Results: A statistically significant association was found between tumor type and cyclin D1 staining, distribution, and intensity. There were fewer cyclin D1-positive (+) FAs than PTCs (52% vs 88% respectively; p<0.001). Stain distribution was greater in PTC than FA (p=0.032). More PTCs were (+) than FCAs (88% vs 61% respectively; p=0.013). FA did not differ significantly from FCA in staining or intensity. There were fewer cyclin D1 (+) FAs than PTCC (52% vs 89% respectively; p=0.003) and PTCFV (52% vs 88% respectively; p=0.023). FCA also differed significantly from PTCC in cyclin D1 staining (61% vs 89% respectively; p=0.044) and intensity (p=0.024). FA had significantly less intense staining than PTCC (p=0.004). No significant associations were found between PTC LN status and any cyclin D1 characteristic.
Conclusions: Frequency and intensity of cyclin D1 expression was increased in differentiated malignant tumors relative to benign ones, and was progressively amplified from FAs to carcinomas (PTC and FCA). Distribution and intensity was significantly higher in (all) PTCs than FA and FCA, especially FA. The heterogeneity in distribution and intensity of staining in all thyroid tumor types disqualifies cylcin D1 as a primary diagnostic marker. However, it may be helpful in distinguishing FA from PTC, especially PTCFV. Cyclin D1 expression by benign and malignant thyroid tumors suggests a possible role in tumor development and progression, which should be investigated further.

593 CDX2 Is Rarely Expressed in Columnar Cell Variant of Papillary Thyroid Carcinoma: A Study of Ten Cases.

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Background: Columnar cell variant is a recognized rare variant of papillary thyroid carcinoma (PTC) associated with an uncertain clinical course. This variant has been regarded as a more aggressive form in comparison to the more common classical and follicular variants. These tumors have morphological resemblance with colonic adenocarcinoma. CDX2, a transcription factor of the caudal homeobox family, plays a key role in intestinal development and differentiation and it is widely used as a marker to detect adenocarcinoma of intestinal and colonic origin. CDX2 has been rarely reported in PTC. Only a single report of three cases of columnar cell variant of PTC has suggested that CDX2 should be considered a novel marker for diagnosis of this entity.
Design: We studied ten cases of columnar cell variant of papillary thyroid carcinoma. The histological, architectural, and cytological features fulfilled the diagnostic criteria of the columnar cell variant of papillary thyroid carcinoma as defined by the current WHO classification including neoplastic follicular cells with basally pseudostratified, hyperchromatic nuclei with eosinophilic-to-clear cytoplasm and supranuclear and/or subnuclear cytoplasmic vacuoles. Ten patients (6M:4F) ranging from 32 to 90 years of age (mean 58.3 years) presented with tumors classified as indolent (4 cases) or aggressive (6 cases); 3 with $BRAF^{V600E}$ mutation. All cases were β-Catenin negative. The Ki-67 proliferative index was up to 50%. All cases were TTF-1 positive.
Using paraffin embedded blocks, immunohistochemistry for CDX2 (mouse monoclonal to CDX2 (CDX2-88), 1:50 dilution; Biogenex Laboratories) was performed to evaluate the reactivity of this antibody to this variant of PTC.
Results: Nuclear positivity for CDX2 was detected in one out of the ten cases studied (10%), the other nine cases did not express CDX2.
Conclusions: Only one of our cases showed nuclear positivity for CDX2, therefore our study failed to confirm this as a marker for columnar cell variant of papillary thyroid carcinoma. The absence of CDX2 in the majority of the cases does not support the theory of CDX2 playing a role in the intestinal phenotype of these tumors.
The use of CDX2 as an indicator of this rare variant of thyroid cancer should be considered with caution based on our study.

Gastrointestinal

594 Effects of Tissue Fixatives on Immunohistochemical Expression of MSI Makers in Colon Adenocarcinoma.

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Background: Colorectal adenocarcinomas with microsatellite instability (MSI) do not respond well to Fluorouracil-based chemotherapy; and do have treatment outcome that differs from that seen in microsatellite stable tumors. In clinical settings, immunohistochemical staining for makers of MSI [Mismatch repair (MMR) gene products] is used to screen for the presence of MSI; and has been shown to have comparable sensitivity and specificity with MSI detection by PCR. However, the effects of tissue fixatives on the immunohistochemical expression of MMR gene products are not known. This study explores the effects of two routinely used tissue fixatives [Dissect Aid and 10% Neutral Buffered Formalin (NBF)] on the immunohistochemical expression of three MMR gene products (MLH1, MSH2 and MSH6).
Design: Study materials consisted of 7 colectomy specimens received for tumor diagnosis and staging. Samples of normal colon and tumor from each specimen were fixed in NBF and Dissect Aid solutions. Matched samples from each fixative were submitted for routine processing and paraffin embedding after fixation for the following day(s): 1, 7, 14, 28, 42, 56, 84 and 112. Immunohistochemistry for MLH1, MSH2 and MSH6 was performed on representative sections of each block. Immunoreactivity scoring was done in a blinded fashion using a semi-quantitative score of 0, 1, 2, 3, and 4. The code for the fixatives was broken after scoring was done.
Results: MLH1 immunoreactivity scores for samples processed in fixative A was at least one score less than that observed for corresponding samples in fixative B; and almost undetectable by the end of week 4 for samples processed in fixative A but remaining strong throughout the study for samples processed in fixative B. Both benign and tumor samples stained strongly positive for MSH2 and MSH6 in all tissue samples processed in fixative B and the strong staining reaction was maintained throughout the study. In comparison, negative staining reaction was observed in both tumor and benign mucosa for all samples processed with fixative A – with as early as 24 hours fixation.
Conclusions: For all three MSI markers investigated, fixative B (10% Neutral Buffered Formalin solution) is the preferred fixative for immunohistochemical assay. Fixative A (Dissect Aid) may produce erroneous negative immunostaining results for MSI markers, even with as little as 24 hours fixation. These findings highlight the need for protocol modifications to separate the mesentery from the colon for fixation of colon in NBF and mesentery in Dissect Aid for optimal processing of both specimen components.

595 HER2 Assessment in Gastric Cancer Surgical Specimens: Proposal of a Work-Flow for Practical Routine Use.

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Background: In gastric cancer (GC) the expression of HER2 is known as a marker of prognosis and recently it has been confirmed as a predictive marker of response to Trastuzumab.
Design: GC specimens of 100 patients were collected. Representative samples from both primary tumors (100 samples) and lymph node metastases (24 samples), were selected. In each case, 4B5 (Ventana), CB11 (kit Oracle Menarini), HercepTest (Dako) antibodies were tested in immunohistochemistry (IHC) and scored as proposed. *HER2* gene status was studied by double probe fluorescence in situ hybridization (FISH) in all cases. Concordance among IHC scoring results of the 3 antibodies and between FISH results and IHC (0/1+ and 2+/3+), independently from the percentage of positive cells, were evaluated using the Cohen-Fleiss' kappa statistic (K). The number of specimens needed to be tested in cases with <10% of HER2 overexpression was assessed. Finally, influence of gain of CEP17 (copies number >3) on the results of FISH ratio was considered.
Results: The 3 antibodies showed a K of 84% (p<0,05). The overall concordance of FISH/IHC was >80% (p<0,05) in the primary tumor and was >85% (p<0,05) when correlated with lymph node metastases for the 3 antibodies. Nine cases showed 2+/3+ in <10% of cells which corresponded to a IHC score value of 0. In 8 of these cases the percentage increased to >10% adding 2 more sections from different tissue blocks of the primary tumor. In our case series, the gain of CEP17 did not influence the final score ratio of FISH analysis.
Conclusions: The HER2 analysis of surgical specimens of GC has to consider the tumor heterogeneity. When the IHC score is 0/1+ on 1 tissue block, we recommend to test 2 more tissue blocks, particularly in the cases were the negative score is related to the low percentage of positive cells (<10%). Our work flow protocol avoids working over-load and solves equivocal cases.

596 TTF-1 and Napsin-A Frequently Positive in Esophageal and Pulmonary Adenocarcinomas: Dispelling Myths and Offering New Insights.

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Background: Because the esophagus is situated in close proximity to the bronchial tree and lungs, invasive adenocarcinomas of these approximated anatomic structures can directly invade the other, creating a diagnostic challenge to determine which is the primary tumor. Tumors which metastasize to the esophagus include the thyroid, stomach, breast, ovary, and most commonly, the lung. Thyroid Transcription Factor-1 (TTF-1) protein is a reliable marker of pulmonary adenocarcinoma, being positive in up to 75% of these tumors. Despite its ubiquitous use in the evaluation of primary pulmonary tumors and distant metastases, its expression in primary esophageal adenocarcinoma has never been thoroughly investigated. We have found that the literature is entrenched with the presumption that esophageal adenocarcinomas lack TTF-1 expression, and subsequently, any TTF-1 positivity should preclude a diagnosis of primary esophageal adenocarcinoma. Other common and uncommon stains such as 34βE12, N-Cadherin, p63, Napsin-A, and IMP3 have also not been used in larger studies comparing these two entities.
Design: We applied these 6 stains (TTF-1, 34βE12, N-Cadherin, p63, Napsin-A, and IMP3) to primary esophageal and pulmonary adenocarcinomas that have been resected or biopsied and evaluate for immunohistochemical positivity. Immunohistochemical staining was scored semiquantitatively. The percentage of stained cells was graded from 0 to 4+: 0, no staining; 1+, 1%-25%; 2+, 26%-50%; 3+, 51%-75%; and 4+, 76%-100%.
Results:

Percent of Positive Cases

Adenocarcinoma Type	TTF-1	Napsin-A	N-Cadherin	P63	34βE12	IMP3
Pulmonary	92%	92%	42%	50%	96%	54%
Esophageal	88%	79%	0%	17%	92%	96%

Conclusions: According to our study, TTF-1 is frequently expressed in primary esophageal adenocarcinomas, contrary to presumptions that have been propogated in multiple literature sources broaching the subject. TTF-1, 34βE12, and Napsin-A fail to discriminate between esophageal and pulmonary adenocarcinomas due to frequent positivity in both tumor types. IMP3 is frequently positive in esophageal adenocarcinomas. Failure to stain with IMP3 should virtually exclude a diagnosis of esophageal adenocarcinoma. N-Cadherin did not stain any esophageal cases. Positive N-cadherin should also virtually exclude a diagnosis of esophageal adenocarcinoma.

597 Morphologic Findings in a Novel Familial Polyposis Syndrome.

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Background: Familial Polyposis Syndromes (FPS) are gastrointestinal disorders characterized by diffuse and regional polyposis and are often associated with systemic manifestations including skin lesions and non-GI malignancies. Known syndromes include Familial Adenomatous Polyposis, Juvenile polyposis, and the harmatomatous polyposes. Specific genetic changes associated with FPS include APC and MYH gene mutations. We now describe the morphologic findings of polyps encountered in family members of an as yet unidentified gastric FPS.

Design: Specimens from patients with gastric polyposis enrolled in an NIH IRB approved protocol were evaluated. Multiple HE sections were reviewed along with p53, MIB-1, and E-cadherin immunostains when available. Six family members have undergone genetic testing using $1x10^6$ SNP array.

Results: Specimens were available in 10 patients (2 male, 8 female), ranging in age from 18 to 64. None had systemic manifestations. 8 had biopsies performed and 2 had total gastrectomy. On endoscopy, 1 had colonic polyps and 6 had gastric polyps and in 3, there were no lesions. Biopsy findings included: fundic gland polyps (1), colonic tubular adenoma (1), colonic hyperplastic polyp (1) and no lesions (5). Gastrctomy specimens from the 2 patients showed similar findings: multiple (>100) fundic gland like polyps with and without dysplasia (low and high grade) and flat mucosa with dysplasia (tubular adenoma like). Dysplastic lesions showed a high proliferative index with MIB-1 and scattered p53 positive cells. E-cadherin stains showed normal staining. Genetic testing using $1x10^6$ SNP array was negative for known genetic abnormalities.

Conclusions: We suggest that fundic gland like polyps with dysplasia and flat gastric mucosal dysplasia are the hallmark of an as yet unidentified novel gastric FPS.

598 Heterogeneity of Clinicopathological Features in Microsatellite Instability-Positive Colorectal Cancers Depending on CIMP Status.

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Background: Microsatellite instability–positive (MSI+) colorectal cancers (CRCs) are divided into CpG island methylator phenotype–positive (CIMP+) and –negative (CIMP-) tumors. The repertoire of inactivated genes in CIMP+/MSI+ CRCs overlaps with but is likely to differ from that of CIMP-/MSI+ CRCs because CIMP+/MSI+ tumors harbor additional genes that are inactivated by promoter CpG island hypermethylation. Because genotypic differences are likely to be manifested as phenotypic differences, CIMP+/MSI+ CRCs are expected to differ from CIMP-/MSI+ CRCs in some clinicopathological features, which are currently understood poorly. We characterized both common and different features between the two subtypes.

Design: A total of 72 MSI+ CRCs were analyzed for their CIMP status with eight-marker panel MethyLight assay. CIMP+/MSI+ and CIMP-/MSI+ CRCs were compared regarding clinicopathologic features and mutation in *KRAS*/*BRAF*.

Results: Eighteen cases (25%) were CIMP+, and this CIMP+ subtype was highly correlated with older age (CIMP+ 94.4% vs. CIMP- 33.3%, age >57) ($p < 0.001$). Polypoid gross appearance without ulceration was observed only in CIMP-/MSI+ CRCs (10%, $p = 0.057$). CIMP+/MSI+ CRCs were closely associated with poor differentiation, sheeting appearance, signet ring cell appearance, and acinar-form appearance, whereas the CIMP-/MSI+ subtype was closely associated with intraglandular eosinophilic mucin and stratified nuclei (all p-values < 0.05). The mean count of CD8+ tumor-infiltrating lymphocytes was higher in CIMP+ (51.3/HPF) than CIMP- tumors (36.1/HPF). Patients with CIMP+/MSI+ CRCs showed worse overall survival than patients with CIMP-/MSI+ CRCs.

Conclusions: Our results demonstrate heterogeneity in the clinicopathological features of MSI+ CRCs depending on CIMP status. The observation that CIMP+ and CIMP- subtypes showed different clinical features may provide a clue for establishing subtype-specific therapeutic strategies for these two subtypes.

599 Prognostic Implications of *ALU* and *LINE-1* Hypomethylation in Gastric Cancer.

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Background: Changes in DNA methylation status in cancer cells are characterized by focal CpG island hypermethylation and diffuse genomic hypomethylation. *ALU* and *LINE-1* repetitive DNA elements constitute about 28% of the human genome, and PCR-based measurements of these repetitive DNA elements can be used as a surrogate for genome-wide methylation content. Although repetitive DNA hypomethylation has been shown to be closely associated with poor prognosis in epithelial malignancies of some tissue types, little is known about the prognostic implications of *ALU* and *LINE-1* hypomethylation in gastric cancer.

Design: In the present study, we analyzed the methylation status of repetitive DNA elements (*ALU* and *LINE-1*) and 16 cancer-specific DNA methylation markers in 195 cases of advanced gastric cancer using combined bisulfite restriction analysis and the MethyLight assay, respectively.

Results: Low methylation status of *ALU* or *LINE-1* was closely associated with poor prognosis of gastric cancer, but multivariate analysis revealed that only *ALU* methylation status is an independent prognostic factor. When the combination of *ALU* and *LINE-1* methylation status was analyzed, low *ALU* methylation status plus low *LINE-1* methylation status defined a subset of gastric cancers that were closely associated with poor prognosis, and this was statistically significant upon multivariate analysis.

Conclusions: These findings suggest that the combination of *ALU* and *LINE-1* methylation status could be used as a molecular biomarker to define a subset of gastric cancer patients with a poor prognosis.

600 Foxp3 Expression in Microscopic Colitides: Clinicopathological Study of 69 Patients.

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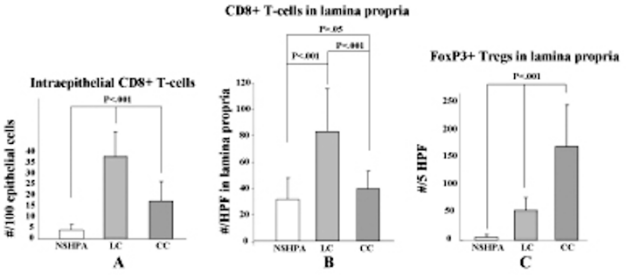
Background: Microscopic colitides, including lymphocytic (LC) and collagenous colitis (CC) are well-described conditions. They show characteristic morphologic changes that distinguish the two and many similarities including expansion of lamina propria predominantly by T lymphocytes. Altered immune response has, therefore, implicated in the pathogenesis of these two entities. It is recently reported that CD8+ T-lymphocytes (CTLs) secret IL2 which stimulates regulatory T cells (Tregs) proliferation and Tregs inhibit CD8+ CTLs induced cytotoxic tissue damage. Foxp3 in Tregs regulates T cell related immune responses. The relationship and distribution of Tregs and CD8+ CTLs in microscopic colitides to date has remained unexplored. The specific aim of this study is to characterize differences in the distribution pattern of Foxp3 in biopsies from LC and CC.

Design: 71 colonic biopsies from 69 patients obtained over a 2 year period with clinical diagnosis of microscopic colitides were retrieved. All biopsies were categorized into one of three diagnoses: no significant histopathological abnormality (NSHPA), LC, or as CC. All biopsies were evaluated by immunohistochemistry using a panel of markers including CD8 and Foxp3. All immunopostive cells were counted within the epithelium (numbers/100 epithelial cells) and in lamina propria (numbers/HPF). Statistical significance was derived utilizing the student's t test.

Results: Microscopic colitides is predominantly noted in females (3:1). Our study demonstrates that there is significant difference in the expression of C8+ T cells both in lamina propria as well as in intraepithelial cells amongst three groups [Fig 1A & 1B]. In contrast to LC, recruitment of Foxp3 + Tregs is significantly more frequently noted in lamina propria of biopsies with CC [Fig 1C]. Interestingly, 2 LC patients demonstrated morphologic changes of CC on follow up with associated conversion in the distribution pattern of Foxp3.



Conclusions: This study shows that differences in immunologic recruitment of Foxp3 + Tregs in colonic mucosa correlates with differences in spectrum of morphologic changes of patients presenting with microscopic colitides, particularly, LC and CC.

601 Relationship of Colorectal Adenoma Recurrence to Stem-Like Cell Populations in Crypt Epithelium and Adenomas.

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Background: Metachronous development, termed recurrence, of colorectal adenomas after polypectomy is a target for prevention strategies. Markers for adenoma recurrence are needed to identify patients with risk of progression to cancer. Colorectal epithelial stem cells in the near-basal region of crypts are the source of the crypt column, and stem-like cells are known to occur in neoplasms. We characterized the population of crypt stem cells in non-neoplastic mucosa for their possible role in the field defect responsible for adenoma recurrence, and stem-like cells in adenomas as a potential marker for metachronous development.

Design: An initial exploratory coded set of 11 non-neoplastic mucosal biopsy specimens and 20 baseline tubular adenoma (TA) polypectomy specimens was selected from patients with and without recurrence in a Phase III prevention trial. Expression of the stem cell marker aldehyde dehydrogenase-1 (ALDH1) was evaluated by immunohistochemistry, and morphometry was used to enumerate stem-like cells in crypt columns and adenomas. An additional 95 coded adenomas from 82 patients were then analyzed for ALDH1 expression using image analysis with a quantitative nuclear algorithm, and logistic regression analysis was performed.

Results: No differences in stem cell populations were found between the non-neoplastic mucosa of patients with and without adenoma recurrence. However, TAs had higher ALDH1 indices in patients with recurrence than those without recurrence in the exploratory set (53.8% vs. 11.9%, p=.002, for deep glands; 34.5% vs. 6.7%, p=.001, for middle glands; 8.2% vs. 2.1%, p=.03, for surface epithelium). In the larger set of specimens, ALDH1 indices in TAs of patients with recurrence (n=45) were statistically significantly higher than in the 37 patients without recurrence (21.6% vs. 15.2%, p=0.03; OR 1.04, 95%CI= 1.00, 1.08).

Conclusions: We report for the first time the association between higher percentages of stem-like cells in baseline colorectal adenomas and the development of metachronous adenomas. These findings suggest that a larger stem-like cell population in a baseline adenoma may be a marker for propensity of patients to develop a recurrent adenoma. Understanding of the factors inducing stem-like cells in adenomas may lead to mechanism-based prevention strategies.

602 Discordance between Molecular and Immunohistochemical Analyses for Lynch Syndrome Assessment.

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Background: Microsatellite instability analysis (MSI) and immunohistochemistry (IHC) for DNA mismatch repair (MMR) proteins are well-accepted clinical approaches to evaluate cancer patients for the possibility of Lynch Syndrome (LS). Some investigators advocate for the use of only one testing method to screen for LS given that the concordance between PCR-based MSI analysis and IHC is typically high. While MSI and IHC discordances have been acknowledged, the prevalence and nature of these cases have not been well-described. We examined the outcomes of a large number of IHC/MSI analyses performed in our laboratory to document discordance and frequency.

Design: The clinical records of 736 cancer patients who underwent MSI and/or IHC testing from 2002 to 2010 were evaluated. Patients with both MSI and IHC analysis (n=628) were subsequently studied in detail. Based on microsatellite marker analysis, 478 were classified as microsatellite stable (MSS), 97 as MSI-high (MSI-H) and 53 as MSI-low. Discordance was defined as a discrepancy between the MSI and IHC results. All IHC and MSI results and pertinent clinical and family history on discordant cases were re-reviewed by two pathologists and one clinical geneticist.

Results: Discordances were identified in 13 of 628 evaluated cases (2.1%). Twelve of 97 MSI-high (MSI-H) cases (12%) were discordant, showing intact IHC expression of the MLH1, MSH2, MSH6, and PMS2 proteins. Nine of these carcinomas were colorectal, 2 endometrial, and 1 small intestinal. 7/12 patients were younger than 50 years at age of diagnosis. 8/12 patients had a first degree relative with an LS-associated cancer. Germline mutational analysis of six patients revealed mutations in four (all colorectal carcinomas; two in *MLH1*, one in *MSH2,* one in *PMS2*). Both patients with *MLH1* mutations fulfilled Amsterdam II criteria, but the others did not. One of 478 MSS tumors showed loss of MLH1 and PMS2 on IHC. Methylation of *MLH1* was not detected in this tumor. No mutations of *MLH1*or *MSH2* were detected on subsequent sequencing or rearrangement analysis.

Conclusions: IHC/MSI discordance is surprisingly common, occurring in about 12% of MSI-H cancers. IHC testing alone would miss a significant number of informative cases given that MMR gene mutations were detected in 67% of the MSI-H patients with positive IHC staining for all markers. Therefore, the use of IHC by itself may yield misleading results that fail to identify potential LS patients.

603 Evaluation of Tumor Regression on Primary Tumors and Corresponding Lymph Nodes of Locally Advanced Rectal Adenocarcinomas after Neoadjuvant Treatment. A Prospective Study of 254 Cases.

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Background: Tumor regression (TR) of locally advanced rectal adenocarcinomas after neoadjuvant treatment gives prognostic information and pathologic complete response (pCR) is associated with better outcome. Major TR may lead to conservative surgery while poor TR can isolate high risk patients. However, most of the studies about TR have concerned the primary tumor without considering lymph nodes evaluation and correlations on response between the 2 sites.

Design: We aimed to evaluate: 1) TR on primary tumor and corresponding lymph nodes after neoadjuvant treatment, 2) the impact of therapeutic options (chemoradiation versus radiotherapy or chemotherapy) on TR. 254 locally advanced rectal adenocarcinomas have been prospectively collected in a database according to standardized reports between 2006 and 2010. TR grade on primary was assessed according to the Dworak (Dw) grading: Dw 0: no regression, Dw 1: dominant residual tumor with morphologic alterations (necrosis, fibrosis, colloid response), Dw 2: dominant morphologic alterations with residual tumor, Dw 3: very few tumors cells with dominant morphologic alterations, Dw 4: pCR. Lymph nodes were classified as: sterilized, metastatic with morphologic alterations, metastatic with no morphologic alterations.

Results: Dw 0, 1, 2, 3, 4 were found in 4, 27, 29, 27 et 13 % of the cases, respectively. Chemoradiation was more often associated with major TR(Dw 2-4), than chemotherapy or radiotherapy alone ($p<0.001$). The rate of metastatic lymph nodes was lower in pCR than in tumors with partial (Dw 1-3) or no response (Dw 0) ($p = 0.014$). Sterilized lymph nodes were found in 12% of negative lymph nodes patients. They were more often encountered among primary tumors with major TR (Dw 2-4 vs Dw 0-1, $p = 0.015$). When primary tumor showed no features of TR, corresponding metastatic lymph nodes displayed no morphologic alterations. Colloid and major colloid (more than 50% of mucin pools) responses were observed in 35 % and 17% of primary tumors, respectively. Colloid response in the primary, whatever the extent of mucin pools, was significantly associated with colloid responses in lymph nodes ($p<0.001$).

Conclusions: These results indicate that TR is more important in case of chemoradiation. TR in primary tumor is significantly associated with TR in metastatic or sterilized lymph nodes. These data, which are not integrated to the available TR classifications, could better reflect the impact of induction treatment and lead to an optimized management of patients.

604 HER2 Testing for Gastric Cancer in Latin America.

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Background: Trastuzumab is a monoclonal antibody indicated for HER2 positive gastric cancer patients. Accurate assessment of HER2 is essential to determine Trastuzumab use. HER2 overexpression/amplification has been seen in 15-22% of gastric cancers. Gastric cancer mortality is high in Chile, Costa Rica and Ecuador and low in Mexico, Puerto Rico and Cuba. Little is known about HER2 in gastric cancer in Latin America. We tested HER2 status by immunohistochemistry (IHC) and fluorescence in situ hybridization (*FISH*) and determined HER2 concordance.

Design: One hundred cases were reviewed (61 Mexico and 39 Costa Rica). Histologic tumor type included 72 intestinal-type, 24 diffuse-type and 4 mixed-type. Thirty four cases were well differentiated, 45 moderately differentiated and 21 poorly differentiated. Fifty nine cases were resections and 41 were biopsies. All tumor specimens were tested by IHC HercepTest™ (Dako, Carpinteria, CA, USA), using scoring system: 0 negative (no staining is observed or membrane staining is observed in <10% of tumor cells), 1+ negative (a faint/barely perceptible membrane staining is detected in >10% of the tumor cells), 2+ equivocal (a weak to moderate complete membrane staining is observed in >10% of the tumor cells, basolateral or U-shape), 3+ strongly positive (a strong complete membrane staining is observed in >10% of tumor cells, basolateral or U-shape). For *FISH*, tumors were tested by HER2 FISH pharmdx™ (Dako, Carpinteria, CA, USA); a HER2:CEP17 ratio of >2 was defined as positive for HER2 amplification.

Results: Among 100 tumors, 7(7%) were 3+, 8 (8%) were 2+ and 85(85%) were 0/1+; 90 (90%) were *FISH* non-amplified and 10 (10%) amplified as shown in Table 1.

Table 1. HER2 IHC-FISH

IHC	FISH NON-AMPLIFIED	FISH AMPLIFIED	TOTAL
0/1+	84	1	85 (85%)
2+	6	2	8 (8%)
3+	0	7	7 (7%)
Total	90 (90%)	10 (10%)	100 (100%)

8 out of 10 amplified cases were highly amplified (≥5). Concordance between IHC and *FISH* was 98.8% for 0/1+ cases, 75% for 2+ cases and 100% for 3+cases.

Conclusions: This study demonstrates a high concordance IHC-*FISH* in 3+ and 0/1+ cases. Discrepancies in HER2 2+ cases are attributed to tumor type, heterogeneity of the tissue and interpretation of basolateral and U-shape staining.

No differences in HER2 expression are seen among countries with high and low incidence of gastric cancer.

605 Determination of KRAS Status in Highly Challenging Rectal Adenocarcinomas after Neoadjuvant Treatment.

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Background: KRAS status is a pre-requisite in patients with metastatic colorectal carcinoma candidate for treatment with monoclonal antibodies targeting EGFR. Indeed, KRAS mutations are associated with resistance to treatment. However, KRAS status determination may be very challenging when tumor cellularity is poor, notably when major tumor regression (TR) is achieved after radiochemotherapy (RCT).

Design: We aimed to determine the most reliable strategy to detect KRAS mutations in poor cellular samples of rectal adenocarcinomas after RCT. 31 tumors with major TR and paired pre-treatment (PT) biopsies were analyzed. Following manual dissection of tumor from surgical specimens and PT biopsies, extracted DNA were submitted to High Resolution Melting (HRM) analysis. DNA displaying an altered shape of the melting curves were submitted to sequencing, as in our daily practice. DNA with altered melting curve without identification of mutation by sequencing were analyzed by an allele-specific PCR assay (a-sPCR). Wild type (wt) KRAS surgical samples, after HRM/sequencing, were microdissected and submitted to the same molecular processes.

Results: Among the 31 manually dissected surgical samples, 7 mutations were identified by HRM/sequencing. 3 additional mutations were detected with a-sPCR. Laser microdissection allowed the detection of 2 supplementary mutations but 2 mutations previously identified with a-sPCR were no more identified. Altogether, 12 surgical specimens displayed a mutated KRAS status. Manual dissection of PT biopsies followed by HRM/sequencing allowed the detection of 12 mutations, of which two were only detected on PT biopsies. Conversely, 2 wt KRAS PT biopsies were found mutated in their paired surgical samples. Overall, when combining the 3 molecular assays and manual/laser dissection, 14 patients displayed a mutated KRAS status. This mutation rate was twice that obtained after our routine procedure.

Conclusions: The recourse to PT biopsies is the best strategy for the management of poor cellular surgical specimens after RCT, allowing the detection of 12/14 mutations. Thus, we recommend a tumor PT biopsy dedicated to molecular diagnosis before any neoadjuvant treatment. If not available, more sensitive assays such as a-sPCR, or laser microdissection, could be helpful in case of wt KRAS status, but at the expense of higher costs and longer delays. These results may impact patients'management, avoiding the use of inefficient, costly and potentially toxic targeted therapy.

606 Leptin and mTOR Are Involved in Appendiceal Mucinous Carcinogenesis.

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Background: In appendiceal mucinous neoplasm, a pathological decision on benignancy or malignancy is a challenging task, since extreme low cellularity and undiscerned layers of appendiceal wall occur commonly due to plethora of mucin. Here, we evaluated signal pathways involved in mucin-producing carcinogenesis, and the putative target for molecular therapy in appendiceal mucinous adenocarcinomas.

Design: Appendiceal mucinous neoplasms were classified into three tumor categories; 32 cases of mucinous adenoma, 23 mucinous tumor of unknown malignant potential and 15 mucinous adenocarcinoma. To study signal pathways involved in mucin-producing carcinogenesis, immunohistochemistry for leptin, leptin receptor, pAkt, mTOR, Erk and pSTAT3 was performed. To propose a candidate for molecular therapy, fluorescence in

situ hybridization for *Her2*, *EGFR* and *ALK*, and immunohistochemistry for mTOR and c-kit were made. Additionally, the previously published our data on MUC2, MUC5AC and c-kit immunoexpression were excerpted.
Results: The expression of leptin, leptin receptor, MUC2, MUC5AC, mTOR and Erk was increasingly high, in order of adenomas, tumors of uncertain malignant potential and adenocarcinomas ($p < 0.05$, respectively). The pSTAT was not expressed in normal epithelium, but in tumors irrespective of tumor categories. As for the relation of examined proteins, leptin was correlated with MUC2, MUC5AC and mTOR ($p < 0.05$, respectively). Univariate analysis showed that mTOR expression was associated with a low rate of disease-free survival of cancer patients ($p < 0.05$). Besides, none of 70 cases showed *Her2* and *EGFR* amplification, *ALK* translocation or c-kit overexpression.
Conclusions: The leptin may collaborate with mucin (MUC2, MUC5AC) on carcinogenesis which the mTOR-dependent, the Erk-dependent, and pSTAT-dependent pathways appear to be involved in. The mTOR is suggested as a therapeutic target for appendiceal mucinous adenocarcinomas. However, *Her2*, *EGFR*, *ALK* and c-kit may not be the target for therapy.

607 Stromal Expression of YKL-40 Correlates with Disease Free Survival in Colorectal Cancer.

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Background: Advances in colorectal cancer (CRC) treatment has generated a need to discover predictive biomarkers. Increased *serum* levels of YKL-40, a secreted glycoprotein, has been inversely correlated with clinical outcome in many cancers, including CRC. To date, limited work has addressed if there is correlation between tissue expression of YKL-40 in CRC and clinical outcomes, such as disease free survival (DFS) and total survival (TS).
Design: Using clinical and tumor registry data we identified 281 patients with Stage 2-4 CRC with a minimum of 2-year follow-up. Immunohistochemistry was performed on archival tissue using a polyclonal YKL-40 antibody. Scores for percent of cells staining and staining intensity were combined for each specimen separately for epithelium and stroma to derive a total score which ranged from 0 to 6. Specimens with a total score ≥4 were classified as "positive (+)" and those <4 classified as "negative (-)". Cox regression was performed to assess the significance of stromal and epithelial expression in CRC in relationship to DFS and TS, controlling for stage and lymphatic invasion. Adjusted Hazard ratios indicate the risk of recurrence controlling for each variable.
Results: Fig 1 shows the crude DFS for patients according to total stromal score cutpoints (+/-). DFS for patient with "-" stromal score was superior to that for patient "+" score. Controlling for other variables in the Cox regression, "+" stromal score was an independent predictor of decreased DFS, ($p<0.05$). As expected, stage was also significantly related to DFS ($p<0.001$). Epithelial score and lymphatic invasion were not significant. Controlling for other variables, patients with "+" stromal scores were over twice as likely to recur (Hazard Ration = 2.1) as those with "-" scores. Stromal and epithelial scores showed no association with tumor stage. Tumor stage was the only predictor of TS by Cox regression.

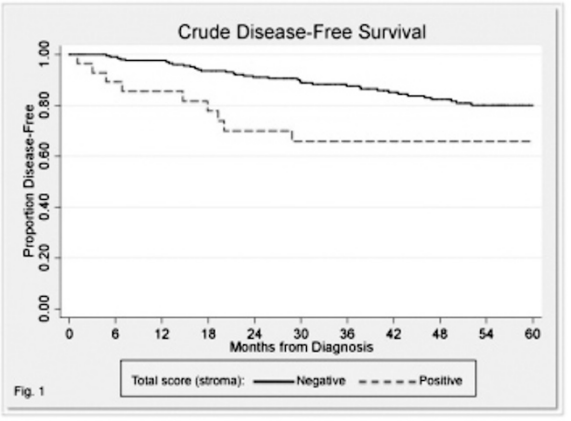

Fig. 1

Conclusions: Our results show a direct correlation between tumor specific stromal expression of YKL-40 and DFS in CRC. Lack of a significant association between stage and stromal score suggest that the latter is not merely a surrogate for the former. Our data suggests that *stromal expression* of YKL-40 predicts recurrence in CRC.

608 HuR Cytoplasmic Expression Is Predictive of Worse Disease-Free Survival in Primary Gastrointestinal Stromal Tumors (GISTs).

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Background: As a protein of the embryonic lethal abnormal vision (ELAV) family, HuR is known to implicate in mRNA stability and turnover and mediate post-transcriptional regulation of several genes involved in carcinogenesis. In a variety of human carcinomas, HuR is highly expressed and its cytoplasmic expression correlates with poor prognosis. However, no study has systematically evaluated the expression status and subcellular localization of HuR in a large, well-characterized cohort of GISTs.
Design: HuR immunostain was performed on tissue microarrays of on tissue microarrays of primary GISTs, yielding 341 cases with assessable results. Among these, mutation variants of *KIT* and P*DGFRA* receptor tyrosine kinase (RTK) genes were determined in 193 cases by sequencing with or without precedent DHPLC screening and dichotomized into two prognostically different groups. Follow-up was obtained in 318 cases with a median of 53 months. Nuclear and cytoplasmic stains of HuR in each case were separately recorded as percentages of tumor cells with positive nuclear and/or cytoplasmic reactivity and correlated with clincopathological variables, NIH risk category, Ki-67 labeling index (LI), RTK genotypes, and disease-free survival (DFS).
Results: HuR nuclear expression was neither associated with any variable examined nor DFS. However, HuR cytoplasmic expression in ≥10% of tumor cells was present in 142 (41.7%) GISTs, which was significantly related to epithelioid histology ($p=0.013$), larger tumor size ($p=0.031$), higher NIH risk categories ($p=0.001$) and Ki-67 LI ($p=0.008$), and decreased DFS ($p<0.001$, univariately). However, it was not related to tumor location, RTK genotypes, and other variables. In multivariate comparisons, HuR cytoplasmic expression remained independently predictive of adverse outcome ($p=0.004$, risk ratio [RR]=2.975), together with high NIH risk category ($p<0.001$, RR=5.261), Ki-67 LI>5% ($p<0.001$, RR=3.615), and non-gastric location ($p=0.001$, RR=2.942).
Conclusions: HuR cytoplasmic expression not only correlates with adverse prognostic variables and cell proliferation but also independently portends worse clinical outcomes in GISTs, suggesting its potentiality as a valuable prognostic biomarker and the possible causative role in conferring an aggressive phenotype.

609 Detection of Human Papillomavirus in Small Cell Carcinomas the Anus and Rectum.

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Background: Anorectal squamous cell carcinomas are often associated with high-risk human papillomavirus infection, whereas most rectal cancers are adenocarcinomas that are not. Small cell carcinomas represent less than 1% of all colorectal/anlal carcinomas and have a poor prognosis. In the uterine cervix, small cell carcinomas are associated with HPV infection. HPV is usually detected by in situ hybridization or other nucleic acid based molecular techniques (i.e. polymerase chain reaction). In HPV infection the oncoprotein E7 inctivates the tumor suppressor Rb leading to upgregulation of p16. In small cell carcinomas, the Rb pathway is often blocked by other mechanisms thus the increased levels of p16 may not be indicative of HPV infection and therefore p16 immunohistochemistry has a limited role.
Design: We have identified 17 cases of anal/rectal small cell carcinomas in our files. Formalin-fixed, paraffin-embedded tissue from small cell carcinomas of the anus (n = 6) and rectum (n = 11) from 17 patients were subjected to immunohistochemistry for p16, CDX2 and p63, followed by in situ hybridization for high risk HPV types using the HPV Inform III family 16 kit (Ventana Medical Sytems, Tucson, AZ) and an HPV-16 genotype specific probe (Dako, Carpintera, CA). At the time of original diagnosis all 17 cases had at least one positive neuroendocrine marker (synaptophysin, chromograninn or CD56) described.
Results:

Number and percent of cases positive according to site

	Anus	Rectum
p16 IHC	6/6 (100%)	11/11 (100%)
HPV in situ	4/5 (80%)	7/9 (78%)
CDX-2 IHC	1/6 (17%)	5/9 (55%)
p63 IHC	5/6 (83%)	6/9 (67%)

IHC: immunohistochemistry

Two cases showed high viral copies, while the majority of cases showed low or very low HPV copy numbers. In 5 cases HPV could only be detected using the HPV-16 genotype specific assay that can detect 1-2 copies of HPV. A tumor was considered CDX-2 or p63 positive if at least 50% of the nuclei were positive irrespective of staining intensity. All but one anal tumor showed weaker p63 staining than the strong nuclear staining observed in the nuclei of anal squamous epithelium. The majority of CDX-2 positive tumors showed diffuse staining.
Conclusions: High risk type HPV can be detected using in situ hybridization in the majority of anorectal small cell carcinomas that are uniformly p16 positive by immunohistochemistry. The majority of tumors also express p63, which is more pronounced in the anal tumors. CDX-2 expression is also observed, predominntly in rectal tumors. HPV targeted therapy could result in better control of these aggressive neopalsms.

610 Mitoses, Genomic Instability and Neoplastic Progression in Barrett's Esophagus.

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Background: Barrett's esophagus (BE)-associated adenocarcinoma (AdCa) develops as a consequence of chromosomal instability which is, in part, related to defects in cell division. A recently proposed theory suggests that Barrett's metaplasia is a successful adaptation to acidic reflux. Intestinal crypt stem cells produce transient amplifying cells that ultimately lead to differentiated cells that function in mucosal defense. The aim of this study was to evaluate epithelial mitoses as a biomarker for AdCa in BE in a large prospective study that included 32 patients who developed cancer.
Design: 3970 mucosal biopsies from 214 BE patients (M/F ratio: 170/44, mean age: 63 years, mean BE length: 5.7 cm), all of whom had a baseline endoscopy and at least one follow up endoscopy (mean follow up: 90.4 months), were evaluated in a blinded

fashion for the presence, number (#), location (surface versus crypts) and type (typical versus atypical) of mitoses in both dysplastic and non-dysplastic epithelium. Weighted Cox regression model was used to assess mitoses and cancer outcome after adjustment for age, gender, and BE segment length. Wilcoxon rank sum test was used to evaluate mitoses and aneuploidy (Yes/No) and ploidy (≤2.7N and >2.7N), and tetraploidy (4N ≤6% and >6%).

Results: Both total # of surface mitoses and mean # of surface mitoses per crypt, in baseline biopsies, were both significantly associated with progression to AdCa ($p<0.0001$ for both comparisons). However, neither total, nor mean, # of crypt mitoses were associated with progression ($p=0.8$ for both comparisons). Regarding genomic instability measured by flow cytometry, a significant association was noted between surface mitoses and aneuploidy ($p<0.0001$), DNA ploidy (> 2.7N versus ≤2.7N, $p=0.0001$), and tetraploidy (4N ≤6% versus >6%, $p<0.0001$). However, no relationship was observed between crypt mitoses and aneuploidy.

Conclusions: Surface epithelial mitoses are a morphologic biomarker of increased risk of progression to AdCa in patients with BE, and are significantly associated with genomic instability. The lack of association of crypt mitoses with these parameters supports the hypothesis that crypt mitoses are a component of the epithelial adaptation to acidic reflux, and may have a protective effect from progression to AdCa.

611 Assessment of *K-ras* Mutational Status Is Influenced by Tissue Archiving: Implications for Routine Molecular Testing.

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Background: *K-ras* mutation analysis has been unambiguously identified as a marker of resistance to cetuximab-based treatment in metastatic colorectal cancer (mCRC) patients. However, most studies have been performed using homogenously archived CRC specimens. The aim of the present study was to evaluate the impact of tissue preservation on the *K-ras* mutational status of mCRC patients.

Design: A series of 131 mCRC tissue specimens were cut into two equal parts. One of the halves was immediately snap-frozen, and the other half was fixed in formol and then paraffin-embedded (FFPE). The samples were first screened by high-resolution melting (HRM), followed by genotyping *via* subsequent direct sequencing to determine the *K-ras* mutational status.

Results: In frozen specimens, HRM clearly identified *K-ras* mutations in 47/131(35.8%) patients, which was consistent with the direct sequencing results. Among the 33 mutated FFPE-paired specimens available, 2 (6%) demonstrated suboptimal template amplification, and 2 (6%) expressed an erroneous wild-type *K-ras* profile by HRM. Using direct sequencing, 6/33(18.1%) samples displayed a wild-type *K-ras* status, 3/33 (9.1%) samples showed discordant nucleic alterations and 2/33 (6%) samples exhibited supplementary artifactual mutations in codon 12/13. Finally, by comparing the FFPE coring vs. the sampling method, no significant discrepancies in *K-ras* genotyping were observed.

Conclusions: Our results indicate that frozen specimen archiving should be used as often as possible in the diagnostic setting. Particular attention should be paid to the prevention of artifacts in routine molecular *K-ras* screening of FFPE specimens by using large amounts of template DNA, performing multiple amplifications, or by using a non-formalin-based fixative.

612 Identifying Low-Risk and High-RiskT1 Esophageal Adenocarcinoma Based on Pathologic Risk Factors.

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Background: Pathologic evaluation of endoscopic resection (EMR) specimens is a critical aspect of the pre-operative staging evaluation for superficially invasive adenocarcinoma of the esophagus and GE junction (stage T1-EAC), helping to direct appropriate treatment. The purpose of this study is to quantify the risk of lymph node metastasis (LNM) associated with known pathologic risk factors in a series of surgically resected T1-EAC.

Design: We reviewed pathology reports and clinical records from a consecutive series of 232 patients who underwent esophagectomy without induction therapy for T1-EAC at the UPMC between 1996 and 2009.

Results: Tumor size (Tsize), angiolymphatic invasion (ALI), submucosal invasion (stage T1b) and histologic grade (poorly differentiated, G3) were all significant risk factors for LNM on univariate analysis. When controlling for all four covariates, Tsize, ALI and grade were independent predictors of lymph node metastasis, but T stage was not (Table 1).

Table 1

Pathologic Risk Factors		LN Metastasis *	
		N/Total (%)	Adjusted OR (95% CI)
T stage	T1b	39/151 (25.8)	1.5 (0.44, 5.1)
	T1a	5/80 (6.3)	
Tumor size, cm (median, IQR)	(2.0, 1.0-3.0)		1.4 (1.1, 1.8)
ALI	+	28/44 (63.6)	10.5 (4.3, 26)
	-	15/181 (8.3)	
Grade	G3	23/65 (35.4)	3.0 (1.2, 7.2)
	G1 or G2	22/163 (13.5)	

* The median number of LN evaluated per patient was 18.5. Using the cumulative number of risk factors with tumor size of 1.0 cm as a cutoff, a simple, unweighted model shows that as the number of pathologic risk factors increases, there is a progressively higher incidence of regional metastasis (Table 2).

Table 2

Number of Risk Factors (T1b, ALI, G3, Tsize>1cm)	LN Metastasis * N/Total (%)
4	18/20 (90.0)
3	14/52 (26.9)
2	8/72 (11.1)
1	4/55 (7.3)
0	1/33 (3.0)

* P<0.001, Chi-squared test

Conclusions: We demonstrate that T stage, when controlling for ALI, grade and tumor size, is not an independent predictor of nodal metastasis and should not be used by itself to determine whether or not a patient can safely be treated with endoscopic resection and ablation therapy. When one accounts for the cumulative number of pathologic risk factors, one can stratify T1-EAC into low-risk and high-risk for LNM. These features can be evaluated pre-operatively and, in combination, be used to select the most appropriate treatment approach (endoscopic vs esophagectomy) and potentially identify patients who will benefit from systemic chemotherapy.

613 Appendiceal Serrated Neoplasms Are More Frequently Associated with Right-Sided Than Left-Sided Colorectal Adenocarcinoma.

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Background: Serrated neoplasms are seen in the colon and appendix. Although significant difference exist, there are morphologic and molecular similarities between serrated neoplasms of the appendix and their colonic counterparts. Colonic sessile serrated adenomas (SSA) are thought to be a precursor of microsatellite unstable (MSI-H) colorectal cancer (CRC), both of which are more common in the right colon than the left colon which mainly habors micosatellite stable (MSS) CRCs. This study addresses the question of whether appendiceal serrated neoplasms are also more commonly found in association with right sided CRC than left-sided CRC and whether there is an association with the MSI status of the CRC.

Design: We studied 3 groups of colectomy specimens which included the appendix in the same operation:(1) right-sided MSI-H CRC (n=65), (2) right-sided MSS CRC (n=80), and (3) all left-sided CRC (n=120). Consensus diagnoses of appendiceal lesions were made by 3 GI pathologists. The microsatellite status was established by a panel of 5 NCI markers for all right-sided CRCs. MUC6, a marker for SSA was assessed by immunohistochemistry.

Results: Appendiceal epithelial lesions were seen in 15 of 265 CRCs with appendectomy. These included 8 serrated neoplasms: sessile serrated adenoma (SSA, n=4), and mixed serrated and adenomatous neoplasm (MSAN, n=4). Other lesions included conventional mucinous cystadenomas (MCA, n=4) and hyperplastic polyps (HP, n=3). All 8 appendiceal serrated neoplasms (SSA and MSAN) were associated with right-sided CRCs and none were encountered in appendectomies that accompanied left-sided CRC.

Table 1

	SSA or MSAN	MCA	HP
Right-sided CRCs (n=145)	8¶	2	2
Left-sided CRCs (n=120)	0¶	2	1

¶p=0.009, Fisher's exact test

Appendiceal serrated neoplasms (SSA and MSAN) were more frequently associated with MSI-H than MSS CRCs, though this result was not statistically significant.

Table 2

	SSA or MSAN	MCA	HP
Right-sided MSI-H CRCs (n=65)	6§	1	1
Right-sided MSS CRCs (n=80)	2§	1	1

§p=0.14, Fisher's exact test

All serrated neoplasms expressed MUC6. Four MCAs and 3 HPs of the appendix were negative for MUC6.

Conclusions: Appendiceal SSAs are commonly associated with right-sided CRCs with a trend toward an association with right-sided MSI-H CRCs. These associations are similar to those of colonic SSA. Our results suggest that there may be a common pathogenesis for colonic and appendiceal serrated neoplasms.

614 Role of Reflux on Eosinophilic Esophagitis: Analysis on Morphologic Feature, Classification and Clinical Correlation with Large Cohort of 273 Patients.

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Background: In adults, eosinophilic esophagitis (EE) is an increasingly recognized disorder. The etiology and pathogenesis is less well defined, probably idiopathic or associated with food allergens.Although, reflux is an important factor involved in the pathogenesis of EE, the definitive role of reflux in EE is not clear, whether a triggering or promoting factor. In the present study, we analyzed a large cohort of 273EE cases to determine the effect of reflux and PPI therapy on EE, mainly focusing on their a) morphological features b)comparison between distalEE and reflux esophagitis andc) correlation of distal predominant EE and diffuse pattern EE with PPI theraupetic effect.

Design: The diagnostic criterion for EE was 15eos/hpf in>3-5 fields or single hpf with>25 intraepithelial eosinophils in at least one biopsy fragment. All of the EE specimens were further classified as proximal predominant,distal predominant and diffuse type (identified in both distal and proximal esophagus). Age, basal cell hyperplasia(BCH) (normal:25%,mild:26-50%,moderate:51-75%,severe>75%), spongiosis/intercellular edema, and ballooning epithelial change (focal/diffuse) were also analyzed.

Results: 546 biopsies from 273 patients(185males,88females) diagnosed as EE were studied, including 188distal,3proximal,and 82diffuse type.Increasing numbers of eosinophils were significantly associated with increasing severity of BCH and

spongiosis($p<0.001$ forboth). Epithelial ballooning change was noted predominantly in reflux (>50%inRE,n=20);but only in 12%distal predominant and nearly 7% in diffuseEE ($p<0.001$). 40 cases of these EE patients were treated with PPI for two months after diagnosis, including 31diffuse, 6distal and 3 proximal type. Morphologically,66% of both diffuse type and distal predominant EE showed significant reduction of eosinophilic infiltrate after PPI treatment($p<0.05$).The maximum count of 50eos/hpf and 100eos/hpf in proximal and distal biopsies, respectively,decreased to 4eos/hpf in both biopsies post treatment. In addition,BCH and spongiosis showed a significant decrease in proximal($p=0.02$), and marked decreased in distal biopsies($p=0.06$).

Conclusions: Our results indicate epithelial ballooning change is a key histological feature that distinguishes RE from EE, and PPI therapy significantly improves EE morphologically in at least 2/3 cases regardless of distal type or diffuse pattern of EE. The results further imply that reflux plays a significant role in the pathogenesis of EE.

615 Staging of Gastroesophageal Junction Adenocarcinoma with 7th Edition AJCC Staging Manual: Accuracy of Tumor Grade Assessment in Biopsy Specimens.

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Background: In the 7th edition of AJCC staging system for esophageal cancer, one of the important changes is the incorporation of histological grade in the stage/prognostic grouping for esophageal carcinoma. For T1-2N0M0 adenocarcinomas, the degree of differentiation is now an independent determinant of stage/prognostic group (Table 1). This study investigated the accuracy of preoperative histopathologic grading and its impact on tumor stage/prognostic grouping.

Design: Preoperative tumor grade was compared to postoperative tumor grade in 427 patients who underwent surgery and without neoadjuvant therapy for esophageal adenocarcinoma. Tumor grade was defined as well (WD), moderately (MD) or poorly differentiated (PD), recording the poorest grade. Cohen's weighted Kappa test for agreement was used as a measurement of accuracy of preoperative tumor grade assessed from biopsies with postoperative stage as gold standard.

Results: The overall accuracy of preoperative tumor grade assessment was 76% (kappa=0.58, $p<0.001$) including all stages. In patients with T1-2N0 stage tumors, 78% were properly staged (Table 1) with a kappa of 0.47 ($P<0.001$); However, 16% were assigned to a lower stage group based on preoperative assessment, whereas 7% were assigned a to higher stage group. This resulted in change of AJCC stage/prognostic grouping in 21% of patients (Table 1). In the T1-2N0 group, sensitivity for detecting a PD tumor was 0.55 (CI 0.39-0.70), whereas specificity was 0.89 (CI 0.83-0.96), e.g. of all PD tumors in this group, 45% were misclassified as WD or MD on preoperative biopsies, leading to an estimated lower prognostic stage.

	Postoperative Stage Grouping			
	IA	IB	IIA	Total
Preoperative Stage Grouping				
IA (T1N0, WD/MD)	64	13	0	77
IB (T1N0, PD or T2N0, WD/MD)	8	32	7	47
IIA (T2N0, PD)	0	1	4	5
Total	72	46	11	129

Conclusions: Factors, which could account for discordance in assessment of tumor grade in biopsies and resections, include sampling issue, technical quality of the specimen, and the experience of the pathologists. With increasing use of neoadjuvant therapy, accuracy of preoperative biopsy assessment becomes increasingly important and the interpretation of tumor grade on biopsies should be performed with caution.

616 Elevated Lipid Peroxidation and Cyclin D1 Levels Correlate with Poor Survival and Disease Recurrence in Stage II Tumours.

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Background: 30% of stage II patients relapse within 5 years with survival similar to Stage III disease. Studies investigating what molecular markers segregate Stage II patients likely to relapse and benefit from treatment are warranted. Using disease specific colorectal arrays, we identified significant alterations in cyclin D1 gene levels and DNA damage markers in this cohort. We hypothesise that expression of Cyclin D1 at the protein level will segregate good and poor Stage II tumours.

Design: We assessed Cyclin D and a lipid peroxidation marker, 4HNE in 260 Stage II cases. Tissue microarrays were constructed using four 0.6mm cores from each tumour and matched normal tissue. The percentage positive staining and intensity of both markers were assessed for epithelial and stroma cells. Results were correlated with patient outcome data.

Results: High levels of nuclear 4HNE expression in epithelium cells of stage II tumours correlated with poor patient survival ($p=0.003$). In contrast to tumour tissue, high 4HNE cytoplasmic expression in epithelium and stroma cells of normal adjacent tissue correlated with good survival ($p=0.000$ and $p=0.010$ respectively). High levels of nuclear Cyclin D1 expression in the stroma in stage II tumours correlated with poor patient survival ($p=0.001$). In addition high levels of Cyclin D1 cytoplasmic and nuclear expression in the epithelium of normal adjacent tissue correlated with poor survival ($p=0.008$, $p=0.038$). A strong association was detected between cyclin D1 and expression of a glycoprotein, Clusterin ($p<0.001$).

Conclusions: High cyclin D1 and lipid peroxidation levels segregates good and poor stage II tumours.

617 Benign Signet Ring-Like Cells in Follow-Up Biopsies of Patients with Gastric Lymphoma in Remission – A Rare but Significant Diagnostic Pitfall.

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Background: Benign signet ring-like cells (SRLC) have been reported in gastric mucosa involved by extranodal marginal zone lymphoma (ENMZL) both in biopsy and resection specimens. The prevalence of SRLC in post therapy follow-up biopsies in patients in clinical remission has not been studied to date.

Design: A pathology database search for gastric lymphoma cases between 1996-2010 was performed. Each biopsy was assessed for presence of SRLC, dysplasia, intestinal metaplasia, inflammation, fibrosis, edema, and regenerative atypia. Review of endoscopic findings and additional studies (AE1/AE3 and mucicarmine) were performed in positive cases.

Results: The study included 28 patients with gastric lymphoma, 17 male and 11 female, median age 62 (range 22-91). 18 had ENMZL and 10 other B cell neoplasms (BCN). In the ENMZL group, 45 of 149 biopsies showed active lymphoma and 104 were post-therapy, negative follow up biopsies (NFB). In the BCN group, 12/26 samples had lymphoma and 14 were NFB. SRLC were identified in 3/104 (3%) NFB samples in the ENMZL group from 2/18 (11%) patients, but not in the BCN group. One ENMZL patient showed SRLC in two non-consecutive follow-up biopsies, at 2 and 4 years after the original lymphoma diagnosis, both interpreted as signet ring carcinoma. A distal gastrectomy was performed following the second diagnosis, no tumor being identified in the resection specimen. Vague nodularity in the area of previous lymphoma and a pre-pyloric ulcer were noted on endoscopy at 2 and 4 years, respectively. The second ENMZL patient showed mucicarmine-negative SRLC at 5 years after diagnosis, with only GEJ nodularity endoscopically. The SRLC were forming small clusters in 2 biopsies and large clusters/sheets in one biopsy, in the latter SRLC being focally positive for mucicarmine and diffusely expressing AE1/AE3. Focal erosion without significant inflammation was seen in one biopsy only but no other significant morphologic findings were associated with SRLC. Both patients had no evidence of lymphoma or carcinoma at last follow up, 2 and 5 years after SRLC finding, respectively.

Conclusions: Epithelial SRLC are a rare but well documented occurrence associated with gastric ENMZL. Our current data suggests that this cellular disaggregation phenomenon can persist for a long time in few patients following eradication of their primary lymphoma, can be found in grossly unremarkable mucosa and are not associated with inflammation. Pathologist's awareness and correlation with clinical history are essential to avoid misinterpretation of SRLC and unnecessary therapy.

618 Metaplasia in the Gallbladder: An Analysis of Clinicopathologic Associations in 1218 Cholecystectomies.

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Background: There is conflicting data regarding the prevalence of intestinal and pyloric metaplasia (IM and PM) in the gallbladder (GB) and their association with GB carcinogenesis.

Design: The prevalence of IM and PM were analyzed in 1218 cholecystectomies from low vs high GB carcinoma (GBC) risk populations (US vs Chile; LR and HR), and non-neoplastic GBs vs dysplasia/early (mucosa confined) carcinomas vs advanced carcinomas. Length of mucosa examined from each case was recorded.

Results: Patients with metaplasia (mean age=55 yrs) were younger than those with GBC (mean=63; $p<0.001$). The frequency of both IM and PM was significantly higher in HR population (28% and 57%) than in LR population (12% and 45%; $p<0.001$). Moreover, the frequency of IM was significantly higher in neoplastic GBs (34%) than in non-neoplastic GBs (%10; $p<0.001$); however, intriguingly, the highest frequency was encountered in dysplasia/early GBCs (50%) vs advanced GBCs (27%) vs non-neoplastic GBs (10%), ($p<0.001$). All associations for IM were determined to be independent of sampling size (after adjusting for mucosal length by logistic regression analyses the p-values remained <0.001). By contrast, similar adjustment for PM attenuated the associations. Most IM was represented as goblet cells while the participation of absorptive cells/brush-border appeared to be very minimal. Areas with goblet cells often also showed significant atypia that qualify as dysplasia.

Conclusions: I. IM occurs in patients who are 5-10 years younger than those with carcinoma. **II.** While PM appears to be ubiquitous, IM is significantly more common in HR population, and in GBs with carcinomatous transformation supporting the role of IM as pre-precursors of carcinoma. **III.** Furthermore, IM is seldom seen in the absence of dysplastic changes and therefore it is advisable to sample/examine the cases with IM more closely. **IV.** That IM is more common in dysplasia/early GBC cases than advanced GBCs may suggest that early and advanced GBCs may not be a mere continuum but rather distinct processes as has been recently discovered in other organs, such as the sequence of athrophy-IM-invasive carcinoma in the stomach associated with intestinal subtype, which warrants further analysis.

619 Measurement of Depth of Invasion, Submucosal and Lymphovascular Invasion (LVI) Predicts Lymph Node (LN) Involvement in T1 Esophageal Adenocarcinoma (EAC). Invasion of Duplicated Muscularis Mucosae (MM) Has Similar Risk of LN Involvement as Mucosal Invasion.

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Background: The major challenge in treatment of T1 EAC is to decide who should be treated by endoscopic mucosal resection or limited esophagectomy. Measurement of depth of invasion has prognostic value in other tumor sites but has not been assessed in

EAC. Recent studies have shown duplicated MM in EAC but potential for LN metastasis in tumors invading the duplicated MM space is yet to be addressed.
Design: H&E from esophagectomy from 91 patients (M/F 84/7, average age 63 yrs.) with T1 EAC were evaluated for tumor differentiation, tumor configuration (exophytic or ulcerated), depth of invasion (mucosa, duplicated MM or submucosa), LVI and depth of invasion in millimeter (from the top of the tumor to deepest focus of invasion). Depth of invasion in millimeter and invasion into three layers were reviewed by second pathologist for interobserver variability. Both pathologists were blinded from LN status. Interobserver variability was assessed by kappa statistics/Pearson coefficient and logistic regression was used to correlate parameters with LN metastases in univariate model.
Results: LN metastases were observed with mucosal invasion (n=2), with submucosal invasion (n=9), and LVI (n=7), none with tumors in duplicated MM space.

Correlation of Pathologic Parameters with LN Status

	N	*p*	Odds Ratio	95% CI*
Depth of Invasion	91	**0.006**	1.04	1.01-1.07
Differentiation		0.07		
Well (reference)	35			
Moderate	42	0.17	4.60	0.51-41.34
Poor	14	0.03	13.60	1.36-135.91
LVI				
No (reference)	71			
Yes	20	**<0.001**	23.00	4.35-121.73
pT		0.15		
Mucosa (LP or Inner MM)* (reference)	27			
Duplicated MM*	37	1.00	0.00	0.00-N/A
Submucosa	27	0.05	5.26	1.00-27.69
pT				
Mucosa* and Duplicated MM* (reference)	64			
Submucosa	27	**0.002**	13.05	2.55-66.79

*CI-confidence interval; LP-lamina propria

Depth of invasion in millimeter, LVI and submucosal invasion were significantly associated with LN metastases while tumor configuration was not. Agreement in depth of invasion in millimeter and invasion into three layers between pathologists were good (k=1, pearson coefficient=0.81 respectively).
Conclusions: Measurement of depth of invasion, submucosal invasion and LVI are predictors of LN metastases in T1 EAC. Patients with invasion into duplicated MM space has similar risk of LN metastasis as those with mucosal invasion.

620 HER2 Protein Over-Expression in Proximal and Distal Gastric Cancers: An Immunohistochemical and Clinicopathological Comparison Study of 957 Cases.

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Background: Gastric cancer is the second most common cause of cancer death in China. Despite advances in treatment, the outcome for advanced gastric cancer remains poor. HER2 gene amplification and over-expression, which represents a potential therapeutic target, was recently reported in 10-40% of gastric cancers. The relative expression of HER2 in proximal and distal gastric cancers remains unknown. The aim of this study was to compare HER2 expression and its correlation with clinicopathological variables between proximal and distal gastric cancers in Chinese patients.
Design: We retrospectively reviewed a prospectively acquired pathology database for gastric cancer resections with HER2 immunostain results over the period from January 2007 through August 2009. We identified 957 consecutive cases including 513 proximal and 444 distal gastric cancers. HER2 immunoreactivity was scored on a 0-3 scale, according to the ToGA study criteria. Correlations between HER2 expression and clinicopathological variables were sought. Chi-square and Spearman correlation tests were used to assess significance.
Results: HER2 expression in both proximal and distal gastric cancers was significantly higher in the Lauren intestinal type ($p<0.001$) and grade 2 ($p<0.001$) gastric cancers as compared to the Lauren diffuse type and grade 3 tumors, respectively. In distal gastric cancers, HER2 expression was significantly higher in stage IV ($p<0.001$) and in patients with distant metastasis ($p<0.001$). No statistically significant difference was found in HER2 over-expression between proximal (11% score 3, 25% scores 2 and 3) and distal (8% score 3, 18% scores 2 and 3) gastric cancers ($p=0.08$).
Conclusions: The rate of HER2 over-expression was similar between proximal and distal gastric cancers. HER2 over-expression significantly correlated with the Lauren intestinal type and grade 2 tumors in the entire series, and with distant metastasis and stage IV in distal gastric cancers only.

621 Desert Hedgehog (DHH) and Sonic Hedgehog (SHH) Overexpression in Colorectal Cancer (CRC): Correlation with AJCC Stage and Overall Survival.

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Background: The Hedgehog signaling pathway has been associated with embryonic development and neoplasia. The prognostic significance of Shh and Dhh expression in CRC has not been widely studied.
Design: Formalin-fixed, paraffin embedded sections from 155 colorectal adenocarcinomas (CRCs) were immunostained by automated methods (Ventana Medical Systems, Inc, Tucson, AZ) with goat polyclonal Dhh and Shh antibodies (Santa Cruz Biotechnology, Inc, Santa Cruz, CA). Cytoplasmic immunoreactivity of each protein was semiquantitatively assessed in the tumor for all cases. Scoring was based on staining intensity and distribution and results were correlated with clinicopathologic variables.
Results: Moderate to marked, diffuse Dhh overexpression was noted in 83% tumors and correlated with advanced AJCC stage (90% of stage III/IV and 76% stage I/II; $p=0.024$), positive lymph node status (92% of node positive and 70% of node negative, $p<0.0001$), and shortened overall survival (88% in those expired and 69% in those alive; $p=0.004$). Intense diffuse Shh overexpression was noted in 28% tumors and correlated with early AJCC stage (39% of stage I/II and 14% stage III/IV; $p<0.0001$); and negative lymph node status (43% of node negative and 14% of node positive, $p<0.0001$). There was significant coexpression of both proteins ($p=0.049$). On multivariate analysis, pathologic stage ($p<0.0001$) and Dhh overexpression ($p=0.031$) were independent predictors of overall survival.
Conclusions: Shh and Dhh are frequently coexpressed in CRC, with Dhh overexpression more directly associated with advanced AJCC stage and positive lymph node status compared to Shh overexpression. The finding that Dhh overexpression was an independent predictor of overall survival further supports the clinical development of Hedgehog pathway inhibitors for the treatment of CRC.

622 Clinicopathologic and Genetic Characterization of Traditional Serrated Polyps of the Colon.

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Background: Traditional serrated adenomas (TSAs) are a type of colorectal polyp with neoplastic potential that are morphologically distinct from sessile serrated adenomas. The clinicopathologic and genetic features of TSAs are uncertain as prior studies have often combined these two entities.
Design: Routinely processed polypectomy specimens and associated clinicopathologic data from 24 patients with TSA were collected. TSA morphology was confirmed for each polyp based on the presence of serrated epithelium, ectopic crypt formations (ECF), cytoplasmic eosinophilia and pencillate nuclei. Unstained sections were immunolabeled for beta-catenin, p53, Ki67 and activated caspase-3. DNA was extracted from paraffin sections and used for PCR amplification and bidirectional sequencing of the *KRAS* and *BRAF* oncogenes.
Results: Among all 24 patients, 16 (67%) were male and 23 (96%) were Caucasian. The mean age was 61.8 yrs (range 40-86). Twenty-one (88%) of TSAs were located in the left colon. The mean size of all TSAs was 0.9 cm (range 0.1 to 2.5 cm). Conventional epithelial dysplasia in a TSA, seen as foci of increased nuclear/cytoplasmic ratio, nuclear crowding and loss of differentiation was present in 15/24 (63%) TSAs; none showed high-grade dysplasia. Abnormal nuclear accumulation of beta-catenin was identified in 7/24 (29%) TSAs, and abnormal nuclear accumulation of p53 in 2/24 (8%) TSAs. Abnormal Ki-67 labeling (defined as labeling of surface epithelium) was observed in 12/24 (50%) TSAs. By contrast, activated caspase-3 labeling was either negative or labeled only rare cells in all TSAs. Sequencing identified activating KRAS mutations in 11 TSAs (46%) and activating BRAF mutations in 9 TSAs (38%). KRAS and BRAF mutations were mutually exclusive and in total accounted for 20/24 (83%) TSAs. There was no difference in the clinicopathologic features of KRAS mutant versus BRAF mutant TSAs. However, nuclear labeling of beta-catenin was more common in TSAs with BRAF mutations (5/9, 56%) versus those with a KRAS mutation (1/11, 9.1%) ($P=0.05$).
Conclusions: Colorectal polyps with morphologic features of TSA are represented by two genetic variants. Wnt pathway activation in TSA predominantly occurs in the setting of BRAF activation. Abnormalities in Wnt signaling and p53 are more common in larger TSAs associated with conventional epithelial dysplasia, suggesting these lesions undergo a genetic progression as described for other colorectal polyps.

623 Immunopathological Mechanisms Operative in Patients with Collagenous Colitis, Ulcerative Colitis and Non-Inflamed, Normal Colon.

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Background: While the understanding of the cellular and molecular mechanisms involved in the pathogenesis of inflammatory bowel diseases (IBD) increased substantially over the past few years, relatively little is known on the mechanisms involved in the aetiology of collagenous colitis. The pathogenetic relationship of collagenous colitis (CC) and inflammatory bowel diseases, particularly, with ulcerative colitis (UC) continues to be debated. In this study we aimed to improve our understanding about the aethiopathogenesis of CC by a detailed phenotypical and functional characterization of the involved cell types. Special emphasis was on the respective contribution of the local innate and adaptive immune system.
Design: We compared the expression of genes related to macrophages, T-lymphocytes and intestinal epithelial cells from CC, UC and healthy control (HC) patients (N=10, each), using RNA isolated from tissue sections of formalin-fixed, paraffin-embedded colonic biopsies for subsequent quantitative realtime PCR.
Results: In UC the expression of the T-cell lineage – specific transcription factors t-bet and GATA3 is increased compared to HC and CC, indicating a higher frequency of effector CD4+ T-cells in UC. Furthermore, the expression of TGFb1 and TGFb3 is exclusively increased in UC and is associated with elevated FoxP3 expression, suggesting a counter-regulation to chronic inflammation through an increased local accumulation of Treg's in UC, but not in CC. Triggering receptor expressed on myeloid cells 1 (TREM1) mRNA is markedly increased in UC, but almost completely absent in CC and HC, whereas TREM2 mRNA expression is comparably low in UC and CC. This may indicate differential macrophage differentiation (M1vs.M2). Transcription of the cytokine TSLP, which is preferentially expressed in intestinal epithelium, is reduced in CC in comparison to HC.
Conclusions: The established methodology is suitable to determine distinct gene expression profiles using paraffin embedded tissues. Preliminary data indicate differences in the gene expression patterns in the myeloid, epithelial and T-cell compartment in the

colonic mucosa of UC, CC, and HC. The cell-type specific isolation of mRNA form colonic tissue sections by laser capture microdissection, also in combination with adapted immunostaining protocols, will further allow to define distinct, cell type-specific pathogenetic pathways operative in CC, UC and HC.

624 High Goblet Cell Density Protects Against Progression to Adenocarcinoma in Patients with Barrett's Esophagus.

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Background: Most Barrett's esophagus (BE)-associated adenocarcinomas (BEA) develop in columnar mucosa with goblet cells (GC). However, 95% of BE patients never develop BEA. One recently proposed hypothesis states that GCs represent a potentially successful adaptive response by producing a thick mucous barrier to acidic reflux which may protect against cancer development, but this has never been tested. The aim of this study was to determine the association of GC density with risk of progression to BEA, and DNA content abnormalities, the latter of which represents a major step in the BE carcinogenic pathway.

Design: 3970 baseline mucosal biopsies from 214 BE patients enrolled in a large prospective surveillance cohort (M/F ratio:170/44, mean age: 63 yrs, mean BE segment length: 5.7 cm) all of whom had a baseline endoscopy and at least one follow-up endoscopy (mean follow up: 90.4 months), were scored in a blinded fashion, for the total # of GCs and crypts, and the # of crypts with ≥1 GC, in both dysplastic and non-dysplastic epithelium, to determine the mean # GC/crypt and the % crypts with ≥1 GC. The relationship between the GC parameters and flow cytometric abnormalities (aneuploidy, tetraploidy) was compared using Wilcoxon rank sum test, and a weighted Cox regression model was used to assess BEA risk, after adjustment for age, gender, and BE segment length.

Results: 32 patients progressed to cancer during follow up. Increased GCs in baseline biopsies were significantly associated with protection from BEA when analyzed for total # of GCs, mean # GC/crypt, # crypts ≥1 GC and % crypts ≥1 GC ($p<0.0001$ for each comparison). GCs in non-dysplastic biopsies were similarly significant for a protective association in all comparisons (p= 0.007, 0.016, 0.0070 and 0.016, respectively). High GC values in all crypts showed a significantly lower association with aneuploidy (p=0.0016), ploidies (>2.7N; p=0.0061), and tetraploidy (elevated 4N≥6%; p=0.0015).

Conclusions: The results of this study show, for the first time, that GC have a protective effect on progression to BEA, and on the development of flow cytometric DNA content abnormalities. GC may participate in mucosal defense and represent a successful adaptative response to carcinogenesis in BE.

625 Sox-9 Immunohistochemistry Demonstrates Abnormal Distribution of Gastrointestinal Stem Cells in Specialized and Non-Specialized Barrett's Mucosa.

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Background: Sox-9 is a gastrointestinal stem cell marker shown to be abnormally expressed in specialized Barrett's mucosa (BM)/intestinal metaplasia. The distribution of stem cell in cardia type mucosa in tubular esophagus (non-specialized BM) is unknown. We quantitatively assessed the stem cell distribution in two types of BM and oxyntic mucosa.

Design: Gastric body biopsies with unremarkable oxyntic mucosa,esophageal biopsies with non-specialized and specialized BM from patients with BM on endoscopy were analyzed. Sox-9 (rabbit antibody,Milipore,CA)immunohistochemistry was performed with an automated immunostainer (Leica Microsystems,IL). The SOX9 positive nuclei were quantitatively assessed by automated image analysis using Aperio's ScanScope XT (Aperio Technologies Inc,CA). Surface epithelium, upper half (body) and lower half (neck and base) of crypt were analyzed as separate overlays in the scanned images at 20X. Three compartments were marked to include only epithelial cells. An automated nuclear quantitation algorithm using the ScanScope was prepared based on color variations to achieve percent positive cells per lesion for each compartment. Mann–Whitney U test and SamplePower 2.0 software used for the statistical analysis.

Results: Twenty two biopsies from 17 adults were analyzed. Sox-9 nuclear staining was observed in lower crypt of the oxyntic mucosa with essentially no staining of the surface epithelium. In contrast, Sox-9 staining was seen in all three compartments of the non-specialized and specialized BM with florid staining in the multilayered columnar epithelium.

Mean±SD Sox-9 positive cells in different compartments of three lesions

	Fundus (n=6)	Non-Specialized BM (n=8)	Specialized BM (n=8)	p value/Power
Total	17.9±9.3	59.6±24.3	66.2±29.3	0.001/0.95[a], 0.001/0.96[b], 0.72[c]
Surface	3.2±2.5	15.1±7.3	18.3±6.1	0.001/0.93[a], 0.008/0.87[b], 0.23[c]
Upper crypt	4.7±1.8	13.9±8.5	19.1±10.2	0.08/0.66[a], 0.008/0.87[b], 0.23[c]
Lower crypt	10.1±6.5	30.6±14	28.8±16.3	0.003/0.85 [a], 0.013/0.68[b], 0.72[c]

a=Fundus vs. Non-specialized BM, b=Fundus vs. Specialized BM, c=Non-specialized BM vs. Specialized BM

Conclusions: Abnormal but similar stem cell distribution in non-specialized and specilaized BM as compared to oxyntic mucosa provides an evidence of biologic similarity between two types of BM.

626 Tumor Deposits in Rectal Adenocarcinoma after Neoadjuvant Chemoradiation Are Associated with Advanced Stage and Tumor Recurrence.

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Background: Pericolonic tumor deposits (TD) in colorectal carcinoma (CRC) have been associated with poor prognosis; however, the nature and impact on outcome of TDs in rectal carcinoma following neoadjuvant chemoradiation (NCR) are unexplored. The aim of this study is to assess the prevalence of TD in rectal cancer patients treated with NCR and the association of TDs with clinical pretreatment tumor extent, clinical outcome, and overall survival (OS). Our hypothesis is that TDs following neoadjuvant therapy are associated with poor outcome in rectal cancer.

Design: This retrospective study included 461 rectal carcinoma resections from 1989-2010 evaluated for TD and history of NCR. TD were defined as irregular tumor deposits in perirectal fat discontinuous from the primary tumor, with infiltrative borders, no surrounding lymphocytes, and close association with large nerves or vessels. Medical records were reviewed for pretreatment clinical stage, total number of positive lymph nodes (pN+), vascular invasion (LVI), perineural invasion (PNI) local recurrence (LR), distant metastases (DM), and OS.

Results: Of 461 cases, 219 were treated with NCR and 233 untreated. Forty-nine (10.6%) contained TD (20 NCR and 29 without NCR). An additional 96 cases contained residual tumor after NCR without TD. Patients with TD after NCR had significantly higher pN+ compared to untreated patients with TD (p=0.001), and higher pretreatment T stage (cT3/T4) compared to untreated patients with TD (p=0.005). Patients with TD after NCR had significantly less LVI (6/20) compared to untreated patients with TD (24/29, p=0.0003). There was no significant difference between these 2 groups in PNI, DM, LR, and OS. Patients with TD after NCR had a significant increase in total number pN+ (p=0.0001), LR (p=0.03), DM (p=0.045), LVI (p=0.03) and PNI (p=0.007) compared to cases without TD after NCR. There was no significant difference in OS between these 2 groups.

Conclusions: The association with deeply invasive (cT3/T4) tumors suggests that many TDs after neoadjuvant therapy result from discontinuous eradication of tumor. While there was no impact on OS, the presence of TD after neoadjuvant therapy is associated with more advanced disease and tumor recurrence (more positive lymph nodes, LVI, PNI, local recurrence, metastases), as compared to patients without TD after neoadjuvant therapy. TDs after neoadjuvant therapy were also associated with higher numbers of involved nodes and higher pretreatment T stage when compared to cases with TDs but without neoadjuvant therapy.

627 Concordance between Microsatellite Instability (MSI) Testing and Mismatch Repair Protein Immunohistochemistry (MMR IHC) and Analysis of Discordant Cases.

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Background: MSI and MMR IHC testing are both widely used to screen patients for Lynch syndrome. Few studies have directly compared the results of MSI and IHC performed with current methodologies (mononucleotide repeat markers, four-protein IHC). Using a series of tumors from patients at elevated risk for Lynch syndrome, we looked at the concordance rate of these tests and analyzed discordant cases to look for trends and to determine if performing both assays provides any benefit over using a single assay.

Design: Cancers were selected on the basis of either histologic or clinical findings suggesting Lynch syndrome. 286 tumors (152 GI, 128 GYN, 2 breast, 1 ea brain, skin, kidney, unknown) were tested with both MSI and IHC. MSI testing used five mononucleotide markers (Promega MSI Analysis System). MMR IHC was performed with stains for MLH1, MSH2, MSH6, PMS2. Agreement between tests is defined as MSS/MSI-L and all proteins present; or MSI-H and any protein absent. For cases with discordance, available clinical history and MMR gene methylation/sequencing results were obtained. Additionally, some cases with absent MSH6 were re-stained with an alternate MSH6 antibody, and neoadjuvantly-treated rectal tumors were re-tested on an untreated sample when available.

Results: Overall, 68 tumors (23.8%) were abnormal by at least one test. This includes 49 tumors (17.1%) that tested abnormal by both methods, and 19 tumors (6.6%) abnormal by only one test. MSI and IHC gave the same result in 266 cases (93%), 14 cases (5.0%) had discordant results, and 6 cases (2.1%) had equivocal IHC results. Of discordant cases, 10 could be resolved with additional testing or history and showed that MSI was correct in 8 of these, while IHC was correct in 2 (TABLE). Of equivocal IHC cases, 2 were shown to have a correct MSI result.

Discordant Cases

	n	MSI correct	IHC correct	Unknown
MSI-H, IHC normal	5	3	0	2
MSS, IHC abnormal	9	5	2	2
Total	14	8	2	4

Conclusions: MSI and MMR IHC have a high rate of concordance when a mononucleotide repeat panel and four-protein IHC are used. However, some patients with defective MMR, including those with Lynch syndrome, are identifiable with only one of these methods. For patients with an elevated suspicion of Lynch syndrome, testing with both MSI and IHC improved sensitivity and specificity over a single test. Additionally, MSI can clarify ambiguous IHC results, such as may be seen post-treatment or with weak MSH6 staining.

628 Evaluation of Peritoneal Elastic Lamina Invasion as a Prognostic Marker in Stage II Colonic Carcinoma.

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Background: The benefit of adjuvant chemotherapy in stage II colorectal cancer remains unclear and it is usually reserved for patients with adverse prognostic features

(including pT4 disease). Distinguishing pT3 from pT4a tumors can be challenging. A recent study demonstrated that peritoneal elastic lamina invasion (ELI) is a predictor of survival in stage II colonic cancer and that survival in pT3N0 cases with peritoneal ELI was comparable to pT4N0 cases (Kojima et al., *Am J Surg Pathol*. 2010; 34:1351-60). We report our own experience with a Movat stain in determining the prognostic significance of peritoneal ELI.

Design: A total of 141 patients undergoing resection of a stage II colonic tumor (114 pT3; 27 pT4) between 1992 and 2006 were identified. Details regarding lymph node count, histologic grade, tumor budding, perforation, lymphatic and vascular invasion were recorded. Peritoneal ELI was assessed in slides closest to the peritoneum with the use of a Movat stain.

Results: The peritoneal elastic lamina was visualized in 82% (93/114) of pT3 cases (elastic lamina of large vessels served as an internal control). Staining intensity was highly variable with continuous, strong staining identified in only 39% (36/93). ELI was seen in 12 of 93 assessable pT3 cases (13%), but showed no significant difference in 5-year overall survival compared to pT3 ELI negative cases. A high tumor budding count and harvesting less than 12 lymph nodes were independently associated with poor survival outcome (p=0.029 and p=0.013, respectively).

Conclusions: Variability in the detection and continuity of the peritoneal elastic lamina may limit the utility of peritoneal ELI as a prognostic marker in routine practice. The prognostic significance of lymph node harvest and tumor budding in stage II colonic cancer is confirmed.

629 Carcinoma in Crohn's Disease: Mucinous Histology Is Not Associated with Microsatellite Instability or Mismatch Repair Protein Deficiency.

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Background: The risk of malignancy in Crohn's disease (CD) depends on disease duration, severity and extent. The proportion of tumors with morphology and molecular features of microsatellite instability which may have better stage for stage outcome has not been described in such patients. We retrospectively reviewed gastrointestinal malignancies occurring in CD patients from the records of a tertiary referral centre for these features.

Design: Only tumors occurring in bowel sites known to be involved by CD were included. Clinicopathological characteristics were recorded and the tumor was tested for immunohistochemical expression of mismatch repair proteins (MLH1, MSH2, MSH6, PMS2) and for microsatellite instability (MSI) at 2 loci (BAT25, BAT26).

Results: 21 tumors in 20 patients were identified (17 resections, 4 biopsies), with equal gender distribution at a median age of 52.0 yrs (range 31-85 yrs). Only 2 tumors occurred in small bowel, and 3 in the right colon. The remainder were all in the sigmoid colon or rectum (11) within sigmoidoscopic range, but 6 occurred in anal fistula tracts. Mucinous predominant (7) or partial differentiation (5) occurred in 60%, with other features suggestive of MSI seen in an additional 2 cases. Of 17 cases available for testing, all 4 MMR proteins were intact and tumors were microsatellite stable in 8 of 10 (80%) cases with and 6 of 7 (86%) cases without MSI-type features. The other 3 tumors were MLH1 and PMS2 deficient and were MSI-high.

Conclusions: Carcinoma complicating CD occurs in younger patients, most commonly in the distal large bowel, and often shows MSI-type features, but usually without concomitant loss of mismatch repair genes or MSI. The relative paucity of small bowel tumors may reflect surgery before malignancy occurs, or may reflect a major change in the distribution of carcinoma in CD.

630 Most Metastatic Colorectal Adenocarcinomas Express Less CDX2 Than Their Primaries.

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Background: CDX2 is an intestine-specific transcription factor and is used diagnostically as a tumor marker of colonic origin. Both CDX2 over-expression and decreased expression in the primary colonic adenocarcinomas have been reported previously. It is still in debate whether CDX2 is a tumor suppressor or an oncoprotein. Loss of CDX2 expression in colonic adenocarcinomas has been found associated with poor prognosis, suggesting its function as a tumor suppressor. Metastasis is a selective process through which only the cancer cell with advantageous phenotypes survives this process and grows in a site away from the primary tumor. Whether CDX2 expression is able to be sustained in the process of metastasis will provide an important clue for its role in the process of carcinogenesis. In this study, we compared CDX2 expression in metastatic and matched primary colorectal adenocarcinomas.

Design: Immunohistochemical staining against CDX2 was performed on a tissue microarray (TMA) (0.6-mm tissue cores) of 27 cases of randomly selected human colorectal adenocarcinoma specimens. Based on CDX2 expression in the primary tumor, 19 pairs of matched primary tumor and metastasis were analyzed. The percentage of cells with nuclear staining and their mean intensity level were scored. Statistical analysis was performed by two-tailed paired T test.

Results: CDX2 is expressed in 68% (19/28) of primary colorectal adenocarcinomas. Among these 19 paired primary and metastatic tumors, expression of CDX2 was completely or significantly reduced (p-value < 0.00004) in 73% (14/19) of metastatic tumors. CDX2 expression in the other 5 pairs showed no significant changes. In the 8 cases that CDX2 were not expressed in primary tumors, no CDX2 was detected in their paired metastases.

Conclusions: While most primary colorectal adenocarcinomas express CDX2, 73% of remote metastasis completely lose or decrease CDX2 expression. This phenomenon should be considered when diagnosing metastatic tumor of unknown origin. The significantly reduced CDX2 expression in metastatic colonic adenocarcinoma also supports the notion that CDX2 behaves as a tumor suppressor. To our knowledge, this is the first study comparing CDX2 expression in primary colorectal adenocarcinoma with its metastasis.

631 Filiform Serrated Adenoma Is an Unusual Pathologic Variant of Traditional Serrated Adenoma.

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Background: Filiform serrated adenoma (FSA) is an uncommon type of polyp that shows dysplastic morphologic features similar to traditional serrated adenoma (TSA). Unlike TSA, FSA is composed predominantly of prominent, thin, elongated filiform projections lined by neoplastic epithelium with serrated contour. However, it is not established that this type of polyp with unique morphology has the same pathogenesis as TSA or not.

Design: Thirteen FSAs were retrospectively identified by review of colonic polyp biopsy which contained the term "serrated" in the final diagnosis between 2001 and 2007. Mutation analysis of BRAF and KRAS genes was performed by using PCR and direct sequencing. Methylation-specific PCR was performed to detect promoter hypermethylation of hMLH1, MGMT, p16, MINT1, MINT2, MINT31 and APC genes. The CpG island methylator phenotype (CIMP) was recorded as negative if ≤1 locus was methylated, low if 2 or 3 loci were methylated, and high if 4 loci were methylated. Microsatellite instability (MSI) was evaluated by using five quasimonomorphic markers. Allelic size variations found in 1-2 microsatellites were classified as MSI-low and ≥3 microsatellites were considered MSI-high. The clinicopathologic and molecular results were compared to our previously published TSA data.

Results: Thirteen polyps were obtained from 13 patients including eight men and five women, with a mean age of 55 years (range: 44-72 y). Twelve polyps were found in the left colon and one in the right colon. Seven patients had more than one polyp in the colon at the time of initial colonoscopy. One patient had metachronous adenocarcinoma detected in the colon at a different anatomic site from the polyp. All but one FSA showed low-grade dysplasia and one with high- grade dysplasia. No carcinomatous transformation was observed. BRAF and KRAS mutation was observed in six (46.2%) and four (30.3%) polyps, respectively, and they were mutually exclusive. Hypermethylation of hMLH1, MGMT, p16, MINT1, MINT2, MINT31 and the APC gene was found in 30.3%, 38.5%, 15.4%, 53.8%, 46.2%, 38.5% and 15.4%, respectively. FSAs were classified as CIMP-negative in three (23.1%), CIMP-low in five (38.5%), CIMP-high in five (38.5%) cases. All polyps were microsatellite stable.

Conclusions: FSA occurs predominantly in the left colon. Unlike TSA, FSA shows more frequent KRAS mutations, and less frequent BRAF mutation and promoter hypermethylation. These molecular findings are similar to TSA located in the left colon. Although the histologic findings are unique, FSA can be categorized as an unusual pathologic variant of TSA.

632 The New AJCC Pathologic T4 Classification Does Not Have a Better Correlation with Outcome.

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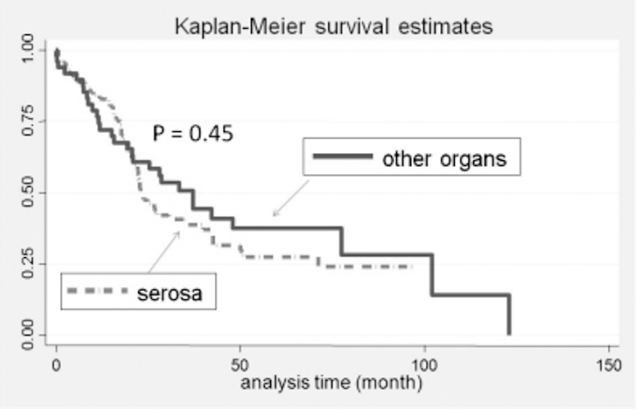
Background: The 7th edition of the AJCC changed its recommendations in January 2010 for stage 4 large colonic carcinomas. The AJCC guidelines recommend classifying tumors invading other organs (previously classified as stage 4a – AJCC 6th edition) as stage 4b. Tumors with serosal involvement (previously classified as stage 4b – AJCC 6th edition) are classified as stage 4a. The AJCC commission based their recommendation on data deposited in the SEER data bank.

Design: One pathologist reviewed all slides for patients diagnosed with "n"pT4 colon cancer at Toronto General Hospital between1999-2008. Clinical data was retrieved from electronic patient record and patients' charts. We grouped tumors by serosal involvement or invasion of other organs. We also grouped tumors by histology type, tumor grade, location and lymph node involvement. The study end points were overall and disease-free survivals. We analyzed differences using Kaplan-Meier survival log rank test.

Results: The study included 140 patients (90 with serosa involvement, and 50 with tumors invading other organs). Overall survival was 22 months for tumors involving the serosa and 24 months for tumors invading other organs, p = 0.45,. Disease free survival was not affected by tumor site (right sided vs left sides), tumor grade, age, or gender.



Conclusions: In this series of operable patients survival is similar between patients with serosal involvement and those with invasion of other organs. Dividing stage T4 into T4a and T4b did not show significant differences in overall survival between the subgroups.

633 Histological Outcome after Treatment in Collagenous Colitis: A Long Term Follow-Up Study of 50 Patients.

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Background: The diagnostic features of collagenous colitis (CC) are well established. However, the long term histologic outcome of CC, and its relationship to clinical response after therapy, remains unknown. The aim of this study was to evaluate histological changes in pre- and post-treatment biopsies in a cohort of CC patients with long term follow up to determine features that may correlate with histologic and/or clinical response.

Design: 404 baseline & 454 post-treatment colon biopsies from 50 patients with CC (M/F;6/44; mean age; 55 yr), all of whom had at least one post-treatment colonoscopy with biopsies obtained ≥2 yrs after initial diagnosis (mean follow up =96 mths; range 25-216 mths), were evaluated in a blinded manner for mean and max. subepithelial collagen thickness, mean and max. intraepithelial lymphocytes (IEL), degree and extent of lamina propria (LP) inflammation and eosinophilic infiltration. Mean number of biopsies obtained at pre- (8.1) and post-treatment (9.1) colonoscopy were similar (p=0.30). Presenting symptoms, colonoscopic findings, treatment details, and response to therapy were recorded by medical chart review.

Results: 10/50 (20%) patients showed complete resolution of both collagen thickening and LP inflammation after treatment. An additional 14 patients (28%) showed partial improvement in collagen thickness (7 with improved LP inflammation and 7 without). LP inflammation improved in an additional 7 (14%) patients without any resolution of collagen thickness. A significant reduction in max. collagen thickness (p=0.05), and both mean (p=0.007) and max. (p=0.002) IEL count was noted after treatment.

Clinical response following therapy was significantly associated with resolution of LP inflammation (p=0.001) and eosinophilic infiltration (p=0.008). Max. collagen thickness after treatment increased by 0.17 μm in patients with no clinical response (n=8), decreased by 5.2 μm in patients with partial response (n=20), and decreased by 14.0 μm in patients with complete response (n=16) but this difference did not reach statistical significance (p>0.05). Use of steroids did not correlate with clinical response or histologic resolution (p=0.48).

Conclusions: Subepithelial collagen thickening in CC is reversible, and a subset (20%) of patients show complete histologic resolution after treatment. Resolution of LP inflammation, but not collagen thickness, is significantly associated with clinical response.

634 Barrett's Esophagus Associated Stem Cells.

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Background: Barrett's esophagus is a squamous to columnar metaplasia that develops as a result of chronic gastroesophageal reflux disease. Barrett's metaplasia is a preneoplastic condition for esophageal adenocarcinoma. A current prevailing hypothesis suggests that cancer is a disease of stem cells or "tumor initiating cells". It would follow that precancerous lesions such as Barrett's esophagus is also a disease of stem cells. Currently, it is unknown where in the normal esophagus the stem cells are located. Popular theory suggests that the stem cells are either within the submucosal glands or the basal cells of the squamous epithelium. Another possibility is that the stem cells are derived from the bone marrow.

Design: In this study, we attempted to locate the stem cells in normal esophagus (n=8), Barrett's esophagus (n=12), Barrett's esophagus with dysplasia (n=4) and esophageal adenocarcinoma (n=24). We used stem cell markers Lgr5, ALDH1, and CD166 for immunohistochemical detection of stem cells in the specimens.

Results: In the normal esophageal mucosa, ALDH1 staining revealed stem cells in the submucosal glands and ducts opening to the lumen of the esophagus. In Barrett's esophagus with or without dysplasia, Lgr5, ALDH1, and CD166 stains revealed stem cells in the base of the crypts of the metaplastic glands and also in submucosal glands. In adenocarcinomas associated with Barrett's, all three markers were expressed in a focal manner, preferentially at the invasive fronts of tumors.

Conclusions: Based on our findings, we believe that the progenitor cells for the Barrett's esophagus are located in the ducts of submucosal glands. Metaplastic glands of Barrett's esophagus harbor stem cells. Gastrointestinal cancers associated with Barrett's do contain stem cells demonstrated by the markers ALDH1, Lgr5, and CD166. Stem cells can be targeted for novel therapies for the prevention of cancers associated with Barrett's.

635 HER2 Scoring in Gastric and Gastroesophageal Junction Adenocarcinoma: Validation of Immunohistochemistry and Silver In Situ Hybridization (SISH).

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Background: HER2 amplification has been shown to occur in a subset of gastric and gastroesophageal junction (GEJ) adenocarcinoma. A recent phase III prospective randomized multicenter trial, ToGA (Bang *et al,* 2010, Lancet 376(9742):659), has demonstrated the efficacy of anti-HER2 therapy with Trastuzumab in advanced gastric adenocarcinoma, in patients with tumour HER2 overexpression by immunohistochemistry (IHC) and amplification by fluorescence in situ hybridization (FISH). As a result, reporting of HER2 status will be clinically indicated in the complete assessment of gastric cancer. IHC and SISH assessment of HER2 status is established in breast cancer, with SISH offering some technical advantages compared to FISH. However, due to differences in scoring criteria from breast cancer, validation of this technique and scoring system is important prior to implementation of routine HER2 IHC and SISH testing in gastric and GEJ cancer.

Design: Consecutive gastric and GEJ formalin-fixed paraffin-embedded biopsy specimens with primary adenocarcinoma were identified from 2009-2010 for this validation study. (Biopsy cases from 1999-2008 have also been identified for inclusion in this ongoing study.) HER2 status was determined by IHC and SISH (Ventana Kits) for all cases with adequate tissue available for analysis, using the HER2 scoring guidelines as applied in the ToGA study.

Results: A total of 51 cases were identified for which 47 had adequate tissue available for analysis (47% intestinal type, 21% diffuse type and 32% mixed morphology). There were 5 (10.6%) SISH positive HER2 amplified cases, 4 of which were scored as IHC positive (3+, 80%) all having intestinal type morphology. One SISH positive case scored as IHC equivocal (2+) also showed intestinal type morphology. The remainder of the IHC 2+ cases (11/47) and all IHC negative (0 or 1+) cases (31/47) were non-HER2 amplified. Only 2/47 cases (4.3%) showed polysomy (CEP17 ≥ 3.0).

Conclusions: Definitive positive and negative gastric HER2 IHC scoring shows excellent agreement with SISH (100%, p<0.00001). HER2 SISH is appropriately indicated for further characterization of HER2 IHC equivocal cases. HER2 IHC overexpression and SISH amplification was seen only in tumors with intestinal morphology in this study. Overall, IHC assessment of HER2 status in gastric and GEJ cancer with the use of SISH for further characterization of equivocal cases is a valid approach for clinical application.

636 Methylation Status of *E-Cadherin, p14, DAB-Kinase* and *THBS1* in Non-Specialized Columnar Metaplasia of the Esophagus.

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Background: Non-specialized columnar metaplasia (gastric metaplasia) of the esophagus is considered as a consequence of gastro-esophageal reflux. Histologically, metaplastic gastric mucosa may display any of those changes commonly observed in normal set gastric mucosa, including *Helicobacter pylori* infection. In addition to aging, increased methylation of certain genes has been also described in chronic gastritis and in premalignant stages of gastric carcinoma. The aim of this study was to explore the methylation status of *E-cadherin* (metastasis and invasion), *p14* (cell-cycle regulation), *DAB-kinase* (apoptosis), and *THBS1* (angiogenesis) genes, as well as the *H. pylori* status, in a group of individuals harboring non-specialized columnar metaplasia of the esophagus.

Design: Sixty-eight subjects (33 females and 35 males), with a mean age of 52 years, were included. Distal esophageal mucosa samples obtained by endoscopy and with confirmed histological diagnosis of non-specialized columnar metaplasia (gastric metaplasia) were thoroughly studied, applying the criteria proposed by the Updated Sydney System for Classification and Grading of Gastritis. DNA was also extracted from paraffin blocks and prepared for studying methylation status by the bisulfite modification method, and *H. pylori* cag A+ status, by PCR.

Results: *Helicobacter pylori* cag A+ was demonstrated in the esophageal metaplastic mucosa of 18 individuals; the *H. pylori* cag+ status was significantly associated with hypermethylation of *DAB-kinase* (p=0.002) and *THBS1* (p=0.028) genes. Methylation status of *E-cadherin* and *p14* genes did not show statistically significant differences between *H. pylori* positive and *H. pylori* negative cases.

Conclusions: Aberrant CpG methylation occurs in non-specialized columnar metaplasia of the esophagus and is closely related to *H. pylori* infection. These findings suggest that a subgroup of patients carrying non-specialized columnar metaplasia of the esophagus, could be at a higher risk for developing more severe lesions.

637 Usefulness of Maspin Immunostaining in Distinguishing Adenocarcinoma from Benign Epithelium in Endoscopic Bile Duct Biopsies.

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Background: Small samples, crush artifact, and a propensity for marked inflammatory and reactive changes following stent placement present challenges in the histologic distinction between benign and malignant epithelium in bile duct biopsies. Overexpression of maspin, a serine protease inhibitor, has been demonstrated in pancreaticobiliary malignancies. However, these studies have largely utilized resection and autopsy specimens. The current study aimed to examine the ability of maspin to distinguish benign from malignant epithelium in endoscopic bile duct biopsies.

Design: A total of 134 endoscopic bile duct biopsies collected between 2006 and 2010 were included in this study. These included 45 adenocarcinomas, 30 atypical cases and 59 benign cases. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissues using a monoclonal antibody to maspin. The staining extent, intensity and pattern were assessed by two observers.

Results: Maspin immunoreactivity was observed in cases in all three diagnostic categories, but was more frequently detected in malignant biopsies than in benign cases (P=0.0016). In comparison with benign cases, malignant biopsies also more frequently showed diffuse (P=0.0063) and strong or intermediate (P=0.0344) staining patterns. In addition, positive staining in malignant biopsies tended to be combined nuclear and cytoplasmic, a pattern less commonly seen in benign biopsies, although this difference

was not statistically significant (P=0.0586). The staining characteristics for atypical biopsies closely resembled those for malignant biopsies, which were largely attributed to 20 (67%) of the cases that were subsequently diagnosed with adenocarcinomas during followup.

Table 1. Results of Maspin Immunostaining in Bile Duct Biopsies

		Malignant (n=45)	Atypical (n=30)	Benign (n=59)
Staining Extent (%)	D	44	27	14
	F	53	67	63
	Neg	2	7	24
Staining Intensity (%)	S	22	20	3
	I	38	37	25
	W	38	37	48
Staining Pattern (%)	N/C	80	73	48
	N	18	20	29

D, diffuse; F, focal; Neg, negative; S, strong; I, intermediate; W, weak; N/C, nuclear and cytoplasmic; N, nuclear only

Conclusions: Bile duct adenocarcinomas more frequently show diffuse, strong or intermediate, and combined nuclear/cytoplasmic staining patterns for maspin in comparison with benign biliary epithelium. In combination with other markers, maspin may prove useful in distinguishing benign from malignant bile duct biopsies.

638 The Clinical Significance of Incidental Chronic Colitis in Children: Are NSAIDS To Blame?

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Background: Chronic colitis is a chronic inflammatory disease of the colon pathologically characterized by the presence of crypt architectural distortion and basal plasmacytosis. In children, a diagnosis of chronic colitis raises a differential diagnosis that includes inflammatory bowel disease (IBD), immunodeficiency states, and longstanding infections. Although previous studies have predominantly focused on the acute effects of NSAIDs, none have linked this drug with histologic changes of chronic colitis.

Design: Patients with chronic colitis with biopsies from the terminal ileum and all five major segments of the colon (cecum, ascending, transverse, descending, and rectosigmoid) on whom follow up lacked definitive etiology are included in this study. Active inflammation is graded as mild (active cryptitis), moderate (crypt abscesses), and severe (erosion/ulceration of surface epithelium).

Results: There were 12 males and 7 females (mean age 10.6 years; range 4-17.2 years). All patients presented with abdominal pain. Follow up (median 21.0 months) resulted in no definitive diagnosis. NSAID intake was documented in 14 patients. Inflammation involved the cecum in 16 patients, ascending in 13 patients, transverse in 5 patients, descending in 4 patients and rectosigmoid in 7 patients. Inflammation involved the right colon (cecum and ascending colon) in 11 patients, left colon (descending and rectosigmoid) in 2 patients, and all 5 segments of the colon in a single patient. Crypt architectural distortion was present in all 19 cases. Basal plasmacytosis was present in 15 cases. In the 4 cases that lacked basal plasmacytosis, significant crypt architectural distortion was present. Biopsies from the ileum were normal in all patients. Eight patients showed mild active inflammation, 7 patients showed moderate active inflammation and 4 patients showed severe active inflammation. In these 4 patients, the histologic features were indistinguishable from active IBD. Three of these 4 patients were taking NSAIDs and there was near total normalization of the colonic mucosa after withdrawal of NSAIDs in 2 patients. In the remaining patients (11 patients), there was normalization of colonic mucosa in 8 patients after withdrawal of NSAIDs.

Conclusions: Our data suggest that the majority of these lesions are likely related to NSAID use. It is important for pathologists and clinicians to be aware of "incidental chronic colitis" to avoid raising other possibilities such as IBD. An important characteristic of these patients is the preferential involvement of the right colon and sparing of the terminal ileum.

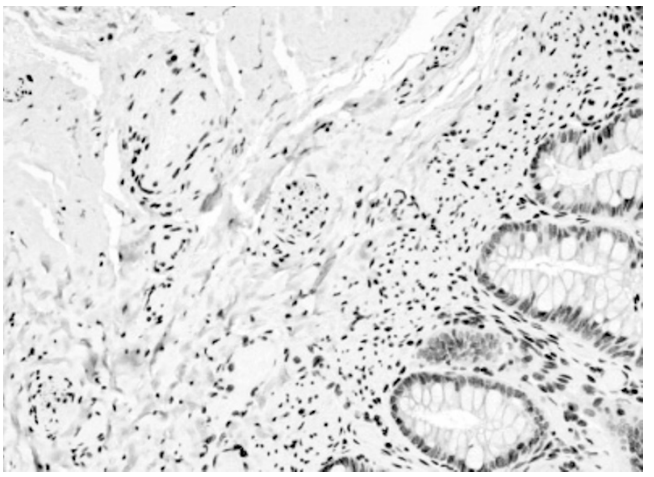
639 Pattern of Calretinin Staining in Hirschsprung Disease; Comparison of Aganglionic Segment to the Transition Zone and Ganglionic Colon.

M Hosseini, AN Husain. University of Chicago, IL.

Background: Hirschsprung disease (HD) is a congenital disorder characterized by absence of ganglion cells involving variable lengths of the colon leading to intestinal dysmotility. It is diagnosed by suction rectal biopsy and is definitively treated by resection. Calretinin immunohistochemical stain is recently described to be useful in its diagnosis. The purpose of this study is to describe the pattern of calretinin staining in nerves and ganglion cells in resected specimens for HD.

Design: A search of archives from 2007-2009, revealed 22 cases of biopsy proven HD with subsequent endorectal pull through. Patients were between 11 days to 17 months old at the time of surgery. Length of aganglionic segment ranged from 3 to 95 cm. Sections representing distal margin, transition zone and proximal margin were identified and slides were immunostained with calretinin.

Results: Distal aganglionic zone lacked any immunostaining except for 3 cases which showed very weak, punctate staining of nerve fibers. Within the transition zone, calretinin staining grew stronger in the nerve fibers in a distal to proximal manner. Darkly staining ganglion cells were present in variable numbers in the transition zone. At the proximal resection margin both ganglion cells and nerve fibers were positive for calretinin. 17 cases showed differential staining of the ganglion cell in the proximal ganglionic zone in that darkly staining ganglion cells were present in the same cluster as non- or weakly staining ganglion cells. Available follow up information in 8 cases indicates that resection has been therapeutic. In the normal ganglionic zone, nerve fibers in 20 cases were strongly positive, 2 cases were weakly positive. In addition mast cells stained strongly with calretinin.



Conclusions: Aganglionic segment in HD can have weak positive calretinin staining of nerve fibers (14% of cases). Based on the limited follow up data, it does not appear that the differential staining of ganglion cells at the proximal margin is an indication of dysfunction. However the relationship of differential staining of ganglion cells at the proximal margin to the function of residual colon needs to be further studied. Positive staining of mast cells with calretinin is a potential diagnostic pitfall.

640 Pancreatic Acinar-Like Adenocarcinoma of the Proximal Stomach Involving the Esophagus in Chinese Patients: A Clinicopathological Study of 41 Cases from a Single High-Volume Hospital in China.

Q Huang, JK Gold, HY Wu, XS Fan, AN Feng, J Shi. Nanjing Drum Tower Hospital, China; VA Boston Healthcare System, West Roxbury, MA; VA Boston Healthcare System, West Roxbury, MS.

Background: We recently reported that some adenocarcinomas of the proximal stomach involving the esophagus (APSE) mimicked pancreatic acinar carcinoma. In the present study, we aimed to systematically investigate clinicopathological characteristics and survival of pancreatic acinar-like adenocarcinoma (PALA) of APSE and to compare these with non-PALA tumors of APSE.

Design: We retrospectively reviewed 137 consecutive APSE resections over the period from May 2004 through October 2009 at a high-volume center in China. PALA tumors were defined by a pancreatic acinar-like morphology with α1-chymotrypsin immunoreactivity. Clinicopathological features of PALA (n=41) and non-PALA (n=96) groups were compared. Differences were tested using the Student's t, Fisher's exact, and Chi-square tests. Survival was compared using the log-rank test.

Results: In the PALA group, the median age was 65 (range: 51-90). The male:female ratio was 5.8. Grossly, tumors were non-encapsulated with a median size of 5.25 cm (range 2-10.5). There were 7%, 10%, 71%, and 12% Borrmann's I-IV tumors, respectively. Frank necrosis and hemorrhage were rare or absent. Lymphovascular invasion (83%) was more common in PALA tumors ($p<0.001$). Microscopically, PALA tumors showed adenocarcinoma (61%), micropapillary (10%), and mixed (29%) patterns. The mixed pattern consisted of neuroendocrine (17%), signet-ring (75%), and adenocarcinoma (100%). No mucinous or adenosquamous differentiation was noted. Nuclei were round-oval with single prominent nucleoli. Mitotic figures were variable. The cytoplasm was dark eosinophilic, granular, and immunoreactive to the α1-chymotrypsin antibody to various degrees. Tumor stroma was non-desmoplastic and fibrovascular. The overall AJCC stage of PALA tumors (pI, 0%; pII, 22%; pIII, 73%; pIV, 5%) was more advanced than that of non-PALA tumors ($p=0.001$), as were pT ($p=0.02$) and pN ($p=0.04$) stages. The median follow-up of survivors was 36 months after surgery. Advanced overall pT stage ($p < 0.001$), pN ($p = 0.005$), pM ($p = 0.01$), and the age >75 ($p = 0.04$) were associated with worse survival. However, the difference in overall survival rates between PALA and non-PALA groups was not statistically significant ($p=0.69$).

Conclusions: PALA of APSE shows distinct clinicopathological features and is associated with advanced pathological stages. PALA should be qualified as a unique type of APSE.

641 Prolapse-Type Changes Can Lead to Overdiagnosis of Sessile Serrated Adenomas of the Rectum: A Histopathologic Study of 75 Rectal Polyps.

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Background: Serrated polyps of the colorectum include hyperplastic polyp (HP), sessile serrated adenoma (SSA) and traditional serrated adenoma (TSA). The differentiation is clinically important as SSA and TSA have higher risk to progress to cancer than HP. We investigated whether architectural distortion due to prolapse-type changes in the rectum could make the distinction between SSA and HP challenging.

Design: 63 cases were identified by searching the archives of an academic and community pathology practice for "sessile serrated adenoma", "hyperplastic polyps with features of sessile serrated adenoma" and "serrated polyp" from January 2006 to July 2010. The cases were reviewed by two GI pathologists and re-classified based on the classically described histologic features of these polyp types. Polyp size was noted.

Results: 63 cases contained one or more polyps for a total of 75 polyps. Initial diagnosis included 21 SSA, 9 HP, 35 HP with features of SSA, 4 tubular adenomas with features of serrated polyp, 3 SP with features of SSA, 2 tubular adenomas arising in the background of sessile serrated adenoma, and 1 tubular adenoma. Polyp size ranged from 2-20 mm. The polyps were reclassified into the following categories: SSA (7), HP (22), HP with features of rectal prolapse (24), serrated polyp favor inflammatory polyps (12), SSA with dysplasia (2), Tubular adenomas (7), and rectal prolapse (1). Many of those cases initially classified as SSA (or having features of SSA) on review were felt to represent HP and/or prolapse change.

Conclusions: Prolapse-type architectural changes such as crypt distortion and exaggerated serrated changes are pitfalls for the overdiagnosis of sessile serrated adenomas of the rectum. When prolapse-type features are identified, cyto-architectural criteria should be strictly applied when making a diagnosis of SSA in the rectum.

642 Geographic Distribution of Eosinophilic Esophagitis in the United States: An Analysis of 6,724 Unique Patients.

JM Hurrell, RM Genta. Caris Life Sciences, Irving, TX; UTSW and Dallas VAMC, TX.

Background: Eosinophilic esophagitis (EoE) is characterized by dysphagia, odynophagia, and marked eosinophilic infiltrates in the esophageal mucosa. The etiology is unknown; leading hypotheses involve antigenic exposure to airborne or ingested antigens. Previous studies have shown geographic variations in the numbers of constitutive eosinophils in the gastrointestinal mucosa. We hypothesized that geographic factors may influence the epidemiology of EoE and designed a study to estimate its prevalence in different U.S. regions.

Design: Demographic, clinical, endoscopic, and histopathologic data from all patients with esophageal biopsies submitted to a national pathology laboratory from 1/2008 through 8/2010 were analyzed and all unique patients with a histopathologic diagnosis of EoE (>15 eosinophils per high-power field) were selected. We then calculated the relative prevalence of EoE within Koppen-Geiger Zones (a widely used climate classification system that categorizes regions based on temperature, precipitation, and aridity) and further analyzed areas of high and low prevalence.

Results: There were 207,496 patients with esophageal biopsies (47% male); 6,724 unique patients had a histopathologic diagnosis of EoE (65% male; OR 2.07; $p<0.0001$). Main indications for endoscopy were dysphagia (55%), odynophagia (8.8%) and GERD (36%). Biopsy specimens were available from upper esophagus (7%); mid-esophagus (72%); lower esophagus (62%). The percentage of patients with EoE varied from 2.08% in Climate Zone A (Tropical) to 3.42% in Zone D (Snow) (OR 1.67; $p<0.001$).The highest prevalence rates (6.7%) were found in the northern Great Basin Desert (UT, ID), whereas the lowest (<1.9%) were in PR, HI, and southern FL (OR 3.39; $p<0.001$).

Conclusions: The wide variations in the prevalence of histopathologic EoE in different regions of the U.S. were inversely correlated with previously reported constitutive eosinophil gradients. Climates with cool summers and abundant snow had significantly higher prevalence than humid tropical regions. Practice patterns, patient populations, and living conditions may affect our findings and need to be explored. However, since the distribution of vegetation is an expression of climate, our results support the possibility that exposure to airborne plant antigens may play a role in the etiology of EoE. Geographic patterns identified in our large patient population may help identify candidate antigens characteristic to high-prevalence areas to be targeted for investigation.

643 Duodenal Adenomas: Clinical, Endoscopic, and Histopathologic Characteristics in 1,132 Patients.

JM Hurrell, RM Genta. Caris Life Sciences, Irving, TX; UT Southwestern and Dallas VA Medical Center, TX.

Background: Duodenal adenomas (DA) are relatively rare lesions, with a reported prevalence of <0.4%. With small numbers available for study in each series, potentially useful associations may escape detection. The purpose of this study was to evaluate the demographic, clinical, endoscopic, and histopathologic characteristics of an unusually large series of adenomatous lesions of the duodenum.

Design: Using an electronic database we extracted demographic, clinical, endoscopic, and histopathologic data from patients who had duodenal biopsies submitted to a national pathology laboratory from 1/2008 to 8/2010. Data from patients with the histopathologic diagnosis of duodenal adenoma were compared to patients without duodenal adenoma. The characteristics of concurrent gastric, small intestinal, and colonic biopsies were also analyzed when available.

Results: There were duodenal biopsies from 148,245 patients with (median age 53 years, 34% male); 1,132 patients (0.8%) had a histopathologic diagnosis of duodenal adenoma (median age 67; 47% male; OR 1.69 1.50-1.90; $p<0.0001$); 24 DAs (2.1%) had high grade dysplasia. There were also 4 duodenal adenocarcinomas, not included in this analysis. Amongst patients with DA, 77 (6.8%%) were identified as having one of the familial polyposis syndromes. The main indications for endoscopy (GERD, pain, and anemia) were not different for patients with and without DA. An endoscopic impression of a polyp or nodule was reported in virtually all patients. Concurrent gastric biopsies were available from 607 DA patients and 106,007 non-DA patients: amongst non-polyposis patients, funding gland polyps were almost 3 times as common in patients with DA than in those without (OR 2.62 95%CI 2.08-3.33; $p<0.0001$). Amongst the 33,355 patients who had a simultaneous colonoscopy, 61.3% of non-polyposis patients with DA had colonic adenomas, in contrast to 29.1% of those without DA (OR 4.34 95%CI 3.23-5.84; $p<0.0001$).

Conclusions: In our series DAs had a higher prevalence (0.8%) than previously reported. They occurred mostly in the 6th and 7th decade, with a distinct male predominance. Virtually all DAs were discovered incidentally during esophagogastroduodenoscopy performed mostly for GERD or dyspepsia. A nodule was seen endoscopically in almost every case. Even in patients with no known polyposis syndromes there was a strong association with gastric fundic gland polyps and colonic adenomas, raising the possibility that some DA patients have unrecognized polyposis syndromes.

644 Iron Deposits in the Gastric Mucosa: Prevalence and Clinicopathologic Associations.

JM Hurrell, SD Melton, RM Genta. Caris Life Sciences, Irving, TX; UT Southwestern and Dallas VA Medical Center, TX.

Background: Iron deposition in the mucosa of the upper gastrointestinal tract is an established complication of oral iron therapy. However, more information about its prevalence and clinicopathologic associations is needed.

Design: We searched the electronic database of a national pathology laboratory to identify patients who had a diagnosis of iron deposition in gastric biopsy specimens between 1/2008 and 8/2010. We then compared their demographic, clinical, endoscopic, and histopathologic data to those of patients who had a diagnosis of reactive gastropathy (RG) with or without erosions, but no evidence of iron deposits, during the same period.

Results: There were gastric biopsies from 326,539 patients (median age 53 years, range 0-101; 38.2% male); 41,317 patients (median age 62 years, range 15-91; 35.1% male) had a diagnosis of RG; 10,083 of these also had erosions or ulcers). Iron deposits were detected by the Prussian Blue stain in 378 patients (median age 66, range 15-91; 39% male). Compared to patients with RG, those with with iron deposition were older (66 *versus* 62 years, $p<0.0001$), were much more likely to have anemia as the main indication for endoscopy (42.8% *versus* 8.8%; OR 6.00 95%CI 4.85-7.43, $p<.0001$), and significantly less likely to present with GERD, dyspepsia, nausea, vomiting, or abdominal pain. Endoscopically, erosions or ulcers were noted in 29% of patients with iron deposition, 19% of RG patients with no histologic erosions, and in 56% of RG patients with histologic erosions. Concurrent *H. pylori* infection was present in 0.03% of patients with RG and in 2.4% of those with iron deposits (OR 9.03 95%CI 4.54-17.95; $p<.0001$). No patients had a concurrent diagnosis of pill esophagitis, but iron deposits were noted in the duodenal mucosa of 12 of 126 patients (9.6%) who had a simultaneous duodenal biopsy.

Conclusions: Iron was identified in 0.1% of all patients with gastric biopsies and in 0.9% of all those with RG. Since only Prussian Blue-confirmed deposits were included, the prevalence of this finding is likely to have been underestimated. A history of anemia was the only significant clinical association detected. In contrast to the notion than oral iron medications induce pain, patients with iron deposits had a much lower prevalence of dyspepsia, nausea, vomiting, or abdominal pain. The clinical significance of this histopathologic finding remains uncertain; studies are needed to determine whether iron is the cause of erosions or simply deposits in existing gastric lesions.

645 Pauci-Eosinophilic Esophagitis: An Under-Recognized Variant of Eosinophilic Esophagitis.

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Background: Symptomatic patients with eosinophilic esophagitis (EE) characteristically present with dysphagia or food impaction. Endoscopically, these patients may show ringed, furrowed or strictured esophagus and tiny white spots involving any part of esophagus but preferentially the mid esophagus. Histologically, markedly increased mucosal eosinophils, >20/hpf, are considered essential for making the diagnosis and differentiating EE from GERD. We describe a variant where the eosinophils are considerably fewer (<10/hpf) than expected, but the endoscopic and other histological features and clinical aspects are similar to classical EE. Failure in recognition of this variant may lead to therapeutic mismanagement.

Design: The endoscopy and clinical database records in our institution were reviewed to select all clinically and endoscopically suspected EE cases from 2002 to 2010 that had biopsy sampling of mid and distal esophagus (total of 208 biopsy events). These included 171 initial biopsies (125 M, 46 F, mean age 23) and post-therapeutic follow-up biopsies in 37/171 patients. Their histological features, endoscopic findings and clinical features were reviewed.

Results: All patients had dysphagia and/or food impaction and many of the following endoscopic findings such as rings, furrows, strictures, minute white spots and preferential mid esophageal involvement. 162/171 (95%) patients with initial biopsies showed histological features of classical EE such as severe basal zone hyperplasia, markedly increased eosinophils (>20/hpf) and focal luminal eosinophilic clusters. 2/171 (1%) patients showed histologically normal squamous mucosa (the endoscopic changes were reinterpreted as spasmodic). 8/171 (4%) patients showed marked basal zone hyperplasia and patchy areas of abrupt keratinization but eosinophils were sparse and maximum of 10/hpf in focal areas. These cases were diagnosed as PEE. Of 37 patients who underwent post-therapeutic endoscopic examination and biopsies, 24/31 (77%) of classical EE and 5/6 (83%) PEE patients showed marked and almost complete histological and endoscopic resolution.

Conclusions: PEE is a variant that has similar clinical presentation and endoscopic findings as EE but lacks the level of eosinophilia that is considered essential for a histological diagnosis of EE. This variant needs recognition since it is unlikely to be histologically diagnosed as EE, but can be clinically managed similar to classical EE. Furthermore, it is important that dilation of a stricture is either avoided or performed cautiously in PEE similar to EE, given the potential risk of fracture.

646 Characterization of HER2 Expression in Primary Gastroesophageal Adenocarcinomas.

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Background: Overexpression of human epidermal growth factor receptor 2 (HER2) protein, a 185 kDa transmembrane tyrosine kinase receptor, has been detected in various solid tumors. Few studies have demonstrated that HER2 may also be overexpressed in

gastroesophageal (GE) adenocarcinomas; however, the clinical significance of HER2 amplification in these tumors remains unclear. Targeted therapy with monoclonal antibody trastuzumab (Herceptin) may potentially be effective in the treatment of HER2 positive GE adenocarcinomas. The aim of our study was to quantify HER2 overexpression in GE adenocarcinomas by both visual and computer software analysis and correlate these findings with histopathologic features of GE adenocarcinomas.
Design: We identified 122 cases of primary gastric and esophageal adenocarcinomas received from 2004-2009. HER2 immunohistochemistry (IHC) was performed on paraffin sections. A visual and computer based analysis (ChromaVision analyzer) was used to quantitate HER2 expression and graded according to Hofmann's proposed HER2 IHC scoring system for gastric cancer. Parameters including TNM staging (AJCC/UICC convention) for resection specimens and histologic grade for resection and biopsy specimens were recorded and correlated with HER2 IHC.
Results: HER2 IHC results are demonstrated in Figure 1. Overall, 12% of GE adenocarcinomas were strongly positive (3+) for HER2, 10% were equivocal (2+), and 78% were weak to negative (18% were 1+ and 60% were 0). To date, 16 cases analyzed via image analysis demonstrated 94% concordance between the IHC score and digital interpretation. Of note, 86% of poorly differentiated (Grade 3) tumors and all tumors stage IIB and above were negative for HER2 IHC.

HER2 Expression in Gastric and Esophageal Tumors by Clinical Stage and Grade

	Esophageal/ GE junction tumors (n=70)	Gastric Tumors (n=52)	HER2 IHC Expression			
			0	1+	2+	3+
Biopsies (n=57)	33	24	28	9	11	9
Resections (n=65)*	37	28	45	13	1	6
Stage						
0	--	1	0	0	0	1
I A	6	6	9	1	0	2
I B	9	6	5	7	1	2
II A	1	7	5	2	0	1
II B	11	1	12	0	0	0
III A	10	1	8	3	0	0
III B	--	4	4	0	0	0
III C	--	1	1	0	0	0
IV	--	1	1	0	0	0
Grade 1†	11	1	7	1	1	3
Grade 2†	15	13	8	11	3	6
Grade 3†	28	35	46	8	4	5

*Staged according to AJCC/UICC TNM, 6th edition; †Total of resection and biopsy specimens, 19 cases did not have an assigned grade

Conclusions: Only a subset of GE adenocarcinomas were found to strongly overexpress HER2. The majority of the poorly differentiated tumors or higher stage tumors appear to be negative for HER2 expression.

647 Biomarkers of Cancer Risk in Ulcerative Colitis (UC) Pouches Post Colectomy: A Pilot Study.

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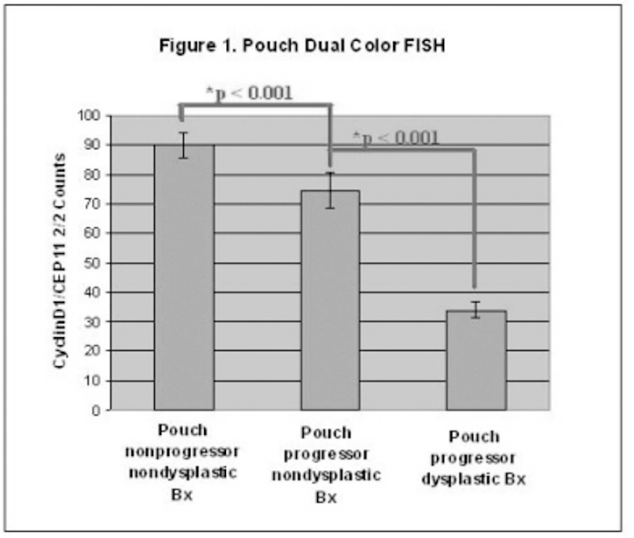
Background: Patients with ulcerative colitis (UC) have an increased lifetime risk of colorectal neoplasia. This risk persists status post proctocolectomy with ileal pouch anal anastomosis (IPAA), but its impact on post colectomy pouch surveillance remains unclear. Currently, it is not possible to reliably distinguish the minority of UC pouch progressors who develop pouch neoplasia from nonprogressors who remain neoplasia free. Genomic biomarkers on native UC rectal biopsies have been reported to distinguish native UC progressors from nonprogressors. Similar better recognition of the pouch patients destined to develop neoplasia is also needed.
Design: Nondysplastic pouch biopsies from 10 randomly selected UC pouch nonprogressors were compared to nondysplastic distant pouch biopsies from 6 pouch progressors with carcinoma (n=4) or high grade dysplasia (n=2) located elsewhere in the pouch. Neoplastic pouch biopsies from two patients were selected as positive controls. Dual color fluorescence in situ hybridization (FISH) for cyclin D1 and its matched centromere (cyclin D1/CEP11) was performed. To enable analysis on paraffin-embedded, fixed archival tissue, methodology was developed using CAM5.2 immunohistochemistry with tyramide chromogen enhancement to isolate epithelial cells for FISH. A total of 50-100 epithelial cells were enumerated for FISH signals in each assay.
Results: Normal 2 arm + 2 centromere FISH signals in 10 UC pouch nonprogressor patients were present in 90% of cells/sample (range 86-96%) [figure 1]. Alternatively, nondysplastic biopsies from 6 pouch progressor patients revealed significantly greater FISH abnormalities, in that the normal FISH counts decreased to an average of 75% (range 68-83%) ($p<0.001$) [figure 1]. Positive control results using actual pouch adenocarcinoma tissues revealed even greater decreases in normal FISH counts to 32% and 36% of cells, respectively ($p<0.001$) [figure 1]. Of note, one of the six pouch progressors did not have native colonic neoplasia, reiterating that native colonic neoplasia status is not a reliable indicator of pouch progression.



Conclusions: This pilot study on UC pouch biopsies provides evidence that FISH alterations on archival paraffin block material holds promise as a cancer risk biomarker in the postcolectomy setting.

648 Loss of CADM1/TSLC1 Expression and Its Clinicopathologic and Prognostic Significance in Colorectal Adenocarcinomas.

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Background: Cell adhesion molecule 1 or tumor suppressor in lung carcinoma 1 (CADM1/TSLC1) is a novel tumor suppressor involving in cell adhesion, proliferation and apoptosis. CADM1/TSLC1 is expressed universally in human tissues and is frequently inactivated in a variety of human carcinomas. However, alterations of expression and clinicopathologic significance in colorectal adenocarcinomas have not been elucidated yet. The aim of this study was to investigate the CADM1/TSLC1 expression and its correlation with various clinicopathological parameters, as well as its effect on patient survival in colorectal adenocarcinomas.
Design: We examined the CADM1/TSLC1 expression in tissue microarrays of 513 colorectal adenocarcinomas by immunohistochemistry. The correlation between CADM1/TSLC1 expression and clinicopathologic characteristics, as well as prognosis, was investigated.
Results: CADM1/TSLC1 was expressed in the cytoplasm of tumor cells. 210 (41%) of 513 colorectal adenocarcinomas showed high CADM1/TSLC1 expression. 185 cases (36%) revealed downregulation of CADM1/TSLC1 expression and 118 cases (23%) showed loss of CADM1/TSLC1 expression. Loss of CADM1/TSLC1 expression was correlated with larger tumor size ($p < 0.001$), mucinous tumor type ($p < 0.001$), lymph node metastasis ($p = 0.020$), higher Dukes stage ($p = 0.038$), and poorer differentiation ($p < 0.001$). In univariate survival analysis, patients with loss of CADM1/TSLC1 expression revealed poorer overall survival and disease-free survival ($p = 0.041$ and $p = 0.036$, respectively, log-rank test). In multivariate survival analysis with the Cox proportional hazards model, CADM1/TSLC1 expression was not an independent prognostic factor of overall survival and disease-free survival ($p = 0.074$ and $p = 0.087$, respectively).
Conclusions: Our results demonstrated that loss or downregulation of CADM1/TSLC1 expression is a relatively frequent event in the development of colorectal adenocarcinomas. We conclude that loss of CADM1/TSLC1 expression may play an important role in cancer progression and aggressive phenotype of colorectal adenocarcinomas and can be a target biomarker for molecular staging or therapeutic approach of patients with colorectal adenocarcinomas.

649 Loss of SIRT1 Histone Deacetylase Expression Correlates with Tumor Progression and Microsatellite Instability Phenotype in Colorectal Adenocarcinoma.

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Background: The class III histone deacetylase SIRT1 is a nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylase that mediate heterochromatin formation. SIRT1 has been reported to serve diverse roles in various biological processes, such as caloric restriction, apoptosis, neuronal protection, cell growth, differentiation, and tumorigenesis. In the view of tumorigenesis, there have been conflicting data supporting whether SIRT1 act as a tumor promoter or as a tumor suppressor.
Design: To elucidate whether SIRT1 is involved in the development and progression of colorectal cancer, we investigated SIRT1 protein expression, determined by immunohistochemistry, in human normal colonic mucosa (n=24), adenoma (n=52), adenocarcinoma (n=497) and metastatic tissue sample (n=126).
Results: All 24 normal colonic mucosa (100%) were positive for SIRT1 with no exception, and 42 (80.8%) of 52 adenomatous polyps were positive for SIRT1 and 10 (19.2%) were negative. However, 208 (41.9%) of 497 colorectal adenocarcinomas were positive for SIRT1 and 289 (58.1%) were negative. Moreover, 45 cases (35.7 %) of 126 metastatic tissue were positive for SIRT1 and 81 cases (64.3%) were negative.

Collectively, the SIRT1 expression was gradually decreased during carcinogenesis and tumor progression. The correlations between SIRT1 expression and clinicopathologic parameters revealed that the loss of SIRT1 expression correlated with proximal tumor location, mucinous histology, and defective mismatch repair protein (MLH1 and MSH2) expression. This result suggests that the loss of SIRT1 expression is associated with microsatellite instability phenotype of colorectal adenocarcinoma. In survival analyses, the loss of SIRT1 expression was significantly correlated with overall survival (p = 0.027, log-rank test) in univariate analysis, but multivariate analysis failed to achieve the significance.

Conclusions: The SIRT1 expression was gradually decreased during normal-adenoma-adenocarcinoma-metastasis sequence, suggesting the possible role of SIRT1 on tumor suppression in colorectum.

650 Slow-Cycling, Treatment-Resistant Cancer Stem Cells of Colorectal Cancer.

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Background: Cancer stem cells are a subpopulation of cancer cells with stem cell properties that are thought to drive tumor growth, resistance to therapy and recurrence. One important property of normal adult stem cells is their quiescent, slow-cycling nature that helps them survive chemotherapy and radiotherapy. This property may also allow cancer stem cells to survive current treatment regimens and consequently leads to recurrence. Our study was designed to isolate slow-cycling cancer cells, identify key regulatory genes and determine cancer stem cell survival after chemo- and radiotherapy.

Design: Colon cancer cell line (HCT116) and primary cancer tissue was labeled with the metabolically activated dye, Carboxyfluorescin succinimidly ester (CFSE), and injected into immunocompromised mice to generate tumor xenografts. After 2-4 weeks of chase period xenograft tissues were harvested and labeled CFSE-positive and negative cells were isolated by fluorescence-activated cell sorting (FACS). RNA was harvested and used for RNA expression analysis. Several genes showed consistent up-regulation in quiescent cancer stem cells among the different tumors. Twenty specimens of colorectal cancer, status post chemo- and radiotherapy, paired with pretreatment initial biopsy were selected from the surgical pathology files of the Dept. of Pathology. Selected blocks with most representative cancer sample and pretreatment biopsy were examined by immunohistochemistry for expression of up-regulated genes p16 and CDK5R1, as well as described stem cell markers, CD44 and CD24. Dworak tumor regression rate was recorded for each post-treatment specimen to determine association of regression with protein expression.

Results: There was an increased population of cancer cells showing expression of p16 and CDK5R1 in residual, post-treatment tumors compared with pretreatment biopsies. No significant changes were observed in expression of purported stem cell surface markers CD44 and CD24. Tumors with low Dworak regression scores tended to have higher expression of p16 in the pretreatment biopsy while tumors with extensive regression showed lower initial staining and increased staining of residual tumor cells.

Conclusions: Expression levels of p16 and CDK5R1 are up-regulated in slow-cycling, CFSE-label retaining cancer stem cells. There is enrichment of p16 and CDK5R1-positive cancer cells after chemo- and radiotherapy suggesting that these genes may enhance the chemo-/radio-resistance of cancer stem cells.

651 IMP3, an Oncofetal Protein, Is a Useful Diagnostic Biomarker for Hepatocellular Carcinoma.

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Background: IMP3, an oncofetal protein, plays an important role in tumor proliferation and invasion. Studies have shown that expression of IMP3 is associated with epithelial dysplasia and invasive cancers. However, little is known about expression of IMP3 in hepatocellular lesions of liver. In this study, we investigated expression of IMP3 in benign and malignant lesions of liver to define usefulness of IMP3 as marker for detection of hepatocellular carcinoma (HCC).

Design: A total of 230 hepatocellular primary and metastatic lesions were retrieved from the surgical pathology files of a large Academic Medical Center. These included resection and biopsy specimens from 7 adenomas, 30 Focal nodular hyperplasia (FNH), 52 cirrhotic livers, 129 HCC and 12 metastatic HCC. All cases were stained with antibody against IMP3 protein.

Results: All benign lesions including cirrhosis (0/52), FNH (0/30) or adenomas (0/7) were negative for IMP3. From the pool of 129 HCC (79/129), 61.24% cases were positive for IMP3. Additionally, 83.3% of metastatic HCC (10/12) were positive for IMP3. In biopsy material, expression of IMP3 was detected in 71.83% (51/71) of HCC cases. However, the sensitivity of IMP3 varied from 52.0% in well-differentiated HCC up to 93.3% in poorly differentiated tumors in biopsy material.

Conclusions: Our data provide important baseline information for IMP3 expression in benign and malignant hepatocellular lesions. IMP3 is a useful biomarker for differentiating benign lesions from HCC in challenging cases. The expression of IMP3 in tumor cells can increase the level of confidence in establishing a definitive malignant diagnosis, especially in biopsy material.

652 Unusual Tumors in Lynch Syndrome Patients: Potential Entities within Tumor Spectrum.

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Background: The well-established group of malignancies commonly associated with Lynch syndrome (LS) includes carcinomas of colorectum, endometrium, renal pelvis, upper gastrointestinal tract, ovaries, as well as tumors of skin and brain. Recently discovered histologic, immunohistochemical (IHC), and molecular signatures of the tumors have significantly facilitated the recognition of the syndrome. These include specific histologic features of the tumors, DNA mismatch repair (MMR) proteins detection by IHC, microsatellite instability (MSI) analysis, and, as the final and the most definitive step, MMR genes germline mutation analysis. Due to progressively increasing availability and precision of the diagnostic tools, more tumor entities are being identified in LS. In this report, we describe 2 rare and 3 not previously reported tumors that occurred in LS patients.

Design: Unusual tumor entities were collected from the pathology and clinical genetics service databases. Clinical information was retrieved from the electronic medical records. Tumor material was evaluated histologically and immunohistochemically.

Results: 5 unusual tumors were identified in LS patients (Table 1). 2 tumors (adrenal cortical carcinoma and lung adenocarcinoma) have been reported in LS patients previously; 3 (pancreatic acinar cell carcinoma, pancreatic endocrine tumor, and mesothelioma), to the best of our knowledge, have not been previously reported.

Table 1

Age	Sex	Germline mutation	Family history (tumor site)	Personal history (tumor site)	Diagnosis	IHC
65	F	MSH6*1312insA	Breast, colon, larynx, brain, pancreas	Breast, skin	Pancreatic acinar cell carcinoma	MSH6 negative
56	M	MLH1*1456insT	Colon	Colon, skin	Peritoneal mesothelioma	MLH1/PMS2 negative
50	F	Not done	Colon, prostate, endometrium	Endometrium, colon	Pancreatic endocrine neoplasm	MSH2/MSH6 negative
29	M	MSH2*1906GC	Colon, breast	Appendix, skin	Adrenal cortical carcinoma	MSH2/MSH6 negative
71	M	MSH2*1906GC	Colon, endometrium	Colon, pancreas	Pulmonary adenocarcinoma	MSH2/MSH6 negative

Conclusions: Our report illustrates the occurrence of rare and previously not reported entities that occurred in LS patients. Given the IHC results being concordant with the genes mutated in these patients, the tumors are likely to be pathogenetically associated with MMR deficiency. Awareness of such occurrences would enhance our ability to identify patients at risk for LS, a genetic disorder that carries profound clinical and familial implications.

653 Clinicopathologic and Outcome Analysis of Serrated Polyps in Inflammatory Bowel Disease.

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Background: Not uncommonly, serrated polyps, which include hyperplastic polyps (HP), sessile serrated polyps (SSP), and traditional serrated adenomas (SA) occur in patients with Inflammatory Bowel Disease (IBD), but the biological significance and neoplastic potential of these polyps in patients with IBD is unknown. The aim of this study was to evaluate the clinicopathologic features and outcome of a cohort of patients with IBD, all of whom had at least one serrated polyp of the colon at index endoscopy.

Design: A search through the pathology files of two major hospitals between the years 1993-2010 detected 16I IBD patients with at least 1 serrated polyp (total # polyps: 188), and these patients formed the study group [ulcerative colitis (UC):109, Crohn's disease (CD):52]. The clinical and pathologic features of the patients and the polyps, and outcome, were evaluated, and also compared between the patient groups.

Results: The IBD patients consisted of 83 males and 78 females of mean age 48 years (range: 8-88 years). Most patients had left (53%) or subtotal colitis (15%), and had inactive (35%) or mildly active (29%) disease. The mean duration of colitis was 18 years (range:1-45 years). Pathologically, of the 188 polyps identified, 182 (97%) were HP (148 microvesicular, 36 goblet cell-type), and 6(3%) were SSP. No SA were identified. 18 patients (11%) had associated neoplastic polyps [sporadic adenoma (12), adenoma-like DALM (6)] at index endoscopy. 4% polyps were identified endoscopically; 60% were identified only by pathologic evaluation of the biopsies. Most polyps occurred in the left colon (63%) and within areas of previous or concurrent colitis. Follow up information was available in 74 patients (mean follow up; 8 years). None of the patients developed adenocarcinoma, adenomas or flat/elevated dysplasia, but 24/72 (33%) of patients developed further polyps (18 HP, 6 inflammatory). There was no association between disease type (UC vs CD), polyp type, or location (within vs outside colitis) and outcome.

Conclusions: Most serrated polyps in IBD represent microvesicular HP and are detected microscopically, not endoscopically. A small percentage are associated with concurrent adenomas or DALMS. Due to the lack of development of neoplasia upon follow up, increased surveillance of these patients is not warranted.

654 P53 Expression and Proliferation Index Measured by Ki-67 Immunostaining in Gastric Pit Dysplasia.

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Background: Despite the wide acceptance of the chronic gastritis-intestinal metaplasia-dysplasia-carcinoma sequence, especially for intestinal type gastric adenocarcinoma, the precise detailed biologic characteristics of gastric pit dysplasia as one of precursor lesions of gastric cancer remains to be delineated.

Design: We have evaluated the surrounding gastric mucosa of 414 gastric cancers for the presence of gastric pit dysplasia (GPD) (dysplasia in pit with surface foveolar maturation). We investigated the p53 expression, Ki-67 immunohistochemistry for proliferation index for the characterization of GPD lesions compared to normal appearing mucosa. For the measurement Ki-67 and p53 expression, we subdivided gastric mucosa into three portions; upper, middle and lower thirds. We evaluated Ki-67 and p53- positive cells by counting the number of positive cells at least at least total number of 200 epithelial cells counted in each portion of gastric mucosa.

Results: We have found 21.0% (n=87) cases of GPD in adjacent gastric mucosa out of 414 gastric adenocarcinomas. p53 expression was evident in lower portion of gastric epithelial cells compared to adjacent normal appearing mucosa. Proliferation index measured by Ki-67 immunostaining was higher in lower third in GPD compared to in middle third in adjacent mucosa.

Ki-67 labelling index and p53 expression in gastric pit dysplasia of 39 cases of gastric adenocarcinoma

	P53 positive cells (%)		Ki-67 positive cells (%	
	Gastric pit dysplasia	Adjacent gastric mucosa	Gastric pit dysplasia	Adjacent gastric mucosa
Upper	0.54±0.10	0.07±0.04	1.26±0.15	1.41±0.17
Middle*	2.05±0.25	0.13±0.07	4.00±0.34	12.85±0.93
Lower*	8.23±1.06	0.20±0.13	14.59±0.92	3.00±0.23

Data means means±standard errors and % of positive cells at least 200 gastric epithelial cells counted. * means p<0.05.

Conclusions: We suggest that gastric pit dysplasia is neoplastic and important candidate precursor of gastric adenocarcinoma and may represent another pathway for the pathogenesis of gastric adenocarcinoma, especially of intestinal type.

655 Peritoneal Elastic Laminal Invasion of Colorectal Cancer: The Diagnostic Utility and Clinicopathological Relationship.

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Background: The distinction of pT3 and pT4a especially in the case which the tumor exhibits wide invasion in the vicinity of the peritoneal surface continues to pose some difficulty for pathologists. Anatomical landmark of peritoneal elastic lamina can provide a clue for making an objective diagnosis.

Design: We performed elastica staining in 564 pT3 and pT4a colorectal cancer cases. Associations between peritoneal elastic laminal invasion of the tumor and clinicopathological features were evaluated. Next, morphology of tumor was compared between cases with and those without peritoneal elastic laminal invasion to estimate morphological alteration that occurs when tumor invades beyond the peritoneal elastic lamina. Next, morphology of tumor was determined and compared between cases with and without peritoneal elastic laminal invasion to estimate morphological alteration that occurs when tumor invades beyond the elastic lamina. Furthermore, morphometric analysis of tumor area beyond the peritoneal elastic lamina was performed and compared with other tumor area to elucidate morphological characteristics of the tumor area beyond the elastic lamina.

Results: Clinicopathological analysis revealed that peritoneal elastic laminal invasion was associated with higher tumor stage, palliative resection, deeper tumor invasion, deeper ulceration, over 5mm of muscular layer elevation and peritoneal surface elevation with fibro-inflammation, higher budding grade, high grade of lymphovascular invasion ($P < 0.01$). Peritoneal elastic laminal invasion associated with recurrence and prognosis in colon cancer and was an independent risk factor for the recurrence of Stage II colon cancer. Furthermore, morphometric analysis revealed that tumor area in subserosal invasive front beyond peritoneal elastic lamina exhibited significantly more prominent fibrosis and tumor budding than other tumor area ($P < 0.01$).

Conclusions: Peritoneal elastic lamina was useful hallmark to determine the level of tumor invasion, and was powerful indicator to predict prognosis in colon cancer. Tumor area beyond the elastic lamina is characterized by extensive tumor budding and fibrosis.

656 A Shift from pStat6 to pStat3 Predominance Is Associated with Inflammatory Bowel Disease-Associated Dysplasia.

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Background: Mucosal inflammatory response in ulcerative colitis(UC) and Crohn's disease(CD) is characterized by Th1, Th2 and Th17 effector cells. Th1 differentiation is dependent on activation of Stat1/4, Th2 on Stat6 and Th17 on Stat3. Activated Stat3 is an important mediator of oncogenesis. This study examined the distribution of activated Stat1(pStat1), Stat 6(pStat6) and Stat3(pStat3) in the continuum of inactive UC and CD to colitis-associated cancer(CAC). Such data may begin to define whether select Stat activation is an early initiator of neoplastic transformation leading to CAC.

Design: Tissue microarrays were constructed with biopsy and colectomy specimens from 67 patients(pts) with UC and CD(1983-2001) and 11 controls without clinical or pathologic diagnosis of colitis or cancer. Analysis of 136 samples included inactive (n=32) and active colitis(n=39), low grade(n=21) and high grade dysplasia(n=19), and CAC(n=25). 2-10 samples were analyzed per patient. Immunohistochemistry(IHC) was used to score pStat1, pStat6 and pStat3 in colonic epithelial(EC) and mucosal immune cells(IC) by two blinded observers(scale 0-3). IHC was correlated with clinical and pathologic data(tumor location, histologic type, grade and node involvement).

Results: pStat3 was more common in UC than CD (34/39 vs 10/18, p<0.02). In chronic colitis without dysplasia, pStat3 was limited to rare IC and EC. Colitis associated dysplasia had intense IC pStat3. In a CAC subset, EC pStat3 was prominent and always in association with intense pStat3 in surrounding IC. IC pStat3 in CAC was greater in low compared to high grade CAC, in lymph node positive compared to lymph node negative cancers, and in patients with underlying UC compared to CD. In contrast, IC pStat6 expression was more common in control patients compared to CAC (8/10 vs. 9/25, p<0.03). pStat1 was detected in only a small subset of pts with UC and CD colitis but not with CAC.

Conclusions: pStat3 is a marker for neoplastic transformation in a subset of pts with UC and CD. A shift from predominant IC pStat6 expression to pStat3 expression may be an early sign of neoplasia. Additional data are needed to define the signals promoting this change and to determine how pStat3 correlates with clinical course and prognosis.

657 Pathological Predictive Factors of Submucosal Invasion in the Pretreatment Biopsies of Early Gastric Carcinoma.

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Background: In an endoscopic resection of early gastric carcinoma (EGC), the presence of submucosal (SM) invasion is one of the most significant risk factors associated with lymph node metastasis and prognosis. Although the diagnostic accuracy rate for SM invasion by endoscopy and ultrasonography has approached to 80%, there are still some cases with SM invasion in the endoscopic resection specimen. Identifying pathologic risk factors to predict SM invasion in the pretreatment biopsy will help selecting cases that are most applicable for endoscopic mucosal/submucosal resection.

Design: Pretreatment biopsies from 60 patients with SM invasive GCs who underwent endoscopic mucosal resection (EMR) between 2002 and 2007 were reviewed. For control, 58 patients with intramucosal GCs during the same period were used. WHO histologic grade, single cell invasion, islands of muscularis mucosa, cribriform pattern, papillary features, desmoplastic reaction, and intraglandular eosinophilic necrotic debris (IEND) were investigated to evaluate pathologic factors associated with SM invasion. Pearson's chi-square test and Fisher's exact test were used for statistical analysis.

Results: The depth of SM invasion was varied from 50 μm to 3000 μm (mean 949 μm). In the biopsy of SM invasive GCs, well to moderately differentiated histology, single cell invasion, islands of muscularis mucosa, cribriform pattern, papillary features, desmoplastic reaction, and IEND were observed in 96.7%, 36.7%, 16.7%, 16.7%, 23.3%, 40%, and 46.7% of cases, respectively, while 100%, 5.2%, 0%, 1.7%, 5.2%, 19%, and 22.4% were observed in the biopsies of intramucosal GCs. WHO histologic grade was not associated with SM invasion. However, single cell invasion, islands of muscularis mucosa, cribriform pattern, papillary features, desmoplastic reaction, and IEND were significantly related to SM invasion in endoscopic mucosal resections ($P < 0.05$). The coexistence of 4 or more of these "SM invasion-associated features" in pretreatment biopsies yielded a 94% sensitivity and 98% specificity in the prediction of SM invasion in the endoscopic mucosal resection specimens.

Conclusions: Our study showed that SM invasion in EGC was not associated with the degree of differentiation. Rather, combined SM invasion-associated features were significant pathologic findings predicting SM invasion and could be used to distinguish SM invasion from intramucosal gastric carcinoma with a high degree of accuracy.

658 Prognosis of Minimally Invasive Adenocarcinoma Arising in Mucinous Cystic Neoplasms of the Pancreas.

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Background: While patients with surgically resected non-invasive mucinous cystic neoplasms (MCN) of the pancreas are cured, the behavior of surgically resected minimally invasive adenocarcinoma arising in MCN is not well-established. We report the follow-up on 11 surgically resected MCN with minimal stromal invasion.

Design: Eleven surgically resected MCN with minimal stromal invasion, defined as unifocal or multifocal microscopic invasive adenocarcinoma into the ovarian stroma without invasion through the capsule into the adjacent pancreatic parenchyma, were identified in the files of our three institutions. Pathological findings were correlated with patient demographics, type of surgery, and long-term follow-up.

Results: Our study included ten females and 1 male ranging in age from 38-66 years (mean 52.6 years). The MCN ranged in size from 3.5-16 cm (mean 9.8 cm) and were all located in the body/tail of the pancreas. Seven cases had unifocal invasion and 4 cases had multifocal invasion. Multifocal invasion was identified in 2 of 4 cases in which the tumors were entirely submitted for histological examination and 2 of 7 cases in which the tumor was not completely submitted. Lymph nodes were negative in all cases. No lymphovascular invasion was present in any cases. The patients were followed for 19-148 months (mean 58.3 months). During the follow-up, only one of the 11 patients had recurrence at 36 months after resection of a MCN with unifocal invasive adenocarcinoma. The recurrent tumor was identified by CT scan and was surgically unresectable. The patient died secondary to complications from the recurrent tumor at 41 months after resection. Upon review, only 5 sections from the tumor, which measured 5.0 cm, were submitted for histological examination in this case; thus, more extensive invasion could not be excluded.

Conclusions: Surgically resected MCN with minimal stromal invasion have an excellent prognosis. None of the completely examined neoplasms with minimal invasion recurred.

The single case in this study which recurred was not completely sampled, possibly leaving areas with more extensive invasion unidentified. Our study demonstrates that the majority of patients with minimally invasive adenocarcinoma arising in MCN are cured by surgery, particularly if the neoplasms are completely examined histologically.

659 Oncofetal Protein IMP3 as a Predictor of Prognosis in Gastric Adenocarcinoma.

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Background: Despite improved long-term survival rates after surgery in association with adjuvant and neo-adjuvant radiation/chemotherapy, gastric adenocarcinoma (GACA) remains a major cause of cancer death worldwide. The aggressiveness of GACA is often difficult to predict based on the pTNM stage alone. One approach to improve stratification of patients for adjuvant and neoadjuvant therapy is to utilize biomarkers that can predict disease outcome. IMP3, an oncofetal protein, is a member of the insulin-like growth factor mRNA binding protein (IMP) family that plays an important role in RNA trafficking and stabilization, cell growth and migration in early embryogenesis. Aberrant overexpression of IMP3 has been associated with malignant transformation and tumor progression in several tumor types. The goal of this study was to evaluate the significance of IMP3 expression for prognosis of GACA.

Design: The study group consisted of 61 resected GACAs, including 32 of diffuse type (mean age 63.9; range 38-82; M:F=15:17) and 29 of intestinal type (mean age 71.1; range 44-92; M:F=20:9). Cytoplasmic IMP3 expression was evaluated immunohistochemically using monoclonal anti-IMP3 antibody (Dako). Immunoreactivity was scored as negative; minimal (staining of 1-10% of tumor cells); moderate (staining of 10-50% of tumor cells) and strong (staining of more than 50% of cells). Overall survival was analyzed using Kaplan-Meier method. The Cox proportional hazards model was used for multivariate analysis.

Results: The results demonstrated a striking difference between tumor types with a significant correlation between survival and IMP3 expression in patients with diffuse GACA. Median overall survival was 22.6 months, (95% CI: 9.55-137.0) in patients with absent/minimal IMP3 staining versus 8.5 months (95% CI: 3.17-14) in patients with moderate/strong IMP3 staining (p=0.006, log-rank test). In multivariate analysis, correcting for the effects of age, sex and pathologic stage, only pathologic stage significantly affected survival (p=0.002). No correlation was observed between survival and IMP3 expression in patients with intestinal GACA. IMP3 staining was negative in 12 normal gastric mucosae serving as control.

Conclusions: Our results suggest that IMP3 may play an important role in refining the progressive potential of GACA and may serve as a helpful prognostic biomarker in evaluating the clinical behavior of diffuse GACA.

660 MGMT (O6-MethylguanineDNA Methyltransferase) Is Inactivated in a Significant Subset of Distal Esophageal Adenocarcinomas: Implications for Neoadjuvant Chemoradiation Therapy.

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Background: Epigenetic silencing of DNA-repair gene MGMT by promoter methylation compromises DNA repair and sensitizes tumors to alkylating chemotherapeutic agents. Temozolomide is an alkylating agent shown to be effective in MGMT-methylated pancreatic endocrine tumors and glioblastomas. Loss of MGMT expression is a surrogate marker of MGMT promoter methylation. The goal of this study was to ascertain the prevalence of MGMT loss in distal esophageal adenocarcinoma (EA) using immunohistochemistry, and to determine whether the immunohistochemical findings correlate with MGMT promoter methylation status.

Design: The study group consisted of 62 patients (mean age 62.4 yrs, M:F = 53:9), all of whom were treated with neoadjuvant platinum-based chemoradiation regimen followed by esophagectomy. MGMT expression was evaluated immunohistochemically in the pretreatment biopsies using monoclonal anti-MGMT antibody (Novus Biologicals). Nuclear staining was graded semi-quantitatively for extent (focal <25%; diffuse >25%) and intensity (weak, moderate, strong). Cases were then scored as 0 (negative), 1 (focal weak), 2 (focal moderate-strong), 3 (diffuse but weak-moderate), and 4 (diffuse strong). MGMT promoter methylation was analyzed using nested methylation-specific PCR. Immunohistochemical findings were correlated with MGMT promoter methylation status and with post-treatment T and N stage in esophagectomy specimens.

Results: Immunohistochemistry performed on pre-treatment biopsies showed complete (score 0) or near complete (score 1) loss of MGMT staining in 14 and 3 cases, respectively (17/62; 27%). Score 2 was present in 3 (5%) cases, score 3 in 12 (19%) cases, and score 4 in 30 (49%) cases. DNA from 8/17 biopsies with loss of MGMT staining was subjected to methylation specific PCR and all 8 (100%) showed promoter methylation. In contrast, 4 of 6 (67%) cases with strong MGMT expression (score 4) showed no methylation, while 2 of 6 (33%) were methylated (p=0.015). Loss of MGMT staining did not correlate with post-treatment ypT and ypN stage in esophagectomy specimens.

Conclusions: A significant subset (27%) of distal esophageal adenocarcinomas shows complete or near complete loss of MGMT protein expression, which correlates with promoter methylation. Immunohistochemical assessment of MGMT may be helpful in selecting patients for future trials to assess the value of alkylating agents in neoadjuvant therapy of EA.

661 Dietary Zinc Inhibits *N*-Nitrosomethylbenzylamine-Induced Esophageal Carcinogenesis in Wild-Type and Tumor Suppressor-Deficient Mouse Strains.

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Background: Zinc (Zn) deficiency in humans is associated with increased risk of developing esophageal cancer, and leads to carcinoma in the upper gastrointestinal tract in laboratory rodents. Previous studies have focused on effect of Zn replenishment in deficient rodents. In this study, we investigated the effect of Zn supplementation on carcinogenesis in Zn-sufficient mice.

Design: Wild type C57BL/6 (B6), *Fhit-/-*, *Fhit-/-Nit1-/-* and *Fhit-/-Rassf1a-/-* mice were used. All mice received *N*-nitrosomethylbenzylamine (NMBA) and half of each then received Zn supplementation. 14-16 weeks final NMBA administration, mice were sacrificed and tumors in forestomach analyzed.

Results: The number of mice with tumors was significantly reduced in groups receiving Zn supplementation (Table 1). Histological analysis also showed significant decreases in severity of preneoplastic and neoplastic lesions in Zn-supplemented cohorts (Table 2).

Table 1. Effect of Zn supplementation on NMBA-induced tumor development in four mouse strains

Genotype	Treatment	# mice	Tumors/mouse (mean ± SE)	# tumors of diameter/mouse		
				≤0.5 mm	~1 mm	≥2 mm
B6	No Zn	24	7.0 ± 1.2	4.1	2.0	0.9
	Zn	29	5.0 ± 0.7*	3.2	1.0	0.8
Fhit-/-	No Zn	34	8.0 ± 0.6	5.4	1.6	1.0
	Zn	29	5.7 ± 1.0*	3.9	1.2	0.6
Fhit-/-Nit1-/-	No Zn	24	9.2 ± 1.6	4.5	3.2	1.5
	Zn	26	5.3 ± 0.8*	3.2	1.3	0.9
Fhit-/-Rassf1a-/-	No Zn	26	9.1 ± 1.5	5.3	2.6	1.2
	Zn	27	5.9 ± 0.5*	3.4	1.5	0.9
* Zn vs no Zn, p value <= 0.01						

Table 2. Histopathological analysis of forestomach lesions

Genotype	Treatment	# Mice	Normal	Mild hyperplasia	Severe hyperplasia	hyperplasia w. dysplasia	Carcinoma
B6*	no Zn	24	0	1	19	11	4
	Zn	29	3	17	8	6	0
Fhit-/-Nit1-/-*	no Zn	24	0	2	16	8	4
	Zn	26	2	6	17	3	0
Chi square test (Zn vs no Zn): p=0.003 in B6 and p=0.002 in Fhit-/-Nit1-/- mice.							

Conclusions: Zn supplementation significantly reduced tumor burdens in mice. When Zn supplementation was begun at 7 weeks after the final carcinogen dose, the reduction in tumor burden was the same as observed when supplementation began immediately after carcinogen dosing, suggesting that Zn supplementation may affect tumor progression rather than initiation, and supporting the hypothesis that Zn has a role in tumor prevention that should be more thoroughly examined and defined in preclinical models.

662 Epithelial Changes Indefinite for Dysplasia in the Pouch and Peri-Pouch but Not Rectal Cuff Is Associated with Inflammation and Warrants Close Follow-Up.

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Background: In idiopathic inflammatory bowel disease (IBD) patients with pouch, surveillance biopsies are regularly obtained from neoterminal ileum (TI), pouch, and rectal cuff and dysplasia classified according to the established criteria for IBD into negative (NEG), epithelial changes indefinite for dysplasia (IND), low-grade dysplasia (LGD), and high-grade dysplasia (HGD). However, as this region consists of three biologically different structures [small bowel, colonized small bowel (pouch), and rectal mucosa], the assessment of dysplasia poses unique challenges. This study was undertaken to compare histologic features in IND pouch surveillance biopsies with NEG biopsies and to determine the interobserver variability in diagnosing IND and its-associated histologic features.

Design: Pouch surveillance biopsies from 18 patients with IND from 2006 to 2010 were reviewed in a blinded fashion by two GI pathologists. Features assessed included neutrophils (0-3), ulceration (0-3), villous blunting (0-3, in TI and pouch), crypt distortion (0-3), mononuclear inflammation (0-3), and eosinophils (0-3). These features were compared between IND and NEG TI/pouch biopsies as well as IND and NEG rectal cuff biopsies.

Results: 25 biopsies originally classified as IND and 47 NEG biopsies were reviewed. One case (4%) was reclassified as LGD, 6 cases (24%) as NEG, one NEG (2.1%) as IND, and one IND could not be agreed upon by the reviewing pathologists. Compared to NEG TI and pouch biopsies (N=38), IND in the TI and pouch (N=9) was associated with neutrophilic inflammation, crypt distortion, mononuclear inflammation, and eosinophilic infiltrate (p=0.01, 0.04, 0.01, 0.04, respectively), and showed a borderline association with ulceration and villous blunting (p=0.06 and 0.05, respectively). In contrast, IND in the rectal cuff (N=7) was not associated with any of these features, when compared to NEG rectal cuff biopsies (N=8). The kappa interobserver agreement was excellent for dysplasia (0.83) and moderate to good for neutrophlic infiltrate (0.67) and crypt distortion (0.43). Follow-up biopsies showed that 1/16 cases with IND progressed to HGD.

Conclusions: IND in the pouch and peri-pouch region presents as a diagnostic challenge, but can be reliably interpreted in majority of the cases. Inflammation and crypt distortion are significantly associated with IND in the TI and pouch biopsies. A larger, long-term follow-up study is required to confirm our current findings.

663 Duodenal Intraepithelial Lymphocytosis in *Helicobacter pylori* Gastritis: A Comparison after Treatment.

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Background: Duodenal intraepithelial lymphocytosis is present in, and indeed necessary for the diagnosis of, many inflammatory and autoimmune conditions, including celiac sprue, Crohn's disease, and post-infectious duodenitis. The isolated presence of increased lymphocytes within the duodenal epithelium without villus blunting, neutrophilic inflammation, or chronic architectural distortion has been noted in patients with *Helicobacter pylori* gastritis. Our aim was to confirm this assertion and compare the degree of lymphocytosis in the duodenal biopsies of patients with active *H. pylori* gastritis, treated/resolved *H. pylori* gastritis, and no *H. pylori* gastritis.

Design: We conducted a retrospective review in our institute to identify duodenal and gastric biopsies obtained between 2006 and 2010. Biopsies from patients with a history of celiac sprue, inflammatory bowel disease, or autoimmune gastritis were excluded. Each duodenal biopsy was reviewed and the intraepithelial lymphocytes were counted in five consecutive villi within the area of the most prominent lymphocytosis. The concurrent gastric biopsy and Genta stain were then evaluated for the presence of *H. pylori* organisms. Biopsies negative for *H. pylori* in the setting of a prior positive *H. pylori* antibody test (IgG by EIA) were considered to be successfully treated.

Results: A total of 108 paired duodenal/gastric biopsies from 108 patients were identified, including 50 biopsies histologically positive for *H. pylori* and 58 biopsies without histologic evidence of *H. pylori*. Eight of the biopsies found to be histologically negative were previously treated following a positive *H. pylori* antibody test (IgG by EIA). The average number of intraepithelial lymphocytes identified in duodenal biopsies with concurrent *H. pylori* gastritis was 25.3 (SD 16.1, SEM 2.3) lymphocytes per villus. Duodenal biopsies taken from patients without *H. pylori* gastritis had an average of 11.3 (SD 7.6, SEM 1.1) lymphocytes per villus. Patients who had laboratory evidence of *H. pylori* subsequently eradicated had an average of 7.9 lymphocytes per villus (SD 3.3, SEM 1.2).

Conclusions: This study confirms previous reports of increased duodenal intraepithelial lymphocytes in patients with concurrent *H. pylori* gastritis($p<0.0001$), an important differential to be considered with this finding. Additionally, our results show that the number of intraepithelial lymphocytes per villus decreases, approximating the number seen in *H. pylori* naive biopsies, following antibiotic therapy and eradication of *H. pylori* ($p=0.0039$).

664 Caveat Emptor: CD10 Is Not a Reliable Marker of Inflamed Small Intestine.

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Background: Ileal pouch-anal anastomosis (IPAA) is a surgical option in patients who undergo total proctocolectomy, often in the setting of ulcerative colitis (UC). IPAA patients are followed clinically for pouchitis as well as for residual UC in the remnant rectum. Distinction between the two is important; pouchitis is treated with antibiotics, while rectal UC may need more aggressive treatment and can develop colitis-associated dysplasia. Crohn's ileitis (CI) may enter the differential diagnosis in patients with severe pouchitis. Inflammatory changes in the small intestinal (SI) mucosa can make it difficult to identify mucosa as being ileal or colonic. CD10 is a cell surface metalloproteinase expressed by small intestinal brush border. Its role in the evaluation of biopsies in IPAA patients has only been rarely examined. This study aims to determine the utility of CD10 immunohistochemistry (IHC) in identifying SI mucosa in the setting of inflammation.

Design: CD10 expression was determined by IHC (clone 56C6; Ventana, Tucson, AZ) on formalin-fixed, paraffin-embedded tissue from surgical resections and endoscopic biopsies in a variety of clinical scenarios. CD10 was scored as positive (complete luminal staining), negative, or patchy, and percent of the epithelium with loss of staining was recorded. The presence or absence of active inflammation was noted.

Results: All (35) colonic specimens were negative for CD10. 27/68 (40%) SI specimens had patchy staining. 6/6 normal SI and 3/3 normal ileocecal valve had uniformly positive CD10 staining. 1/12 (8%) ileostomy (without CI), 1/6 (17%) enteroenteric anastomosis (EEA; without CI), 3/7 (43%) ileocolonic anastomosis (ICA; without CI or UC), 7/16 (44%) ileal pouch, 6/8 (75%) backwash ileitis (BWI), and 9/10 (90%) CI cases had patchy staining. Percentage of CD10 loss varied from 20-90% in BWI (mean=55%; all with active inflammation), 10-50% in pouches (mean=36%; all with active inflammation), and 10-80% in CI (mean 32%; all with active inflammation). In the ileostomy with patchy staining, there was <10% loss and active inflammation. The 3 ICA all had <10% loss and 1/3 had active inflammation. The EEA had 10% loss and active inflammation. Loss of staining was accentuated in intact epithelium surrounding ulcers.

Conclusions: While CD10 is a reliable marker of SI mucosa in the absence of inflammation, caution must be exercised when interpreting CD10 IHC in inflamed mucosa, especially when biopsy material is limited. SI mucosa seems particularly susceptible to CD10 loss in the microenvironment of BWI, suggesting that the mucosa in this setting may be undergoing phenotypic alteration.

665 Extensive Characterization of EGFR Pathways May Help in Integrating the Use of EGFR-Targeted Therapies in Patients with Squamous Cell Anal Cancer.

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Background: Chemo-radiation is the standard treatment for local squamous cell anal cancer (SCAC). In locally advanced head and neck squamous cell carcinomas, monoclonal antibodies (MoAbs) targeting EGFR have proven to improve survival in combination with radiotherapy. In colorectal cancer, patients benefit from anti-EGFR MoAbs in presence of EGFR gene copy number gain (CNG) and absence of mutations in EGFR downstream members (except for PIK3CA exon 9 mutations).

Design: We described alterations of EGFR pathway in SCAC biopsies, to evaluate whether anti-EGFR MoAbs can be integrated in the management of this condition. Thirty-five SCAC biopsies were collected in the Departments of Pathology in Locarno, Legnano and Novara. EGFR gene status was assessed by fluorescent in-situ hybridization, whilst K-Ras, BRAF and PIK3CA mutations by direct sequencing.

Results: EGFR CNG was observed in 5 cases (20%). No BRAF mutations were detected. K-Ras was mutated in 1 case (3%, G12V change), PIK3CA in 8 cases (23%, 6 changes in exon 9 and 2 in exon 20). EGFR CNG was concomitant either to K-Ras or to PIK3CA exon 9 mutations (in 1 case each). The 3 remaining EGFR CNG cases did not show any other EGFR downstream signal alterations. Overall 4 out of 36 SCAC patients (11%) might have a proficient pattern for anti-EGFR MoAbs treatment

Conclusions: EGFR gene deregulation, K-Ras and PIK3CA mutations are involved in SCAC carcinogenesis. Our results suggest a possible role of EGFR-targeted agents in integrated treatment of SCAC, and clinical trials involving anti-EGFR MoAbs in this disease should consider an early analysis of these factors to identify patients who might benefit from these drugs.

666 Loss of Short Chain Fatty Acid Receptor GPR43: A Common Molecular Event in Colorectal Carcinoma.

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Background: GPR43 is a G protein-coupled, short chain fatty acid (SCFA) receptor that activates signal transduction pathways and cellular responses. SCFAs affect colonocyte transport and metabolism, growth and differentiation, hepatic control of lipids and carbohydrates, and provide energy to colonic epithelium, muscle, kidney, heart and brain. The role of SCFAs, particularly butyrate, in colon cancer therapy has been extensively studied, however the role of its receptor GPR43 is unknown. Recently, we found GRP43 frequently loses expression in colonic carcinoma, probably as a tumor suppressor. Here, we analyzed GPR43 expression in cultured human colonic adenocarcinoma and normal cell lines and colorectal resections in order to investigate its functional role in response to SCFAs in regulating tumor cell growth with or without GRP43.

Design: GRP43 expression was determined using 11 cultured cell lines; 10 human colonic adenocarcinomas and 1 normal human colonic epithelial cell line NCM460 using real-time PCR, Western blot and immunohistochemical (IHC) approaches. Colon tissue microarrays (TMA) were created for IHC use and included 15 cases from colonic resection specimens with normal, primary and metastatic colonic adenocarcinoma from each patient, 5 tubular adenomas (TA), and 5 adenocarcinomas arising in TA. The staining intensity was classified as no staining, low intensity staining, or high intensity staining. All tumor cell lines were studied for their response to butyrate using an MTT assay.

Results: High intensity expression of GRP43 in NCM460 cells was determined at both mRNA and protein levels, however only 1 cancer cell line (LS180) of 10 showed GRP43 expression in western blot assays and only the HT29 cell line displayed GRP43 expression using real-time PCR. Decreased response to butyrate-induced inhibition of cell proliferation and differentiation correlated with GRP43 expression in cells. Parallel IHC study on TMAs showed high intensity expression in normal colonic epithelium (n=15) and 65% (13/20) of adenocarcinomas exhibited loss of expression ($p<0.01$), with moderately/poorly differentiated carcinomas showing no expression (n=13). All TAs (n=5) demonstrated identical levels of expression as in normal mucosa.

Conclusions: This study demonstrates that loss of GPR43 expression is a common molecular event in less differentiated colonic adenocarcinomas and cultured cell lines, indicating it may serve as a useful malignant biomarker. Loss of expression is correlated with response to SCFA treatment, implying regulation of GRP43 can be a potential target for SCFA therapy.

667 Gain of Expression of Aldo-Ketoreductase Family 1 B10 (AKR1B10) Protein in Pancreatic Adenocarcinoma: A Potential Therapeutic Target.

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Background: Aldo-keto reductase family 1 B10 (AKR1B10) protein acts as an enzyme capable of reducing aliphatic aldehydes and ketones; it detoxifies reactive free radical carbonyl compounds and is involved in retinoid metabolism, thus modulating cell proliferation, differentiation and tumorigenesis. Expression of AKR1B10 protein is normally expressed in the intestine and over-expressed in liver and lung carcinomas. Several studies have identified upregulation of AKR1B10 in the normal respiratory epithelium of smokers, suggesting cigarette smoking as an inciting event. The development of pancreatic cancer is firmly linked to cigarette smoking, however the

role of AKR1B10 in pancreatic ductal adenocarcinoma is not known. Here, we studied the expression of AKR1B10 in pancreatic epithelium and showed increased expression in pancreatic ductal adenocarcinomas.
Design: Normal and tumor pancreatic tissue microarrays were created for immunohistochemistry use and included 36 cases of pancreatic ductal adenocarcinoma and adjacent non-neoplastic pancreatic tissue from Whipple resection specimens. Immunohistochemistry was performed using a monoclonal mouse AKR1B10 antibody and the avidin-biotin-peroxidase approach. The staining intensity was classified as no staining or increased expression.
Results: AKR1B10 was expressed as cytoplasmic staining in pancreatic ductal adenocarcinoma compared to adjacent non-neoplastic pancreatic tissue. Increased expression of AKR1B10 was identified in 64% (n=23) of pancreatic ductal adenocarcinomas. The majority of the tumors expressing AKR1B10 were well and moderately differentiated (65 and 30%, respectively). In addition, 70% (n=16) of the tumors expressing AKR1B10 were identified to be from patients with a smoking history.
Conclusions: The over-expression of AKR1B10 in well and moderately differentiated pancreatic adenocarcinomas demonstrates that AKR1B10 may contribute to disease development via effects of smoking by altering retinoid homeostasis with dysregulated apoptosis and cellular proliferation, and should be considered as a candidate for further therapeutic investigations.

668 Gastrin-Releasing Peptide Receptor Pathway Immunoexpression Profile in Pancreatic Endocrine Tumors.

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Background: GRPR is known to be constitutively expressed in pancreatic islet cells, though its role in pancreatic endocrine tumors (PET) has not been studied. We herein evaluate a series of PET by immunohistochemistry (IHC) for GRPR, its downstream target pSTAT3, and also assess potential crosstalk with EGFR via TGF-a transactivation.
Design: A tissue microarray was constructed from 45 paraffin embedded tumors from 40 patient (cored in triplicate). Sections were then immunostained for GRPR, EGFR, pSTAT3, and TGF-a, as well as previously reported prognostic markers, CK19, and Ki-67. Ki-67 was scored as a percentage, while other markers were assigned an H-score (Product of staining intensity [0-3] and percentage. Range: 0-300). Results were correlated with various clinicopathologic parameters. For subgroup comparison, Ki-67 was dichotomized to <2% and ≥2%, while other markers were dichotomized based on 50th percentile of staining distribution.
Results: Table 1 summarizes correlations (Kaplan Meier method, log rank comparison). A Ki-67 ≥ 2% correlated with worse PFS and OS. While GRPR, pSTAT3, and TGF-a were frequently expressed in PET, there were no significant correlations with outcomes. Tumors with low GRPR did have a slight tendency to behave more aggressively. EGFR staining was not noted in any of the tumors. CK19 also showed no correlation with outcome.

		N	5-year PFS (%)	p value	5-year OS (%)	p value
Tumor Extent	limited to pancreas	23	87	0.049	95	0.043
	peripancreatic invasion	6	50		67	
Necrosis	no	8	100	0.008	100	0.04
	yes	1	0		0	
Metastatic	no	26	96	<0.001	96	0.038
	yes	6	*		67	
T stage	1	16	100	0.018		
	2	10	70			
	3	6	50			
GRPR	<#	9	78	0.080	89	0.229
	≥#	13	100		100	
pSTAT3	<#	12	74	0.176	91	0.913
	≥#	15	93		93	
TGF-a	<#	13	85	0.984	92	0.949
	≥#	13	84		92	
CK19	<#	10	90	0.590	100	0.297
	≥#	16	81		88	
Ki67	<2%	22	95	0.006	100	0.006
	≥2%	6	50		67	

= 50th percentile; *all cases censored at 48 months, 17% surviving
Conclusions: GRPR is expressed and retained in the majority of PET, underscoring its potential role as a novel therapeutic target in PET. Decreased GRPR expression trends toward a more aggressive phenotype. However, other tested pathway components do not seem to have any prognostic value. Unlike Ki-67, CK19 has no prognostic value in this cohort.

669 Role of Inflammation in Barrett's Esophagus-Related Cancer Development.

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Background: Cancer in Barrett's esophagus (BE) develops via a metaplasia-dysplasia-carcinoma sequence, which is initiated by reflux-induced inflammation and associated epithelial damage. However, the role of inflammation in the development of cancer in BE has never been evaluated. The aim of this case-control study was to determine whether the degree, and type, of inflammation in the esophagus is related to cancer risk in BE.
Design: All esophageal mucosal biopsies (N=3,240) obtained from endoscopies at baseline until the patient's final outcome (cancer versus no cancer) were evaluated from 38 BE patients enrolled in a long term prospective surveillance program (mean follow up: cases; 24.4 months, controls; 56.7 months). Cases consisted of 19 patients who developed cancer (mean age: 68 years, M/F ratio:18/1, mean BE length 5.8 cm, mean # biopsies/patient:92) and 19 without cancer (mean age: 68 years, M/F ratio:18/1, mean BE length 5.0 cm, mean # biopsies/patient:83). The biopsies were evaluated in a blinded fashion for the mean # of eosinophils and the mean # of mononuclear cells per high power field (HPF) in the lamina propria, and the degree of active inflammation, measured on a four-point scale (0=none, 1=<50% of mucosa involved, 2=>50% of mucosa, 3=ulceration). The results were compared between cases and controls.
Results: Overall, the mean # of mononuclear cells/HPF and mean # of eosinophils/HPF were statistically similar in the cases (33.2±24.8, 4.7±4.7, respectively) and controls (30.4±24.6, 5.4±5.7, respectively) (p>0.05 for both comparisons). No differences were noted in the presence, or degree, of active inflammation between the cases (mean score: 0.83±0.77) and controls (mean score 0.86±0.75, p>0.05). The degree of eosinophilic and mononuclear inflammation, and the activity scores, did not differ significantly in individual biopsies that contained BE without dysplasia compared to those with either low or high-grade dysplasia, in either of the two patient groups.
Conclusions: In this case-control study, the degree of mononuclear, eosinophilic, and active inflammation were not risk factors for cancer development in BE. Once inflammation-induced epithelial damage and columnar metaplasia has occurred, further progression to cancer may be unrelated to the degree of inflammation.

670 Eosinophilic Gastritis: Histopathologic Characterization and Quantification of the Normal Gastric Eosinophil Content.

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Background: There are no published criteria for the diagnosis of eosinophilic gastritis, and information about normal eosinophil counts in the gastric mucosa is limited. Therefore, pathologists are often uncertain as to when and how increased eosinophils in gastric biopsies should be reported. The aims of this study were: 1) to determine the normal gastric eosinophil content in histologically unremarkable gastric biopsies; and 2) to investigate the clinicopathologic features of eosinophilic gastritis.
Design: From an electronic database we extracted 60 patients (median age 50; range 4-83; 34 females) whose gastric biopsies over a 2-year period were diagnosed as having elevated mucosal eosinophils. For each study patient we then identified 2 or 3 age-, sex-, and ZIP code-matched control subjects who had unremarkable gastric biopsies during the same period. We reviewed all slides and counted eosinophils in the lamina propria in 5 high-power fields. Involvement of the *muscularis mucosae* or submucosa and sheets of eosinophils were noted, and infiltration of the gastric epithelium was scored on a scale of 0 to 2. Results are reported in eosinophils/mm^2.
Results: Eosinophil counts from gastric biopsies of study patients and controls are listed in Table 1.

	Study patients	Controls
	n = 60	n = 135
Median density (eos/mm^2)	180	6
Mean density (eos/mm^2)	218	4.9
S.D.	139	5.3
Range	4 - 665	0 - 34.6

The normal eosinophilic count in this geographically diverse sample of the US population was ~6 eos/mm^2. There was no significant difference between eosinophil counts in biopsies from the antrum and corpus, and no significant variation by age or geographic location. Amongst study patients, the average count was 218 eos/mm^2; sheets of eosinophils were seen in 38 patients; 27 had involvement of the *muscularis mucosae* or submucosa; and 52 had intraepithelial eosinophils. None showed evidence of *H. pylori* by immunohistochemistry. No distinct presenting symptoms or endoscopic features were identified.
Conclusions: This study suggests that the normal gastric eosinophilic counts in the US population are usually <12 eos/mm^2 and specimens with >40 eos/mm^2 (or >30 eos/HPF) in at least 5 high-power fields represent a definite departure from normal (mean + 7SD). We recommend using the term "histologic eosinophilic gastritis" for the diagnosis of such gastric biopsies. Clinical correlations may emerge if sufficient numbers of cases are diagnosed and analyzed.

671 Superficial (pT1b) Basaloid Squamous Cell Carcinoma of the Esophagus: Its Clinicopathologic Findings.

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Background: Basaloid squamous cell carcinoma of the esophagus (BSCCE) is a rare distinct variant of esophageal cancer. It is known to be made up of several kinds of tissue components, of which the ductal differentiation (DD) component has the lowest frequency. Recently, some cases of BSCCE with ductal, myoepithelial, and adenoid cystic features type have been reported, and it has been recommended to distinguish it from adenoid cystic carcinoma of the esophagus (ACCE). All of these reports are of advanced cases, and the early phase this tumor is not yet well known. We present three cases of this rare type tumor of the superficial case.
Design: Case 1 is a 71-year-old man who was admitted for an esophageal tumor detected during a routine medical examination. The tumor was located in the lower intrathoracic esophagus, and an esophagectomy was performed. Case 2 is a 56-year-old man who was admitted for hematemesis after alcohol consumption. The tumor was located 31 to 35 cm from the incisor, and an esophagectomy was performed. Case 3 is a 63-year-old man who was admitted for sense of incongruity of the pharynx. The tumor was located 26cm from the incisor, and an endoscopic mucosal dissection (ESD) was performed.
All the lesions were cut in a by 4mm (esophagectomy)/2mm (ESD) width, and histological and immunohistochemical examination was performed.
Results: Grossly, two cases were of a slightly depressed type and a flat type. The depths of all the cases were pT1b. One esophagectomy case was pN2 and the other was pN0.

Histologically, all the cases showed the squamous cell carcinoma component in the epithelium, and DD component in the epithelium to submucosa. The DD component consisted of an outer layer and an inner layer of epithelium.
Immunohistochemically, the squamous cell carcinoma component was positive for CK5. In the DD component, the outer layer of epithelium was positive for CK5 and p63 overexpression. Inner epithelium was positive for CK7, human gastric mucin (HGM) and MUC5AC, and was negative for SMA, MUC2 and MUC6. The CK14 positive cells were stained in the epithelial basal to parabasal layers, and in the DD component, positive cells appeared in the inner layer and both of the outer layers. Case 2 showed some NSE/NCAM positive tumor cells, but were negative for chromogranin A and synaptophysin.
Conclusions: BSCCE is an extremely rare tumor of the esophagus and may show the DD suggesting involvement of the basal to parabasal layeres of the squamous epithelium. Cases of BSCCE with NCAM positivity need to be distinguished from small cell carcinoma.

672 Tumor-Infiltrating CD45RO+-Cell Density, but Not CD3+, CD8+, or FOXP3+-Cell Density, Has a Prognostic Role in Colorectal Cancer, Independent of Molecular Features.

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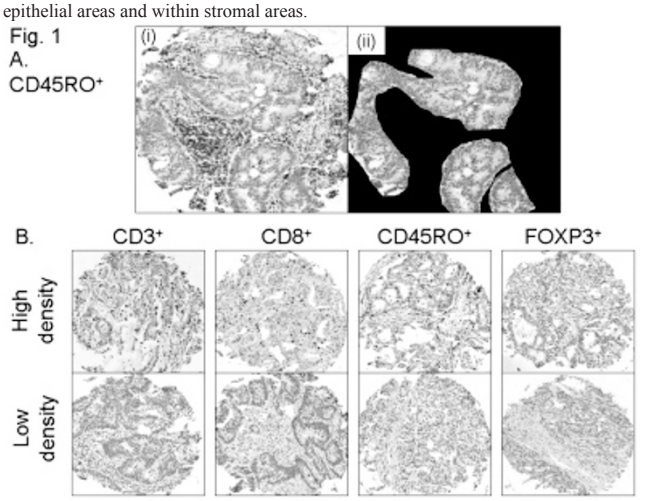
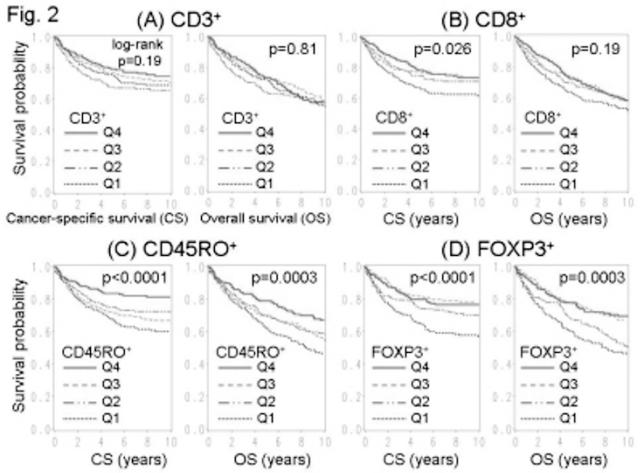
Background: The abundance of tumor-infiltrating T-cells is associated with microsatellite instability (MSI) and good prognosis in colorectal cancer. Because molecular features including MSI, the CpG island methylator phenotype (CIMP), and *BRAF* mutation have been associated with prognosis, potential confounding by these molecular features needs to be controlled when assessing the prognostic significance of T-cells.
Design: We utilized a database of 768 colorectal cancers with clinical and molecular annotations. Using tissue microarray and automated Ariol image analysis system, we quantified densities of $CD3^+$, $CD8^+$, $CD45RO^+$ and $FOXP3^+$-cells within neoplastic epithelial areas and within stromal areas.



We used Cox proportional hazard models to compute mortality hazard ratio (HR), adjusting for clinical and molecular features including *KRAS*, *BRAF*, and *PIK3CA* mutations, MSI, CIMP and LINE-1 hypomethylation.
Results: The densities of $CD45RO^+$ and $FOXP3^+$-cells in epithelial areas (and stromal areas) were significantly associated with patient survival in Kaplan-Meier analyses (log-rank $p<0.0004$) (Q1-Q4 indicating the lowest to highest quartiles in Fig. 2) and univariate regression analyses ($P_{trend}<0.0001$).



In multivariate analyses, tumor-infiltrating $CD45RO^+$-cell density, but not $CD3^+$, $CD8^+$ or $FOXP3^+$-cell density, was associated with survival (multivariate HR=0.51; 95% CI, 0.32-0.80; p=0.0032). In multivariate linear regression, MSI-high ($p<0.0001$) and tumor LINE-1 hypermethylation (p=0.0013) were independently associated with $CD45RO^+$-cell density. Nonetheless, the survival benefit associated with $CD45RO^+$-cells was independent of MSI and LINE-1 status.
Conclusions: Tumor-infiltrating $CD45RO^+$-cell density is a prognostic factor, associated with longer survival of colorectal cancer patients independent of tumor molecular features.

673 Collagenous Ileitis – A Clinicopathological Study of Thirteen (13) Cases.

BH O'Brien, K McClymont, IS Brown. Royal Brisbane and Women's Hospital, Brisbane, Australia; Sullivan Nicolaides Pathology, Brisbane, Australia.
Background: Collagenous ileitis (CI), characterised by sub-epithelial collagen deposition +/- inflammation in the terminal ileum, is an uncommon condition. The few cases reported to date have been associated with collagenous colitis (CC), collagenous gastritis (CG), collagenous sprue (CS) or lymphocytic colitis (LC). There are no previous reports of CI occurring in the absence of collagenous or inflammatory changes elsewhere in the gastro-intestinal tract (GIT).
Design: CI cases were retrieved retrospectively from Sullivan Nicolaides Pathology over a 9 year period from June 2001–2010. Clinical data recorded were symptoms, medications and co-morbidities. Histological parameters recorded were maximum sub-epithelial collagen thickness, percentage of tissue involved by sub-epithelial collagen, intra-epithelial lymphocyte (IEL) density, degree of lamina propria chronic inflammation, eosinophil and neutrophil infiltration and degree of villous atrophy. Findings in colonic, gastric and duodenal biopsies were noted where available.
Results: There were 13 cases (7 females, 6 males) with age range 39-72 (mean 64). Diarrhoea was the presenting symptom in 11 cases. 2 patients (13%) had gluten-sensitive enteropathy (GSE). Collagen thickness was 15-100µm (mean 32µm) and involved 5-80% (mean 35%; <50% in 8 cases) of the sub-epithelial region of the biopsies. 6 cases had >25 IEL/100 epithelial cells. 11 cases showed villous blunting (subtotal n=1, moderate n=4 and mild n=6). Lamina propria chronic inflammation was present in 9 cases (marked n=3; mild or moderate n=6 cases). Eosinophil infiltration was common and >10/HPF in 1 case. Focal neutrophilic infiltrate was observed in 3 cases. 7/13 colonic biopsies showed CC. 4/9 gastric biopsies showed CG. 2/10 duodenal biopsies were abnormal with CS (n=1) and partial villous atrophy and increased IEL (n=1) (both GSE related). In 3 cases, stomach, duodenum and colon were normal. Follow-up was 3-96 months (median 17 months) with 1 case showing collagen disappearance.
Conclusions: Two clinical subgroups of CI exist: 1) CI associated with collagenous or lymphocytic disease elsewhere in the GIT; and 2) CI as an isolated process. We did not identify a cause for the latter group. Diarrhoea is the typical presentation of CI. Histologically, there is overlap between the clinical subgroups. Lamina propria chronic inflammation is often present and generally associated with villous blunting. The collagen deposition is often focal. An increased IEL density is identified in 46%. GSE may be an occasional association.

674 Histologic Characteristic of Human Intestinal Spirochetosis Is Evident Only Where Colonic Surface Epithelium Has Microvilli.

S Ogata, I Ohara, K Sato, J Matsuzaki, M Higashiyama, K Nakanishi, T Kawai. National Defense Medical College, Tokorozawa, Saitama, Japan; Japan Self Defense Forces Hospital Yokosuka, Yokosuka, Kanagawa, Japan; Japan Self Defense Forces Central Hospital, Setagaya, Tokyo, Japan.
Background: Human intestinal spirochetosis (HIS) is a colorectal infectious disease caused by *Brachyspira* species bacteria. Its histologic characteristic is the so-called fringe formation of spirochetes on the colonic surface epithelium, while its cytologic one is spiral organisms floating within the mucus. Previously, we demonstrated that for the detection of spirochetes, imprint cytology was more powerful than histology (Hum Pathol 2010).
Design: We compared these two methods by a large-scale trial of their routine practice (435 endoscopy cases). We investigated the microvillus-spirochete relationship ultrastructurally in 16 HIS cases.
Results: Fifty-eight cases (13.3 %) were histology-positive (fringe formation). Seventy-seven (17.7 %) were cytology-positive (spiral organisms), of which 22 (5.1 % of the 435 cases) were histology-*negative*. The two major ultrastructural findings were: 1) a single spirochete was frequently surrounded and enclosed by several microvilli, and 2) when microvilli were sparse, spirochetes attached to the cell surface only where microvilli were present.
Conclusions: In the present large-scale study, histologic diagnosis of HIS was difficult in some cases. Since our ultrastructural study suggests that robust microvilli must be present on the epithelial surface for spirochetal attachment, the density of microvilli may determine whether a fringe formation is seen under the light microscope.

675 Multivariate Negative Binomial Regression Identifies Length of Colorectal Resection, Proximal Tumor Location, T3N0M0 Stage, Academic Setting, and Tumor *PIK3CA* Mutation as Independent Predictors of Lymph Node Count.

S Ogino, N Tanaka, C Fuchs. Brigham and Women's Hospital, Boston, MA; Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.
Background: Lymph node examination in a colorectal cancer resection specimen is important for accurate staging. The number of recovered nodes is associated with improved prognosis. However, little is known on how the lymph node count is influenced by demographic, clinical, pathologic and molecular variables.
Design: Utilizing a database of 1124 colorectal cancer patients, multivariate negative binomial regression was used to identify factors associated with the total and negative node counts.

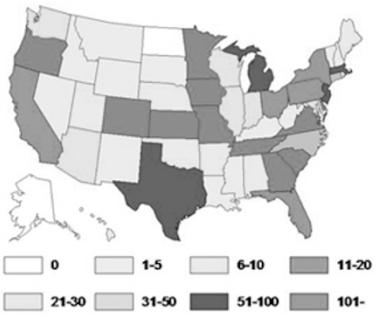


Fig. 1. Number of cases (by State) with available data on tumor molecular features and node count. Our cases distributed throughout the 48 States except for North Dakota and Alaska. The data indicate that our results would not have been influenced by a few outlier surgeons or pathologists.

Variables used were sex, age, body mass index, family history of colorectal cancer, hospital setting, year of diagnosis, tumor location, resected colorectal length, TNM stage, tumor grade, mucin, signet ring cells, lymphoid reactions, MSI, CpG island methylator phenotype (CIMP), LINE-1 hypomethylation, *BRAF*, *KRAS* and *PIK3CA* mutations.
Results: Because node counts showed gamma-poisson distribution, we used negative binomial regression analysis.

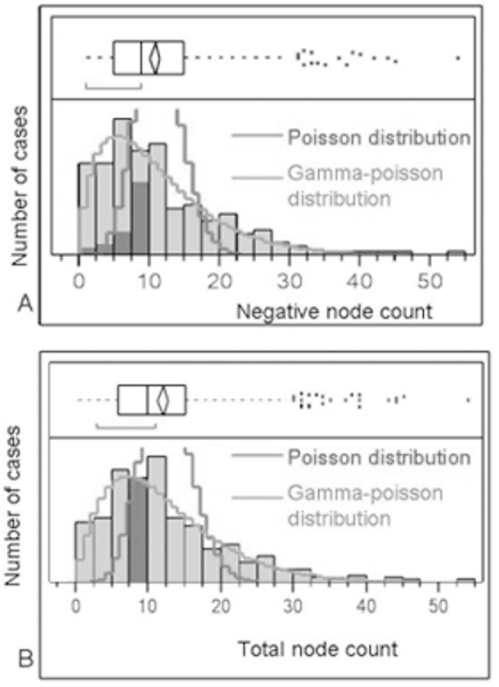


Fig. 2. Distribution of negative node count (A) and total node count (B) in 1124 colorectal cancer resection specimens. Both negative and total node counts show gamma-poisson distribution, rather than poisson distribution. Thus, we utilized negative binomial regression models to fit the raw node count data.

Independent predictors of the negative node count included resected colorectal length (1.27 times more by 20-cm increment, p<0.0001), proximal tumor location (1.36 times, p<0.0001), T3N0M0 stage (1.22-1.72 times, vs. other stages, p<0.0001), and academic hospital setting (1.23 times, p=0.0071) and *PIK3CA* mutation (1.22 times, p=0.0080). Independent predictors of the total node count were similar. The multivariate logistic regression analysis utilizing the total node count as a binary outcome variable (>=12 vs. <12) showed similar predictors.
Conclusions: *PIK3CA* mutation and the length of resected colorectum are new independent predictors of the recovered node count. Length of colorectal resection should be examined in future studies to evaluate adequacy of lymph node examination for colorectal cancer.

676 LINE-1 Extreme Hypomethylator Is a New Distinct Molecular Subtype Associated with Young Age and Poor Prognosis: Bioinformatic Analysis of 1190 Colorectal Cancers.

S Ogino, C Fuchs, C Huttenhower. Brigham and Women's Hospital, Boston, MA; Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA; Harvard School of Public Health, Boston, MA.

Background: Global DNA hypomethylation plays a role in carcinogenesis. LINE-1 constitutes 15-25% of the human genome, and its methylation level correlates with global DNA methylation status. LINE-1 hypomethylation in colon cancer is strongly associated with poor prognosis. However, whether there is a distinct group of LINE-1 hypomethylators remains uncertain.
Design: Utilizing a database of 1190 colorectal cancers with clinical, pathologic and molecular annotations, we quantified LINE-1 methylation by Pyrosequencing. We conducted multivariate linear regression analysis for LINE-1 methylation, using age, sex, body mass index, family history of cancer, smoking status, tumor location, TNM stage, pathologic features, the CpG island methylator phenotype (CIMP), MSI, *KRAS, BRAF*, and *PIK3CA* mutations.
Results: Tumor LINE-1 methylation levels ranged from 23.1-90.3 of 0-100 scale (mean 61.4; median 62.3; SD 9.6), and distributed normally, except for extreme hypomethylators [LINE-1 methylation <40; N=27 (2.3%), which were far more than what could be expected by normal distribution]. LINE-1 extreme hypomethylators were significantly associated with young age of onset (p=0.0017), left colon location (p=0.0068), and CIMP-negative (p=0.0067). Residual plot by multivariate linear regression showed that the residuals of the LINE-1 extreme hypomethylators clustered as a distinct group, separate from the main tumor group.

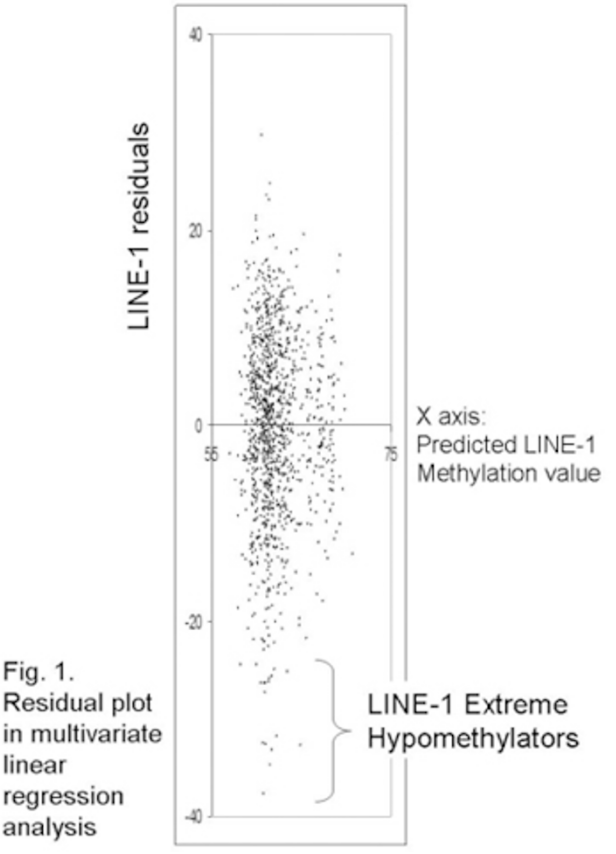


Fig. 1. Residual plot in multivariate linear regression analysis

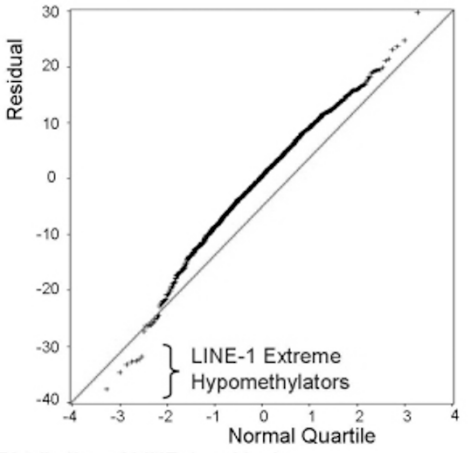


Fig. 2. Distribution of LINE-1 residuals

In survival analyses, LINE-1 extreme hypomethylators showed aggressive tumor behavior (multivariate mortality hazard ratio=3.29; 95% CI, 1.62-6.68; p=0.0010; log-rank p=0.0002).

Conclusions: LINE-1 extreme hypomethylators constitute a previously unrecognized subtype of colorectal cancers with young age of onset and aggressive tumor behavior.

677 CDH17, GPA33 and MS4A12: Three Intestine-Specific Cell Surface Proteins as Potential Diagnostic Tools or Therapeutic Targets.

NC Panarelli, RK Yantiss, R Chiu, LJ Old, Y-T Chen. Weill Cornell Medical College, New York, NY; Ludwig Institute for Cancer Research, New York, NY.

Background: Lineage-specific cell surface proteins are useful diagnostic tools and represent potential targets for monoclonal antibody therapy in cancer treatement. We identified three transmembrane proteins: CDH17, a member of the cadherin superfamily; GPA33, a cell surface differentiation antigen that belongs to the immunoglobulin superfamily; and MS4A12, a transmembrane calcium-channel protein with sequence homology to CD20, via a literature review and in-silico analysis for genes with intestine-restricted expression. The purpose of this study was to examine expression of these markers in cancers and normal tissues from the gastrointestinal (GI) tract in order to assess their potential as diagnostic markers and immunotherapeutic targets.

Design: Immunostains for CDH17, GPA33 and MS4A12 were performed using tissue microarrays (TMA) of 247 GI cancers, including esophageal adenocarcinoma (n=40), squamous cell carcinoma (n=19), diffuse-type (n=33) and intestinal-type (n=14) gastric adenocarcinoma, and colonic adenocarcinoma (n=141). A TMA of normal tissues from various organs was also tested. Membranous staining for each marker was interpreted as positive.

Results: Benign colonic and small-intestinal epithelium showed apical and basolateral membranous staining for CDH17 and GPA33, whereas MS4A12 showed apical membranous staining in normal colon but not in small intestine. All other normal tissues were negative for all three proteins. Colon cancers showed frequent expression of CDH17 (100%) and GPA33 (93%), but infrequently expressed MS4A12 (16%). CDH17 was more commonly expressed in adenocarcinomas of the esophagus (78%) and diffuse-type (91%) and intestinal-type (86%) gastric cancers than GPA33 (34%, 50%, 36%, respectively), and all GPA33-positive cases were CDH17-positive. MS4A12 was negative in all non-colonic adenocarcinomas, and esophageal squamous cell carcinomas were negative for all three markers.

Conclusions: CDH17 is a sensitive marker for adenocarcinoma of the GI tract, whereas GPA33 is preferentially expressed in colorectal cancer. These two markers could supplement existing stains, such as CDX2 in demonstrating intestinal differentiation in cancer. Their expression in >90% of colorectal cancers and in many adenocarcinomas of the upper GI tract suggests their potential as therapeutic targets. In comparison, MS4A12 is of limited diagnostic or therapeutic potential due to its low frequency of expression in cancer.

678 Serosal Scraping of Cancer Specimens Aids the Detection of Serosal Penetration by Invasive Carcinoma.

NC Panarelli, SM Brandt, RK Yantiss. Weill Cornell Medical College, New York, NY.

Background: American Joint Committee on Cancer (AJCC) staging guidelines for abdominal colon cancers require perforation by tumor or histologic documentation of tumor cells on the serosa for pT4a designation. We, and others, believe that tumor cells within <1mm of the serosa that are associated with an inflammatory reaction likely represent peritoneal involvement by cancers that would otherwise be categorized as pT3. The purpose of this study was to determine whether cytologic preparations obtained from the serosal surface aid the detection of serosal penetration.

Design: Serosal scrapings with the edge of a glass slide were obtained from 16 abdominal colon cancer specimens prior to opening the specimen. The material was smeared on a glass slide, stained with hematoxylin and eosin, and evaluated for the presence of malignant cells. Colon cancers were routinely processed with four sections obtained from the area of deepest penetration, and the tumors were staged according to the AJCC 7th edition staging manual. The findings in cytology specimens were compared to the histologic extent of disease.

Results: There were 7 men and 9 women in the study group (mean 61 years) which included 2 pT1, 1 pT2, 8 pT3, 4 pT4a, and 1 pT4b tumors. Cytology preparations from all pT1 and pT2 cancers contained mesothelial cells and red blood cells and were negative for malignancy, whereas all 5 pT4 cancers had positive cytology specimens. Five of eight (63%) scrape preparations from pT3 tumors were negative for malignant cells, but 3 contained carcinoma. Histologic sections from all 3 latter cases showed carcinoma cells within <1mm of the serosal surface which also showed an inflammatory reaction. All 5 pT3 tumors with negative cytology specimens contained tumors >2mm from the serosa, which was normal histologically.

Conclusions: Cytology preparations obtained from serosal scrapings enhances detection of serosal penetration by cancer cells, and is superior to currently accepted histologic criteria for pT4a designation. Our results support the belief that colon cancers in close proximity to the serosa, in association with a fibroinflammatory reaction, have violated the peritoneum and should be designated as pT4a, not pT3, tumors.

679 EBV Infection and Mismatch Repair Deficiency Mediated by Loss of hMLH1 Expression Independently Contribute to the Development of Multiple Synchronous Gastric Carcinomas.

HY Park, GH Kang, GE Bae, SE Lee, N Yoon, K Park, CK Park, K Kim. Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; Sanggye Paik Hospital, Inje University, Seoul, Korea.

Background: Multiple synchronous gastric carcinomas (MSGCs) are variously described in 1~10% of patients. MSGCs occur typically in elderly men, intestinal in histologic type, and early in tumor stage. The incidence of MSGCs appears to be higher in Epstein-Barr virus (EBV)-associated GC than in EBV-negative carcinomas. To explore pathogenesis and the relationship between carcinomas in MSGC, we evaluated EBV infection with expression of hMLH1 and epidermal growth factor receptors.

Design: From 2004 to 2009, 141 cases of MSGCs were retrieved from 7,598 gastrectomies for carcinoma. For comparison, consecutive 161 single GCs during recent 3 months were used. In situ hybridization for EBV and immunohistochemical analyses for hMLH1, HER2, and EGFR were performed. For statistical analysis, Pearson's chi-square test and Fisher's exact test were used.

Results: MSGCs were closely associated with male preponderance (81.6% were male; P=0.039), older ages (P=0.007), and lower TNM stages (P=0.003) compared to single GCs. EBV-positivity was found in 31 MSGCs (21.9%) and 12 single GCs (7.5%) and this difference was statistically significant (P=0.000). In 31 EBV-positive MSGCs, EBV was concurrently positive in 18 cases (58.1%) and 13 cases (41.9%) were positive in a single carcinoma. Loss of hMLH1 expression was significantly frequent in MSGCs than single GCs (24.1% *versus* 6.7%, P= 0.004). Expression of HER2 and EGFR showed no significant differences between MSGCs and single GCs.

In MSGCs, all 31 EBV-positive MSGCs were exclusively found in male patients and the mean age was 59.4 years, which are younger than EBV-negative MSGCs (mean 65.0 years; P=0.017). Loss of hMLH1 expression was more frequently observed in EBV-negative MSGCs (29.1%) than EBV-positive MSGCs (6.5%) (P=0.009). In EBV-positive MSGCs, concordant immunoreactivity between separate carcinomas for hMLH1, HER2, and EGFR was 96.9%, 87.1%, and 96.9%. In EBV-negative MSGCs, concordance for hMLH1, HER2, and EGFR was 91.7%, 69.1% and 86.4%, respectively. Although concordance rate between carcinomas within MSGCs was slightly higher in EBV-positive MSGCs, there was no significant difference.

Conclusions: EBV infection and mismatch repair deficiency mediated by loss of hMLH1 expression may independently contribute to the development of MSGCs. The concordant immunoreactivity found in separate carcinomas of MSGC suggests that multiple carcinomas occur in the tumor-prone genetic background.

680 Utility of Lethal Giant Larva-2 (Lgl2) Gene Expression and MUC Proteins in Distinguishing Barrett's Gastric-Type Dysplasia from Reactive Gastric Cardiac Mucosa in GERD.

DT Patil, AE Bennett, D Mahajan, MP Bronner. Cleveland Clinic.

Background: Barrett's gastric-type dysplasia is characterized by non-stratified, basally oriented but enlarged nuclei with mild pleomorphism. Nuclear stratification and surface predominant atypia with non-crowded, villiform architecture are features that most reliably distinguish marked reactive cardiac atypia in GERD from gastric-type Barrett's dysplasia on histology {Patil D 2010}. Recent reports demonstrate that Lgl2, a *Drosophila* gene with a putative tumor-suppressor function in mammals, is either aberrantly expressed or lost in gastric dysplasia and adenocarcinoma and retained in reactive gastric mucosa. We sought to evaluate the utility of Lgl2, MUC2, MUC5AC and MUC6 proteins in separating Barrett's gastric-type dysplasia from reactive cardia in GERD.

Design: A total of 3,698 biopsies from 461 Barrett's patients were reviewed to identify 43 patients (80 biopsies) with Barrett's gastric-type dysplasia (13 LGD,30 HGD) using previously defined criteria. These were compared to biopsies from 60 GERD patients with markedly inflamed and reactive cardiac mucosa. Ten cases from each cohort were immunostained for Lgl2 and the pattern (basolateral membranous, cytoplasmic) and intensity of staining were scored. Cytoplasmic expression or apical membrane staining was considered aberrant expression. Cytoplasmic expression was considered as positive staining for MUC2, MUC5AC and MUC6.

Results: Moderate to strong basolateral membranous staining of the surface epithelial cells was seen in all cases of reactive cardia in GERD (100%). In contrast, 8/10 (90%) cases of Barrett's gastric-type dysplasia showed either no Lgl2 expression or weak cytoplasmic expression (1/10;p=0.03). Strong MUC5AC expression in the surface epithelium but not in the deeper glands was seen in all cases of reactive cardia and Barrett's dysplasia. The opposite staining pattern was noted with MUC6 in that 8/10 (80%) cases of reactive cardia and 9/10 (90%) cases of Barrett's dysplasia revealed strong expression in the deep glands only. Only 10% of reactive cardia (1/10) versus

90% of Barrett's dysplasia (9/10) showed MUC2 expression in 5%-50% of the epithelial cells (p=0.011).
Conclusions: Loss or aberrant Lgl2 and gain of MUC2 expression are useful adjunct tools to differentiate Barrett's gastric-type dysplasia from reactive cardiac atypia in GERD. The complementary staining patterns of MUC5AC and MUC6 in the superficial and deep glands confirm the gastric phenotype of Barrett's dysplastic epithelium; however, does not differentiate reactive cardia from Barrett's gastric-type dysplasia.

681 FISH as a Cancer Risk Biomarker on ThinPrep Rectal Cytologic Brushings in Ulcerative Colitis (UC): A Promising Alternative to Biopsy-Based FISH.

DT Patil, J Lewis, B Shadrach, M Settle, B Ozimek, N Ray, J Brainard, T Pua, M Bronner. Cleveland Clinic.
Background: Neoplastic progression in inflammatory bowel disease results in widespread mucosal chromosomal alterations detected by FISH on mucosal biopsies, including nondysplastic rectal samples. Rectal brushing may be an attractive alternative compared to colonoscopic biopsies, since it does not require endoscopy and may be less subject to sampling error. In this study, we evaluate the feasibility of FISH as a neoplastic biomarker on nondysplastic UC rectal brushings.
Design: Nondysplastic rectal brushings using standard cytology brushes and corresponding biopsies were obtained from surgical resections from 2 UC-progressors [(P), with multifocal high-grade and low grade-dysplasia and one with multifocal cancer in addition], 3 UC-nonprogressors (NP) without neoplasia and 2 non-UC controls. Two techniques were compared on ThinPrep (Hologic) processed rectal brushings: A) FISH without epithelial isolation, and B) FISH with epithelial isolation using tyramide-enhanced fluorescent cytokeratin immunohistochemistry plus FISH. Epithelial isolated touch preparations from corresponding rectal biopsies were also processed for FISH. Dual Vysis FISH probes targeting cyclin D1 and its matched CEP11 centromere were used and 100 epithelial cells/slide were enumerated per sample. A Pap-stained slide was additionally prepared for each brushing sample to assess for inflammatory activity and lack of neoplastic change.
Results: All UC cases had active rectal inflammation. The number of cells with FISH abnormalities (arm or centromere gains or losses) was significantly higher in UC-P (touch prep and ThinPrep with and without epithelial isolation) compared to UC-NP and non-UC controls (18%, 46%, 34% vs. 9%, 6%, 8% vs. 4%, 4%, 4%, p=0.0001). While all three methods distinguished UC-P's from UC-NP's, the difference was most pronounced with the ThinPrep method compared to biopsy touch preparations (p= 0.0001 vs 0.02), irrespective of whether epithelial isolation was used or not on the ThinPrep.
Conclusions: FISH chromosomal gains and losses on rectal cytologic brushings from UC patients shows promise as a biomarker and may be an improvement over biopsy testing. ThinPrep brushings may also potentially eliminate the need for epithelial isolation. This technique must still be adapted to in vivo patient brush rectal sampling, as these preliminary results were obtained from ex vivo surgical resections. The findings are nonetheless novel and suggest that far less invasive cytology preparations could better target UC patients at highest risk of neoplastic progression using FISH biomarkers.

682 Can a Three-Marker Panel (NR-21, BAT-25 and MONO-27) Accurately Detect Microsatellite High (MSI-H) Colorectal Tumors without Control DNA?

DT Patil, MP Bronner, TP Plesec, CR Fraser, X Liu. Cleveland Clinic.
Background: Microsatellite testing (MSI) testing is performed on tumor samples to screen for Lynch Syndrome (LS). Previously, we demonstrated that a 3-marker panel of NR-21, BAT-25, and MONO-27 (Promega MSI kit) accurately determines MSI status when read with paired control DNA {Portier BP 2009}. This study was undertaken to determine if this 3-marker panel without paired control DNA accurately detects MSI-H tumors.
Design: A total of 478 MSI tests performed were divided into a test group [colorectal cancer (CRC),n=172] and a validation group (179 CRCs,127 adenomas). The size of control alleles for each marker from the test group was recorded, their mean and median size calculated, and a range (median size ± 3 bp) was generated. Each marker was considered unstable either when the difference between tumor allele and mean control allele size (generated from test group) was ≥3 bp or when the tumor allele size was beyond the range generated from test group. Using the 3-marker panel, a tumor was interpreted as MSI-H when ≥ 1 marker was found to be unstable. The performance of this panel was compared to the 5-marker panel with control non-tumor DNA; MSI-H defined as ≥ 2 unstable markers (gold standard).
Results: In the test group, the mean size of normal alleles for NR-21, BAT-25 and MONO-27 was 101, 124, and 152 bp; their median and range were 102 and 99-105 bp, 124 and121-127 bp, 152 and 149-155 bp, respectively. Overall, this 3-marker panel identified all MSI-H tumors but overcalled 2.7% cases (mean ± 3 bp) and 3.3% cases (median size ± 3 bp) of MSS tumors as MSI-H.

			Compared to 5-marker panel	
	MSI-H	MSS	Sensitivity (%)	Specificity (%)
Test group (n=172)				
5-markers + control DNA	36	136	-	-
3-markers (mean ± 3 bp)	40	132	100	97
3-markers(median ± 3 bp)	43	129	100	95
Validation group(n=306)				
5-markers + control DNA	47	259	-	-
3-marker (mean ± 3 bp)	52	254	100	98
3-markers(median ± 3 bp)	56	239	100	96
Entire cohort (n=478)				
5-markers + control DNA	83	395	-	-
3-markers (mean ± 3 bp)	96	382	100	97
3-markers (median ± 3 bp)	99	379	100	96

Conclusions: This 3-marker panel simplified from the Promega MSI kit with MSI-H defined as ≥ 1 unstable marker, without paired control DNA, determines MSI status with 100% sensitivity. The rare false positive cases can be clarified either by mismatch repair protein immunohistochemistry or by repeating PCR with paired control DNA. This approach will miss extremely rare non-monomorphic alleles, but will greatly decrease the MSI testing cost and facilitate universal LS screening in CRCs.

683 EBV-Associated Gastric Carcinoma Displays Type I Viral Latency, Regardless of Host Inflammatory Response Pattern.

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Background: EBV can be identified in approximately 10% of gastric carcinomas (GC). The patterns of host inflammatory response in EBV associated GC (EBV-GC) that resemble lymphoepithelioma-like carcinoma (LELC) and Crohn's disease-like lymphoid infiltrate (CLR) are associated with better prognosis than those which resemble conventional adenocarcinoma (CA). EBV associated neoplasms are associated with distinct types of viral latency, classified as type I, II, or III, based on expression patterns of EBV latency proteins. The aim of our study was to determine if the type of EBV latency correlates with the pattern of host inflammatory response in EBV-GC.
Design: A consecutive series of 1080 GC cases at a single large academic hospital in a region endemic for GC was subjected to screening by in-situ hybridization to detect EBER-positive GC. Cases with 100% EBER-positive tumor cells were classified as EBV-GC and were included in this study. Inclusion criteria for this study included pathologic stage 1B through IV (AJCC 2002), curative resection, and complete clinical follow-up data. All tumors were classified histologically into LELC, CLR, or CA based on host inflammatory response pattern using previously published criteria. A tissue microarray (TMA) was constructed using two representative cores from each case. Cores from normal gastric tissue were included as controls in the TMA. TMA slides were immunostained histochemically for EBV latency proteins LMP-1 and EBNA-2 in order to stratify each case into type I, II, or III latency.
Results: 106 EBV-GC (mean age: 56; range: 32-76 yrs; M/F ratio 76:30) formed the final study group. The pattern of inflammatory response in this group was classified as LELC in 39/106 (36.8%), CLR in 27/106 (25.5%), and CA in 40/106 (37.7%). Although EBER positivity was present in a 100% of cases, only 1/106 (0.9%) case was positive for EBNA-2, and this case showed a CLR pattern of inflammation. All cases were negative for LMP-1 expression by immunohistochemistry.
Conclusions: Our findings suggest that EBV-GC is almost universally associated with a type I EBV latency regardless of host inflammatory response pattern. Differences in inflammatory response and prognosis in EBV-GC are, therefore, more likely to be due to genotypic differences in the tumor cells rather than in type of EBV latency, which should be explored in future studies.

684 Surface Hyaline Keratosis Is a Highly Significant Marker of Reflux vs. Eosinophilic Esophagitis: A Multivariate Analysis Study.

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Background: Gastroesophageal reflux disease (GERD) and eosinophilic esophagitis (EoE) are distinct clinicopathologic entities with overlapping clinical, endoscopic and histologic findings but different management approaches. Although certain histologic features may favor one diagnosis, inadequate specificity and poorly standardized criteria often result in uncertainty. We evaluated putative histologic aids in this distinction including surface hyaline keratosis (SHK), a feature that had hitherto received little attention.
Design: Retrospectively identified consecutive patients (n=216) with esophageal biopsies were divided into 3 groups: (1) GERD (n=63): typical GERD symptoms/endoscopic findings (heartburn, vomiting, hiatal hernia, erosion/ulcer) and concordant histology (basal hyperplasia, papillomatosis, blood lakes, <15 intraepithelial eosinophils/HPF); (2) EoE (n=62): typical EoE symptoms/endoscopic findings (food allergy, atopy, asthma, food impaction, peripheral eosinophilia, esophageal furrows/rings) and concordant histology (>20 intraepithelial eosinophils/HPF with superficial distribution/degranulation/microabscesses, submucosal fibrosis); (3) uncertain (n=91): discordant clinical, endoscopic and histologic findings. H&E sections were blindly and semiquantitatively graded for 16 histological features including SHK, defined as a surface layer of hypereosinophilic squamous epithelium ≥3 cells thick devoid of inflammation, erosion or ulcer.
Results: In univariate analysis, features significantly more common in EoE vs. GERD included eosinophil degranulation (p<0.001), microabscesses (p<0.001) and superficial distribution (p<0.001), lamina propria fibrosis (p=0.05) and higher mean lamina propria eosinophils (p=0.002). GERD cases had more frequent squamous ballooning (p<0.001), blood lakes (p<0.001), neutrophils (p=0.04) and SHK (21% vs. 2%; p=0.006). In multivariate logistic regression of these variables, SHK emerged with the highest odds ratio (>10^4) followed by blood lakes and eosinophil degranulation. In the uncertain group, SHK was present in 17 (19%) cases. Clinical post-biopsy follow-up by an EoE specialist revealed that 10 (59%) of these cases could be classified as GERD, 2 as EoE and 1 as EoE with possible GERD overlap; follow-up could not be obtained in 4 cases.
Conclusions: SHK, when strictly defined, is a histologic marker significantly associated with GERD that may be useful in discriminating it from EoE when other clinical, endoscopic, and histologic features are inconclusive.

685 Universal Screening for Lynch Syndrome in Newly Diagnosed Colorectal Cancer Significantly Increases the Detection Rate .

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Background: Lynch syndrome (LS) is the most common hereditary form of colorectal cancer (CRC). In our institute, prior to universal testing of all colorectal cancers, LS screening rates and MSI-H detection rates in newly diagnosed CRCs were 35.6% and 8.5% using the revised Bethesda guidelines and histomorphology in patients 60 years or older, indicating the inadequacy of these approaches for LS screening in newly diagnosed CRC. The degree to which universal screening has improved these results forms the subject of this report.

Design: All CRC cases surgically resected since the initiation of a universal testing paradigm (7/2009 to 7/2010) were retrieved from the pathology database and the MSI testing rates and results were determined. Exclusionary criteria included: recurrent or metastatic CRCs, idiopathic inflammatory bowel disease, familial adenomatous polyposis, and known LS patients.

Results: 267 newly diagnosed CRCs resected during the study period met our inclusion criteria. The cumulative reflex MSI testing rate was 69.3%, or 183 total CRCs, including 139 of 185 (75.1%) untreated CRCs, 43 of 75 (57.3%) CRC resections following neoadjuvant treatment, and 1 of 14 (14.3%) CRC re-excisions following polypectomy. The MSI-H detection rate among the screened cases was 16.9%. The LS screening rate in this cohort (69.3%) was far from universal, but was significantly higher than our previously reported rate prior to the institution of the universal paradigm of 35.6% ($p<0.00001$). Of the 185 CRCs without neoadjuvant therapy, polypectomy and transanal resection specimens accounted for 10 out of 40 (25%) untested, and 7 of 145 (4.8%) tested ($p=0.0004$). Universal LS screening in newly diagnosed CRCs significantly increased the detection rate of MSI-H CRCs (29 out of 185 newly diagnosed CRCs without neoadjuvant treatment, 15.6%) compared to our previously reported rate of 8.5% using only revised Bethesda guidelines and histomorphology in patients 60 years or older ($p=0.01$).

Conclusions: The institution of universal LS screening in newly diagnosed CRCs has significantly improved the LS screening rate at our institution and the detection of potential LS patients. CRCs diagnosed in polypectomy and transanal resection specimens were less likely to be screened for LS, as were those treated by neoadjuvant therapy, which are target areas for quality improvement.

686 Incomplete Goblet Cell Maturation: A Distinctive Form of Flat Dysplasia in IBD.

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Background: Nuclear atypia is a hallmark of dysplasia in inflammatory bowel disease (IBD), however, a notable exception is incomplete goblet cell maturation (IGCM), a type of dysplasia characterized by unexplained absence or near-absence of goblet cells, uniformly eosinophilic cytoplasm, minimal or absent nuclear atypia in most cases, and near-normal crypt architecture without a polypoid component. Because IGCM is underrecognized due to the lack of nuclear atypia and resemblance to regenerative mucosa, we evaluated its association with conventional dysplasia and its significance as a risk factor for colorectal cancer.

Design: Our GI Database was queried for a diagnosis of IGCM between 1994-2010. The diagnoses were correlated with synchronous and metachronous dysplasia using chi-square statistics. IGCM was usually graded indefinite for dysplasia (IND), mainly due to the difficulty of excluding regenerative change with certainty, however, diagnoses of indefinite probably negative (IND-N) or probably positive (IND-P) were rendered when regeneration seemed more or less likely based on the inflammatory surroundings. A minority of cases were interpreted as low-grade dysplasia (LGD) or high-grade dysplasia (HGD) based on criteria for conventional dysplasia.

Results: IGCM was reported in 80 patients (51 males, 29 females, ages 22-81y). It accounted for 4.7% of all biopsies with definite or indefinite dysplasia (9% IND-N, 52% IND, 19% IND-P, 17% LGD, and 2% HGD). A significant correlation was observed between the grade of dysplasia assigned to IGCM and the presence of synchronous conventional dysplasia: no biopsies with conventional dysplasia were observed in the same procedure as IND-N compared to 29% in procedures with IGCM of grade IND or higher ($p=0.008$). Among 56% of patients with IGCM who had undergone previous biopsies, no significant differences were noted in the prevalence of previous dysplasia.

Among 56 IGCM patients with long-term follow-up, LGD and carcinoma were more prevalent among 48 with IGCM graded IND or higher (15 and 10, respectively) than among 8 graded IND-N (0 and 0, respectively), though statistically insignificant. Among these 56 patients, IGCM persisted in subsequent procedures in 13 (23%). Of 21 patients who had IGCM as their first and only dysplastic finding and adequate follow-up, 6 (29%) developed conventional dysplasia including 4 LGD, 1 HGD and 1 carcinoma.

Conclusions: IGCM, a distinctive type of flat dysplasia in IBD, should be considered when an unexplained absence of goblet cells is noted in the setting of IBD and should be managed similarly to conventional types of dysplasia.

687 Prevalence and Prognostic Significance of HER2 Amplification in Adenocarcinoma of the Esophagus and GE Junction.

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Background: The ToGA trial showed that trastuzumab (a monoclonal antibody targeting HER2) with conventional chemotherapy improves survival in advanced gastric or GE junction (GEJ) adenocarcinoma, underscoring the therapeutic importance of HER2 amplification and overexpression. There are conflicting reports of the prognostic significance of HER2 amplification in esophageal or GEJ adenocarcinoma (EAC). In this study we assess the association of HER2 amplification with pathologic features and survival in a large series of EAC treated by esophagectomy.

Design: 82 patients who underwent esophagectomy from 2000-2004 for EAC were prospectively evaluated for HER2 amplification. Dual-color FISH using a HER2 region probe and a chromosome 17 centromeric probe (CEP17) (Pathvysion, Abbott Molecular) was performed on paraffin sections of the resected tumor (N=79) or pre-operative biopsy (N=3). Pathologic, clinical and survival data was obtained for all patients by review of the medical record.

Results: Twenty-seven of 82 (32.9%) patients had HER2 amplification using a HER2/CEP17 ratio cutoff of 2.2. The HER2/CEP17 range for amplified cases was 2.2 – 42.6. The distribution of these results is summarized in Table 1.

HER2 FISH Results

HER2/CEP17 Ratio	Number of Cases (%)
> 4.0	10 (12.2)
2.2-4.0	17 (20.7)
1.8-2.2	4 (4.9)
1.5-1.8	10 (12.2)
< 1.5	41 (50.0)
Total	82 (100)

There were no significant differences between HER2 amplified and non-amplified cases with respect to age, gender, TNM stage, tumor grade, perineural and angiolymphatic invasion, intestinal metaplasia, columnar dysplasia, or margin status. The median recurrence free survival was 18.3 mo for HER2 amplified cases and 24.9 mo for non-amplified cases, but the survival functions were not significantly different (Kaplan-Meier, log rank test). HER2 amplification had no significant effect on overall survival (Kaplan-Meier, log rank test).

Conclusions: The frequency of HER2 amplification in EAC in this series (32.9%) is consistent with what has been reported for EAC (15-54%). In contrast to other reports, we saw no association with tumor grade, nor other conventionally reported pathologic variables. We also failed to observe any significant adverse effect on survival in univariate analysis, consistent with the larger of two previous studies. The number of cases may be insufficient to detect a small effect. HER2 protein overexpression may be a better prognostic marker based on comparison to the literature.

688 Gastric Fundic Gland Adenocarcinoma: Clinical, Histological and Immunohistochemical Characterization.

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Background: Gastric fundic gland adenocarcinoma (FGA) is a newly recognized entity reported in the Japanese population. We report the first series of U.S. cases and describe the tumor's clinical, histological and immunohistochemical characteristics.

Design: Three patients were diagnosed with FGA in consultation within a 1-year interval. The clinical records were reviewed and paraffin-embedded tissue was obtained for further study. All lesions underwent immunohistochemical staining for the following: pepsinogen II (Novus Biologicals); sodium channel antigen ACCN5 (Sigma-Aldrich); MUC2, MUC5A/C and MUC6 (Dako); and Dolichos biflorus lectin (DBA, Vector).

Results: The patients, all females between 48-81y of age, underwent upper endoscopy for nonspecific symptoms. None had any history of gastric neoplasia or gastrointestinal polyposis. One was on chronic PPI therapy. Endoscopically, each presented a sessile polyp of 3-8mm in diameter in the gastric body or fundus. Two patients underwent complete endoscopic polypectomy and the 3rd underwent a biopsy followed by surgical wedge resection without lymph nodes. The referring diagnoses were inconclusive, proposing an unusual gastric adenoma or atypical glandular proliferation. Microscopically, each of the tumors comprised anastomosing tubular and cribriform glands arising directly from the deep fundic gland compartment and infiltrating the submucosa. The neoplastic epithelium featured 2 distinct phenotypes, a predominant chief cell-like component, i.e, columnar cells with basophilic cytoplasm and round basal nuclei, and a smaller component of interspersed parietal-like cells, i.e., pyramidal eosinophilic cells with round central nuclei. Nuclear atypia ranged from mild to moderate. Abundant mitotic activity with MIB1 index of 20% was noted in 1 case. In 2 patients the adjacent and overlying mucosa were unremarkable, whereas the 3rd, who was taking PPIs, had superimposed features of a fundic gland polyp with parietal cell hyperplasia. Immunohistochemically, the neoplastic chief cell-like epithelium was positive for MUC6 and pepsinogen II and negative for MUC2 and MUC5AC, whereas the parietal-like cells were positive for ACCN5 and/or DBA. One lesion was negative for chromogranin but variably positive for synaptophysin. All the patients are clinically tumor-free after 3-15m of endoscopic follow-up

Conclusions: FGA is a distinct but as yet poorly recognized type of well-differentiated gastric carcinoma. Although conservative modalities may afford adequate therapy, further experience with this entity may better define its clinical behavior.

689 Two-Antibody Screen with PMS2 and MSH6 Is Equally as Effective as Four Antibodies for Identification of Mismatch Repair Defects in 1000 Colorectal Cancers.

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Background: Analysis of DNA mismatch repair (MMR) status in colorectal cancers (CRCs) is commonly undertaken utilizing immunohistochemical (IHC) analysis of MMR protein expression. Because MMR proteins function as heterodimers, abnormalities in MLH1 or MSH2 may be identified by corresponding loss of PMS2 or MSH6 respectively. We undertook this study to determine whether testing for MMR defects with only PMS2 and MSH6 is effective in identifying MMR defects in all four proteins.

Design: 1153 consecutive biopsies diagnosed with colorectal cancer at Caris Life Sciences from March 15 through August 31, 2010 were eligible for DNA MMR testing. 152 were excluded due to inadequate tissue (21), declination by clinician (73), or the presence of other conditions (13). IHC stains for MLH1, PMS2, MSH2, and MSH6

were reviewed by a single observer (MR). MSH6 and PMS2 stains were reviewed first, and scored as either Intact (retained nuclear expression) or Deficient, including 1) absent expression (no nuclear staining) or 2) markedly reduced expression (<20% of nuclei with 2-3+ staining). MLH1 and MSH2 were independently scored according to the same criteria.

Results: 887 (84.8%) of 1046 CRCs had intact PMS2 and MSH6 expression, and 159 (15.2%) had abnormal PMS2 or MSH6 expression, including 135 (12.9%) with absent PMS2, 18 (1.7%) with absent MSH6, 2 (0.2%) with markedly reduced MSH6, and 4 (0.4%) with absent PMS2 and absent or markedly reduced MSH6. Subsequent scoring of the additional MMR IHC stains revealed that all 887 cases with intact PMS2 and MSH6 also had intact MLH1 and MSH2, while 147 of the 159 cases with PMS2 or MSH6 abnormalities also had absent MLH1 or MSH2 expression. The only CRCs with discrepant results were 6 (0.6%) cases that had absent PMS2 only and 6 (0.6%) cases that had absent MSH6 only.

PMS2/MSH6 vs. MLH1/MSH2 MMR IHC Testing

Number of CRCs	PMS2/MSH6 Result	MLH1/MSH2 Result
887 (84.8%)	Intact	Intact
147 (14.0%)	Deficient	Deficient
12 (1.2%)	Deficient	Intact

Conclusions: IHC analysis of PMS2 and MSH6 is as sensitive in identifying defective mismatch repair protein expression as a panel that also includes MLH1 and MSH2. MLH1 and MSH2 are only required for further characterization of those CRCs with PMS2 or MSH6 defects, respectively. Of note, 12 MMR deficient CRCs would not have been identified by screening with MLH1 and MSH2 alone. These results strongly support the less costly use of PMS2 and MSH6 only in the initial screening for MMR defects.

690 "Pediatric-Type" Gastrointestinal Stromal Tumors in Adults: Distinctive Histology Predicts Genotype and Clinical Behavior.

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Background: Gastrointestinal stromal tumors (GISTs) rarely affect children, mainly girls. Pediatric GISTs typically arise in the stomach and show epithelioid morphology, multinodular architecture, lymph node metastases, an absence of KIT and PDGFRA mutations, and indolent behavior. Occasional GISTs in adults show similar features. Such tumors are not widely recognized.

Design: GISTs in patients >18 yrs with a multinodular growth pattern were retrieved from surgical and consult files. H&E slides were reviewed, immunohistochemistry (IHC) was performed, and KIT (exons 9, 11, 13 and 17) and PDGFRA (exons 12, 14 and 18) genes were screened for mutations. Clinical follow-up was obtained.

Results: 16 cases were identified, affecting 13 women and 3 men (mean age, 34 yr; range, 19-56 yr), all gastric. Mean tumor size was 5.4 cm (range, 1.8-11 cm); 4 were multifocal. All showed an infiltrative architecture and epithelioid (3) or mixed (13) morphology. Five tumors had vascular invasion; 6 focal necrosis. Mitoses ranged from 3-156 per 50 HPF (8 tumors had ≤5). Using AFIP risk stratification, categories were: none (2), very low (3), low (3), moderate (3), and high (5). By IHC, all tumors were positive for KIT, 82% DOG1, 72% CD34, 18% caldesmon, 9% S100, 8% SMA, and 0% desmin. All were wild-type for KIT and PDGFRA in the exons screened. At primary resection, 8 patients had lymph node and 3 liver metastases. Follow-up ranged from 16 mo – 16 yr (median, 5 yr). Only 3 of 11 patients treated with imatinib responded. Two tumors recurred locally, and 8 subsequently metastasized, to lymph nodes (5), liver (3), and peritoneum (3). Resected tumors from 3 patients who developed metastases were AFIP low or very low risk for recurrence. All patients are alive at last follow-up.

Conclusions: Gastric GISTs in adults with a multinodular/infiltrative growth pattern and epithelioid or mixed morphology are similar to pediatric GISTs. Unlike conventional adult GISTs, this distinctive subset predominantly affects women, often metastasizes to lymph nodes, and lacks mutations in KIT and PDGFRA. Current risk assessment criteria do not reliably predict behavior for this group. Although metastases are common and most tumors are imatinib-resistant, they pursue an indolent clinical course. Recognition of "pediatric-type" GISTs in adults is critical to guide mutational testing and appropriate therapy and follow-up.

691 Does Histology of Gastroesophageal Junction (GEJ) Biopsies Predict Future Development of Barrett's Esophagus (BE).

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Background: Mucosal biopsies of the GEJ in patients with GERD, but without endoscopic evidence of columnar-lined esophagus (CLE), often show abnormalities such as increased inflammation, multilayered epithelium (ME), squamous islands, and elongation of mucosa consisting of pure mucous glands. It is unclear if these changes represent an early manifestation or precursor of CLE. The aim of this study was to determine if histologic features of GEJ biopsies in patients without endoscopic evidence of CLE are predictive of future development of CLE upon follow up.

Design: 204 GEJ mucosal biopsies from 102 GERD patients (M/F ratio: 39/63, mean age: 54 years) without CLE or goblet cells were evaluated for a variety of histologic features, including the presence or absence of ME, type of glands, degree of inflammation, presence or absence of squamous islands, and presence or absence of subsquamous columnar epithelium, among others. The findings were correlated with the histologic features and endoscopic appearance of the esophagus at follow up endoscopy (mean follow up: 60.5 months). All patients had at least 1 follow up endoscopy.

Results: Of the 102 study patient index biopsies, 14.7% had ME, 79.4% mucous or mixed mucous and oxyntic glands, 46.1% pure oxyntic glands, 24.5% active inflammation, 77.5% chronic inflammation, 32.4% active inflammation of the squamous mucosa, 15.7% squamous islands, and 34.3% subsquamous columnar epithelium. None of these histologic features changed significantly in frequency upon follow up endoscopy. 24 of 102 (23.5%) patients developed goblet cells [involving <10% of crypts in 16 (15.7%) and 10-50% of crypts in 8 (7.8%)]. Of all the features evaluated in the index biopsy, none were significantly predictive of the development of CLE or goblet cells upon follow up except the presence of ME, which was significantly associated with the future development of CLE (p=0.046).

Conclusions: The data supports the theory that ME represents a precursor to CLE, and is predictive of the future development of this condition when detected in biopsies of the GEJ region.

692 BMI-1 Nuclear Overexpression Correlates with Low Tumor Grade and Lengthened Overall Survival in Colorectal Adenocarcinoma (CRC).

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Background: Bmi-1 protein expression plays a vital role in cell cycle regulation and senescence, and has been implicated in lymphangiogenesis and carcinogenesis. Bmi-1 oncogene overexpression has been previously identified in several human malignancies including hematologic malignancies, as well as carcinomas including CRC; however, clinicopathologic and prognostic significance of Bmi-1 expression has not been fully elucidated.

Design: Formalin-fixed, paraffin-embedded sections from 153 colorectal adenocarcinomas (CRCs) were immunostained by an automated method (Ventana Medical Systems; Tucson, AZ) using mouse monoclonal Bmi-1 (clone F6; Millipore, Burlington, MA). Nuclear and cytoplasmic immunoreactivity were semi quantitatively evaluated based on both intensity (weak, moderate and intense) and distribution (focal <10%, regional 10 to 50% and diffuse >50%) and results were correlated with clinic-pathologic variables.

Results: Bmi-1 nuclear immunoreactivity was over-expressed in 100/153 (65%) of CRC, while cytoplasmic over-expression was observed in 62/153 (41%). Nuclear overexpression correlated with low tumor grade (77% grade 1 vs 72% grade 2 vs 49% grade 3, p=0.016) and lengthened overall survival (80% alive vs 59% expired, p=0.008). There were no other significant correlations. On multivariate analysis, only pathologic stage at diagnosis independently predicted patient survival.

Conclusions: Bmi-1 overexpression is associated with lower grade CRC's and significantly correlates with increased overall survival. These findings indicate that Bmi-1 over-expression may be a significant prognostic biomarker that could play a role in the planning of therapy in CRC. Further study of Bmi-1 expression in CRC appears warranted.

693 Osteopontin Overexpression Is Associated with Improved Survival Time in Patients with Gastric Adenocarcinoma.

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Background: Osteopontin (OPN) is an important mediator in tumor progression in several cancers. Through its interactions with CD44 and Integrins, OPN enhances the ability of tumor cells to metastasize, however, regulatory mechanisms are poorly understood. Alterations in p53 are well known factors in tumorigenesis. We evaluated the expression of OPN, CD44, and p53 and their association with outcome in gastric adenocarcinoma.

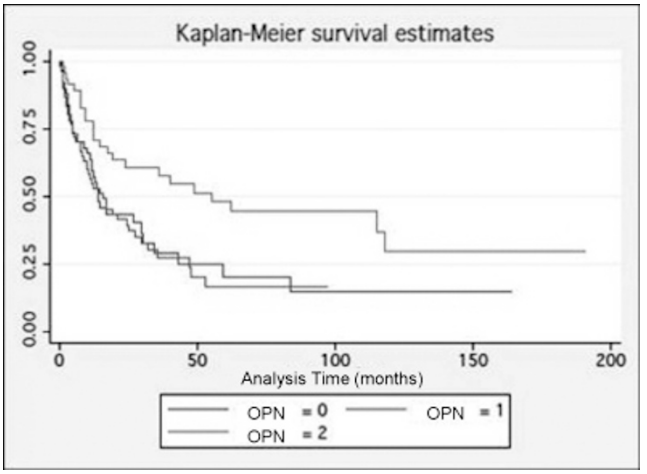
Design: Gastric adenocarcinomas (159) with available tissue and clinicopathologic data including patient age, gender, tumor grade and outcome were reviewed. Tissue microarrays with 1 mm cores were constructed, stained with antibodies for OPN, CD44 and p53, and graded as strongly positive (2), weakly positive (1) and negative (0). Controls were adjacent uninvolved stomach in 30 cases. Statistical analysis was performed using student's t-test, Chi-Square, Logistic Regression Analysis for correlation with differentiation and Cox proportional hazards analysis for survival.

Results: There was no association between patient age, gender or any marker and poor tumor differentiation. Expression of OPN, CD44 and p53 in tumors is shown in the table. Controls were negative with all markers.

OPN, CD44 and p53 Staining in Tumors

	Negative	Weak	Strong
OPN	50 (31.4%)	61 (38.4%)	48 (30.2%)
CD44	59 (37.1%)	46 (28.9%)	54 (34.0%)
p53	66 (41.5%)	33 (20.8%)	60 (37.7%)

Expression of p53 correlated with both OPN (P=0.03) and CD44 (P=0.002), while OPN and CD44 were not associated with each other. By multivariate analysis, poor differentiation was associated with shorter survival with Hazards ratio of 1.93 (95% CI 1.26, 2.95; P=0.002). Negative and weak expression of OPN was associated with shorter survival with Hazards ratio of 1.79 (95% CI 1.08, 2.94; P=0.002) and 1.97 (95% CI 1.23, 3.18; P=0.005), respectively.



Conclusions: Although OPN and CD44 were associated with p53, they did not correlate with each other suggesting complex interactions in gastric adenocarcinoma. Surprisingly, our results suggest that strong OPN expression in gastric adenocarcinoma was associated with longer survival. Additional studies may help elucidate the role of OPN in tumorigenesis.

694 Expression of MDM2, MDM4 and p53 and Association with Outcome in 162 Cases of Gastric Adenocarcinoma.

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Background: Gastric adenocarcinoma (GA) is one of the leading causes of cancer-related death worldwide. Polymorphisms in *p53* have been associated with risk for development of GA, and *p53* mutations have been identified in a significant proportion of these tumors. MDM2 and MDM4 are critical regulators of p53 protein levels, function and subcellular location. Promoter polymorphisms and mutations of *MDM2* and *MDM4* can deregulate p53 leading to impaired cell cycle arrest and apoptosis. Novel small molecule inhibitors that disrupt the MDM2-p53 interaction are being studied in some cancers. We evaluated the expression of MDM2, MDM4 and p53 and correlated them with outcome in GA.

Design: Gastric adenocarcinomas with available tissue and clinicopathologic data including patient age, gender, tumor grade and outcome were reviewed. Controls were obtained from uninvolved adjacent stomach in 31 cases. Tissue microarrays with 1 mm cores were constructed, stained with antibodies for MDM2, MDM4 and p53, and graded as strongly positive (2), weakly positive (1) and negative (0). Statistical analysis was performed by Chi-Square, Logistic Regression Analysis for correlation with poor differentiation and Cox proportional hazards analysis for survival.

Results: Mean age was 65.8 years with a male to female ratio 1.2:1. No association was seen between age or gender and poor differentiation. Controls were negative for markers except 4 cases with weak staining for MDM4.

MDM2, MDM4 and p53 Staining Intensity in Tumors

	Negative	Weak	Strong
MDM2	117 (72%)	16 (10%)	29 (18%)
MDM4	74 (46%)	66 (40%)	22 (14%)
p53	68 (42%)	34 (21%)	60 (37%)

By multivariate analysis, strong expression of MDM4 was correlated to poor tumor differentiation (Odds ratio 4.4; 95% C.I. 1.26, 16.67; $P = 0.02$). No association was identified between MDM2, MDM4 and p53. Weak expression of MDM2 was associated with shorter survival time (Hazards ratio 2.06; 95% C.I. 1.16, 3.65; $P = 0.01$). In addition, poor differentiation was associated with shorter survival (Hazards ratio 2.1; 95% C.I. 1.38, 3.20; $P = 0.001$).

Conclusions: Strong expression of MDM4 correlated with poor differentiation, while weak MDM2 was associated with shorter survival. MDM2 and MDM4 were expressed by many GA (28% and 54%, respectively), emerging as potential targets for small molecule inhibitors currently being investigated in other neoplasms.

695 SDHB Staining in Sporadic GISTs: A Report from a Population-Based Italian Study (REGISTER Study).

S Rossi, L Toffolatti, D Gasparotto, G Gallina, E Scaramel, A Marzotto, L Messerini, I Bearzi, G Mazzoleni, C Capella, G Arrigoni, C Gnocchi, P Casali, R Maestro, AP Dei Tos. General Hospital, Treviso, Italy; C.R.O., Aviano, Italy; University School of Medicine, Firenze, Italy; University School of Medicine, Ancona, Italy; General Hospital, Bolzano, Italy; Ospedale di Circolo e Fondazione Macchi, Varese, Italy; Novartis Farma, Origgio, Italy; Istituto Nazionale Tumori, Milano, Italy; S. Raffaele, Milan, Italy.

Background: It has been recently reported that GISTs of Carney triad and a subgroup of pediatric GISTs show loss of expression of succinate dehydrogenase B (SDHB), a subunit of mitochondrial complex II, whose inactivation is involved in tumorigenesis. In order to understand whether SDHB inactivation is also involved in the pathogenesis of sporadic GISTs, we investigated SDHB expression in the REGISTER series.

Design: Immunohistochemistry for SDHB (1:750, pH9 WB, clone 21A11 0, ABCAM) was performed on tissue microarray from 712 GISTs including 2 pediatric cases and 7 NF1 cases. Tumour cells were considered positive only if there was granular cytoplasmic staining, even weak or focal, and reliable only in presence of an internal positive control. Molecular analysis for *KIT/PDGFRA* was performed in a fraction of SDHB-negative cases. Clinico-pathological features of this group were compared to the whole series.

Results: Among 700 assessable cases, immunostain for SDHB was negative in 22 (3.1%). Clinico-pathological features of the latter group are shown in Table 1. Interestingly, in the SDHB negative subgroup, median age was slightly younger. One of the 2 pediatric GISTs was SDHB negative. SDHB-negative cases occurred more often in stomach, featured a higher mitotic activity and more frequently a mixed/epithelioid morphology. Unexpectedly, males were more than females. No difference in median tumor size was found. None of the NF1 cases belonged to the SDHB negative group. Despite the high proliferation activity, GIST progression occurred only in 5 out of 22 cases (median follow-up of 7.5 years). Six out of 9 (66%) SDHB negative GISTs were *KIT/PDGFRA* WT.

Conclusions: Loss of SDHB is observed in a small fraction of sporadic GISTs, mainly WT. Possible prognostic and predictive implications should be investigated.

		Register series	SDHB negative cases
Age	Median	66.0	61.5
	Range	12-95	15-92
Gender	Male	56.5%	63.6%
	Female	43.5%	36.3%
Site	Stomach	60%	81.8%
	Other	40%	18.2%
Size	Median	5.5	5.0
	Range	0.1-50	0.6-16
Mitoses/50HPF	Median (range)	3.0 (0 - 262)	5.0 (0 - 146)
	Mean (+/-SD)	12.1 (28.73)	20.5 (35.9)
Cell morphology	Spindle	60.3%	50%
	Epithelioid	14.1%	22.5%
	Mixed	25.5%	27%

696 Mucosal Predominant Lymphoplasmacytic Inflammation May Be Associated with but Is Not Specific for Autoimmune and Distal Obstructive Biliary Pathology: An Analysis of 998 Cholecystectomies and Proposal for Diffuse Chronic Superficial Cholecystitis as the Category Designation.

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Background: Diffuse mucosal-predominant lymphoplasmacytic inflammation (MPLI), once considered specific for primary sclerosing cholangitis (PSC) and termed "diffuse lymphoplasmacytic sclerosing cholecystitis", has recently been proposed to be a marker of downstream obstruction, occurring also in "lymphoplasmacytic sclerosing" (autoimmune) pancreatitis (LPSP) and in patients with ampullo-pancreatic carcinomas (APC).

Design: Inflammation & other pathologic mucosal changes were evaluated in 998 cholecystectomies (34 PSC; 13 LPSP; 57 APC; and 894 ordinary chronic cholecystitis-OCC).

Results: Although MPLI appeared to be more closely associated with autoimmune conditions and distal biliary tract obstruction, detected in 16/34(47%) of PSC, 6/13(46%) of LPSP, and 28/57(47%) of APC, when all-comers were considered the most common cause of MPLI was non-specific CC (70/120, 58% of the MPLI cases) and this did not seem to be associated with lithiatic obstruction: 31/246 (12%) of non-obstructive calculous CC, 18/163(11%) of acalculous CC and 4/75(5%) of obstructive calculous CC showed MPLI. The mucosal changes accompanying MPLI had some clinical associations: **1)** *Atrophic form* with flattened mucosa, lighter inflammation, less activity, mucous gland hyperplasia were features of PSC; **2)** *Active type* with significant intraepithelial PMNs and mural thickening with inflammatory nodular-sclerosis were more in keeping with LPSP; **3)** *Hyperplastic changes* with elongated back-to-back folds with edematous tips showing lymphangectasia was more typical of APC. However, overlaps were common, and any of these patterns were ultimately seen more commonly in cases with OCC. IgG4 immunostain was helpful in identifying LPSP but not entirely discriminatory, with 3/13 APC and 3/32 OCCs showing >10/HPF IgG4+ cells.

Conclusions: Although MPLI is one of the prominent patterns of injury in patients with distal biliary tract obstruction and autoimmunity, it is by no means specific; most MPLI occur in association with non-obstructive ordinary CC. Therefore, the term lymphoplasmacytic sclerosing cholecystitis with its autoimmune implications may not be appropriate for this entity, and thus we propose the term diffuse chronic superficial cholecystitis (DCSC). In reporting of DCSC, we recommend documentation of the mucosal changes so that clinical correlative determination of the disease process can be initiated.

697 Is the "Pragmatic Approach" for *H. pylori* Diagnosis Effective?

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Background: Controversy exists over the most cost-efficient approach for *Helicobacter pylori* diagnosis. Since 2004, to reduce costs, our group routinely screened gastric biopsies with sections stained only with hematoxylin and eosin (H&E). Special stains (Giemsa, Warthin Starry, or immunohistochemistry) were performed at the discretion of the pathologist. The goal of this study was to evaluate the "pragmatic approach" in a surgical pathology setting. In addition, we sought to evaluate how having biopsies from only one site (either antrum or oxyntic) might affect the diagnostic accuracy.

Design: We randomly chose from our files biopsy specimens from 93 patients who had at least antral and oxyntic biopsies taken specifically for *H. pylori* diagnosis. All cases were interpreted during regular sign out following the "pragmatic approach". We then stained each block with a silver stain and *Helicobacter* immunohistochemistry stain. Slides were coded, randomized, and reviewed independently by four pathologists and one trainee. Observers had no information on the clinical status of the patients. We used

a visual analogue scale graded from 0 (absent) to 5 (maximum) to score *H. pylori* and inflammation on each slide. We did not consider any stain as the "gold standard" for identifying *H. pylori*. We considered a biopsy specimen positive when all observers agreed that bacteria with the characteristic morphologic features of *H. pylori* were present in at least one of the special stain slides.

Results: There were 28 *H. pylori*-positive and 65 *H. pylori*-negative patients. Following the "pragmatic approach" 3 of 28 (11%) *H. pylori* positive patients were interpreted as negative at regular sign out (sensitivity 89%). Most of these biopsy specimens had minimal chronic inflammation. *Helicobacter* were identified at both sites in 57% of patients, in the antrum only in 25%, and in the oxyntic mucosa only in 18%. Of the 65 *H. pylori*-negative patients one was interpreted as positive for the infection (specificity 98%).

Conclusions: When the "pragmatic approach" is used, pathologists tend to over-estimate the minimal amount of inflammation that can be seen with *H. pylori* infection. This suggests that pathologists need to lower their threshold of when to order special stains, or get special stains routinely. A second potential reason for under diagnosis of *H. pylori* could be when biopsies are only taken from one site. In this study, biopsies from the antrum or body only would have resulted in a significant false negative diagnosis. The "pragmatic approach" using H&E sections only, leads to inconsistent results..

698 Analysis of c-KIT Expression and *KIT* Mutations in Gastrointestinal Melanomas: A Multicentric Study from Istituto Toscano Tumori (ITT).

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Background: Malignant melanoma involving the gastrointestinal (GI) tract is mainly related to metastatic disease while primary mucosal melanomas are exceedingly rare. Despite multimodal therapeutic approach, primary GI melanomas are associated with a poor prognosis. Recently it has been emphasized that mucosal melanomas may harbour *KIT* mutations with increased frequency, thus allowing targeted therapies with tyrosine kinase inhibitors in affected patients.

Design: Forty-two GI melanomas observed over the past two decades were retrospectively analyzed. c-KIT protein expression was investigated by immunohistochemistry, whereas *KIT* gene mutations were analysed by PCR amplification and DNA sequencing of exons 11, 13 and 17. Seven cases with clear cell features were submitted to FISH analysis to exclude the presence of a t(12;22)(q13;q12) translocation (r/o clear cell sarcoma).

Results: There were 24 females and 18 males, ranging from 45 to 89 years of age. The most common anatomical location was the anorectal region (n=26), followed by the large bowel (n=5), small bowel (n=5), stomach (n=2) and oesophagus (n=2). Two patients presented with multiple lesions, involving different and/or distant gastrointestinal tracts and the omentum. Based on the morphological appearance and available clinical history, the cases were tentatively classified as primitive (n=8), metastatic (n=8) or uncertain (n=26). KIT cytoplasmic staining was demonstrated in 35 (83%) cases. Among the KIT-positive cases, 24 (57%) showed a percentage of positive cells greater than 51% and 28 (66%) displayed moderate to strong immunoreactivity. Nine cases (4 primary and 5 of uncertain origin) exhibited *KIT* gene mutations. Interestingly, none of the metastatic cases was mutated. With the exception of one case, tumours harbouring *KIT* mutations showed moderate to strong staining in a high percentage of cells.

Conclusions: Due to their rarity and lack of conventional histopathological prognosticators, GI melanomas present a difficult diagnostic and therapeutic challenge. Our preliminary results of this ongoing study support the finding that *KIT* mutations presumably activating the tyrosine kinase activity of c-KIT can be found in a subgroup of GI melanoma patients who may benefit of tyrosine kinase inhibition. Immunohistochemical expression of c-KIT can be considered as a useful tool to select cases for additional *KIT* genotyping.

699 Analysis of Full-Thickness Gastric Body Sections: Normal Values for Gastric Motility Disorders.

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Background: Due to growing interest in gastrointestinal motility and neuromuscular disorders, the Gastro 2009 International Working Group (IWG) created guidelines for assessing histologic sections in these patients. However, recommendations originated from studies of small intestinal and colonic specimens. The aim of this study was to examine full thickness gastric body sections from patients without symptoms of gastroparesis, as controls.

Design: Full-thickness H+E sections from the gastric margin, approximately 8 cm proximal to the pylorus, were evaluated from 17 patients who underwent a Whipple procedure at Temple University Hospital between 2005 and 2010. There were 17 patients (11 male, 7 female); average age of 60.2 (range 40 to 76) years; 7 patients had diabetes mellitus, type II. Masson trichrome stain and immunohistochemical stains for CD45, CD3, CD20, CD68, NSE, S-100, GFAP and c-kit were also performed. Sections were evaluated for fibrosis, ganglia, ganglion cells, interstitial cells of Cajal (ICC), inflammatory cells and glia in the muscularis propria.

Results: All 7 diabetics had mild fibrosis of the muscularis propria. B lymphocytes were confined to the mucosa; 3 patients had >5 periganglionic T lymphocytes (IWG criteria for myenteric ganglionitis); 2 intraganglionic T lymphocytes were noted in one specimen. In the intermyenteric plexus (IMP), there were 1.0±0.4 (SD) ganglia/HPF with 2.3±1.9 (SD) ganglion cells/HPF. S-100 showed strong staining of the Schwann cells; GFAP staining showed very focal weak positivity in close proximity to ganglia in two cases; c-Kit staining revealed 3.7±2.6 (SD) ICC in the inner circular layer, 1.3±1.1 (SD) ICC in the outer longitudinal layer, and 1.0±0.7 (SD) ICC in the IMP. There was no difference between diabetics and non-diabetics in number of ICC in any layer.

Conclusions: These results provide control data for the evaluation of tissue from patients with gastroparesis. Further studies to detect glial cells by immunohistochemical staining are necessary. Myenteric ganglionitis in 3 specimens may be due to inflammation secondary to prolonged surgery, as there was a prominent infiltrate of neutrophils and macrophages in several cases. ICC control data is presented; however, the wide standard deviation indicates patchy distribution of these cells in the gastric body. The lack of difference in ICC distribution in diabetic and non-diabetic patients suggests that some trigger may play a role resulting in decreased ICC in diabetic gastroparetic patients, or, the decreased ICC in diabetic gastroparetic patients may be dependent on the portion of stomach sampled.

700 Immunohistochemical Staining for DNA Mismatch Repair Proteins in Intestinal Tract Carcinoma: How Reliable Are Biopsy Samples?

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Background: In recent years, immunohistochemistry (IHC) has emerged as an efficient tool in the detection of DNA mismatch repair (MMR) proteins in colorectal cancer (CRC). At the current time, the IHC test is mainly applied to CRC resection specimens. Detection of MMR abnormality at the biopsy stage carries obvious clinical importance as it would allow more informed decision about the extent of surgery (segmental resection versus total colectomy, prophylactic hysterectomy etc.) and, in the case of treated rectal carcinoma with no residual tumor, a means for MMR IHC. However, whether biopsy samples can be used reliably or not remains to be determined.

Design: Paired biopsy and resection specimens of adenocarcinomas of the gastrointestinal (GI) tract were analyzed for IHC staining patterns for MLH1, MSH2, MSH6 and PMS2. Abnormal staining was defined as total loss of protein in the tumor with appropriate controls. Cases with focal and weak staining, defined as staining present in <10% of the tumor with weak staining intensity, were recorded.

Results: Of a total of 70 GI tract cancers (3 from small bowel, 36 right colon, 15 left colon, and 16 ano-rectum), both biopsy and resection specimens detected the same 29 patients as having loss of staining for at least 1 MMR protein, 14 affecting MLH1/PMS2 and 15 affecting MSH2/MSH6. Focal and weak staining was most commonly seen for MLH1 stain in biopsy specimens (4/70, 6%), followed by MSH6 stain in biopsy specimens (3/70, 4%). Concordant staining patterns between biopsies and resections were reached in all 70 cases for MSH2 and PMS2, whereas discordant patterns were identified in 3 cases (3/70, 4%) for MLH1 and in 2 (2/70, 3%) for MSH6. None of the discordant patterns affected the final interpretation of whether MMR IHC was normal or abnormal in either the biopsy or the resection.

Conclusions: This study provides data suggesting that biopsy samples are as reliable as resection in the immunohistochemical detection of MMR protein abnormality in intestinal cancers. Our study also illustrates the various staining variations that can occur in both biopsies and resections. Awareness and further understanding of such staining variabilities will enhance the utility of IHC, a commonplace tool that is being increasingly used in the screening workup for Lynch syndrome.

701 Evaluation of Esophageal Biopsies for Squamous Intraepithelial Neoplasia: Differences between Japan and the West.

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Background: Compared to dysplasia in Barrett esophagus, limited attention is given to squamous intraepithelial neoplasia (IEN). Also, variation in the evaluation of early squamous neoplasms between Japanese and Western observers has been noted. Our aim was to test the Western and Japanese's evaluations of esophageal squamous neoplasia by comparing grading between these two groups.

Design: Digitalized H&E slides from 50 esophageal biopsies covering all recognized grades from benign to malignant lesions were circulated among 5 Japanese and 5 Western pathologists. The participants classified the lesions as: negative or indefinite for neoplasia, low- or high-grade IEN or squamous cell carcinoma (including probably invasive). Consensus diagnoses were defined as the opinion of the majority and interobserver variability was assessed by kappa statistics.

Results: A majority diagnosis (in which more than 6 agreed) was reached in 21 cases (42%). Seven more cases had a majority diagnosis when high-grade IEN and squamous cell carcinoma where combined. A unanimous diagnosis was reached in only 5 cases (all benign). Within the Japanese group, the overall level of agreement was moderate to almost perfect (kappa:0.54 to 0.9). The intragroup agreement among Western pathologists was moderate to good (kappa:0.5 to 0.79). The intergroup agreement between Japanese and Western observers was moderate to excellent (kappa:0.41 to 0.81) Eight cases diagnosed as low grade IEN by a majority of Western pathologists were diagnosed as at least high grade IEN by a majority of Japanese observers. The Japanese pathologists as a group diagnosed 45% of cases a category higher than Western pathologists. After excluding the 5 cases with perfect agreement, the average increase in consensus diagnosis between Japanese and Western pathologists equaled one full grade higher.

Conclusions: Japanese pathologists tend to diagnose esophageal squamous IEN more aggressively than Western pathologists. The interobserver variability is caused by the lack of universally accepted criteria. An international effort is needed to harmonize the criteria and analyze possible therapeutic implications.

702 The Diagnostic Utility of Hepatocyte Antigen, GPC3, and IMP3 in Distinguishing between Hepatocellular Carcinoma and Benign Hepatic Lesions.

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Background: Histopathologic distinction between hepatocellular carcinoma (HCC) and benign hepatocellular adenoma (HA) and focal nodular hyperplasia (FNH) can sometimes be challenging, especially in small biopsy samples. Hepatocyte Specific Antigen (HSA) staining has been demonstrated consistently in the vast majority of HCC. More recently, insulin growth factor messenger RNA binding protein 3 (IMP3) expression has been identified in multiple malignant neoplasms including HCC. Glypican-3 (GPC3), a cell surface proteoglycan have been shown to be overexpressed in HCC, but not in HA and FNH. The aim of this study is to determine the usefulness of these markers in the differential diagnosis of hepatocellular mass lesions.

Design: 29 surgical resected or biopsied specimens of well- to moderately- differentiated HCC (25 resections, 5 biopsies), eight HA (all resections) and twenty one FNH (15 resections, 6 biopsies) were obtained from University of Ottawa Medical Center. Immunohistochemistry was performed using HSA (Abcam), IMP3 (Abcam) and GPC3 (Santa Cruz). Cytoplasmic staining was considered positive for HSA and IMP3 and cytoplasmic and/or membranous staining was considered positive for GPC3. The percentage of positively stained tumor cells was recorded and the staining intensity was graded as weak (1+), moderate (2+), or strong (3+).

Results: Strong 2+ to 3+ HSA reactivity was detected in 20 (95.2%) of FNH, all HA, and 26 (89.7%) of HCC. Staining for IMP3 was observed in 20 (95.2%) of FNH and all HA and all HCC. IMP3 showed a stronger (3+) staining at the periphery of the tumors. GPC3 was negative in all FNH and HA whereas 20 (69%) HCC showed strong (3+) reactivity for GPC3. Neither HSA nor IMP3 could differentiate between the 3 lesions. However, GPC3 could distinguish HCC from either HA ($p=0.001$) or FNH ($p<0.001$).

Conclusions: IMP3 (Dako) was previously reported as being positive in HCC and negative in HA and non-neoplastic hepatic tissues. However in our study all HA and the vast majority of FNH expressed IMP3.

Our findings suggest that 1) Immunohistochemical detection of GPC3 but not IMP3 is a useful diagnostic tool in segregating well differentiated HCC from HA or FNH, in particular when limited material from a needle biopsy is evaluated. 2) GPC3 expression in HCC can be focal, and thus, the lack of GPC3 staining does not exclude the diagnosis of HCC. 3) IMP3 may be more frequently expressed in HCC than HSA. 4) Morphology is still the gold standard in separating FNH from HA. Larger studies are needed for further validation of these findings.

703 Abnormal Cytoplasmic Localization of p27 (KIP) Protein Is Associated with Poor Survival in Gastric Carcinoma.

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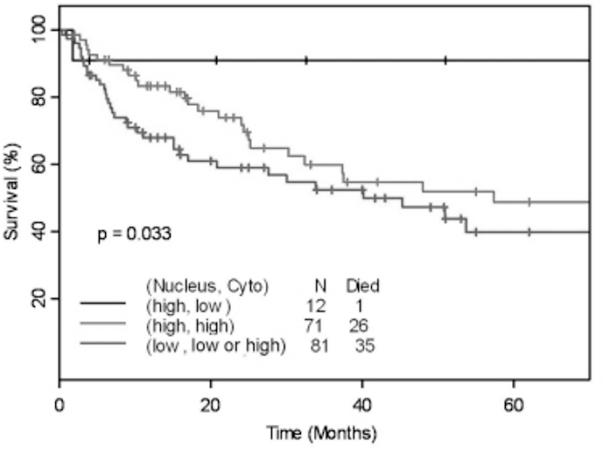
Background: The p27(Kip1) protein is a negative cell-cycle regulator and a potential tumor suppressor gene. Reduced nuclear p27(Kip1) protein expression is associated with increased mortality in gastric carcinoma. Recent studies have suggested that p27 phosphorylation-mediated cytoplasmic localization can confer a novel oncogenic role for the p27protein. We have demonstrated that *H. pylori* promotes cytoplasmic p27 expression and p27 phosphorylation at T157 and T198 in gastric cancer cells in vitro. Our aim was to investigate p27 protein expression in gastric carcinoma and to correlate its expression with patient outcome.

Design: Tissue microarrays were constructed for 164 cases of gastric carcinoma involving the antrum or body excluding the cardia. The extent (percentage) & intensity (0-3) of immunohistochemical p27 expression was assessed in the nucleus and cytoplasm of the tumor cells.The stage, tumor subtype and 5-year survival were noted from Hospital Cancer Registry data.

Results: Normal gastric mucosa showed only nuclear p27 expression in the lower 1/3-1/4 of the glandular crypts with no cytoplasmic p27 expression. Regression analysis identified 3 groups of p27 expression: 1) ↑ nuclear, ↓ cytoplasmic, 2) ↑ nuclear & ↑cytoplasmic, & 3) ↓ nuclear & ↑ or ↓ cytoplasmic. There was a significant difference in overall survival in these groups($p=0.033$). Group 3 with ↓ nuclear p27 expression had the worst survival. Group 1 with ↑ nuclear & ↓ cytoplasmic p27 expression had the best overall survival. Group 2 with both ↑ nuclear and ↑ cytoplasmic p27 protein also had a poor survival, similar to group 3. In multivariate analysis cytoplasmic P27 expression was an independent predictor of survival.



Conclusions: Gastric carcinoma cells exhibited abnormal p27 protein expression including loss of nuclear and gain of cytoplasmic p27 protein. We confirmed that low nuclear p27 expression is associated with poor survival and demonstrated for the first time that high cytoplasmic p27 expression is also independently associated with poor survival in gastric carcinoma.

704 PCR Based Detection of B1/NAP-1/027 Strain of Clostridium Difficile: Decrease in Prevalence and Clinical Severity?

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Background: Clostridium difficile (C. difficile) infections are an increasing cause of healthcare associated infections. The B1/NAP-1/027 (PFGE type B1/NAP1, also called ribotype 027) strains carry mutations in the negative regulator tcd C gene, leading to greatly increased toxin A and B production (up to 20% more) with pronounced colonic injury. Though this strain has been associated with the rise in C. difficile associated disease (CDAD), recent reports in Europe indicate that the prevalence is actually decreasing. We report on the prevalence and clinical features of this strain in our population.

Design: We developed a PCR based Light Cycler assay to detect this mutation on 1,979 C. difficile PCR positive samples. Additionally, we examined tissue and stool samples from 24 patients with a histologic diagnosis of pseudo-membranous colitis. The overall morbidity and mortality of the mutated strain is compared to the non-mutant strain.

Results: There were 544 C. dificile PCR positive cases in 2008, 984 in 2009 and 451 cases to date in 2010. The percentage of positive C. difficile cases is 10 %. The percentage of the ribotype 027 strain in the PCR positive C. difficile cases is 9% for each year since 2008. The mortality rate of the non-ribotype 027 strain group was 11% which was significantly higher than the ribotype 027 group. Additionally, There were no ribotype 027 strains detected in tissue or stool samples patients with pseudo-membranous colitis.

Conclusions: The prevalence of C. difficile B1/NAP-1/027 strains is stable and is not increasing in the Central Texas population. Additionally, this strain is not associated with greater mortality and morbidity compared to the wild-type strain.

705 Duodenal Serrated Adenomas: Evidence for Serrated Carcinogenesis in the Proximal Small Intestine.

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Background: Primary sporadic duodenal serrated adenomas (SA) are extremely rare. The clinicopathologic and molecular features of sporadic (non-FAP associated) duodenal SA have never been reported. The aim of this study was to evaluate the morphological, immunohistochemical and molecular features of sporadic duodenal SA, and to compare them to conventional tubular adenomas (TA) of the duodenum.

Design: 10 patients, each with a sporadic duodenal SA, were identified via a 5 year search through the pathology files of 3 academic hospitals. Clinical and endoscopic information was obtained by chart review. Immunostaining for MLH-1, MGMT, p53, beta-catenin, SMAD4 and Ki-67 was performed in all cases. DNA from formalin-fixed tissue was analyzed in 7 TaqMan PCR allelic discrimination assays for detection of common *KRAS* mutations and in two allele-specific SYBR Green PCR assays for detection of *BRAF* V600E mutation. 14 consecutive conventional TA (11 sporadic; 3FAP) were analyzed as a comparison (control) group.

Results: The 10 patients included 5 males and 5 females, mean age 70.5 yrs, with sessile or pedunculated polyps in the first (n=4) or second (n=6) part of the duodenum. Size range was 0.8-4.6cm. All duodenal SA were morphologically similar to traditional SA of the colon. 8 duodenal SA showed low grade dysplasia and 2 showed high-grade dysplasia (HGD), 1 of which also had an invasive adenocarcinoma.

Nuclear staining for MLH-1, MGMT and SMAD4 was intact in all cases (100%). p53 overexpression was not identified in any polyp (0%). A high proliferative index (>50% positive cells) was present in 1 case (10%) associated with carcinoma. Nuclear beta-catenin was present in 1 duodenal SA (10%) in an area of HGD. *KRAS* mutations [(G12D (n=4); G13D (n=1)] were present in 5/10 (50%) duodenal SA, and a *BRAF* V600E mutation was detected in 1 case (10%).

Compared to duodenal SA, conventional TA were present in younger patients (mean age; 62 yrs), more often involved the 2nd or 3rd part of duodenum (86%), and showed significantly higher nuclear beta-catenin staining (86%; p=0.001), higher proportion of cases with >50% proliferative index (93%; p<0.0001) and a lower frequency of *KRAS* mutation (7%; p=0.009).

Conclusions: This study provides evidence that the serrated pathway of carcinogenesis is also involved in the development of a subset of primary duodenal carcinomas. Duodenal SA are morphologically and molecularly distinct from conventional TA of the duodenum.

706 An Oncofetal Protein IMP3, a New Molecular Marker for the Detection of Dysplasia and Carcinoma of the Gallbladder.

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Background: Gallbladder adenocarcinoma is a very aggressive cancer with a 13% 5-year survival rate. Clinically, it is crucial to recognize epithelial dysplasia, a premalignant lesion in the gallbladder. However, distinguishing dysplasia from reactive atypia in cholecystectomy specimens often presents a diagnostic challenge to pathologists. A reliable biomarker that can unambiguously identify dysplasia from reactive changes will be very useful for histological diagnosis of dysplasia and adenocarcinoma. IMP3, an oncofetal protein, plays an important role in carcinogenesis in different cancers. Several studies have shown that IMP3 is a cancer specific biomarker. The aim of this study was to establish the expression pattern and diagnostic value of IMP3 in dysplasia and carcinoma of the gallbladder.

Design: A total of 95 cases (cholecystectomy specimens) with 139 lesions (invasive adenocarcinoma, n=25; high grade dysplasia, n=8; low grade dysplasia, n=26; benign gallbladder, n=80) obtained from the surgical pathology files of a tertiary Medical Center were examined by immunohistochemistry for IMP3 expression. ≥5% of epithelial/tumor cells stained with IMP3 antibody was considered as positive staining. Focal staining was defined as IMP3 positivity in 5-50% and diffuse staining as IMP3 positivity in >50% of lesional epithelia/glands.

Results: IMP3 showed dark brown cytoplasmic staining. IMP3 expression was found in 24/25 (96%) of invasive carcinomas, 8/8 (100%) of high grade dysplasia, and 23/26 (88%) of low grade dysplasia. Diffuse IMP3 positivity was found in 18/25 (72%) of cases with invasive carcinoma, 6/8 (75%) of cases with high-grade dysplasia and 9/26 (35%) of cases with low grade dysplasia. In contrast, all benign gallbladder (n=45) and benign epithelia adjacent to the cancer or dysplasia (n=35) were negative for IMP3 staining.

Table 1

	# Categories	Positive, diffuse	Positive, focal	Negative
Invasive	25	18(72%)	6(24%)	1(4%)
High-grade dysplasia	8	6(75%)	2(25%)	0(0%)
Low-grade dysplasia	26	9(35%)	14(54%)	3(12%)
Benign epithelia	80	0(0%)	0(0%)	80(100%)

Conclusions: IMP3 is highly expressed in dysplasia and adenocarcinomas of the gallbladder but not in benign epithelia. The data suggest that IMP3 may play an important role in malignant transformation in gallbladder cancer. IMP3 is a highly sensitive and specific biomarker for the detection of dysplasia and carcinomas of the gallbladder. Positive staining for IMP3 can increase the level of confidence in establishing a definitive malignant/dysplasia diagnosis of the gallbladder.

707 Fatty Acid Synthase Overexpression in Gastrointestinal Stromal Tumor (GIST): Emphasis on Prognostic and Therapeutic Relevance.

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Background: Fatty acid synthase (FASN), a critical enzyme catalyzing synthesis of palmitate into long-chain saturated fatty acids, is frequently overexpressed in a variety of aggressive cancers, including high-risk and metastatic GISTs. In vitro targeting of FASN has shed light on its potential therapeutic application in some carcinomas. However, little is known about the associations of FASN expression in GISTs with clinical outcomes, *KIT* and *PDGFRA* receptor tyrosine kinase (RTK) genotypes, and the efficacy of treatment with pharmacological inhibitors.

Design: FASN immunostain was performed on tissue microarrays of primary GISTs, generating 370 cases with interpretable results. Of these, RTK genotypes were determined in 223 GISTs by sequencing with or without screening by DHPLC. FASN mRNA was quantified for 40 independent fresh samples by real-time RT-PCR coupled with laser microdissection. The expression levels of FASN mRNA and immunostain were correlated with clincopathological variables and disease-free survival (DSS). GIST48 and GIST430 cell lines, both imatinib-resistant and FASN-expressing, were subjected to XTT, Western blotting, and caspase-3/7 activity assays to evaluate their susceptibility to FASN inhibitor (Orlistat).

Results: FASN overexpression was present in 100 (27%) GISTs and significantly associated with epithelioid morphology, unfavorable genotypes, larger tumor size, and higher mitotic count, NIH risk category, and Ki-67 labeling index (LI) (all p≤ 0.001). In multivariate analysis, FASN overexpression independently portended inferior DFS (risk ratio [RR]=1.735, p=0.0226), along with intermediate- (RR=2.782) or high-risk (RR=4.816) category (p<0.001), high Ki-67 LI (RR=3.125. p<0.001), non-gastric site (RR=1.930, p=0.008), and unfavorable genotypes (RR=1.763, p=0.0260). The endogenous FASN mRNA expression was significantly more abundant in high-risk GISTs than the non-high-risk counterpart (p=0.0007). In both GIST48 and GIST430 cells, treatment with Orlistat at a concentration of 300 uM could remarkably result in activation of caspases and apparently declined cell viability.

Conclusions: FASN overexpression in GISTs not only confers clinical aggressiveness but also represents a potential alternative therapeutic target in high-risk, imatinib-resistant cases.

708 Proposal for a More Applicable and Clinically Relevant Staging Evaluation of Ampullary Carcinomas.

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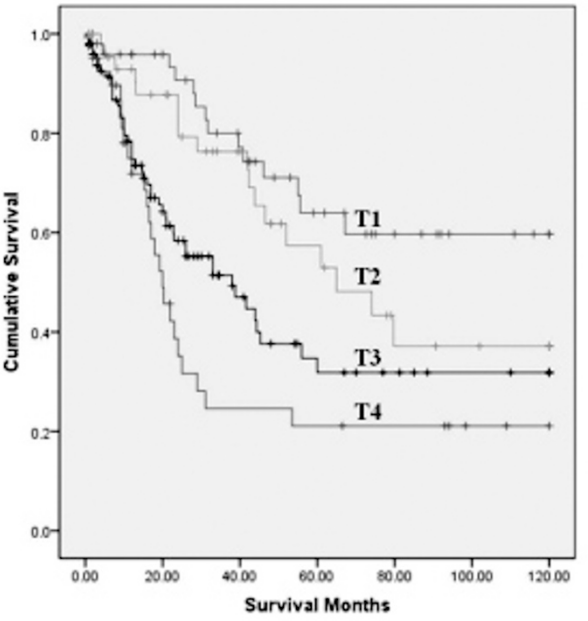
Background: Due to its anatomic complexity and lack of uniform definitions, staging of ampullary carcinomas (ACs) is highly problematic, as evidenced by the survival data provided within the AJCC-2010 manual, which shows reverse prognosis for T1 vs T2. Since ACs tend to have a large preinvasive component, the importance of size of the invasive component may have been underestimated.

Design: A revised staging system was devised by 1) modifying the problematic aspects of T-stage, and 2) by incorporating invasion size (i-size). Since the current distinction between AJCC T1 vs T2 is highly subjective and sampling-protocol dependent, we classified these early tumors confined to muscle (Oddi/duodenal musculature complex) based on size: T1, < 1 cm and T2, ≥ 1 cm. We defined T3 as spread beyond the muscles into either the periduodenal soft tissue (at the groove area) or < 0.5 cm into the pancreas, and/or i-size of ≥ 2 cm but < 4 cm. T4 was defined as invasion (perforation) of duodenal serosa, deep (> 0.5 cm) invasion into the pancreas, and/or i-size ≥ 4 cm. This staging scheme was tested in 249 well-characterized and strictly defined ACs.

Results: The AJCC stage rendered in the original pathology report was different than the AJCC stage assigned in our study in 37% of the cases, highlighting the challenges in employing AJCC, while our proposed staging system was applied with relative ease, regardless of sampling protocol. Moreover, our proposed system has strong prognostic value (p<0.0001; Fig.1).



Conclusions: Current AJCC-2010 T-staging of ACs is difficult to apply and prognostically irrelevant. Our modified stage is relatively simple for practicing pathologists to use, circumvents anatomic complexities of the region and the corresponding sampling protocol differences, and incorporates biologic characteristics of ACs. Most importantly, it has very strong prognostic value.

709 Crypt Apoptotic Count Reproducibility in Small Bowel Allograft Biopsies.

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Background: Enumeration of crypt apoptotic bodies (AB) is one feature in the schema for the histologic diagnosis of acute cellular rejection (ACR) in small bowel allografts, with ≥6 AB in the proper background qualifying as mild ACR. Similar cases with fewer AB are often called indeterminate. When a quantified variable is used for diagnosis, clear definition and reproducibility are important. Some consider only well-formed "classical" exploding crypt cells as AB while others are more "liberal" in their descriptions, including clusters of pyknotic debris. Due to differing definitions, there is a potential for variation in AB numbers between pathologists that may impact diagnosis. To our knowledge, this is first study specifically examining intra- and interobserver reproducibility in AB counts.

Design: Thirty mucosal biopsies from small bowel allografts were obtained, selected by original diagnosis: 10 screening biopsies negative for ACR, 10 indeterminate, and 10 mild ACR. Three transplant pathologists blindly reviewed each case twice with randomization before each read. They were given written and pictorial descriptions of classical and liberal AB and counted the maximal number per 10 epithelial cells.

A difference of >2 AB qualified as discrepant. "Diagnoses" (assuming the proper histologic background) were characterized by AB count as follows: negative-0-2, indeterminate-3-5, and mild ACR-≥6.

Results: Intraobserver kappa values for overall classical and liberal AB counts ranged from 0.749-0.914 and 0.814-0.933, respectively and interobserver kappa values were 0.478 and 0.686 (P<.05, each). Intraobserver diagnosis agreement based on classical AB number occurred in 79 of 90 instances (88%) and in 57 of 90 using the liberal definition (63%, P<.01). Total interobserver diagnosis agreement using an average of both individuals' counts based on the classical AB description was reached in 9 of the 10 negative, indeterminate, and mild ACR cases. Using the liberal definition, total diagnostic agreement occurred in 9 negative, 6 indeterminate, and 7 mild ACR cases (P=0.13).

Conclusions: There was substantial intraobserver and moderate to substantial interobserver agreement in overall crypt AB counts using classical and liberal definitions. Slightly better agreement was seen using the latter. However, both intra- and interobserver variability would have led to diagnostic discrepancies in a small subset of cases. Better diagnostic agreement was achieved when counting classical AB.

710 Potential Signature Molecular Biomarkers of Prognosis of Gastric Adenocarcinoma: A Study of 114 Cases Using Genome-Wide Technique and FISH.

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Background: Accumulated evidence suggests that multiple genetic alterations are involved in the complex carcinogenetic process of gastric adenocarcinoma (GAC). Although a number of genetic changes has been reported in GAC, including amplification of *CMET and FGFR2*, mutation of *E-cadherin and KRAS*, and loss of heterozygosity on 5q and 18q, the molecular events leading to GAC and its progression remain largely unknown. To assess global molecular changes in GAC, we use whole genomic assay to evaluate human GAC samples.

Design: Oligonucleotide array comparative genomic hybridization (aCGH) was performed on 46 GAC samples using a high-density (244K) aCGH system (Agilent Technologies). For each aCGH probe, each sample was classified as having normal, gained, or lost DNA copy number based on log 2 ratio thresholds of 0.15. An independent set of tissue arrayed samples (n=68) was further validated by fluorescent in-situ hybridization (FISH) by using probes visualizing 19q13.3 (red signal) and the centromere (green signal). Amplification of 19q13.3 was defined if the ratio of 19q13.3 to centromere is greater than 2.2. The mean patient's survival follow-up time was 58 months.

Results: aCGH identified 1271 genes with DNA copy loss and 1449 genes with DNA copy gain in gastric cancer. Among these identified genes, 11 deleted and 198 amplified genes were observed to have significant association with patient's survival. Forty-eight of amplified genes were specifically located on chromosome 19q13.3, including *CRX, DACT3, DKKL1, EHD2, EMP3, HIF3A, HRC, IGFL2, IGFL3, KPTN, LIG1, PNKP,* and *PTOV1*. Compared with all other patients, those (n=14) with gene amplification on 19q13.3 had a significantly poorer prognosis (p<0.01), independent of other conventional prognosis factors including TNM stage. These results were further confirmed by FISH method and amplification of 19q13.3 was identified in 18 cases with unfavorable clinical outcome.

Conclusions: This genome-wide study identified a panel of critical genes associated with progression of GAC. Amplification of the genes on chromosome 19q13.3, a possible signature event in gastric carcinogenesis, represents a potentially useful prognostic biomarker for this aggressive malignancy. Further functional studies are needed to confirm the potential value of these genes in the management of gastric cancer.

711 Osteoclastic Giant Cell Tumor of the Pancreas: Clinicopathological Correlation Study of Twelve Cases.

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Background: Osteoclastic giant cell tumor of the pancreas (OGCTP) is a rare subtype of pancreatic cancer. It has been variably described in case reports as pancreatic cancer with pleomorphic, anaplastic, spindle cell, undifferentiated or mixed histology. Clinical and pathologic data regarding this tumor are very limited.

Design: We conducted a retrospective review of patient records with the above histologies at a single institution from 1994 to 2009. Archival pathology specimens were independently reviewed by two surgical pathologists (WP, DT).

Results: Twelve patients with OGCTP were identified. Demographics: 9 males, median age = 66 (range 49-78), 10 Caucasian, 6 smokers. Median CA 19-9 was 70 IU/mL (range = 1.0-28,373). Site: 6 located in the body or tail, 5 locally advanced, 6 metastatic. For most of the cases (10/12), a large, locally infiltrative mass was frequently seen on imaging studies. Pathologic examination revealed a histologically distinct entity consisting of a heterogeneous but characteristic mixture of bland osteoclast-like giant cells, mononuclear ovoid to spindle cells, large pleomorphic cells with bizarre nuclei, and a more typical adenocarcinoma component. This pattern was seen in all of the examined specimens. Treatment: 2 underwent surgical resection, 7 received gemcitabine or fluoropyrimidine chemotherapy, 3 received radiation. Response: Stable disease or partial response in 4 patients, 1 with long-term response to capecitabine. Median survival of 16.5 months (range = 2-58) was higher than usually noted historically with adenocarcinoma.

Conclusions: This is the largest reported series of OGCTP and identifies this tumor as a distinct clinical and pathological entity. We are conducting additional pathologic studies to further characterize OGCTP.

712 An Objective Assessment of Ki67 Proliferative Index Quantification in Gastroenteropancreatic Neuroendocrine Tumors (NETs).

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Background: The pathologic prediction of prognosis of NETs is challenging. The proliferative rate is important for prognosis and is often assessed using Ki67 immunohistochemistry. A limitation to Ki67 assessment is the lack of uniformity and consistency in quantification. This is accentuated in well-differentiated NETs since differences of 1-3% can change the tumor grade with resultant treatment implications.

Design: Different Ki67 scoring techniques included: a) digital image analysis (DIA), b) manual counting (MC) of >2000 cells, c) "eyeballed" estimate (EE). 20 pathologists were involved in EE assessment. 45 Ki67 images were selected and analyzed as above. Statistical analyses were performed to evaluate: a) the concordance between methods, b) intra- and inter-observer consistency, and c) correlation of Ki67 scores with the NET grades. Agreement between scores was assessed by intraclass correlation (ICC).

Results: DIA and MC exhibited a concordance of ICC=0.98. ICC between DIA and EE on average was 0.88. Discordance occurred between observers on all cases quantified by EE (ICC 0.13). The intra-observer consistency ICC was 0.39 ± 0.26. Kappa statistic of EE demonstrated low to moderate agreement on all tumor grades proposed by ENETS (Table 1). EE was 93%±2 correct with Ki67 <1%, and 55%±7 with Ki67 of 2-3%. Incorrect assessment resulted in upgrading of all G1 group tumors (n=14); in the G2 group, downgrading of 41% occurred (n=11) when Ki67 was <5% and upgrading of 59% occurred (n=16) when Ki67 was >5%.

Table 1. Correlation of EE Assessment of Ki67 and Tumor Grades (using DIA as gold standard)

	ICC	Kappa Value
All Grades (29)	0.13 (CI 0.05-0.37)	0.24 (CI 0.23-0.25)
Ki67 ≤ 2%, (ENETS G1), n=14	0.01 (CI 0-0.96)	0.12 (CI 0.09-0.16)
Ki67 3-20%, (ENETS G2), n=29	0.13 (CI 0.04-0.39)	0.20 (CI 0.07-0.26)
Ki67>20%, (ENETS G3) n=2	0.05 (CI n/a)	0.13 (CI n/a)

Conclusions: DIA and MC are the gold standards for Ki67 assessment. Given the inherent discordance in determining the grade (especially for NETs bordering between low and intermediate grade), the use of an "eyeballed" Ki67 index alone requires critical reevaluation. Determination of therapeutic strategies should be guided by an amalgamation of clinicopathological characteristics of an individual tumor.

713 Expression of Aldehyde Dehydrogenase in Dysplastic Lesions Arising from Inflammatory Bowel Disease.

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Background: The cancer stem cell hypothesis proposes a relatively small population of neoplastic cells have the unique ability to initiate and sustain tumoral growth. Detection of cancer stem cells has been performed with a variety of markers, most notably aldehyde dehydrogenase (ALDH), responsible for catalyzing the irreversible oxidation of aromatic aldehydes to their corresponding carboxylic acids. Prior work has shown increased ALDH expression in carcinomas is an independent predictor of poor prognosis. We hypothesize cancer stem cells are present in pre-neoplastic lesions as well, and examined this within the context of dysplasia arising from inflammatory bowel disease (IBD). The distinction between inflammatory change and dysplasia can be difficult, and has significant patient management implications. Increasing levels of ALDH may help identify dysplasia, and confirm the role of stem cells in cancer progression.

Design: Fifty-four surgical resections of IBD were studied. All diagnoses were confirmed by at least two experienced pathologists. The diagnostic categories were as follows: 13 high-grade dysplasia / adenocarcinoma, 19 low-grade dysplasia, and 22 inflammatory atypia / negative for dysplasia. Immunohistochemical staining for ALDH was evaluated in the cytoplasm of epithelial cells both on intensity (0-3+), and percentage of cells staining. Criteria for positive staining was modeled after previous work, requiring at least 1+ staining in >10% of cells to be considered positive.

Results: Positive staining was seen in 100% of cases with high-grade dysplasia / adenocarcinoma (13/13), and 95% (18/19) of cases with low-grade dysplasia. Staining was homogeneous in the cytoplasm of dysplastic mucosa. Adjacent benign mucosa showed patchy focal positivity mainly in the crypt base. Staining of invasive adenocarcinoma was strongest in the advancing front. Cases with inflammatory atypia / negative for dysplasia showed positive staining in 32% (7/22) of cases. The sensitivity and specificity of ALDH for dysplasia was 97% and 68%, respectively.

Conclusions: Our study demonstrates ALDH is significantly expressed in dysplastic lesions arising from IBD. ALDH-expression in cancer stem cells suggests an important causative role in the progression to cancer in IBD. Although we found high sensitivity for dysplasia, the specificity was poor. In addition to neoplasia, ALDH-expressing stem cells proliferate in response to chronic inflammation, accounting for the cases of inflammatory atypia with positive ALDH expression.

714 Morphological and Molecular Evidence for Target Transformation during the Evolution of Gastric Carcinoma (GC).

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Background: GC is a heterogenous disease with several epidemiologic, anatomic, and histopathologic characteristics. However, it continues to be grouped and treated as a single malignancy. The recognizable morphologic alteration in GC may be evidence for pathogenic or molecular transformation to more aggressive disease. We investigated the correlation of morphologic evolution and biomarker expression in GC subtypes.

Design: 72 GCs with heterogenous features were evaluated and grouped by specific morphologic variants: 1) moderately differentiated (MD) tubular carcinoma, 2) poorly differentiated (PD) tubular carcinoma, 3) diffuse carcinoma, and 4) others (Table 1). 136 foci with designated morphologic subtypes were investigated by immunohistochemistry on tumor sections and on tissue microassays. Immunoreactivity was interpreted based on features of individual antibodies.

Table 1 Biomarker Expression in Morphologic Variants of Gastric Carcinoma

	p-MET	EGFR	IGFR	VEGF	p-ERK	p53	E-Cad Loss
All Variants (136)	40%	15%	25%	25%	45%	25%	25%
MD Tubular (67)	63%	60%	78%	87%	55%	42%	17%
PD Tubular (41)	35%	25%	13%	7%	23%	30%	37%
PD Diffuse (26)	2%	15%	9%	6%	22%	22%	44%
Other (2)					2%	6%	2%

Results: Uniform expression/loss of any biomarkers in individual tumors was <10%. Growth factors and receptors (including Her2Neu) were expressed in significantly higher levels in MD tubular carcinomas (P<0.005); and they were markedly decreased in diffuse type when compared with tubular type as one entity (P<0.001). When individual markers were assessed in cases with biphasic morphologic subtypes present in the same tumor, 71% p-Met, 72%, IGFR, and 74% VEGF expression were lost in the PD component whereas they were expressed in the corresponding better differentiated component (p<0.01). E-cadherin loss was strongly associated with PD carcinoma of either tubular or diffuse subtype (p<0.001). There was no significant differential expression of p53 and pERK in tumor subtypes.

Conclusions: GC exhibits morphologic diversity, which is associated with both pathogenic evolution and genetic transformation. It appears that growth factors/receptors act as carcinogenesis drivers in early malignancy and in better differentiated tumors, whereas loss of this pathway may be associated with additional cell cycle dysfunction that may be responsible for morphologic transformation and associated aggressiveness of PD carcinomas. This dynamic series of regulatory/dysregulated events may explain why effective targeted therapeutic strategies are difficult to develop given the evolving molecular targets in GC.

715 PTEN Loss of Heterozygosity Correlates with Decreased PTEN Expression and Increased p53 Expression in Intraductal Papillary Mucinous Neoplasms of the Pancreas.

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Background: Proposed functions of the tumor suppressor PTEN include a role in cellular senescence via interaction with p53. Mutations of PTEN appear to occur late during tumorigenesis. Previous studies have shown that PTEN loss of heterozygosity (LOH) occurs in a significant proportion of pancreatic adenocarcinoma cases. We sought to determine the frequency of PTEN LOH in pancreatic intraductal papillary mucinous neoplasms (IMPN), and to determine the relationship between PTEN LOH and expression of PTEN and p53 by immunohistochemistry (IHC) using tissue microarrays (TMA).

Design: We analyzed 36 cases of IPMN. DNA was extracted from paraffin-embedded tissue. LOH was assessed by PCR amplification using primers corresponding to a polymorphism within the PTEN locus (RefSNP# rs34421660). TMA's consisting of 3 representative cores per case were constructed. Expression of PTEN and p53 was assessed by IHC. PTEN expression in lesional epithelium (compared to normal epithelium) was scored as normal (2 points), decreased (1 point), or negative (0 points). Expression of p53 was scored as a product of intensity (0-3 points) multiplied by proportion of lesional epithelium with positive staining. Average scores for PTEN and p53 staining were calculated for each case.

Results: Of our 36 cases, 17 showed heterozygosity for PTEN by PCR, and 19 showed homozygosity. Of the 17 heterozygous cases, 7 showed LOH; DNA was not extracted from lesional epithelium of one case. The LOH cases showed significantly lower expression of PTEN (average score = 1.12/2.00) compared to cases without LOH (average score = 1.58/2.00). Additionally, the LOH cases showed significantly higher expression of p53 (average score = 0.31/3.00) compared to cases without LOH (average score = 0.03/3.00).

Conclusions: Of 36 IPMN cases, 7 showed PTEN LOH. Cases with PTEN LOH showed significantly lower PTEN expression, and significantly higher p53 expression by IHC. These findings are consistent with patterns of expression of these markers in other tumor types, and provide insight into changes in regulatory processes that occur in IPMN. Limitations of the current study include potentially low sensitivity for LOH, resulting from assessment of a single polymorphism. Future directions include assessment of LOH using additional polymorphisms, FISH analysis to evaluate PTEN deletions, and assessment of changes in expression of additional markers.

716 Analysis of KRAS, NRAS, BRAF and PIK3CA Mutations in Matched Primary and Metastatic Colorectal Carcinomas.

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Background: Activating KRAS mutations have emerged as a major negative predictor of EGFR inhibitor efficacy in patients with metastatic colorectal carcinoma (CRC). Retrospective studies have also shown a lack of response to EGFR inhibitors among patients with BRAF and NRAS mutations, whereas preliminary studies of PIK3CA have been conflicting. However, the appropriate tumor site for genotyping studies is unclear. Some have suggested analyzing metastases, though the latter tissue is not always available. Thus, we analyzed the mutational profiles of matched primary CRC and metastases to investigate the reasons for the reported discrepancies.

Design: Fresh frozen tissue was analyzed for mutations in KRAS exons 2-4, NRAS exons 2-3, BRAF exon 15 and PIK3CA exons 9 and 20 using a Sequenome MALDI-TOF mass spectrometry-based genotyping assay. All tumors were checked for misidentification using a multiplexed PCR/MS-based genetic fingerprinting assay. Formalin-fixed paraffin embedded (FFPE) tissue from cases showing discrepant results was subjected to Sanger sequencing after microdissection.

Results: We analyzed fresh frozen tissue from 82 matched pairs of primary and metastatic CRC. Initial sequencing identified 41 (50%) KRAS (37 in exon 2, 1 in exon 3 and 3 in exon 4), 2 (2.4%) NRAS (exon 3), 5 (6.1%) BRAF (4 V600E and 1 K601E) and 15 (18.2%) PIK3CA (11 exon 9 and 4 exon 20) mutations. KRAS, NRAS and BRAF mutations were mutually exclusive. Discordant mutational profiles were found in 10 cases (12.2%); 4 of these had a relatively low tumor content in the frozen tissue and concordant mutational profiles were detected after sequencing of FFPE tissue. Three cases were found to be unrelated based on the fingerprinting assay, suggesting errors in tissue identification. One case had multiple colon primaries, raising the possibility that the metastatic focus was not derived from the genotyped primary. No clinical history was available in the remaining two discrepant cases.

Conclusions: Primary and metastatic CRC show a high rate of concordance in KRAS, NRAS, BRAF and PIK3CA mutations. The majority of discrepant results are likely to be due to technical reasons, such as sample mislabeling and low tumor content, rather than gain or loss of mutations during tumor dissemination. Barring technical limitations, genotyping of the primary carcinoma can correctly characterize the genetic lesions associated with metastatic disease. Metastatic foci should be genotyped in patients with multiple primaries, since in these cases it is unclear as to which primary tumor is associated with disease progression.

717 Chromogranin A Expression and Ki67 Index in Residual Rectal Cancer Treated with Preoperative Therapy.

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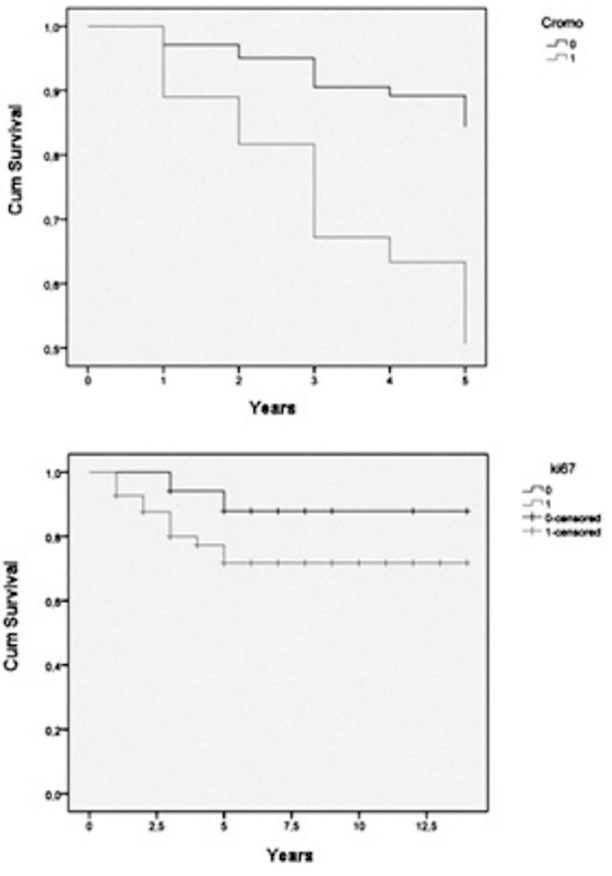
Background: This study was conducted to determine the significance of chromogranin A expression and Ki67 proliferative index in residual rectal cancer with good response after neoadjuvant therapy.

Design: The study population consisted of fifty-five patients with locally advanced rectal cancer treated with preoperative therapy and classified as Tumor Regression Grade (TRG) 3 according to the Mandard Score (Cancer 1994;73:2680-86), defined as regression greater than 50% with fibrosis outgrowing the tumor mass. Tumors expressing Chromogranin A were classified as either absent/low expression (≤30% cells staining positive) or high expression (>30% cells staining positive). The same cut-off was used to estimate the proliferative index by Ki67. The influence of these factors on disease-free survival was evaluated using Kaplan-Meier curves and Cox proportional hazards modeling.

Results: Of the fifty-five patients [33M/22F, mean age 58.3 (range 25 to 76)] 41 (75%) showed a Ki67 index > 30% and 14 (25%) showed a Ki67 index <30%. 10/55 (18%) expressed chromogranin A >30% and 45/55 (82%) expressed chromogranin A <30%. In multivariate analysis, a model for prediction of disease-free survival (five-ten years follow up) including the covariates chromogranin A expression >30%, age and Ki67 was found to be consistent with the assumption of proportional hazards. Statistical significance was reached for Chromogranin A (p=0.041) and age (p=0.019), while Ki67 displayed a "trend" (p=0.084) to be confirmed in a larger series.



Conclusions: This study demonstrates that expression of Chromogranin A is a predictive factor of disease-free survival in rectal cancer treated with neoadjuvant therapy in a regression model also including patient age and Ki67. Accordingly, chromogranin A expression may be considered as a variable useful for a better prognostic assessment of patients with TRG3 rectal cancer.

718 Status of Helicobacter pylori Infection in Fundic Gland Polyps.

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Background: Fundic gland polyps (FGPs) account for approximately 50% of gastric polyps and are present in approximately 0.8% to 1.9% of all patients undergoing gastroscopy. There is limited literature reporting that patients with FGPs have a low prevalence of H. pylori infection.The aim of this study is to assess prevalence and associations of FGPs and H. pylori infection at our institution.

Design: We conducted a retrospective search of the surgical pathology database from 2007 to 2010 for all FGPs and non-FGPs gastric biopsies. The status of H.pylori was assessed by immunohistochemical staining in all the gastric biopsies.

Results: A total of 8390 gastric biopsies were performed in which 345 (4.1%) FGPs and 8045 (95.9%) non FGPs gastric biopsies were identified. In 405 of 8390 (4.8%) gastric biopsies H. pylori infection was present. 343 of 345 (99.4%) cases of FGPs showed no presence of H.pylori with the microorganism identified in only 2 (0.6 %) cases of FGPs. Of the 405 H. pylori positive cases; 347 (77.9%) were associated with active chronic gastritis, 45 (11.1%) with chronic gastritis, 8 (2.0%) with gastric ulcer, 1 (0.2%) with adenocarcinoma and 4 (1.0%) with non-pathologic diagnosis.

Conclusions: FGPs were present in 4.1% of gastric biopsies analyzed in this study. The prevalence of H. pylori infection in FGPs was very low (0.6%) compared to non-FGP gastric biopsies (4.8%). Our results question the value of doing IHC staining for H. pylori in gastric biopsies of FGPs.

719 The Significance of IGG4 in Inflammatory Bowel Disease.

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Background: IgG4 has emerged as a diagnostic marker for autoimmune pancreatitis (AIP). Several prior studies have noted an elevated risk of inflammatory bowel disease (IBD) in patients with AIP, and the majority of these patients have ulcerative colitis (UC). Based on this data, and the observations that both UC and AIP show robust Th2 type responses, we hypothesized that IgG4 stain may assist in distinguishing UC from Crohn's disease (CD), the latter being associated with a Th1/Th17 CD4 response.

Design: We evaluated consecutive patients with UC (n= 42), and CD (n=34). The biopsies we evaluated were obtained at their initial presentation and prior to therapy. The median follow-up of this cohort was 52 months. Four cases of AIP with IBD were also evaluated, as were 16 cases of lymphocytic/collagenous (microscopic colitis, MC). The inflammatory activity in these biopsies was graded as 1 (cryptitis only), 2 (crypt abscesses), and 3 (ulceration). IgG4 and IgG immunostains were performed. A single high power field (HPF) with the highest number of IgG4 positive plasma cells was enumerated. The same HPF was located on the IgG stain and the number of immunoreactive plasma cells counted.

Results: UC cases showed significantly more IgG4 positive plasma cells than CD ($p<0.0001$) and also showed higher IgG4 to IgG ratio ($p=0.001$).

IgG4 in inflammatory bowel disease

Disease	Mean IgG4/HPF (median/SD)	Mean IgG4/IgG ratio
Ulcerative colitis	10.6 (6/13)	0.2
Crohn's disease	2.1 (1/2.7)	0.06

At a cut point of 5 IgG4 positive plasma cells, this stain distinguished UC from CD with a specificity of 87% and sensitivity of 54%. Within the UC cohort, patients with pancolitis showed higher IgG4 counts than cases without pancolitis (mean 13 vs. 6; $p=0.05$). Interestingly, UC cases whose biopsies showed >5 IgG4 positive cells were significantly more likely to be associated with grade 3 inflammatory activity ($p=0.01$). All 4 AIP cases with IBD showed IgG4 positive cells (mean 9) in their colonic biopsies. Cases of microscopic colitis showed significantly fewer IgG4 positive cells than UC ($p=0.0001$), but similar number of these cells as CD ($p=0.37$).

Conclusions: IgG4 shows promise as a biomarker in distinguishing UC from CD, albeit with a low sensitivity, and in the rare instances of overlap between microscopic and ulcerative colitis. It's significance as a marker of severe disease requires further study.

720 The Usefulness of New Preparatory Procedures for EUS-FNAB To Diagnose Gastrointestinal Stromal Tumor.

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Background: Endoscopic ultrasound-guided fine needle aspiration biopsy (EUS-FNAB) is very useful for the diagnosis of submucosal tumor of the stomach. At present, immunohistochemical staining is necessary to diagnose and confirm the malignant potential of mesenchymal tumors. However, the preparation of a sufficient amount of a paraffin-embedded block is often difficult, because samples often contain a large amount of blood. The bloody contents make it difficult to detect the tumor or interpret the results of immunohistochemistry. We herein established a new preparatory method using culture medium for EUS-FNAB specimens and evaluated its utility.

Design: Between October 2008 to August 2010, 24 patients diagnosed with a submucosal tumor of the stomach and 2 patients with that of the duodenum underwent EUS-FNAB. Specimens were obtained by physicians using EUS with a 22-gauge needle. All specimens were immediately put into 10 ml of culture medium in a Petri dish (Hanks solution, Nissui Pharmaceutical, Tokyo, Japan). The dish was transferred to the laboratory, and then we separated tissue samples and mucus from the clot. The tissue samples were put into a fine mesh bag, and were processed routinely for paraffin sections and HE staining. After the pathologist diagnosed the tumors based on HE staining, immunohistochemical staining for c-kit (CD117), CD34, smooth muscle actin, Ki-67, and S-100 was performed for mesenchymal tumors.The reactivity for each immunohistochemical stainings was compared with tissues from surgically resected specimens.To evaluate the volume of the collected tissue samples, the area of positive staining for c-kit was measured using on image analyzing system (WinROOF, Mitani Co., Tokyo, Japan).

Results: Sixteen cases were diagnosed as GIST and 3 cases as adenocarcinoma, 6 cases as ectopic pancreas, and 1 case as schwannoma. All cases involved a high yield of tissues, and so we could diagnose them histologically. Among them, 10 cases of GIST, 1 case of adenocarcinoma, and 1 case of schwannoma were operated on. In all 10 surgically resected cases of GIST, the diagnoses and the immunoreactivity of EUS-FNAB specimens were consistent with those based on surgical specimens. In 16 GISTs, the average area of c-kit-positive tissue was 5.15 mm^2 (2.1 to 9.7) in paraffin-embedded specimens.

Conclusions: Our methods using culture medium ensure a sufficient amount of histological specimens for immunohistochemical staining.

721 Heterogeneity in Signaling Pathways of Gastrointestinal Neuroendocrine Cell Tumors: A Critical Look at Notch Signaling Pathway.

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Background: The molecular pathogenesis of gastrointestinal neuroendocrine tumors (GI-NET) is largely unknown, as are their signaling pathways. We hypothesize that GI-NETs are heterogeneous with regards to these signaling pathways and the differences could have a significant impact on the outcome of clinical trials. Herein, we explore differences in the signaling pathways of GI-NETs emphasizing heterogeneity in the Notch signaling pathway.

Design: We selected 120 well-differentiated NETs including tumors originating in pancreas (n=74), ileum (n=31), and rectum (n=15). Immunohistochemistry was performed on tissue microarrays using the following antibodies: Notch-1, Hes-1, Hey 1, pIGF1R, and bFGFR. Tumor mRNA were extracted from formalin-fixed paraffin-embedded tissue blocks after macro-dissection. Gene profiling study was performed by using human genome U133A 2.0 array and data were analyzed using Array Studio (Omics Soft, NC). The gene profiling results were selectively confirmed by using quantitative reverse transcription polymerase chain reaction (qRT-PCR).

Results: Initial immunohistochemical analysis showed Notch-1 was uniformly expressed in rectal NETs (100%), a subset of pancreatic NETs (34%), and negative in ileal NETs. Similarly, a downstream target of Notch-1, Hes1 was preferentially expressed in rectal NETs (64%), a subset of pancreatic NETs (10%), and uniformly negative in ileal NETS. Messenger RNAs for Notch-1, Hes 1 and Hey 1 were 2.32, 2.44 and 2.39 folds higher in rectal NETs as compared to ileal NETs. Global gene expression profiling showed more than 100 genes were differentially expressed in small intestinal vs. rectal

NETs, with changes as high as 50 fold. These genes were concentrated in several signal transduction pathways including cancer endocrine pathway and cell growth/proliferation pathway. The differential expression of selected genes including ISL LIM homeobox 1, cathepsin B, FGF receptor 3, glucagan, and tryptophan hydroxylase 1 were confirmed by qPCR and immunohistochemistry.
Conclusions: Our results confirm the heterogeneity in signaling pathways of GI-NETs. We demonstrate an active Notch-1 signaling in rectal NETs, conversely, ileal NETs lack such activity. Notch-1 inhibitors are unlikely to provide benefit in ileal NETs, conversely, their efficacy in rectal NETs needs further study. Further analysis of signaling pathways is critical for designing clinical trials in GI-NETs.

722 SIRT1 Is a Useful Marker for Epithelial Dysplasia and Neoplasia in Barrett's Esophagus.

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Background: Documenting the presence of epithelial dysplasia and subsequently grading the dysplasia in Barrett's esophagus are clinically significant for patient's follow-up and management. However, histological diagnosis for these lesions is often challenging to general surgical pathologists and even GI pathologists. Some biomarkers, such as p53 and alpha-methylacyl-coenzyme A racemase (AMACR), have been proven to be helpful, but they show significant overlap among these lesions. We found that SIRT1, the mammal homologue of silent information regulator 2 in budding yeasts, is overexpressed in colonic tubular adenomas. The goal of this study is to investigate the value of SIRT1 in histologically confirmed Barrett's esophagus.
Design: 20 biopsies of Barrett's esophagus without epithelial dysplasia, 11 Barrett's with low-grade dysplasia, 4 Barrett's with high-grade dysplasia, and 8 invasive adenocarcinoma were included in this study. The nuclear staining of SIRT1 was blindly reviewed and graded as 0-3 by two histopathologists, and the staining patterns were also recorded. Fisher exact test was used for the statistic analysis.
Results: Most Barrett's esophagus cases without epithelial dysplasia or low-grade dysplasia (29/31) showed weak nuclear stain at the base of the crypts, and surface epithelium was negative for SIRT1. One low-grade dysplasia showed 2+ surface epithelial nuclear staining. One Barrett's esophagus without dysplasia had 2+ surface nuclear epithelial staining, however, this patient had previous diagnosis of low-grade dysplasia one year ago. Most high-grade dysplasia and invasive adenocarcinomas cases (10/11) had 2-3+ diffuse nuclear staining including surface epithelium. One invasive carcinoma case had 1+ nuclear staining. Using 2+ nuclear staining as the cutoff value, high-grade dysplasia and carcinoma (10/11) had significant higher SIRT1 staining than Barrett's esophagus without dysplasia and low-grade dysplasia (2/31) (p=0.0001).
Conclusions: SIRT1 is overexpressed in high-grade dysplasia and invasive carcinoma and can be used for diagnosis of high-grade dysplasia. There is no significant difference of SIRT1 expression between Barrett's esophagus without dysplasia and low-grade dysplasia. A large scale study and long-term follow-up are needed to find out the significance of low-grade dysplasia with 2+ SIRT1 expressions.

723 The Impact of Dropping the Requirements for Goblet Cells for the Diagnosis of Barrett's Esophagus (BE).

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Background: Currently the American Gastroenterological Association (AGA) requires two elements for the diagnosis of BE: endoscopic evidence of columnar mucosa within the esophagus, and the presence of goblet cells (GC) in biopsies from the columnar lined portion. In contrast, the British Society of Gastroenterology only requires that columnar mucosa be documented by biopsy, regardless of the presence of GC. The AGA is presently strongly considering dropping the requirement for GC.
Design: Our pathology database was searched from 1990-2008 for all cases in which esophageal biopsies were performed to identify BE mucosa in patients without a previous diagnosis of BE. Information from the clinical history, endoscopy reports, biopsy findings and any follow-up were collected.
Results: A total of 708 patients without a previous diagnosis of BE underwent biopsies of endoscopically evident columnar mucosa. In 276, GC were identified and BE was diagnosed, while in 379 no GC were identified (53 had columnar mucosa visualized but only squamous mucosa on biopsy and were excluded). The length of the endoscopically apparent columnar mucosa was greater in patients with GC (X=4.6 cm) than in those without (X=1.6 cm). Patients with GC had more biopsies taken (X=5) compared to those without (X=4). A total of 125 patients (33%) without GC underwent at least one additional endoscopy. In 14 (11%) of these patients, GC were subsequently identified, requiring a mean follow-up of 4.9 years and 2.07 more procedures in which a mean of 6.2 more biopsies were obtained. In the other 111, who underwent a mean of 2.8 procedures (average follow-up 5.4 years) and a mean of 7 more biopsies, GC were still not identified. The mean total number of biopsies from all procedures was 11 for this subset of patients. The mean length of the columnar lined segment in the 14 patients in whom GC were identified upon repeat endoscopy was 4.1 cm, compared to a mean of 1.6 cm in the 111 without GC. GC were identified in only 9% of all patients with columnar mucosa <2 cm or in whom an irregular Z-line suspicious for Barrett's esophagus was reported. No patient without GC developed dysplasia, followed over a range of 1-23 years (X=5.4).
Conclusions: In this study, dropping the requirement for the identification of GC would have increased the initial diagnosis of BE by 53%. Among patients with a short segment of columnar mucosa, subsequent endoscopy generally does not reveal GC, suggesting that the columnar mucosa may in fact represent proximal gastric mucosa. Dropping the requirement for GC may cause many of these patients to be inaccurately labeled as BE.

724 Cyclin E Amplification and Overexpression in Esophageal Carcinoma and Dysplasia by SNP Microarray and Immunohistochemisty Studies.

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Background: Cyclin E, an activator of CDK2, accumulates in later G1 phase and marks the transition from G1 to S phase. It plays an important role in cell cycle progression and cell proliferation. Though a few reports have examined cyclin E expression in esophageal adenocarcinoma(EAC) and squamous cell carcinoma(SCC), the results were not consistent, and the relationship of cyclin E expression and the patients' survival is unknown.
Design: Genomic DNA were analyzed from 116 EAC patients (95 M and 21 F) using Affymetrix SNP 6.0 arrays. All analysis was done in Nexus 5.0 Copy number software. The protein expression level of cyclin E was detected by immunohistochemistry (IHC, Santa Cruz; CA) from tissue microarrays (TMA) including Barrett's esophagus (BE), low- (LGD) and high-grade dysplasia (HGD), columnar cell change (CC), squamous mucosa (SE), EAC and SCC. Patients' survival data, demographic, histological diagnoses and tumor staging data were collected. The intensity (0-3) and percentage of the cyclin E expression in TMA slides were scored by two pathologists (JY and ZZ). Fisher exact tests and Kaplan-Meier methods were used to analyze data.
Results: By genomic analysis, cyclin E1 was amplified in 19% of the EAC samples. By IHC, Cyclin E is strongly overexpressed in 15% EAC (18/117), 34% SCC (12/35), 36% HGD (5/14) and 19% LGD (4/21). However, cyclin E positivity is less than 5% in BE (2/34), CC (3/81), and SE (2/86), which usually have weak, patchy staining. The differences of cyclin E immunostaining between most neoplastic groups (including EAC, SCC, HGD and LGD) and benign groups (including BE, CC and SE) are statistically significant ($p < 0.05$). Interestingly, cyclin E overexpression groups in both SCC and EAC patients show better survival time than non-overexpression groups. The difference of survival time in SCC group is statistically significant ($p = 0.01$), but not in EAC group ($p = 0.11$). No association also were found between genomic amplifications and overall survival time (p=0.21) in EAC patients with genomic analysis (median follow up 26.8 months).
Conclusions: Cyclin E is significantly amplified and overexpressed in a subset of EAC patients, and significantly overexpressed in SCC, HGD and LGD patients comparing to non-dysplastic process. Cyclin E overexpression in EAC and SCC patients shows favorable prognosis. Further study is needed to explore and verify the effect of cyclin E on esophageal carcinoma.

725 Genotypic Heterogeneity May Influence Tumor Immunologic Response in DNA Mismatch Repair Deficient Colorectal Carcinoma.

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Background: DNA mismatch repair (MMR) deficiency in colorectal carcinoma (CRC) has been associated with tumor behavior and clinical outcome. It is believed that the mutator phenotype results in an increased immune response which may then account for the better outcome observed in MMR-deficient CRCs. It is unclear whether the nature and extent of the immune response differ among different genotypic alterations (e.g., germline mutation versus somatic change, or the specific gene that is mutated). Clarification of these issues may provide insights into the biology of MMR deficient CRCs and carry prognostic and therapeutic implications.
Design: Histological features with an emphasis on the type of inflammatory response were analyzed among MMR-deficient CRCs from 2 groups of patients: 1) Lynch syndrome (LS) cases with a defined pathogenic mutation in 1 of the 4 MMR genes; and 2) sporadic cases defined by personal/family history and/or *MLH1* hypermethylation without germline mutation. For statistical analysis, proportions were compared by chi-square analysis and means compared by ANOVA.
Results: Group 1 had 33 cases (13 with mutations in *MLH1*, 15 in *MSH2*, 2 in *MSH6* and 3 in *PMS2)* and group 2 had 23. No significant difference was seen between the 2 groups in terms of tumor stage, histologic type, presence of medullary, mucinous or heterogeneous components. Differences were seen regarding the presence of peritumoral lymphoid aggregates (PTLA) (group1 vs 2, 39% vs 4%, p=0.03), tumor associated neutrophils (TAN) (49% vs 17%, p=0.017), and tumor-infiltrating lymphocytes (TIL)/10HPFs (mean, 167 vs 88; p=0.057). Group 1 tumors were also more likely to have an expansile tumor border (82% vs 52%, p=0.018). Cases with *MLH1* mutation differed from those with *MSH2* mutation in PTLA (92% vs 60%, p=0.049).
Conclusions: Among the MMR-deficient CRCs studied, there was a more prominent immunologic response, manifested by PTLA, TANs and TILs, in LS cases than in sporadic ones. A difference in peritumoral lymphocytes was also seen between *MLH1*-mutated and *MSH2*-mutated LS CRCs. Such observations warrant further investigation about the effect of genotypic heterogeneity on the biology and clinical behavior of MMR-deficient CRCs.

726 KRAS Mutational Testing in Tumor Samples Obtained before and after Chemoradiation in Rectal Cancer Patients: Different Protocols on the Basis of Available Tissues.

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Background: KRAS testing represents the prerequisite for administration of EGFR-targeted therapies to metastatic colorectal cancer patients. The choice of tumor blocks for

KRAS testing is fundamental. In patients with locally advanced rectal cancer (LARC), chemoradiation is generally offered before curative surgery, therefore only small tumor amounts are available at diagnosis.
Design: We tested the KRAS mutational status (using two methods) on biopsies and tumor blocks obtained before and after chemoradiation to evaluate whether differences occurred between the two materials, and the two methods. Tumors samples obtained before (diagnostic biopsy) and post (surgery specimen) neoadjuvant chemoradiation from 61 LARC patients were analyzed. Tumor microdissection was performed. KRAS status was evaluated by direct sequencing (DS, sensitivity of 10-20%) and by mutant-enriched PCR (ME-PCR, sensitivity of 0.01%).
Results: In pretreated biopsies, DS revealed KRAS mutations in 24 cases, ME-PCR in 28. In post-treated tumors, DS found KRAS mutations in 13 cases and ME-PCR in 24. Eight cases showed a discordant pattern between pre and post-therapy tissues: six patients showed the KRAS mutation limited to the pre-treated biopsy (in 4 cases detected by DS and ME-PCR, in 2 cases only by ME-PCR), two patients showed the mutation limited to the post-treated tissue (detected only by ME-PCR).
Conclusions: The use of ME-PCR enhances the detection of KRAS mutations in LARC. Discrepancies between pre- and post-chemoradiation tissues may occur. If the pre-treatment biopsies are available, DS can be routinely used. Although carefully microdissected, post-chemoradiation biopsies must be evaluated only by high sensitive methodologies, such as ME-PCR.

727 Distinct *KRAS* Mutation Spectrum in Colorectal Carcinoma and Lung Adenocarcinoma Implicates Different Mutagenesis.

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Background: Mutations in *KRAS* oncogene result in constitutive activation of the MEK/ERK signaling pathway and are frequently present in human cancers, including colorectal carcinoma (CRC) and lung adenocarcinoma (LAC). The identification of *KRAS* mutations is useful for prediction of resistance to anti-EGFR therapies and prognosis. We analyzed and compared the prevalence and spectrum of *KRAS* mutations in CRC and LAC.
Design: The detection of *KRAS* mutations in exon 2 was performed on genomic DNA extracted from archived paraffin embedded tissue using target PCR followed by direct sequencing in a cohort of 173 CRC (99 primary and 74 metastatic) and 109 LAC (95 primary and 14 metastatic). Primary and metastatic tumors were not from the same patient in any case. The status of *KRAS* mutation was correlated with potential risk factors, including tobacco and alcohol use, diabetes mellitus, hypertension, hyperlipidemia, previous cancer history and family cancer history. The specific types of *KRAS* mutations in CRC and LAC were compared. Fisher's exact test was used.
Results: *KRAS* mutations were detected in 58 of 173 (33.5%) CRC and 52 of 109 (47.7%) LAC. *KRAS* mutations were associated with tobacco use in LAC ($P < .0001$), but not in CRC. No significant association was found between *KRAS* mutation and any of the other risk factors evaluated. All 8 frequent mutations previously described involving codons 12 and 13 were detected. A novel mutation, Val8Ile involving codon 8, was also identified in one metastatic CRC. Double mutations affecting codons 12 and 13 were detected in three cases. The specific types of *KRAS* mutations were different between CRC and LAC. In CRC, the G > A transitions (29/56, 51.8%) and the G > T and G > C transversions (27/56, 48.2%) occurred with a similar frequency. In contrast, in LAC, the G > T and G > C transversions (38/52, 73.1%) were more common than G > A transitions (14/52, 26.9%) ($P = .0107$).

KRAS mutations and tobacco use in LAC and CRC

KRAS Mutation	LAC (n = 52)	CRC (n = 58)
Smoker	47 (90.4%)	25 (43.1%)
Non-Smoker	5 (9.6%)	33 (56.9%)

Transversion and transition mutations of *KRAS* in LAC and CRC

KRAS Mutation	LAC (n = 52)	CRC (n = 56)*
Transversion	38 (73.1%)	27 (48.2%)
Transition	14 (26.9%)	29 (51.8%)

*Two CRC carried double mutations not classified as transition or transversion.
Conclusions: *KRAS* mutations were common in both CRC and LAC and were associated with tobacco use in LAC. Transversion mutations of *KRAS* were more common in LAC than in CRC which may be attributed to a different mutagenesis/mutagenic exposure between these types of carcinoma.

728 Her2 Status in Gastric Adenocarcinoma: A Comparison of Immunohistochemistry (IHC), Fluorescent In-Situ Hybridization (FISH) and Dual Color Silver In-Situ Hybridization (SISH) Methods.

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Background: Testing of Her 2 status of gastric adenocarcinoma is now being requested as a routine pathology test as trastuzumab is a potential treatment option for Her2 positive gastric adenocarcinoma. However, reports about Her 2 testing in gastric adenocarcinoma are few and standards for Her2 testing in gastric adenocarcinoma are not yet established.
Design: A total of 87 patients with gastric adenocarcinoma either from the British Columbia Cancer Agency tumor registry or from an ongoing prospective clinical trial were identified. Formalin fixed biopsy or resection tissues containing gastric adenocarcinoma were used for IHC staining for Her2 protein expression (Ventana®,4B5 antibody), for FISH testing (Abbott, PathVysion®) and for dual color SISH testing (Ventana®, Inform Her2).
Results: When the ASCO/CAP guideline for Her2 testing in breast cancer is used, Her2 protein expression positive rate is 12.7% (IHC 3+, 7 cases; and IHC 2+ with gene amplification by FISH or SISH, 4 cases), Her2 gene amplification rate by FISH is 14.9% (13/87 cases) and by SISH is 13.8% (12/87 cases). When the modified ASCO/CAP guideline, as defined by the manufacturer's package insert is used, Her2 protein expression positive rate is 19.5% (IHC 3+, 11 cases; and IHC 2+ with gene amplification by FISH, 6 cases) or 18.4% (IHC 3+, 11 cases; and IHC 2+ with gene amplification by SISH, 5 cases), Her2 gene amplification rate by FISH is 18.4% (16/87 cases) and by SISH is also 18.4% (16/87 cases). There is good concordance between FISH and SISH, IHC and FISH and IHC and SISH (table 1).

Table 1: Concordance between Analysis Methods

	FISH vs. SISH	IHC vs. FISH	IHC vs. SISH
Negative concordance	95.6%	98.0%	96.4%
Positive concordance	90.0%	81.8%	81.8%

Compared to FISH, dual color SISH has some technique advantages as it uses bright field and has shorter turn around time (1 day vs. 7 days).
Conclusions: Her2 status of gastric adenocarcinoma can be reliably tested using the ASCO/CAP guideline or the modified ASCO/CAP guideline for Her2 testing in breast cancer. SISH and FISH are highly concordant. Combined with IHC, either FISH or SISH can be used as a reliable method for testing of Her2 gene status in gastric adenocarcinoma.

729 The Clinical Significance of Active Ileitis and Chronic Active Ileitis: Clinicopathologic Study of 72 Cases.

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Background: Biopsy of the terminal ileum (TI) is a common procedure for evaluating diarrhea, abdominal pain, anemia, and rectal bleeding. Ileitis may be seen in the setting of ischemia, medication effect, infectious disease, and inflammatory bowel disease, among others. The pathologist usually renders a differential diagnosis, and clinicopathologic correlation is very important. The aim of the study was to correlate TI and associated colonic findings with the final clinical impression to assess the significance of these patterns of inflammation.
Design: Using our institutional database, 93 patients were identified with at least one TI biopsy diagnosed as either active ileitis (AI) or chronic active ileitis (CAI) within a five year period (2005-2010). TI and any concomitant colonic biopsies were reviewed by two pathologists. The inflammation pattern was evaluated in the TI. Colonic biopsies were noted to have either normal or abnormal histology. Up to 5-year clinical follow up was available for 72 cases and the clinical, endoscopic, and radiographic findings were noted.
Results: Of 93 patients (mean age 41 years, 55% female) with TI biopsies, the final clinical diagnostic information was not available in 21, resulting in 72 patients with 28 diagnosed as AI and 44 diagnosed with CAI. The final clinical diagnoses for patients with AI were 21 Crohn disease (CD) (75%), 3 medication effect (11%), 1 ulcerative colitis (UC) (4%), 1 prior intestinal surgery (4%), and 2 unknown (7%). The final clinical diagnoses for patients with CAI were 36 CD (82%), 1 medication effect (2%), 2 UC (5%), 2 prior intestinal surgery (5%), 1 graft versus host disease (GVHD) (2%), and 2 cytomegalovirus (CMV) (5%). Incorporating the colonic histologic findings with the TI diagnoses, 35 patients were excluded due to missing final clinical diagnoses and/or colonic biopsies, resulting in 58 patients. Diagnoses of the 6 patients with AI and normal colons were 4 CD (67%), 1 medication effect (17%), and 1 unknown (7%). The 15 patients with AI and abnormal colons had 10 CD (67%), 2 medication effect (14%), 1 UC (7%), 1 prior intestinal surgery (7%), and 1 unknown (7%). Diagnoses of the 8 patients with CAI and normal colons were 6 CD (76%), 1 medication effect (13%), and 1 prior intestinal surgery (13%). Those with CAI and abnormal colons included 24 CD (83%), 2 CMV (7%), 2 UC (7%), and 1 GVHD (3%).
Conclusions: These results show that the majority of final clinical diagnoses in patients with AI or CAI regardless of the colonic findings represent CD. Other diagnoses are less likely but should remain in the differential.

730 Prognostic Impact of Jass's Proposed Molecular Classification of Colorectal Cancer Based on MSI, CIMP, MGMT, KRAS and BRAF Status.

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Background: In 2007, Jeremy Jass proposed a new molecular classification for colorectal cancer based primarily on 5 features: microsatellite instability status (MSI), CpG Island Methylator Phenotype (CIMP), O-6-methylguanine-DNA methyltransferase (MGMT), KRAS and BRAF gene status. The aim of this study was to validate this classification and its impact on prognosis.
Design: 404 patients were included in this study. MSI-H was defined as instability in ≥2 Bethesda-panel markers. CIMP-high was defined as methylation in ≥4/5 loci including CACNA1G, CDKN2A, CRABP1, MLH1, and Neurog1. Mutation of KRAS (codons 12/13) and BRAF (codon V600E) and methylation of MGMT were investigated. The 5 proposed Jass groups were tested; patients were subsequently re-classified based on 10-year overall survival (OS).
Results: CIMP-high was linked to right-sided location ($p<0.001$), higher tumor grade ($p=0.005$), BRAF mutation ($p<0.001$), MSI-H ($p<0.001$), MGMT methylation ($p=0.022$), and worse survival ($p=0.053$). 123 (40.7%) patients could not be assigned to any proposed Jass groups. The frequencies of patients successfully classified did not show the expected trends. After re-classification, BRAF best classified OS, followed by CIMP, KRAS, MSI and MGMT. Six prognostic subgroups were identified, the largest comprised of patients with BRAF WT/CIMP-H or -low (n=39.1%; OS: 38%), the worst OS was observed for patients with BRAF mutation (n=9.9%; OS:10%), independently of CIMP, MSI, or MGMT.

Conclusions: Although MGMT may have limited prognostic value, BRAF, CIMP, KRAS and MSI status may interact with each other to modify clinical outcome. These features will likely play a significant role in a future molecular classification of colorectal cancer.

731 CDX2 Expression as a Potential Surrogate Marker for CpG Island Methylator Phenotype in Colorectal Cancers.

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Background: CpG Island Methylator Phenotype (CIMP) is currently being investigated for its role in colorectal cancer pathogenesis and impact on clinical outcome. However, the assessment of CIMP in routine diagnostic pathology is complicated by the technical challenge and costs associated with its assessment. The aim of this study was to identify a protein marker capable of predicting CIMP status from a panel of 50 potential candidates.

Design: 404 patients were included in this study. MSI-H was defined as instability in ≥2 Bethesda-panel markers. CIMP-high was defined as methylation in ≥4/5 loci including CACNA1G, CDKN2A, CRABP1, MLH1, and Neurog1. Using tissue microarrays, 50 tumor and immune cell markers were investigated by immunohistochemistry.

Results: Only 7/50 markers were associated with CIMP-high including increased numbers of granzyme B+ (p=0.002; AUC:0.65), and CD8+ (p<0.001; AUC:0.66) cells, increased tumor cell expression of nuclear MST1 (p=0.012; AUC:0.67), and loss of cytoplasmic RKIP (p=0.007; AUC: 0.64), membranous/cytoplasmic EphB2 (p=0.007; AUC:0.7), and cytoplasmic CK20 (p=0.002; AUC:0.67). However, loss of cytoplasmic CDX2 was the strongest predictor of CIMP-high (p<0.001; AUC: 0.81). Of the 206 tumors with diffuse CDX2 staining, 202 (98%) were CIMP-negative/low. Similarly, of the 23 CIMP-high cases, 19 (82.6%) showed a loss of CDX2 expression.

Conclusions: CIMP-high colorectal cancers show a considerable loss of CDX2 expression. Since loss of CDX2 has previously been associated with BRAF mutation and MSI-H, two features closely related to CIMP, these preliminary results suggest that CDX2 may be useful as a possible surrogate marker for the determination of CIMP status.

Genitourinary

732 Molecular Genetic Abnormalities in Regulators of Cell Cycle and Apoptosis in High Grade Urothelial Carcinoma of Bladder.

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Background: Cell cycle dysregulation and inhibition of apoptosis both drive tumorigenesis in multiple malignancies, including urothelial carcinoma (UC). Copy number alteration (CNA) is a well-known mechanism for dysregulation of these cardinal functions. Deletion of *CDKN2A* and *TP53* and amplification of *MDM2* has been observed in UC, but the exact frequency and functional consequence of such alterations is less known. We sought to define the frequency of amplification, deletion and mutations of genes that regulate cell cycle or apoptosis in a panel of 96 cases of high-grade UC (HGUC) of bladder.

Design: DNA was isolated from 96 frozen samples of HGUC (including 10 cases of small cell carcinoma of bladder) and analyzed for CNA through comparative genomic hybridization (CGH) using a 1 million oligonucleotide probe array from Agilent. The targeted genes included *TP53*, *MDM2*, *CCND1*, *CCNE1*, *CDKN2A/B*, *E2F3* and *Rb1*. Traditional Sanger sequencing to screen for mutations within select genes (*TP53*, *Rb1*, and *CDKN2A*) was also performed.

Results: Table 1 depicts the frequency of CNA and mutations found within the studied genes.

Table 1. Copy number alterations and mutations in genes of cell cycle regulation or apoptosis

Gene	Abnormality (n)
CCND1	Amp (11)
CCNE1	Amp (4)
CDKN2A/B	Del (13), Amp (1), Mut (2)
E2F3	Amp (13)
RB1	Del (5), Mut (1)
TP53	Mut (12), Del (9)
MDM2	Amp (4)

Amp: amplification; Del: deletion; Mut: mutation

Overall, 54 of the 96 cases (56%) showed some CNA (45) or mutation (13). Deletion of *CDKN2A/B* and amplification of *E2F3* were the most common CNA events identified within cell cycle regulatory genes, occurring in 13 samples each (14%), followed by amplification of *CCND1* in 11 samples (11%). There was no co-amplification of *CCND1* and *CCNE1* in any of the samples. *Rb1* deletions were observed in 5 samples. CNA in *E2F3* and *Rb1* were mutually exclusive in 14 of the 16 samples (88%) and were both present in 2 samples only. Mutations in *TP53* were noted in 13 samples and deletions in 9 samples. Amplification of *MDM2* was noted in 4 samples, none of which overlapped with *TP53* deletions or mutations. Overexpression of *E2F3* was significantly more common in small cell carcinoma (5/11) compared to conventional UC (8/85), $p = 0.006$.

Conclusions: Regulators of cell cycle and apoptosis are amplified, deleted or mutated in more than half of cases (56%) of high grade urothelial carcinoma. The overwhelming majority of these abnormalities are nonoverlapping. Amplification of *E2F3* seems to be overrepresented in small cell carcinoma of the bladder.

733 Increased Phosphorylated 4EBP1 Expression in Minute Prostatic Adnocarcinoma.

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Background: Phosphorylation of 4EBP1 results in the release of eukaryotic initiation factor 4E and increased cap-dependent translation of a set of proteins involved in G_1-S-phase. Reduced phosphorylated 4EBP1 expression was significantly associated with dramatically shortened survival in prostatic cancer. We aimed to evaluate phosphorylated 4EBP1 expression in minimal (insignificant) prostate adenocarcinoma (MinPCa), defined as tumors with insufficient virulence to threaten survival.

Design: Tissue microarrays were constructed from 33 consecutive radical prostatectomy spemcimens containing MinPCa. Each tumor and paired benign tissue was represented by up to triplicate,1mm, spots. Standard immunohistochemistry analysis for phosphorylated 4EBP1 was performed. Staining pattern was evaluated as nuclear vs. cytoplasmic, Percentage of positive cells (extent) and intensity (0 to 3+) of staining was assigned in each spot. A final H-score (product of intensity x extent) was calculated per spot and averaged among spots representing a single sample.

Results: Cytoplasmic and nuclear p4EBP1 expression (H scores) were significantly higher in MinPCa cancer tissues compared to benign tissues (p=0.0001 and p=0.0003 respectively). Only a minorty of MinPCa tumors (4/34; 11%) revealed cytoplasmic p4EBP1 levels lower than the mean level of their paired benign glands (Hscore <25).

p4EBP1 Hscore in benign prostatic tissue vs. adenocarcinoma

	No cases	Cytoplasmic p4EBP1 Hscore mean (range)	nuclear p4EBP1 Hscore mean	p value
Benign prostatic tissue	34	25 (0-100)	96 (10-230)	0.0001
Adenocarcinoma	34	136 (0-300)	162 (0-300)	0.0003

Conclusions: We found significantly increased cytoplamic and nuclear expression of phosphorylated 4EBP1 in our cohort of MinPCa compared to paired non neoplastic prostate tissue. Our finding of decreased phosphorylated 4EBP1 levels only in a minority of tumors in this cohort of clinically insignificant minimal prostate carcinoma is in line with the previously suggested aggressive prognostic implication for marked reduction in phosphorylated 4EBP1 expression.

734 PTEN and Phosphorylated S6 Expression in Clinically insignificant Prostate Adenocarcinoma: Correlation with *ERG* Fusion Status.

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Background: Loss of PTEN leads to activation of mTOR pathway and has been linked to poor survival in patients with prostatic cancer (PCa). Phosphorylated S6 (pS6) expression is a potential predictive marker of response in mTOR targeted therapy. Minimal or insignificant prostatic adenocarcinoma (MinPCa) is defined as PCa with Gleason Score 6 and tumor volume < 0.5 CC. Studies assessing mTOR pathway status in MinPCa are lacking. The current study evaluates PTEN and pS6 expression in MinPCa in correlation with previously assessed *ERG* fusion status.

Design: Tissue microarrays (TMA) were constructed from 45 consecutive prostatectomies performed in our hospital (2002-2003) and diagnosed as MinPCa. Each tumor and paired benign tissue was represented by up to triplicate 1mm spots. Standard immunohistochemistry analysis for mTOR pathway members PTEN, pS6 was performed. H-score was generated for each marker as a product of intensity (0 to 3+) x percent of positive cells. FISH analysis was previously performed using break-apart probes for 5' and 3' regions of *ERG*.

Results: PTEN expression was retained in 28/29 (97%) evaluable MinPCa while pS6 positivity was present in 9/29 (31%). We found a significant correlation between pS6 expression and *TMPRSS2-ERG* fusion status (p<0.05) with 77% of pS6 positive tumors showing *ERG* fusion. Surprisingly, of 9 tumors demonstrating pS6 expression, 8 (89%) did not show associated loss of PTEN tumor suppressor gene suggesting an alternative mechanism controlling pS6 activation in MinPCa.

PTEN and pS6 expression vs. ERG rearrangement in minute protatic carcinoma

	No. cases	No ERG Rearangement	ERG Rearangement	p value
PTEN loss	1	0 (0%)	1 (100%)	p=1
No PTEN loss	28	14 (50%)	14 (50%)	
pS6 negative	20	13 (65%)	7 (35%)	p=0.014
pS6 positive	9	2 (22%)	7 (77%)	

Conclusions: In our cohort of MinPCa, loss of PTEN was only a rare event (1/29 tumors). Loss of PTEN was not associated with *ERG* fusion. The latter is in contrast to prior studies suggesting a collaborative role of PTEN loss and *ERG* fusion in early prostate cancer development. *ERG* rearrangement was associated with pS6 expression independent of PTEN loss. This finding suggests an alternative signaling mechanism controlling pS6 activation in MinPCa and call for further analysis.

735 Initial High Grade Prostatic Intraepithelial Neoplasia (HGPIN) with Carcinoma on Subsequent Prostate Needle Biopsy: Findings at Radical Prostatectomy.

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Background: There are only a few small studies on men with an initial biopsy showing HGPIN who later have cancer on repeat biopsy and then undergone radical prostatectomy. It is unknown whether this scenario impacts the prognosis of the subsequent radical prostatectomy.

Design: We compared radical prostatectomy findings in 45 men with an initial diagnosis of HGPIN who subsequently were diagnosed with cancer to 18,450 men diagnosed with cancer who lacked a prior diagnosis of HGPIN. All cases were retrieved from our institution between 1993 and 2008.

Results: The mean patient age was 60.2 years and the mean serum PSA value was 9.0 ng/mL. for the 45 men with an initial HGPIN diagnosis. 21/45 (46.7%) men were