design: We measured PD-L1 protein using quantitative immunofluorescence (QIF) in a retrospective collection of 302 stages I-IV GCs represented in tissue microarray format. PD-L1 was detected using two monoclonal antibodies targeting different protein domains (clones E1L3N and E1J2J, Cell Signaling Technology) and measured using the AQUA method of QIF. Cutpoints for PD-L1 protein levels were generated using the visual detection signal threshold and by a permutation Jointpoint regression analysis.
Results: PD-L1 protein signal was located predominantly in the tumor compartment with a perinuclear/membranous staining pattern.Elevated PD-L1 protein was detected in 10.5% and 9% of GCs using the two different antibodies and a high correlation between the assays was seen (Regression coefficient [R^2]=0.83). There was no association between the levels of PD-L1 protein, patient age, the tumor anatomic location, and histological subtype. A trend for lower PD-L1 levels was seen in stage IV tumors. Elevated PD-L1 protein was associated with longer overall survival (log-rank P<0.05).
Conclusions: Elevated PD-L1 protein levels occur in ~10% of GCs and is associated with better outcome. Increased PD-L1 protein could be mediated by gene amplification, especially in EBV-associated GCs.

757 Adding Wide Area Transepithelial Sampling With Computer-Assisted 3-Dimensional Analysis (WATS3D) To Forceps Biopsies (FB) Augments Detection of Intestinal Metaplasia (IM) Following Ablation of Barrett's Esophagus (BE)

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Background: BE is a premalignant condition. Endoscopic ablation decreases the risk of progression to adenocarcinoma. Post-ablation surveillance involves 4-quadrant FB through the original BE segment. WATS^{3D} uses brushes to sample a significantly larger mucosal area, with computer-assisted analysis. Prior studies demonstrated the benefit of adjunctive use of WATS^{3D} for detection of IM and dysplasia. Our aim was to determine if extent of IM can predict discordance between WATS^{3D} and FB results.
Design: Patients undergoing follow-up endoscopy after BE ablation between June 2012 and May 2014 were evaluated. WATS^{3D} samples were obtained using the standard 2-brush technique (CDx Diagnostics, Suffern, NY). The squamocolumnar junction and the tubular esophagus were sampled by a single gastroenterologist with expertise in BE treatment and WATS^{3D}. WATS^{3D} samples were analyzed by a central laboratory. Four-quadrant FB were obtained simultaneously and reviewed by an expert GI pathologist. The biopsy specimens were evaluated for number of fragments, fragments with IM and goblet cells (rare, few, abundant).
Results: Post-ablation biopsies were obtained in 112 cases (78% male, mean age 62 years). Diagnostic agreement between WATS^{3D} and FB was seen in 88 cases (80%). Of the 28 cases in which IM was detected, only 4 (14.3%) had positive results on both WATS^{3D} and FB. One case had high grade dysplasia on WATS^{3D} but not on FB. Fourteen cases were detected by FB alone; goblet cells were graded as rare or few, and IM was seen on a single tissue fragment. Of the10 cases seen by WATS^{3D} alone, at least 4 tissue fragments were present on FB.
Conclusions: Residual or recurrent IM detected following BE ablation was found in 25% of cases. When IM was present, goblet cells were detected on both WATS^{3D} and FB in only 14.3% of cases. Although WATS^{3D} covers a significantly larger area compared to FB, these results demonstrate that the brush technique does not sample all post-ablation mucosa, or that biopsy samples deeper tissue. Additionally, it shows that when goblet cells are sparse, either technique is equally likely to not sample them. Further improvements in WATS^{3D} brush technology and technique may further augment its benefit in post-ablation surveillance, although for now it cannot replace FB for single method BE sampling.

758 Foveolar Dysplasia: An Interobserver Study

Stefano Serra, Rola Ali, Adrian Bateman, Kaushik Dasgupta, Vikram Deshpande, David Driman, David Gibbons, Andrea Grin, Sara Hafezi-Bakhtiari, Vajpeyi Rajkumar, Kieran Sheahan, Amitabh Srivastava, Shaun Walsh, Lai Mun Wang, Runjan Chetty. University Health Network, Toronto, Canada.
Background: This study examined the strength of agreement between pathologists in foveolar dysplasia diagnosis.
Design: Whole-slide scanned images of H&E stained slides of 9 gastric biopsies and 2 resections were circulated and assessed by 13 gastrointestinal pathologists. Each pathologist recorded the type of lesion (reactive vs dysplastic), the degree of dysplasia (low-grade vs high-grade) and the type of dysplasia (foveolar vs adenomatous). Results were entered into a standardized data collection form and analyzed using k– statistics.
Results: The cases were selected by and the diagnoses agreed on by SS and RC. Four cases were reactive, 5 had low-grade dysplasia (4 foveolar, 1 adenomatous), and two had high-grade dysplasia (both foveolar). The results were as follows: reactive vs dysplastic and low-grade vs high dysplasia: slight to substantial agreement (K= 0.2 to 0.72) with slight-fair agreement for 7 pathologists and moderate agreement for 5 pathologists; agreement with respect to the type of lesion, foveolar vs adenomatous, ranged from less than chance to substantial (K=-0.08 to 0.67) with moderate agreement for 7 pathologists

Observer	K for reactive vs dysplastic	K for type of dysplasia
Pathologist 1	0.31	0.02
Pathologist 2	0.45	-0.08
Pathologist 3	0.17	-0.37
Pathologist 4	0.31	0.27
Pathologist 5	0.56	0.53
Pathologist 6	0.72	0.57
Pathologist 7	0.32	0.67
Pathologist 8	0.32	0.43
Pathologist 9	0.44	0.58
Pathologist 10	0.02	0.25
Pathologist 11	0.45	0.46
Pathologist 12	0.30	0.16
Pathologist 13	0.58	0.56

Conclusions: This study showed that there is poor to moderate agreement in separating reactive change from foveolar dysplasia, in grading dysplasia and in distinguishing between foveolar and adenomatous dysplasia.

759 Reporting of Gastric Foveolar Dysplasia: A Survey of Gastrointestinal Pathologists

Stefano Serra, Rola Ali, Adrian Bateman, Kaushik Dasgupta, David Driman, David Gibbons, Andrea Grin, Sara Hafezi-Bakhtiari, Vajpeyi Rajkumar, Kieran Sheahan, Amitabh Srivastava, Eva Szentgyorgyi, Shaun Walsh, Lai Mun Wang, Runjan Chetty. University Health Network, Toronto, Cape Verde.
Background: There is considerable variability in the criteria used for diagnosing gastric foveolar dysplasia (FD) amongst pathologists.
Design: The diagnostic criteria for separating FD from reactive change and adenomatous dysplasia (AD) were tested by an online questionnaire that was circulated to 13 gastrointestinal (GI) pathologists (Canada, UK and USA) The following was ascertained:
1. How often do you make a diagnosis of FD per year?
2. List the diagnostic criteria you use for FD.
3. List the criteria separating low-grade (LGD) from high-grade (HGD) FD
4. How do you distinguish LGD FD dysplasia from reactive change?
5. How do you distinguish FD from AD dysplasia?
6. Do you use immunohistochemistry in the diagnosis of FD? If so, what stains?

Results: Eight out of 13 pathologists never or rarely (1-2 times /year) make the diagnosis of FD, while 5 pathologists make it 4-12 times/year. The criteria used for the diagnosis of FD include: eosinophilic pale cytoplasm, cytoplasmic features of foveolar type mucin, nuclear size, round monomorphic nuclei, prominent nucleoli, minimal or no nuclear stratification. Criteria used to separate LGD from HGD include: membrane irregularities, loss of polarity, higher N/C ratio, architectural complexity, nuclear size. The distinction between reactive and dysplastic lesions is based on the presence of inflammation, surface maturation, lack of architectural complexity and gradual merging with the surrounding cells, nuclear hyperchromasia and enlargement. Rounded nuclei with minimal atypia and stratification, and lack of intestinal metaplasia are used to distinguish FD from AD. Immunohistochemistry (CDX2, MUC5AC and CD10, AMACR and p53) is used by 6 out 13 pathologists in difficult cases.
Conclusions: Gastric foveolar dysplasia is rarely diagnosed in this survey of GI pathologists and the criteria used are variable and inconsistently applied. There is a need to standardize criteria, particularly with regard to separating reactive change from LGD.

760 ARID1A in Gastric Adenocarcinoma: Clincopathologic Features and Association With Expression of p53 and MMR Proteins

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Background: *ARID1A* mutations, mutually exclusive with *TP53* mutations, have been described in ovarian and uterine carcinomas. In recent years, mutations in *ARID1A* have been reported in a subset of esophageal, gastric and colonic adenocarcinomas and an association with MLH-1 loss and/or microsatellite instability has been proposed. The aim of our study was to determine the clinicopathologic characteristics of ARID1A-deficient gastric adenocarcinomas (GAC) and investigate its association with p53 and DNA mismatch repair protein (MMR) expression.

Design: Tissue microarrays (TMAs) were constructed from 146 GAC treated at MGH between 1988 and 2007. Clinical and pathologic data was obtained by chart and/or histologic review of slides. ARID1A and MMR proteins were recorded as deficient when there was complete loss of staining in the tumor cells. Diffuse/strong, confluent p53 staining or complete loss of p53 staining (with positive stromal or inflammatory background cells) was scored as a mutant pattern of staining. Logistic regression analysis was done to determine significant clincopathologic parameters associated with loss of ARID1a expression.

Results: The mean age of patient cohort was 68.3 yrs (range 22-104 yrs) and the M:F ratio was 1:1.5. ARID1a expression could be evaluated in 144/146 (99%) tumors and loss of ARID1A was seen in 22/144 (15.3%) cases. ARID1A-deficient GAC were seen more commonly in individuals ›70 years of age (p= 0.027). Loss of ARID1A expression was significantly associated with MMR deficiency (MLH1 loss in 33%; p=0.0013). Mutant pattern of p53 expression show no relationship to loss of ARID1A staining (13% vs. 19% , respectively). Although GAC with ARID1A loss showed no difference in stage ³pT2 (16% tumors with loss vs. 13% of ARID1a intact tumors), but were associated with a lower frequency of lymph node disease (8% of ARID1a-deficient tumors had N stage ³1 vs. 22% of ARID1a-intact tumors, p 0.045). ARID1A loss was not associated with gender, T stage, M stage, tumor grade, WHO histologic type, lymphovascular invasion, perineural invasion or tumor size.

Conclusions: ARID1A-deficient GAC are more prevalent in older patients and show an association with DNA MMR deficiency, low risk of nodal involvement and are not mutually exclusive of p53 overexpression.

761 Test Algorithm for Protein-Expression Based Classification of Gastric Adenocarcinoma Encompassing TCGA and "Mesenchymal" Subtype

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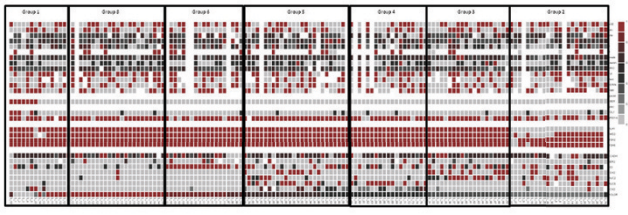
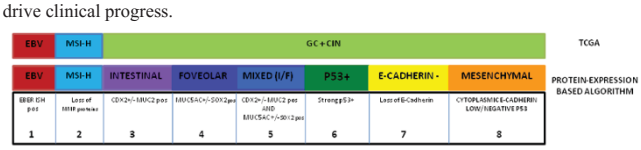
Background: Recently, comprehensive molecular classification by TCGA identified 4 distinct groups of gastric adenocarcinoma: EBV, MSI, genomically stable (GS), and chromosomally instable (CIN)[Nature 513, 202-9; 2014]. While comprehensive molecular-genetic classification drives progress, it is currently impractical from a routine diagnostic perspective. Building on the TCGA data, here we employed techniques available in routine diagnostic practice to devise a novel classification of gastric adenocarcinoma.

Design: Tissue microarrays from 146 tumor resections performed between 1988-2007 were assessed using 14 diagnostic biomarkers. We defined the following diagnostic groups. Pattern of protein expression was assessed using supervised cluster analysis followed by comparison of clinical phenotype and outcome analysis.

Results: Supervised analysis allowed classification into EBV positive (1) and MSI-H (2) whereas the GS/CIN group separated into: intestinal (3), foveolar (4), mixed (intestinal/foveolar) (5), strong p53 expression (6), loss of E-Cadherin (7) and mesenchymal (8).



By phenotyping, group 8 demonstrated significantly higher number of cases with cytoplasmic E-cadherin (P 0.02)and absent p53 (<0.0001), characteristic of "mesenchymal" subtype by gene expression profiling (Gastroenterology 145, 554-65, 2013). Proteomic groups differed by clinical phenotype and the MSI group (2) showed longer whereas the foveolar (4) and mesenchymal groups (8) showed shorter overall survival times. A straightforward test-algorithm allows clinical implementation to drive clinical progress.



Conclusions: We propose a novel classification for gastric adenocarcinoma based on supervised analysis of protein expression. This analysis is an important first step in providing large-scale datasets amenable to routine clinical evaluation and therapeutic design.

762 Western Series of Early Gastric Cancer: Comparison With Eastern Series and Evaluation of Risk Factors for Lymph Node Metastasis

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Background: Early gastric carcinoma (EGC) has a favorable prognosis unless associated with lymph node metastasis. Prognostic differences have been noted with shorter survival in western patients compared to Asian, particularly Japanese patients. Epidemiologic and biologic differences are present between the Eastern and Western population. The objective of this study was to investigate the differences in EGC in western vs. an eastern study group (i.e., Korean) and identify the features predictive of lymph node metastasis in a Western cohort of EGC.

Design: The western study cohort consisted of 52 cases that underwent gastrectomy and lymph node dissection without neoadjuvant therapy at MGH between 1995 and 2011. The data was compared against similarly treated Korean study cohort of 86 cases treated at Pusan National University Hospital in 2010. Demographic and clinicopathologic features were recorded by medical chart and slide review. Additional data including measurement of tumor thickness and submucosal depth of invasion by classic and previously described alternate method was also recorded. Contingency testing and survival analysis employed t-tests, Fisher's exact, Chi-square and log-rank test as applicable; statistical significance was defined as P<0.05.

Results: The mean age in our study cohort was significantly older than the Korean study group (mean age, 69.8+-11.2 yrs vs. 59.9+-9.8 yrs, P 0.0001), with equal distribution in both genders (M:F 1.2:1 vs. 2.9:1, P 0.06) and a homogenous distribution of different gross types of EGC (Type0I:Type0II:Type0III-2.1:1.8:1 vs. 2.5:1:9, P <0.001). No significant difference in lymph node metastasis was seen in between the two groups (our: Korean- 1.05:1, P 1). Despite the small number of cases with lymph node metastasis (n-10), the rate of lymphovascular invasion was higher in lymph node positive EGC cases (P 0.0004). No significant correlation was seen between the presence of node metastasis and other histologic parameters or overall survival.

Conclusions: Although there are some clinicopathologic differences in characteristics of EGC in Western and Korean study groups, the rate of lymph node metastasis and overall survival are similar. Correlation of lymphovascular invasion and nodal involvement confirms that presence of any level of lymphatic involvement carries prognostic significance. Furthermore, our overall results counter the myth of worse prognosis in western patients.

763 PTEN Protein Expression Is Reduced in Eosinophillic Esophagitis (EoE) Biopsies From Patients With PTEN Gene Mutations (PTEN MT)

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Background: We recently reported an increased incidence of eosinophilic gastrointestinal disorders (EGID) in patients who have PTEN gene mutations (JPGN 2014; 58:553). We hypothesized that PTEN protein expression is reduced in biopsies showing EoE, the most common form of EGID, in patients who have PTEN MT compared to those who do not (PTEN NL).

Design: Distal esophageal biopsy slides were stained with PTEN antibody (Abcam, Cambridge, MA, 1:150). Staining intensity was scored using a 4 point scale (0=normal; 3=most intense). Stain distribution was scored also using a 4 point scale (0=none; 3=staining in more than two-thirds of the total epithelial thickness). EoE biopsies showed ³15 eosinophils per high field and were obtained prior to EoE-targeted therapy. Student's t test was used to compare the results and significance was set at P£0.05.

Results: PTEN protein was expressed in basal layer squamous epithelial cell cytoplasm of normal uninflamed esophageal biopsies; staining intensity of such biopsies from PTEN NL patients (1.3±0.5, range 1-2, N=10) did not differ from uninflamed esophageal biopsies from PTEN MT patients (1.3±1, range 0-3, N=6, P=0.93). However, PTEN protein-staining intensity in PTEN MT EoE biopsies was significantly reduced compared to PTEN NL EoE (0.7±1, range 0-2, N=3 vs 2.5±1, range 0-3, N=8, P=0.03). In addition, staining occurred in significantly less depth of epithelium in EoE biopsies from PTEN MT patients compared to EoE biopsies from PTEN NL patients (0.3±0.6, range 0-1 vs 1.8±0.9, range 0-3 P=0.03).

Conclusions: We show, for the first time, that PTEN protein is expressed in normal esophageal squamous epithelium, and expression is significantly reduced in EoE biopsies of patients who have mutated PTEN genes. These observations suggest that reduced PTEN protein expression contributes to the increased incidence of EGID in PTEN MT patients.

764 Is Grossly Cryptic Early Gastric Carcinoma a Distinctive Phenotype Rather than a Stage?

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Background: The vast majority of gastric carcinomas are advanced. In the WHO classification, early gastric carcinoma (EGC) is not a subtype but carcinoma limited to the mucosa or submucosa regardless of the nodal status. In endoscopic/clinical classifications (Japanese Endoscopic Society, Paris Classification), EGCs are classified by the macroscopic appearance as polypoid, elevated or ulcerated. However, none of these classification schemes apply when EGC is grossly cryptic or extends beyond a small grossly visible lesion. We studied resected grossly cryptic EGCs for their clinical, endoscopic and pathological characteristics.

Design: Between 1994 and 2014, 16 cases of EGC were retrieved from the clinical/pathology archives at our medical center. Only cases with a prior biopsy and subsequent gastrectomy were included. We reviewed each case for clinical presentation, endoscopic appearance, gross appearance of the resected specimen, microscopy of tumor and surrounding stomach, stage and available follow-up.

Results: There was a female predominance (68%) and a mean age of 62. The most common clinical presentation were epigastric pain (7), weight loss (5), anemia (2) and gastrointestinal bleeding (2). Endoscopic findings included small mucosal irregularity (9), small shallow ulcer/erosion (3), and normal stomach (4). After biopsy, all cases underwent partial or subtotal gastrectomy. Determining the extent of the tumor was challenging in 15 of 16 cases since the tumor was either grossly unidentified or cryptic tumor extended considerably beyond the confines of a shallow erosion. Eighteen to 58 blocks were required for evaluation. Microscopic invasive tumor was either well to moderately differentiated intestinal (7), poorly differentiated (7) or mixed (2). The stomach in the background showed chronic gastritis (10), intestinal metaplasia (6) and Helicobacter organisms (3). All cases except one (pT1N2) were stage pT1N0. More than 6 months of follow-up was available in 12 cases and ranged from 7 months to 18 years. A disease-free state without recurrent tumor was seen in 8/12 (66%) cases.
Conclusions: The grossly cryptic EGCs show female preponderance and have clinical, endoscopic and pathological characteristics that do not conform to any prototypical gastric carcinoma classification schemes. EGCs also show mixed microscopic subtypes and predisposing conditions. Gross evaluation, sampling and sizing of EGC is challenging. Nodal metastasis is rare and prognosis is excellent. Rather than just an early stage, our findings raise the possibility that EGC may signify a distinctive and overlooked phenotype.

765 Usage of Helicobacter pylori Immunohistochemistry Is Not Associated With the Diagnostic Rate of Helicobacter pylori Infection

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Background: There are no accepted guidelines for the optimal utilization of Helicobacter pylori immunohistochemical testing. Some institutions perform this test on every gastric biopsy (or perform an alternative special stain by protocol). Our institution leaves it to the discretion of the pathologist, who orders immunohistochemistry on a self-defined "as needed" basis. Special stains are infrequently used in our institution for this purpose. We sought to determine if pathologists who ordered immunohistochemistry more frequently had higher rates of detecting Helicobacter pylori infection.
Design: Following approval by our IRB, we queried our database for all gastric biopsies received in calendar year 2013 (excluding biopsies of the cardia/GEJ). Included biopsies were sorted by pathologist and we subsequently calculated the rate of Helicobacter pylori diagnosis and the rate of immunohistochemical staining. Preliminary data was gathered for 3 general surgical pathologists, and 1 gastrointestinal pathologist (all with >5 years' experience).
Results:

	GI	SP1	SP2	SP3
Total number of gastric biopsies	769	187	298	155
Total H.pylori positive biopsies	97	16	42	35
Percentage of H.pylori positive biopsies	12.61%	8.56%	14.09%	22.58%
Total HP IHC use	269	67	43	36
Percentage of HP IHC use	34.98%	35.83%	14.43%	23.23%
Number of IHC ordered per case	0.52	0.53	0.22	0.33
Average number of IHC ordered for 1 positive HP case	4.34	5.58	1.43	1.44

LEGEND:
GI = GI Pathologist
SP1,2,3 = Surgical Pathologists
HP = Helicobacter pylori
IHC = Immunohistochemistry.

Conclusions: In our preliminary investigations, pathologists with the highest rates of diagnosing *Helicobacter pylori* infection had the lowest rates of utilization of immunohistochemistry. This suggests that the use of immunohistochemistry is not independently associated with diagnosis of infection. False positives are an unlikely confounder given our rigorous quality assurance program. This suggests the use of *Helicobacter pylori* immunohistochemistry should be restricted to cases with a reasonable level of suspicion based on routine stain.

766 DNA Mismatch Repair Deficient Tumors Are Not More Prevalent Among Interval Colon Cancers When Matched for Tumor Location

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Background: Interval colorectal cancers (CRC) are reported to be more likely than non-interval CRC to exhibit DNA mismatch repair (MMR) deficiency, suggesting a faster progression via the serrated instead of the conventional pathway of CRC carcinogenesis. This finding is potentially confounded by tumor location as both interval and MMR-deficient CRC tend to predominate in the right colon. We compared the clinicopathologic characteristics and MMR expression in interval and non-interval CRC matched for tumor location.
Design: All CRC diagnosed during 2007-2011 in our institution were identified. Interval CRC was defined in two ways: (i) CRC diagnosed prior to the next recommended colonoscopy and at least 1 yr after last colonoscopy; (ii) CRC diagnosed <5 yrs but >1 yr after last colonoscopy. DNA MMR status was determined using a two-antibody screen (PMS2 and MSH6) which has been validated to be as effective as a four-antibody panel (PMS2, MSH6, MLH1, MSH2). Interval CRC and non-interval controls were matched 1:1 on age, gender and tumor location. Associations of interval CRC with MMR deficiency and other clinicopathologic characteristics were evaluated using McNemar's test and conditional logistic regression, as well as Chi-square test for any unmatched comparison.
Results: Fifty-four of 1094 tumors were interval CRC (4.9%) detected prior to next recommended colonoscopy. Interval CRC were more likely than non-interval CRC to occur in the right colon (57.4% vs. 35.8%; p=0.006) and in patients older than 70 years (54.6% vs. 34.8%; p=0.002). There was no significant difference in aberrant MMR expression between interval CRC and matched controls (18.5% vs. 16.7%; p=0.80). MMR-deficient tumors were seen mostly in women, with 89% involving the right colon and 74% being ^{3}pT3. A greater proportion of interval CRC than matched controls presented as >pN1 at diagnosis (14.8% vs. 5.6%). Tumor size or other clinicopathologic characteristics showed no significant difference between interval CRC and matched controls. All associations remained significant when analyses were restricted to interval CRC diagnosed <5 yrs after last colonoscopy (n=42).
Conclusions: Interval CRC are more prevalent in the right colon and show no difference in aberrant MMR expression when compared to non-interval CRC matched for tumor location. Interval CRC appear more likely to develop from missed lesions or incompletely excised precursors rather than rapid tumor growth related to DNA MMR deficiency.

767 Comparison of Mismatch Repair Protein Immunohistochemistry in Matched Colorectal Adenocarcinoma Biopsy and Resection Specimens

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Background: Lynch syndrome (LS) is an autosomal dominant disorder due to germline mutations in mismatch repair (MMR) genes which lead to an increased risk of multiple neoplasms, including colorectal adenocarcinoma. The most commonly mutated MMR genes are: *MLH1*, *MSH2*, *MSH6*, and *PMS2*. It is the loss of the protein products of these genes which are routinely examined for by immunohistochemistry (IHC) on surgical specimens as a screening tool for LS. This study aims to compare MMR IHC results in paired biopsy and resection specimens of colorectal adenocarcinomas.
Design: The surgical pathology archives at our institution were examined for colorectal resection specimens with abnormal MMR IHC from 2010 through 2014. We identified 23 cases with abnormal MMR IHC on the resection specimen which had a matching biopsy specimen available for MMR IHC. A comparative cohort of 20 colorectal adenocarcinoma resection specimens with intact MMR IHC expression and available biopsy specimens was also examined. IHC testing was performed against MLH1, MSH2, MSH6, and PMS2 (Biocare Medical) and nuclear staining was considered positive. Concordance between biopsy and resection specimen IHC staining was determined.
Results: Forty (93%) of the biopsy specimens demonstrated concordance with their matched resection specimens for all MMR proteins. All cases (100%) with retained MMR IHC expression on resection demonstrated IHC expression of all four MMR proteins on biopsy specimen. Of the 23 cases will loss of staining of at least one of the MMR proteins on resection, 20 (87.0%) of the cases demonstrated complete concordance in MMR IHC staining between the two specimens. The three cases that did not have complete concordance still demonstrated loss of at least one of the MMR proteins in both specimens.
Conclusions: MMR IHC testing of colorectal adenocarcinomas is already recognized as an important screening modality for the identification of patients at risk for LS. As such, the identification of abnormal MMR IHC testing at time of endoscopic biopsy is critical for the surgical management and genetic counseling of these patients. We demonstrated a greater than 90% concordance rate between the MMR IHC staining profile of matched biopsy and resection specimens. Of note, those cases where there was not complete concordance, at least one of the MMR proteins on IHC was lost, flagging the patient for further workup. MMR IHC has been shown to be a useful tool in LS screening and the use of MMR IHC testing on endoscopic biopsy specimens prior to resection should be considered.

768 Increasing Incidence of Gallbladder Dysplasia and Malignancy at Younger Ages: A Case Series

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Background: Adenocarcinomas of the gallbladder are presumed to arise through inflammation-induced intestinal metaplasia and dysplasia. Though uncommon, the identification of clinically unsuspected dysplastic and malignant lesions is well recognized in the examination of cholecystectomy specimens in routine pathology practice. In our practice, there is a perception that an increasing proportion of cholecystectomies are performed in younger patients, and that the rates of incidentally identified dysplasia are also rising.
Design: We evaluated cholecystectomy specimens from a 10 year period, 2004-2014. The laboratory information system was queried for total number of gallbladders and for those having diagnoses of intestinal metaplasia (IM), indefinite for dysplasia (IND), low grade dysplasia (LGD), high grade dysplasia (HGD) and adenocarcinoma (CA).
Results: A total of 2340 gallbladders were resected over the 10 year period, from 1574 females (67.3%) and 766 males (32.7%), with an average age of 50.8 years. 1305 (55.8%) were younger than 50 years. Diagnoses of IM, IND, LGD, HGD and CA were made in a total of 70 cases (2.99%) (Table 1). Lower grade lesions were also noted in the background of higher grade lesions (20 patients with IM in the background of LGD/HGD/CA, 6 with LGD in cases of HGD and 2 with HGD in cases of CA). The average age of patients increased with progression toward malignancy, correlating with the decreasing proportion of younger patients, however, pre-malignant and malignant lesions were identified in a large proportion of younger patients.

Conclusions: Pre-malignant lesions such as IM and dysplasia are not uncommon in gallbladder resections, and these seem to be increasing in younger patients. This may reflect the growing rate of obesity in North America, which significantly increases the risk of gallstones and their complications.

	Number	% of all Cholecystectomies	Average age	% in patients 50 years old
Intestinal Metaplasia	11	0.47	48.6	63.6
Indefinite for dysplasia	16	0.68	51.2	43.7
LGD	21	0.89	51.4	38.1
HGD	9	0.38	56.8	33.3

769 Pathologic Mechanism of Late Onset Gastrointestinal Complication Following 90Y Microsphere Radioembolization for Unresectable Hepatobiliary Cancer

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Background: Yttrium-90 (^{90}Y) microsphere radioembolization is a therapeutic technique permanently implanting resin or glass microspheres containing Yttrium-90, a beta-emitting isotope with a radiation range of 2 mm, into hepatic tumors via the hepatic artery. ^{90}Y has half-life of 64 hours and the effective radiation is delivered within 14 days post implant. The etiology of delayed gastric ulceration developing months after the treatment in some patients remains unclear. We investigate the histopathological changes of the gastric mucosa in patients with late onset gastrointestinal complication following ^{90}Y microsphere radioembolization for unresectable hepatobiliary cancer.

Design: Patients who have been treated with ^{90}Y microsphere radioembolization for unresectable hepatobiliary tumors were followed up in three different institutions. Three patients who presented with late onset gastrointestinal complication were examined by upper gastrointestinal endoscopy and biopsies. Biopsy tissues were processed for morphologic studies, special chemical stains and immunohistochemical stains for detection of infection and neoplasm.

Results: Focal gastric mucosal ulcers were diagnosed within 4 months to 12 months post ^{90}Y microsphere radioembolization. Microscopy showed multiple, monophorphic, round, purplish, ^{90}Y microspheres scattered within the lamina propria and submucosa. Most of the microspheres were distributed in a linear fashion, consistent with an intravascular location. The vascular lumen and endothelial cells were not clearly identified. The microspheres were surrounded by fibrotic tissue infiltrated by numerous plasma cells, eosinophils, lymphocytes and rare neutrophils. Foreign body giant cells, viral inclusions, Helicobacter pylori organisms and neoplastic cells were not identified. Epithelial granulation without pititis and pitabscesses, and miniaturized glands with intervening fibrosis were noted. The histopathological changes were consistent with localized gastric ulcer resulting from chronic ischemic gastritis due to thromboemboli of ^{90}Y microspheres.

Conclusions: Our findings suggest that late onset gastric ulcer following ^{90}Y microspheres radioembolization of hepatobiliary malignancies is a secondary injury due to chronic ischemia resulting from small vessel damage by ^{90}Y microsphere thromboemboli that were inadvertently rerouted to the gastric mucosa by way of the gastrohepatobiliary microvascular arcade.

770 The Lauren Classification Highlights the Role of Epithelial-Mesenchymal Transition in Gastric Carcinogenesis: An Immunohistochemistry Study of p-Stat3 and Adhesion Molecule Expression

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Background: Gastric cancer is a major health concern worldwide. According to Lauren classification, gastric diffuse type carcinoma is composed of poorly differentiated, small, round, dissociated tumor cells, which suggests epithelial-mesenchymal transition (EMT) process. Signal transducer and activator of transcription 3 (Stat3) is involved in EMT and its expression has been reported in gastric carcinoma. We sought to establish a link between Stat3 and EMT and highlight its expression according to Lauren classification. We performed immunohistochemical assessment of an activated form of Stat3 (pStat3^{Tyr705}) and adhesion molecules (E-caderin, α and β-catenin) in the two main types of gastric cancer defined by Lauren classification.

Design: FFPE tumor fragments from 195 patients with gastric cancer were retrieved from 3 pathology departments. All cases were independently reviewed by two pathologists to confirm diagnosis of intestinal or diffuse gastric cancer according to Lauren classification. Tissue microarray blocks were prepared and immunohistochemical staining was performed with antibodies directed against pStat3^{Tyr705}, E-cadherin, α-catenin and β-catenin. Statistical analysis was conducted using Student t test. Data were analyzed using Prism 5.0b (GraphPad Software Inc., La Jolla, CA).

Results: pStat3 expression was mainly present in diffuse type gastric cancer (median 31.2, standard deviation (SD) 41.2, p=0.003). For adhesion molecules, expression was decreased in the same type of gastric tumor: E-cadherin – median 62.0, SD 54.5, p<0.0001; α-catenin – median 38.1, SD 46.3, p<0.0001; β-catenin – median 47.9, SD 47.1 and p<0.0001.

Conclusions: Morphological features may help identify molecules involved in carcinogenesis. Immunohistochemical comparison between intestinal and diffuse types of gastric cancer has shown significant difference for pStat3, E-cadherin, a and b-catenin, suggesting the key role of EMT in carcinogenesis. Targeting molecules involved in EMT in aggressive gastric tumors could represent a novel therapeutic alternative.

771 Pancreaticobiliary Histologic Phenotype Is Predictive of Worse Overall Survival in Ampullary Carcinoma

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Background: Surgical resection for ampullary adenocarcinomas is associated with widely diverse survival outcomes, complicating clinical management for these patients. The different histologic lineages (intestinal, biliary or pancreatic) of tumors arising in the periampullary region can contribute to varied biological behavior of ampullary tumors, with histologic and immunohistochemical sub-classification proposed as an important factor for determining prognosis and possible response to adjuvant therapy.

Design: Sixty-seven cases of ampullary adenocarcinoma resected at our institution from 1995-2010 with known survival outcomes (up to ten years follow-up) were reviewed by three gastrointestinal pathologists. Hematoxylin and eosin (H&E) evaluation was performed to determine histologic subtypes of intestinal (IN), pancreaticobiliary (PB) or mixed (M, at least 10% of both components). Histologic subtypes were dichotomized (IN+M) versus (PB) and evaluated in relation to clinicopathologic variables and survival.

Results: There was concordant histologic subtyping by all three pathologists in 55 of 67 (76%) cases with good interobserver agreement for IN and PB subtypes (κ=0.79 and 0.70, respectively). PB subtype positively correlated with high grade differentiation and high pT status (pT3+pT4 vs. pT1+pT2) (p=0.001 and 0.045, respectively, Chi-square test), but did not correlate with other variables (age, sex, tumor size, pN, perineural, lymphovascular). Univariate analyses revealed statistically significant worse overall survival for pN1 (HR=2.27, 95% CI 1.06-4.89), AJCC Stage 2+3 (HR=2.59, 95% CI 1.11-6.08) or PB subtype (HR=2.37, 95% CI 1.14-4.92). In multivariate analysis, PB subtype was an independent predictor of overall survival (adjusted HR=2.28, 95% CI 1.09-4.75), as was pN1 status (adjusted HR=2.19, 95% CI 1.01-4.72). Other clinicopathologic variables were not significant in multivariate analysis. Patients with PB subtype had a median overall survival of 31.7 months versus >120 months for the IN+M subtype (log rank p=0.018).

Conclusions: Reliable H&E histologic subtyping can be performed for a majority of ampullary carcinomas to yield important prognostic information useful in clinical care and evaluation of clinical trials exploring better treatment regimens. Addition of immunohistochemical markers for classification should improve subtyping for cases with ambiguous patterns of differentiation.

772 Clinical, Histologic, and Immunophenotypic Features of Serrated Polyps in Patients With Inflammatory Bowel Disease

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Background: Colorectal serrated polyps (SPs) include hyperplastic polyps (HP) and sessile serrated adenomas (SSA). SPs in normal subjects have been widely studied. However, the classification and biological behavior of SPs in the setting of inflammatory bowel disease (IBD) are of continuous debate. Here we describe the clinical and histologic features; as well as Ki67, BRAF and b-catenin immunohistochemical (IHC) staining patterns of SPs in IBD screening biopsies.

Design: IBD biopsies with a diagnosis of HP or SSA from 2004 to 2013 were reviewed. Rectal and histologically poorly-oriented polyps were excluded. Ki67, b-catenin, and BRAF (IHC) were performed, and staining patterns were compared to a control group of SPs in non-IBD patients (25 HPs, 15 SSAs). Polyps were classified based on Ki67 staining patterns previously reported by Tarlokovic et all. Regular extension of Ki67 proliferative zone up to ½ of the crypt was designated as HP-like pattern, whereas irregular Ki67 staining with skip areas and extension of more than ½ of the crypt as SSA-like pattern.

Results: The IBD group consisted of 35 polyps. These were re-examined by two pathologists and classified to either SSA (n=12) or HP (n=23) in a blinded fashion. Average IBD duration was 13.1 years. Comparison of SSAs and HPs' location (right vs left), Ki67 and BRAF IHC readings between IBD and non-IBD patients are listed in table1. B-catenin did not show aberrant nuclear staining in the SSAs or HPs in either the study or the control groups. P values were calculated by Fisher's exact test, and no statistically significant differences were seen in any of these variables.

	Study Group		Control Group	
	SSA	HP	SSA	HP
SITE				
Right	66%	56%	60%	40%
Left	34%	44%	40%	60%
Ki-67				
SSA-like	58%	13%	60%	40%
HP-like	42%	87%	40%	60%
BRAF				
Positive	75%	28%	80%	24%
Negative	25%	72%	20%	76%

Table 1: Site, Ki67 and BRAF comparisons.

Conclusions: Serrated polyps in IBD patients have similar distribution, histologic features, and Ki67, BRAF and b-catenin immunohistochemical staining patterns compared to serrated polyps in non-IBD patients. Our findings suggest that the lesions in both groups are likely to represent similar entities.

773 Comprehensive Genomic Profiling of Esophageal Squamous Cell Carcinoma Reveals a High Frequency and Complex Landscape of Clinically Relevant Genomic Alterations

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Background: Esophageal squamous cell carcinoma (ESCC) is an aggressive form of malignancy with initial clinical presentation at high grade/stage and is often associated with poor outcomes. We queried whether ESCC also featured clinically relevant genomic alterations that could lead to targeted therapies for patients with relapsed and metastatic disease.

Design: DNA was extracted from 40 microns of FFPE sections from 54 clinically advanced ESCC. Comprehensive genomic profiling was performed on hybridization-captured, adaptor ligation based libraries to a mean coverage depth of 719X for 3,230 exons of 182 cancer-related genes plus 37 introns from 14 genes frequently rearranged in cancer. The results were evaluated for all classes of genomic alterations (GA) including base substitutions, short insertions and deletions, copy number alterations and fusions/rearrangements. Clinically relevant GA were defined as GA linked to drugs on the market or under evaluation in mechanism driven clinical trials.

Results: There were 29 female and 25 male patients with a mean age of 63.3 years. Two (3.7%) of the ESCC were grade 1, 22 (40.7%) grade 2 and 30 (55.6%) grade 3. There were 12 (22.2%) stage III and 42 (77.8%) stage IV tumors at the time of sequencing. A total of 397 GA alterations were identified (7.4 GA/ESCC), with 50 (92.6%) ESCC patients harboring at least 1 clinically relevant GA i (2.7 CRGA/tumor). Alteration of PI3K/mTOR pathway were found in 31 (57%) of patients involving 9 genes (*AKT1*, *AKT2*, *FBXW7, KIT*, *PIK3CA*, *PTEN*, *RICTOR*, *STK11*, *TSC2*). The most frequent clinically relevant GAs included *CCND1* (39%), *CDKN2A* (31%), *PIK3CA* (28%), *NOTCH1* (22%), and *PTEN* (13%). Frequent non-clinically relevant GA identified included TP53 (91%) and *FGF4*, *FGF3*, *FGF19* (39% each). There were no *ERBB2* amplifications or mutations identified in the ESCC cases. The 22% *NOTCH1* clinically relevant GA frequency was higher than that seen in previously reported literature. HPV was identified in 3 cases (5.6%) with 2 HPV-16 and 1 HPV-18 positive cases.

Conclusions: ESCC are a distinct and aggressive form of malignancy which with comprehensive genomic profiling harbor a diverse landscape of genome derived drug targets. These alterations can serve as targets for therapies, either approved or in clinical trials for the majority of patients and have the potential to improve outcomes for patients with this aggressive form of malignancy.

774 Increased HER 1, 2 and 3 Expression From Low To High Grade Dysplasia in Barrett Esophagus Neoplasia: Mechanistic and Therapuetic Implications

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Background: Increased HER2 expression is a neoplastic driver of a variety of adenocarcinomas, including those arising in Barrett esophagus. Other members of the Her2 family, Her1 and Her3, are known to have a similar carcinogenic role. The goal of our study is to quantitatively assess the expression and inter-relationships of all three proteins in low and high grade dysplasia (LGD and HGD) in Barrett esophagus. Evaluating the level of expression of Her 1, 2, and 3 along neoplastic progression pathways adds to our mechanistic understanding of esophageal adenocarcinoma (EAC).

Design: By a Cerner database search, 42 LGD and 62 HGD were retrieved for the study. HER2 staining was performed with the HercepTest on Dako autostainer. EGFR (Clone H11; Dako; 1:50 dilution), Her3 (Clone RTJ.2; Santa Cruz; 1:30) were done on Leica Bond-III instrument. Immunoreactivity was then graded for % and intensity (0-3+) according to which H score was calculated in each case. One-sided t-test for the difference in means between two samples was used. Correlations among HER1, HER2, and HER3 expression within LGD or HGD was determined by the Pearson correlation coefficient.

Results:

		HER1	HER2	HER3
Means	HGD	18.82	3.23	80.81
	LGD	9.86	0.14	59.05
	Difference	8.96	3.09	21.76
	Std. err.*	4.69	1.66	9.34
	p-value**	0.030	0.042	0.011
Observations	HGD	62	15	59
	LGD	42	42	42

* Standard error of the difference in mean scores

** One-sided t-test, assuming unequal variances

The table summarizes the analysis of the differences in mean HER1, HER2 and HER3 H scores, between LGD and HGD. Within HGD, HER1 and HER3 scores were positively correlated, r=0.059 (p=0.658), and HER2 and HER3 scores were negatively correlated, r=-0.136 (p=0.642). Within LGD, HER1 and HER2 scores were positively correlated, r=0.135 (p=0.398); HER1 and HER3 scores were positively correlated, r=0.199 (p=0.213), and HER2 and HER3 scores were negatively correlated, r=-0.108 (p=0.497). However, these coefficients are not statistically significant.

Conclusions: Her1, Her2 and Her3 expression are increased in higher grade dysplasia. Although there is a trend of various coexpression of these proteins, the differences are not statistically significant. HER2 is expressed in only 10-20% of EAC, but rarely expressed in LGD and HGD. In contrast, HER1 and HER3 are frequently expressed in dysplasia, particularly HGD. We hypothesize that HER1 and HER3 are early driver events in neoplastic progression in the esophagus and offer additional avenues for targeted therapies.

775 The Prognostic Significance of ErbB-1 (EGFR), ErbB-2 (HER2) and c-MET Overexpression in Resectable Gastric Carcinoma (GC)

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Background: EGFR, HER2 and c-MET are tyrosine kinase growth factor receptors implicated in numerous malignancies, including GC. The aim of this study was to investigate the prognostic value of EGFR, HER2 and c-MET overexpression by immunohistochemistry (IHC) in GC and correlate it with clinicopathological characteristics.

Design: This retrospective study included 120 consecutive GC resected between 2002-2008. Tissue Microarray (TMA) blocks containing 4 cores/tumor were constructed, stained for EGFR, HER2 and c-MET by IHC, and scored independently by 3 pathologists, from 0-3+, based on membranous and cytoplasmic staining intensity respectively. Overexpression by IHC was defined as 2+ and 3+ for EGFR and c-MET; 3+ and 2+/ FISH amplified for HER2. Biomarker expression was compared with clinicopathological characteristics and overall survival (OS).

Results: Of the 113 interpretable cases, median age was 65 (range 29-94), 72% were male, histologic types: intestinal 76%, diffuse 14%, mixed 10%. Mean follow up time was 80.0 months, with 6 patients lost to follow-up. Median OS was 29.0 months 95%CI [21.7-47.9]. Biomarker correlation with OS:

IHC (N=113)	N/%	Median OS (mo)		HR [95% CI], p-value
		Positive	Negative	
EGFR	17/15	15	30	1.60, [0.89-2.87], p=0.11
HER2	14/12	85	26	0.51, [0.22-1.18], p=0.12
c-MET	65/58	28	48	1.17, [0.74-1.87], p=0.49
EGFR+/c-MET+ vs. Rest	12/11	13	35	2.58, [1.21-5.50], p=0.003

Univariate analysis showed tumor location, T, N, clinical stage and complete resection (R0) as correlated with OS. Only T and N stage remained significant in the biomarker adjusted model.

Conclusions: In our cohort of GC, HER2+ conferred a better prognosis while c-MET+ and EGFR+ emerged as negative prognostic markers. EGFR+ concomitant with c-MET+ status was significantly associated with poor OS. c-MET+/EGFR+/HER2- seem a stronger predictor for survival, however the group was very small. Larger studies to confirm our findings are warranted, since targeted therapy with dual or triple inhibition may provide a major therapeutic advance.

776 Expression of PDL1 in Gastric Adenocarcinomas and Associated Tumor Infiltrating Lymphocytes

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Background: Gastric adenocarcinoma is a leading cause of cancer mortality. Cases often present at an advanced stage for which prognosis is poor and standard chemotherapy offers minimal benefit, making identification of novel treatment strategies of paramount importance. Immunomodulatory therapy targeting Programmed Death Ligand-1 (PDL1) has shown promise in other tumors, including non-small cell lung carcinoma and malignant melanoma, but little is known regarding its expression in gastric tumors. Here we report the status of PDL1 expression on gastric adenocarcinoma and associated tumor infiltrating lymphocytes (TIL).

Design: 34 cases of invasive gastric adenocarcinoma were stained for PDL1 and the tumors and associated TILs were scored for PDL1 expression. Tumors with greater than 5% membranous staining were considered PDL1 positive. TILs were scored as no significant staining (0), less than 50% (focal) or greater than 50% (high). PDL1+ tumors were evaluated by DNA in situ hybridization (ISH) for EBV. Clinicopathologic data were collected for all cases.

Results: Our gastric adenocarcinoma cohort had an average age of 64 with 53% men and 47% women. 56% of tumors were intestinal subtype and 44% diffuse. 4/34 tumors (12%) showed membranous PDL1 expression. Of the PDL1 positive tumors, one was positive for EBV by DNA ISH. TILs were primarily present in an interface pattern between tumor and stroma. Overall, 45% of gastric adenocarcinomas showed some level of PDL1 expression among TILs with 27% showing focal interface expression and 18% showing high expression. 55% of TILs in had no expression of PDL1. High PDL1 expression on TILs correlated with lower tumor stage at presentation, while deaths occurred in a higher percentage of patients with PDL1 positive tumors than negative tumors.

Conclusions: We demonstrate that a small percentage of gastric adenocarcinomas and a significant proportion of gastric TILs express membranous PDL1. While limited in sample size, suggestive differences were present in survival and tumor stage based on

the status of tumor and TIL PDL1 expression. These findings indicate the potential importance of PDL1 expression in the gastric tumor microenvironment and support the targeting of PDL1 as potential therapy for gastric adenocarcinoma.

777 Assessment of Melanocyte Density in Anal Mucosa for the Evaluation of Surgical Margins in Primary Anal Melanoma

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Background: Histologic evaluation of surgical margins for resections of anorectal melanoma (AM) can be challenging as there are few guidelines on the normal density of melanocytes in the anorectal mucosa. We aimed to study the density of anal melanocytes using H&E morphology and commonly used immunohistochemical (IHC) markers for melanocytic differentiation, MiTF and HMB45. We also compared the density of melanocytes in normal anorectal mucosa to anorectal mucosa adjacent to AM.

Design: 24 cases of anorectal squamocolumnar mucosa without melanoma (no pathologic findings n=8, hemorrhoids n=10, dysplasia/colorectal carcinoma n=6) and 11 cases of AM were retrospectively analyzed. MiTF and HMB45 stains were performed. The number of MiTF- and HMB45-positive melanocytes was counted in three 400X high-power fields (HPF) with highest density of staining. Density of melanocytes in the squamous mucosa was defined as melanocytes per HPF whereas density in the glandular mucosa was defined as melanocytes per glandular epithelial cell. Statistical analysis was performed using two-tailed t-tests (P-value <0.05 considered significant).

Results: Non-AM patients had an average age of 45 years, male-to-female (M:F) ratio of 19:5, and racial demographic of white (n=19), black (n=3), and Asian (n=2). The melanocyte density in the glandular epithelium was 0.014 melanocyte/epithelial cell by MiTF and 0.004 melanocyte/HPF by HMB45. The density in the squamous epithelium was 3.633 by MiTF and 3.069 by HMB45. No significant difference in melanocyte density was identified between white and non-white patients.

AM patients had an average age of 67 years and M:F ratio of 3:5; all patients were white. AM cases had a significantly higher density of melanocytes in the mucosa adjacent to AM and/or melanoma in-situ with 0.278 melanocytes/epithelial cell in the glandular mucosa by MiTF and 0.245 by HMB45 ($p < 0.05$) and 32.2 melanocytes/HPF in the squamous mucosa by MiTF and 36.4 by HMB45 ($p < 0.05$).

Conclusions: Our study quantifies the density of melanocytes in normal anorectal squamous and glandular mucosa using IHC. We identified a significantly higher density of melanocytes in the mucosa adjacent to AM/melanoma in-situ which lack cytologic and architectural features of malignancy, consistent with hyperplasia of benign activated melanocytes. Benign melanocyte hyperplasia should be recognized when evaluating surgical margins for resection of primary AM.

778 Gastrointestinal Cytomegalovirus Infection Detectable in Biopsies Only By Immunostaining: Clinical and Pathological Features

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Background: Cytomegalovirus (CMV) infection is a great concern when diagnosing gastrointestinal (GI) biopsies of immunocompromised patients, and immunostaining is used liberally in such cases. We review the rare cases in which CMV infection was found only by immunostaining, but not in routine sections, and attempt to determine their clinical and pathological features.

Design: We performed a computer search for the GI biopsies from the University of Minnesota from 2004 to 2014 that were immunostained for CMV, and identified the cases in which CMV was found only by immunostaining (Group 1). We collected equal numbers of controls in which CMV infection was found in routine sections (Group 2) and those in which both the routine sections and the CMV stains were negative (Group 3). We quantified the pathological and clinical features of all cases and controls, correlated them with patient survival, and performed statistical comparison between the groups.

Results: We identified 8 cases in Group 1, 6% of all cases that had been immunostained for CMV. The mean patient age was 48 (range, 1-71) years. All had received solid organ or bone marrow transplants. The factors adversely associated with survival were extensive granulation tissue in the biopsy (p=0.026) and inclusion by the clinicians of the history of CMV infection in the clinical information (p=0.022). Compared with Group 2, Group 1 had fewer CMV-affected tissue pieces per biopsy (p=0.018), fewer CMV-affected cells (p=0.036), and fewer CMV nuclear and cytoplasmic inclusions (both p=0.036). All other clinical, laboratory and pathological features of Groups 1 and 2 were similar. Compared with Group 3, Groups 1 and 2 had much higher serum CMV quantitation results (Gr. 1 vs. Gr. 3 p=0.005 ; Gr. 2 vs. Gr. 3 p=0.009) and a greater degree of apoptosis (Gr. 1 vs. Gr. 3 p=0.033 ; Gr. 2 vs. Gr. 3 p=0.002) and edema (Gr. 1 vs. Gr. 3 p=0.04 ; Gr. 2 vs. Gr. 3 p=0.01) in the biopsies. There was no difference in other inflammatory and reactive changes between Groups 1 and 2 and Group 3.

Conclusions: Cases in which CMV can be found only by immunostaining are clinically and pathologically similar to those with more obvious CMV changes, differing only in the number of affected cells, making CMV hard to find on routine stains. Increased CMV DNA in the serum and the degree of mucosal apoptosis and edema distinguish CMV-affected cases from those in which CMV immunostains are likely to be negative.

779 Clinicopathologic and Molecular Analysis of Colorectal Carcinomas With POLE Hotspot Mutations

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Background: The Cancer Genome Atlas (TCGA) project identified a subset of colorectal carcinomas (CRC) that are mutated in the exonuclease domain of DNA polymerase epsilon (POLE-exo*). These tumors show an ultra-high somatic mutation incidence but little is known about them. In this study we examined their clinicopathologic and molecular features.

Design: Twelve POLE-exo* CRC cases described in the TCGA and one case identified at our institution were included in the study. Twenty eight microsatellite unstable (MSI-H) and 30 microsatellite stable (MSS) CRC cases in the TCGA were used as control groups. Virtual whole slide images (www.cbioportal.org) were examined for a number of morphologic features including presence and number of tumor infiltrating lymphocytes (TILs). Clinical (available in 9 cases) and molecular data were obtained from the cBioPortal.

Results: The majority (56%) of patients with POLE-exo* tumors were male (compared to 25% and 63% of patients with MSI-H and MSS tumors) and 44% of tumors occurred on the right side (vs 96% and 43% MSI-H and MSS cases). Five (38%) POLE-exo* tumors showed areas of poor differentiation, similar to MSI-H tumors (50%), while only 1 (7%) case had a typical medullary component compared to 7 (25%) MSI-H cases. Villous architecture was seen in 5 (38%) cases, compared to 2 (7%) and 0 MSI-H and MSS tumors. Mucinous differentiation was seen in 4 cases (31%) similar to MSI-H tumors (28%). Five (38%) cases showed high number of TILs (>10TILs/medium power field), compared to 71% and 3% of MSI-H and MSS tumors. All 13 POLE-exo* tumors were MSS and had mutations in *APC*. Seven (54%) cases had a mutation in *KRAS* or *NRAS* with the majority of mutations (4/7) found in *KRAS* exon 4. No case had a *BRAF* V600E mutation. Mutations in *PTEN*, *PIK3CA* and *ARID1a* were more common in POLE-exo* cases compared to MSI-H and MSS cases (54% vs 7% vs 0, 62% vs 25% vs 7%, 38% vs 7% vs 0).

Conclusions: POLE-exo* tumors are a morphologically heterogeneous group that shows histologic overlap with MSI-H tumors including frequent mucinous and poor differentiation. They are, however, more likely to have villous architecture and less likely to show typical medullary type histology and increased numbers of TILs, compared to MSI-H tumors. They are also more likely to occur on the left side compared to MSI-H CRC. Molecularly they appear to arise in the context of *APC* mutations, do not show *BRAF* V600E mutations, but harbor frequent *RAS, PTEN*, *PIK3CA* and *ARID1a* mutations.

780 Celiac Disease-Associated Small Bowel Adenocarcinomas: Clinico-Pathologic, Phenotypic and Molecular Study of 11 Cases

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Background: Primary non-ampullary small bowel adenocarcinoma (SBA) is a remarkably rare tumor in the general population. Celiac disease (CD) is associated with an increased risk of developing SBA. Published data on the morphologic, phenotypic and molecular features of CD-associated SBAs (CD-SBAs) are very limited.

Design: We analyzed 11 SBAs arisen in the setting of CD collected from four different Institutions. Immunoreactions for intestinal markers (MUC2, CDX2, CD10) and for gastric markers (MUC5AC, MUC6) were performed on representative sections of each SBA. In all cases, non-tumoral small intestinal mucosa was also examined. Additionally, we investigated the frequency of *KRAS*, *NRAS*, and *BRAF* somatic mutations in 9 out of 11 cases.

Results: The patients, 9 females and 2 male, had an average age of 56 (range 38-72). Three tumors were stage III or IV and 1 patient died after 6 months from diagnosis. One tumor was localized in the duodenum (second part), the remaining 10 were in the jejunum. On histologic examination, three neoplasms were high grade poorly differentiated carcinomas, one of which showing a poorly cohesive component with signet ring cells, while 8 were low grade intestinal-type adenocarcinomas. Ten cases were positive for CDX2, 7 for MUC2 and 7 for CD10. One case showed 15% MUC5AC-positive cells. No case had MUC6. Sequencing analysis of *KRAS*, *NRAS*, and *BRAF* hot spot regions revealed *KRAS* mutations in 3 of 9 cases: we found mutations in codon 12 exon 2 in 2 cases and in codon 61 exon 3 in the other one. We found no mutations in the hot spot regions of *NRAS* (exons 2, 3, 4) or *BRAF* (exon 15).

Conclusions: CD-SBAs affect more often younger people (especially female) than other SBAs and may behave aggressively. In about 30% of cases they display a high grade histology. In our series, most CD-SBAs express structural and phenotypic patterns of intestinal differentiation, including CD10 (a marker of the enterocyte brush border). Unlike SBAs associated with Crohn's disease, CD-SBAs are seldom positive for gastric markers. From a molecular standpoint, we could identify *KRAS* mutations in a subgroup of tumors.

781 Dysplasia in Sessile Serrated Polyps and Serrated Polyposis Syndrome: Clinocopathologic Characteristics

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Background: Sessile serrated polyps (SSP) and serrated polyposis syndrome (SPS) have emerged in recent years as precursors of microsatellite instability (MSI) colorectal cancer (CRC). Dysplasia in SSPs (SSPD) is a well-recognized but poorly understood pathologic finding and progression of SSP to CRC remains ill defined. The clinicopathologic features of SSPD and any association with SPS have not been extensively studied.

Design: A retrospective search of the pathology database was performed to identify all patients with SSPD between 2011-2014. For each patient, we recorded age, gender, and SSPD characteristics (endoscopic location, size, pathologic features). Each patient was evaluated for SPS using the WHO 2010 criteria. History of other polyps, CRC or other carcinoma, MSI status and family history of CRC were also noted. Patients with coexisting inflammatory bowel disease were excluded.

Results: We identified 61 patients with SSPD (26 men, 35 women, average age 63.8 years). Most patients (98%) had 1 SSPD, mostly in the right colon (79%) and most had low grade SSPD (LG-SSPD; 85%). 1 patient had 2 LG-SSPD, 1 each in right and left colon. 9 (15%) patients had SSPD with advanced neoplasia (SSPD-AN) at index colonoscopy (1 with high grade dysplasia, 4 with intramucosal carcinoma and 4 with invasive carcinoma). Patients with SSPD–AN were significantly younger (56.6 years versus 65 years, P = <0.05) than those with LG-SSPD. 18 (30%) patients had prior or concurrent SSP and 2 (3%) met WHO criteria for SPS; 1 had SSPD and high grade dysplasia and the second SSPD and intramucosal carcinoma (negative for MSI by IHC/PCR). Of the 7 other patients with SSPD associated carcinoma, 1 had MSH2/MSH6 loss, diagnostic of Lynch syndrome while another showed MLH1/PMS2 loss and BRAF V600E mutation. The remaining 3 patients in this group tested negative for MSI in their CRC and in 2 the MSI status was unknown.

Conclusions: We found 15% of patients with SSPD also had advanced neoplasia, supporting the role of SSP in carcinogenesis. Interestingly, the patients with SSPD-AN were younger than patients with LG-SSPD. 3% of the overall SSPD patient cohort and, more significantly, 22% of patients who had SSPD-AN met WHO criteria for SPS, confirming the relationship between SPS and increased CRC risk. Majority of our patients with SSPD and CRC tested negative for MSI, including 1 patient who met SPS criteria, suggesting alternate pathways of serrated carcinogenesis. Our data suggests that perhaps advanced neoplasia in SSP may be a minor criterion for SPS.

782 Hypoxia Induced HIF-1a Expression Promotes Angiogenesis, Tumor Budding Cell Survival and Cell Proliferation Arrest in High-Grade Tumor Budding Colorectal Carcinomas

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Background: Tumor budding (TB) in colorectal cancer (CRC) is an unfavorable histologic feature that correlates with advanced tumor stage, lymphovascular invasion, and metastasis. Hypoxia, one of the most important microenvironmental changes in solid tumors, has been shown to activate HIF-1a, a key player in the intracellular hypoxia pathway, and promotes tumor progression/metastasis and induces radio- and chemotherapy resistance. However, the association of hypoxia and HIF-1a activation in TB cells and their impact on CRC biology remains unclear. The goal of the study was to evaluate the role of hypoxia and HIF-1a activation in the tumor microenvironment.

Design: 10 cases of CRC with high-grade (>10 buds) and 9 low-grade budding (0-9 buds) were included in this study. Immmunostains for pancytokeratin AE1/3, HIF-1a, Ki-67 and CD31 were performed on representative sections. Cultured HCT116 human CRC cells were used for *in vitro* study and were exposed to hypoxia. Western blot for HIF-1a and phosphor-AKT and flow cytometry for cell cycle arrest were performed on these cells with appropriate controls.

Results: HIF-1a was strongly expressed in TB cells at the tumor invasion front and was also significantly increased in HCT116 cells after hypoxia exposure. The microvascular density (MVD) was significantly increased at the invasive front of high-grade TB group than the low-grade TB group (114.95±36.01 *vs* 58.43±15.84, p=0.015). p-AKT, a cell survival serine/threonine protein kinase, was strongly expressed in TB cells and HCT166 cells after hypoxia exposure. The proliferation index (Ki-67) of TB cells is significantly lower compared to the glandular epithelium of the tumor at the invasive front (6.7%±2.0 *vs* 73.7%±9.4, p=0.001). Similarly, the hypoxic HCT166 cells showed increased cell cycle arrest (G0/1 stage) compared to controls (61.90%±2.45 *vs* 39.36%±1.73, p=0.003).

Conclusions: Our results suggest that exposure of TB cells to hypoxia leads to increased angiogenesis as shown by increased HIF-1a expression *in vivo* and *in vitro* experiments. Increased MVD may facilitate lymphovascular invasion and metastasis. Increased expression of p-AKT can promote cell survival in a low proliferation state and hypoxic microenvironment, and hence also confer resistance to chemo-/radiotherapy. HIF-1a activation may be a key factor in cancer cell survival, angiogenesis and therapy resistance in CRC, and also explains aggressive behavior of CRCs with high TB.

783 A Molecular Signature for the Prediction of Recurrence in Colorectal Cancer

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Background: Several clinical and pathological factors have an impact on the prognosis of colorectal cancer (CRC), but they are not yet adequate for risk assessment. We aimed to identify a molecular signature that can reliably identify CRC patients at high risk for recurrence.

Design: Two hundred eighty-one CRC samples (stage II/III) were included in this study. A two-step gene expression profiling study was conducted. First, gene expression measurements from 81 fresh frozen CRC samples were obtained using Affymetrix Human Genome U133 Plus 2.0 Arrays. Second, a focused gene expression assay, including prognostic genes and genes of interest from literature reviews, was performed using 200 fresh frozen samples and a Taqman low-density array (TLDA) analysis.

Results: An optimal 31-gene expression classifier for the prediction of recurrence among patients with stage II/III CRC was developed using logistic regression analysis. This gene expression signature classified 58.5% of patients as low-risk and 41.5% as high-risk (P<0.001). The signature was the strongest independent prognostic factor in the multivariate analysis. The five-year relapse-free survival (RFS) rates for the low-risk patients and the high-risk patients were 88.5% and 41.3% (P<0.001), respectively.

Conclusions: We identified a 31-gene expression signature that is closely associated with the clinical outcome of stage II/III CRC patients.

784 What Is the Clinical Significance of Intestinal Metaplasia in Stomach Biopsies of Pediatric Patients?

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Background: Intestinal metaplasia (IM) of the stomach in adults has been associated with an increased risk for gastric cancer. IM is sometimes encountered in pediatrics gastric biopsies but there is very limited information in the literature regarding its clinical significance. The aim of the study was to determine the clinical significance and associations of gastric IM in pediatric patients.

Design: We searched our database for IM in gastric biopsies from pediatric patients (<18 years-old) from 2000-2014. The clinical presentation, endoscopic and histologic findings were reviewed. We randomly selected age-, demographics- and clinical presentation-matched control group. Follow-up biopsy findings and clinical presentations were reviewed, when available. Chi-square test statistical analysis was used to evaluate any association.

Results: We identified 23 patients with pediatric gastric IM and selected 46 patients in the control group. All IM were in the gastric antrum. 5 patients (21.7%) with IM were <10 years old and 2 (8.7%) were <5 years old. 56.5% of the patients were females; 78.3% were white. Follow-up biopsies in 6 patients (26%) >1year after original IM showed no IM. There was no statistically significant difference (i.e. <.05) in gender (p = 0.73) or race (p = 0.13) in the IM and the control group.

Clinical presentation / Endoscopic findings / Pathologyfindings	Gastric Intestinal Metaplasia N=23	Control Group N=46	p-value
Dysphagia	4 (7%)	7 (15%)	0.82
Clinical Reflux Diagnosis	11 (48%)	22 (48%)	1
Reflux changes on endoscopy	4 (17%)	12 (26%)	0.42
Antral gastritis	13 (56%)	17 (37%)	0.12
Helicobacter pylori infection	1 (4%)	0 (0%)	0.15
Allergies	10 (43%)	30 (65%)	0.08
Asthma	5 (22%)	10(22%)	1
Proton pump inhibitor Rx	18 (78%)	30 (65%)	0.27
Sucralfate	8 (35%)	7 (15%)	0.06
Inflammatory bowel disease	1 (4%)	9 (19%)	0.09

Conclusions: There is no significant difference in the demographics, clinical presentation and medications between the patients with IM and the control group. It appears that IM in pediatric patients does not have the same association or pathway to carcinogenesis as IM in adult. Larger cohort with longer follow-up, perhaps into adulthood may be required to determine the clinical significance, if any of IM in pediatric patients.

785 Development of a Comprehensive Registry of Gastrointestinal Stromal Tumor (GIST) Patients at a Single Canadian Institution

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Background: GIST are the most common mesenchymal neoplasms of the gastrointestinal tract. The development of targeted therapies, specifically imatinib, has dramatically changed the management and outcome of patients with these tumors. However, there are very few reports of long-term prognosis from a Canadian population in the post imatinib era.

Design: All patients diagnosed with GIST at a single institution between 2001 and 2013 were registered. Collected data included clinicopathological characteristics: tumor size, location, histologic classification, mitoses; demographics, outcomes and therapy. A standard immunohistochemical (IHC) panel was performed: CD117, DOG1, CD34, vimentin, S100, desmin, SMA, caldesmon, and ki-67. Biological risk was assessed using AFIP criteria. PCR amplification and sequencing was done for exons 9, 11, 13, 17 of KIT and exons 12 and 18 of PDGFRA genes. Univariate analysis was performed using the Chi-square and Mann-Whitney tests to identify clinicopathological variables associated with progressive disease, defined as metastases or local recurrence.

Results: 167 patients were registered; median follow-up was 41 months (range 0–162). Five patients received neoadjuvant imatinib, 161 had surgery and 49 received adjuvant imatinib. The majority (60%) were in the stomach. The median tumor size was 7.23 cm (range 0.3–35 cm) and 68% were spindle-type. By IHC, 100% and 63% were CD117 and DOG1 positive, respectively. Biologic risk included: 19 very low risk (11%), 71 low risk (43%), 20 intermediate risk (12%), and 52 high risk (31%). Of 56 GIST sequenced, mutations included: 44 in KIT and 12 in PDGFRA; 15 were wild-type. Progressive disease included: 27 metastases and 8 local recurrences. Median time to progression was 14 months (range 0–125). Of 29 patients who died, 16 died of disease with metastases. Univariate analysis showed that gender (p=0.02), tumor size (p<0.001), mitoses/50 HPF

($p<0.001$), and ki-67 ($p<0.001$) were associated with increased risk of metastases. Most metastases developed in high risk GIST: 24/27 (89%). In contrast, only 24/52 (46%) high risk GIST developed metastases and 7 (4%) had local recurrence. Of these high risk GIST, only ki-67 was significantly associated with metastases ($p=0.003$).
Conclusions: In this Canadian registry with long term follow up, standard pathological variables were effective at identifying patients with low risk GIST. However prognostication of high-risk GIST may be improved by adding ki-67 to usual IHC panels.

786 Idelalisib Is a Novel Biologic Agent That Can Cause Severe Gastrointestinal Toxicity

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Background: Idelalisib is an inhibitor of the PI3KΔ isoform used to treat patients with B-cell lymphoma and leukemia. Unfortunately, this protein is also necessary for intestinal mucosal integrity and, thus, some patients develop gastrointestinal toxicity with treatment. We performed this study to characterize the histologic features of idelalisib-induced colonic injury in biopsies obtained from patients who developed gastrointestinal symptoms.
Design: We evaluated the clinical features of 50 lymphoma/leukemia patients treated with idelalisib for at least 3 months. We reviewed hematoxylin and eosin (H&E) stained sections from colonic biopsies of those patients with symptoms severe enough to warrant colonoscopy. Cases were assessed for histologic patterns of injury and distribution of inflammation. The nature of inflammatory infiltrates was evaluated using immunostains for CD3, CD4, CD8, CD25, CD30, CD20, S100, CD1a, and CD207.
Results: Twenty-two (44%) patients experienced variably severe watery diarrhea during treatment with idelalisib, including 14 who underwent colonoscopy. Nine patients underwent colonoscopy at our institution and had available biopsy material. All 9 were adults with chronic lymphocytic leukemia. Most had elevated stool lactoferrin indicating the presence of fecal leukocytes; stool cultures, ova and parasite evaluation, and *Clostridium difficile* testing were negative. Endoscopy revealed colitis in 6 (67%) patients, although 8 (89%) patients had variably severe colonic injury upon biopsy analysis in the form of intraepithelial lymphocytosis with crypt cell apoptosis and active colitis with occasional ulcers. These features were present in variable combinations, such that 4 cases simulated lymphocytic colitis; apoptosis was often striking. Immunohistochemistry demonstrated the intraepithelial mononuclear cells to be CD8+ T-cells. Six patients required cessation of idelalisib and systemic corticosteroid therapy, 1 was maintained on idelalisib and corticosteroids, and 2 continued on the drug with resolution of their symptoms.
Conclusions: Idelalisib commonly causes diarrheal illness in patients undergoing therapy for B-cell neoplasia. The drug causes a pattern of injury characterized by intraepithelial lymphocytosis and apoptosis, reminiscent of either lymphocytic colitis or graft versus host disease.

787 Somatic Mutation Profiles of MSI and MSS Colorectal Cancer Identified By Targeted Next Generation Sequencing

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Background: Microsatellite instability (MSI) is an established prognostic marker in colorectal cancer; MSI-H tumors have a better prognosis than those that are microsatellite stable (MSS). The mutational status of *BRAF* is also prognostically important in MSS cancers; those with *BRAF* V600E mutations are associated with decreased survival compared to those that are *BRAF* wildtype. However, the prognostic importance of *BRAF* mutations in MSI-H cancers is not clear. We investigated the genomic profiles of MSI-H tumors with, and without, *BRAF* V600E mutations using targeted Next Generation Sequencing (NGS) and compared them to variants seen in MSS colorectal cancers.
Design: We performed targeted NGS on DNA extracted from 34 routinely processed colorectal cancer specimens, including 21 presumably sporadic MSI-H (12 *BRAF* mutants and 9 *BRAF* wildtype) and 13 MSS tumors. All cases were interrogated for cancer-related mutations in 50 genes using the Ion Torrent Personal Genome Machine and data were analyzed using the variant caller 4.0 software.
Results: Mutations in *KRAS* were detected in 41% of cases, including 4 (44%) MSI-H/*BRAF* wildtype tumors, and were mutually exclusive with *BRAF* mutations. A greater number of variants were detected in theMSI-H/*BRAF* wildtype group (mean: 4.9) compared to MSI-H/*BRAF* mutants (mean: 3.25), and the difference was statistically significant ($p=0.02$). *PIK3CA* variants were most prevalent in the MSI-H/*BRAF* wildtype tumors (56%) compared to MSI-H/*BRAF* mutants (33%, $p=0.01$) and MSS tumors (15%, $p=0.01$). *PDGFRA* variants were marginally higher in MSI-H/*BRAF* wildtype tumors compared to MSS tumors (33% and 8%, $P=0.06$). Not surprisingly, *APC* mutations clustered in the MSS group. Mutations in *TP53* were similarly detected in all three groups. The only *PTEN* deletion occurred in a MSI-H/*BRAF* wildtype tumor.
Conclusions: Colorectal cancers with MSI-H and wildtype *BRAF* display a larger number of variants in cancer-related genes, such as *PIK3CA* and *PDGFRA,* than MSH-H/*BRAF* mutants and MSS tumors. These findings indicate that MSI-H cancers with, and without, *BRAF* mutations develop via heterogeneous mechanisms.

788 Expression of Wnt/Beta-Catenin Signaling Pathway in Pancreatic Neuroendocrine Neoplasms

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Background: The Wnt/β-catenin signaling pathway plays an important role in tumor growth and proliferation. Without an activating ligand, a protein complex, including APC, enhances degradation of β-catenin. Ligand binding leads to nuclear localization of β-catenin and transcription of target genes. This study assessed the expression of β-catenin and APC in pancreatic neuroendocrine neoplasms.
Design: Eighty-seven pancreatic neuroendocrine neoplasms and corresponding unremarkable pancreas in a TMA were evaluated by immunohistochemistry for expression of β-catenin and APC. B-catenin membrane staining (intensity=0-3 scale, in ≥ 25% of cells) and nuclear staining (+/-, in ³25% of nuclei), as well as APC cytoplasmic staining (+/-, in ³10% of cells) were scored. Scores were correlated with clinicopathologic parameters using Fisher's exact test and Cox-regression analysis with Breslow method.
Results: Strong β-catenin membranous expression was seen more frequently in higher stage pancreatic neuroendocrine tumors when compared to those of lower stage. Strong membranous staining was observed in 100% of Stage III/IV neuroendocrine tumors (13/13 cases) when compared to 47% of Stage I/II tumors (35/74 cases, $p<0.001$). In addition, β-catenin nuclear staining was seen in 13% of Stage III/IV neuroendocrine tumors (2/13 cases) when compared to 0% of Stage I/II tumors (0/74 cases, $p<0.05$). APC downregulation was also shown to play a role in pancreatic neuroendocrine tumors. While 86% of normal pancreatic islets in this study strongly expressed APC (30/35 cases), only 30% of the neuroendocrine neoplasms in this study retained strong APC expression (24/80 cases, $p<0.001$). Stage III/IV disease demonstrated a significantly worse survival (hazard ratio 10.4, $p<0.05$). However, β-catenin and APC expression were not associated with disease-specific survival.
Conclusions: This study provides new evidence of dysregulation of the Wnt/β-catenin pathway in pancreatic neuroendocrine tumors when compared to normal pancreatic islet cells. The majority of neoplasms in this study downregulate APC, increasing their β-catenin signaling capacity. The data also demonstrate increased membranous and nuclear β-catenin expression in high stage tumors. Our findings lend support for the development of therapies targeting the Wnt/β-catenin pathway in treating pancreatic neuroendocrine neoplasms.

789 Clinical and Histopathologic Characterization of Polyp Burden in Patients With MUTYH-Associated Polyposis

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Background: MUTYH-associated polyposis (MAP) often presents with an attenuated polyposis phenotype. Diagnosis traditionally has been based on the presence of multiple adenomatous polyps, although serrated polyps have recently been described as comprising a subset of polyps in these patients.
Design: 58 unique patients with genetically documented MAP were identified. All polypectomy/biopsy specimens were reviewed as well as surgical excision specimens. We determined types of polyp(s) present, location of polyps, and number of polyps. Surgical excision specimens were reviewed for the presence of adenocarcinoma, number of grossly identified polyps, and serrated polyps (SSPs, HPs, and TSAs).
Results: Specimens were available for review for 46 separate patients (27 M, 19 F). The mean age at presentation was 48.5 years (range 16-79 years). Regarding polypectomies/biopsies, specimens included gastric polyps (14/44 patients; 11 FGP, 2 FGP LGD, 2 HP), duodenal polyps (9/44; 12 TA), proximal colonic polyps (26/44; 338 TA, 13 HP, 8 SSP, 4 IP, 1 TSA), distal colonic polyps (27/44; 115 TA, 26 HP, 2 TSA, 1 SSP), rectal polyps (26/44; 63 TA, 27 HP, 16 TSA, 1 SSP LGD), and one anal polyp (mucosal prolapse). 23 separate patients had documented evidence of a colectomy, of which 17 cases were available for review. Invasive adenocarcinoma was found in 10/17 cases, while 7/18 cases showed only multiple polyps. By gross report, estimated polyp burden ranged from 12 to over 100. Routine sampling of polyps in most cases revealed multiple adenomas; one specimen also had an SSP with low grade cytologic dysplasia and three separate specimens revealed multiple hyperplastic polyps. In total, adenomas were present in 45/46 patients. 11 separate patients harbored dysplastic polyps in the upper gastrointestinal tract (9 patients with duodenal adenomas, 2 patients with gastric FGP LGD). Serrated polyps in the form of SSP's or TSA's comprised a component of the polyp burden in 12 patients (26%).
Conclusions: MAP patients almost universally harbor adenomas; however, a spectrum of other types of polyps, including SSPs, HPs, and TSAs, not uncommonly comprise a portion of the polyp burden. Polyps in these patients appear to be greatest in number in the proximal colon. MAP should remain a consideration in patients presenting with dysplastic polyps of the upper GI tract, especially if the overall polyp burden is less than expected for FAP.

790 Expression of ARID1A and p53 in Patients With a Consensus Diagnosis of Barrett's Foveolar and Intestinal-Type Dysplasia

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Background: Recent studies have identified *ARID1A* as a tumor suppressor gene that is lost in several cancers, including Barrett's-related adenocarcinoma, with a possible inverse relationship to *p53* mutation. As its role in precursor lesions is unclear, we evaluated expression of ARID1A and p53 in biopsies with a consensus diagnosis of Barrett's-related dysplasia and correlated their expression with morphologic subtype [intestinal (INT) vs foveolar (FOV)] and grade of dysplasia.

Design: A total of 288 biopsies from 181 patients with Barrett's esophagus (BE) diagnosed between 2007 and 2014 were independently reviewed by 8 GI pathologists for grade [negative (NEG), low-grade (LGD), high-grade (HGD), HGD with marked glandular distortion; cannot exclude adenocarcinoma (HGD-MAD)/intramucosal adenocarcinoma (IMC)] and subtype of dysplasia (INT/FOV/mixed) using published criteria. Immunohistochemistry for p53 (clone DO-7, 1:20 dilution, Dako, Carpinteria, CA) and ARID1A (HPA005456, 1:100 dilution, Sigma, St. Louis, MO) was performed on a subset of 41 cases (10-N, 9-LGD, 20-HGD, 12-HGD-MAD/IMC) with consensus diagnosis (agreement by 5/8 pathologists). Overall, 21 FOV and 27 INT dysplasia biopsies were analyzed for these two markers. Nuclear staining of any intensity was considered as intact ARID1A expression. Complete loss or overexpression (overexpression defined as moderate or strong expression in >50% of dysplastic nuclei) of p53 was considered as an aberrant pattern.
Results: The 41 patient cohort included 32 men and 9 women (mean age 65 yrs, range 49-83). Weak to strong ARID1A expression was seen in all biopsies with FOV (21/21; 100%) and INT dysplasia (27/27; 100%). Aberrant p53 pattern was observed in majority of biopsies with FOV (20/21; 95%) and INT-type (26/27; 96%) dysplasia. With respect to the grade, there was no difference in ARID1A expression between dysplastic and non-dysplastic biopsies. Compared to all biopsies with LGD, HGD, and HGDMAD/IMC, none of the NEG biopsies showed aberrant p53 expression.
Conclusions: As ARID1A expression is retained in all precursor lesions, irrespective of the morphologic subtype or grade of dysplasia, molecular alteration of *ARID1A* appears to be a late genetic event in Barrett's carcinogenesis. Aberrant p53 expression does not separate INT from FOV-type dysplasia, but serves as a promising biomarker of BE-related dysplasia, especially in patients with a consensus biopsy diagnosis.

791 LncRNA-mRNA Expression Profiling Identifies Potential Prognostic Biomarkers in Gastric Cancer

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Background: Gastric cancer (GC) is the most common type of gastrointestinal tumor and accounts for 700,000 cancer deaths per year. Improved prognostic markers are needed to predict prognosis and could potentially aid in the treatment. Long non-coding RNAs (lncRNA) are non-protein coding RNAs with regulatory functions and their differential expressions have been described in GC. We compared the lncRNA-mRNA expression profiles in GC from patients to identify specific genes with useful prognostic associations.
Design: RNA was extracted from 8 fresh frozen GC specimens. lncRNA and mRNA expression profiling was performed using microarrays. Potential core regulative lncRNAs and target mRNAs were identified by integrating bioinformatic, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses and then validated by qRT-PCR in the training group (n=66). A prognostic lncRNA-mRNA signature was developed from the training group using univariate and multivariable Cox regression analyses, which were then validated in a test group (n=78).
Results: LncRNAs and mRNAs showed significantly altered expression levels in the GC tissues. The constructed lncRNA-mRNA-pathway correlation network revealed 37 potential core regulative lncRNAs and 20 target mRNAs were significantly enriched in 15 different pathways, of which the PI3K-Akt signaling pathway was the most significant. From the training group, we identified a TCONS_00015570-TIMP-1 signature that classified the patients into two groups with significantly different overall survival rates (median survival >60 months vs 15 months, p<0.0001). The signature was applied to the test group and exhibited similar prognostic values (median survival >60 months vs 24 months, p=0.0015). Multivariable Cox regression analysis indicated that the signature was an independent prognostic factor for the GC patients. A receiver operating characteristic (ROC) analysis suggested that the signature was prognostic in the clinical stages.
Conclusions: Our data provides a comprehensive bioinformatics analysis of lncRNAs, functions, and pathways which may be involved in the pathogenesis of GC. The identified TCONS_00015570-TIMP-1 signature may potentially be a powerful prognostic marker of GC, as well as a robust tool for the prediction of post-surgical outcomes.

792 Plasma Cell Paucity in the Gut Following Stem Cell Transplant

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Background: Graft versus host disease (GVHD) and opportunistic infections of the gut are common clinical concerns in patients presenting with gastrointestinal (GI) symptoms in the post allogeneic stem cell transplantation (SCT) period. Histologic criteria for GVHD and infections are well established. However, the spectrum of histologic changes unrelated to these clinical entities is not well understood. We report 4 patients who present with unusual plasma cell paucity in the gastrointestinal tract in the post-SCT period.
Design: Clinicopathologic features recorded included hematologic diagnosis, initial therapy, prior transplant history, degree of HLA match, conditioning regimen, time to engraftment, interval to and indication for GI biopsy, prior or subsequent graft versus host disease, immunosuppressive drugs and patient outcome.

Patient	Age, gender	Diagnosis	Initial Therapy	Conditioning Regimen	type of SCT	Drugs
1	63 M	AML	Cytarabine, Idarubicin	Melphalan/ Fludarabine	Related	Tacrolimus, Sirolimus
2	57 F	Diffuse Large B-cell Lymphoma	EPOCH-Rx6	Pentostatin/ Radiation	Unrelated	Cellcept, Tacrolimus
3	53 M	Follicular Lymphoma	CHOPx9	Cytoxan/ Fludarabine/ Rituxan	Related	Tacrolimus
4	64 M	Angioimmunoblastic T-cell Lymphoma	CHOPx6	Pentostatin/ Radiation	Related	Cellcept, Tacrolimus

Results: All patients had 10 of 10 HLA-matched donors, reduced-intensity chemotherapy prior to SCT and demonstrated engraftment within 30 days. GI biopsies were performed 31, 389, 132, and 89 days post SCT, respectively. Two patients presented with diarrhea. Patients 1,2 and 4 biopsy indication was rule-out GVHD. Patient 3 had duodenal erythema/edema on endoscopy. Histologic examination of small bowel or colon biopsies revealed a paucicellular lamina propria with near absence of plasma cells, without villous blunting or architectural change. Features of GI GVHD or infection were not seen. 2 patients developed subsequent GVHD (2 and 3) and died. Patients 1 and 4 are alive without GVHD. None of the patients had pre-SCT GI biopsies for comparison.
Conclusions: We identified 4 patients with remarkable paucity of lamina propria plasma cells of the gut in the post SCT period. Similar histologic findings can be seen in association with immune deficiency disorders. We speculate that the features observed are the result of acquired immune deficiency in the setting of post SCT immunosuppression. The clinical significance of this finding is unclear, but may contribute to enteropathy-type digestive symptoms, including diarrhea, and possibly its own clinicopathologic entity.

793 Impact of Chemokine Receptor CXCR3 on Tumor-Infiltrating Lymphocyte Recruitment and Favorable Prognosis in Advanced Gastric Cancer

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Background: Little is known about the correlation of chemokine receptor CXCR3 with tumor-infiltrating lymphocytes (TILs) and prognosis of advanced gastric cancer(GC).
Design: 192 GC specimens and 48 para-cancerous (≥5 cm from GC) tissues were collected at Zhongnan Hospital,Wuhan University,China between 2008 and 2013. None of the patients received preoperative chemotherapy.Clinicopathologic features of tumor and follow-up procedures were obtained.Tumors were histologically classified as intestinal or diffuse type and staged according to American Joint Committee on Cancer guidelines.Immunohistochemical (IHC) staining was carried out by using primary rabbit anti-human CXCR3 polyclonal antibody (BA0759,1:200 dilution;Boster Biological Technology,Ltd.,Wuhan,China),rabbit anti-human monoclonal antibody CD8(1:50 dilution,SP16) and mouse anti-human CD4 monoclonal antibody (1:50 dilution,UMAB64),Zhongshan Golden Bridge Biotechnology,Beijing,China. Staining was scored as 0 (negative,≤5% cells positive),1+(6–25% cells positive),2+(26–50% cells positive),and 3+(>50% cells positive).The final score for each slide was represented by the average of three representative high-power fields (hpf, ×400).
Results: The positive staining of CXCR3 protein was located in the cytoplasm of cells.Of all GC,36 (18.8%) scored 0,44 (22.9%)1+,60 (31.2%) 2+ and 52 (27.1%) 3+,while 26 (54.2%) para-cancerous tissues score 0,15 (31.3%) 1+,5 (10.4%) 2+ and 2 (4.2%) 3+.Compared with para-cancerous tissues,GC was more likely to have CXCR3 expression ^{3}2+ (58.2% vs 14.58%,P<0.001).Higher CXCR3 expression was associated with more CD8 and CD4 TIL in GC(P=0.032 and P<0.001,respectively),while there was no significant association between CXCR3 expression and CD8 or CD4 infiltrations in para-cancerous tissues (P=0.211 and P=0.146,respectively).CXCR3 expression was also significantly higher in GC with lesser tumor invasion depth or lack of lymphnode metastasis than that with more invasion depth or lymph node metastasis (P =0.002 and P =0.001,respectively).In addition, higher CXCR3 expression and higher CD8 TIL were associated with longer overall survival (log-rank test,P<0.001 and P=0.002,respectively). Multivariate analyses indicated that only CXCR3 expression was an independent prognostic factor (P =0.002).
Conclusions: CXCR3 expression is overall up-regulated in advanced GC.Higher CXCR3 expression is associated with increased CD4 and CD8 TIL and improved overall survival.

794 Molecular Analysis of Mixed Adenoneuroendocrine Carcinomas (MANEC) Signifies a Common Clonal Origin of Adeno and Neuroendocrine Components

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Background: Neuroendocrine carcinomas (NECs) of the gastrointestinal tract are rare but highly aggressive tumors. Little is known about the genetic alterations and origin of these tumors. Approximately half of the NECs show admixed adenoma/

adenocarcinoma components (MANECs). In this study we evaluated whether the adenoma/adenocarcinoma and NEC components of MANECs are clonal, suggesting that NECs may originate from adenomas or adenocarcinomas.
Design: In a collection of 27 MANECs of the upper and lower gastrointestinal tract, CD56, synaptophysin and NSE stains were used to identify and distinguish the neuroendocrine from the adenoma/adenocarcinoma components. These two components then were microdissected, their DNA isolated, and their KRAS and TP53 genes analyzed for mutations by sequencing. Mutational profiles of both components then were compared.
Results: We identified KRAS mutations in 37% of MANECs. These KRAS mutations were identical in the adenoma/adenocarcinoma and NEC components in all cases. In addition, TP53 mutations were found in 70% of these tumors. Two thirds of them were identical in both components. Importantly, a large majority of the remaining third of TP53-mutations was found within the NEC component only, whereas a minority was exclusive to the adenoma/adenocarcinoma component.
Conclusions: The finding of identical KRAS and TP53 mutations in the adenoma/adenocarcinoma and the NEC components of MANECs strongly suggests a common clonal origin. Furthermore, the neuroendocrine component more frequently harbors additional mutations in TP53 compared to the adenoma/adenocarcinoma component. These results strongly suggest that NECs, rare aggressive tumors of the gastrointestinal tract, may arise from pre-existing adenomas or adenocarcinomas, a much more common entity.

795 Clinical Significance of Duodenal Mucosa With Normal Villous Architecture and Slightly Increased Intraepithelial Lymphocytes in Pediatric and Adult Populations

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Background: Duodenal mucosa with normal villous architecture and increased intraepithelial lymphocytes (IEL) can be seen in celiac disease, NSAIDs use, post-viral gastroenteritis, Crohn's disease, H. Pylori infection, among other etiologies. Currently, 30 IELs/100 epithelial cells is the accepted cutoff value for a diagnosis of increased IELs, and when met, a pattern diagnosis of "duodenal mucosa with normal villous architecture and slightly increased intraepithelial lymphocytes" (DMSIL) is made. However, a formal count is not always performed in practice. This prompted some doubts about the cases with DMSIL diagnosed and that we retrospectively investigated in both pediatric and adult populations.
Design: Of the 5071duodenal biopsies performed from 2010 to 2011, 320 (6.3%) were reported as DMSIL. Among those, 308 cases were available for review. Two pathologists independently counted IELs per 50 epithelial cells at the tip of 5 well-oriented villi and an average of IELs/100 epithelial cells was calculated. Clinical features including age, sex, indications for biopsy, initial celiac serology, EGD findings, and followups were compared in both populations.
Results: The cohort included 112 pediatric (£18 years) and 196 adult cases. Female to male ratio was 1.3:1 and 2.9:1 in pediatric and adult group, respectively. Mean age was 10.8 in pediatric and 46 years in adult population. The most common indications for biopsy were abdominal pain, reflux, and IBD in kids, and anemia, diarrhea, and epigastric pain in adults. Duodenal mucosa was normal in 93% of kids and 94% of adults. Before the biopsy was taken, only 17 kids had an initial TTG serology test and 7 were positive, with 1 case showed <20% IELs. Thirty eight adults were initially tested for TTG and 17 were positive, with 2 cases showed <20% IELs. Interestingly, using a 30% IEL cutoff and counting IELs within 5 well-oriented tips of villi, only 35% of original DSMIL would meet a diagnosis of DMSIL in this study (30% in adults; 43% in kids). Ultimately, only 9% of all patients with DMSIL were confirmed to have celiac disease.
Conclusions: DMSIL is a non-specific histological finding in duodenal biopsies and is overdiagnosed in practice in both pediatric and adult populations. Counting IELs within 5 well-oriented tips of villi can increase the diagnostic stringency and avoid unnecessary clinical followups.

796 Colonic Lamina Propria Lymphatics Are Associated With Inflammatory Bowel Disease

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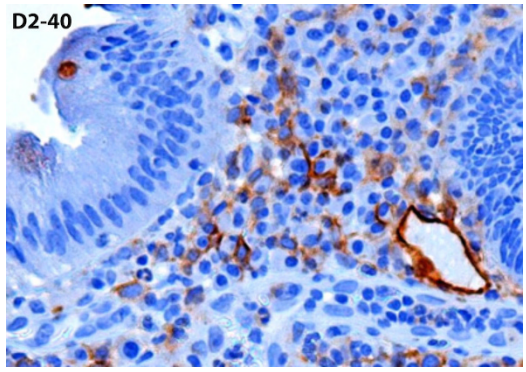
Background: Dogma has it that colonic mucosa lacks lymphatic vessels. The current AJCC staging system for colonic carcinoma treats *in situ* and intramucosal carcinoma as stage 0. Nevertheless cases are occasionally noted in which lymphatics are found adjacent to intramucosal carcinoma in the setting of inflammatory bowel disease (IBD), including both Crohn's disease (CD) and ulcerative colitis (UC).
Design: Our pathology archives were searched to identify the following categories: quiescent CD and UC, mildly active CD and UC, moderately active CD and UC, severely active CD and UC, and dysplasia/intramucosal carcinoma arising with CD or UC. Histologically normal colon sections were chosen as control cases. H&E slides were reviewed to identify sections that best represent each category. Immunostaining with D2-40 was performed and then each case was blindly scored for the following: (1) extent of lamina propria lymphatics (0-none, 1-rare, 2-focal, 3-moderate, and 4-diffuse) and (2) distribution of lymphatics within the lamina propria (0-none, 1-basal involvement, 2-lower half, 3– 50 to 75% of the mucosal thickness and 4- >75% of the mucosal thickness). The sum of these categories was calculated for each case. Scoring was not performed in areas of ulceration or near lymphoid aggregates.
Results: Fifty-five cases were reviewed (IBD, n=50 and control, n=5). Lamina propria lymphatic scores ranged from 0 to 8. Control cases had rare lymphatics (mean score of 2.0). As the severity of IBD progressed, the extent and distribution of lymphatics increased. Student t-tests were performed comparing the control group to each IBD category. The t-test was statistically significant for the following categories:

	Quiescent CD	Moderate CD	Moderate UC	Severe CD	Severe UC	CD with Dysplasia	UC with Dysplasia
Mean Score	4.8	4.4	6.6	5.8	5.2	4.8	5.9
p-value	0.011	0.050	0.001	0.006	0.013	0.034	0.001



Conclusions: Both CD and UC are associated with a greater distribution and extent of lamina propria lymphatics as the disease severity progresses. The presence of lymphatics within the mucosa in IBD warrants further investigation as it may alter the classification and staging of colonic intramucosal carcinoma.

797 Clinical Relevance of Cancer Stem Cell on Residual Disease After Preoperative Chemoradiotherapy for Rectal Cancer: A Retrospective Single Institution Study

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Background: The cancer stem cell (CSC) hypothesis has critical clinical significances for cancer therapeutics because CSCs may be result in chemoresistance. We aimed to assess the clinical relevance of CSCs on residual disease after preoperative chemoradiotherapy (CRT) in patients with rectal cancer.
Design: The surgical specimens 146 rectal cancer patients with residual disease after preoperative CRT were analyzed. The proportions of putative CSCs with CD44+ or ALDH1+ were determined by immunohistochemistry in whole section tissues, and their scores were dichotomized as positive or negative. For CD44 and ALDH1, cases with ³1% positive staining were regarded as positive.
Results: Of the 146 cases, positivities of ALDH1 and CD44 were observed in 80.8% (n=118) and 47.9% (n=70), respectively, and expression of both markers showed significantly positive correlation (rho=0.282, $P<0.001$). CD44 positivity inversely correlated with tumor regression grade (TRG) ($P=0.004$), whereas ALDH1 positivity had no association with any clinicopathologic variables, including sex, age, residual tumor size, yTNM stage, lymphatic invasion, nerve invasion, and venous invasion, TRG (all $P>0.05$). On univariate analysis, ALDH1 positivity was related to shorter disease-free survival (DFS) ($P=0.010$) and recal cancer-specific survival (RCSS) ($P=0.012$). In contrast, there was no association between CD44 positivity and DFS ($P=0.790$) as well as RCSS ($P=0.367$). In multivariate Cox regression analysis, ALDH1 positivity was an independent worse prognositc factor for DFS (Hazard ratio=4.551, 95% confidence interval=1.406-14.725, $P=0.011$) along with venous invasion ($P<0.001$) but not RCSS ($P=0.961$).
Conclusions: Although further extensive validation studies are warranted, ALDH1 expression may serve as a predictive prognostic biomarker for DFS in patients with residual disease after CRT. Our findings would be support the understanding of targeting CSCs therapeutic approaches in rectal cancer receiving preoperative CRT.

798 MiRNA Expression Profiling of Sorafenib Response and Resistance in Hepatocellular Carcinoma

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Background: The tyrosine kinase inhibitor (TKI) sorafenib is the first line treatment for metastatic hepatocellular carcinoma (HCC) by inhibiting Raf, VEGFR-1, 2, -3, PDGFR-β and kit kinases. It significantly improves overall survival of HCC patients. Similar to other VEGF targeting compounds, sorafenib administration eventually results in drug resistance. This process was reported to be reversible. Epigenetic mechanisms behind TKI resistance are suspected. miRNAs have been described to be involved in developing resistance to the anti-angiogenic therapies. Our aim was to investigate miRNA expression changes in sorafenib response and resistance in an in vivo xenograft model.
Design: We developed an orthotopic model using human Hep3B cells injected into the liver parenchyma of CB17 SCID mice. Mice treated with sorafenib showed initial tumor size reduction followed by tumor rebound. Tumor tissues from control, responder and resistant mice were harvested, total RNA was extracted and miRNA expression profiles were determined by Illumina next-generation sequencing.

Results: We found miR-210 and miR-6089 expression alteration in responder tumors compared to the controls; and 26 other miRNAs' dysregulation in resistant tumors.
By creating a miRNA-target interaction network we identified miR-106a-5p, miR-34a/c-5p, miR-141-3p and miR-181d/a-5p participating in sorafenib resistance. Pathway analysis of the miRNA targets identified the "PTEN Signalling", "STAT3 Pathway" and "Regulation of the EMT pathway" signalling to be influenced by these miRNAs.
Conclusions: Unveiling the role of miRNAs in the reversible sorafenib resistance helps to understand the mechanisms behind the resistance to anti-angiogenic therapies, therefore can lead to identification of potential new biomarkers and drug targets, and improve therapeutic strategies.

799 'Gestalt' Assessment of Intraepithelial Lymphocytosis in Duodenal Biopsies Overcalls Normal as Abnormal, Falsely Raising the Possibility of Celiac Disease

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Background: Duodenal biopsies are considered the gold standard for the diagnosis of celiac disease (CD). Samples are examined for intraepithelial lymphocytes (IELs), with the upper limit of normal reported from 18-25 IELs per 100 enterocytes, with no commonly used guidelines. At our institution we have observed that many pathologists will rely on a 'gestalt' method of assessing IELs. We set to determine whether the lack of standardized counting of IELS overcalls normal biopsies as abnormal, and to establish a normal range for duodenal IELs at our institution.
Design: Surgical pathology files were reviewed between 2012 and 2013 for duodenal biopsies with a diagnosis of lymphocytosis, matched by an equal number of normal controls. In total, 110 duodenal biopsies were reviewed, equally split between diagnoses of lymphocytosis and normal. The biopsies were reviewed separately by a GI pathologist and resident, both blinded to the original diagnosis and any clinical history. In each case an averaged villous score (counting the number of IELs per 100 enterocytes from 3 evaluable villi) was calculated.
Results: The IEL counts for the 110 cases were tallied and averaged between the two reviewers. The cases were divided into two groups: biopsies that contained 20 IELs or less, and those above that, which were considered true lymphocytosis.

	Original Diagnosis	After Review
Lymphocytosis	55	37
Normal	55	73

Thirty-seven of the 110 biopsies had lymphocytosis with counts of more than 20 IELs, whereas 55 had been originally designated as increased. A chi-squared analysis shows the difference between the original 'gestalt' diagnosis versus IEL counting diagnosis is statistically significant at 0.0139 ($P<0.05$). There were only 5 cases where a difference in IEL count between reviewers led to a different categorization of the biopsy (kappa score 0.901). On review of available clinical history, 49 biopsies were confirmed to come from true normal patients. The average IELs on these cases was 9.6, with a range of 3.55 to 19.85.
Conclusions: Without using standardized counting of IELs, normal biopsies are overcalled as abnormal. The findings of this study lend credence to the argument that using 'gestalt' method may be mislabeling a significant subpopulation of patients with CD. The data allowed us to establish a normal range for our institution, which coincides with published data and is below the proposed 20 cut-off.

800 Analysis of Expression of HER2 in a Cohort of Gastric and GEJ Carcinomas From a Single Institution of Spain. Correlation Between HER2 Expression and Polysomy of CHR17

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Background: HER2 is a key driver of tumorigenesis and its protein overexpression, as a result of gene amplification has been observed in several solid tumours. In breast cancer it is a well-established therapeutic target. There is increasing evidence that HER2 is an important biomarker in gastric (GC) and gastro–esophageal junction tumours (GEJ) being overexpressed in 7–34% of GC.
HER2 expression in gastric carcinomas may show incomplete, basolateral, or lateral staining in addition to complete membrane staining and also more heterogeneous staining.
Immunohistochemical (IHC) expression is scored as IHC 0 (negative), IHC 1+ (negative), IHC 2+ (equivocal) or IHC 3+ (positive). Samples scored as IHC 2+ must be retested with fluorescent or silver *in situ* hybridization (FISH or SISH).The definition of ISH positivity in those tumors is a HER2/CEP17 ratio ^{3}2.0.
Design: We have reviewed 170 cases of GC and GEJ carcinomas from our Institution (Hospital del Mar, Spain) from 2010 to 2014. Both endoscopic biopsies and surgical samples have been included.
We have analysed the status of HER2: Her2 overexpression (Ventana anti-HER-2/neu(4B5) Antibody) and/or amplificaction (SISH/FISH (Ventana Medical Systems Inc. /Abbott Molecular) and the level of polysomies and correlated with age, tumour localization and differentiation.
Results: We found 16.37% of cancers that showed HER2 positivity.
The median age was 71.9 years (range: 26-100).
The expression was more frequently detected in carcinomas from GEJ compared to gastric location (25.8 vs 14.2%).
The majority of HER2 positive cases (77.27%) presented intestinal pattern, being 18.18% mixed and 13.63% diffuse.
All the positive cases were amplified by ISH.
In borderline cases, we have demonstrated amplification in 18.33% and in the negative cases analysed with ISH 9.52%.
Chromosome 17 polysomy was found in 5 of 9 cases 1+ analysed (55.5%), in 28 of 60 cases 2+ (46.6%) and in 2 of 9 cases 3+ analysed.
From those 2+ polysomic cases, 14.28% were amplified and 85.72% non amplified.
Conclusions: There is a good correlation between HER2 IHC expression and the level of gene amplification using ISH techniques.
We found correlation between borderline IHC expression and the presence of chr.17 polysomy, mostly in non amplified *HER2* cases. The clinical value of these non amplified polysomic cases needs further analysis.

801 Clinical and Immunohistochemical Features of Non-Pancreatic Gastrointestinal Neuroendocrine Neoplasms

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Background: Non-pancreatic gastrointestinal neuroendocrine neoplasms are classified into three grades, using mitotic counts and Ki-67 indices (WHO 2010). It has been suggested that immunohistochemical expression of p16/p53 and loss of chromogranin may provide additional prognostic information.
Design: Non-pancreatic GI neuroendocrine neoplasms were identified in a retrospective search of the surgical pathology database of a large academic medical center from 1992-2013, and were compiled in a tissue microarray. H&E and Ki67-stained sections were examined to classify the lesions as well-differentiated neuroendocrine tumors, including WHO grades 1 and 2 (WDNET), versus poorly-differentiated neuroendocrine carcinomas, WHO grade 3 (PDNEC). Immunohistochemistry for p16, p53 and chromogranin was performed. Kaplan-Meier analysis was used to calculate overall survival (OS). Cox proportional hazards models were constructed to assess significance of differentiation, p16, p53 and chromogranin with survival.
Results: 178 non-pancreatic GI neuroendocrine neoplasmswere identified. Median age was 58.5 years, 94 (53%) were female, 169 (95%) were WDNET, 9 (5%) were PDNET. The majority of cases were p16/p53 negative and chromogranin positive. The 5-year OS rate for the entire cohort was 75%. Of the PDNECs, p16/53 positive cases had worse OS. Univariate Cox regression analysis demonstrated that PDNEC was associated with an increased hazard of death with a HR of 17.7 ($p<0.0001$), as was p53 expression with a HR of 8.6 ($p<0.0001$). Only PDNEC maintained this association in a bivariate Cox analysis ($p<0.001$).
Conclusions: In a population of non-pancreatic GI neuroendocrine neoplasms, patients were young and had WDNET. As expected, PDNEC had poorer OS compared to WDNET. Of PDNEC, p16/53 positive cases had poorer OS compared to p16/53 negative cases. In univariate Cox regression analysis, both PDNEC and p53 expression were associated with increased hazard of death. In bivariate Cox analysis, only PDNEC maintained this association. Our analysis was limited by low case numbers for PDNEC and p16/53 expression. Further studies are needed to determine if p16, p53 and chromogranin need to be incorporated into the WHO grading system for neuroendocrine neoplasms.

802 BRAF/KRAS Mutation, MSI/MSS Status and Tumor Budding in Colorectal Carcinoma

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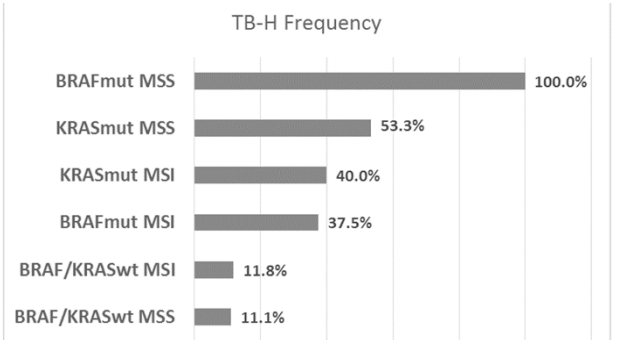
Background: BRAFmut (but not KRASmut) has been identified as adverse and MSI as favorable prognostic molecular biomarkers in colorectal carcinoma (CRC). Among histological prognostic markers tumor budding (TB) appears to have independent adverse prognostic significance. In the present study we addressed the strength of association between TB and BRAF and KRAS mutation and MSI/MSS status in CRC.
Design: All colorectal cancers (n=279) screened for BRAF, KRAS and MSI status over the period 2009-14 at our insitution were identified; 30 (10.8%) were BRAFmut and 51(18.3%) were MSI. All cases based on availability of resection H&E slides and additionally, sections of a random sample of 27 of the total of 214 BRAFwt, (17 KRASmut and 9 KRASwt) MSS cases, were reviewed for the presence of peritumoral TB, and classified as TB-High or TB-Low respectively, using the methodology of Giger OT et al. (Mod Path 2012, 25:1048-53). Chi^2 or Fisher Exact Test, as appropriate, were used to assess statistical associations among the categories.
Results: Tumors that were BRAFmut or KRASmut showed TB-H in 18/33 (54.5%) compared to 3/26(11.5%) of BRAF/KRASwt tumors [p=.0006]. There was no difference in frequency of TB-H in KRASmut (10/20) vs BRAFmut (8/13) in CRC studied. TB-H was identified in 14/29 (48.2%) of MSS compared to 7/30(23.3%) of MSI tumors [p<.05]. However, among BRAF/KRASwt MSI tumors (n=17) the frequency of TB-H (11.8%) did not differ from that in BRAF/KRASwt MSS tumors (11.1%). Figure 1 illustrates the relative frequencies of TB-H across the BRAF/KRAS/MSI/MSS matrix.



Conclusions: Mutation of BRAF or KRAS is strongly associated with TB-H in CRC, independently of MSI/MSS status. The findings suggest the hypothesis that RAS-RAF MAPK signalling may be directly related to the epithelial–mesenchymal transition that underlies TB in CRC.

Genitourinary Pathology (including Renal tumors)

803 Analysis of Presence and Extent of Adverse Pathologic Characteristics in Small Clear Cell Renal Cell Carcinomas

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Background: Tumor size has been used for decision making in the management of patients with Renal Cell Carcinoma (RCC). Active surveillance in selected patients is now increasingly common and tumor size is being used as threshold for enrollment. Adverse pathologic features have been described in renal tumors and these features correlate well with prognosis in RCC. The aim of this study was to determine the frequency and extent of adverse pathologic characteristics in small RCC.

Design: A computer search of a single institution database for partial and radical nephrectomies performed for RCC identified a total of 898 consecutive cases of RCC with available clinical follow-up. Tumor slides were reviewed for histologic correlation for the following adverse conditions: presence and proportion of high nuclear grade, necrosis, rhabdoid features, sarcomatoid features and lymphovascular invasion (LVI). Relationships between the variables were examined by Kruskal-Wallis tests, Wilcoxon tests, Chi-square tests and logistic regression.

Results: Every tumor characteristic evaluated was significantly related to size in all RCC. The frequency of adverse tumor histopathologic characteristics based on size of 629 cases of clear cell RCC is provided in Table 1 (p values by Chi-square test). 15% of cases of clear cell RCC ≤ 4 cm have some component of high grade nuclear features compared with 45% in cases > 4cm. 21% of cases ≤ 4 cm have areas of necrosis compared with 46% in cases > 4cm. 3% of cases ≤ 4 cm have LVI compared with 17% in cases > 4cm.

	Size ≤4cm(n=288)	Size >4cm(n=341)	p value
High grade	43 (15%)	153 (45%)	~ 0
Necrosis	60 (21%)	157 (46%)	~ 0
Rhabdoid	3 (1%)	27 (8%)	.0001
Sarcomatoid	3 1%)	21 (6%)	.01
LVI	9 (3%)	58 (17%)	~ 0

Conclusions: Adverse histopathologic characteristics in small clear cell RCC may have clinical implications for tumor sampling and clinical management, especially in patients with tumors ≤ 4cm for whom active surveillance is considered.

804 A Prospective Investigation of PTEN Loss and ERG Expression in Lethal Prostate Cancer

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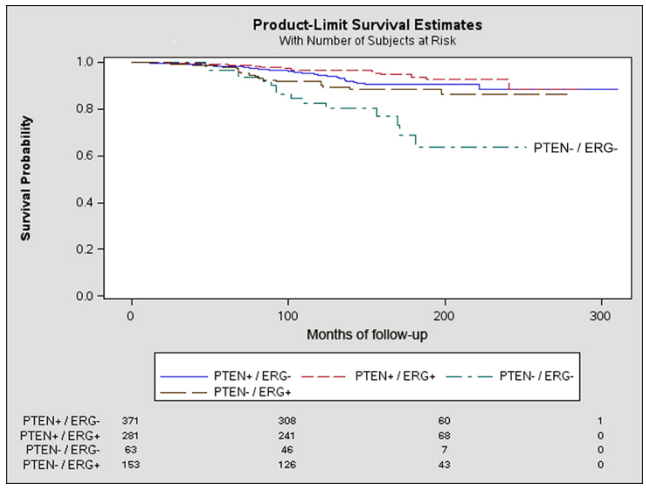
Background: PTEN is a tumor suppressor frequently deleted in prostate cancer that may be a useful prognostic biomarker. However, the association of PTEN loss with lethal disease has not been tested in a large, predominantly surgically-treated cohort.

Design: We studied 1045 men in the Physicians' Health Study and Health Professionals Follow-up Study diagnosed with prostate cancer from 1986 – 2009, treated with radical prostatectomy (95%) and followed through 2012 for biochemical recurrence and cancer and all-cause mortality. Genetically validated and dichotomously scored PTEN and ERG immunohistochemistry (IHC) assays were performed on tumor tissue microarrays (TMA). Cox proportional hazards models adjusting for age and BMI at diagnosis, Gleason grade, and clinical/pathologic stage were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) of the association with lethal disease.

Results: On average, men were followed 11.6 years, during which there were 81 lethal events. 16% of tumors had homogeneous PTEN loss in all TMA cores, 9% had heterogeneous PTEN loss, and the remaining 75% had intact PTEN expression. Homogeneous PTEN loss was significantly associated with ERG expression, higher Gleason grade, and higher pathologic stage. In multivariate analysis, cases with homogeneous PTEN loss (HR=1.9; 95% CI=1.2-3.0), but not cases with heterogeneous PTEN loss (HR=1.2; 95% CI=0.5-2.6), were associated with lethal progression. Homogeneous PTEN loss was more strongly associated with lethal progression among tumors with Gleason score ≤ 7 (HR=2.7; 95% CI=1.4-5.5) compared to Gleason score 8-10 (HR=1.6; 95% CI=0.9-3.1). Finally, any PTEN loss (homogeneous or heterogeneous) was significantly associated with lethal progression only in ERG negative tumors (HR=2.8; 95% CI=1.5-5.2) in multivariate analysis.



Conclusions: PTEN loss is associated with increased risk of lethal progression in prostatectomy samples independent of clinical-pathologic parameters, particularly in the ERG negative subgroup. These validated and inexpensive IHC assays may be useful for routine risk stratification in low and intermediate risk prostate cancer.

805 A Retrospective Analysis of the Outcome for Patients With ASAP Diagnosis in the Trans-Rectal Ultrasound (TRUS)-Guided Prostate Biopsies at Pennine Acute Hospitals

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Background: In patients with high PSA or clinically suspicious patients the trans rectal ultrasound (TRUS)-guided prostate biopsy became the gold standard in the diagnosis of the tumours. In a small percentage of cases the pathologist has insufficient data to make a definitive diagnosis. This entity called ASAP, is defined as a "focus of small acinar structures formed by atypical epithelial cells". According to the literature the cancer detection rate in the second biopsy after a first diagnosis of ASAP varies between 21% and 60%. The reason of the conflicting results found by different authors is the subjectivity of the diagnostic criteria.

Design: We retrospectively reviewed all the TRUS-guided biopsy reports of patients that underwent to TRUS-guided biopsy in Pennine Acute Trust between 2007 and 2012. Of the 4631 cases 270 (5.8%) were diagnosed as ASAP, 215 (4.6%) of which were reported in the first biopsy specimen. All cases were stained for racemase (AMCR), p63 and high-molecular weight cytokeratin.

Results: The mean age of the patients that were diagnosed with ASAP was 66.8 (39-86). 98 (45.6 %) patients with persistent clinical suspicion underwent another TRUS-guided biopsy. 59 (60%) of the subsequent biopsies showed a hyperplastic process or prostatitis with no tumour. In 8 (13.7%) of this patients a High Grade PIN has been identified. 39 (39.8%) of the subsequent biopsies showed a primary adenocarcinoma. In 3 cases the tumour was diagnosed only in the third biopsies after 2 to 3 years from the first diagnoses. The median time elapsed (MET) between the first and second biopsy was 13.6 month for the cases subsequently reported as tumour and 9 month for the one with no tumour. 23 ASAP cases were subsequently reported as Gleason 6 (MET: 14.34 month), 12 as Gleason 7 (7 cases 3+4 and 5 cases 4+3) (MET: 14.58 months), four cases as Gleason ≥ 8 (MET: 9.5 months) (3 cases 4+4 and 1 case 5+4).

Conclusions: In our data the prostate cancer has been found subsequently in 18.1% of all the cases with an ASAP diagnosis. This rate is increasing to 39.8 % in ASAP cases when a repeat biopsy had been performed. This results together with the relatively shorter MET in patients diagnosed as tumour in the repeat biopsy are suggesting that a multidisciplinary approach involving the clinicians and the radiologist are extremely important to identify rapidly the patients that needs a subsequent biopsy for a definitive diagnosis of tumour.