Design: We studied a series of 60 lymph node metastases of papillary carcinoma for BRAF, RAS and RET/PTC mutations. In 47 cases frozen tissue and in 13 cases paraffin-embedded tissue from the lymph nodes involved with metastatic carcinoma were available for the study. DNA and RNA were extracted and the PCR and RT-PCR assays were used to detect the mutations.
Results: Among 47 metastatic papillary carcinomas where frozen tissue was available, 21 (45%) were found to have BRAF point mutations, 12 (26%) had RET/PTC-1 or 3 rearrangement, and only 2 (4%) had RAS point mutations. Another 12 lymph node metastasis had no detectable genetic alterations. Among 13 cases where paraffin tissue was available, 8 (62%) revealed BRAF mutations and 3 (23%) revealed RAS mutations, while no RET/PTC rearrangement was detected. By combining the two groups, 48% of papillary carcinomas metastatic to lymph nodes revealed BRAF mutation, 20% RET/PTC rearrangements and 8% RAS mutations, whereas 24% of lymph nodes demonstrated none of these genetic alterations. All 5 metastatic tumors harboring RAS mutations were classified as the follicular variant of papillary carcinoma.
Conclusions: Our data demonstrates that the majority of lymph node metastases from papillary thyroid carcinoma contain either BRAF point mutations or RET/PTC rearrangements. A higher prevalence of BRAF mutations in metastases than in primary tumor nodules supports the previously suggested association between BRAF and more aggressive behavior of papillary carcinomas. A lower frequency of RAS mutations in lymph node metastases correlates with the recent findings demonstrating the association between RAS and the follicular variant of papillary carcinoma, which has an overall lower prevalence of lymph node metastasis.

Gastrointestinal

452 "Seedling" Mesenchymal Tumors (Gastrointestinal Stromal Tumors and Leiomyomas) Are Very Common in the Esophagogastric Region

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Background: Gastrointestinal stromal tumors (GIST) are the most common non-epithelial neoplasm of the gastrointestinal tract and show a predilection for the stomach and esophagogastric junction (EGJ). Most are detected because of symptoms, but some are incidental findings at autopsy or surgery for other reasons. Incidental GIST tend to be smaller at diagnosis, but even small (<1 cm) GIST have been shown to harbor activating *KIT* mutations at rates similar to advanced GIST. However, the prevalence and characteristics of small GIST in surgical resections of the EGJ remains unclear.
Design: We studied 150 esophagogastric resections for esophageal or EGJ carcinomas (100 with pre-operative chemoradiation and 50 untreated cases) that had been extensively embedded for histologic examination (mean 30 sections/case). Number, size, morphology, and location of all GIST and leiomyomas were recorded. All potential GIST were evaluated with CD117 (c-kit) and CD34 immunohistochemistry, and a subset (6) leiomyomas with smooth muscle actin.
Results: 19 incidental GIST were found in 15 (10%) patients; 4 patients harbored 2 separate lesions. Prevalence of GIST was identical in treated (10/100) and untreated (5/50) cases. All (100%) showed diffuse positivity for both CD117 and CD34 and all were of spindle cell morphology. Lesions ranged from 0.2-3.0 mm in size (mean 1.3 mm). Eight (42%) were present in the outer muscularis propria, 8 (42%) in inner muscularis, and 3 (16%) between the muscle layers. Lesions clustered near the EGJ, with 9 (47%) on the gastric side, 9 (47%) on the esophageal side, and 1 (5%) undetermined due to overlying ulceration. Leiomyomas were even more common than GIST, occuring in 47% of patients (44% of treated and 52% of untreated, p= 0.39), with a mean of 3 leiomyomas/patient (range 1-13) and mean size of 1.7 mm (range 0.2-12 mm). Unlike colorectal leiomyomas, most (91%) EGJ leiomyomas were located in the inner muscularis propria and only rarely (1%) in muscularis mucosa.
Conclusions: Both GIST and leiomyomas are common incidental "seedling" lesions of the EGJ, found in 10% and 47% of patients, when carefully evaluated. The universal positivity for c-kit in small incidental GIST suggests that additional genetic or epigenetic alerations are needed for neoplastic progression. Further, the large difference in prevalence between incidental and clinically significant GIST suggests that most do not progress.

453 Duplication of the Muscularis Mucosae and Adenocarcinoma Staging in Barrett's Esophagus

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Background: Depth of invasion is one of the most important prognostic indicators in esophageal adenocarcinoma. Unlike other regions of the gastrointestinal tract, the esophagus in Barrett's metaplasia frequently develops a duplicated muscularis mucosae (MM). The effect of this MM duplication on appropriate staging of superficially invasive adenocarcinoma, however, is unclear.
Design: The study was carried out in two parts. First, 50 resections for high grade dysplasia or T1 adenocarcinoma in Barrett's esophagus (BE) were evaluated for the presence and histologic characteristics of MM duplication, including 1) % of BE segment involved by MM duplication, 2) origin of the duplicated muscle layer, and 3) appearance of the tissue between duplicated MM. Next, we studied 30 resections for BE that had superficial invasion confined to regions of MM duplication. These cases were classified as to 1) depth of invasion (inner MM, space between duplicated MM, or outer MM), 2) angiolymphatic invasion, and 3) rate of lymph node metastasis. For comparison, we used recently published data (*Am J Surg Pathol* 2005;29:1079-85) for cases of lamina propria invasion and superficial submucosal invasion, respectively.
Results: 46 of 50 (92%) BE resections showed MM duplication, involving 5% to >90% of the Barrett's segment. In 5 (10%) cases MM was focally triplicated. The outer MM was in continuity to a single muscle layer beneath squamous epithelium, suggesting that outer MM represents the "original" muscle layer. The space between duplicated MM predominantly consisted of loose fibrovascular tissue similar to submucosa; in 15 (30%) cases, there were also areas of fibrosis or thin muscle strands joining the 2 MM layers. Of 30 adenocarcinomas invading duplicated MM, 10 (33%) invaded only inner MM, 12 (40%) invaded between the MM layers, and 8 (27%) into outer MM. Angiolymphatic invasion was seen in 5 (17%) cases, and nodal metastases in 3 (10%, 1 case each of inner MM, between MM, and outer MM invasion).
Conclusions: MM duplication is a characteristic finding in BE but can pose difficulty in proper staging of superficial adenocarcinoma. The rates of angiolymphatic invasion (17%) and nodal metastases (10%) in cases with invasion into duplicated MM are higher than published rates for lamina propria invasion (each 0%) but similar to those for superficial submucosal invasion (25% and 8%, respectively), suggesting that these tumors can behave aggressively despite their technically "intramucosal" location.

454 True Smooth Muscle Neoplasms of the Stomach: A Clinicopathologic Study

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Background: Gastric leiomyomas and leiomyosarcomas are distinct from the more common c-kit positive gastrointestinal stromal tumors (GIST). Due to their rarity, a comprehensive review has not been undertaken to evaluate their incidence, histopathologic features and clinical behavior. The objective of this study was to analyze true gastric smooth muscle neoplasms, define their clinicopathologic features and criteria for malignancy.
Design: All gastric spindle cell tumors diagnosed at two institutions between 1980-2005 were evaluated. Immunohistochemical stains including c-kit, SMA, desmin, S-100, CD34, caldesmon and vimentin were performed. Tumors that were c-kit negative, >2 cm in size and expressed at least one smooth muscle differentiation marker were included in the study. Mitotic rate, atypia, coagulative necrosis and presence of mucosal ulceration were evaluated and follow-up was obtained. Sequencing of KIT exons 9, 11, 13, and 17 and PDGFRA exons 12 and 18 was performed in selected cases.
Results: Of 1881 tumors evaluated, 16 met the inclusion criteria. The mean tumor size was 5.4 cm (2-12 cm) and the mean age was 53 years (5-88 yrs.). Using gastric GIST criteria, 5 tumors were classified as benign, 7 as having uncertain malignant potential and 4 as malignant. The appearance of the benign lesions was homogeneous and resembled smooth muscle neoplasms at other sites. None showed more than 1 mitosis per 50 HPF. The malignant tumors showed significant heterogeneity, pleomorphism, an increased mitotic rate (12-22/50 HPF) and coagulative necrosis. 3/4 malignant tumors showed adjacent organ invasion, and one had metastasized to the liver at diagnosis. KIT and PDGFRA analysis revealed no mutations in 7 cases. Follow-up was available in all but one case (mean 67 months, range 2-207). One tumor recurred widely throughout the peritoneum during the follow-up. None of the patients died of their disease.
Conclusions: Less than 1% of all gastric spindle cell neoplasms are true smooth muscle tumors. Despite a large mean tumor size (5.4 cm), the majority of tumors behaved in a benign fashion. The only reliable criteria for malignancy was the presence of an increased mitotic rate and adjacent organ invasion. These results suggest that the criteria for malignancy in gastric smooth muscle tumors is likely to be different from those in c-kit positive GIST's. Evaluation of additional cases is needed to confirm this observation.

455 Expression of Annexin A1 in Esophageal and Esophagogastric Junction Adenocarcinomas: Association with Poor Outcome

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Background: The prognosis of patients with esophageal and esophagogastric junction (EGJ) adenocarcinoma is poor even after a curative surgical resection. Annexin A1 (ANXA1) is a calcium binding protein involved in arachidonic acid metabolism and EGFR tyrosine kinase pathway. ANXA1 has been implicated in early esophageal carcinogenesis, particularly in squamous cell carcinoma. However, the role of ANXA1 in esophageal adenocarcinoma and its clinical significance is not clear.
Design: Expression of ANXA1 was assessed by immunohistochemistry and quantitated by percentage of tumor cells with positive staining in tissue microarrays constructed from triplicates of 1 mm tumor tissue cores of 104 (11 stage I, 24 stage II, 53 stage III, and 16 stage IV) surgically resected esophageal and EGJ adenocarcinomas. ANXA1 expression was defined by ≥25% of the tumor cells with positive staining, and was correlated with clinicopathologic features, recurrence-free survival and overall survival.
Results: ANXA1 has site-specific expression in the esophagus and stomach, being expressed in normal squamous epithelium but not in normal columnar esophageal glands or gastric mucosa. ANXA1 expression was detected in 39% (41/104) of adenocarcinomas and was associated with higher T stage (46%, 36/78 in T3 tumors vs 19%, 5/26 in T1/T2 tumors, p=0.03) and with the presence of distant metastasis (63%, 10/16 with distant metastasis vs 35%, 31/88 without metastasis, p=0.04). There was no association between ANXA1 expression and tumor differentiation (p=0.65), or presence of lymph node metastasis (33/76 in N1 vs 8/28 in N0, p=0.17). ANXA1 expression correlated with decreased recurrence-free (p=0.003) and overall survivals (p=0.003) in univariate analysis. In multivariate analysis ANXA1 expression (p=0.008 and p=0.02) and N stage (p=0.001 and p<0.001) were prognostic factors for recurrence-free and overall survivals independent of T stage.
Conclusions: ANXA1 is expressed in a subset of esophageal and EGJ adenocarcinomas. ANAX1 expression is associated with higher T stage and distant metastasis, and is an independent prognostic factor for patient survival.

456 Expression of Epidermal Growth Factor Receptor in Squamous Cell Carcinomas of the Anal Canal Is Independent of Gene Amplification

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Background: Epidermal Growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase whose activation initiates an intracellular pathway that regulates cellular proliferation, differentiation and angiogenesis. With the availability of anti-EGFR monoclonal antibodies for treatment of several solid neoplasms, assessment of EGFR status has become a frequent clinical question. We have previously determined that EGFR expression is readily detected by immunohistochemistry (IHC) in squamous cell carcinoma (SCC) of the anal canal. However, *EGFR* gene amplification in this tumor type has not been investigated.

Design: A total of 38 SCCs of the anal canal (31 biopsies and 7 resection specimens) were studied. Hematoxylin and eosin (H&E) and immunostained slides against EGFR (clone 31G7; Zymed Laboratories, Inc.) were re-examined to confirm the original diagnosis and EGFR expression status. Dual-color fluorescence *in-situ* hybridization (FISH) assays were performed using paired EGFR (home brew) and centromere 7 (CEP 7) probes (Vysis). Copy numbers of *EGFR* and chromosome 7 probes were assessed in at least 100 tumor nuclei per case.

Results: In 4 (11%) cases, FISH was non-interpretable and these cases were excluded from further analysis. Of the remaining 34 cases, there was disomy 7 (2 copies) in 65%, polysomy 7 (chromosomal gains) in 32%, and monosomy 7 (loss) in 3% of the cases. None of the cases had *EGFR* gene amplification. The distribution of FISH patterns was not associated with age or sex of the patients, nor the grade or stage of the tumors. Increased gene copy numbers due to polysomy 7 were more frequent in keratinizing than in non-keratinizing SCCs (55% vs. 22%), but this difference did not reach statistical significance. Polysomy 7 was present in 39% (7/18) of the cases with positive EGFR immunostaining. Among the 16 cases with negative EGFR immunoreactivity, 4 (25%) had polysomy 7. The majority of the tumors (56%) with positive EGFR immunoreactivity showed a normal disomy 7 pattern by FISH. One case with loss of chromosome 7 had strong positive EGFR expression (3+) by IHC.

Conclusions: Our data show that there is no association between EGFR expression by IHC and gene copy numbers by FISH. This suggests that the high protein expression levels encountered in a subset of SCCs of the anal canal is due to other molecular mechanisms, such as transcriptional upregulation, activating EGFR mutations, increased coexpression of receptor ligands, decreased receptor turnover, and/or heterodimerization with other heterologous receptor systems.

457 Identification of Genes Associated with Colon Carcinoma Metastasis Using "In Silico" Microdissection

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Background: A well recognized problem in studying the gene expression profiles of various tumors is the contamination with normal tissue. We developed an algorithm that estimates the liver tissue contamination in samples of metastatic colon carcinoma, and can generate a "liver-free" gene expression profile. We used this method to identify genes with a potential role in tumor metastasis.

Design: HG-U133A microarrays were used to profile 147 primary colon carcinomas, 26 liver metastases and 11 samples of normal liver. Primary tumors were divided in indolent (stage 1-2, no recurrence at 5 yr -34 cases) and aggressive (stage 4 with synchronous liver metastases -39 cases). A list of 25 liver-specific genes was generated using stringent criteria: >50 fold difference between normal liver and primary carcinoma and $p < 0.001$. The selected genes are not known to play a role in tumorigenesis, thus their presence in a liver metastasis most likely indicates contamination. Estimates of liver contamination (C%) were computed based on the level of expression of liver-specific genes in metastases (M) compared to normal liver (L) and primary colon cancer (P) utilizing the formula C%= (M-P)/(L-P). For each liver metastases the entire expression profile was recalculated using the formula M'= (M-L*C%)/(1-C%). Genes differentially expressed in metastatic versus primary carcinoma were identified using both the raw and "liver-free" expression values and compared with list of genes overexpressed in aggressive versus indolent primary carcinomas.

Results: Estimated average contamination was 7% (0.1-80%). Clustering of the initial data revealed that 16 metastases clustered with normal liver samples and 10 with primary carcinomas. Using the "liver-free" profile, all 26 colon metastases clustered with primary carcinoma. Using the raw and adjusted data, a list of 61 and 18 genes respectively were found overexpressed in liver metastases. Only 4 genes from the first list were potentially associated with tumor spread as opposed to 11 genes from the "liver-free" data. Osteopontin was found overexpressed in both aggressive primary as well as metastatic tumors only using the "liver-free" expression profile.

Conclusions: "In-silico" microdissection enabled us to extract a list of metastasis-specific genes from contaminated samples. Osteopontin which has a known role in tumor metastasis was detected only in the "liver-free" data set, supporting the validity of this method. This method can be applied to any contaminating tissue type.

458 P16 Immunoexpression and HPV Status in Anorectal Epithelial Malignancies

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Background: P16 is a cyclin D-dependent kinase inhibitor tumor suppressor gene that regulates the transition from the G1 to the S phase of the cell cycle. Overexpression of p16 has been reported in squamous cell carcinomas (SCC) as well as in adenocarcinomas (AD) of the cervix and association with high risk subtypes of human papilloma virus (HR-HPV). While it is known that SCC of the anal canal is also associated with HR-HPV, little data exist about the anorectal adenocarcinoma. Additionally, so-called cloacogenic (CC) carcinomas usually behave more aggressively than the more common squamous cell carcinomas of the anorectum. It has been suggested that this aggressive behavior may reflect a different pathogenesis.]This study was undertaken to examine the potential role of HR-HPV in the pathogenesis of anorectal AD and CC using p16 immunoreactivity and HR-HPV status as reference parameters.

Design: A total of 23 biopsy and resection cases from the anorectal region were retrieved from our files and reviewed by two pathologists. 16 were squamous cell carcinomas, of which 6 exhibited a cloacogenic pattern, and 7 were anorectal adenocarcinomas. Immunoperoxidase staining for p16 was performed. P16 was considered positive when nuclear and/or both nuclear and cytoplasmic staining was seen and the positivity for p16 was categorized as follows: 0; + ; + +; + + +; and + + + +. In Situ Hybridization (ISH) was performed utilizing the GenPoint High Risk HPV Biotinylated DNA Probe Cocktail and GenPoint Tyramide Signal Amplification System (Dako Corp. Carpinteria, CA) as per manufacturer's recommendations, with appropriate controls. ISH preparations were evaluated for presence of HR-HPV without knowledge of p16 results and recorded as negative, positive for integrated DNA (punctate nuclear staining), or positive for episomal DNA (diffuse nuclear staining).Correlation between tissue diagnosis, p16 immunoreactivity and HR-HPV ISH signal was made.

Results: (1) All AD were HR-HPV negative and p16 either negative or positive in scattered cells; (2) SCC were 60% positive for HR-HPV and all positive for p16; and (3) Cloacogenic variety of SCCs were 83% positive for HR-HPV and all positive for p16. Of all the carcinomas positive for HR-HPV 7 showed episomal and integrated forms, 3 only integrated HR-HPV, and 1episomal pattern.

Conclusions: 1. Adenocarcinomas of the anorectum are not associated with HR-HPV, thus suggesting a different pathogenesis. 2. Cloacogenic carcinoma pattern is associated with HR-HPV thus suggesting a pathogenesis similar to that of SCC.

459 MGMT Deficient Colorectal Cancer: A Distinct Subset of Mismatch Repair Intact Tumors with Potential Chemosensitivity to Alkylating Agents

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Background: O^6-methylguanine-DNA-methyltransferase (MGMT) removes O^6-alkyl adducts from normal cells and protects tumor cells from alkylating agents. Methylation of specific CpG regions of the MGMT promoter silences gene expression. MGMT promoter methylation is common in colorectal cancer (CRC) and often associated with promotor-methylation of the mismatch-repair (MMR) gene MLH1, which renders cells resistant to alkylating agents. We examined the expression pattern of MGMT, MLH1 and MSH2 in a series of CRCs to determine the frequency and clinicopathologic characteristics of MGMT deficient-MMR intact tumors.

Design: Routinely processed tissue sections from 370 consecutive CRC resection specimens were stained by immunohistochemistry for MGMT, MLH1 and MSH2. Nuclear staining was scored as intact or absent; staining of adjacent non-neoplastic crypt epithelial nuclei served as an internal positive control to assess staining quality. Staining frequency and clinicopathologic parameters were compared by Chi square test.

Results: MGMT staining abnormalities were present in 78 (21%) CRCs, including 63 (17%) with loss throughout the entire tumor section (considered MGMT deficient) and 15 (4%) with admixed foci of intact nuclear staining. MLH1 loss was found in 38 (10%) CRCs, and although it was significantly associated with MGMT deficiency (13/25 MLH1 deficient compared to 50/282 MLH1 intact were also MGMT deficient; $p<0.01$), CRCs with MGMT deficiency alone comprised a larger proportion of all CRC (50/370, 13%). Compared to MLH1 deficient CRC, MGMT deficient (MLH1 intact) CRCs were less common over the age of 75 ($p<0.01$), less likely to be female ($p<0.05$), more frequent in the left colon ($p<0.001$) and less likely to be mucinous ($p<0.01$). MSH2 was lost in 6 (1.6%) CRCs and was not present in association with MLH1 or MGMT loss.

Conclusions: MGMT loss is common in CRC and is occasionally associated with MLH1 silencing. However, the majority of MGMT deficient tumors are MLH1 intact, and they do not share many of the clinicopathologic features typically associated with sporadic MLH1 deficient CRC. These findings suggest differences in the etiology and pathogenesis of MGMT and MLH1 silencing, and highlight the existence of a considerable subset of MGMT deficient MMR-intact CRC that might potentially benefit from chemotherapy with alkylating agents.

460 Is Microsatellite Instability the Main Prognostic Factor in Poorly Differentiated Colon Cancer?

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Background: Colorectal carcinoma (CRC) with microsatellite instability (MSI) is thought to have a better prognosis than microsatellite stable (MSS) carcinoma at comparable stages, despite frequent poorly differentiated histology in MSI CRC. Histopathologic features associated with poor prognosis in CRC include high grade, lymph node (LN) metastases, positive neuroendocrine markers and galectin-3 expression. Peri-tumoral Crohn-like reactions and cytotoxic T-lymphocyte infiltrates are associated with MSI CRC and also reportedly associated with a more favorable prognosis, as is expression of ectopic gastric mucin MUC5AC. This study describes the association of these prognostic markers with microsatellite status in poorly differentiated CRC.

Design: Standard PCR markers were used to determine microsatellite status in 22 cases of poorly differentiated CRC (< 5% gland formation, <5% extra cellular mucin, no signet-ring cells), with no known hereditary association. An H&E and immunohistochemical (IHC) stains for CD8, MUC5AC, galectin-3 and synaptophysin were reviewed. The intensity of IHC staining and density of CD8+ T-cell infiltrate were scored as per Schoeppner et al (Cancer 1995; vol 75:2818-26). IHC staining at ≥5% of maximum intensity score was considered positive. Chi-square, Fisher exact and Mann-Whitney tests were utilized as appropriate.

Results: Poorly differentiated MSI and MSS CRC have a variety of growth patterns and morphologies. The frequency of LN metastasis is significantly lower in MSI cases, while the density of peri and intra-tumoral CD8+ T-cells is higher. The total number of positive LNs in MSI cases is also significantly lower, with no difference in the total

number of LNs sampled in each group. MUC5AC expression and lack of synaptophysin expression are significantly more frequent in MSI cases, while Galectin-3 expression appears to be independent of microsatellite status. (Table 1)

Conclusions: The low frequency of LN metastasis, cytotoxic T-cell infiltrate, (+) MUC5AC and (-) synaptophysin, all favorable prognostic factors, are significantly more common in sporadic MSI CRC than in MSS cases. If these factors are dependent on microsatellite status, MSI may be the main prognostic factor in non-hereditary poorly differentiated CRC.

	Results		
	MSI (n=12)	MSS (n=10)	Stat. Significant difference
Cases with (+) LNs	3/12	8/10	Yes
Total No. of (+) LNs	26	127	Yes
Total No. of LNs isolated	183	208	No
CD8+ T-cell infiltrate	8/12	3/10	Yes
MUC5AC (+)	8/12	0/10	Yes
Synaptophysin (+)	1/12	6/10	Yes
Galectin-3 (+)	11/12	9/10	No

461 Gastric Adenocarcinoma with Low Histologic Grade Simulating Intestinal Metaplasia

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Background: Gastric adenocarcinomas with extremely well-differentiated and low-grade histologic appearances are very rare. Some of them are reportedly reminiscent of complete-type intestinal metaplasia (complete-type intestinal metaplastic carcinoma: CIMC; Endo, Hum Pathol 1999). However, only a small number of such cases has been reported until now. We experienced several cases of gastric adenocarcinoma with low histologic grade and features simulating intestinal metaplastic epithelium of the stomach.

Design: Gastrectomy specimens of nine patients retrieved from our institution and referring hospitals were subjected to the histopathological examination. They contained 10 adenocarcinomas with the above-mentioned histologic features entirely or partially, including three intramucosal lesions being designated as carcinoma based on their structural atypia similar to other invasive carcinomas. Their phenotypic features were determined by immunohistochemistry with monoclonal antibodies (MUC2, MUC5AC, MUC6, HIK1083, CD10, and villin).

Results: The patients were all men of ages ranging from 50 to 78 years (mean 63 years). The tumor sizes were from 1.5 cm to 10 cm in the largest diameter, and the carcinomas were located in various sites of the stomach. Three of them (one limited to the mucosa, one submucosal invasion, and one serosal invasion) mostly showed complete intestinal phenotypes (almost negative for MUC5AC, MUC6, and HIK1083, and positive for MUC2, CD10 and villin) and were consistent with CIMC. The remaining seven carcinomas (two limited to the mucosa, two submucosal invasions, one proper muscle invasion, and two serosal invasions) showed a mixed intestinal and gastric phenotype (variously positive for MUC5AC, MUC6, and HIK1083 as well as MUC2, CD10, and villin). Of those seven carcinomas, one submucosally invasive carcinoma included a focus of intramucosal poorly differentiated adenocarcinoma, and in the invasive area, two carcinomas with invasion to the proper muscle and to the serosa showed mucinous adenocarcinoma and poorly differentiated adenocarcinoma (scirrhous invasion), respectively.

Conclusions: Although our study is comprised of a limited number of cases, the results indicate that gastric adenocarcinomas with low histologic grade and intestinal metaplastic features include CIMCs and carcinomas with a mixed intestinal and gastric phenotype. The latter might be more prone to progress into aggressive histological types than the former.

462 Immunohistochemical Expression of p16 and Ki-67 Correlate with Degree of Anal Intraepithelial Neoplasia (AIN)

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Background: AIN is a human papilloma virus (HPV) related lesion. Expression of p16 and Ki-67 has been correlated with severity of dysplasia in the uterine cervix. Few studies have evaluated the expression of these markers in AIN. The objective of this study is to correlate the expression of p16 and Ki-67 with the degree of dysplasia in the anal canal and to determine the efficacy of these markers in diagnosing high grade AIN.

Design: A search of the computerized database identified 76 anal specimens from 55 patients (37 males; 18 females; mean age: 48 years; median: 44 years; range 25-96 years). They included 36 normal/reactive lesions, 23 low grade (LG) AIN (I and condyloma), and 17 high grade (HG) AIN (II and III). H&E sections were reviewed to confirm the original diagnoses. Five cases were excluded due to lack of squamous mucosa on deeper sections. Immunostaining for p16 (clone: 16P04; dilution 1:100; Cell Marque, Hot Springs AR) and Ki67 (clone: MM1; prediluted; Ventana; Tuscon AZ) was performed. Positive p16 staining was defined as the presence of diffuse/continuous staining in more than 1/3 of the thickness of epithelium. Positive Ki67 staining was defined as the presence of nuclear staining in more than 25% of the cells in more than 1/3 of thickness of epithelium.

Results: Expression of p16 in AIN correlated with that of Ki-67. ($p< 0.001$) The expression of both p16 and Ki67 correlated with the degree of dysplasia. ($p<0.01$) HG AIN often demonstrated p16 staining in more than one-thirds of thickness of the epithelium in a diffuse/continuous fashion. p16 expression in LG AIN was often restricted to the lower 1/3 of the epithelium and/or was focal and discontinuous. Normal/reactive epithelia were either negative or demonstrated rare (<5%) wispy cytoplasmic staining. Table 1 summarizes the sensitivity and specificity of p16 and Ki67 in the detection of HG AIN.

Conclusions: Expression of Ki67 and p16 correlate with degree of the squamous dysplasia in anal lesions. Both antibodies are specific and moderately sensitive markers in the detection of high grade anal dysplasia. A negative staining pattern with both markers strongly supports the absence of a high-grade AIN.

Table 1. Operation Characteristics of Ki-67 and p16 in Detecting High Grade AIN

Biomarkers	p16	Ki67
HG AIN positive	76% (13/17)	71% (12/17)
Normal/LG AIN positive	16% (8/50)	18% (9/50)
Sensitivity	77%	71%
Specificity	86%	85%
Negative predictive value	93%	91%
Positive predictive value	62%	57%
Likelihood	24:1	19:1

463 Mismatch Repair Protein Deficiency and Precursor Intraepithelial Serration in Colorectal Adenocarcinomas

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Background: Colorectal adenocarcinoma (CRA) develops via a stepwise accumulation of genomic alterations. Most CRAs develop through the well-established "adenoma-carcinoma" sequence, however it is currently speculated that some fraction of familial and sporadic CRAs may develop through a precursor lesion termed the "sessile serrated adenoma" (SSA). CRAs that develop through this putative precursor are believed to develop through deficiencies of mismatch repair (MMR) proteins. One piece of evidence for this pathway has been the finding of prominent intraepithelial serration adjacent to right-sided CRAs. We performed this study to investigate whether intraepithelial serration adjacent to CRAs was predictive of MMR protein loss.

Design: We searched our database for cases of CRA which had intact precursor lesions. 47 cases (1999 to present) were found. Patient age and sex and lesion location were noted. Cases were assessed for the following histologic features: size and architecture of the precursor lesion, the presence of intraepithelial serration, quantity of serration, differentiation and the presence of mucinous features. Immunohistochemical staining for hMLH1, hMSH2, and hMSH6 was performed and was assessed as either intact or lost with positive internal control required for assessment. Fisher's exact test was employed to assess the relationship between serration and MMR status.

Results: The 47 cases identified exhibited the following characteristics: age (range 32-83; median 65), sex (27 male, 20 female), location (cecum/right-sided 9, transverse colon 2, sigmoid/rectum/left-sided 23), precursor size (range 4-28 mm; median 11 mm), precursor architecture (tubular 26, villous 20), presence of serration (overall 10/47; >20 glands 6; <20 glands 4), differentiation of malignancy (well-differentiated 2, moderately differentiated 39, poorly differentiated 6), presence of mucinous features (8/47). 4 cases demonstrated loss of MMR protein (concurrent loss of MSH2 and MSH6 in 1 case, loss of MLH1 in 3 cases). 10% of cases showing serration (1 case) and 8.1% of cases without serration (3 cases) demonstrated MMR loss. No statistically significant relationship was demonstrated between the two variables ($p=1$).

Conclusions: Our limited study suggests that the presence of intraepithelial serration does not predict MMR status in adjacent CRAs. A larger study, with a greater number of cases and MSI testing may help to confirm these findings. These findings may also call into question some aspects of the proposed SSA-adenocarcinoma pathway.

464 Endoscopic Mucosal Resection Does Not Improve Lingering Differences in the Evaluation of Gastric Neoplasia between Eastern and Western Pathologists

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Background: Variations in the evaluation of gastric biopsies for neoplasms between Eastern and Western pathologists have led to considerable efforts to understand the causes of the disagreement and improve interobserver agreement. Our goal was to test the Eastern and Western interobserver agreement in light of an increased awareness in the differences and the now common use of endoscopic mucosal resection (EMR) as a diagnostic tool.

Design: Ten pathologists (5 from the Far-East; 5 from the West) reviewed, blindly, 41 paired EMR specimens and corresponding pre-operative biopsies. The cases were classified as: 1) reactive/normal; 2) low grade dysplasia; 3) high grade dysplasia; 4) intramucosal carcinoma; and, 5) invasive carcinoma. The results were analyzed for concordance between raters and to evaluate whether the interobserver agreement was better for EMR specimens than biopsies. Descriptive analysis included intraclass correlation (ICC) and Kendall's coefficient (KC) of concordance for ordinal data. These interobserver agreement rates were compared between the two methods using the Wilcoxon Signed-Rank test.

Results: The ICC and KC for the biopsies was 0.945 (95% CI = 0.905-0.954) and 0.677, respectively. The ICC and KC for the EMRs was improved at 0.962 (95%CI=0.933-0.971) and 0.753. Wilcoxon analysis showed that the interobserver agreement for EMR specimens was not significantly better than that of biopsy specimens (one sided p-value>0.1) In sum, this analysis shows that pathologists are not more likely to agree on a diagnosis of an EMR specimen than they are on a biopsy.

Conclusions: Gastric EMRs do not translate into higher interobserver agreement and concordance among all observers is only marginal. However, the interobserver agreement is better among Eastern than Western pathologists. The cause remains centered around the diagnosis of dysplasia in the West, which is a lesion frequently interpreted as carcinoma in the East because of the different definitions of carcinoma in each system. It is sobering that despite much interest in the litterature, no significant improvement has been made in the rapprocehement of the Eastern and Western point of views in the evaluation of gastric mucosal malignancies.

465 Protein Expression of Tetraploidy-Associated Genes, Mitosin, CDC2, and CENPA, Is Upregulated in Neoplasia of Barrett's Esophagus

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Background: Molecular markers of cancer development in Barrett's esophagus (BE) include 17p(p53) LOH, tetraploidy and aneuploidy. We have previously identified a more than five-fold increase in the expression of some genes [mitosin (CENPF), CDC2, CENPA, HNF3 and Cyclin B1], by mRNA microarray, in p53-/- tetraploid (G2/4N) epithelial cell strains established from BE biopsies (Barrett *et al.,* Cancer Res. 15;63(14):4211-7).The aim of this study was to evaluate the expression of these tetraploidy-associated proteins in in BE associated neoplasia and to correlate their expression with degree of dysplasia, ploidy status and 17p (p53) LOH.

Design: 43 mucosal biopsies [21 negative for dysplasia (ND), 4 with low grade dysplasia (LGD), 8 with high grade dysplasia (HGD) and 10 with cancer (Ca)] from esophagectomy specimens of 14 BE patients (mean age; 65 years, M/F ratio=13:1) were divided into two halves. One half was formalin fixed for subsequent immunostaining with mitosin, CDC2, CENPA, HNF3 and Cyclin B1. The other half was purified into 2N, 4N and aneuploid epithelial fractions using Ki67/DNA content flow cytometric cell sorting and the DNA extracted from these fractions was evaluated for 17p (p53) LOH using short tandem repeat markers. The immunohistochemical staining results (expressed as % positive cells/ total cells) were correlated with grade of dysplasia, DNA content and 17p (p53) LOH data for each biopsy in a blinded fashion.

Results: Mitosin, CDC2 and CENPA expression were all significantly increased in both HGD (mitosin; 21%, CDC2; 21%, CENPA; 31%) and Ca (mitosin; 27%, CDC2; 13%, CENPA; 31%) compared to all other diagnoses (mitosin; 10%, CDC2; 6%, CENPA; 8% , $p<0.01$ for all comparisons). Mitosin, CDC2 and CENPA were also significantly increased in biopsies that were aneuploid in comparison to biopsies that contained either diploid or tetraploid populations ($p<0.05$). Only Cyclin B1 was significantly increased in biopsies that were tetraploid (76%, $p<0.025$) compared to non-tetraploid epithelium (52%). HNF3 did not correlate with the degree of dysplasia, or ploidy status. None of the proteins examined correlated with 17p (p53) LOH.

Conclusions: mRNA microarray technology is a valuable method of identifying proteins involved in carcinogenesis in BE. Mitosin, CDC2, and CENPA appear to be promising candidates for further evaluation as markers of neoplastic progression in BE.

466 Mucinous Carcinoma Peritonei as the Pathologic Counterpart of Pseudomyxoma Peritonei: 101 Patients with Primary Appendiceal Neoplasms, Uniformly Treated at One Institution. A Clinicopathologic Analysis with Literature Review

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Background: Pseudomyxoma peritonei is a clinical syndrome of mucinous/gelatinous ascites, usually secondary to an appendiceal tumor. The pathologic classification of pseudomyxoma peritonei, and its associated appendiceal tumors, has been plagued with controversy and confusing terminology.

Design: In an effort to clarify this, we reviewed the pathology of 101 patients, all treated at our institution from 1993 to 2005, with pseudomyxoma peritonei of appendiceal origin. As the largest pathologic series of gelatinous ascites related to primary appendiceal neoplasms, all patients were uniformly treated with our standardized protocol. The cases were assigned, according to previously published criteria, to the categories of Disseminated Peritoneal Adenomucinosis (DPAM), Peritoneal Mucinous Carcinomatosis (PMCA), or PMCA with Intermediate (well-differentiated) features (PMCA-I), with the exception that any case with a signet-ring cell component was considered PMCA and not PMCA-I.

Results: By histologic category, 58 patients had DPAM, 23 were PMCA, and 20 were PMCA-I. One-, three- and five-year survival outcomes were not significantly different between DPAM and PMCA-I. DPAM and PMCA-I also exhibited a roughly equal incidence of parenchymal (beyond the serosa) organ invasion. Survival outcomes were significantly worse for PMCA, compared to PMCA-I and DPAM. After reviewing our data and the literature, Mucinous Carcinoma Peritonei- Low grade (MCP-L) is applied to the low grade histology of pseudomyxoma peritonei, including those cases referred to by some as DPAM in the same category with PMCA-I. Cases that are moderately- to poorly- differentiated are classified as Mucinous Carcinoma Peritonei- High grade (MCP-H).

Conclusions: The lowest grade of clinical behavior within this disease (MCP-L) includes a subset of the carcinomatous histology that had previously been placed with a higher grade by other authors. We suggest that the previously confusing terminology be replaced by mucinous carcinoma peritonei, low or high grade.

467 KOC (K Homology Domain Containing Protein Overexpressed in Cancer) Is Expressed Late in the Malignant Transformation of Barrett's Esophagus

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Background: KOC (K Homology Domain Containing Protein Overexpressed in Cancer) is an oncofetal RNA-binding protein expressed in pancreatic adenocarcinoma and its precursors, but not in benign ducts. Its expression has not been studied in other GI malignancies that develop via morphologically distinct stages, such as Barrett's esophagus (BE), which may progress to glandular dysplasia and adenocarcinoma. Thus, the aim of this study was to evaluate KOC expression in BE-associated neoplasia in order to 1) evaluate its expression in benign and malignant glandular lesions of the distal esophagus and 2) assess its role in the neoplastic transformation of BE.

Design: Mucosal biopsy specimens from 41 patients with BE and/or BE-associated neoplasia were reviewed. The glandular foci were classified as cardiac-type mucosa (CTM, n=35), BE (n=22), low-grade dysplasia (LGD, n=10), high-grade dysplasia (HGD, n=9), and invasive adenocarcinoma (ADCA, n=8). Each case was stained with a monoclonal antibody to KOC using standard techniques. The intensity (weak, moderate, strong) and extent of the staining reaction in each of the glandular elements was recorded.

Results: The results are summarized in Table 1. Moderate to strong, diffuse staining for KOC was present in 8/8 (100%) foci of ADCA and 3 (33%) foci of HGD showed patchy staining of weak or moderate intensity. All foci of CTM, BE, and LGD were negative for this marker ($p<0.001$ compared to ADCA).

Table 1. KOC Expression in Barrett's Esophagus and Barrett's Esophagus-Associated Neoplasia

	Extent of KOC Staining in Lesional Epithelium			
Type of Epithelium	Absent	<25% of Cells	25-75% of Cells	>75% of Cells
Cardiac-Type Mucosa	35/35 (100%)	0/35 (0%)	0/35 (0%)	0/35 (0%)
Barrett's Esophagus	22/22 (100%)	0/22 (0%)	0/22 (0%)	0/22 (0%)
Low-Grade Dysplasia	10/10 (100%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
High-Grade Dysplasia	6/9 (67%)	2/9 (22%)	1/9 (11%)	0/9 (0%)
Esophageal Adenocarcinoma	0/8 (0%)	0/8 (0%)	1/8 (13%)	7/8 (87%)

Conclusions: KOC expression occurs late in the neoplastic transformation of Barrett's esophagus. It is a highly sensitive marker of esophageal adenocarcinoma and distinguishes it from low-grade dysplasia and non-neoplastic glandular epithelium. The potential role of this marker in diagnostic pathology should be evaluated in future studies.

468 Diagnostic Accuracy in Ulcerative Colitis Neoplasia

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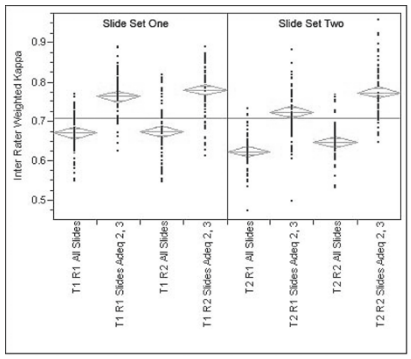
Background: Dysplasia in UC, despite limitations, remains the gold-standard for cancer risk management. Diagnostic reproducibility has not been rigorously evaluated since publication of provisional criteria over 20 years ago. To address this, a multi-institutional study was carried out.

Design: 266 slides covering the full neoplastic spectrum was assembled from consecutive cases at 12 institutions. Slides were blindly block randomized into 2 sets, coded and circulated twice each. Two independent readings for set 1 occurred without prior discussion of diagnostic criteria. Two readings for set 2 occurred after a consensus conference to define uniform criteria. Neoplasia, inflammation and biopsy adequacy were scored. Four levels of biopsy adequacy reflected the pathologist's diagnostic confidence as: uncertain/guess, somewhat certain, fairly certain, and certain. Given the ordinal nature of the data, weighted kappa was used to measure rater agreement.

Results: Based on consensus diagnoses, there were 47 + 36 negative, 27 + 32 indefinite, 35 + 38 low-grade, 22 + 15 high-grade dysplasias and 9 + 5 carcinomas in sets 1 and 2, respectively. Intra and inter-rater agreement did not improve after the consensus conference, even after stratifying by level of inflammation, adequacy and fixation. Biopsy adequacy scores had a large effect on agreement; this was evident in all four rounds for both inter (see figure), and intra-rater agreement.



Inter-rater agreement for the four readings (T1/T2=slide set 1/set 2; R1/R2=reading 1/2; Adeq 2,3=adequacy fairly certain or certain)

Conclusions: These results are clinically significant, since they reveal that GI pathologists can achieve substantial diagnostic reproducibility (weighted kappa > 0.6), which could not be improved upon by consensus criteria. More significantly, the adequacy of the biopsy specimen strongly influences diagnostic agreement.

469 Enteroendocrine Cell Dysgenesis and Malabsorption, a Histopathologic Characterization

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Background: Enteroendocrine cell dysgenesis was observed in three patients with intestinal failure of unknown cause. Enteroendocrine cell dysgenesis is a congenitally acquired life threatening malabsorption condition with a unique clinical phenotype paired with a histologically identifiable disease pattern. The clinical phenotype was unusual in that the affected patients demonstrated a profound malabsorption to all nutrients except water. The small intestine in each patient demonstrated almost no abnormality except a near absence of endocrine cells in the mucosa. Known causes of congenital malabsorption and inflammatory and infectious causes of diarrhea were excluded. This work describes the first pathological characterization of enteroendocrine cell dysgenesis using routine techniques. The pattern of injury is distinct from other

histopathologically assessed congenital malabsorptive conditions such as microvillus inclusion disease and abetalipoproteinemia, and inflammatory conditions such as allergy, gluten sensitive enteropathy, and inflammatory bowel disease (IBD).
Design: Small intestine and colon biopsies/resection specimens from three patients with the same clinical phenotype of profound malabsorption were examined using hematoxylin and eosin staining as well as immunohistochemical staining for chromogranin, synaptophysin and NSE (neuron-specific enolase). They were then compared to normal small intestine/colon controls to determine the normal number of endocrine cells/crypt.
Results: We found that the small intestine and colon samples from all three patients either had a complete lack of or a markedly decreased number of endocrine cells in their crypts when compared to normal controls.
Conclusions: We present the first histologic characterization of enteroendocrine cell dysgenesis as a newly described pathologic entity that can be added to the differential diagnosis of childhood malabsorptive disorders.

470 Stroma-Rich Hyperplastic Polyps: A Clinicopathologic and Immunohistochemical Study of 18 Cases

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Background: Recent studies suggest that "hyperplastic" polyps (HP) probably represent a heterogeneous group of lesions with different biologic properties and neoplastic potential. Anecdotally, we have noted that some otherwise typical HPs contain a prominent spindle cell stroma, but the significance of this finding is unclear. The purpose of this study was to evaluate the clinical, pathologic, and immunohistochemical features of 18 "stroma-rich" hyperplastic polyps (SHP) identified from a search of 494 consecutive HPs (prevalence rate=3.6%) at our institute.
Design: The clinical and pathologic features of 18 SHPs excised from 17 patients (M/F ratio: 7:10, mean age: 58 years) were evaluated and compared to a control group of 135 consecutive conventional (non-stroma-rich) HPs (M/F ratio: 58:77, mean age: 58 years). SHPs were also immunostained for desmin, SMA, CD34, S-100, EMA, claudin-1, and c-kit in an effort to identify the nature of the stromal cells and provide insight into the etiology of SHPs.
Results: All SHPs, except 1, were located in the rectosigmoid region, which was significantly different from controls (94% vs. 31%; $P < 0.001$). However, the mean size of the polyps (3 mm) and the prevalence of associated conventional HPs (24%) and adenomas (35%) were similar in SHPs and controls (4 mm, 23%, and 32%, respectively). Histologically, the epithelium in all SHPs was microvesicular. In all cases, the lamina propria contained a cellular stroma composed of cytologically bland spindle cells containing oval to slightly elongated nuclei and pale eosinophilic cytoplasm. In 5 (28%), the spindle cells showed a whorling pattern around entrapped crypts. The remaining 13 (72%) showed a more haphazard growth pattern of spindle cells. By immunohistochemistry, 3 distinct patterns were recognized. In 13 (72%), the stromal spindle cells were positive for SMA and desmin, 5 of which also showed rare S-100-positive cells. In 2 (11%), they were positive for EMA and claudin-1 (characteristic of perineurial cells), and in 3 (17%) all immunomarkers were negative.
Conclusions: SHPs represent a phenotypically heterogeneous group of lesions that occur almost exclusively in the rectosigmoid. The stroma of most SHPs shows features of smooth muscle, or myofibroblasts, and may represent early prolapse-associated changes, whereas a smaller subset are probably fibroblastic in nature, without a distinctive immunophenotype. Finally, rare cases show perineurial differentiation and may represent early perineuriomas.

471 Low Rate of BRAF Mutations in Cholangiocarcinoma

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Background: BRAF mutations have been reported in a variety of solid organ tumors, including melanoma, colon cancer and thyroid cancer. There are conflicting results as to the frequency of BRAF mutations in cholangiocarcinoma (CC). A German study (Tannapfel A, et al. Gut 52:706, 2003) found BRAF mutations in 22% of intrahepatic cholangiocarcinomas (ICC), while an American study (Goldenberg D, et al. Mod Path 17:1386, 2004) found no evidence of BRAF mutations in extrahepatic (ECC) or ICC. Based on these conflicting results, we tested a group of ICC and ECC for BRAF mutations.
Design: 50 CC (22 ICC and 28 ECC) were analyzed. DNA was extracted from microdissected, formalin-fixed tissue and was subjected to PCR for BRAF exon 15. The PCR amplicon product was then used for a cycle sequencing reaction using the BigDye Terminator kit and an automated sequencer (ABI, Foster City, CA). The sequence was analyzed for the characteristic T1799A point mutation (V600E).
Results: For ICC, the mean tumor size was 6.6 cm with a median grade of 2 (of 3), T stage of 3 and N stage of 1. For ECC, the mean tumor size was 3.4cm with a median grade of 2 (of 3), T stage of 3 and N stage of 0. Most ICC were mass-forming tubuloglandular CC while most ECC were periductal infiltrating CC. Two of 50 (4%) CC (1 ICC and 1 ECC) had BRAF V600E mutations: the ICC was 15cm, grade 2, of tubular type and had extensive intrahepatic metastases; the ECC was 1.7 cm, grade 2 and was of tubular type.
Conclusions: 4% of cholangiocarcinomas (1 ICC and 1 ECC) demonstrated BRAF mutations. There were no clinicopathologic features that were particular to the cases with BRAF mutations. Our data support the study that BRAF mutations are uncommon in CC (both ICC and ECC) in the United States.

472 Expression Profiling of Primary and Metastatic Pancreatic Cancers Indicate Maintenance of Gene Regulation with Tumor Progression

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Background: Previously reported expression profiles of primary pancreatic cancers indicate that reproducible changes in gene expression occur that are associated with pancreatic carcinogenesis. However the changes in gene expression associated with pancreatic cancer metastasis are unknown.
Design: Two samples each of normal pancreas, chronic pancreatitis, normal liver, normal lung, normal pancreatic ductal epithelium and five samples of primary pancreatic cancers were collected from resection specimens. In addition, 24 samples of primary infiltrating or metastatic pancreatic cancer to liver, lung, peritoneum or lymph node were obtained from the collections of the GI Cancer Rapid Medical Donation Program. Labeled cDNA was prepared for each sample and hybridized to Affymetrix Human Genome U133 Plus 2.0 GeneChips®. Profiles were analyzed for patterns related to carcinogenesis, resected vs. unresectable cancer, and cancer metastasis. The expression of selected genes was validated by qPCR and immunohistochemistry.
Results: Hierarchical cluster analysis accurately classified normal tissues, resected pancreatic cancers and rapid autopsy derived cancers. Among the autopsied primary or metastatic pancreatic cancers, each primary carcinoma or metastasis was most similar to all other samples derived from that same patient, irrespective of sample location. Comparison of normal pancreas and pancreatitis tissues to all cancers identified 128 genes as differentially expressed in pancreatic cancers including NOTCH1, DUSP6 and ERBB3. However, comparison of resected primary carcinomas and autopsy derived primary carcinomas specifically did not identify significant differences in gene expression, nor did a comparison of the autopsied primary carcinomas and their matched metastases. Furthermore, genes associated with metastasis in published breast cancer expression profiles were not confirmed in our subset of pancreatic cancers. The consistent expression among primary carcinomas and their matched metastases of a subset of genes was confirmed by qPCR and immunohistochemistry.
Conclusions: Unlike pancreatic carcinogenesis that is associated with robust changes in gene expression, additional changes in gene expression do not occur with the development of pancreatic cancer metastasis as described for other tumor types. These findings suggest that alternative mechanisms such as host factors or post-translational processing may play a role in modulating metastatic ability of pancreatic cancers.

473 Evaluation of Microvascular Density in Barrett's Mucosa

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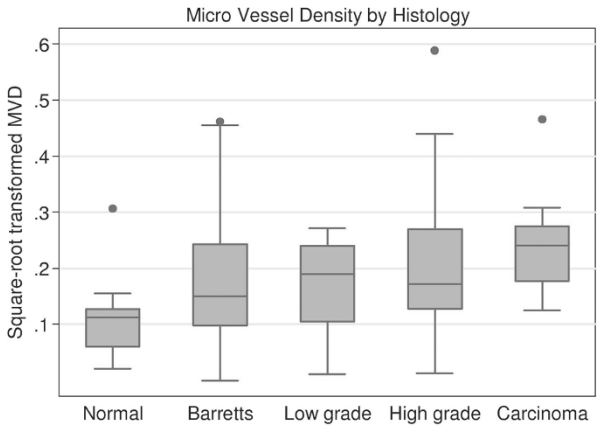
Background: Angiogenesis is an important component of the neoplastic process in a variety of tumor types, including Barrett's-related adenocarcinoma. Using the latest generation of high-resolution endoscopes, it appears that dysplastic foci in Barrett's mucosa (BM) may be more easily identified by increased neovascularization, rather than by pit pattern. The aim of our study was to examine foci of BM, low-grade dysplasia (LGD), high-grade dysplasia (HGD), and invasive carcinoma (CA) to determine if microvascular density (MVD) is indeed associated with progression in these lesions.
Design: From the files in the Department of Pathology, endoscopic mucosal resection specimens containing BM and Barrett's-related lesions were selected. Each H&E slide was reviewed, and areas of BM, LGD, HGD, and CA were selected for study. Sections were stained with anti-CD31 (Dako, 1:40), and the slides were then digitally scanned. Using Chromavision® software, each confirmed focus of BM, LGD, HGD, and CA was evaluated for MVD. Gastric cardia mucosa served as normal, control tissue.
Results: In total, 178 foci were evaluated. There was a statistically significant trend (p=0.003) of increasing MVD across the five histologic groups (Figure 1). MVD was also statistically significant in the pair-wise comparisons between BM and CA (p=0.043) and LGD and CA (p=0.042).



		Pair-wise comparisons			
	Normal	BM	LGD	HGD	CA
Normal	—	—	—	—	—
BM	0.11	—	—	—	—
LGD	0.10	0.83	—	—	—
HGD	0.007	0.19	0.59	—	—
CA	0.002	0.043	0.042	0.14	—

expressed as p-values

Conclusions: We have demonstrated a significant stepwise increase in MVD in Barrett's-associated neoplastic progression. Consistent with previous studies, our results show a significant difference in MVD between BM and CA. In addition, we have shown a significant difference in MVD between LGD and CA. These data suggest that neovascularization is a biologically important factor throughout the metaplasia-dysplasia-carcinoma sequence in Barrett's mucosa. These findings also suggest that modern endoscopes may accurately discriminate CA from BM and LGD, but not between other Barrett's-related lesions, based on vascular pattern.

474 Eosinophilic Esophagitis Versus Reflux Esophagitis: Correlation of Clinico-Pathologic Features, Expression of Eotaxin-2 and Chemokine Receptor-3

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Background: Differentiating gastro-esophageal reflux disease (GERD) from eosinophilic esophagitis (EE) is important given pathogenetic and therapeutic differences. EE is considered an allergen-mediated disorder characterised by a dramatic increase in intraepithelial eosinophils. Eotaxins are chemokines involved in the activation and recruitment of eosinophils mediated by the chemokine receptor-3 (CCR-3). Eotaxin and CCR-3 have been implicated in the pathogenesis of bronchial asthma. The aim of this study was to compare the expression of Eotaxin-2 and CCR-3 mRNA in esophageal biopsies from patients with EE, GERD and normal controls.

Design: This was a retrospective study using formalin-fixed paraffin embedded blocks, all biopsies, from 11 cases of EE, 17 cases each from GERD and normal esophagus controls. Parameters studied included clinical /endoscopic features, histological findings including intraepithelial eosinophil counts, and expression for Eotaxin-2 and CCR-3 mRNA by quantitave RT-PCR.

Results: All 11 EE cases were males, age 2-49 years, with a mean eosinophilic count of 62.75/HPF (range 34.5-140.75). The 17 cases of GERD (10 males and 7 females), had a mean eosinophil count of 4.75/HPF and 17 normal controls. EE cases had symptoms of dysphagia, food getting stuck, abdominal pain etc. Endoscopic appearance included friability, furrows, ridging, cracking, white spots etc. 11/11 cases of EE as opposed to 7/17 cases of GERD had luminal distribution of eosinophils; 5/11 of EE had eosinophilic microabscesses versus only 1 case of GERD. Basal cell hyperplasia was more prevalent in EE and papillary elogation was more common in GERD. The mean expression of Eotaxin-2 mRNA among EE, GERD and normal controls was 5.44 (range 0.66-19.9), 1.24 (0.0-5.62), and 0.46 (0.0-1.7) pg/ng, respectively. The difference was significant with p value of 0.012 between EE and GERD cases, and 0.003 between EE and normal controls. Mean expression of CCR-3 mRNA among EE, GERD and normal controls was 4.18 (range 0-27.1), 3.47 (range 0-0.59), and 0.14 (range 0-0.76) pg/ng respectively. Both Eotaxin-2 and CCR-3 showed a strong expression in EE cases.

Conclusions: Our results highlight the important role of Eotaxin-2 and CCR-3 in the pathogenesis of EE. Based on the findings, agents that block Eotaxin-2 and CCR-3 may have a promising role in the management of EE by preventing damage caused by the influx of eosinophils.

475 NF kappa-B and Bcl-2 in Epstein-Barr Virus Positive Gastric Carcinomas

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Background: The mechanism by which Epstein-Barr virus (EBV) contributes to the carcinogenesis of gastric mucosa remains unanswered, while the involvement of NF-kappaB p65 (Rel A) and bcl-2 has been elaborated in EBV-positive B-cell lymphoma.

Design: In situ hybidiztion for EBV-encoded small RNAs, immunohistochemistry for NF-kappaB and bcl-2, and TUNNEL method were performed on tissue array slides of gastric carcinomas. In situ hybridiztion for EBV-encoded small RNAs, RT-PCR for EBV transcripts, and Western blot for bcl-2 were done in stomach cancer cell lines, lymphoma cell lines and lymphoblastoid cell line .

Results: NF-kappaB nuclear positivity was more frequent in EBV-positive gastric carcinomas (n=55) than those in EBV-negative gastric carcinomas (n=72) ($p<0.001$). In EBV-positive gastric carcinomas, NF-kappaB nuclear positivity was associated with a good prognosis in univariate analysis ($p<0.05$), although the pathological tumor stage was the only independent prognostic factor. The apoptotic index (AI) showed no difference between NF-kB positive group (AI: 0.33) and NF-kB negative group (AI: 0.31). NF-kappaB immunopositivity was more frequent at an earlier pathological stage of tumor with a marginal impact on statistical significance ($p= 0.069$). EBV-positive gastric carcinoma showed rare bcl-2 positivity (1 case). Western blot showed bcl-2 to be irrespective of EBV status in stomach cancer cell lines. However, bcl-2 was highly expressed in EBV-positive lymphoma or EBV-positive lymphoblastoid cell lines. The BARF1 transcript was confirmed in both EBV-positive stomach cancer and EBV-positive lymphoma, suggesting tissue type-specific bcl-2 activation by BARF1.

Conclusions: NF-kappaB may be associated with oncogenesis in EBV-positive gastric carcinomas. EBV-positive gastric carcinomas showed rare bcl-2 involvement. The characteristic expression of proteins may relate to both EBV and tissue type.

476 CK7 Expression in Normal, Regenerative and Neoplastic Intestinal Epithelium: Uncovering the Identity of Potential Human Intestinal Progenitor Cells

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Background: Cytokeratin (CK)7 is an intermediate filament that is generally believed to be absent in normal intestinal epithelium. Our previous study has shown that it is aberrantly expressed in primary small intestinal adenocarcinomas. Herein, we report our new observations on the intriguing CK7 expression in normal and abnormal intestinal epithelium.

Design: A total of 25 tissue blocks selected from different portions of the intestinal tract that contained mucosa with no identifiable histopathologic abnormality (5 each from the duodenum, jejunum, ileum, right and left colon) and a total of 35 sections containing ulcerated mucosa of various causes (19 from the small intestine and 16 from the colon) were included in this study. Immunohistochemical staining for CK7 was performed and cytoplasmic staining was considered positive. For quantitative analysis of CK7-postitive cells in normal small intestine, we counted 100 villous and 100 crypt cross-sections for each specimen. For colon, 100 crypt cross-sections were counted for each case.

Results: Scattered individual CK7-postive cells were identified throughout the intestinal tract in an order of decreasing frequency from the duodenum (135 ± 80.1/100 crypts; 89 ± 11.4/100 villi) to the left colon (28 ± 20.9/100 crypts; $P<0.0001$). However, No statistically significant difference was detected among the different parts of the small intestine or between the right and left colon. In the small intestine, these cells were more frequently seen in crypts than in villi ($P<0.004$). CK-expressing cells in both small and large intestines were uniformly small and angulated in shape with scanty cytoplasm and lymphocyte-like condensed nuclei, and almost always situated on the basement membrane. In ulcerated mucosa, the number of CK7-positive cells was dramatically increased near the ulcers. They not only formed aggregates in the crypts, but also replaced entire crypts and extended to the surface forming long stretches of villous or lining epithelium. Morphologically, CK7-positive cells near the ulcers exhibited regenerative features including nuclear enlargement with prominent nucleoli. Interestingly, increased CK7 expression was also noted in normal-appearing small intestinal epithelium adjacent to adenocarcinoma.

Conclusions: We identified a population of CK7-expressing cells in normal intestinal mucosa. Their unique morphology and anatomic distribution, and their potential roles in epithelial regeneration and tumorigenesis suggest that they may represent the long-searched intestinal progenitor cells.

477 Vascular Endothelial Growth Factor-A and Tissue Factor in Distal Esophageal Adenocarcinomas and Precursors: An IHC Tissue Microarray Analysis

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Background: Over 10,000 adenocarcinomas of the esophagus are diagnosed annually in the U.S. Barrett's changes and dysplasia precede adenocarcinoma. Prognosis is poor with 20% overall 5-year survival. Early stage detection can improve survival up to 80%. Elucidating mechanisms of progression may lead to earlier diagnosis and more effective management.

Design: The data included 21 esophageal resections (ENH 1999-2004) for adenocarcinoma arising within 3 cm of the gastroesophageal junction. Patients included 16 (76%) males and 5 (24%) females, mean age 67 years, 5/21 (24%) stage 0/1, 6/21 (29%) stage 2, and 10/21 (47%) stage 3. Mean follow-up was 23 months. A tissue microarray with 3 cores (0.6mm) each of Barrett's epithelium (BE), high grade glandular dysplasia (HGGD), and adenocarcinoma (AdCa) was created from archived paraffin-embedded blocks. Immunohistochemical (IHC) results for tissue factor (TF), vascular endothelial growth factor-A (VEGF-A), Ki-67, p53 and CDX-2 were interpreted by two pathologists (IAC & CDS).

Results: Strong VEGF-A IHC reactivity was noted in 76% of AdCa, 29% of HGGD and none of BE cases (Mantel-Haenszel p-<0.0001). Strong p53 overexpression was seen in 33% of BE, 47% of HGGD and 67% of AdCa (p-<0.0001). High proliferation fraction (Ki-67 >50%) was demonstrated in none of the BE, 12% of HGGD, and 43% of AdCa. Weak TF positivity was noted in 3 AdCa. CDX-2 reactivity was noted in 90% of AdCa. No individual IHC marker correlated to lymph node metastases or survival.

Conclusions: Recent studies suggest a correlation between VEGF expression and esophageal carcinogenesis. Our data indicate a statistically significant increase in VEGF-A expression along the BE->HGGD->AdCa pathway. This finding supports investigating the use of targeted VEGF therapies in patients with esophageal adenocarcinomas and precursors. Our novel data show high specificity of TF in AdCas, compared to HGGDs; although only 14% of AdCas were TF positive. Studies of VEGF-A and TF expression in metastatic esophageal AdCas are suggested as associations between TF expression and metastsis are known in colonic and hepatocellular carcinomas.

478 Frequency of p53 Mutation and Overexpression in Esophageal Adenocarinoma: A Critical Reassessment

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Background: p53 alterations occur commonly in esophageal adenocarcinoma (EAC), and immunohistochemical staining (IHC) for p53 overexpression has been postulated as a potential marker in monitoring patients with Barrett's dysplasia. However, the reported frequency of p53 alterations in EAC varies widely even in recent reports, from <20% (Modern Pathol. 17:1323, 2004) to >95% (Br. J. Surg. 89:824, 2002), presumably affected by whether protein overexpression or gene mutation was analyzed, as well as

by specific techniques used to detect mutation. A critical re-assessment of p53 alterations in EAC is thus warranted.
Design: Forty cases of EAC that underwent esophagectomy were identified, and a representative formalin-fixed paraffin block was obtained from each case. Sections were prepared and stained with anti-p53 antibody; >10% nuclear staining was considered expression. The adjacent sections were used for laser microdissection, followed by DNA extraction, PCR amplification, and DNA sequencing of exons 4, 5, 6, 7, 8 of p53 and mutation analysis for *k-ras*. The sequencing data were analyzed using Mutation Surveyor™ software.
Results: IHC staining showed unequivocal nuclear staining of p53 in 23 of 40 (58%) EAC, with almost all (22/23) positive cases showing staining of >50% of cells at 2+ to 3+ intensity. The frequency of positive staining did not correlate with the histological grade of the EAC. Mutational analysis is currently ongoing and has been completed in 17 of the 40 EACs. In addition to a *k-ras* mutation found in a single case (1/17), p53 mutations that would lead to protein sequence changes were found in 11 of 17 (65%) EAC. Seven of these 11 cases were also p53-positive by IHC; 2 of 4 mutation-positive, IHC-negative cases were nonsense mutations that would lead to truncated p53, known to be undetectable by anti-p53 antibodies. On the other hand, 6 of 17 cases examined showed no p53 mutation (4 cases) or silent mutations (2 cases). Of these 6 cases, 5 were also negative by IHC staining. The basis for the single mutation-negative, IHC-positive case is unclear and may be due to mutation in exons outside of exons 4 to 8.
Conclusions: Both IHC and mutational analysis following microdissection estimated the rate of p53 alterations in EAC at the range of ~60-65%. This indicates that p53, evaluated by either method, is of inadequate sensitivity to be used as the sole screening marker for the monitoring of Barrett's progression. However, as a frequent and specific marker, it could be useful as part of a monitoring panel.

479 Prevalence-Driven Comparative Histological Analysis of Recent and Archival Esophageal Biopsies for Eosinophilic and Reflux Esophagitis

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Background: Eosinophilic esophagitis (EE), first systematically described in 1995, is now being diagnosed rather frequently. Pre-1995 EE cases were usually (mis)interpreted as severe gastroesophageal reflux (GERD). While the etiology of EE is unknown, novel food and airborne allergens have been implicated as contributors raising the suspicion that the prevalence of EE is increasing. It remains controversial whether the prevalence of EE is truly increasing, or if enhanced awareness alone is responsible for the increasing numbers of currently diagnosed cases. To investigate this, we performed a retrospective comparative histological analysis of our recent and pre-1995 archival esophageal biopsy specimens.
Design: The recent group consisted of a total of 170 cases diagnosed either with EE or GERD during year 2004 (age range 3 months – 86 years, males 101 and females 69) and the archival group consisted of a total of 128 cases diagnosed with GERD at our institution between January 1993 and December 1995 (age range 1 month – 89 years, males 82 and females 46). The archival esophageal biopsies were retrospectively reassigned as EE if two or more of the following histological features were present: >20 eosinophils per HPF, the presence of luminal eosinophilic clusters, and severe basal zone hyperplasia. Statistical analyses were performed to determine any differences in the frequency and other characteristics between EE and GERD between the archival and recent cases.
Results: There was no statistically significant difference in the frequency of EE between the archival group and the recent material (Chi-square test, p=0.731). The patients with EE were consistently younger than those with GERD (by 16-22 years) in both groups and commonly diagnosed as having severe GERD in the archival material.

Total Cases	EE	GERD	EE age	GERD age	
Pre-1995	128	16 (13%)	112 (87%)	Mean 17.5	Mean 32.9
2004	170	26 (15%)	144 (85%)	Mean 19.9	Mean 42.8

Conclusions: The prevalence of EE is not increasing, only awareness. Hence, it appears that the cause of EE is not related to any newly introduced etiological agents as some have proposed. The perceived increase in prevalence is due to the fact that EE went unrecognized for many years and was earlier diagnosed as severe GERD. Apart from similar prevalence, the mean age of patients affected with EE remains similar in archival and recent cases.

480 p4EBP1 in Colorectal Carcinomas Is Associated with Tumor Stage and Reflects the Real Oncogenic Role of the mTOR Pathway

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Background: Mammalian target of rapamycin (mTOR) controls initiation of translation through regulation of ribosomal p70S6 kinase (S6K1) and eukariotic translation of initiation factor-4E (eIF4E) binding protein (4E-BP). Activation of translation complex eIF4F is essential for the genesis and maintenance of the malignant phenotype in colorectal carcinoma (CRC). Nonetheless, p4EBP1 can be regulated by other pathways including MAPK, p53, and can reflect the real oncogenic role of this pathway in colorectal tumors.
Design: p4EBP1 expression from 133 formalin-fixed, paraffin-embedded CRC was analyzed in order to asses the clinicopathological utility of the levels of expression of p4EBP1 protein. Tissue microarrays representative of normal mucosa and carcinoma of all cases were performed. When present, additional microarrays from liver or lymph node metastases where done. Levels of expression were evaluated as percentage and intensity of stained tumor cells (Hscore). Results were correlated with clinicopathological parameters and survival.
Results: Cytoplasmic as well as nuclear staining of p4EBP1 were significantly higher in carcinomas (74.1%) than in normal mucosa (34.1%) (p=0.001). Left-sided and rectal tumors had higher levels of p4EBP1 expression than right-sided ones (p=0.024). Levels of expression correlated with the presence of lymph node metastasis (p=0.005) and depth of tumor infiltration (pT) (p=0.022). Cytoplasmic expression of p4EBP1 was higher in liver metastasis than in synchronous primary colorectal tumors (p=0.012), while there were no significant differences of expression between the primary tumor and their lymph node metastases (p=0,785). Survival was not related to p4EBP1 expression.
Conclusions: With these results we conclude that p4EBP1 expression in CRC is associated with tumor infiltration and metastasis. Since p4EBP1 is activated by several cell signaling transduction pathways, we propose that p4EBP1 expression may reflect its more aggressive behavior and the real oncogenic role of this biochemical pathway in CRC. Further studies have to be done to confirm whether p4EBP1 expression can be used as a potential marker of progression risk in CRC.

481 CDX2 Staining Is Decreased in Microsatellite Unstable Colorectal Carcinomas

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Background: CDX2 is an intestine-specific nuclear transcription factor and useful marker for intestinal adenocarcinomas. However, some studies have suggested that colorectal tumors with microsatellite instability (MSI) might actually exhibit loss of CDX2 expression. We compared the frequency of CDX2 staining in MSI colorectal carcinomas to staining in microsatellite stable (MSS) tumors to determine whether the sensitivity of CDX2 for colorectal carcinomas was decreased in MSI tumors.
Design: Thirty-three MSI-high (H), 8 MSI-low (L), and 29 MSS colorectal carcinomas were retrieved from archival files. Tumors had been previously assessed for MSI using a modified Bethesda panel of microsatellite markers (BAT25, BAT26, D18S69, D2S123, and D5S346). Tissue microarrays were made from formalin-fixed, paraffin-embedded blocks (2 cores per block, 2 mm each). Sections were stained with a monoclonal antibody against CDX2 and evaluated by two pathologists. Convincing nuclear staining in either core was considered positive and controls stained appropriately. Statistical analysis was performed using a Chi-square test and Fisher's exact test.
Results: CDX2 was positive in 9/24 (37.5%) MSI-H tumors, 5/8 (62.5%) MSI-L tumors, and 19/29 (65.5%) MSS tumors. CDX2 staining was significantly decreased in MSI-H as compared with both MSI-L tumors (p=0.007) and MSS tumors (p=0.003). There was no significant difference in staining between MSI-L and MSS colorectal cancers.
Conclusions: CDX2 staining is significantly decreased in MSI-H colorectal cancers when compared to MSS or MSI-L tumors. There is no difference in the frequency of staining when MSI-L tumors were compared with MSS tumors. Therefore, MSI-H colorectal cancers may show a loss of CDX2 expression and in this subset of tumors, CDX2 may not be a sensitive marker for intestinal differentiation.

482 Autoimmune Pancreatitis: A Systemic IgG4 Immune Complex Mediated Disease

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Background: Autoimmune pancreatitis (AIP) is a mass forming inflammatory disease. Recent reports have alluded to the multisystemic nature of this condition. Increased numbers of tissue IgG4 positive plasma cells have been documented. In this study we explore the systemic nature of this disease, ultrastructurally evaluate for immune complexes and perform a systematic study of tissue IgG4 immunoperoxidase, and assess its diagnostic utility in AIP.
Design: The study group consisted of 36 cases of AIP, 21 of whom underwent a Whipple procedure. The extrapancreatic mass lesions included bile duct (n=3), salivary glands (n=3), lung (n=2) and kidney (n=4). The clinical and radiological data was recorded. Based on the pattern of inflammation, the pancreatic involvement were subtyped in ductocentric (AIP-D) and lobulocentric (AIP-L)types. Immunohistochemistry for IgG4 was performed on both the pancreatic and extrapancreatic samples and the numbers of IgG4 positive plasma cells were semiquantitatively scored. A control cohort composed of pancreatic adenocarcinoma (n=19) and pancreatitis-NOS (n=14) were also stained. Seven cases of AIP and four kidneys were evaluated ultrastructurally.
Results: The pancreas, bile duct, gall bladder, salivary gland, kidney and lung lesions were characterized by dense lymphoplasmacytic infiltrates with reactive fibroblasts and venulitis. IgG4 positive plasma cells were identified in all pancreatic specimens and extrapancreatic sites. The AIP cases showed significantly more IgG4 positive plasma cells than pancreatitis-NOS or adenocarcinoma cohort (p=0.001). However IgG4 cells were identified in 57.1% of pancreatitis-NOS and 47.4% of ductal adenocarcinoma. Fifteen cases were subtyped as AIP-D, and 6 as AIP-L, the latter showed significantly more IgG4 positive plasma cells than the former (p=0.02). Additionally the AIP-D cohort was statistically more likely to present with obstructive jaundice (p=0.03). Ultrastructurally electron dense deposits were identified in the pancreatic basement membranes of 7 of the 9 AIP cases and in three of the four renal biopsies evaluated.
Conclusions: This study provides support for the concept of two overlapping conditions, a pancreato-centric and multisystemic immune-complex mediated IgG4 diseases. Clinical and immunological findings justify the recognition of pancreatic lobulocentric and ductocentric subtypes. Documentation of increased numbers of IgG4 positive plasma cells may provide ancillary evidence for a diagnosis of AIP, particularly in extrapancreatic locations.

483 Are Colonic Mucosal Eosinophils Affected by Seasonal Influences?

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Background: Previous studies have shown that the number of eosinophils present in the colonic mucosa varies geographically, leading some to suggest that "normal values" reflect regional exposures to environmental antigens. However, this hypothesis is based on limited studies and concrete data regarding the normal distribution of colonic mucosal eosinophils are lacking. The aims of this study were to 1) establish the number of eosinophils normally present in colonic mucosal biopsies with respect to anatomic site and 2) determine whether their numbers vary with environmental stimulation, such as allergen exposure.

Design: We retrospectively identified 340 asymptomatic adult patients who underwent routine screening colonoscopy during a 12 month period. Of these, 198 had mucosal biopsies of endoscopically normal areas in the ascending (AC) or descending (DC) colon and these patients comprised the study group. H&E stained slides from each case were reviewed. The total number of eosinophils present in four consecutive intercrypt spaces was recorded as well as the number present within the epithelium of four consecutive, well-oriented crypts. Cases were grouped according to the month of the procedure, the means were calculated, and the results compared across the 12 groups.

Results: There were 97 AC biopsies [male/female ratio (M/F): 2/1, mean age: 58 years] and 101 DC biopsies (M/F: 2/1, mean age: 57 years). The mean number (±SE) of AC stromal eosinophils was 34±2 compared to 11±1 in the DC ($p<0.0001$). Stromal eosinophils were most numerous during the spring (April, May) with a second peak in the autumn (September, October, November), although the differences were not statistically significant.

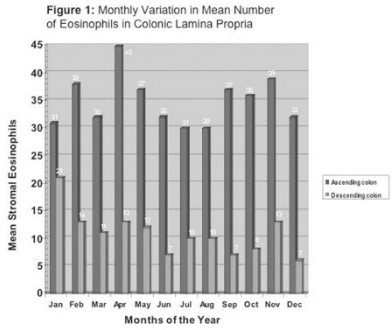

Figure 1: Monthly Variation in Mean Number of Eosinophils in Colonic Lamina Propria

Intraepithelial eosinophils were uncommon in the crypts of both the AC and DC; no more than 4 were identified in the crypts evaluated at either site.

Conclusions: Eosinophils are commonly present in the colonic lamina propria, particularly in the proximal colon, and tend to increase during the months of highest seasonal allergen exposure. Pathologists should not over-interpret the significance of this finding in the absence of other evidence of mucosal injury.

484 Perineural and Vascular Invasion in Colorectal Carcinoma: Comparison of Detection Methods to Clinical Outcome

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Background: Vascular invasion (VI) has been suggested by many, but not all, as an adverse finding in colorectal carcinoma (CRC) with perineural invasion (PNI) in more question as to its significance. How best to make these assessments is not well studied. Our goal was to determine if re-review of hematoxylin/eosin stained (HE) slides or S-100 and CD31 enhancement to stain nerves and vessels would alter finding PNI or VI.

Design: HE slides of 49 resections of CRC were re-reviewed for the presence of PNI and VI and the results were compared to the findings from the original report and all tumor containing slides stained with S-100 and CD31. All patients were followed for 5 years or until death. PNI was defined as tumor involvement of the perineural space. VI was defined as the presence of tumor within vessels, either lymphatic or blood, large or small. Statistical analysis of differences in survival were calculated with the log-rank test with a significance of $P < .05$.

Results: The 49 tumors originated in the colon (40) and rectum (9). PNI was identified in 14% (12/49) of tumors in the original reports, 39% (19/49) of the authors' re-review of HE slides, and 65% (32/49) when slides were stained with S100. VI was identified in 24% (12/49) of tumors in the original reports, 20% (10/49) of the authors' re-review of HE slides, and 47% (23/49) when slides were stained with CD31. False-negative results for PNI and false-positive results for VI were most common in the original reports. VI was significantly associated with death at 5-year follow-up when using data obtained by HE re-review ($P =.001$) or by CD 31 stained material ($P =.008$). PNI was significantly associated with death at 5-year followup when using data obtained by HE re-review ($P =.02$) but did not reach our defined level of significance using S-100 material ($P =.09$). Disease-free survival (DFS) was not associated with VI ($P =.55$) or PNI ($P =.97$) determined by any method. Any tumor that exhibited invasion in large blood vessels also had tumor in small vessels.

Conclusions: Focused re-review of HE slides increased finding PNI and decreased false positive cases of VI in CRC. Staining with S-100 and CD31 further improved the detection of both PNI and VI. DFS was not associated with either PNI or VI. 5 year survival was associated with VI using HE or CD 31 stained slides but PNI was found to be significantly associated with survival only when using HE slides.We suggest that careful review of routinely stained material will yield meaningful survival data in CRC.

485 Improved Diagnoses of Corpus Gastric Atrophy: A Comparison with Sera PGI/PGII Ratio

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Background: Gastric adenocarcinoma develops through a cascade of events that involves multifocal atrophic gastritis (MAG or separate foci of atrophy). The updated Sydney System incorporates MAG stating that the patchy nature of atrophy makes it difficult to determine its extent. We recently confirmed the notion that corpus atrophy is a continuous process that progresses proximally and towards the greater curve. This study was desinged to compare a sampling and histopathologic approach for the evaluation of corpus atrophy and the Sydney system.

Design: Patients had 8 gastric biopsy specimens obtained from defined locations (4 corpus, 4 antral). Each was sectioned separately. Sites were designed to capture the atrophic border as it expands proximally. 2 corpus sites correspond to Sydney system recommendations. Sections were scored for *H. pylori*, intestinal metaplasia, and gastric atrophy on a visual analog scale (0-5). Atrophy was defined as loss of normal glandular components with or without its replacement with intestinal metaplasia and/or pseudo-pyloric metaplasia. The overall grade of atrophy was designed to evaluate the stomach globally; it was scored on a scale of 0-3 based the degree of atrophy within each slide, the number of corpus biopsies with atrophy, and the location of corpus biopsy with atrophy. Patients sera were examined for pepsinogen I, and pepsinogen II.

Results: 180 patients were examined (139 *H. pylori* positive). Corpus atrophy was present in 73 patients (56 with grade 1, 14 grade 2, and 10 grade 3). There was a significant inverse relationship between the grade of corpus atrophy and the PGI/PGII ratio ($p < 0.01$). Atrophy in patients with grade 1 and grade 2 was present in distal corpus biopsies located proximal to the normal antrum-corpus junction (lesser curve > greater curve) which are not included in the Sydney system recommendations. More severe atrophy was present in more proximal biopsies (lesser curve > greater curve) which correspond to those recommended by Sydney system. Atrophy was never present in the proximal biopsies with sparing of the distal corpus biopsies. Corpus atrophy was significantly under diagnosed using the Sydney system ($p<0.001$).

Conclusions: The current system for evaluating corpus atrophy is a global one that is more appropriate for the clinical evaluation of patients.

486 Analysis of DNA Mutations in Stool Is a Novel Method To Detect Neoplasia in Patients with Inflammatory Bowel Disease

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Background: Patients with ulcerative colitis (UC) or Crohn's colitis (CC) undergo regular colonoscopic surveillance to detect dysplasia or early carcinoma. However, there are many limitations with regard to surveillance colonoscopy, such as sampling error, that limit its efficacy. Newer methods to identify patients at risk for neoplasia are needed. Therefore, we performed this pilot study to examine the utility of DNA stool assays in detecting early neoplastic lesions in patients with UC or CC.

Design: DNA from stool samples collected from 13 UC patients and 3 CC patients (M/F ratio: 10/6, mean age: 50.1 years, mean duration of illness: 19.6 years) was extracted, amplified with PCR and analyzed for 21 mutations within the K-ras, APC and p53 genes, deletions in BAT 26 and for DNA integrity (DIA). DIA identifies long DNA strands (1300-2400 bps, non-apoptotic DNA), which are distinct from short strands (200 bps) that develop as a result of normal programmed cell death. DNA results were correlated with the pathologic findings in either the patient's biopsy (n=5) or resection (n-11) specimens.

Results: Of the 16 patients, 5 had colorectal cancer (CRC), 5 had flat low-grade dysplasia (LGD), 1 had polypoid LGD and 5 patients had no evidence of dysplasia or CRC on surveillance colonoscopy (n=3) or colectomy (n=2). Overall, 11/11 (100%) study patients with dysplasia or CRC had a molecular abnormality compared to only 2/5 (40%) controls ($P=0.018$). The DIA assay was positive in 7/11 (64%) patients with either previous or current dysplasia or CRC compared to 2/5 (40%) control patients without neoplasia. K-ras mutations were detected in the four DIA negative study patients with either flat LGD (K12p2, K13p2), polypoid LGD (K13p2) or CRC (K13p2, K12p1) but in none of the 5 control patients. None of the study or control patients had mutations in APC or p53, or deletions in BAT 26.

Conclusions: Evaluation of DNA mutations and alterations in stool is a promising new technique that may serve as a useful adjunct to colonoscopic surveillance for the detection of dysplasia or CRC in patients with UC or CC. Larger studies are needed to determine the sensitivity and specificity of this approach.

487 Sessile Serrated Polyp/Adenoma: Challenging Discrimination from Other Colonic Polyps

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Background: Sessile serrated polyp/adenoma (SSA) has been proposed as a precursor for microsatellite unstable colorectal carcinomas. However, the distinction between hyperplastic polyp (HP), SSA, and traditional serrated adenoma (SA) remains problematic. The aim of our study was to evaluate interobserver agreement for the diagnoses of serrated polyps and determine which features were used most often to make this distinction.

Design: 187 serrated polyps (106 right or transverse colon; 81 left) at least 5 mm in size (original diagnoses: HP 93; SSA 74; SA 20) removed between July 2003 and June 2005 were included. Three GI pathologists were provided with lists of architectural and cytologic features characteristic of SSA (such as dilatation of $\geq$ 10% crypt bases, basilar serration, horizontal crypts, increased upper/middle crypt mitoses, round nuclei,

etc.) and SA (nuclear hyperchromasia, stratification, hypereosinophilic cytoplasm, etc.). All polyps were reviewed without clinical and demographic data and classified as HP, SSA, or SA. Three features most helpful in reaching their diagnosis were recorded for each case by all observers. Concordance among 2 and 3 observers was evaluated and compared to original diagnoses. Concordance was also examined relative to polyp size (< 10 mm vs. ≥ 10 mm) and location.

Results: There was a complete agreement among 3 observers in 113/187 (60%) and among 2 or more observers in 184/187 (98%). The overall interobserver agreement was moderate ($k = 0.56$). When polyps were stratified by location and size, concordance among the 3 observers was worst (20/56; $k = 0.26$) for small right-sided polyps. Of 93 original HP diagnoses, 72 (77%) were confirmed by 2 observers and 44 (47%) by three; 10 (11%) were reclassified as SSAs by 3 observers. Of 74 original SSAs, 47 (64%) were confirmed by at least 2 observers and 30 (41%) by three; seven (9%) were reclassified as HP and 5 (7%) as SA. Conversely, 19 of 20 (95%) original SA diagnoses were confirmed by at least 2 observers and 17 (85%) by three. All observers relied more often on architectural features (crypt dilatation, horizontal orientation, etc.) than cytologic criteria to distinguish SSAs from HPs.

Conclusions: The distinction between HP, SSA, and SA remains challenging, particularly among small, right-sided polyps. Small polyp size and lack of consensus regarding the relative importance of individual pathologic features may lead to variable assessment of serrated polyps. These obstacles continue to impede defining SSA as a distinct, reproducible entity.

488 Utility of p53 and β-Catenin Staining in Separating Dysplasia-Associated Lesion/Mass [DALM] from Sporadic Adenoma [SA] in Colitis Patients

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Background: Dysplasia is present in up to 70% of chronic inflammatory bowel disease [IBD] patients with carcinoma. Dysplasia in IBD is categorized as either flat or associated with a raised lesion or mass [DALM]. However, since many IBD-associated dysplasias resemble SA, distinguishing these two lesions often requires histologic and endoscopic correlation. Although there are histologic criteria that define the lesions, immunohistochemical analysis with p53 and β-Catenin has been proposed to help differentiate SA from adenoma-like DALM.

Design: We evaluated polypoid dysplastic lesions from 28 patients with IBD, and correlated the morphologic features of these lesions with clinical, endoscopic, and follow-up data. Lesions were classified as probable SA if the lesion arose outside an area of histologically proven colitis. A lesion was classified as a probable DALM if it developed within an area of colitis, particularly if there was associated flat dysplasia. Using commercially available polyclonal antibodies to p53 (Ventana, 1:2000) and β-Catenin (Transduction, 1:1000) we performed immunohistochemistry (IHC) on a series of colonic biopsies and correlated those finding with H&E histopathology.

Results: There were 26 M and 2F aged 22-75 years (mean, 52; median 52). Of these, 6 had ulcerative colitis, 15 had Crohn's disease and 7 had unclassified colitis. Twenty-five patients had a single lesion, 2 had 2 lesions, and 1 had 3 lesions. Lesions involved the transverse colon (10), rectum (6), descending (5), cecum (4), ascending (4), sigmoid (2), and ileum (1). There were 21 lesions classified as DALM, 8 as SA, and 3 as inconclusive. Immunohistochemical staining results are tabulated.

Conclusions: In our material, p53/ β-catenin immunohistochemistry was useful in confirming an impression of DALM v SA in 12/28(43%) of studied cases, but added no additional information in cases which were inconclusive on clinicopathologic correlation. Lesions classified as adenomas displaying p53 tended to be advanced and all other lesions with strong p53 otherwise met clinicopathologic criteria for DALM. This confirms previous results indicating that p53/ β-catenin immunohistochemistry has a role but is often inconclusive in separating these lesions.

p53 & βCatenin in DALM v. SA

	p53 Strong	p53 Weak	p53 Negative
βCatenin Strong	SA 1/8; INC 1/3	SA 4/8	
βCatenin Weak			SA 1/8
βCatenin Negative	DALM 5/21; SA 1/8	DALM 8/21 INC 1/3	SA 1/8; INC 1/3
No βCatenin	DALM 7/21	DALM 1/21	

489 Synchronous and Metachronous Gastrointestinal Polyps in Patients Diagnosed with Sessile Serrated Adenoma

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Background: Sessile serrated adenomas (SSA) have gained recognition in recent years following growing evidence of an association with microsatellite unstable colorectal neoplasia. Due to a lack of consistent diagnosis of these lesions prior to 2003, their relationship with other clinicopathologic features of patients is incompletely understood.

Design: All patients diagnosed with an SSA from the time period of 2003-2005 in routine specimens from a large teaching hospital were identified, and all prior and subsequent lower GI biopsy or resection specimens for these same patients subjected to histologic evaluation. Patients with hyperplastic polyposis or inflammatory bowel disease were excluded. Clinicopathologic information was collected for each patient, including age, race, gender, and concurrent and subsequent gastrointestinal polyps (both neoplastic and nonneoplastic).

Results: A total of 117 patients were identified. The average age was 61.0±12.0 years, 63 patients were male (54%) and 98 (84%) patients were white. A total of 382 polyps were diagnosed in these 117 patients over an 18 year period, of which 150 were SSAs, 133 were tubular adenomas (TA), 50 were mixed adenomatous-hyperplastic polyps (MAHP), 4 were serrated adenomas (SA), and 94 were hyperplastic polyps (HP). SSAs and MAHP were more frequently located in the right colon (n=158) versus left colon (n=41). Nineteen of 117 patients (16%) had >1 SSA at diagnosis and 40/117 (34%) had ≥1 TA at diagnosis. TAs in patients with SSA were evenly distributed throughout the colon, whereas HPs were 2X more common in the left colon. Of those patients with short term clinical followup, 9 of 14 (65%) had a subsequent diagnosis of SSA, and 8 of 12 (66%) had a subsequent diagnosis of TA (mean followup interval 24 mo).

Conclusions: As the majority of patients diagnosed with SSA had a recurrent SSA or TA on follow-up, and SSAs may occur with and without an associated TA, these results support similar management of SSA and traditional sporadic adenomas as proposed (Am J Clin Pathol. 2005 1233:349-59).

490 Small Colonic Microsatellite Unstable Adenocarcinomas and High Grade Epithelial Dysplasias in Sessile Serrated Adenoma Polypectomy Specimens: A Study of Eight Cases

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Background: The morphology of advanced sessile serrated adenomas associated with malignant transformation and their relationship with traditional serrated adenomas are unknown. Eight right colon SSA, polypectomies with focal invasive adenocarcinoma or high grade dysplasia were studied.

Design: Six study cases had invasive adenocarcinoma and two had only high grade dysplasia. All eight malignancies were microsatellite unstable-high and had absent hMLH1 nuclear immunoreactivity. The cytologic features of the adjacent dysplasia and polyp were characterized ("serrated dysplasia") and compared to the cytologic features of control group tubular adenomas from FAP syndrome patients ("APC-dysplasia").

Results: Mean polyp maximum dimension was 8.5 mm (range, 6–12 mm). High grade serrated-dysplasia was present in all eight cases. The majority of each polyp was non-malignant SSA. All eight had an abrupt transition from benign to high grade in situ or invasive malignancy. In the six invasive adenocarcinomas, the neoplasm invaded directly down into the submucosa without lateral intramucosal spread. The mean maximum dimension of the invasive adenocarcinoma was 2.9 mm (range, 2-4 mm). Crypts adjacent to malignancy had moderately enlarged nuclei, irregular nuclear membranes, and overly prominent nucleoli. Serrated dysplasia began in the mid or basilar crypt region and extended towards the surface. High grade serrated dysplasia shared some cytologic features with APC-dysplasia. Adjacent SSA crypts were lined by a variety of gastric-type cells, no single cell type predominated. None of the 8 study cases had APC-type dysplasia. SSA and high grade serrated dysplasia (advanced SSAs) areas were morphologically identical to traditional serrated adenoma.

Conclusions: High grade serrated dysplasia is cytologically distinct from APC-type dysplasia and arises in the basilar or mid crypt regions. Proximal SSAs can transform into MSI-high adenocarcinomas when small and without a component of APC-type (adenomatous) dysplasia. The intermingled morphology of SSA, high grade serrated dysplasia, and malignancy in these lesions is evidence of their relationship. The morphologic similarity of these cases and traditional serrated adenoma suggests the latter are merely late serrated neoplasia pathway lesions, not a distinct and separate entity, whereas SSAs without high grade dysplasia are earlier serrated pathway lesions. In keeping with contemporary clinical terminology, SSAs with high grade dysplaia are "advanced-SSAs".

491 Loss of Extracellular Ep-CAM in Invasive Colorectal Carcinoma Cells

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Background: In rectal cancer, an infiltrative tumor growth pattern, also referred to as tumor budding, is closely connected with higher recurrence rates and poorer survival as compared to circumscribed growing tumors. The epithelial cell adhesion molecule (Ep-CAM) is a homotypic and homophilic CAM which is expressed on the membranes of glandular epithelial cells and over-expressed in adenocarcinomas, but we observed loss of expression at areas of tumor budding.

Design: Immunohistochemistry was performed using 2 different monoclonal antibodies, one against the intracellular part of EpCAM, the other against the extracellular part. A third polyclonal antibody was used as well. RNA-expression for EpCAM was studied using mRNA in situ hybridization on parallel sections of paraffin embedded tissue. Cases from a randomized trial on the effects of preoperative radiotherapy for rectal cancer were stained for extracellular EpCAM and the results were correlated with the clinical data.

Results: Immunohistochemical analysis on human tissue using specific antibodies directed towards the membrane distal and membrane proximal extracellular Ep-CAM domain revealed a loss of the extracellular part only. This was observed in isolated tumor cells and small cell clusters at the infiltrating margin of diffuse growing carcinomas, but not in circumscript growing tumors. Tumor cells lacking the extracellular epitope revealed a cytoplasmic staining with the polyclonal antibody and had an equal degree of Ep-CAM mRNA expression as tumor cells with extracelullar domain. This suggests posttranslational modifications of Ep-CAM. Importantly, reduction of Ep-CAM staining of rectal tumor specimens from patients (n=133) from a randomized clinical trial correlated with an increased risk on local recurrence (5-year 34% versus 13%, $p = 0.02$), overall recurrence (73% versus 49%, $p = 0.03$).

Conclusions: Absence of extracellular Ep-CAM is related to tumor budding, is due to posttranslational modifications and may enable tumor cell migration by reduced cell-cell adhesion. Loss of Epcam predicts poor prognosis due to increased local and distant recurrence.

492 Expression of Her1 and Her2 in MSI Positive and Negative Colonic Adenocarcinoma

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Background: Her1 (EGFR) and Her2/neu receptors are members of a family of four closely related growth factor receptors. The expression of Her2/neu has been widely used in breast carcinoma as a standard test, both as a prognostic factor and as predictor of response to Herceptin. Assessment of Her1 and Her2/neu status in colonic malignancy has gained wide interest in view of the possible use of targeted therapies Cetuximab and Herceptin. Recent studies have shown that there is expression of Her2/neu in colorectal carcinoma that varies from 4% to 83% and Her1 expression from 25% to 82%.

Design: We have examined 20 cases of negative microsatellite instability (MSI) colorectal adenocarcinoma and 23 MSI positive cases. Sections were stained with the monoclonal antibodies for Her1 (EGFR, Zymed, Carlsbad, California) and for Her2/neu (CB11, NovoCastra, Newcastle, England) using standard procedures. 10% was used as the cut off for positivity.

Results: 23/23 cases of MSI positive colonic adenocarcinoma were negative for both Her1 and Her2 whereas 18 (90%) cases of MSI negative colonic carcinoma were positive for Her1 and one case was positive for Her2/neu as well. Her2/neu oncoprotein overexpression was associated with Her2/neu gene amplification using in situ hybridization technology. In 17 of the 18 cases normal epithelium adjacent to the tumor was also positive.

Conclusions: The study shows that there is a different biological profile for MSI positive vs. MSI negative adenocarcinoma. The wide expression of Her1 in MSI negative cases as well as the presence of 5% of cases with Her2/neu overexpression indicates that these types of cancer could be treated with anti-Her1 and Her2 humanized antibodies available in clinical practice while MSI positive cases will not benefit from either Herceptin or Cetuximab therapy. Testing for Her1 and Her2 could be a useful adjunct in characterizing colonic adenocarcinomas and to tailor the treatment approach to a disease that morphologically may appear to be homogeneous.

493 Helicobacter Heilmannii Gastritis: Need for Reappraisal, Collaboration and Guidelines

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Background: It is well recognized that Helicobacter heilmannii (Hh) gastritis has low prevalence (0.1% to 6% worldwide) and distinctly different morphology compared to Helicobacter pylori (Hp). It is also reported that Hh is poorly culturable, zoonotic, ensemble of multiple different Helicobacter species and displays fewer organisms and higher rate of MALT lymphoma (1.47%). We highlight issues such as prevalence accuracy, potential diagnostic oversight, delayed urease reactivity, lack of physician awareness and lack of recommended official guidelines.

Design: 8 cases of Hh (ages 7-74, 4 males and 4 females) and 998 cases of Hp were identified at our institution among 6771 gastric biopsies and 5486 cases of gastritis from 2000 to mid-2005. All gastric biopsies with gastritis were stained by Giemsa stain. In patients with Hh, the following were checked: local prevalence, ease of histological detection, urease reactivity time (4/8), MALT lymphoma and clinical follow-up. Trainee and attending gastroenterologists and pathologists were quizzed about published data on Hh with a 10-point questionnaire.

Results: Hh prevalence was calculated variously as 0.11% of all gastric biopsies, 0.14% of all gastritis and 0.8% of all Helicobacter gastritis. In all 8 Hh cases, there were relatively few organisms and in 2/8 cases the diagnosis was initially missed and only made on review after markedly delayed urease positivity (24-48 hours). None of our Hh cases showed MALT lymphoma. In 3/8 cases, there was no enquiry about domestic animal contact in the clinical history or follow-up, in 2/8 cases, there was history of patient contact with sick cat and in 3/8 cases, there was no patient contact with domestic animal, probably indicating species variation. Physician awareness and knowledge about Hh was unsatisfactory.

Conclusions: Prevalence of Hh is overall quite low and multi-institutional collaboration is needed for study of enough cases to accurately assess the course of Hh gastritis, particularly if there is suspicion of higher risk for MALT lymphoma. A significant number of cases may remain undetected due to low organisms load and markedly delayed urease reactivity. There may be need for species identification to predict viability or lack of zoonotic transmission and appropriate management. There is a great need for national official guidelines regarding Hh gastritis in matters such as prevalence calculations, standardizing urease reactivity time and addressing zoonosis and physician education.

494 Incidentally Detected Focal Intramucosal Signet-Ring Cell Gastric Carcinoma: An Unrecognized Variant Posing Diagnostic, Therapeutic and Prognostic Challenges

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Background: In Western patients, gastric signet-ring cell carcinoma is usually advanced with few symptoms and poor endoscopic distensibility. Biopsy diagnosis is usually followed by endoscopic ultrasound (EUS) and/or gastrectomy. The prognosis is poor in most cases. An incidentally detected variant is described in routine gastric biopsy that shows focal intramucosal signet-ring cell carcinoma without clinical or endoscopic suspicion of tumor. This poses challenges in work-up, therapy and prognosis.

Design: 4 patients were identified (ages 45-75, 3 males and 1 female) with incidentally found infiltrating signet-ring carcinoma cells (cytokeratin AE1/AE3 positive) in gastric biopsies performed routinely for gastritis. An external second opinion on biopsy diagnosis was sought in 2/4 cases due to the clinical non-suspicion. Their clinical histories and endoscopic reports were reviewed. All patients then underwent repeat endoscopy, EUS and partial (3/4) or total gastrectomy (1/4). The gastrectomies were reviewed and the patients were followed-up for 1-8 years.

Results: All patients presented with epigastric pain and had no weight loss or early satiety. Endoscopically, the mucosa showed non-erosive gastritis and no visible mass, ulcer or giant folds. Random biopsies of antrum and/or corpus showed Helicobacter (2/4) and chronic gastritis (2/4) and small focus of infiltrating keratin positive signet-ring cells in the mucosa. This was confirmed on second opinion. Repeat endoscopy and EUS failed to show any visible tumor. No tumor was grossly seen in gastrectomies and only 3/4 gastrectomies showed a small focus of mucosal tumor (0.2 to 0.4 cm) after extensive random sampling of the specimen. The lymph nodes were negative. Follow-up 1-8 years showed no recurrent tumor.

Conclusions: While the biopsy diagnosis of gastric infiltrating signet-ring carcinoma usually forebodes poor prognosis, an incidentally found small mucosal focus appears to portend excellent outcome. This variant needs to be recognized since it presents special challenges such as demand for external second opinion, frustrating lack of visible lesion on repeat endoscopy and EUS, therapeutic submission to imprecise gastrectomy and need for extensive random sampling of the resected specimen to recover the initially diagnosed carcinoma focus. While this variant may potentially exemplify the earliest phase of early carcinoma, exclusive signet-ring cell morphology, lack of any visible erosion and minute mucosa-alone location are unique features of this variant.

495 Atrophic Autoimmune Pangastritis: A Distinctive Form of Antral and Fundic Gastritis Associated with Systemic Autoimmune Disease

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Background: Two major recognized forms of atrophic gastritis are autoimmune and environmental atrophic gastritis. These differ in their topography, histology, and etiology. Autoimmune atrophic gastritis results from immune-mediated destruction of oxyntic glands, is restricted to the body/fundus, and shows charcateristic neuroendocrine hyperplasia. Environmental atrophic gastritis is associated with longstanding *Helicobacter* infection and preferentially involves antrum/transitional zone. In this study we describe a distinctive form of atrophic gastritis that differs markedly from both these classic variants.

Design: We studied 7 cases of unusual atrophic gastritis from two institutions. H&E-stained slides of gastric body (all 7 patients) and antrum (6 patients; one patient had a remote history of antrectomy for peptic ulcer disease) were reviewed, and mononuclear inflammation, neutrophilic inflammation, intestinal metaplasia, and glandular atrophy were separately graded in accordance with the Updated Sydney System. Immunostains for chromogranin and gastrin were performed to evaluate neuroendocrine populations of the body and antrum. *Helicobacter* infection was excluded by immunohistochemistry.

Results: The atrophic gastritis was characterized by: 1) Intense mucosal inflammatory infiltrates, persisting even into the phase of severe glandular atrophy, 2) Pangastric distribution with involvement of both body and antrum, 3) Lack of association with *Helicobacter pylori*, and 4) Lack of neuroendocrine hyperplasia. The 7 patients ranged from 1 – 75 years and showed a female predominance (5F:2M). All had systemic autoimmune and/or connective tissue diseases including autoimmune enterocolitis (n=3), systemic lupus erythematosus, refractory sprue, autoimmune hemolytic anemia, and disabling fibromyalgia. Positive serum autoimmune markers were documented in 6 of 7 (86%) patients, but serologies for anti-parietal cell and anti-intrinsic factor antibodies were undertaken in only one patient each and were negative. One patient, a 19-year-old woman, developed low-grade gastric dysplasia.

Conclusions: We propose that the distinctive histology of this form of atrophic pangastritis and its association with systemic autoimmune disease suggest an autoimmune process directed against multiple cell lineages in the stomach. The development of multifocal low-grade dysplasia in one of the patients underscores its neoplastic potential and clinical significance.

496 Gastric Hyperplastic Polyps in Post Transplant Patients: A Clinicopathologic Study

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Background: Gastric hyperplastic polyps (HP) in organ transplant recipients has been recently described; however, the clinical significance of HP in this setting remains unclear. The aim of this study is to further characterize the clinical presentation and histopathology of HP in organ transplant recipients as compared to HP in non transplant patients.

Design: All gastric HP diagnosed in our institute from 1999 to 2005 were retrieved. Clinical data including endoscopic findings were reviewed. 20 cases without history of transplantation were randomly selected for a control population. H/E and Genta stains were reviewed.

Results:

Clinicopathologic Features		
	Transplant (n=14)	Non-transplant (n=20)
Age (average years)	46	60
M:F	1.3	0.33
Single Polyp	8(57%)	15(75%)
Antral	7(47%)	14(70%)
Body	3(20%)	4(20%)
Cardiac	2(13%)	2(10%)
Site not specified	3(20%)	0
Size (average)	1.37 cm	1.65 cm
Active Inflammation	5(36%)	15(71%)
Intestinal Metaplasia	3(21%)	7(33%)
H.pylori	0	1(5%)

96 cases of gastric HP were identified. 14 (15%) had a history of solid organ (1 liver/

kidney, 4 livers, 1 kidney, 1 kidney/pancreas, 2 hearts) or bone marrow transplantation (5). The average time after transplantation was 24.5 months. Signs/symptoms leading to endoscopy were more frequently nausea/vomiting and diarrhea in transplant patients as compared to bleeding/hematemesis/anemia in non-transplant patients. The transplant patients tended to be younger with a reversed M:F ratio, but age was the only demographic factor that was statistically significant. There was no difference in polyp size, location and number. Histologically, no difference was observed in the frequency of active inflammation or intestinal metaplasia. Dysplasia was not present in any of the cases. None of the patients had a history of polyposis syndrome.
Conclusions: A significant percentage of gastric HP (15%) were from organ transplant patients, further suggesting a strong association of HP with transplantation. The younger age in the transplant group may be explained by the nature of the cohort qualified for transplantation. While no statistically significant differences in histopathologic features were found between transplant and non-transplant groups, analysis was limited by small case numbers. Overall, gastric HP in the post transplant setting is a common, but under-recognized entity and merits further clinicopathologic analysis.

497 Pathological Correlates of Microsatellite Instability in IBD-Associated Colorectal Carcinomas

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Background: Defective DNA mismatch repair manifested by microsatellite instability (MSI) is involved in the pathogenesis of approximately 15% of sporadic colorectal carcinomas (CRCs). High-level MSI (MSI-H) correlates with certain pathological tumor characteristics, including location in the proximal colon, mucinous histology, high histological grade, intratumoral lymphocytosis and Crohn's like peritumoral inflammation. We have recently shown that whereas CRCs complicating chronic inflammatory bowel disease (IBD-CRC) are similar to sporadic CRCs with respect to the overall prevalence of MSI-H, their specific mutational and promoter methylation profiles are distinct. However, it is not yet known whether correlations exist between MSI status and the pathological characteristics of IBD-CRC.
Design: We reviewed the pathological characteristics of 42 IBD-associated CRCs of known MSI status, 7 MSI-H, 9 low-level MSI (MSI-L) and 26 microsatellite stable (MSS), and compared them with respect to the following parameters: patient age, gender, tumor location, multiplicity, size, stage, grade, histological subtype, heterogeneity, intratumoral lymphocytosis, peritumoral Crohn's-like inflammatory reaction, lymphovascular invasion, and the presence or absence of peritumoral dysplasia. Data were analyzed by chi-square methodology.
Results: No significant differences were observed between MSI-L and MSS tumors and they were combined for statistical comparisons. Of the parameters examined, only intratumoral lymphocytosis, Crohn's-like inflammation and high histological grade were significantly more prevalent in MSI-H CRCs than in the other MSI categories ($p<0.05$).
Conclusions: Despite underlying differences in molecular pathogenesis, IBD-associated CRC resembles sporadic CRC with respect to certain pathologic correlates of MSI-H status, specifically intratumoral lymphocytosis, Crohn's-like inflammation and high histological grade. However, no such correlation exists with respect to the prevalence of mucinous histology, a subtype that is disproportionately common in IBD, or location in the proximal colon. The lack of correlation with these parameters probably reflects overriding factors related to the unique molecular pathogenesis of IBD-CRC and the inflammatory setting in which it arises.

498 PKC-theta Expression in the Gastrointestinal Stromal Tumor

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Background: Gastrointestinal Stromal Tumor (GIST) is the most common mesenchymal neoplasm of the digestive tract and most GIST is characterized by c-kit expression and gain of function mutation of the kit gene. But in up to 15% of cases, c-kit expression is negative although histologic findings of GIST are most suspicious. Recently, a gene expression study revealed constant over-expression of protein kinase C theta (PKC-theta) on GISTs.
Design: In this study, we studied expression of PKC-theta in the GISTs and correlated their expression with other immunohistochemical profiles of GISTs and evaluated their usability as a diagnostic marker. For this purpose, 227 cases of GISTs, 20 leiomyomas, 11 Schwannomas, 1 each case of leiomyosarcoma, and MPNST were used. Immunohistochemical staining was performed using EnVision kit (DAKO) after antigen retrieval with microwave for 15 minutes using monoclonal antibodies from BD Bioscience.
Results: All leiomyomas, leiomyosarcona, MPNST were negative and 3 Schwannomas showed focal weak positivity for PKC-theta. Among 227 GISTs, 207 cases (91.2%) were positive for PKC-theta in the cytoplasm of tumor cells with diffuse staining pattern. CD117 was positive in 95.6% of the GISTs. Among 207 PKC-theta positive GISTs, 8 cases were c-kit negative. All the cases of GISTs with dot-like expression of c-kit protein were positive for PKC-theta and two of them showed dot-like expression of PKC-theta in the cytoplasm of tumor cells. Among PKC-theta positive cases, CD34 was positive in 166 cases, SMA was positive in 62 cases, and S-100 protein was positive in 54 cases. According to the site of GISTs, PKC-theta expression was more frequently expressed in the stomach and colon than small intestine.
Conclusions: These findings suggest that unlike c-kit, PKC-theta expression is variable according to the site and histologic differentiation of GISTs. With its high specificity and sensitivity, PKC-theta immunostaining can be used as a diagnostic tool in the pathologic diagnosis of the GISTs in addition to the c-kit protein expression.

499 Mucin Expression and Its Relationship with Clinicopathologic Charateristics of 343 Gastric Adenocarcinomas – Emphasis on the Aggressive Behavior of Null Phenotype

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Background: Gastric adenocarcinoma can be subdivided by mucin expression phenotype. It has been suggested that biologic characteristics of gastric adenocarcinoma are different on the basis of different mucin expression.
Design: The purpose of this study was to investigate mucin expression and its relationship with clinicopathologic characteristics in 343 gastric adenocarcinomas. We classified gastric adenocarcinomas as gastric (G), intestinal (I), mixed gastric-intestinal (GI) and null (N) phenotype as result of immunohistochemistry for MUC5AC, MUC6 (gastric phenotype markers) and MUC2, CD10 (intestinal phenotype markers).
Results: 343 Gastric adenocarcinomas showed expression of MUC5AC, MUC6, MUC2 and CD10 in 182 (53.1%), 60 (17.5%), 157 (45.8%) and 61 (17.8%) cases, respectively and they were classified into G-phenotype (114; 33.2%), GI-phenotype (88; 25.7%), I-phenotype (92; 26.8%) and N-phenotype (49; 14.3%). The N-phenotype was associated with more corporeal location than GI- and I-phenotype ($p=0.009$ and $p=0.007$, respectively), larger size than I-phenotype ($p=0.007$), a higher proportion of advanced gastric cancer than G-, GI- and I-phenotype ($p=0.003$, $p<0.001$ and $p<0.001$, respectively), more neural invasion than G-, GI- and I-phenotype ($p=0.076$, $p=0.003$ and $p=0.003$, respectively) and more lymph node metastasis than GI phenotype ($p=0.017$).
Conclusions: These results suggest that null mucin phenotype might be one of factors that suggest aggressive behavior in gastric adenocarcinomas.

500 Origin of Multifocal Neuroendocrine Tumors of the Enteropancreatic Axis

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Background: Neuroendocrine tumors of the enteropancreatic axis are often multifocal. The genetic relationships among these multicentric tumors are uncertain. We investigated the pattern of allelic loss and X-chromosome inactivation in a set of multifocal carcinoid tumors and multifocal pancreatic endocrine tumors to assess whether noncontiguous tumors arise independently or whether they originate from a single clone with subsequent intramural or intrapancreatic spread.
Design: We examined 24 patients with multifocal neuroendocrine tumors including 16 patients with multifocal carcinoid tumors and 8 patients with multifocal pancreatic endocrine tumors. All patients had multiple grossly separate tumor nodules (ranging from 2 to 6). Genomic DNA samples were prepared from formalin-fixed, paraffin-embedded tissue sections using laser-capture microdissection. Loss of heterozygosity assays were performed utilizing markers for putative tumor suppressor genes located on chromosomes 9p (p16), 11q (SDHD), 16q, and 18q. X-chromosome inactivation analysis was performed on tumors from 8 female patients.
Results: Fifteen of 16 (94%) cases of multifocal carcinoid tumors and 7 of 8 (88%) cases of multifocal pancreatic endocrine tumors showed allelic loss in at least one tumor focus. Two of 24 (8%) cases showed the same LOH pattern in every individual tumor. Eleven of 24 (46%) cases exhibited a different LOH pattern for each tumor. Additionally, 9 of 24 (38%) cases demonstrated different LOH patterns among some of the coexisting tumors while other coexisting tumors displayed the same allelic loss pattern. A concordant pattern of non-random X-chromosome inactivation was observed in 3 of 5 informative cases of multifocal carcinoid tumors and in 1 informative case of multifocal pancreatic tumors with a discordant pattern of non-random X-chromosome inactivation observed in 2 of 5 cases of multifocal carcinoid tumors.
Conclusions: Our data suggest that some multifocal neuroendocrine tumors of the enteropancreatic axis arise independently whereas others originate as a single clone with subsequent local and discontinuous metastasis. Additionally, some patients manifest both of these phenomena by possessing some tumors that are clonally related and others that are genetically independent. These findings may be relevant in planning an appropriate treatment strategy and in assessing prognosis.

501 Morphologic & Immunohistochemical (IM) Changes of Gastrointestinal Stromal Tumors (GIST) after Gleevec Treatment: A Study of 16 Cases

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Background: GIST constitutes the majority of GI mesenchymal tumors. Currently Gleevec, a tyrosine kinase inhibitor, is considered the treatment of choice for recurrent cases, inhibiting growth & eventual apoptosis; however, the histologic changes of GIST post-treated with Gleevec have been rarely described.
Design: We analyzed the histopathologic & IM changes of metastatic & recurrent GISTs treated with Gleevec & compared them with their corresponding primaries. 16 cases of GISTs diagnosed & treated between Sep.1999 & Nov. 2004 were retrieved from the Pathology files at the Asan Medical Center.
Results: There were 11 males & 5 females with a mean age of 52 years. The primary sites of malignant GIST included the small intestine (SI, 9 cases), stomach (5), esophagus (1), & omentum (1). All cases showed IM proven c-kit expression. Besides

c-kit, 8 cases were positive for CD34, 3 for SMA, & 5 for S-100. The mean tumor size was 13.0 cm (range, 4.3-22.0). The mean mitotic number was 31.4/50HFFs (1-90). The interval between the resection of the primary & metastasis or recurrent was 28.6 months (7-51). The metastatic & recurrent sites were the liver (4), SI (3), SI & omentum (3), & 1 each in omentum, retroperitoneum, mesentery, spleen, azygoesophageal recess, & stomach, & the size ranged from 1.3 to 13.0 cm (mean, 6.1). The morphologic changes of the post-treated GISTs included decreased cellularity (13/16, 81.2%), followed by hyalinization (11/16), hemorrhagic necrosis (6/16), cystic & myxoid changes (6/16), fibrosis (4/16), & calcification (2/16). Among them, 6 cases revealed extensive hyalinization, leaving a few residual spindle cells with pyknotic nuclei, & were initially interpreted to be no residual tumors. However, all these cases were positive for c-kit, confirming focal residual GISTs. Mean mitotic rate was significantly decreased to 12.1/50hpfs (range, 0-60) after treatment. The immunoprolifes were similar to those of initial tumors, except for significantly decreased Ki-67 positivity (24.0 vs. 11.4, p<0.001).

Conclusions: Post-treated GISTs show variable morphologic features, which are different from their primary tumors & can easily be mistaken as an absence of residual tumor because of the decreased cellularity & rare mitoses. Therefore, careful examination of post-treatment GISTs with IM staining for c-kit is required to accurately assess the residual tumor cells as well as residual tumor volume.

502 Lymphatic Microvessel Density as Prognostic Marker in Colorectal Cancer

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Background: Metastasis to regional lymph nodes is a major prognostic indicator for disease progression and crucial for therapeutic strategies in colorectal carcinoma. Recent studies showed that D2-40 is a specific lymphatic marker. There is limited data evaluating the significance of lymphatic microvessel density (LMD) as a prognostic marker. In this study, we investigated tumor lymphangiogenesis as a predictive marker for the risk of lymph node (LN) metastasis and its relation to other prognostic parameters in colon cancer patients.

Design: Resected colorectal carcinoma from 90 patients was examined, including 30 patients without LN metastases, 30 with only LN metastases and 30 with liver metastases (22 cases also had positive LNs). Cases were immunostained for CD31, D2-40 and vascular endothelial growth factor (VEGF). Positively staining microvessels (MV) were counted in densely vascular/lymphatic foci (hot spots) at x400 field in each specimen (=0.17 mm^2) by 2 pathologists. In addition, intensity of staining for D2-40 and VEGF was scored on a three-tiered scale. Results were correlated with other prognostic parameters.

Results: D2-40 LMD demonstrated significant correlation with CD31 counts (20+/-9 vs 18+/-6/0.17 mm2 field, P<0.05) and VEGF expression (P< 0.01). VEGF and D2-40 were expressed in 59/90 and 26/90 cases, respectively. D2-40 identified lymphatic tumor invasion in 53/90 cases which was greater than with CD31 (43/90) and H and E (29/90). There was a positive significant correlation of D2-40, CD31 counts and VEGF expression with the presence of angiolymphatic invasion and lymph node metastases (P<0.05). Only D2-40 LMD and lymphatic tumor invasion correlated significantly with liver metastases, positive vascular pedicle lymph nodes, and depth of invasion (P<0.05), while intensity of staining by tumor cells with D2-40 showed significant correlation with liver metastases (P<0.001).

Conclusions: Immunostaining with D2-40 increases the frequency of detection of lymphatic invasion relative to conventional H and E staining and the commonly used pan-endothelial marker, CD31. D2-40 LMD showed prognostic significance with positive correlation with angiolymphatic invasion, depth of invasion and metastases to lymph nodes and liver. D2-40, by staining lymphatic vessels and detecting lymphatic invasion in colon carcinoma, is a more useful immunohistochemical marker for tumor progression than the commonly used panendothelial marker, CD31.

503 Low-Grade Appendiceal Neoplasms: Mutational and Allelotypic Analysis

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Background: Low grade appendiceal epithelial lesions are often found incidentally and typically appear as mucinous lesions, rather than as conventional adenomas (CA). While the adenoma-carcinoma and serrated polyp (SP) pathways have been extensively analyzed in the colon, precursor lesions of appendiceal adenocarcinoma and "pseudomyxoma peritonei" have not been well studied. We correlate the morphologic features with genetic abnormalities of primary lesions of the appendix.

Design: 30 primary appendiceal lesions from 25 patients were analyzed. DNA was extracted from microdissected, formalin-fixed tissue. PCR was performed using fluorescently labeled primers for polymorphic microsatellite makers at the following loci: APC/5q (D5S659, D5S346), p16/9p (D9S251, D9S1748), p53/17p (D171844) and DPC,DCC/18q (D18S364, D18S34, D18S487). Peak heights from informative alleles were compared in a ratio between the lesional and normal tissue. Ratios that were <0.5 or >2 were indicative of allelic imbalance (loss of heterozygosity). Fractional allelic loss (FAL) was calculated as the number of loci with allelic imbalance/total number of informative loci. Tissue was also tested for KRAS (exon 1) and BRAF (V600E) mutations.

Results: There were 9 high grade lesions (7 adenocarcinomas, 2 adenomas; Group1) and 21 low grade lesions (9 mucinous cystadenomas, 9 SP and 3 CA; Group 2). The FAL results are in Table 1; there were no significant differences in the remaining loci. KRAS mutations were detected in 56% of the cases in Group 1 and 30% of the cases in Group 2 (p=NS), but 100% of the CA had KRAS mutations compared to 18% of low grade mucinous lesions (p≤0.01). No BRAF mutations were identified.

Conclusions: Benign-appearing mucinous lesions, including serrated polyps and cystadenomas, are more common than conventional adenomas in the appendix. With an overall FAL of 30% and KRAS mutation rate of 30%, all should be considered to be low grade neoplasms. Appendiceal adenocarcinomas may not arise through the classic adenoma-carcinoma sequence, since the adenocarcinomas did not accumulate additional mutations compared to the low grade lesions. In addition, low grade appendiceal lesions appear to be different than colonic serrated polyps and adenomas due the absence of BRAF mutations and the low rate of 18q imbalance.

Results: FAL Values (%)

Group	Overall*	D5S659**	18q*	D18S364***
1 (High grade)	20^	80	11	0
2 (Low Grade)	30^^	18	16	29^^^

^At least one locus lost in 67%; ^^At least one locus lost in 71%. *p=NS; **p≤0.025; ***p≤0.1. ^^^All losses were in SP.

504 Histologic Characteristics and Follow-Up of 37 Cases of Barrett's Esophagus with High Grade Dysplasia and Intramucosal Adenocarcinoma Treated with Photodynamic Therapy

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Background: Photodynamic therapy using porfimer sodium (Ps-PDT) is a novel endoscopic treatment associated with ablation of high grade dysplasia(HGD) or superficial adenocarcinoma(ACA) in 77% of cases and complete elimination of specialized Barrett's mucosa (SBM) in 44%. Successful ablation limits the need for surgery.

Design: This study of consecutive patients treated with Ps-PDT for HGD or ACA from 8/2001 to 4/2004 included patients referred to our institution. All available pathology slides of biopsies pre and post PDT were reviewed by at least 1 gastrointestinal pathologist for the presence of SBM, architecture and extent of HGD, ACA and squamous epithelial overgrowth (SEO). HGD was defined as focal (single focus involving 5 crypts or less) or diffuse (>5 crypts involved in a single biopsy or HGD in >1 biopsy fragment). Architecture of HGD was classified as tubular and/or papillary. Mucosal location was classified as involving the crypt and/or surface.

Results: A total of 37 study patients (33M,4F; 47-84yrs) were treated with Ps-PDT (32 HGD, 5 ACA). All patients had follow-up (F/U) endoscopies by a single endoscopist, range 1-10 (mean 5.7). Interval from PDT to most recent endoscopy ranged from 1 to 46 (mean 23) months. Extent, architecture and location of HGD in pre-PDT biopsies could be assessed in 10 cases. HGD was diffuse in 7 cases and focal in 3. Architecture of HGD was tubular in 8 cases, tubulopapillary in 2 and papillary in 1. HGD involved the crypts in all 10 cases, and in 7 cases also involved the surface epithelium. F/U post-PDT biopsies on patients with HGD showed SBM in 13/32 cases (41%), HGD in 2 cases(6%) and ACA in 2 cases(6%). F/U biopsies on patients with ACA showed ACA in 2/5 cases (40%), SBM in 1 (20%), and no dysplasia or carcinoma in 3 (60%). After PDT SEO on SBM was present in at least 1 biopsy in 10/13 cases (76%) during the F/U period. After PDT, 2 cases with recurrent/residual ACA and 1 with HGD showed SEO. Pre-PDT biopsies on 4 patients also showed SEO (1 SBM, 2 HGD, 1 ACA).

Conclusions: 1) PDT is highly effective in ablating HGD and mucosal ACA arising in SBM (94% HGD; 60% ACA). 2) SEO is common after PDT, but can occur in patients not treated with PDT. 3) Diffuse HGD as defined in this study usually involved the crypt and surface epithelium and is effectively ablated by PDT.

505 Different Histopathological Features between Gastrointestinal Stromal Tumors with KIT and PDGFRA Mutation

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Background: Activating mutations of KIT and platelet-derived growth factor receptor-α(PDGFRA) are mutually exclusive genetic changes in the gastrointestinal stromal tumor(GIST), however, the histologic differences according to mutation type have not yet been clarified. The aim of this study is to identify the histopathological characteristics of GISTs according to the activating mutation type.

Design: 57 cases of GIST which were diagnosed on the basis of histologic, immunohistochemical findings and mutational analysis were included in this study. Mutation analyses for KIT and PDGFRA were preformed. Histologic parameters including histologic type (spindle, epithelioid or mixed), mitotic count, necrosis, pleomorphism, alveolar pattern, perinuclear vacuoles, intratumoral lymphocytes, nuclear palisading, cellularity, myxoid change, cystic degeneration, and size were evaluated on routine sections. The differences in the histologic parameters between GISTs with KIT and PDGFRA mutation were evaluated using the chi-square and Fisher's exact tests.

Results: Among the 57 GISTs, KIT and PDGFRA mutations were detected in 47 cases(82%) and 6 cases(10%), respectively. PDGRFA mutations were associated with epithelioid or mixed histologic patterns (p=0.007), moderate to severe cellular pleomorphism (p=0.03), and moderate to severe intratumoral lymphocytic infiltration (p=0.008). KIT mutations were correlated with higher cellularity (p=0.003) and nuclear palisading (p=0.035). There was no significant difference between KIT and PDGFRA-mutant GISTs with regard to perinuclear vacuole, myxoid change, size, mitosis and necrosis.

Conclusions: GIST shows different morphologic changes according to the activating mutation type. These findings can be used for the differential diagnosis and as predictive biomarkers.

506 Microsatellite Instability (MSI) in Colorectal Carcinomas – Prospective Correlation of Clinicopathologic Features with MSI Status

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Background: MSI arises from a defect in DNA mismatch repair and occurs in 10-15% of all colorectal carcinomas. MSI is a useful screening test for hereditary nonpolyposis colorectal cancer syndrome (HNPCC) and a prognostic indicator for sporadic colorectal cancer. Several clinicopathologic predictors of MSI have been reported, including poor tumor differentiation, mucinous change, right-sided location, age <50 years, increased tumor infiltrating lymphocytes (TIL), Crohn's-like lymphoid reaction and absence of dirty necrosis. However, data to guide prospective clinical testing are scarce.

Design: Between December 1, 2004 and August 31, 2005, colorectal carcinomas observed to have any of the above 7 parameters were prospectively tested for MSI on paraffin-embedded tissue. The PCR-based MSI Analysis System® (Promega, Madison, WI) was used to analyze 5 standard mononucleotide markers. A total of 34 colorectal carcinomas were tested. According to the Bethesda consensus criteria, cases were considered microsatellite unstable (MSI-H) if at least 2/5 markers displayed MSI.

Results: Microsatellite testing was informative in all 34 cases. 12 cases (35.3%) were MSI-H. Frequencies of individual clinicopathologic features in MSI-H cases were calculated, as were the frequencies of combined features:

Conclusions: 35.3% of tested colorectal carcinomas in our population were MSI-H. 100% of MSI-H cases in our series showed at least 2 of the features previously associated with MSI-H. These data may help identify the most appropriate tumors to test for MSI.

Frequency of Individual Predictors in MSI-H cases

Age < 50 years (n=5)	20%
Right-sided location (n=14)	29%
>2 TIL / hpf (n=28)	36%
Crohn's-like response (n=26)	38%
Mucinous change (n=16)	50%
Absence of dirty necrosis (n=16)	50%
Poor differentiation (n=9)	56%

Frequency of Combined Predictors in MSI-H cases

1 feature (n=1)	0%
2 features (n=9)	22%
3 features (n=10)	20%
4 features (n=10)	50%
5 features (n=2)	100%
6 features (n=2)	50

507 Aberrant CpG Island Methylation, *BRAF/KRAS* Mutation, Microsatellite Instability, and Genotypes of *MTHFR* in Colorectal Carcinomas and Their Relationships

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Background: CpG island methylation (CIM) is common in colorectal cancer (CRC), associated with silencing of various tumor suppressor genes, and plays an important role in carcinogenesis. *Methylenetetrahydrofolate reductase* (*MTHFR*) is involved in the metabolism of the methyl donor and is polymorphic within codon 677, 1298, and 2756. These variants are thermo-labile and their decreased enzyme activity may induce DNA hypomethylation. *KRAS* or *BRAF* mutations were recently shown to be associated with aberrant CIM. We aimed to investigate the relationship of epigenetic changes with mutation pattern of *KRAS/ BRAF*, and polymorphism of *MTHFR* in CRC.

Design: The study included 141 CRC patients (56 proximal and 85 distal CRCs) and 146 normal controls. Methylation status of promoter of *APC*, *COX-2*, *DAP-kinase*, *E-cadherin*, *GSTP1*, *hMLH1*, *MGMT*, *p14*, *p16*, *RASSF1A*, *THBS1*, and *RUNX3* was analyzed in CRC using methylation specific PCR. Mutation analysis of *BRAF* and *KRAS* and genotyping of *MTHFR 677* and *1298* and *MTR 2756* was performed using PCR-restriction fragment length polymorphism method. Microsatellite instability was analyzed using 5 microsatellite markers.

Results: CIM was detected in 98% of CRC (range, 1-10 genes; average, 4 genes). CIM was significantly frequent in proximal colon cancer, mucinous type, and older patients (p=0.004, 0.03, and 0.02, respectively). *KRAS* and *BRAF* mutations were evident in 34% and 4.3% of CRC, respectively. Tumors with *KRAS* mutation showed lower hypermethylation frequencies of *APC*, *GSTP1*, and *RUNX3* and higher hypermethylation frequency of *MGMT* than those without *KRAS* mutation (p=0.005, p=0.04, p=0.04, and p=0.002. respectively). Tumors harboring CT or TT variant of *MTHFR 677* and AG or GG variant of *MTR 2756* were frequently methylated in *p16* promoter (p=0.007 and 0.05, respectively). Case/control approach indicated that individuals with CT or TT variant of *MTHFR 677* had a significantly higher risk of developing distal colon cancer compared with subjects with CC genotype (odd ratio, 2.039; 95% CI, 1.107-3.756).

Conclusions: CIM of *APC*, *GSTP1*, *MGMT*, and *RUNX3* might have an association with mutation of *KRAS* in CRC. Polymorphism of *MTHFR 677* might be a risk factor for distal colon cancer and affect the methylation status of CpG islands during colorectal carcinogenesis.

508 Overexpression of Fatty Acid Synthase in Gallbladder Carcinoma Is an Independent Marker of Poor Survival

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Background: Gallbladder carcinoma (GC) is an aggressive cancer that is difficult to cure by conventional procedures. It is desirable to identify novel markers for assessing prognosis and potential targeted therapy. Fatty acid synthase (FASE), responsible for de novo synthesis of saturated fatty acids, is overexpressed in several cancers, and FASE inhibitors have shown antitumor activity by halting cell proliferation and inducing apoptosis in xenografts. However, the expression and significance of FASE have never been exmained in GCs.

Design: We assessed the expression and prognostic value of FASE and its correlations with clinicopathological variables and Ki-67 labeling index (LI) by tissue microarrays from 86 GCs. Strong cytoplasmic staining of FASE in >30% of tumor cells in cases with ≥2 preserved cores was regarded as overexpression.

Results: There were 33 men and 53 women with ages ranging from 39 to 91 years (median, 70). Follow-up was obtained in 85 cases with a median of 21 months. Fifty, 44, and 52 GCs presented with deep invasion (T3, 4), higher grade (grade 2, 3, 4), and advanced stage (stage 2, 3, 4), respectively. Among GCs with scoring data, FASE overexpression was observed in 48/83 cases and high Ki-67 LI (>50%) in 34/82 cases. FASE expression was positively related to Ki-67 (p<0.0001, r=0.387), but not associated with stage or grade. The cumulative rates of disease-free survival (DFS) and disease-specific survival (DSS) at 2 years were 28% and 34%, respectively. FASE overexpression was significantly predictive of adverse DFS (p=0.0028) and DSS (p=0.0119) at the univariate level, together with higher T status, advanced stage, and high Ki-67 LI. In multivariate analyses, FASE expression was the strongest independent predictor for DFS (p=0.001, RR=3.06), followed by stage (p=0.003, RR=2.63) and Ki-67 LI (p=0.05, RR=1.89). Apart from stage (p=0.001, RR=3.305) and Ki-67 LI (p=0.041, RR=1.99), FASE expression remained statistically independent for DSS (p=0.006, RR=2.81).

Conclusions: FASE is overexpressed in a high proportion of GCs and represents an independent prognostic marker that correlates with Ki-67 expression. The findings substantiate the significance of FASE overexpression in tumor aggressiveness and cell proliferation of GCs and suggest a potential role of FASE inhibitors in treating refractory GCs.

509 Remarkable Prognostic Heterogeneity Justifies Subdividing High-Risk Gastrointestinal Stromal Tumors (GIST) of NIH Consensus Scheme into Two Different Risk Groups

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Background: The clinical spectrum of GISTs is very broad, resulting in various classification systems. For prognostication, NIH consensus meeting recently defined 4 risk groups (very low [VL], low [L], intermediate [I], and high [H]) based on the size and mitotic figures (MF). In H-risk GISTs, we have noted that cases with high MF behaved worse than large tumors with low MF, prompting us to correlate clinicopathologic findings with outcomes of a large cohort to refine the NIH scheme.

Design: We identified 291 primary localized GISTs with follow-up(FU)≥6 months, confirmed the diagnosis by CD117 and/or PKC-θ stains, and evaluated histologic features, including NIH risk categories. We also compared the efficacy of a modified scheme that segregated a very high (VH)-risk subset with size>5 cm and MF>10/50HPF from the rest of original H-risk (Hr) GISTs. The endpoint analyzed was disease-specific survival (DSS).

Results: 291 patients (M=141, F=150) ranged in age from 23-87 years (median, 61) with a median FU of 41 months. At last FU, disease-specific death was seen in 0/18, 0/92, 3/89, 14/60, and 21/32 of VL-, L-, I-, Hr-, and VH-risk GISTs. The 5-year DSS rates for all 289, VL/L-, I-, all H-, Hr-, and VH-risk cases were 82%, 100%, 96%, 51%, 67%, and 25%, respectively. By log-rank tests, old age (p=0.009), high cellularity (p=0.001), large size, epithelioid histotype, nuclear atypia, high MF, and higher risk groups of both NIH and modified schemes (p<0.0001 for the latter 6) were all adverse factors of DSS. Significant difference in DSS between VL/L- and I-risk GISTs only emerged in the modified scheme (p=0.041). This prognostic difference was even stronger in both I- vs. Hr-risk (p<0.0001) and Hr- vs. VH-risk (p<0.0001) GISTs.Thus, only the modified scheme and other significant factors except size and MF were entered for multivariate analyses. The modified scheme was the strongest independent adverse factor for DSS (p=0.000, RR=8.93 for Hr and RR=22.40 for VH compared to VL/L/I), followed by epithelioid histotype (p=0.011, RR=2.41) and nuclear atypia (p=0.049, RR=7.74).

Conclusions: significant prognostic heterogeneity exists in H-risk GISTs of NIH criteria, which is not as effective as the modified scheme. Primary localized VH-risk GISTs in the modified scheme are highly lethal and may represent candidates for adjuvant imatinib trial.

510 Distinct Expression Pattern of Trefoil Peptides TFF1 and TFF3 in Early Gastric Carcinoma

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Background: Trefoil peptides (TFFs) are a group of small secretory peptides with trefoil structural motif/s. Functionally, TFFs are involved in epithelial defense, regeneration/migration process, apoptosis and oxidative stress, and carcinogenesis. TFFs exhibit cell- and tissue-specificty, mainly in mucus-secreting gastrointestinal epithelia. Although several studies evaluate the expression status of TFFs in cancer and normal tissues, the detailed immunohistochemical analysis of TFF1 and TFF3 in gastric carcinoma, particularly in early stages of carcinoma, remains to be investigated further.

Design: Twenty-eight cases of early gastric carcinoma, adjacent mucosa and intestinal metaplasia were analyzed. Early gastric carcinoma was defined according to WHO criteria that carcinoma is limited to mucosa or mucosa and submucosa regardless of nodal status. Paraffin sections were labeled with anti-TFF1 and TFF3 antibodies (Santa Cruz, CA) using heat-treated antigen retrieval and appropriate positive and negative controls. The avidin-biotin-peroxidase method was used in all labeling. Cases were scored as positive if cytoplasmic labeling was seen.

Results: For TFF1, 27 of 28 (97%) early gastric adenocarcinomas exhibited positive labeling, including all 19 intestinal types, and 8 of 9 diffuse types. Positive

immunolabeling were confined to the cytoplasm of carcinoma cells as a diffuse pattern. In adjacent gastric mucosa, all of 27 intestinal metaplasia showed strong immunolabeling as perinuclear pattern (Golgi region). Four hyperplastic foveolar epithelia also displayed perinuclear staining. Morphologically normal antral and fundic mucosa were negative. For TFF3, 20 of 28 (71%) early gastric adenocarcinomas exhibited positive labeling, including17 of 19 intestinal types and 3 of 9 diffuse types. Positive staining signals in carcinomas showed weak intensity. In adjacent gastric mucosa, neck zone epithelia of fundic mucosa and pyloric glands were strong positve. Positve staining in both carcinomas and gastric epithelia presneted as diffuse cytoplasmic patterns. All intestinal metaplsia were negative.

Conclusions: TFF1 immunostaining profiles of non-neoplastic, intestinal metaplasia and early carcinomas appear to be significantly different and indicate that it is a biomarker of intestinal metaplasia and carcinoma.The different staining pattern in intestinal metaplasia and carcinoma of TFF1 aids to differentiate these two lesions. TFF3 appears to be a marker of neck zone epithelia and pyloric glands.

511 Bacterium-Macrophage Interaction in Gastroesophageal Reflux Disease

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Background: Gastroesophageal reflux disease (GERD) is a defined risk factor for the development of adenocarcinoma in the distal esophagus, for reasons unclear. *Streptococcus mits* predominates the normal esophageal bacterial biota while gram-negative anaerobes comprise the majority of the biota in GERD. Inflammatory cells infiltrate the esophageal mucosa in reaction to acid reflux. Our hypothesis was that esophageal bacteria regulate expression of genes related to inflammation and redox stress.

Design: We examined the responses of human macrophage cell line THP-1 to the predominant bacterial species found in the normal esophagus and GERD and acid treatment. Heat-inactivated bacteria were mixed with THP-1 cells at various ratios. After incubation at 37 °C in 5% CO_2 for 72 or 96 hours, whole cell lysates were used as antigens to assess gene expressions by Western Blots.

Results: Both *S. mitis* and *Veilonella atypica* induced COX-2 expression and upregulated NQO1 expression in THP-1. Acid treatment alone had no effect on the expression of either of the two genes. Acid treatment diminished the bacterium-induced NQO1 expression but enhanced COX-2 expression. Both *S. mitis* and *V. atypica* down-regulated Bcl-2 expression and promoted the cytoplasm to nucleus translocation of NFkB but not that of Nrf2.

Conclusions: Studies of bacterium-macrophage interaction in the *in vitro* cell culture system generated several interesting hypotheses that can be tested in GERD. Commensal bacteria may participate in complex gene regulation processes related to inflammation and redox stress in GERD. Esophageal bacteria may: (i) in synergy with acid reflux, induce cytoprotective reactions via COX-2 expression in inflammatory cells, (ii) upregulate cellular capacity of processing oxygen redicals via NQO1, and (iii) shorten the duration of inflammation by the induction of apoptosis of inflammatory cells through down-regulation of Bcl-2.

512 MLH1 and PMS2 Protein Expression in 103 Colorectal Carcinomas with *MLH1* Promoter Methylation and without *MLH1 or PMS2* Germline Mutation

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Background: Promoter methylation is an epigenetic phenomenon that can prevent normal transcription of genes. Methylation of the *MLH1* promoter and subsequent loss of mismatch repair function can lead to the development of colorectal carcinoma (CRC). These tumors show microsatellite instability (MSI) and are associated with a better prognosis than stage matched microsatellite stable CRC. We assessed CRC with methylation of the *MLH1* promoter using immunohistochemistry (IHC) to determine how often MLH1 and its heterodimer partner PMS2 protein expression were lost and to determine if MLH1 IHC could be used as a marker for MSI.

Design: All patients diagnosed with CRC since 1/99 were offered participation in the Columbus-area HNPCC study regardless of age or family history. Tumors were evaluated for MSI using a modified Bethesda panel of microsatellite markers (BAT25, BAT26, D18S69, D2S123, and D5S346). Methylation status of the *MLH1* promoter in a region close to exon 1 was evaluated in tumor DNA by the combined bisulfite restriction analysis method (COBRA). All patients with MSI tumors underwent mutation analysis of *MLH1*, *MSH2* and *MSH6* genes by full sequencing of germline DNA and multiplex ligation probe amplification and IHC for MLH1, MSH2, MSH6, and PMS2.

Results: From a total of 264 MSI-high or -low CRC cases, 103 showed *MLH1* promoter methylation. Of these, 98 (95%) were MSI-high and 5(5%) were MSI-low. No deleterious germline mutations in *MLH1*, *PMS2, MSH2 or MSH6* were found. 97 (94%) showed absence of MLH1 expression by IHC (89 of these 97 cases also showed absence of PMS2). In 6 (6%) cases MLH1 protein expression persisted. Of the 6, 4 (67%) were MSI-low and 2 (33%) were MSI-high. PMS2 but not MLH1 expression was lost in 1 of these 6 tumors.

Conclusions: Loss of MLH1 and PMS2 staining were seen in most CRCs with methylation of the *MLH1* promoter. Therefore, IHC for MLH1 will detect most cases and can be used to estimate MSI status for prognostic purposes. Persistent protein expression of MLH1 in spite of promoter methylation was seen rarely and most cases were MSI-low. The mechanism by which protein expression persists is unclear, but the MSI-low status of most suggests that *MLH1* promoter methylation in these cases led to incomplete disruption of transcription (and thus translation) of MLH1. Of course, if IHC for MLH1 is used to estimate MSI status, patients with MSI tumors due to germline mutations in *MSH2*, *MSH6* and possibly *PMS2* would be missed.

513 Amsterdam Criteria, Bethesda Guidelines and Histologic Findings in 34 Patients with Hereditary Nonpolyposis Colorectal Cancer

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Background: The detection of microsatellite unstable (MSI) colorectal carcinoma (CRC) has prognostic value and can help screen for Hereditary Nonpolyposis Colorectal Cancer (HNPCC). Population based screening for all with CRC has been proposed. Histologic features are associated with MSI tumors and include a conspicuous host lymphoid reaction, mucinous or signet ring cell carcinoma or a medullary growth pattern; and are included in the revised Bethesda Guidelines in those under 60 years old. We studied 34 CRC patients with germline mutation confirmed HNPCC and determined whether patients met Amsterdam Criteria and/or Bethesda Guidelines with/without histologic features to determine whether patients would have escaped detection without CRC population based screening.

Design: All CRC patients with a confirmed HNPCC deleterious mutation were identified from the Columbus-area HNPCC study. Immunohistochemistry for MLH1, MSH2, MSH6 and PMS2 and molecular analysis was available on all including MSI status, methylation of the *MLH1* promoter, full sequencing of genomic DNA for *MLH1*, *MSH2* and *MSH6*, and mutiplex ligation probe amplification. Patient age at diagnosis, tumor site in the colon, other cancer history, and family history were noted. Histologic endpoints included tumor type (mucinous, signet ring, or medullary growth pattern), tumor infiltrating lymphocytes (0-3), and Crohn's-like reaction (0-2).

Results: Histologic findings associated with MSI CRC were seen in 22 of 34 cases (64.7%). Of the 34, 8 (23.5%) met Amsterdam Criteria, 27 (79.4%) met the less stringent Bethesda Guidelines, and 7 (20.6%) did not meet either clinical criteria without assessing tumor histology. Of the 7 that did not meet clinical criteria, 4 had histologic features consistent with MSI positivity. However, one of these tumors was in an 82 year old. Therefore, 3 additional cases would have been detected by including those diagnosed under age 60 whose tumors met histologic criteria. Overall, 4 out of 34 cases (11.8%) would have been missed using Amsterdam Criteria and Bethesda Guidelines including histologic features.

Conclusions: Amsterdam Criteria and Bethesda Guidelines identify many CRC patients with HNPCC, and the addition of histology helps detect a few more cases. Histologic findings associated with MSI CRC are not seen in all cases and many lack specificity. As a result, screening for HNPCC among all newly diagnosed CRC patients is suggested to identify all patients with this condition.

514 Significance of Focally Enhanced Gastritis in the Pediatric Population

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Background: Localized inflammation involving a single or several gastric glands has been referred to as focally enhanced gastritis (FEG). The inflammation consists mainly of lymphocytes and histiocytes with occasional eosinophils, neutrophils and/or plasma cells. Initially described in Crohn's disease (CD) patients, it has also been reported in patients with ulcerative colitis (UC). A recent study from our own institution found that FEG was a nonspecific diagnosis in adults that was rarely associated with IBD. Our anecdotal experience, however, suggested that FEG was often associated with IBD in children. Hence the following study was undertaken.

Design: We reviewed stomach biopsies from patients under the age of 18 with a previous diagnosis of FEG at our institution from 2000-2005. Biopsies were identified using a free text computer search. Histologic criteria were an isolated focus or foci of foveolar, pit, or gland inflammation with associated epithelial injury. We recorded the type of inflammatory cells, presence of granulomas, number of foci and number of glands involved. Follow-up data were obtained by review of pertinent medical records.

Results: 25 patients were identified (12 males, 13 females) with a median age of 12 (range, 2-16). FEG lesions were most often multifocal (56%) with a mean of 2.3 foci (range, 1-7) and involved 8.2 glands per patient (range, 1-24) on average. All lesions contained lymphocytes and histiocytes; in addition, plasma cells were present in 76% followed in frequency by eosinophils (44%) and neutrophils (36%). Granulomas were present in 32% of biopsies. An additional 32% of cases contained histiocytic aggregates. On follow-up, 20 of 25 patients had evidence of IBD; 52% CD, 12% UC, 12% indeterminate colitis and one (4%) lymphocytic colitis. The positive predictive value (PPV) of FEG for IBD was 80%. Of the five FEG patients without IBD, one had celiac disease, one had DiGeorge's syndrome with a Nissan fundoplication and possible autism and one was autistic.

Conclusions: The presence of FEG is highly predictive of IBD in pediatric patients. In this study, FEG was most often associated with Crohn's disease. This is in stark contrast to a previous study from our institution that found a PPV of IBD of only 5.9% for FEG in adults. This large difference probably reflects differing indications for undergoing endoscopy between these two patient groups.

515 Squamous Cell Papillomas of the Esophagus: A Study of 19 Lesions for Human Papillomavirus by In Situ Hybridization and the Polymerase Chain Reaction and p16 Immunoprofile

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Background: Esophageal squamous papilloma (ESP) is an uncommon benign lesion. ESP have been related to different factors including gastroesophageal reflux, irritants, and several studies have focused in human papilloma virus (HPV) infection. High prevalence of cervical HPV has been demonstrated in mexican population; however, little is known about esophageal lesions HPV-related. The aim of this study was to investigate the presence of HPV in ESP and also determine p16, p53 and Ki67 expression in a Mexican cohort.

Design: Nineteen cases diagnosed as ESP corresponding to 18 patients were reviewed from Department of Pathology-UPAEP University Hospital files during a 12-year period. H&E and immunostains for P16INK4a, p53 and Ki67(DAKOCytomation)

were studied. HPV detection was performed by amplified chromogenic in situ hybridization (Genpoint/DAKOCytomation) (ACISH) using the wide spectrum-cocktail probe (DAKOCytomation) in 16/19 cases and PCR-RFLP in 14/19 cases. MY09/MY11 were used as consensus primers and PCR products detected by agarose gel electrophoresis.

Results: The mean age at presentation was 46.3 years with a female predominance (14 F, 4 M). ESP was an incidental finding in all cases. A history of HPV-related diseases was absent. ESP was located from: upper third (11), middle third (3) and unknown location (5). All except one case were present as a single lesion. The size ranged from 3-7 mm. Microscopically exophytic lesions with basal cell hyperplasia and vacuolated cells in upper layers were present in all cases. No dysplastic changes were detected. Immunohistochemical studies showed basal and focal expression of p53 in 17 cases. p16 was positive in 8 cases (basal layer and focally in medium third), with a low rate (<20%). Ki67 index ranged from 10-30%. HPV was detected in 14/16 cases (87.5%) by ACISH: 12 diffuse nuclear pattern and 2 granular pattern. HPV DNA was identified by PCR in 12/14 cases (85.7%). Low-risk HPV types were detected in the most of the cases.

Conclusions: Our study provide identification of HPV infection in almost 80% of ESP cases using either ACISH or PCR. Diffuse pattern by ACISH indicates episomal HPV infection. p16 and p53 were not overexpressed in all cases related to HPV infection. We also suggest ACISH as an alternative diagnostic tool for HPV detection in ESP with acceptable sensitivity.

516 Buried Dysplasia and Early Adenocarcinoma Arising in Barrett Esophagus (BE) after Photodynamic Therapy (PDT): Not Uncommon Findings

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Background: BE with or without dysplasia after squamous restoration following PDT is raising concern because of a risk of malignant transformation. However, the prevalence and characteristics of post-PDT buried neoplasm have not been fully evaluated.

Design: 52 BE patients with high-grade dysplasia (HGD;19), intramucosal carcinoma (IMC;28) and invasive carcinoma (InvCA;5) were treated with PDT. Four quadrant every 2 cm biopsies were taken before PDT. Post PDT, the same protocol surveyed the site of the original BE, independently of squamous restoration. The presence of buried BE and/or neoplasm pre and post PDT was correlated with several clinicopathological features.

Results: Buried BE was seen in 11 patients (21.2%) on 13 biopsy levels pre PDT and in 27 patients (51.9%) on 78 biopsy levels post PDT. Buried BE was more frequent after (78/609, 12.8%) than before PDT (13/240, 5.4%) ($p<0.01$). Buried neoplasms were seen in 5 patients (9.6%) with 7 foci (InvCA;1, IMC;3, HGD;3) pre PDT and in 17 patients (32.7%) on 36 levels (InvCA;2, IMC;11, HGD;21, LGD;2) post PDT. The prevalence of buried neoplasms was 4.0% (7/173) and 14.0% (36/258) in pre- and post-PDT positive biopsies ($p=0.001$). Twenty six buried neoplasms were from unremarkable squamous mucosa and 10 from either nodule, erythema or stricture. There was no prevalent site for buried neoplasms with relation to the original length of BE. InvCA was more frequently buried (2/6 positive post-PDT biopsy levels) in comparison to lower neoplastic grades (IMC;11/69, HGD;21/164, LGD;2/19). There was no correlation between the presence of pre- and/or post-PDT buried neoplasms and the diffuseness of neoplasms before PDT. At the end of follow-up, 3 of 5 patients with pre-PDT buried neoplasms had persistent disease compared to 9 of 47 patients with no buried neoplasms ($p=0.074$). Of 37 non-responders to one course of PDT, 17 showed buried neoplasms. After additional therapy, 5 of the 17 patients had persistent disease versus 7 of 20 patients with only surface neoplasms ($p=0.544$).

Conclusions: Buried BE and neoplasms are common after PDT. Although buried lesions respond to additional therapy in the same way as surface neoplasms, their detection is difficult without reliable clinicopathologic characteristics suggestive of an increased risk. Thus, systematic endoscopic surveillance and thorough histologic evaluation are important to avoid overlooking buried neoplasms that may progress over time.

517 Barrett Esophagus Associated Neoplasms Treated by Photodynamic Therapy: Determination of Limiting Factors

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Background: Photodynamic therapy (PDT) is being evaluated for the treatment of superficial neoplasia arising in Barrett Esophagus (BE). Several reports have demonstrated marked reduction of neoplastic tissue post PDT, but only a few have included a systematic pathologic assessment.

Design: 52 BE patients (M:41;F:11) with high-grade dysplasia (HGD, n=20), intramucosal carcinoma (IMC, n=28) and invasive carcinoma (InvCa, n=4) were treated with PDT using porfimer sodium as photosensitizing agent. Jumbo biopsies were taken from 4-quadrant every 2 cm over the length of BE. During follow-up, the protocol surveyed the length of the original BE, including areas of squamous re-epithelialization.

Results: Mean length of BE was 6.45 cm (range; 1-13). Mean follow-up was 30.1 months (range; 6-89). PDT was successful after one session in 15 patients (29%; HGD 7, IMC 8) with no recurrence (mean follow-up; 19.9 months, range; 7-32). Thirty five patients were treated with additional PDT alone (n=4), in combination with mucosal fulguration (n=17) or fulguration alone (n=14). Two patients declined additional treatment. In toto, neoplasias were eradicated in 40 patients (77%) (HGD 16, IMC 22, InvCa 2) with a mean disease free interval of 15.3 months (0-39). The neoplastic lesions were downgraded in 2 patients (HGD to LGD, IMC to LGD), unchanged in 9 (HGD 3, IMC 4, InvCa 2), or progressed in one (IMC to InvCA). Among 28 patients with neoplasias spanning over less than 4 cm, 17 (61%) were successfully treated by one course of PDT and 25 (89%) responded to the treatment at the end of follow-up. Conversely, only 2 of 24 patients (8%) with neoplasia spanning over 4 cm were treated by one course of PDT and 15 (63%) responded to the treatment. Residual neoplasias were more common in the distal (n=18) than in the proximal (n=4) BE. Patches of BE covered by squamous epithelium were found in 27 patients (52%), and foci of buried neoplasia were found in 18 (35%) after PDT, while they were observed in only 11(21%) and 4(8%) before PDT, respectively.

Conclusions: Post PDT detailed pathologic evaluation reveals a high frequency of persistent neoplasia after one course of PDT; however, additional treatment improved response rate to 77%. Diffuseness and distal location of the lesions are associated with treatment failure. Residual neoplasia is often covered by squamous epithelium. These characteristics underline the importance of close follow-up.

518 Post-Gastric EMR Surveillance Biopsies: Evaluation of Mucosal Changes and Recognition of Potential Mimics of Residual Adenocarcinoma

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Background: Endoscopic mucosal resection (EMR) offers curative treatment for patients with node-negative early gastric carcinoma, and is now the standard therapy in Japan and in some Western centers. An important step in the follow-up of patients is the evaluation of post-EMR biopsies performed to ensure the absence of residual neoplasia. However, the spectrum of mucosal changes that can be observed in the biopsies has not been systematically evaluated.

Design: The goal of this study was to categorize the various reactive pathologic changes that might be difficult in differentiating from residual adenocarcinoma. We retrospectively investigated 39 post-EMR biopsies after 1-16 (mean 6.7) days from 33 patients who underwent gastric EMR.

Results: The results were shown in Table 1. Residual adenocarcinomas were noted in 4 of 34 cases, and consistently showed high N/C ratio with high glandular density. Among various reactive changes, glands showing clear cell degeneration and/or signet-ring cell-like change were great mimics of residual carcinoma; however, they were usually embedded in a non-desmoplastic stroma and showed anisonucleosis of glandular epithelia.

Conclusions: Given the raising popularity of EMR, pathologists should be aware of the difficulties in the evaluation of post-EMR mucosal changes, some of which may lead to a potential misdiagnosis of residual adenocarcinoma. Mimics of residual adenocarcinoma, namely clear cell degeneration and signet-ring cell-like change should be judiciously assessed to avoid unnecessary surgery.

TABLE 1. Pathologic Features of the post-EMR biopsies

Features	Biopsies, n=39 (%)
Architectural Changes	
Foveolar hyperplasia	28 (72.0)
Villiform configuration	8 (20.5)
Lobular glandular proliferation	3 (7.7)
Architectural changes	2 (5.1)
Cytological Changes	
Increased mitoses *	20 (51.3)
Anisonucleosis	18 (46.2)
Atypia	22 (56.4)
Mucin depletion	4 (10.3)
Apocrine-hobnail type change	1 (2.6)
Clear cell degeneration	5 (12.8)
Signet-ring cell-like change	2 (5.2)
Ulceration	
Fibrinopurulent exudate	18 (46.2)
Granulation tissue	5 (12.8)
Stromal Changes	
Edema	38 (97.4)
Inflammation	38 (97.4)
Ischemia	18 (46.2)
Ectatic blood vessels	22 (56.4)

*: Increase mitoses >2 mitoses/foveolae

519 Evaluation of Immunohistochemical Expression of p16(INK)4A and Oncogenic Human Papillomavirus in Anal Biopsies from HIV-Positive Males

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Background: Immunohistochemical expression of the cyclin-dependent kinase inhibitor p16INK4a has been demonstrated in human papillomavirus (HPV)-associated neoplasms of the anogenital squamous epithelium. P16INK4a expression may therefore be a useful marker of neoplastic squamous lesions that pose diagnostic difficulty. Because of the prolonged survival of HIV-positive patients, HPV- associated neoplasms of the anal squamous epithelium are emerging as a frequent complication. Accurately identifying these lesions in a preinvasive state allows for appropriate local treatment to prevent the development of invasive lesions with their inherent morbidity and mortality.

Design: HIV-positive men with a history of receptive anal intercourse underwent high-resolution anoscopy. Biopsies were taken of abnormal-appearing areas. All patients underwent anal cytological smears that were collected in liquid medium. HPV testing for oncogenic types was carried out on the cytology material using the Hybrid Capture technique (Digene Diagnostics Inc.). All biopsies were evaluated by two pathologists. From this patient base, 300 biopsies were selected; 100 from each of the following categories where consensus diagnosis had been reached: negative for neoplasm, low grade squamous intraepithelial lesion (LSIL) and high grade squamous intraepithelial lesion (HSIL). Sections were stained for p16INK4a using a standard immunohistochemistry technique. The p16INK4a slides were considered positive if there was nuclear staining in some or all of the squamous epithelial cells.

Results: The p16INK4a positivity rates for HSIL, LSIL, and negative were, respectively, 99%, 69%, and 26%. HSIL expression tended to be diffuse within the lesion whereas LSIL expression was confined to the basal region or lower third. The distribution of expression in histologically negative cases was variable but many were diffusely positive. Oncogenic HPV was present in 98% of patients with HSIL or LSIL and in 86% where the biopsy was negative. Most (12/14) of the HPV- negative cases with negative histology were p16INK4a positive.
Conclusions: The immunohistochemical expression of p16INK4a in anal biopsies is highly sensitive in detecting HSIL but considerably less sensitive for LSIL. Its diagnostic utility is limited by the observation that benign, reactive, HPV-negative processes are often diffusely and strongly p16INK4a positive.

520 There Is No Relationship between Loss of 8P and Overexpression of Clusterin in a Consecutive Series of 81 Sporadic MSS Colorectal Carcinomas

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Background: Colorectal carcinoma (CRC) has been shown to arise through at least two disctinct pathways : one involving microsatellite instability (MSI) and the other involving chromosomal instability. Tumors characterized by chromosomal instability demonstrate a high frequency of allelic imbalance, the most common involved chromosomal arms being 5q, 17q, 18q, and 8p. Previous studies of acquired loss of heterozygosity (LOH) in CRC have suggested that a tumor suppressor gene may lie within the short arm of chromosome 8, but a candidate tumor suppressor gene has not yet been identified, and its precise localisation remains to be determined. The aim of this study is to investigate whether CLU, encoding for clusterin, having anti-apoptoic function, and known to reside on chr 8p21, could be this putative suppressor tumor gene.
Design: Allelic deletion on the short arm of chr 8 was studied in a consecutive series of 81 sporadic MSS colorectal carcinomas, MSI phenotype was eliminated by molecular biology, and immunohistochemistry for hMLH1 and hMSH2. We elected to confirm this deletion on the protein level by immunostaining of clusterin, in relation to clinicopathologic parameters, after 5 years of patient follow-up.
Results: Fifty one tumors showed loss of heterozygoty of 8p, whereas clusterin expression was seen in 20 cases (25%), from which only 15 showed loss of 8. Neither LOH of 8p, nor clusterin expression showed a significant relationship with all clinicopathologic parameters, particularly tumor staging, and vascular permeation at diagnosis.
Conclusions: Our findings rule out the potential role of CLU located on chr 8p21, as a putative tumor suppressor gene in the tumorigenesis of CRC. The only explanation for the overexpression of clusterin in some CRCs in our series, is the presence of dominant mutation, without relation to the genotype, through the activation of other genes, probably those related to apoptosis. This hypothesis needs to be confirmed by additional studies.

521 Low Rectal Cancer; a Call for a Change of Approach in Abdominoperineal Resection

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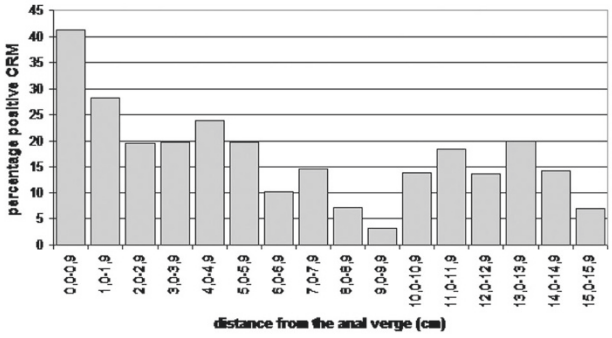
Background: Decreasing local recurrence rates in low rectal cancer still remain a challenge despite the major improvements that have been made due to Total Mesorectal Excision (TME) and the the role of pathologists in quality control.
Design: By investigating pathological factors from a prospective randomized rectal cancer trial in which surgeons had undergone training in TME those factors responsible for the poor outcome were determined and a new method for assessing the quality of surgery by pathologists was tested.
Results: Survival differed greatly between abdominoperineal resection (APR) and anterior resection (AR) (38.5% versus 57.6%, $p = 0.008$). Low rectal carcinomas have a higher frequency of circumferential margin involvement (26.5% versus 12.6%, $p < 0.001$). More positive margins were present in the patients operated with APR (30.4%) compared to AR (10.7%, $p = 0.002$). Furthermore, more perforations were present in these specimens (13.7% versus 2.5%, $p < 0.001$). The plane of resection lies within the sphincteric muscle, the submucosa or lumen in over 1/3 of the APR cases and in the remainder lay on the sphincteric muscles.
Conclusions: We systematically described and investigated the pathological properties of low rectal cancer in general and APR in particular in a prospective randomized trial including surgeons who had been trained in TME. The poor prognosis of the patients with an APR is ascribed to the resection plane of the operation leading to a high frequency of margin involvement by tumor and perforation with this current surgical technique. The clinical results of this operation could be greatly improved by adopting different surgical techniques and possibly greater use of radiochemotherapy.



522 Reevaluation of Intraglandular Necrotic Debris in Gastric Specimens

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Background: Intraglandular necrotic debris (IND) in gastric biopsy specimens has been recently reported as a diagnostic clue to non-invasive high-grade dysplasia or invasive carcinoma (Ann Diagn Pathol 2001;5:141-147). It is defined as an eosinophilic material with necrotic epithelial fragments within the lumen of a dilated atypical gland. So far, no detailed immunohistochemical studies on IND in the gastric specimens have been reported.
Design: We reviewed 34 gastric cases (32 neoplastic cases and 2 non-neoplastic cases) with IND. Immunohistochemical analysis was performed for these cases with the following antibodies: pancytokeratin, E-cadherin, p53, Ki-67, and CD68 to clarify the origin of IND. To avoid discrepancies between Western and Japanese criteria of gastric adenocarcinoma, the Vienna classification was used (Gut 2000;47:251-255). In addition, the pattern of IND in benign cases were evaluated.
Results: IND was found both gastric biopsy specimens and surgical specimens. With the relation to the histological type, moderately differentiated adenocarcinmas showed the highest incidence of IND. Interestingly, IND was found in 1 case of well-differentiated adenocarcinoma mimicking complete-type intestinal metaplasia (Hum Pathol 1999;30:826-832). Immunohistochemical staining were performed on 32 gastric specimens, and evaluated in 28 of these, since 4 cases showed poor staining of the area of IND due to necrosis. Six cases (21%) revealed the presence of p53 positive cells, 13 cases (46%) of Ki-67 positive cells, 12 cases (43%) of E-cadherin positive cells and 25 cases (89%) of CD68 positive cells. Eight cases (29%) showed that only CD68 was positive in IND. In 2 cases of non-neoplastic cases, the distribution of IND was more extensive than that seen in adenocarcinomas and they were closely related to severe ischemic change.
Conclusions: Our study indicates that IND may not contain necrotic epithelial fragments and only macrophages are present in IND in some cases of gastric adenocarcinoma. This is probably due to complete necrosis of epithelial fragments within a dilated atypical gland, which is eventually replaced by macrophages. Even in such cases, IND is still useful to make a diagnosis of gastric adenocarcinomas. Althoug IND may be a clue to a rare type of well-differentiated adenocarcinoma mimicking complete-type intestinal metaplasia, careful attention is required in cases with severe ischemia since such cases may show extensive IND.

523 Distinct Molecular Features of Colorectal Carcinoma with Signet Ring Cell Component and Colorectal Carcinoma with Mucinous Component

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Background: Signet ring cell carcinoma and mucinous carcinoma are distinct subtypes of colorectal adenocarcinoma. The morphologic and molecular spectra of colorectal carcinomas with various signet ring cell components and colorectal carcinomas with various mucinous components, compared to non-mucinous adenocarcinomas, have not been examined.
Design: The study groups consisted of 39 carcinomas with a signet ring cell component ("the signet group"), 167 carcinomas with a mucinous component ("the mucinous group"), and 457 non-mucinous non-signet-ring-cell carcinoma. We visually estimated the amounts of signet ring cell and mucinous components in tumors, and subclassified the signet and mucinous groups, according to the amount of each component (≤19%, 20-49%, and ≥50%). We sequenced *BRAF* and *KRAS*, analyzed for microsatellite instability (MSI), 18q loss of heterozygosity (LOH), and performed immunohistochemistry for TP53, COX2, MLH1, MGMT, p16 (CDKN2A) and fatty acid synthase (FASN).
Results: Signet ring cell carcinoma (≥50% signet ring cell tumors) and ≤49% signet ring cell tumors showed similar molecular features. Except for MSI and MGMT, ≥50% mucinous tumors and ≤49% mucinous tumors also showed similar molecular features. *BRAF* mutations, MSI, and MLH1 loss were more frequent in both the signet and mucinous groups than non-mucinous carcinoma. More frequent *KRAS* mutations and less frequent p16 loss and TP53 positivity were observed in the mucinous group than non-mucinous carcinoma. 18q LOH was less common in the signet group than non-mucinous carcinoma. FASN levels were highest in the mucinous group, followed by

non-mucinous carcinoma, and lowest in the signet group. COX2 overexpression was less common in the signet group than non-mucinous carcinoma.
Conclusions: A minor (≤49%) signet ring cell or mucinous component in colorectal carcinoma suggests similar molecular features to ≥50% signet ring cell or mucinous carcinoma, respectively. Signet ring cell carcinoma and mucinous carcinoma are related subtypes of colorectal adenocarcinoma, but have distinct molecular features from each other.

524 Quantitative DNA Methylation Analysis Determines CpG Island Methylator Phenotype (CIMP) as a Distinct Subtype of Colorectal Cancer

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Background: Transcriptional silencing by gene promoter methylation is an important carcinogenic mechanism. Extensive promoter methylation in colorectal cancer has been termed CpG island methylator phenotype (CIMP). However, controversy exists whether CIMP represents a distinct subtype of colorectal cancer or is merely within the spectrum of a predicted distribution of stochastic methylation events. Previous studies on CIMP used methylation-specific PCR, which may detect biologically insignificant low levels of methylation.
Design: Using sodium bisulfite treatment and quantitative real-time PCR (MethyLight), we quantified DNA methylation (PMR, "percent of methylated reference") in promoter regions of *MLH1*, *MGMT*, *CDKN2A* (p16), *CACNA1G*, *CRABP1*, and *NEUROG1* in 460 colorectal cancers. The number of methylated loci among the 6 genes (CIMP panel) was determined for each tumor. Methylation data were stratified according to microsatellite instability (MSI) and 18q loss of heterozygosity (LOH) status (as a surrogate of chromosomal instability).
Results: Using PMR = 4% as a cutoff, 94 (20%) or 129 (28%) of the 460 tumors showed ≥ 4 methylated loci or ≥ 3 methylated loci, respectively, among all 6 loci. A bimodal distribution of the number of methylated loci was not evident. As a single locus, *MGMT* methylation had the lowest sensitivity (62%) and specificity (66%) for predicting CIMP status. When *MGMT* was excluded from the 6-gene CIMP panel, a bimodal distribution of the number of methylated loci was quite evident, and 78 (17%) or 109 (24%) tumors showed ≥ 4 or ≥ 3 methylated loci, respectively, among the remaining 5 loci. A bimodal distribution is particularly evident among MSI-H tumors (N = 75), with almost all tumors showing either ≥ 4 methylated loci (76%), or ≤ 1 methylated loci (21%). 18q LOH status among microsatellite stable tumors did not significantly affect the distribution of the number of methylated loci.
Conclusions: CIMP is a distinct subtype of colorectal cancer, and may be less frequent than previously reported. Quantitative DNA methylation analysis should be used to determine CIMP status to exclude biologically insignificant low levels of methylation. MGMT methylation should be evaluated with caution when determining CIMP status.

525 Incipient DNA Mismatch Repair Deficiency in Large Non-Dysplastic Serrated Polyps of the Colorectum

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Background: Non-dysplastic serrated polyps of the colorectum are the frequent precursors of sporadic colorectal cancers (CRCs) that have MLH1 silencing due to CpG island promoter hypermethylation, with associated DNA mismatch repair (MMR) deficiency and high frequency microsatellite instability. We previously showed that foci of mixed hyperplastic polyp (HP) and serrated adenomatous epithelium adjacent to these cancers are also MLH1 deficient, supporting a direct role in tumor progression. We undertook this study to investigate MLH1 deficiency in large non-dysplastic serrated polyps in an effort to identify features that may better predict their risk of progression.
Design: We identified 73 HPs ranging in size from 0.6-1.9 cm (median 0.9 cm) and removed by polypectomy between 1999-2001 (prior to use of the diagnostic term sessile serrated polyp (SSP)/sessile serrated adenoma). Clinicopathologic characteristics were reviewed, and polyps were classified as either typical HPs or having features suggestive, or diagnostic of SSP. Cases were immunostained for MLH1 and considered deficient when there was total loss of nuclear expression in at least one crypt.
Results: Large HPs were equally common in men and women, but were more common in the right colon (48 right-sided vs. 25 left-sided). Histologic features of SSP were present in 35/73 (48%) polyps and were associated with size (10/30 between 0.6-0.8 cm vs. 25/40 >0.8 cm had SSP histology; p<0.025) but not with sex or site. MLH1 deficient crypts were found in 6/73 (8%) polyps, and ranged from 2-14 involved crypts. MLH1 deficient polyps were: more common in the right colon (6/6 deficient polyps vs. 42/67 intact polyps were right-sided; p<0.10 NS), larger in size (1/33 polyps <0.9 cm vs. 5/40 polyps >0.8 cm were MLH1 deficient; p<0.20 NS), and more frequently associated with SSP histology (6/6 MLH1 deficient vs 29/67 MLH1 intact polyps had SSP histology; p<0.01). Right-sided polyps measuring >0.8 cm, with SSP histology, and obtained from women had the highest frequency of MLH1 deficiency (3/13, 23%).
Conclusions: Large HPs are more frequent in the right colon and are more likely to have SSP histology with increasing size. Foci of MLH1 deficiency are identifiable in these polyps, and are associated with increasing size, right-sided location and SSP histology. These foci are the likely precursors of progression to dysplasia and CRC, therefore size, location and histologic features may have utility as risk markers. Additional studies are required to fully understand the natural history of these incipient MMR deficient foci.

526 Immunohistochemical Staining for p63 Is Useful in the Diagnosis of Anal Squamous Cell Carcinomas

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Background: p63 is a good marker of squamous differentiation in carcinomas of the head and neck, esophagus and uterine cervix. Poorly differentiated carcinomas in the anal region can present a diagnostic challenge, especially with small biopsy specimens. The differential diagnosis includes poorly differentiated colorectal adenocarcinomas, carcinoid tumors/neuroendocrine carcinomas, and anal squamous (cloacogenic) cell carcinomas. Since therapy for squamous cell carcinoma is primarily non-surgical, accurate diagnosis is imperative. Furthermore, apart from differential cytokeratin staining, there has been no widely used positive immunostain for these tumors in the anus. We undertook the following study in order to test the diagnostic utility of p63 staining in neoplasms of the anus and colorectum.
Design: Slides from twenty-four anal squamous cell carcinomas (eleven of which were poorly differentiated), seven rectal carcinoid tumors and a tissue microarray of 49 colorectal adenocarcinomas (including six poorly-differentiated carcinomas) were stained with anti-p63 antibody using the Dako automated staining system. Only nuclear staining was regarded as positive. A section of prostate tissue was utilized as a positive control (basal cell staining).
Results: All twenty-four (100%) of the squamous cell carcinomas were strongly positive for nuclear p63 staining, while neither the adenocarcinomas (0/49; 0%) nor the carcinoid tumors (0/7; 0%) had nuclear staining. There was cytoplasmic staining of the associated skeletal muscle in specimens where it was present, but not of the smooth muscle. There was nuclear staining of the basal layers of the associated squamous epithelium, both in non-neoplastic epithelium and in anal intraepithelial neoplasia.
Conclusions: Immunohistochemical staining for p63 had 100 percent sensitivity and specificity as a diagnostic marker for squamous differentiation in invasive carcinomas of the anal region, including poorly-differentiated squamous cell carcinomas. Once the diagnosis of invasive carcinoma is made, p63 can be an important confirmatory stain to ensure that the patient receives proper therapy.

527 Analysis of BRAF-V600E Mutation in Gastric Carcinoma

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Background: BRAF, a member of the RAF family of serine/threonine kinases, mediates cellular responses to growth signals through the RAS-RAF-MAP kinase pathway. In colorectal carcinoma (CRC), BRAF-V600E mutation has been shown to be strongly associated with epigenetic silencing of MLH1, but not with germline mutation of mismatch-repair (MMR) genes. Its detection has been proposed as a reliable, fast, and low cost strategy which simplifies genetic testing for HNPCC. Gastric carcinoma is the third most frequent cancer in this syndrome. Approximately 20% of non-selected gastric carcinomas have MMR deficiency. The frequency of BRAF mutation and its association with MMR genes alterations in gastric carcinoma have not been extensively studied.
Design: 116 consecutive gastric carcinomas were retrieved from our files. We performed a microsatellite instability (MSI) status analysis using BAT26, and immunohistochemistry for MMR proteins. Tissue cores from formalin-fixed, paraffin-embedded donor blocks were arrayed to create a tissue microarray. Sections were stained with monoclonal antibodies against MLH1, MSH2 and MSH6. BRAF-V600E mutation detection was performed in a ABIPRISM 7500 based on the design of two TaqMan probes labelled with a different fluorescent tag (FAM and VIC), specific for the wildtype allele and the mutant allele. We selected for BRAF mutation analysis all MSI(+) (n=22) cases, and as control group 44 negative tumors. A CRC cell line (HT29) with known BRAF-V600E mutation was included as a positive control.
Results: MSI was found in 22 of 116 (19%) of cases. Among MSI cases, 86.4% (19/22) had loss of MLH1 expression. MSI phenotype was more frequently seen in tumors of intestinal type (86% vs. 63%, p=0.04), and those presenting in females (64% vs. 33%, p=0.008), and at older ages (74 years vs. 55 years, p=0.018). None of the tumors (0/66; 0%) showed BRAF-V600E mutation.
Conclusions: In our series of gastric carcinoma with known MSI status, BRAF-V600E mutation was not found. Therefore, our results suggest that in contrast to CRC, BRAF-V600E analysis can not be used as a screening strategy to exclude HNPCC syndrome.

528 Comparison of Three Commercially Available Immunohistochemical Tests for EGFR Expression in Colorectal Cancers. Is Immunohistochemistry Reliable?

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Background: IHC appears to be the required test for the selection of patients for a monoclonal antibody based targeted treatment with C225 (Erbitux®). In an attempt to standardize the immunohistochemical detection of EGFR, we have retrospectively evaluated three commercially available EGFR kits or antibodies and analyzed the discrepancies between the tests in terms of percentage of positive cells, intensity, cut off value and fixatives.
Design: A series of 232 samples of metastatic colorectal cancer were tested for EGFR expression using the FDA-approved Dako pharmaDx kit, the Zymed kit and the Ventana 3C6 antibody. Different cut off values were tested and intensity was scored 0, 1+, 2+, 3+.
Results: The percentages of positive cases varied from 75 to 93% with a cut off value of 1% of positive cells, from 61 to 80% with a cut off value of 5% and from 48 to 72% with a cut off value of 10%.

	Dako	Ventana	Zymed
Cut off >1%	75%	93%	86%
Cut off >5%	61%	80%	78%
Cut off >10%	48%	72%	60%

Both Ventana and Zymed tests performed better than the Dako test in terms of percentage of positive cells (Ventana > Dako; $p<10^{-7}$, Zymed > Dako; $p=2.10^{-6}$). However, no difference was noted between Ventana and Zymed tests (p=0.75). The three tests reacted similarly for high intensities. The use of a scoring system combining percentage of positive cells and intensity was not useful for Zymed and Ventana as the intensity of staining is linked to the percentage of positive cells: Ventana ($p<10^{-6}$) and Zymed ($p<10^{-5}$). No interaction with staining was identified for any of the fixatives, nor with the nature of samples.

Conclusions: These data showed a higher percentage of positive cells detected by Ventana and Zymed tests, no matter what the cut off value for positivity was. No scoring system shows, to date, their accuracy, and more studies must be conducted with an evaluation of response to cetuximab, and perhaps linked to FISH amplification in colorectal carcinoma. More data concerning correlation between IHC and FISH and CISH will be shown at the meeting

529 Strong PDGFRA Positivity Is Seen in GISTs but Not in Other Intraabdominal Mesenchymal Tumors: Immunohistochemical and Mutational Analyses

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Background: Mutation of the *PDGFRA* gene has been well documented as an alternative oncogenic mechanism in a subset of gastrointestinal stromal tumors (GISTs) lacking *c-kit* mutations. However, the role of PDGFRA immunohistochemistry in the diagnosis of GISTs has not been well studied.

Design: We investigated PDGFRA immunoreactivity in GISTs and in other intraabdominal mesenchymal tumors, and correlated PDGFRA expression with CD117 positivity and with the mutational status of *PDGFRA* and *c-kit* genes. In addition, expression of pAKT, an activated downstream molecule in the PDGFRA and c-kit signaling pathways, was correlated with PDGFRA and CD117 status. A total of 39 GISTs and 20 other mesenchymal tumors in the abdomen were included in this study.

Results: Thirty-five of 39 GIST cases (89.7%) were positive for PDGFRA and 19 of these 35 positive cases were strongly positive. Five of 20 non-GIST lesions (25%) were positive for PDGFRA, but none of these cases were strongly positive. With one exception, PDGFRA positive cases were also positive for CD117. pAKT positivity was not associated with the immunoreactivity or mutation of *PDGFRA* and *c-kit,* suggesting that the activation of AKT is probably independent from the activation of PDGFRA and c-kit in GISTs. Of 14 GISTs assayed, 4 had mutations in *c-kit* at exons 11 or 17, and 4 had mutations in PDGFRA at exons 12 or 18. Three of 4 GIST cases with *PDGFRA* mutations show epithelioid morphology and strong PDGFRA immunoreactivity with prominent perinuclear dot-like accentuation (so-called Golgi pattern).

Conclusions: In conclusion, strong PDGFRA positivity with Golgi pattern is a useful adjunct in the diagnosis of GISTs with *PDGFRA* mutation.

530 "Juvenile" Polyps in Adults: A Clinicopathologic Study

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Background: Juvenile polyps (JPs), the most common colorectal polyp in children, are rarely reported in adults. The pathogenesis of JPs remains undefined, although nuclear beta catenin accumulation is common in JPs from children, and some cases of JPS are due to germline smad4 mutations. We compared JPs in adults to those in children, to determine whether such polyps in adults are histologically and clinically similar to those in children in both juvenile polyposis syndrome (JPS) and sporadic JP.

Design: Cases coded as JP were retrieved from the pathology archives from 1982-2005. All 29 adult cases were reviewed and available blocks (27) stained for beta catenin (BD Transduction Laboratories, 1:200 dilution) and smad4 (Santa Cruz Biotechnology, 1:50 dilution). Comparison groups consisted of all pediatric cases with multiple JPs (≥3 polyps) (34 cases, 29 with available blocks) and 27 random pediatric cases with single JPs. Medical records were reviewed for presenting symptoms, colonoscopic findings, and follow-up.

Results: Age range for adults was 23-75 years (mean, 44), compared to 4 years for sporadic JP and 6 years for multiple JP patients. Adult JPs were histologically similar to pediatric JPs, with a rounded polypoid configuration and abundant inflammatory stroma with dilated crypts. No polyp showed adenomatous change. All children with JPs presented with rectal bleeding. Adults with JPs were presented less often with overt rectal bleeding (10); 7 had heme + stool, 5 had abdominal pain, and 2 polyps were found on screening colonoscopy. 4 adult patients had malignancies (1 colon cancer). 3 adults with >20 polyps were judged to have JPS. 16 adults had single JPs. 8 children had 10 or more polyps and were considered JPS. Loss of smad4 was not seen in any case. Nuclear beta catenin staining was less common in JPs from adults and was usually limited to reactive-appearing crypts just beneath the ulcerated surface.

Nuclear Beta Catenin Staining in Juvenile Polyps

% + Epithelial Cells	Adults	Children with Single JPs	Children with Multiple JPs
0	12/27 (44%)	6/27 (22%)	10/29 (34%)
10-25%	7/27 (26%)	11/27 (41%)	6/29 (21%)
26-50%	5/27 (19%)	4/27 (14%)	9/29 (31%)
51-100%	2/27 (7%)	5/27 (19%)	5/29 (17%)

Conclusions: JPs histologically similar to those in children also occur in adults; most are probably sporadic, but adults may present with JPS and this diagnosis should be considered when multiple polyps are present. Lack of nuclear beta catenin in many JPs in adults suggests that translocation of beta catenin to the nucleus is not intrinsic to polyp formation in this setting.

531 Does an Intensive Pre-Resection Biopsy Protocol Improve Correlation with Resection Diagnosis in Patients with Superficial Esophageal Adenocarcinoma (SEA)?

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Background: Continued surveillance has been advocated by some for patients with Barrett's esophagus (BE)-related high-grade dysplasia (HGD). This approach assumes reliable distinction between HGD and intramucosal (IMC) or submucosal (SMC) carcinoma in biopsy specimens as well as assurance of adequate sampling. This study sought to examine if an intensive pre-resection biopsy protocol accurately predicts the level of invasion in SEA esophagectomy specimens.

Design: In patients with SEA lacking an endoscopically visible lesion, the immediate pre-resection biopsies were reviewed individually by seven GI pathologists and scored as HGD, IMC, or SMC. Based on endoscopy reports, the group was divided into those who had intense surveillance (IS) and those who did not (NIS). The IS group all had four quadrant jumbo biopsies taken every 1-2 cm of BE, and the NIS group did not. The mode consensus diagnosis was compared with the resection diagnosis.

Results: There were 29 and 21 patients in the IS and NIS groups, respectively. Age, gender, and length of BE did not significantly differ between the two groups. The IS group had 12/29 (41.4%) patients with discrepancies between pre-resection and resection diagnoses. Of these, 9 HGD cases were IMC on resection, 2 IMC cases were HGD on resection, and 1 IMC case was SMC on resection; therefore 10/29 (34.5%) cases had a more advanced lesion on resection than was seen in the pre-operative biopsy specimens. The NIS group had 9/21 (42.9%) patients with discrepancies. Of these, 5 HGD cases were IMC on resection, 2 IMC cases were SMC on resection, and 1 HGD case was SMC on resection. The two groups showed no significant differences in frequency of discrepancies (p=.57).

Conclusions: Despite intensive pre-operative biopsy sampling, a more advanced lesion is found in up to 34.5% of cases in resection specimens for BE-related SEA. This calls into question the advisability of continued surveillance for HGD without an endoscopically visible lesion.

532 Chasing Colonic "Polyps": Features that Predict Pathologic Lesions which Account for Endoscopically Apparent Polyps

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Background: Pathologists often encounter colonic mucosal biopsy specimens that carry a clinical diagnosis of "polyp, rule out adenoma". Initial tissue sections may fail to demonstrate a pathologic lesion to account for the clinical findings, but guidelines regarding the handling of these cases are lacking. Previous studies have shown that deeper sections and/or re-orientation will reveal a lesion in 10-30% of such cases, leading many authors to propose that all initially non-diagnostic biopsies be examined further. However, many polyps identified in these studies were clinically inconsequential; thus, the time and costs required to uncover them may not be justified. The aim of this study was to identify any clinical parameters that predict the likelihood of ultimately finding adenomas in initially non-diagnostic biopsies.

Design: We retrospectively identified 101 colonic mucosal biopsies from 94 patients (female/male ratio: 2/1, mean age: 58.9 years) submitted with the clinical impression of a "polyp", but no pathologic diagnosis on initial tissue sections. For each case, the original H&E stained slides were reviewed, the paraffin blocks melted, the tissues re-oriented, and 3 additional levels obtained. Information regarding patient age, gender, polyp size and site was obtained from the endoscopy and pathology reports.

Results: A new diagnosis was reached upon re-orientation in 21/101 (21%) cases including 11 (11%) hyperplastic polyps, 9 (9%) adenomas, and 1 (1%) inflammatory polyp. Only 3/75 (4%) polyps measuring ≤5 mm were ultimately diagnosed as adenomas, compared to 6/26 (23%) that were >5 mm in size (p=0.01). None of the clinically apparent polyps <3 mm (n=21) proved to be adenomas. A new diagnosis of adenoma was reached in 8/88 (9.1%) samples from the abdominal colon, whereas 1/13 (7.7%) lesion from the rectum was found to be an adenoma following reorientation (p>0.05). There was no association between patient age or gender and a new diagnosis of adenoma.

Conclusions: In this study, we found that re-orientation and additional tissue sections of initially non-diagnostic colonic biopsies resulted in a new diagnosis in 21% of cases when there was a clinically apparent polyp. However, most of these lesions (57%) were non-neoplastic and clinically inconsequential, particularly when small (≤5 mm). Thus, pathologists may consider re-orienting only those samples taken from polyps larger than 5 mm, since they are more likely to be adenomas than smaller "polyps".

533 Macrophage and Dendritic Cell Activation in the Rectal Mucosa in Acute Cholera, Shigellosis and *Campylobacter* Colitis

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Background: Mononuclear phagocytic cells are thought to play a key role in defense against bacterial pathogens, but little is known about their in vivo response to acute bacterial infections in humans.

Design: We studied the ultrastructural features of all mononuclear phagocytic cells in rectal mucosal biopsies from patients with acute cholera(n=10), shigellosis(n=6) and *Campylobacter* spp infection(n=5), and in controls(n=5) with no diarrhea or inflammatory disorders. The mean duration of illness at the time of biopsy was 1.4, 3.6 and 1.2 days for patients with cholera, shigellosis and *Campylobacter* infection. The chi square and Mann-Whitney U tests were used for comparison of data.

Results: Mononuclear phagocytic cells in the superficial mucosa affected by cholera and *Campylobacter* spp.showed an increase in nucleocytoplasmic ratio (p=.046), more prominent phagocytic activity (p=.0096), increased lysosomes (p=.0000) and more lipid bodies(p=.0184) than in controls. Macropinocytosis was seen in 15% of these

cells from patients with acute infections, but not controls. Mononuclear phagocytic cells in shigellosis showed an immature phenotype with less prominent organelles (p=.003), less phagocytic activity (p=.0003) and a lower prevalence of lipid bodies (.0001) than controls, irrespective of the duration of illness at the time of biopsy. Monocytes were also more frequent in shigellosis than in controls or patients with cholera or *Campylobacter* spp. infection (p=.0326, .0000 and .0003 respectively). Mononuclear phagocytic cells with convoluted nuclei, numerous dendritic processes and only mild phagocytic activity, suggestive of dendritic cells, were prominent in the mucosa of 2 of 5 patients with colitis due to *Campylobacter* spp.(41 and 45% of all mononuclear phagocytic cells), and occasionally present in 4 of 10 patients with cholera (4 to 22 % of cells).

Conclusions: This is the first in vivo ultrastructural study to show that mononuclear phagocytic cells in the rectal mucosa of patients with cholera and *Campylobacter* spp. infection appear activated. In shigellosis, the macrophage population appeared to be replaced by a less mature population. Prominent activation of dendritic cells in the rectal mucosa of some patients with acute *Campylobacter* colitis is a novel finding and suggests potent activation of the mucosal immune response. The presence of dendritic cells in the rectal mucosa of patients with cholera suggests that activation of the mucosal immune system may be more widepread than expected.

534 The Prognostic Role of RKIP and STAT3 in Gastric Adenocarcinomas; a Tissue Microarray Study

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Background: The Raf Kinase Inhibitory Protein (RKIP) is a member of the phosphatidylethanolamine-binding protein family and is a negative regulator of the MAPK cascade initiated by Raf-1. RKIP is also considered to play a pivotal role in cancer, regulating apoptosis induced by drugs, metastasis or immune-mediated stimuli. As opposed to RKIP, the signal transducer and activator of transcription 3 (STAT3) is a transcription factor that plays a critical role in cytokine and growth factor signaling and is frequently activated in human tumors, thus favoring tumor growth. Significantly, cytokine-mediated activation of Stat 3 in human tumor models results in 26S proteosome-dependent loss of RKIP expression. The purpose of our study was to determine the expression and prognostic significance of RKIP and STAT3 expression in gastric adenocarcinomas.

Design: Tissue microarrays were created from paraffinized samples from 136 patients with gastric adenocarcinomas, 60 intestinal subtype and 76 diffuse or mixed subtype. The microarrays were immunohistochemically stained for RKIP and STAT3 and the intensity of staining was semiquantitatively scored.

Results: Forty percent of the intestinal type tumors stained strongly for RKIP and 21% for STAT3. Twenty nine percent of the diffuse type tumors stained strongly for RKIP and 14% for STAT3. RKIP expression was inversely correlated with STAT3 expression in the intestinal type tumors (p=0.02, OR=0.19). RKIP expression correlated positively with patient survival whereas STAT3 was negatively associated with survival. Multivariate analysis revealed that RKIP, STAT3 and stage to be significant independent predictors of survival (p=0.031, p=0.037 and p=0.001, respectively) in the intestinal type tumors. In the diffuse type, stage was the only significant predictor of survival (p=0.004).

Conclusions: These findings suggest a protective role for RKIP expression and a deleterious role for STAT3 expression in patients with the intestinal type of gastric adenocarcinoma.

535 EGFR Expression in Advanced Colorectal Adenocarcinoma: A Study of 415 Cases

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Background: The expression of EGFR in tumors tends to correlate with poor prognosis and, in colorectal adenocarcinoma (CRC) is currently used as a predictor of response to Cetuximab, an anti EGFR monoclonal antibody that has shown to improve survival in patients with advanced CRC. However, absence of correlation between EGFR expression levels and response to Cetuximab in CRC, as well as the observation of response in patients apparently lacking EGFR expression, have hampered the role of EGFR testing.

Design: 424 either paraffin blocks or sections from 415 patients with advanced CRC were sent to our laboratory from 37 different institutions. 402 were surgical specimens and 22 biopsies. 366 samples were from primary tumors and 58 from metastases. In 9 patients, matched samples from primary tumours and metastases were received. 424 sections were immunostained with a standardized anti EGFR diagnostic kit. The intensity of membrane staining (0-3+) and the percentage of positive cells were assessed. The results were correlated with tumour grade, specimen type and primary vs. metastatic. Possible biases related to specimen handling were also investigated.

Results: 368 patients (88.6%) were EGFR + and 47 (11.3%) EGFR -. 1+ cells were found in 85.3%, 2+ cells in 62.6%, and 3+ cells in 31.3% of cases. The occurrence of 3+ cells correlated with higher grades (p=0.06). The incidence of negativity was similar in both resected (11%) and bioptic specimens (13.6%). However, the incidence of 3+ cells was significantly lower in biopsies (9% vs 32.5%; p=0.02). Negative cases were equally represented in both primary (10.8%) and metastatic tumours (13.8%). However only 5/9 primary/metastatic matched samples showed concordant EGFR expression. Both negative and 3+ cases were not equally distributed in the different institutions.

Conclusions: 1. EGFR 3+ cells in CRC correlate with higher grades. **2.** Specimen type does not affect the incidence of negative results. **3.** Lack of a consistent concordance in the matched primary/metastatic cases questions the adequateness of assessing EGFR status in primary tumors. **4.** The uneven distribution of both negative and EGFR 3+ cases from different institutions clearly points to the critical role played by pre-analytical conditions.

536 The Majority of Intraepithelial T Cells in Celiac Disease Are CD5 Negative: An Extensive Immunohistochemical Analysis

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Background: Celiac disease (CD) is an immune-mediated inflammatory disorder primarily affecting the small intestine, characterized histologically by villous blunting, crypt hyperplasia, and increased CD8+ intraepithelial lymphocytes (IEL). Patients with CD are at increased risk for enteropathy-type T cell lymphoma (ETL). In recent years, an extended panel of antibodies directed against T cell and NK cell antigens has become available for use in paraffin-embedded tissue sections. However, many of these markers have not been systematically evaluated in CD. The purpose of this study was to characterize the phenotype of IELs in CD using a broad panel of immunohistochemical markers.

Design: 10 duodenal biopsies from patients with serologically confirmed CD and typical histologic features (all Marsh type 3) were immunostained for CD3, CD20, CD4, CD8, CD5, CD7, CD2, CD25, CD30, TCRβF1, TIA-1, perforin, granzyme, CD56, and CD57. The extent of immunoreactivity was graded according to the percentage of positive IELs as follows: 0, no cells; 1, <5% of IELs; 2, 5-25%; 3, 26-50%; 4, 51-75%; and 5, 76-100%. The results were compared to 5 control biopsies of normal duodenal mucosa.

Results: In the CD biopsies, the mean number of IELs/100 epithelial cells was 73, compared to 22 in controls. All IELs in CD were CD3+, CD8+, and CD7+, and most were CD2+ (mean score 3.7), with variable expression of TIA-1 and perforin (mean scores 3.1 and 1.6, respectively). Interestingly, in all cases except one, <25% of IELs in CD were CD5+ (mean score 1.4), and a similar subset were immunoreactive for TCRβF1 (mean score 1.9). Granzyme+ IELs were seen in only 2 biopsies (mean score 0.4). IELs in CD were uniformly negative for CD4, CD25, CD30, CD56, and CD57. Similar results were observed in control biopsies, with the exception that no perforin expression was seen.

Conclusions: In normal duodenal mucosa and CD, IELs are CD3+, CD8+, and CD7+ T cells showing variable reactivity for the cytotoxic molecules TIA-1 and perforin. Expression of perforin seems to be increased in IELs in CD compared to normal mucosa. CD5+ T cells represent a small subset of IELs, which correlate with the fraction of cells reactive for TCRβF1. Thus, most IELs appear to be CD5-negative T cells of gamma-delta type. These findings have implications for the evaluation of IELs in refractory CD and early ETL. Specifically, since the majority of IELs are negative for CD5, lack of expression of this antigen cannot be used as an indicator of neoplastic progression in this context.

537 Impact of Neoadjuvant Therapy on the Lymph Nodes Status after Mesorectal Excision for Rectal Adenocarcinoma

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Background: The number and status of lymph nodes analyzed is crucial for optimal tumor staging and adjuvant treatment. The impact of preoperative radiotherapy on the number and status of lymph nodes retrieved in rectal specimen is still controversial

Design: The aim of this study was to define influence of preoperative treatment on the number of both lymph nodes retrieved and positive lymph nodes after rectal excision for cancer.

Results: From January 1994 to December 2004, 495 patients with rectal adenocarcinoma underwent rectal excision. Preoperative radiotherapy was given in 332 patients (248 with concomitant chemotherapy), whereas 163 patients were treated by surgery alone. Influence of clinical and pathological variables on nodal status (total number and positive lymph nodes) was assessed by univariate and multivariate analyses.The mean number of lymph nodes retrieved per specimen was 15. It was independently influenced by preoperative radiotherapy, tumor size, tumor parietal infiltration (pT) and type of surgery. The number of lymph nodes retrieved was lower after preoperative radiotherapy than after surgery alone (13 *vs* 17). The mean number of positive lymph nodes was 1.6. It was independently influenced by preoperative radiotherapy, tumor size, tumor parietal infiltration (pT), tumor differentiation and vascular invasion. The number of positive lymph nodes was lower after preoperative radiotherapy than after surgery alone (1.2 *vs.* 2.3). The dose of radiation influenced the number of lymph nodes retrieved.

Conclusions: Compared to surgery alone, preoperative radiotherapy decreased by 24% the number of lymph nodes retrieved and by 48% the number of positive lymph nodes after mesorectal excision. Further studies are necessary to assess the impact of the number of lymph nodes retrieved on survival in patients treated by preoperative radiotherapy for rectal cancer

538 Novel Indices of Spatial Distribution, Orientation and Complexity Applied to Barrett's Esophagus, for the Classification and Grading of Epithelial Dysplasia

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Background: The grading of dysplasia in Barrett's Esophagus (BE) provides the basis for treatment decisions. The current grading system incorporates nuclear features (i.e. pleomorphism, enlargement, and hyperchromasia) and spatial features (orientation and stratification). Yet, considerable interobserver variability in grading is encountered. The goal of this study was to try to objectively differentiate between the following BE related classes: negative for dysplasia (ND), low-grade dysplasia (LGD), or high-grade dysplasia (HGD) using computerized morphometry for analysis of nuclear size, shape, and spatial features.

Design: Nineteen cases of BE (ND, n=7; LGD, n=5; HGD, n=7) were collected. Seventy five representative images (ND, n=28; LGD, n=25; HGD, n=22) were obtained for each case and have been used as a training set for the multivariate predictive statistical model. For the computerized image analysis, a total of 6,325 nuclei from the superficial

epithelium were analyzed. Each nucleus was analyzed for size, shape, texture, pleomorphism, symmetry, self-similarity with neighbors and spatial distribution. Univariate and multivariate analyses were applied and predictive formulas were created. The predictive ability of these resulting formulas was validated on 30 new Barrett's esophagus cases with and without dysplasia.

Results: Computerized morphometry revealed the following results: For a differentiation between ND and LGD, a predictive formula using 2 spatial descriptors (stratification, orientation) and one textural index (margination), correctly classified 99% of the training and 96% of the testing cases. Low grade dysplasia was differentiated from HGD by a predictive formula using spatial (nuclear crowding), symmetry and self-similarity descriptors, correctly classifying 95% of the training and 86% of the testing cases. To differentiate ND from HGD, size, shape, and textural symmetry descriptors were used to correctly classify 96% of the training set of images.A similar accuracy was obtained for the testing set of images.

Conclusions: Quantitative evaluation of nuclear features through the use of computerized image analysis can be a valuable tool for predicting the grade of dysplasia in BE.

539 Colonic Mucosal Neurofibromatous Polyps: Clinicopathological Evaluation

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Background: Most primary mesenchymal spindle cell neoplasms of the gastrointestinal tract are submucosal and classified as stromal, smooth muscle, or autonomic nerve tumors, each with benign and malignant counterparts. Frequently submucosal tumors of neural origin arise from the peripheral nerve sheath, autonomic system, or gastrointestinal plexuses and are associated with systemic neurofibromatosis type I. We describe 13 cases of incidentally identified benign neural polyps located superficially in the colonic mucosa and *not* associated with neurofibromatosis, to which we give the moniker 'mucosal neurofibromatous polyp' or MNP. Such colonic mucosal neurofibromatous polyps have not been previously described.

Design: Unusual colonic polyps with spindled cells were identified in 13 patients: 8 women and 5 men (ages 27-67 years, mean 56.1). CD117, S-100, and smooth muscle actin immunostains were performed. Clinical findings were reviewed including indications for colonoscopy, presence of coexistent polyps, and systemic disease, especially neurofibromatosis. The endoscopic size, appearance, and location of the polyps were noted.

Results: MNPs show a polypoid growth of bland monomorphic spindled cells in the mucosal lamina propria with crypt displacement and no submucosal extension. Cells are arranged in tight fascicles without necrosis or mitoses and stain positive for S-100 and negative for CD117 and smooth muscle actin, reminiscent of neurofibroma. Some contain a few ganglion cells. All MNPs were small (0.1-1 .0 cm), non-bleeding, and found in the distal colon (1 rectosigmoid, 5 sigmoid, 1 descending, 1 ascending colon, 5 unspecified colon). There was no multiplicity and most patients had other concomitant colonic adenomatous and/or hyperplastic polyps (8/13). MNPs were not recognized endoscopically as distinct from adenomas. All 13 patients were asymptomatic, none had clinical evidence of systemic neurofibromatosis, and the MNPs were removed during colonoscopy for screening or surveillance.

Conclusions: We describe previously undocumented, incidental, small solitary colonic mucosal neurofibromatous polyps not associated with clinical neurofibromatosis. In contrast to other gastrointestinal mesenchymal tumors which are submucosal, MNPs occur in the mucosa, endoscopically mimicking adenoma-like epithelial growth. Often MNPs coexist with other adenomas and are sampled during screening or surveillance. The natural history of the MNP is unknown, but it is important to recognize this entity to avoid oversight as mucosal fold or misdiagnosis as stromal tumor or colonic neurofibromatosis.

540 Unexpected Morphologic Changes of Crohn's Disease in Asymptomatic Patients Undergoing Routine Screening Colonoscopy

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Background: Crohn's disease is typically diagnosed in symptomatic patients with abdominal pain, diarrhea, hematochezia, weight loss, anemia or perianal disease. In such setting, endoscopic and histological findings such as aphthous erosions, fissures, cobblestoned edematous mucosa, patchy active inflammation with cryptitis and occasional granulomas are considered diagnostic of Crohn's. We report rare occurrence of endoscopic and histologic findings consistent with Crohn's in asymptomatic patients undergoing routine screening colonoscopy for colon cancer risk. Such occurrence poses crucial challenges in diagnosis, management and surveillance.

Design: 3102 adult patients underwent cancer-screening colonoscopy in our institution from 2003 – mid 2005. In this cohort, 7 asymptomatic patients (ages 48-79, 3 men and 4 women) had unexpected ulcerated areas endoscopically, which were biopsied. 1/7 patients was re-scoped and re-biopsied per year for 2 years. Clinical features including family history of IBD and NSAID use, laboratory data such as hemoglobin and vitamin B12 levels, endoscopic reports and images and H&E stained biopsy sections were reviewed.

Results: All 7 patients were asymptomatic and none had family history of IBD. 3/7 patients used baby aspirin (81 mg) for cardiac prophylaxis. Hemoglobin and B12 levels, available in 4/7 patients, were normal. Endoscopically, the lesions were in terminal ileum (4/7), ascending colon (2/7) or ileum, cecum and ascending colon (1/7) and described as discrete, 3-4 cm friable solitary or multiple areas showing fissuring or aphthous ulceration reminiscent of Crohn's disease. Histologically, biopsies from these areas showed patchy chronic active inflammation with aphthous and fissuring ulcerations, cryptitis and crypt abscesses and no granuloma or dysplasia. In the patient with repeat colonoscopy, the changes were persistent and unchanged over two years.

Conclusions: In rare asymptomatic patients undergoing routine screening colonoscopy, endoscopic and histologic changes typical of Crohn's disease may be incidentally found (prevalence 0.2%). Such changes occur typically in the terminal ileum or proximal right colon and appear to persist without causing symptoms, functional abnormality or morphologic worsening or improvement over prolonged periods. Whether these patients need IBD-related follow-up and surveillance is unknown. The presence of these lesions underscores the familiar discordance between morphologic disease, function and symptoms.

541 Rectal Adenocarcinoma with Oncocytic Features: Possible Relationship to Pre-Operative Chemoradiotherapy

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Background: The introduction of pre-operative chemoradiation into the treatment protocol of rectal adenocarcinomas has impacted on the microscopic morphology seen in the subsequent resection specimens. The constellation of histopathological changes are varied and well documented. In this paper we wish to describe oncocytic change in rectal cancers that have been treated with chemoradiation prior to surgery.

Design: Cases of colorectal cancer with pre-operative chemoradiation were accessed. Clincal details and treatment information were noted.

Results: Eight of 55 rectal cancer cases were identified with a history of chemoradiation specifically directed to the rectal tumours in the form of fractionated 4500 to 5000cGy of radiation and 5-fluorouracil). Six of these 8 cases were composed of oncocytes which constituted 30-80% of the cancers. There were 4 males and 2 females ranging in age from 62 to 73 years, all with T3 N0 tumours. The intervals between chemoradiation and resection varied from 3 to 12 weeks. The tumor cells conformed to oncocytes morphologically (large size with abundant, granular eosinophilic cytoplasm, vesicular nuclei and prominent acidophilic nucleoli), immunohistochemically (positive for CEA, CK20 and CDX-2, but negative for both chromogranin and synaptophysin) and ultrastructurally (large cells demonstrating tight junctions and cytoplasmic engorgement by mitochondria and absence of neurosecretory granules).

Conclusions: These cells are different to the previously described endocrine cell change encountered in pre-treated rectal cancers. It is felt that oncocytic change in this particular clinical context occurs as a reflection of cytotoxic damage and/or cellular hypoxia induced by chemoradiation resulting in cell degeneration and the oncocytic phenotype. Oncocytic change may be an under-recognized histopathological change in rectal cancers receiving pre-operative chemoradiation.

542 Sessile Serrated Adenomas with High-Grade Dysplasia and Early Carcinomas; an Immunohistochemical Study of Serrated Lesions "Caught in the Act"

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Background: Sessile serrated adenomas (SSA) are lesions of the colon showing serrations typical of hyperplastic polyps, but often arising in the right colon, and displaying architectural differences. They differ from traditional serrated adenomas (SA) by lacking traditional dysplasia. They are thought to be precursors to colorectal cancers (CRC) arising through the microsatellite instability (MSI) pathway, predominantly via loss or promoter methylation of DNA mismatch repair genes. Loss of MLH1, a mismatch repair gene, has been demonstrated in such lesions from patients with sporadic MSI CRC, whereas germline loss of any of several mismatch repair genes is the hallmark of syndromic MSI CRC (Hereditary Non-Polyposis Colorectal Carcinoma/HNPCC). SSA with foci of high-grade dysplasia (HGD) or early invasive carcinoma are seldom biopsied and thus are not well-studied.

Design: SSA with HGD or early carcinomas were identified from the files of a large teaching hospital and consultation files. Immunohistochemical analysis for MLH1, MSH2, MSH6 and PMS2 (mismatch repair genes) was performed. Avidin/biotin blocking was performed prior to addition of secondary antibodies; detection was with a labeled streptavidin-biotin complex. The SSA and HGD/carcinoma components were assessed for nuclear staining, using normal colon for internal controls.

Results: Colon biopsies from 9 patients (age 54-87; M=4, F=5) showing SSA with HGD (N=5) or focal invasive carcinoma (N=4) were analyzed. Lesions were located in the ascending colon (3), transverse colon (1), descending colon (2) and rectosigmoid (3). All 9 cases showed nuclear staining for MSH2 and MSH6 in the SSA component, foci of HGD/carcinoma, and in background normal mucosa. In contrast there was tandem loss of MLH1 and PMS2 in both zones of HGD (3/5) and early carcinoma (3/4; with concordant loss in associated HGD) but retention in SSA areas (9) and normal mucosa (9). SSA areas showed reduced surface labeling, as did normal mucosa. One focus of traditional SA showed retention of all 4 markers. No patient was known to have HNPCC.

Conclusions: There is progressive loss of MLH1 and PMS2 as SSA enters the dysplasia-carcinoma sequence in a high percentage of tested cases (66.7%). As expected in sporadic lesions, these displayed retention of MLH2 and MLH6. This study offers additional strong evidence that SSA is truly a precursor to at least a subset of sporadic microsatellite unstable CRC.

543 Genomic Aberrations in Metaplastic Barrett's Esophagus (BE) from Patients with and without Carcinoma: Array-Based Comparative Genomic Hybridization (A-CGH) Study

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Background: Genomic aberrations occur in chronic inflammatory gastrointestinal disorders including BE. The association of genome-wide aberrations with carcinoma development in BE is unknown. We analyzed non-dysplastic mucosa from patients with long-standing uneventful BE and from those with concurrent carcinoma to assess

whether a distinct pattern of abnormalities may be associated with dysplasia development.
Design: We studied 22 non-dysplastic biopsy- or esophagectomy-derived BE samples from patients without evidence of dysplasia (ND; n=11; in surveillance for a minimum of 3 years) and with BE-associated adenocarcinoma (CA; at least intramucosal; n=11). Whole genomic DNA extracted from epithelial isolates from freshly frozen mucosa containing minimum of 60% goblet cells was labeled by random priming using Cy3 (sample DNA) and Cy5 (normal human reference DNA). Labeled DNA was co-hybridized to microarrays containing triplicate spots of approximately 19,000 genomic RPCI-11 bacterial artificial chromosome (BAC) clones (Roswell Park Cancer Institute, Buffalo, NY). The fluorescence ratios and their log-transformed values were analyzed using the fluorescence ratios and their log-transformed values were analyzed using DNAcopy as well as customized software.
Results: The patients included 17 men and 5 women ages 53-74 years (mean: 62 years). A spectrum of genomic abnormalities was detected among both groups of patients, with potential copy number aberration differences occurring at several chromosomal loci including: 1q25, 1q31, 2p25, 3q, 8p22, 8p23, 12p13, 17p11, 17p12, 18q11-18q12. Further reassessment of false discovery rate and verification of selected targets by fluorescence in situ hybridization is currently underway.
Conclusions: A spectrum of genomic changes can be seen in non-dysplastic intestinalized BE mucosa in both patients with and without carcinoma. Several recurrent alterations in potential "hot-spot" regions throughout the genome were seen in patients with concurrent carcinoma that did not occur in long-term BE patients without dysplasia in this pilot cohort of samples. Further study is needed to assess the utility of these changes as markers of BE-associated neoplasia.

544 Hyperplastic/Serrated Polyposis in Inflammatory Bowel Disease: A Case Series of a Previously Undescribed Entity

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Background: Patients with inflammatory bowel disease (IBD) are at increased risk for neoplasia. Herein, we describe the clinical, pathologic and immunohistochemical features of 3 unique patients with IBD, all of whom had numerous discrete colonic polyps with features similar to those described in the hyperplastic/serrated polyposis syndrome (HSPS).
Design: Routinely processed tissue sections (2 colon resection specimens, 1 endoscopic biopsies) from 3 patients with IBD [2 with ulcerative colitis (UC) and one with Crohn's Ileo-colitis (CC)] were evaluated for a variety of clinical (including follow up data and family history of polyposis) and histologic features including the type, location and number of polyps in the colon. Immunohistochemical staining for hMLH-1, hMSH-2, MGMT and p53 were performed on polyps and adjacent flat mucosa from each case.
Results: All 3 patients (2 males, 1 female, ages; 45, 47 and 51 years) had moderate pancolitis of more than 10 years duration. None of the patients had evidence of polyps in the upper GI tract. All patients had > 20 colonic polyps (1 had > 100). Pathologically, a combination of conventional hyperplastic polyps and sessile serrated polyps were present in all 3 cases. In addition, serrated adenomas were present in 2 and conventional adenomas in 1. 2 patients also had adenocarcinoma. The CC patient also had conventional (non-serrated) dysplasia. All 3 cases showed retention of hMLH-1 and hMSH-2, and a membranous beta-catenin staining pattern, in the polyps, flat mucosa and in the carcinoma, when present. However, 2 cases showed loss of MGMT in several hyperplastic/serrated polyps, 1 of which also showed loss in adjacent non-dysplastic epithelium. p53 was positive in 1 adenocarcinoma. Upon follow up (minimum 1 year), both patients with cancer had no evidence of recurrence or metastasis, and the other had no evidence of cancer on imaging studies.
Conclusions: Rarely, patients with IBD may develop multiple hyperplastic/serrated polyps similar to the HSPS. Loss of MGMT expression in some of these lesions is consistent with silencing by promoter methylation. The finding of MGMT loss in non-dysplastic epithelium in one case raises the possibility of a distinct pathogenesis in the setting of IBD. Additional studies are needed to determine whether these cases represent the simultaneous occurrence of HSPS and IBD, or whether the polyps developed as a direct result of the underlying IBD.

545 Goblet Cell Tumors of the Appendix: An Immunohistochemical and Outcome Analysis of 63 Cases

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Background: Appendiceal goblet cell tumors (GCT) represent a heterogeneous group of neoplasms with variable malignant potential. Unfortunately, morphologic categorization of these tumors has had limited success in predicting their behavior. The aim of this study was to evaluate the efficacy of immunophenotypic markers in predicting outcome in GCTs.
Design: Routinely processed tissue sections from 63 GCTs of the appendix (33M:30F, mean age: 55.2 yrs) were immunostained for (1) MUC antigens (MUC1, MUC2, MUC5AC, MUC6), (2) intercellular adhesion marker E-cadherin (3) markers of tumor suppresor genes (beta-catenin, p53, SMAD4), and (4) markers of cell proliferation and cell cycle regulation (Ki67, p16 and cyclin D1). The immunostains were graded and the results correlated with a variety of morphologic features and with outcome (alive with no disease (AND), alive with disease (AWD), dead of disease (DOD) and alive with unknown disease (AUD) status).
Results: 17.4%, 100%, 44.4% and 9.5% cases were positive for MUC1, MUC2, MUC5AC and MUC6, respectively. 12.6% of cases showed either focal or complete loss of membrane staining with E-cadherin. Nuclear beta-catenin accumulation was seen in 7.9% of GCTs; 22.2% showed nuclear positivity for p53 but loss of SMAD4 was not seen in any case. Ki67 showed a high proliferative index in 49.2% cases; cyclin D1 was positive in 26.9% and p16 in 7.9%. Most notably, increased MUC5AC (p=0.007) and MUC6 (p=0.05) staining, loss of E-cadherin (p=0.02), p53 positivity (p=0.006) and an increased Ki67 proliferation rate (p<0.001) correlated significantly with traditional morphologic features of potential aggressive behavior, such as lymphovascular invasion. In addition, p53 positivity (p=0.06) and increased Ki67 proliferation rate (p=0.06) showed a strong trend towards an association with poor outcome (AWD or DOD) in the 46 patients in whom outcome data was available.
Conclusions: Immunostaining with MUC5AC, MUC6, E-cadherin, p53 and Ki67 may be useful to predict aggressive behavior, and poor outcome, in patients with GCT of the appendix.

546 Predictive Value of Discrete Histologic Features for Risk of Cancer in Barrett's Esophagus

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Background: Currently, categorization of dysplasia into low- (LGD) or high-grade (HGD) is the most commonly used indicator of an increased risk of cancer (Ca) in Barrett's esophagus (BE). However, the discrete histologic features that have traditionally defined the LGD and HGD categories have never been tested. The aim of this study was to systematically evaluate the relative risk of Ca according to the presence or absence of discrete histologic features in a large prospective surveillance cohort of patients with BE that began in 1983.
Design: 3778 routinely processed biopsies from 151 BE patients (M/F ratio: 134/17, mean age: 62 years, mean length of BE: 6.5 cm, mean surveillance duration: 55.9 months), 63 of whom eventually developed Ca, were evaluated for more than 30 discrete pathologic features, such as villous change, crypt branching, and loss of cell polarity, in dysplastic epithelium, and the results were correlated with Ca outcome. The mean number of biopsies per patient was 25 and the mean number of crypts per biopsy was 24.7. Data were analyzed using a Cox regression model to account for follow-up intervals and censored data.
Results: The maximum diagnosis in the study cohort was metaplasia in 57, indefinite in 1, LGD in 29, HGD in 64. Ca subsequently developed in 63 patients. Among the many histologic features evaluated, villous change, crypt branching and crowding, marked loss of cell polarity and the presence of mitoses in the surface epithelium, showed a relative risk of Ca development of 5.5, 6.7, 5.1, 7.6 and 8.3 by univariate Cox regression model ($p < 0.0001$ for all variables). Other features, such as the presence of round/oval ("non-adenomatous") nuclei, as well as a variety of inflammatory features, showed a relatively low risk of Ca. Some features, such as villous change and crypt branching, or loss of polarity and surface mitoses, were found to be independent and unrelated predictors (p values < 0.001). None of the histologic features were detected exclusively in patients who ultimately developed Ca.
Conclusions: Our data indicates that evaluation of specific discrete histologic features may prove useful in helping to stratify BE patients into low- and high-risk groups, and may represent an alternative method of basing management decisions in high-risk BE patients.

547 Mismatch Repair Protein Status of Colorectal Small Cell Neuroendocrine Carcinomas

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Background: Small cell neuroendocrine carcinoma (SCNC) of the colorectum is a rare and highly aggressive malignancy. It can be associated with conventional-type adenocarcinoma, and an overlying adenoma can often be identified. A disproportionate number have been noted to arise in the right colon. Although some phenotypes (e.g., mucinous adenocarcinoma) have been shown to be associated with deficient mismatch repair (MMR) and thus micro-satellite instability (MSI), the MMR protein status of colorectal SCNCs has not been investigated. This study investigated the status of three MMR proteins, hMLH1, hMSH2, and hMSH6, in SCNCs of the colorectum.
Design: 14 SCNCs were identified based on the WHO histologic criteria for the diagnosis of pulmonary small cell carcinoma and immunohistochemical evidence of epithelial and neuroendocrine differentiation. Patient age and sex and tumor size and location were recorded. Immunohistochemistry was performed with antibodies the pancytokeratin (cocktail), CD56, neuron specific enolase, synaptophysin, chromogranin, hMLH1, hMSH2, and hMSH6. Staining with antibodies to MMR proteins was assessed for the presence of internal controls.
Results: Patients' ages ranged from 62-87 (mean age=75) and there were 8 men and 6 women. Tumors were located in the right colon (6), sigmoid colon (4) and rectum (3) (the location of 1 case was not recorded) and ranged in size from 4-15 cm in greatest dimension (mean=7.4 cm). All tumors showed immunoreactivity with antibodies to pancytokeratin and with antibodies to at least 1 neuroendocrine antigen. MMR proteins were intact by immunohistochemistry in all but a single case which had neither an identifiable precursor lesion nor positive internal control (hMLH1 loss).
Conclusions: Colorectal SCNCs are rare and are often right-sided. They are aggressive and tend to occur in older individuals. Most colorectal SCNCs have intact MMR proteins, suggesting that they develop secondary to chromosomal instability rather than MSI. Our single case showing potential MMR protein loss suggests that this phenotype may be independent of the developmental pathway (i.e., chomosomal instability vs MSI). This may explain how rare cases of SCNC have been identified in both patients with familial adenomatous polyposis and in patients with hereditary non-polyposis colon cancer.

548 How Many Plasma Cells Are Too Many for Gastric Antrum in Children?

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Background: Although gastric mucosal biopsies are common and encountered in everyday sign-outs, the number of inflammatory cells in the gastric mucosa is not well defined, especially in children. Previous reports of inflammatory cells in the stomach

lack information on differences noted among infants, toddlers, and adolescents. A preliminary survey at our pediatric tertiary institution showed that 30–50% of GI biopsies are reported with no abnormality. It is, therefore, likely that a substantial number of 'normal' children undergo endoscopic examination for GI symptoms, and that a study is warranted to help clarify and estimate normal values for endoscopically obtained material. We focused on the number of plasma cells in the gastric antrum.

Design: We reviewed 678 GI endoscopic examinations performed in 1/03-4/03. 141 'normal' cases (ages: 2wk–19y) were selected as the experimental cohort which met the following criteria: a) no previous history of GI diseases, b) normal endoscopic findings throughout the GI tract, and c) reported as no histologic abnormalities in all specimens submitted. Cells recognized as plasma cells on HE stained slides were counted in the entire biopsy area, excluding ambiguous cells. Counts were normalized for biopsy area (mean $1.16\pm0.07mm^2$) by image analysis of captured digital photomicrographs. Numerical values are expressed as mean cells/$mm^2\pm$95% confidence level. Cases of chronic gastritis with *H. pylori* infection and Crohn disease were similarly evaluated for reference.

Results: Gastric antrum plasma cells in *H. pylori* gastritis (463 ± 132) and Crohn disease (375 ± 179) are significantly greater than for aggregates of non-disease states (39.7 ± 6.1). With age stratification of normal patients, the lowest values are observed for infants less than one year (4.69 ± 2.06). Indeed, significant increases are also evident with advancing age with comparisons among groups <1yr, 1-2yrs (22.4 ± 6.82), and those ≥8yrs (55.4 ± 10.5).

Conclusions: The obtained values for presumed normal patients were distinct from the patients with two representative GI diseases. Moreover, age stratification among 'normals' yielded evidence for increased plasma cell density and gut surveillance occurring concomitantly with advancing age. It is hoped that with these data, guidance for the interpretation of plasma cells populations in pediatric gastric biopsies is possible.

549 Serrated Colorectal Polyps: Morphology and Expression of Mismatch Repair Gene Proteins and P504S

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Background: Serrated colon polyps (SP) are emerging as possible precursor lesions of colon cancer via the microsatellite instability (MSI) pathway; however, their morphological distinction from conventional hyperplastic polyps (HPP) is problematic. The aim of this study was to determine: 1. Whether expression of immunohistochemical markers of microsatellite instability (MSI) could help identify precursor SP. 2. Whether P504S (AMACR) can reliably differentiate SP from HPP. 3. Whether polyps coded as "serrated" in a general pathology practice are histologically different than a control group of HPP.

Design: 85 colon polyps accessioned between 1/1/2003 and 6/30/2005 and diagnosed as SP were identified. 15 HPP consecutively diagnosed in 6/2005 comprised the control group. The specimens came from 95 patients [M/F ratio: 0.8, mean age (M/F): 54.8/61.5] and were evaluated for size, location, basilar crypt serration (BS), >10% crypt dilation (CD), inverted crypts (IC), superficial mitotic figures (Mit), round/oval nuclei (RO), prominent nucleoli (Nuc) and cytoplasmic eosinophilia (Eos). Each specimen underwent immunostaining with antibodies to polyclonal P504S and the MSI proteins MLH1, MSH2, and MSH6. 6 patients had prior or subsequent colon carcinoma and the same four stains were applied to the tumors.

Results: Only 1/85 SP showed loss of expression of any MSI protein (loss of MLH1staining). No loss of expression was seen in any HPP.One of 6 invasive carcinomas showed loss of MLH1 expression; the SP from the same patient showed no MSI. P504S staining of varying intensity was seen in more SP (46/81, 4 cases not interpretable) than HPP (4/15) (p=.04). SP with the cellular features of Mit, RO, and Eos showed greater P504S expression.Only BS, CD, and RO were more frequent in SP than in HPP (41 vs 6.2%, 39 vs 0%, 68 vs 25%). SP were larger (mean 8.8mm) than HPP (mean 4.3mm) (p=0.001). More SP were right sided than HP, but this was not statistically significant.

Conclusions: BS, CD, and RO were the only histological features which were more frequently present in SP than HPP. SPP were larger than HPP. P504S stained SP more frequently than HP, especially in cases demonstrating Mit, RO, and Eos. Immunochemical markers for MSI are not useful in separating SP from HP.

550 Partial Acinar Cell Differentiation of Type-I Gastric Carcinoid Tumors Induced by Achlorhydria Secondary to Hypergastrinemia

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Background: Gastric carcinoid tumors (GCTs) have been classified by their association with (Types I, II) or without (Type III) hypergastrinemia ; the former can be further subclassified as achlorhydria (Type I) and hyperchlorhydria (Type II). Both Type I and Type II GCTs represent gastrin-dependent proliferations of histamine-secreting enterochromaffin-like (ECL) cells. The pathogenesis of Type III GCT is largely unknown.

Design: We evaluated 29 GCTs of all subtypes for pancreatic acinar differentiation using immunohistochemistry for trypsin, chymotrypsin, and lipase. The subtype of GCT was determined by the combination of clinicopathologic criteria and by chromogene in situ hybridization for histidine decarboxylase (CISH-HDC).

Results: The clinicopathologic features of GCTs are shown in Table 1. Trypsin immunoreactivity was present in 94% of Type I tumors. Immunoreactivity was variously present in 5-75% of tumor cells with moderate to strong staining intensity. The trypsin immunoreactivity was evident not only in tumor cells but also in hyperplastic endocrine cells in the neighboring mucosa. In contrast, 2 gastrin-producing G-cell tumors showed no reactivity for trypsin, nor did the 10 type III GCTs. No chymotrypsin or lipase immunoreactivity was identified in any GCTs. However, all three enzymes were expressed in foci of pancreatic acinar metaplasia in the atrophic gastric mucosa outside the carcinoids. The ECL cell nature of the tumors was confirmed by CISH-HDC. No pancreatic exocrine enzymes were expressed in carcinoid tumors of duodenum (3), ileum (6), or rectum (6).

Conclusions: Partial pancreatic acinar transformation of HDC-positive neuroendocrine cells is associated with hypergastrinemia secondary to achlorhydria. Non-ECL neuroendocrine tumors are not associated with this transformation. Thus, in addition to endocrine markers, trypsin immunoreactivity may be utilized to delineate the pathogenesis of GCTs.

Type of Carcinoid	# cases	Fundus / Antrum	Gastrin (pg/ml)	Trypsin IHC	Chymotrypsin IHC	Lipase IHC
Type I	18	18/0	1892±306	17/18	0/18	0/18
G-Cell Tumor	2	0/2	31000	0/2	0/2	0/2
Type III	9	8/1	128±51	0/9	0/9	0/9
Non-Gastric Carcinoids	20	N/A	N/A	0/20	0/20	0/20

551 Basaloid Squamous Carcinoma of the Esophagus – Pathological and Clinical Considerations

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Background: Basaloid squamous cell carcinoma (BSCC) of the luminal GI tract is rare. In the esophagus, it represents 5% of squamous cell carcinoma (SCC). Due to its histologic pattern and morphologic heterogeneity, BSCC may resemble high grade neuroendocrine carcinoma (HGNECA) or low grade adenoid cystic carcinoma (ACC). These issues are of concern regarding differential diagnosis in surgical and clinical management.

Design: The clinical characteristics of 8 cases of BSCC were compared with 57 cases of typical SCC and 12 cases of HGNECA of the esophagus. Cases of BSCC were further evaluated by immunohistochemistry.

Results: The clinical features and the prognosis of BSCC and typical SCC were comparable, and HGNECA had the worst clinical outcome among all three types of tumor (Table 1). Distal esophageal location was more common in HGNECA (92%) than BSCC (50%) and SCC (42%), respectively. In situ carcinoma was identified in most of BSCC (75%) and SCC (82%). An ACC-like histologic pattern occurred either exclusively (1/8) or was admixed with the basaloid pattern (2/8); cylinder-like space were present, but true lumina were not. Immunohistochemical studies demonstrated that the BSCCs exhibited more consistent positive immunoreactivity for p63 than any other markers investigated (Table 2). In addition, they were usually positive for high MW cytokeratin and negative for low MW keratin and CK20; CK7 immunoreactivity was variably present. In contrast to ACC, myoepithelial makers SMA and Calponin revealed rare and inconsistent immunoreactivity. The highly proliferative rate of BSCC was demonstrated by Ki67 staining; and endocrine markers were consistently negative.

Conclusions: BSCC represents a variant of SCC and should be clinically managed as such. It should be differentiated from low grade ACC of peri-esophageal glands or upper aerodigestive tract and high grade neuroendocrine carcinoma of the esophagus. An immunohistocfhemical panel is useful in the differential diagnosis.

Table 1. Features of BSCC, SCC, and HGNECA of the Esophagus

Tumor Type	# Cases	Upp Esoph	Mid Esoph	Dist Esoph	In situ Carcinoma	ACC-like Pattern	Survival (mean mons)
BSCC	8	2/8	2/8	4/8	6/8	3/8	34.2
SCC	57	7/57	26/57	24/57	47/57	N/A	32.3
HGNECA	12	0/12	1/12	11/12	4/8	N/A	14.4

Table 2. Immunoprofile of BSCC

	CK7	34BE12	SMA	Calponin	P63	Chrom/Synap	Ki67
Staining Distribution	5-80%	30-90%	0-20%	0-50%	90-95%	0-1%	60-80%
Staining Intensity (of 3+)	2-3+	2-3+	2+	1-2+	3+	2+	-

552 Identification of Histidine Decarboxylase (HDC) Positive Endocrine Cells in Subtypes of Gastric Carcinoid Tumors Using Highly Sensitive and Specific Chromogen In Situ Hybridization (CISH)

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Background: Both Type I and Type II gastric carcinoid tumors (GCTs) represent gastrin-dependent proliferations of histamine-secreting enterochromaffin-like (ECL) cells. Type III GCT is not specifically related to known endocrine cells. While the generic endocrine makers such as chromogranin and synaptophysin are useful in identifying carcinoid tumors in general, a more specific and sensitive detection of the ECL cell population would enable the elucidation of the cell type and the subclassification of GCTs.

Design: Two non-overlapping 48bp oligonucleotide probes were designed from a human HDC mRNA sequence. Both antisense and sense probes were labeled with digoxin via a chemical cross linker at the 3' end. Twenty GCTs were identified and characterized in the archived pathology material from 1982 to 2005. Formalin-fixed and paraffin-embedded tissue sections were prepared for HDC-CISH. The hybridized products were detected using a method designed for digoxigenin detection.

Results: HDC expression was identifiable by CISH in normal ECL cells in the fundic-type gastric mucosa; and its expression was correlated with the histologic pattern of ECL-cell hyperplasia and neoplasia. The HDC expression by CISH was specific as confirmed by detection of positive signals with anti-sense and negative results in the presence of unlabeled anti-sense probes or with sense probes. Despite the suboptimal nucleic acid preservation with formalin fixation and paraffin embedding, the HDC expression by CISH was detectable in specimens archived for up to 23 years. All 10 Type I GCTs, which were defined by clinical and pathologic criteria (i.e., hypergastrinemia secondary to atrophic gastritis and achlorhydria) demonstrated HDC expression. Two tumors of known G-cell origin were negative for HDC expression. Of particular interest was that one of eight (1/8) previously classified Type III GCTs exhibited positive HDC expression. This result suggests a gastrin-independent ECL cell neoplasm, which has not been previously recognized.

Conclusions: HDC expression by CISH is highly sensitive for the detection of a subtype of gastric endocrine cells, i.e., ECL cells. This technique is more specific in comparison

to detection by immunohistochemistry using generic endocrine markers. The development of HDC-CISH will provide an opportunity for the further investigation of pathogenesis of subtypes of gastric carcinoid tumors.

553 Histologic Assessment of Margins in Endoscopic Mucosal Resections for High-Grade Dysplasia

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Background: Endomucosal resection (EMR) is an alternative treatment for high-grade dysplasia (HGD) in selected patients. The efficacy of pathologic assessment of margins in EMR has not been studied.

Design: We studied 31 EMR specimens from 27 patients with HGD, 13 arising in Barrett esophagus (5 women, 7 men, 68 ± 11 years), and 18 arising at the esophagogastric junction or proximal stomach (3 women, 12 men, 69 ± 13 years). Three patients had photodynamic therapy (2 before EMR, and one between EMR and subsequent biopsy). Margins were assessed as positive or negative for high-grade dysplasia, or indeterminate.

Results: Follow-up consisted of esophagogastrectomy with or without further biopsy (9 patients); further biopsy only (15 patients); 3 were lost to follow-up. Margins were interpreted as positive (9 patients), negative (7 patients), and indeterminate (11 patients). Final diagnoses in patients with positive margins (mean follow-up, 9 months) were HGD (1), intramucosal carcinoma (IMCA, 2), invasive carcinoma (1), and negative for HGD (3); 2 were lost to follow up. Specimens were: esophagogastrectomy (4), and repeat biopsy (3). Final diagnoses in patients with negative margins (mean follow-up, 18 months) were HGD (3), IMCA (1), invasive carcinoma (1), negative for HGD (1), lost to follow-up (1); all specimens were biopsies without surgical resection. Final diagnoses in EMR with undetermined margins (mean follow-up, 18 months) were HGD (4), IMCA (2), invasive carcinoma (1), no residual HGD (4). Specimens were esophagogastrectomies (5), and repeat biopsies (6).

Conclusions: We conclude that although the margin status affected clinical decision, there was no apparent difference in the rate of residual HGD, intramucosal or invasive carcinoma in subsequent biopsy material in short-term follow-up. The use of margins in EMR needs to be more carefully assessed in order to determine if they are relevant in deciding when surgical treatment for HGD can be delayed.

554 Emergence of Eosinophilic (Allergic) Esophagitis: Increased Incidence or Increased Recognition?

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Background: Eosinophilic esophagitis (EE) has been recently described and there have been a number of reports that its incidence is on the rise. EE has distinct histologic features (prominent intraepithelial eosinophils (IEEs), particularly superficial with clustering) and characteristic endoscopic features ("trachealization"; white nodules or plaques). The presence of IEEs had been recognized since 1982 as indicative of reflux esophagitis (RE) but little attention was initially paid to their numbers or location. Recent publications have generally used a cutoff of more than 20 to 24 IEEs per high power field (hpf) for the diagnosis of EE. It had been our impression that EE was being seen more frequently, perhaps due to some environmental change (possibly the widespread use of proton pump inhibitors (PPI) in recent years).

Design: We analyzed a similar group of cases from 2005 (n=150) as compared to 1990 (n=115). Consecutive patients with mucosal esophageal biopsies from May through June of the respective years were included in the analysis. Patients with Barrett's metaplasia or with carcinoma were excluded. The highest density of IEEs for each patient was recorded as the number of IEEs per single hpf. The patients were categorized by the number of IEEs per hpf with those cases with greater than 20 representing EE.

Results: There was no difference in the incidence of EE between 1990 and 2005.

IEEs per hpf	0	1 to 9	10 to 19	>20
1990	58%	34%	1%	7%
2005	53%	37%	3%	7%

Conclusions: The apparent increased incidence of EE is apparently largely a result of an increase in recognition rather than an increase in incidence of the disease due to an environmental factor.

555 Duodenal Bulb Carcinoids: Association with H Pylori Gastritis and Long-Term Treatment with Proton Pump Inhibitors

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Background: Apart from genetic susceptibility, there is little knowledge about the possible etiological factors involved in the pathogenesis of duodenal carcinoids. The aim of this study was to evaluate the association between H. pylori gastritis and/or long-term use of proton pump inhibitors (PPI) and duodenal bulb carcinoids.

Design: A computer search was performed to identify all patients from a single institution who underwent endoscopic examination for upper GI symptoms in the last five years. Demographic information as well as presence/absence of gastritis, H. pylori infection, carcinoid tumor, and history of H2 blockers or PPI use were recorded. Statistical analyisis using Chi-square and Fisher exact tests were used to compare categorical variables. A logistic regression model with presence of carcinoids as the outcome variable was constructed using backward selection of relevant variables, with a p-value of 0.10 or less needed for inclusion in the model. Additionally, immunohistochemical staining for gastrin was performed on duodenum of all patients with carcinoid tumor, as well as 10 normal controls (no history of acid suppression therapy) and 10 patients with long-term PPI therapy. Morphometric analysis to assess G-cell density was performed and expressed as number of cells per square millimeter. The mean numbers of G cells for each group were compared using Wilcoxon rank sums.

Results: Of the 1454 patients studied, 11 patients had duodenal bulb carcinoid. Table 1 summarizes the characteristics of patients with and without duodenal carcinoids. The presence of H. pylori infection and use of PPI were significantly associated with the presence of carcinoids and remained significant in a logistic regression model adjusted for age, race/ethnicity, and sex, with p-values of 0.002 and 0.03, respectively. There was a significant difference in the mean number of G-cells between the normal control and patients with long-term PPI use (p=0.029).

Conclusions: The associations between H. pylori, PPI usage and duodenal carcinoids appear strong enough to warrant further investigation. The presence of G-cell hyperplasia in patients with carcinoids and long-term PPI use suggests that carcinoids may evolve from such proliferative changes.

Table 1

	H.pylori present	H. pylori absent	Rx with H2-B	No Rx with H2-B	Rx with PPI	No Rx with PPI
Normal duodenum	20.4%	79.6%	17.7%	82.3%	38.1%	61.9%
Carcinoid	63.6%	36.4%	18.2%	81.8%	80%	20%

Rx, treatment; H2-B, histamine receptor blocker

556 Sporadic Duodenal Gastrinomas: Clinicopathologic Profile of 18 Recently Diagnosed Cases

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Background: The incidence of gastrointestinal carcinoids is on the rise. While increasing use of endoscopy may be a major factor, it is speculated that changing diet and environmental exposure may account for a true rise in the incidence. Our aim was to study the clinicopathogic profile of a series of recently diagnosed sporadic duodenal gastrinomas, to evaluate if disease associations with etiopathologic significance could be identified.

Design: We retrospectively reviewed 18 cases of recently diagnosed sporadic duodenal gastrinomas, and compared their clinical and pathological features. Morphological examination to assess G-cell hyperplasia (diffuse, linear, micronodular) and morphometric analysis to assess G-cell density was performed on immunostained sections and expressed as number of cells per square millimeter. The mean numbers of G cells were compared with normal controls using Wilcoxon rank sums.

Results: 17/18 cases were incidentally recognized during evaluation for upper GI symptoms, one was identified at autopsy. 10 patients had H. pylori gastritis and history of long-term use of proton pump inhibitors (PPI) (no gastritis or PPI use was identified in 4 other patients; data was not available in the remaining 4 patients). All 18 tumors were located in the duodenal bulb {lamina propria (n = 4) or submucosa (n = 14)}, showed an insular morphology and ranged in size from 2-10 mm (mean: 5.4). No angioinvasion, mitotic activity (<1/10 hpf) or necrosis was identified in any case. Immunohistochemically, the tumors were positive for chromogranin, synaptophysin and gastrin. Two tumors additionally were positive for somatostatin. 15/18 (83%) showed focal diffuse or linear hyperplasia of G-cells in the adjacent mucosal crypts. By morphometric assessment, there was a significant difference in the mean number of G-cells between the normal control and patients with carcinoids. There was no evidence of metastasis in any case (by CT scan and Octreotide scan). The follow-up ranged from 4-60 months (mean: 21). There were no deaths due to disease; only one patient had local recurrence 37 months after EUS surgery.

Conclusions: High incidence (55%) of sporadic duodenal gastrinomas in patients with H.pylori gastritis and long-term use of PPI suggests an association that needs to be further explored. Presence of G-cell hyperplasia in the nontumorous crypts suggests that gastrinomas may originate from a proliferative phase, similar to the hyperplasia/neoplasia sequence seen in other endocrine neoplasms.

557 P16 and Ki67 Immunostains Decrease Interobserver Variability in the Diagnosis and Grading of Anal Intraepithelial Neoplasia (AIN)

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Background: Significant intra- and interobserver variation exists in the interpretation of anal biopsies for AIN that impacts patient management. P16 and Ki67 have been shown to be biomarkers for high grade AIN. In a recent study of anal biopsies, we found that a band-like pattern of p16 immunostaining and Ki67 positivity in >50% of the lesional cell nuclei were strongly associated with high grade AIN. This pilot study was designed to determine whether p16 and Ki67 immunostaining improves intra- and interobserver variability in the diagnosis and grading of AIN.

Design: H&E stained slides of 30 anal biopsies from individuals at risk for AIN were retrieved from the pathology files and reviewed by two pathologists. Diagnostic differences were resolved by discussion and/or review by a third pathologist and consensus diagnoses were achieved. The slides, designated 1 to 30, were diagnosed independently, and without knowledge of prior diagnoses, by three additional pathologists who routinely sign out anal biopsies. Diagnoses were recorded as: (a) negative; (b) low grade AIN (condyloma and/or AIN I); or (c) high grade AIN (AIN II and III). One month later, the cases were renumbered and the same 30 H&E stained slides were re-evaluated in conjunction with the corresponding p16 and Ki67 immunostains by the same three pathologists. The diagnoses were compared statistically for intra- and interobserver variability.

Results: After initial H&E examination, all three pathologists agreed on the diagnosis in 13 (43%) cases. With immunostains this number increased to 19 (63%) cases. Each pathologist changed a substantial percent (23%, 30%, and 50%, respectively) of the diagnoses after evaluation of the immunostains. These changes reflected increases as well as decreases in the severity of diagnoses rendered by each pathologist. Addition of the immunostains also increased the percent of cases in which all three pathologists

agreed with the consensus diagnosis from 33% to 60%. The multirater simple kappa coefficient improved from 0.427 (95% CI = 0.280 to 0.574) to 0.622 (95% CI = 0.474 to 0.770).

Conclusions: Our findings indicate that addition of p16 and Ki67 immunostains decreases variability in diagnosis and grading of AIN in a manner that may affect patient management. Thus, we propose that these immunostains be incorporated into the evaluation of anal biopsies for AIN.

558 P16 and Ki67 Immunoreactivity Is a Reliable Indicator of High Risk Human Papilloma Virus (HR HPV) in Anal Intraepithelial Neoplasia (AIN)

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Background: Since intra- and interobserver variability in interpretation of AIN can affect clinical management, patients would benefit if reliable biomarkers for HR HPV could be applied to the diagnosis and grading of AIN. We previously found that a band-like pattern of p16 immunostaining and Ki67 positivity in >50% of lesional cell nuclei were strongly associated with high grade AIN while spotty or negative p16 staining and Ki67 positivity in <50% of lesional nuclei was seen in most low grade AIN and benign biopsies. Integration of HR HPV DNA into the host cell genome is believed to be a requisite for high grade AIN. This preliminary study correlates p16 and Ki67 immunoreactivity with the presence of HR HPV as detected by in-situ hybridization (ISH).

Design: Slides of 23 anal biopsies were retrieved from our files and reviewed by three pathologists. Based on H&E, p16, and Ki67 stained slides, consensus diagnoses were: negative, 3 cases; low grade AIN (condyloma/AIN I), 11 cases; and high grade AIN (AIN II and III), 9 cases. Sections of the biopsies and appropriate controls were subjected to ISH utilizing the GenPoint High Risk HPV Biotinylated DNA Probe Cocktail and the GenPoint Tyramide Signal Amplification System (Dako Corp. Carpinteria, CA) as per the manufacturer's recommendations. ISH preparations were evaluated for HPV DNA without knowledge of the p16/Ki67 results and recorded as negative, positive for integrated DNA (punctate nuclear staining), or positive for episomal DNA (diffuse nuclear staining). ISH results were compared with consensus diagnoses and p16/Ki67 reactivity.

Results: Thirteen biopsies were negative for both p16 and Ki67 and all were negative for HR HPV DNA. These included 2 negatives, 8 low grade AINs, and 3 high grade AINs. Five cases were positive for both p16 and Ki67 and all were positive for HR HPV DNA. These included 3 high grade AINs all with integrated HPV and 2 low grade AINs with episomal HPV. Five cases were positive only for p16 or Ki67 and their HPV DNA results were variable (3 positive and episomal, 2 negative).

Conclusions: Our findings indicate that a band-like pattern of p16 immunostaining coupled with Ki67 positivity in >50% of lesional nuclei is a reliable marker for the presence of HR HPV DNA. Absence of both markers indicates absence of HR HPV DNA. Positivity of one with negativitiy of the other marker merits further evaluation of the HPV DNA status (negative vs. integrated vs. episomal) since this may be relevant to patient management.

559 Expression of Aspartyl (Asparaginyl) β-Hydroxylase in Colon Cancer and Its Prognostic Value in Stage II Colon Cancer

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Background: Overexpression of aspartyl β-hydroxylase (AAH) and its truncated homolog, Humbug, has been demonstrated in various tumors including cholangiocarcinoma, pancreatic, and hepatocellular carcinoma. In vivo and in vitro experiments demonstrated that AAH has an important role in regulating motility and invasiveness of neoplastic cells, and recent studies have shown that high levels of AAH expression correlate with poor prognosis of cholangiocarcinomas. The present study examines the expression of AAH and Humbug in colon cancer specimens to determine if these molecules could serve as biomarkers to predict behavior of TNM stage II colon cancer.

Design: 109 cases of TNM stage II colon carcinoma were identified in the pathology archives of the Rhode Island Hospital. Tissue microarrays (TMAs) were immunostained with the FB-50 monoclonal antibody that was generated to human recombinant AAH, but also recognizes Humbug. In addition, paired fresh frozen samples of colon adenocarcinoma and adjacent normal mucosa were analyzed to measure levels of AAH and Humbug mRNA expression by real time quantitative RT-PCR.

Results: FB-50 immunoreactivity was detected in 81% of the neoplasms whereas normal colonic mucosa was negative. FB-50 reactivity was localized to the cytoplasm and it had a distinct perinuclear distribution. The intensity of FB-50 expression was graded as high, low, or absent. 25% of the colon carcinomas had high-level, whereas 56% had low-level, and 19% had no detectable FB-50 immunoreactivity. Statistical analysis demonstrated that high-levels of FB-50 staining positively correlated with tumor grade ($P = 0.006$), and inversely correlated with survival ($P = 0.043$). Finally, the real time RT-PCR studies demonstrated up-regulation of Humbug but not AAH in 90% of colon carcinomas relative to adjacent normal mucosa (P<0.0001).

Conclusions: This study demonstrates that high levels of FB-50 immunoreactivity in colon carcinomas correlate with tumor grade and patient survival, suggesting that FB-50 can serve as a biomarker for colon cancer. In addition, the accompanying molecular studies demonstrated that the increased levels of FB-50 detected was due to Humbug overexpression. Future studies will determine if AAH overexpression in colon cancer is mediated by increased stabilization of the protein due to alterations in the phosphorylation state of AAH.

560 Loss of Heterozygosity of p16 Gene May Predict Response to Chemoradiation Therapy in Advanced Gastric Cancer

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Background: Tumor suppressor gene p16 plays an important role in the development of cancer. It has been found that patients with loss of heterozygosity (LOH) of p16 have a poor prognosis in lung cancer, brain astrocytic tumor, leukemia and adult soft tissue sarcoma. The alterations of p16 gene have been reported in the gastric cancer. However, the correlation of these alterations with patient survival is still unclear. Our present study investigates the predictive values of p16 LOH in patients with advanced gastric cancers.

Design: Advanced gastric cancers from 260 patients entered into a Southwest Oncology Group (SWOG S9008) protocol are studied. None of the patients received treatment (chemo- or radiation therapy) prior to the surgery, and all were randomized to either observation or post-surgical chemoradiation. Tumor and matched normal tissue from each patient were microdissected from H&E stained slides. DNA was isolated from the microdissected tissues. Miscrosatellite marker D9S171 for p16 was amplified by PCR and LOH analyzed by ABI310 automatic sequencer.

Results: Thirty of p16 LOH (13.6%) and 43 MSI (19.5%) cases were identified among 220 informative cases. In the observation arm, the median survival for LOH, MSI and normal was 38, 14 and 27 months respectively, while in the treated arm the medians were 20, 80 and 38 months respectively. A test of interaction between p16 alterations and patient treatment was significant (p=.04) suggesting that the patterns of survival between p16 alterations differed by treatment group. Patients with p16 LOH fared worse on treatment than those without (p=.02). Although these same patients appeared to have higher median survival in the observation arm, these differences were significant. This may be due to relatively small sample sizes in the treatment subsets.

Conclusions: This study suggests that p16 LOH may serve as a potential marker in the evaluation of patient prognosis and chemoradiation therapy in the future. Validation studies are needed to further assess these results.

561 Prevalence and Clinicopathologic Features of Colorectal High-Grade Neuroendocrine Carcinomas Associated with Overlying Adenoma

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Background: High-grade (poorly differentiated) neuroendocrine carcinomas (NECs) of the colorectum are characterized by their aggressive behavior, high propensity for distant metastasis, and poor response to conventional surgical and chemotherapeutic treatment modality. Reportedly, a subset of high-grade NECs arises from an overlying adenoma, but their prevalence and clinicopathological features remain unclear.

Design: Sixty-six consecutive cases of high-grade NEC of the colorectum diagnosed between 1985 and 2005 were included. Low-grade neuroendocrine tumors/carcinomas were excluded. There were 26 cases with biopsy specimens and 40 with resection specimens. Histological sections were reviewed to determine the presence or absence of a coexisting adenoma. Neuroendocrine differentiation was confirmed by immunohistochemistry for chromagranin or synaptophysin. The clinicopathological features of NECs associated with or without an overlying adenoma were then compared.

Results: All 66 cases had high-grade NEC component that had immunoreactivity to one or both neuroendocrine markers (30/66 to synaptophsyin alone, 27/66 to both synaptophysin and chromagranin, and 9/66 to chromagranin alone). In 8 cases, a distinct gland-forming adenocarcinoma component was also present. Tubular or tubulovillous adenoma was present in 26% (17/66) of high-grade NECs. An adenocarcinoma was more frequent in high-grade NECs with an adenoma (29%, 5/17) than in NECs without an adenoma (6%, 3/49, p=0.02). In contrast, high-grade NECs without an adenoma were associated with more advanced tumor stage (96%, 24/25, of resected specimens had tumor invasion into pericolic adipose tissue) and distant metastasis (90%, 35/39) as compared to NECs with an adenoma (45%, 5/11, p<0.001; and 65%, 11/17, p=0.03, respectively). There were no differences in presence of lymphovascular invasion (82%, 9/11, in NECs with an adenoma and 93%, 27/29, in NECs without an adenoma) or lymph node metastasis (75%, 6/8, in NECs with an adenoma and 85%, 22/26, in NECs without an adenoma).

Conclusions: A subset of colorectal high-grade NECs is associated with an overlying adenoma. Neuroendocrine carcinomas with and without adenoma may represent distinct subsets of NECs with different clinicopathologic features.

562 Characterization of T Cells in Colorectal Carcinomas

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Background: The presence of a prominent intratumoral lymphocytic reaction is a characteristic feature of colorectal carcinomas arising via the microsatellite instability (MSI) pathway. Other carcinomas, such as breast medullary carcinoma, breast infiltrating ductal carcinoma, and undifferentiated nasopharyngeal carcinoma, are also associated with prominent lymphocytic infiltrates, yet the immune response does not seem to be beneficial in reducing tumor load. The accumulation of CD4+, CD25+ regulatory T cells in murine fibrosarcomas suppresses proliferation of CD8+ T cells and depletion of the CD4+ cells by use of a monoclonal antibody results in an effective CD8+ immune response and elimination of these tumors (Yu *et al*., *J. Exp. Med.,* 2005). Regulatory CD4+, CD25+ T cells may play a role in colorectal carcinomas as well. Since regulatory T cells are important in suppressing the anti-tumor effect of CD8+ cells, we hypothesize that there may be a reduced number of these regulatory T cells in MSI colorectal carcinomas. This may explain the better prognosis reported for MSI compared to microsatelite stable (MSS) tumors (Gryfe *et al*., *NEJM*, 2000).

Design: We selected 17 invasive colorectal carcinoma cases with prominent lymphocytic infiltrates. Using paraffin embedded tissue, we stained for FoxP3 (transcription factor for regulatory T cells), CD4, and CD8. 8 of the 17 cases showed MSI either by microsatellite analysis or via negative staining for MSH2 and MLH1. T cells were counted manually and on scanned digital images using an automated cell imaging system (ACIS, Clarient).
Results: We observed a significantly greater number of CD8+ T cells in the MSI (76/HPF) *vs*. the MSS tumors (25/HPF). The ratio of CD4+ to CD8+ T cells was lower in MSI tumors using cell counts (CD4/CD8=1.0, SD=3.8 *vs*. CD4/CD8=3.4, SD=3.4; p=0.07) and via computerized pixel counts of digitized slides (CD4/CD8=2.1, SD=2.1 *vs*. CD4/CD8=4.3, SD=3.5; p=0.14). There was no difference in the number of regulatory T cells between MSI and MSS tumors, nor was there a negative correlation between the percent CD8+ T cells and the percent regulatory T cells.
Conclusions: An increase in the number of CD8+ T cells occurs in MSI versus MSS colorectal carcinomas. These CD8+ T cells, which are known to have an anti-tumor effect in some tumor types, may explain the better prognosis of MSI versus MSS tumors.

563 A Quantitative Study of the Lymphocytic Response in Esophageal Biopsies: Gastroesophageal Reflux Disease Versus Eosinophilic Esophagitis

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Background: Gastroesophageal reflux disease (GERD) and eosinophilic esophagitis (EE) often show similar findings on esophageal biopsy. Intraepithelial eosinophil count in esophageal biopsies is the most frequently used histologic feature to separate GERD from EE, with sometimes imperfect disease stratification. To test the hypothesis that intraepithelial T-cell counts may further help separate GERD from EE, we evaluated H&E stained and CD3 immunostained esophageal biopsies from normal (NL), GERD and EE patients. Two unique subsets were also analyzed: patients with normal histology but clinical response to GERD therapy (NL → GERD), and patients with EE by eosinophil count, but clinical response to GERD therapy (EE → GERD).
Design: H&E stained and CD3 immunostained slides from 102 patients were reviewed. Classification of cases was based on medical record review and therapy response. CD3+ T-cells per 5 consecutive 400x high power fields (hpf) and the maximum number of eosinophils per 400x hpf were counted for each case, and the means calculated. The ratio of mean T-cell to the mean maximum eosinophil count was then compared among the cases of GERD, EE, and EE → GERD and analyzed via the General Linear Model (ANOVA).
Results: The mean number of T-cells was increased in cases of EE vs. both NL and GERD (p = <.0001 for both). Similarly, the mean maximum number of eosinophils was increased in EE vs. GERD. Therefore, a higher ratio of T-cells to eosinophils existed for GERD cases (33.3) compared to EE cases (6.64) (p = <.0001). EE → GERD cases showed a ratio of T-cells to eosinophils (6.64) similar to true EE cases (6.99) (p = 0.99).

Case Type	Mean CD3+ T-cells/5 hpf	Mean Max. Eosinophils /hpf	Ratio (CD3+ T-cells : Eos)
NL (n=26)	46.6	0	
GERD (n=32)	99	2.97	33.3
EE (n=27)	175.1	26.37	6.64
Nl → GERD (n=8)	32.8	0	
EE → GERD (n=9)	178.3	25.5	6.99

Conclusions: The quantity of CD3+ T-cell infiltration of the esophageal mucosa is significantly different between EE and GERD, with an even more striking difference in the ratio of CD3 +T-cells to eosinophils between the two diseases. Therefore, in addition to the traditional intraepithelial eosinophil count, the quantity of intraepithelial CD3 + T-cells may help further distinguish EE from GERD, with only occasional overlap in the CD3+ T-cell to eosinophil ratio (9 EE → GERD cases). Cases with histologic and clinical overlap may represent simultaneous disease.

564 Differential Expression of HLA-G in Crohn's Disease and Ulcerative Colitis

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Background: HLA-G is a non-classical major histocompatibility (class 1b) molecule characterized by its restricted tissue distribution and limited polymorphism. The function of HLA-G is best established in placental intermediate trophoblasts, where it plays a part in inhibition of the maternal anti-fetal immune response. The scope of HLA-G's role in mediating immune-responsiveness is expanding, having recently been implicated in various tumors and immunologically-mediated disorders. Due to the disregulation of immune system activity in inflammatory bowel disease (IBD) we have chosen to compare HLA-G expression in patients with Crohn's disease (CD) and ulcerative colitis (UC).
Design: Sections from areas of active colonic disease from formalin fixed, paraffin embedded resection specimens of 19 UC patients and 20 CD patients were utilized for immunohistologic (IHC) studies. A 1:500 dilution of monoclonal mouse HLA-G antibody (clone 4H84, *Pharmingen*), which recognizes all 7 isoforms (membrane bound and soluble), was applied for one hour following antigen unmasking in citrate buffer. Granular cytoplasmic and membranous reactivity in intermediate trophoblasts in a section of placenta was used as the positive control. Reactivity in colonic sections was scored as 0 (negative), 1 (focal positivity) or 2 (positive).
Results: Immunoreactivity in the colonic sections was limited to surface and crypt epithelial cells (cytoplasmic and membraneous) and was usually quite strong. In 8 of 20 positive cases reactivity was focal. Sections from CD patients were significantly more likely to exhibit HLA-G reactivity than UC patients (15/20, 75% vs. 5/19, 26.3% respectively; Fisher's exact test p = 0.004). A significant association between HLA-G expression and IBD diagnosis remained when considering focal positivity as a value between a negative and positive result (Wilcoxon rank-sum, p = 0.0006). In patients immunoreactive for HLA-G there was a 10.45 fold increase in the odds of having CD, while patients without reactivity had a 2.95 increased odds of having UC.
Conclusions: Expression of HLA-G by IHC is significantly more common in Crohn's disease patients than in those with ulcerative colitis. Given the role of HLA-G in the regulation of IL-10 and its importance in local immune tolerance, differential expression in IBD suggests a pathophysiologic role for this molecule. This possibility is now being explored via population based genetic linkage studies of HLA-G polymorphisms in IBD cohorts.

565 Can GEWF Solution Improve the Retrieval of Lymph Nodes from Colorectal Cancer Resections?

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Background: Lymph node (LN) status is an important prognostic factor in colorectal cancer (CRC) staging. An inaccurate pathologic examination may miss positive LNs and under stage the patients, therefore, depriving them from possible adjuvant chemotherapy. The National Cancer Institute and College of American Pathologists have suggested that a minimum of 12 LNs need to be examined for accurate staging of CRC specimens. A number of adjunctive methods have been developed to increase LN yield including alcohol treatment, clearing solution, and more recently revealing solution such as GEWF (glacial acetic acid, ethanol, distilled water, and formaldehyde). The purpose of this study was to evaluate whether GEWF solution increases LN retrieval.
Design: A prospective study was conducted on 85 CRC specimens between July 2004 and May 2005. LN dissection was carried out on 40 of these specimens using the standard method (serial sectioning, inspection and palpation), while 45 cases were evaluated using GEWF solution (which highlights LNs as firm, white chalky nodules). An intense educational effort regarding guidelines for minimum LN retrieval was emphasized to the residents grossing these specimens, just before the beginning of the current study. The number of LNs recovered from the prospective study was then compared to that of 63 CRC specimens examined in the previous year (July 2003 to June 2004), using the standard method, but before the educational effort. Independence samples T-test was used to analyze the data.
Results: The mean number of retrieved LNs using GEWF was slightly higher than the standard method (19.96 vs. 18.3), however, there was no statistical significance (p = 0.53). The mean number of LNs recovered in the current study, however, was significantly higher than that of the previous year (19.17 vs. 11.51, p < 0.001)
Conclusions: GEWF solution did not significantly increase the yield of LN retrieval from CRC specimens. Education regarding guidelines for minimal number of LNs required for adequate CRC specimens, however, was found to be a significant factor in increasing LN retrieval.

566 Excellent Interobserver Agreement on Grading the Extent of Residual Carcinoma Following Preoperative Chemoradiation in Esophageal Carcinoma: A Reliable Predictor for Patient Outcome

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Background: Previous studies by our group have demonstrated that following preoperative chemoradiation the extent of residual carcinoma in resected esophageal cancer specimens is an independent predictor of survival according to 3 groups: P0 (0% residual carcinoma), P1 (1-50% residual carcinoma) and P2 (>50% residual carcinoma). We, therefore, assessed the interobserver variation of this classification among pathologists from different institutions to determine the reproducibility of this grading system.
Design: Histologic H&E slides of 60 coded cases of esophageal adenocarcinomas treated with pre-operative chemoradiation followed by esophagectomy were independently reviewed by 6 gastrointestinal pathologists from 4 different institutions for extent of residual carcinoma and ypT stage. Interobserver agreement was analyzed by kappa (k) statistics, and the grading of the extent of residual carcinoma and ypT stage based on individual and consensus gradings were correlated with patients' survival.
Results: The interobserver agreement was excellent for extent of residual carcinoma (k 0.84 $\pm$ 0.02, Kendall's W 0.92; p<0.000001) and was good for ypT stage (k 0.71 $\pm$ 0.02, Kendall's W 0.88; p<0.000001). Agreement was excellent for all categories of residual carcinoma: P0 (k 0.87 $\pm$ 0.03); P1 (k 0.81 $\pm$ 0.03), and P2 (k 0.85 $\pm$ 0.03). Extent of residual carcinoma was a significant predictor for overall survival based on grading by each individual pathologist (all p values <0.03). Extent of residual carcinoma based on consensus grading (13 with P0, 32 with P1, and 16 with P2) was a significant predictor in univariate analysis (p=0.004), and was independent of ypT and ypN stages in multivariate analysis (p=0.02). In contrast, ypT and ypN stage were not significant predictors for overall survival.
Conclusions: Our results indicate that in esophageal cancer following preoperative chemoradiation there is excellent interobserver agreement among pathologists from different institutions on grading the extent of residual carcinoma. The extent of residual carcinoma is a reliable and reproducible predictor of survival; this grading system may allow a novel means to compare outcomes in different neoadjuvant treatment regimens.

567 Assessment of BRAF and MSI Status in Microdissected Combined Serrated Adenomas and Hyperplastic Polyps and in Carcinomas with Residual Serrated Adenoma Indicate MSI Is a Late Occurrence in the Serrated Polyp Neoplasia Pathway

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Background: MSI-High carcinomas represent up to 15% of sporadic colorectal cancers and frequently exhibit a BRAF mutation, a potential marker of the serrated polyp neoplasia pathway in the colorectum. The aim of the present study was to test the hypothesis that among serrated adenomas and carcinomas, while BRAF mutations were present in precursor lesions, MSI occurred late in this pathway.

Design: Eight (8) cases of BRAF mutated serrated adenomas with residual/contiguous hyperplastic polyp (HPSA) and 7 BRAF mutated colorectal carcinomas with residual serrated adenoma (SACA) were selected from a tissue bank that included 32 SAs and 71 colorectal carcinomas with residual adenoma. Laser capture microdissection using the Arcturus PixCell IIe system was used to harvest DNA samples from the respective HP, SA and CA components of the combined lesions. AS-PCR was used to detect BRAF codon 600 mutation. MSI PCR was performed with fluorescent labeled primers for BAT25 and BAT26 and capillary electrophoresis was performed using an ABI Genetic Analyzer. MSI status was classified as follows - both markers negative: MSS; one marker positive: MSI-Low; both markers positive: MSI-High.

Results: Wild type BRAF was found in 6/6 samples of contiguous normal mucosa. BRAF mutations were present in both the HP and SA components of 8/8 HPSAs and both the SA and CA component of 7/7 SACAs. HP and SA components in 8/8 HPSAs were MSS. Among SACA 6/7 carcinomas (85.7%) were MSI-H and 1/7 MSS; 2/7 (28.6%) of contiguous SAs were MSI-H (concordance with CA 2/2); 2/7 (28.6%) were MSI-L (concordance with CA 0/2) and 3/7 (42.8%) were MSS (concordance with CA 1/3).

Conclusions: In BRAF mutated HPSAs and SACAs this mutation is invariably present in both the precursor and more advanced components, consistent with a role early in the serrated polyp neoplasia pathway. By contrast, our findings suggest that MSI status occurs late in the serrated polyp neoplasia pathway at the transition of SA to CA and appears unlikely to play a role in the earlier transition of HP to SA.

568 Molecular Analysis of "Filiform" Serrated Adenomas: High-Level Microsatellite Instability Does Not Play a Role in the Pathogenesis of These Distinct Neoplasms

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Background: We recently described an unusual type of adenomatous polyp, termed "filiform serrated adenoma" (FSA), that shares some morphologic and immunohistochemical features with serrated adenoma (SA), but is characterized by prominent, thin, elongated "filiform" projections of neoplastic epithelium with a serrated contour. We reported that these polyps demonstrated preservation of hMLH1 and hMSH2, and variable abnormalities in MGMT, p53, and B-catenin, similar to SA. The aim of this study was evaluate the molecular features of FSAs and specifically, microsatellite instability (MSI), in an effort to gain insight into their pathogenesis.

Design: Adenoma samples with at least 50% neoplastic cellularity were microdissected from routinely processed polypectomy specimens obtained from 14 patients (male/female ratio: 4/10, mean age 61.5 years) with FSAs. DNA was extracted, amplified by PCR, and analyzed at ten microsatellite loci (BAT26, BAT25, BAT40, BAT34C4, D5S346, D10S197, D17S250, D18S55, ACTC and MYCL). MSI status was determined using previously defined criteria. Five cases also contained sufficient non-neoplastic tissue for dissection and DNA extraction, allowing analysis of loss of heterozygosity (LOH) at informative loci.

Results: All of the polyps were located in the left colon and had a mean size of 1.6 cm. PCR amplification and microsatellite analysis was successful in 12/14 cases. None (0%) showed a high level of MSI (MSI-H), 5 (42%) showed a low level of MSI (MSI-L), and 7/12 (58%) were microsatellite stable (MSS). Four of 5 cases (80%) revealed LOH at the loci evaluated, including one at D5S346 (APC gene), one at D17S250 (p53 gene), and two at MYCL (chromosome 1p34). FSAs with LOH were both MSS and MSI-L (2 cases each).

Conclusions: "Filiform" serrated adenomas may represent a distinct clinicopathologic subtype of adenoma that occurs exclusively in the left colon. None of these polyps are MSI-H, supporting our previously reported immunohistochemical results, and providing further evidence that mismatch repair deficiency does not play a role in the development of these lesions. The presence of LOH indicates that most of these polyps likely develop as a result of underlying chromosomal instability. Future studies evaluating the role of DNA methylation in the evolution of "filiform" serrated adenomas are necessary to better define their relationship to serrated adenomas.

569 Reinterpretation of High-Grade Dysplasia in Barrett's Esophagus: A Multicenter International Phase III Trial in 485 Patients

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Background: A significant tendency exists among pathologists to over diagnose high-grade dysplasia (HGD) in Barrett's esophagus. The magnitude of this problem has not been evaluated. Given the serious clinical implications, we sought to examine its prevalence in patients being screened for a therapeutic trial of Barrett's with high-grade dysplasia.

Design: Esophageal biopsies from 485 patients carrying a local institution diagnosis of Barrett's esophagus with high-grade dsyplasia were evaluated by three study pathologists as part of a phase III multicenter international trial. Patients were enrolled from both academic and private centers at 25 study sites from throughout the USA, 2 sites from Canada, 2 sites from the UK, and 1 site from France. The original diagnostic slides were reviewed and a repeat protocol endoscopy was performed on each patient by a study gastroenterologist, obtaining 4-quadrant jumbo biopsies every 2-cm throughout the Barrett's segment and additional biopsies of any visible lesion, for an average of 20 biopsies per patient.

Results: Of the 485 screened patients, only 248 (51%) had confirmed high-grade dysplasia. In 193 patients (40%), high-grade dysplasia was believed to have been overcalled. The remaining patients had Barrett's adenocarcinoma (n=43; 9%) or another condition (n=1; <1%). Inter-rater agreement per endoscopy for high-grade dysplasia, carcinoma and Barrett's esophagus was 88%, 96% and 99%, respectively. Intra-rater agreement for high-grade dysplasia was 94% (95% CI 86,97) with a kappa statistic of 0.85 (near perfect)

Reinterpretation of Biopsies in 193 Screened Patients

Reinterpretation	No. of Patients	Percentage
Gastric only	18	9%
Barrett's esophagus, negative for dysplasia	35	18%
Barrett's esophagus, indefinite for dysplasia	61	32%
Barrett's esophagus, low-grade dysplasia	79	41%

Conclusions: These results document that over diagnosis of high-grade dysplasia in Barrett's esophagus is prevalent, identified in 40% of studied patients. In order to avoid unnecessary esophagectomy with its high morbidity and mortality, these data re-enforce the advisability of diagnostic confirmation by GI pathologists who see a high and continuous volume of Barrett's biopsy material.

570 Are All Esophageal Diverticula Secondary to an Underlying Esophageal Motility Disorder (EMD)? The Utility of Esophageal Muscle Biopsy in the Evaluation of Patients with Esophageal Diverticula

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Background: Esophageal epiphrenic diverticula are false diverticula of the distal esophagus and are the result of abnormal intramural pressure applied to an area of weakness of the esophageal musculature. These diverticula have been associated with a variety of EMD. For the past several years at our institution, esophageal muscle biopsies have been procured at the time of diverticulectomy in order to more thoroughly evaluate morphologic changes within the myenteric plexus, since myenteric plexus alterations can be identified in some EMD.

Design: From a large institutional esophageal surgery database, we selected cases that had all of the following: 1) esophageal diverticulum specimen; 2) esophageal muscle biopsy specimen procured from the opposite esophageal wall at the time of diverticulectomy, and 3) clinical diagnosis, typically utilizing endoscopy, barium esophagogram and manometry as part of a thorough evaluation for an EMD. For each esophageal muscle biopsy specimen, we recorded: 1) ganglion cell (GC) number, semiquantified as normal, decreased or absent, and 2) myenteric inflammation (MI) (present or absent).

Results: 23 pts met the inclusion criteria. Clinical evaluation confirmed an EMD in 15 pts (65%), including achalasia (n=8), nutcracker esophagus (n=2) and a nonspecific motility disorder (n=5). 8 pts (35%) had no evidence of an EMD. Decreased/absent GC were noted in 87% of pts with an EMD, compared to 25% with no EMD.

Table 1

	GC			MI	
	Normal	Decreased	Absent	Present	Absent
No EMD (n=8)	6 (75%)	1 (12.5%)	1 (12.5%)	6 (75%)	2 (25%)
Nutcracker esoph (n=2)	2 (100%)	0	0	2 (100%)	0
Nonspecific EMD (n=5)	3 (60%)	2 (14%)	0	4 (80%)	1 (20%)
Achalasia (n=8)	3 (37.5%)	4 (5%)	1 (12.5%)	6 (75%)	2 (25%)

MI was present in 75% of pts with an EMD, but was also found in 80% of those without an EMD. Both pts with nutcracker esophagus had normal GC with MI.

Conclusions: GC abnormalities are extremely common (87%) in pts with an EMD, especially achalasia, where 62% of pts had decreased/absent GC. Since MI is found in 75% of pts without an EMD, MI does not discriminate between those with and without an EMD. Both pts with nutcracker esophagus had normal GC and MI. This study suggests that esophageal muscle biopsy specimens provide useful information in the evaluation of pts with esophageal diverticula to determine the presence of an underlying EMD.

571 Sentinel Lymph Node Mapping in Colorectal Cancer — Benign Mechanical Transport of Epithelial Cells Is Not a Significant Factor

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Background: Sentinel lymph node mapping (SLNM) of colorectal cancers, with multilevel sections (MLS) and cytokeratin immunostaining (IHC) of sentinel lymph nodes (SLN), upstages 15-20% of patients (pts). Since SLNM in breast cancer has been reported to induce benign mechanical transport (BMT) of epithelial cells to axillary nodes (potential false positives), we questioned whether SLNM in colorectal cancer might induce BMT.

Design: A total of 225 consecutive pts with colorectal cancer underwent SLNM by intraoperative subserosal injection of 1-3 ml 1% Lymphazurin. The first 1-5 blue-stained SLN were suture-tagged by the surgeon. They were sectioned grossly at 2-3 mm and totally embedded. Five sections of each were cut at 20-40 micron intervals, 4 for hematoxylin and eosin (H+E) stain, and 1 for IHC (AE-1). The remaining specimen was examined by standard methods. To assess whether SLNM could displace epithelial cells into SLN, 37 pts underwent a second SLNM of normal bowel at least 20 cm. from the primary tumor, with identification of 1-2 blue non-tumor SLN, evaluated also by MLS and IHC.

Results: A total of 225 pts (173 colon, 52 rectum) underwent SLNM. Of these, 161 colon and 51 rectal pts had invasive adenocarcinomas, including 37 T1, 41 T2, 120 T3, and 14 T4 tumors. The remaining 12 colon and 1 rectal pts were Tis or T0 (no residual tumor status post-polypectomy); no epithelial cells were demonstrated in any of the 39

SLN in these 13 pts. Separate SLNM away from the tumor site in 37 pts yielded 40 non-tumor related SLN; again, none had identifiable epithelial cells. Of 212 pts invasive tumor undergoing SLNM, 15 pts had 19 SLN positive for micrometastatic tumor less than 2 mm identified by H+E stain, while13 pts had 16 SLN positive for isolated tumor clusters seen only by IHC. Thus, in summary, for 50 total pts with SLNM either away from the main tumor or without invasive cancer, epithelial cells were absent in 79 SLN examined by MLS and IHC. In contrast, in 212 pts with invasive colorectal cancer who underwent SLNM at the primary tumor site, 28 pts had 35 nodes positive by IHC only or with micrometastasis (<2 mm). The difference between these groups is significant (Chi square; $p \leq 0.025$ comparing either pts or nodes).
Conclusions: SLNM alone does not induce BMT. While BMT in pts only with invasive tumor is not completely excluded, the rate of such passive transfer would be low. In summary, BMT is not a significant factor in SLNM of colorectal cancers.

572 Intraepithelial Lymphocytosis with Normal Villous Architecture in Proximal Small Bowel of Morbidly Obese Patients

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Background: Intraepithelial lymphocytosis with normal villous architecture (IELNVA) in the proximal small bowel may be seen in a variety of conditions besides gluten-sensitive enteropathy, including tropical sprue, food allergy, primary immunodeficiency, H. pylori gastritis, viral enteritis, Giardiasis, blind loop syndrome, Crohn's disease, autoimmune diseases, NSAIDs and irritable bowel syndrome. Morbid obesity is an increasingly common condition in Western cultures that is often treated surgically. One popular approach is the laparascopic roux-en-y gastric bypass, which entails removing a short segment of proximal jejunum after stapling the distal enteroenteric anastomosis. We have frequently observed IELNVA in these surgical specimens.
Design: We searched our surgical pathology database for patients who had jejunum removed as part of their gastric bypass surgery for morbid obesity (MO) in 2004-2005. Based on information from the treating surgeons, only one of these patients was taking any medications (NSAIDs) and another patient had irregular bowel movements. A single H+E slide from each specimen was reviewed and T-lymphocytes were stained for CD3. As controls, a similar number of Whipple procedures were similarly evaluated. The number of CD3-positive intraepithelial lymphocytes (IELs) per 100 epithelial cells was counted in three separate villi and the distribution was assessed for base-predominance, tip predominance or even distribution, as well as for epithelial sublocalization (subnuclear, internuclear or both).
Results: Slides from 16 patients who underwent gastric bypass surgery and 15 controls were evaluated. Eleven (69%) in the MO group had IEL counts >25. Of these, 6 (38%) had counts of 40-60 and 4 (25%) had counts >60. The IELs were evenly distributed throughout the villi. Most of the IELs were subnuclear. In the control group, 13 (87%) had counts <25 and 2 (13%) had counts of 25-40 (p <0.01).
Conclusions: IELNVA is frequently encountered in the proximal small bowel of a subset of morbidly obese patients. The etiology is uncertain but warrants further investigation. Its elucidation may contribute to our understanding of the link between inflammation and morbid obesity.

573 Barrett's/Cardiac High Grade Dysplasia Is Not a Strong Marker for Concurrent Carcinoma, Unless Architectural Changes Suspicious for Adenocarcinoma Are Also Present

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Background: Recommendations for therapy of high grade dysplasia (HGD) in Barrett's esophagus are based on the historical finding of carcinoma (CA) in 30-50% of esophagi resected for HGD, implicating HGD as a marker for concurrent CA. This implication was confirmed recently, but based on biopsies that were not reviewed. Studies of conservative management of HGD have found CA developing in about 15%—lower than expected based on resection data. This study was undertaken to ascertain the prevalence of CA in esophagi resected for HGD using current diagnostic thresholds, and to evaluate histologic features in HGD that may be predictive of concurrent CA.
Design: 127 esophagectomies performed for columnar HGD, or high grade dysplasia suspicious for carcinoma (HGD/S), were studied. The corresponding biopsies in 69 cases were reviewed and reclassified as either negative for dysplasia, low grade dysplasia, HGD, HGD/S or CA by consensus of a group of gastrointestinal pathologists using established criteria, and a set of architectural features, including complexity, necrotic debris in tubules, and irregular dilated tubules, to classify biopsies as HGD/S.
Results: Based on original diagnoses, CA was present in the resection specimen of 16.9% (15/89) patients with HGD, and 73.7% (28/38) of those with HGD/S. Based on reclassification of 69 cases, only one of 21 (4.8%) patients with HGD on biopsy was found to have carcinoma in the subsequent esophagectomy specimen, and this carcinoma was only intramucosal. Of the 25 patients whose biopsies were reclassified as HGD/S, 18 (72%) had carcinoma in the resection specimen, as did 73.9% (17/23) of patients whose biopsies were reclassified as adenocarcinoma.
Conclusions: Based on current criteria, fewer than 10% of esophagectomies for Barrett's HGD harbor CA. In contrast, when HGD/S is diagnosed based on architectural features, CA is found in nearly 75%, comparable to the rate in patients with diagnoses of CA. Management guidelines for patients with Barrett's HGD should be based on up to date resection and follow up data. The lower rate of concurrent CA in patients with Barrett's HGD, and our reclassification of biopsies from HGD to HGD/S or CA highlights the evolution of diagnostic criteria for Barrett's dysplasia that has resulted from experience with these cases and their outcomes.

Genitourinary

574 Plasmacytoid Urothelial Carcinoma of the Urinary Bladder

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Background: Plasmacytoid urothelial carcinoma (PUC) is a rare tumor of the urinary bladder. Its clinical and histopathological features have not yet been characterized. In this study, we report six cases of PUC at one instituition.
Design: Cases of invasive urothelial carcinomas (UC) at the Ottawa Hospital over a period of 7 years were reviewed. Those cases with plasmacytoid features were selected for review of the slides, along with the clinical history. Representaive sections from each case were submitted for immunohistochemical studies.
Results: There were total of seven cases of PUC among 260 cases of invasive UC. In five cases, common UC was either absent, or present in focal areas. In the remaining two cases, common UC formed the predominant component. Cases with extensive PUC showed coarse mucosal foldings and indurations of the thickened bladder walls, with no grossly identifiable tumor. Catheterized urine showed scanty number of atypical single cells, frequently without tumor diathesis, leading to a shortfall in the positive cytological diagnosis. Histologically, PUC appeared as dyshesive, plasmacytoid cells with eccentric nuclei, extending widely into the bladder walls and extensively into adjacent pelvic organs. The diagnosis of the entity in biopsy was based on the presence of urothelial carcinoma in situ, and by immunocytochemistry typical of UC. The typical clinical presentation for PUC included advanced disease at presentation with the absence of hematuria, late onset of lower urinary tract symptoms, indurated mucosal surface on endoscopy, and radiographic findings of bladder wall thickening and hydronephrosis. The disease followed an ominous course with recurrence in all the patients, and death in 6 patients.
Conclusions: PUC is a distinct clinical and pathological entity of urothelial carcinoma, unique by its frequently late clinical presentation, plamacytoid appearance and poor clinical outcome.

575 Separation of Chromophobe Renal Cell Carcinoma from Oncocytoma and Clear Cell Carcinoma: An Optimal Immunohistochemical Panel for Differential Diagnosis

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Background: The separation of chromophobe renal cell carcinoma (chromophobe RCC), oncocytoma, and conventional (clear cell) renal cell carcinoma (clear cell RCC) using light microscopy remains problematic. The aim of this study was to determine a useful immunohistochemical panel for the differential diagnosis of chromophobe RCC.
Design: After reviewing the literature, we chose a panel of markers for the differential diagnosis of chromophobe RCC, including vimentin, GST-alpha, CD10, CD117, CK7, and EpCam. We employed the tissue microarray technique to study the immunohistochemical profile for these markers in 20 chromophobe RCCs, 11 oncocytomas and 36 clear cell RCCs
Results: We found that vimentin and GST-alpha expression were exclusively observed in clear cell RCC. CD10 staining was more frequently detected in clear cell RCC (92%, 33/36) than in chromophobe RCC (45%, 9/20) and oncocytoma (27%, 3/11). CD117 was preferentially expressed in chromophobe RCC (80% of cases, 16/20) and oncocytoma (100 %, 11/11), whereas none of the cases of clear cell RCCs was immunoreactive. Diffuse CK7 staining was observed in 17 of 20 chromophobe RCCs (85%) while focal staining was noted in the remaining 3 cases (15%). All CK7-positive oncocytomas (64%, 7/11) showed only single scattered cell positivity. EpCam protein was diffusely expressed in all 20 chromophobe RCC (100%) whereas all EpCam-positive oncocytomas (82%, 9/11) displayed only scattered and focal positivity in single cells or small cell clusters. EpCam showed diffuse staining in 6 of 36 clear cell RCCs (17%) and focal staining in 8 of 36 clear cell RCCs (22%). Using the combination of three markers (vimentin, GST, and EpCam), we achieved 100% sensitivity and 100% specificity for the differential diagnosis of chromophobe RCC, oncocytoma, and clear cell RCC. The pattern of "Vimentin-/GST-" effectively excludes clear cell RCC, and diffuse EpCam expression confirms the diagnosis of chromophobe RCC rather than oncocytoma. CD117 and CK7 were also useful markers and could be used as second line markers for the differential diagnosis, with high specificity (100%) and high sensitivity (87%, 85%).
Conclusions: We identified three markers (vimentin, GST, and EpCam) for the differential diagnosis of chromophobe RCC, oncocytoma, and clear cell RCC. These markers appear to provide complete accuracy in separation of these renal neoplasms.

576 Clusterin Expression Differentiates Wild Type-*VHL* from *VHL*-Defective Sporadic Clear Cell Renal Cell Carcinomas

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Background: Approximately 70% of sporadic clear cell renal cell carcinomas (CCRCC) show somatic inactivation of both Von Hippel Lindau (*VHL*) gene alleles. The most well characterized function of *VHL* is its ability to regulate HIF-2α (hypoxia inducible factor). Clusterin is a ubiquitously expressed glycoprotein known to regulate apoptosis. Recently, our group showed that clusterin expression is decreased in CCRCC cell lines that lacked wild type *VHL,* including a line transfected to express Type 2C pVHL which is associated with normal HIF function and no increased risk for CCRCC. The results suggest that clusterin may serve as a marker for HIF-independent *VHL* activity.