Conclusions: 1) Xp11.2 translocation RCC is not an uncommon neoplasm. The incidence in this group of 120 patients is 9.2% in adults and up to 20% in patients between 18-40 years. 2) TFE3(P-16) IHC is a valuable assay for the diagnosis Xp11.2 translocation RCC. The strong nuclear TFE3 stain is most likely indicative of Xp11.2 translocation. The weaker nuclear stain appears to be due to expression of full length TFE3 protein, rather than chimeric fusion protein due to translocation.

925 *TMPRSS2-ERG* Gene Fusion in Prostate Cancer of Different Ethnic Groups

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Background: Prostate cancer (PCa) exhibits significant differences in prevalence and mortality among different ethnic groups. The genetics underlying these differences is not well understood. The *TMPRSS2-ERG* fusion is a common recurrent chromosomal aberration in this malignancy and is thought to represent an early event in the prostate carcinogenesis. In this study, we examined the frequency of *TMPRSS2-ERG* gene fusion in PCa from Caucasion, Asian and African American patients.

Design: A tissue microarray of PCa from 51 Caucasian, 22 Asian and 59 African American patients who underwent radical prostactomies was contructed. PCa Gleason score (GS) was 7 in all Caucasian patients; GS 6 in 31 and GS 7 in 28 African American patients; and GS 6 in 4, GS 7 in 8 and GS≥8 in 10 Asian patients. The *TMPRSS2-ERG* fusion status was determined using a multi-color interphase fluorescence in situ hybridization assay for *ERG* break-apart. A nucleus without ERG rearrangement has 2 pairs of juxtaposed red and green (yellow) signals. A nucleus with an *ERG* break apart (indicative of a *TMPRSS2-ERG* fusion) show split-apart of one juxtaposed red-green signal pair resulting in a single red and green signal for the transolcated *ERG* allele and a still combined (yellow) signal for the nontranslocated *ERG* allele in each cell. The telomeric green signal may be lost due to deletion and results in one yellow and one red signal in a nucleus with *ERG* rearrangement through deletion.

Results: *TMPRSS2-ERG* gene fusion was present in 45.1% (23/51) Caucasian, 13.6% (3/22) Asian, and 33.9% (20/59) African American patients (p=0.034 by chi-square test). The gene fusion through translocation, deletion or both occurred in 56.5% (13/23), 39.1% (9/23) and 4.3% (1/23) in Caucasian, 66.7 (2/3), 33.3 (1/3) and 0% (0/3) in Asian and 20%, 60% (12/20) and 20%(4/20) in African American patients, respectively (p=0.098 by chi-square test).

Conclusions: The frequency of *TMPRSS2-ERG* was determined to be significantly different in PCa of Caucasion, Asian and African American patients. This is a small study so the potential limitations to this study include why the patients were screened as they came from different populations. Further studies are needed to address whether such difference may account for the difference in the prevalence and mortality among different ethnic groups.

926 Atypical Cribiform Lesions of the Prostate: Implications for Diagnosis in Prostate Biopsies

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Background: Atypical cribiform lesions of the prostate (ACL) are cribiform structures consisting of cytologically malignant cells with partial or complete basal cell lining. They can represent "intraductal carcinoma" which is invariably associated with high grade prostate carcinoma (PCa) (cancer associated ACL, ACL-PCa), or cribiform high grade PIN which can be an isolated finding not associated with PCa (non-cancer associated ACL, ACL-nonPCa). We report the histological differences of these 2 lesions on radical prostatectomy (RP) and implications for diagnosis in prostate biopsies (NBX).

Design: RP was reviewed for ACL. The presence of basal cells was confirmed by basal cell immunostaining. ACL intermixed with, or within 2 mm from the border of PCa was categorized as ACL-PCa. The following histological features were reviewed: number of ACL/prostate gland, size, glandular contour (round, irregular, branching), architectural pattern (trabecular, cribiform, solid), comedonecrosis, nuclear feature (round and uniform, round with varying sizes, pleomorhpic, giant nuclei [≥6x adjacent nuclei]).

Results: 35 cases with ACL-PCa and 17 cases with ACL-nonPCa were studied (Table). The mean number of ACL per prostate was 25.3 for ACL-PCa and 2.4 for ACl-nonPCa (p=0.008). The size ranged from 0.2-6.5 mm for ACL-PCa, and 0.2-1mm for ACL-nonPCa. The branching contour was present in 29/35 ACL-PCa, but in only 1/17 ACL-nonPCa (p<0.001). The architectural patterns were not different between the two (p=0.21). Comedeonecrosis was present in 14/35 ACL-Pca, and none of ACL-nonPCa (p=0.002). The pleomorphic nuclei or giant nuclei that were ≥6X of the adjacent nuclei were present in 9 of ACL-PCa, but none of ACL-nonPCa (p=0.049).

	ACL-PCa	ACL-nonPCa	P value
# Cases	35	17	
Mean # of ACL/prostate gland	25.3	2.4	0.008
Size (range, mm)	0.2-6.5	0.2-1	
Branching glandular contour	29/35	1/17	<0.001
Comedonecrosis	14/35	0/17	0.002
Nuclear features: pleomorhpic or giant (x6 adjacent nuclei)	9/35	0/17	0.049

Conclusions: NBXs demonstrating several features of large foci, architectural complexity with large branching glands, pleomorphic or giant nuclei, or comedonecrosis are invariably associated with invasive cancer. A note indicating such should be included in the biopsy report. As there is overlap in histological appearance between ACL-PCa and ACL-nonPCa, the presence of atypical cribriform lesions in NBX should prompt repeat biopsy to rule out concomitant PCa.

927 Establishment of Laminin and Pankeratin Dual Immunostain for the Evaluation of Urothelial Carcinoma

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Background: Incorrect staging due to misinterpretation of lamina propria invasion is one of the most commonly described diagnostic errors in bladder specimens. Lamina propria invasion can be difficult to assess due to the presence of only a small microinvasive focus or fragmentation artifact. Immunohistochemical assessment for basement membrane or epithelial cells may help corroborate the histologic impression of lamina propria involvement. We sought to create a dual immunostain containing a basement membrane marker (laminin) and an epithelial marker (pankeratin) to facilitate the interpretation of lamina propria invasion.

Design: 504 cases of paraffin embedded urothelial carcinoma were obtained, including 58 whole tissue sections (cystectomies, transurethral resections and biopsies) and 446 cases using tissue microarray. The tissue was incubated with laminin primary monoclonal antibody (1:25) with 3,3'-diaminobenzidine as the chromogen followed by pankeratin cocktail incubation (prediluted) using Vulcan fast red as the secondary chromogen. Pankeratin was semi-quantitatively analyzed as 0, < 5% of cells stained; 1+, 5-10% of cells stained; 2+, 11-50% of cells stained; or 3+, >50% of cells stained. Invasive and noninvasive urothelial components as well as blood vessels were assessed for laminin.

Results: Pankeratin stained benign and malignant urothelial cells in the vast majority of the 504 cases (0, 2%; 1+, 1; 2+, 1%; 3+, 96%) with intense staining and little background, allowing for clear visualization. Within the 58 whole tissue sections, 40 contained only invasive carcinoma, all of which completely lacked laminin expression surrounding tumor. 18 of the 58 tissue sections contained a noninvasive component, demonstrating laminin separating the urothelium from the lamina propria. Dual staining highlighted vascular invasion in 5 of the 58 cases. One of the 5 cases containing vascular invasion was not diagnosed in the corresponding pathology report.

Conclusions: We created a dual immunostain of laminin and pankeratin. Invasive carcinoma was strongly positive for pankeratin and lacked laminin. Noninvasive components showed dual expression. The dual immunostain of laminin and pankeratin shows promise as a tool to facilitate the interpretation of invasion in bladder specimens. Additionally, this dual stain highlights vascular invasion, allowing for the assessment of both lamina propria and vascular invasion on the same slide.

928 MicroRNA Expression Profiling Distinguishes Conventional and Chromophobe Renal Cell Carcinomas from Normal Renal Parenchyma

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Background: MicroRNAs (miRNA) are small non-coding RNAs that modulate expression of protein-encoding genes by binding and inactivating messenger RNA. Renal cell carcinoma (RCC) subtypes are traditionally distinguished by morphologic, immunophenotypic, and cytogenetic characteristics. miRNA expression patterns have not been previously reported for RCC subtypes. The aims of this study are to 1) determine if unique miRNA expression profiles are present among 2 RCC subtypes (conventional (CON) and chromophobe (CHR)) and normal renal parenchyma (NRP) and 2) identify specific miRNAs that may contribute to underlying mechanisms of renal tumorigenesis.

Design: Sixteen specimens of frozen, banked human renal tissue with 4 in each group (CON, CHR, NPR) yielded high integrity RNA that was labeled without amplification and hybridized on microarrays (Exiqon, Denmark) covering all annotated miRNAs in the miRBase (1168 probes with 4 replicates/array). Statistically significant differential expression was determined by the Significance Analysis of Microarray (SAM) multiclass test (Q=0.03) and post-hoc t-test of individual transcripts.

Results: Differential expression was observed in 32 miRNAs among the 3 specimen classes. CON specimens exhibited 14 miRNAs increased from 1.6 to 8.4 fold compared to NRP. These miRNAs were not differentially expressed between CHR and NPR. However, CHR samples revealed an additional 14 distinct miRNAs elevated (2.3 to 9.4 fold) versus NPR specimens that were not differentially expressed between CON and NPR specimens. These miRNAs were all expressed at significantly different levels between CON and CHR samples. *miR-21* was among the identified miRNAs increased in CON, consistent with upregulation in other malignancies, including breast, colorectal and cervical carcinoma.

Conclusions: The expression pattern obtained from the RCC tumor samples provided an exclusionary signature for each CON and CHR tumor class when compared to NRP, and from each other. Our data suggest that post-translational regulation may contribute to RCC tumorigenesis as well as to the molecular differentiation pathways resulting in histologic subtypes of RCC. We are currently assessing miRNA profiles in papillary RCC and oncocytoma and evaluating messenger RNA profiles in tumors from the corresponding cases to extend the molecular characterization of miRNA expression in renal neoplasia.

Gynecologic

929 Metastatic Pancreatic Adenocarcinoma to the Ovary: A Clinicopathologic Review of Twenty Nine Cases

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Background: Metastatic pancreatic adenocarcinoma to the ovary (MPAO) can represent a diagnostic dilemma. The number of studies on this topic is limited. In this study, we present the clinicopathologic features of twenty nine such cases seen at our institution.

Design: Twenty-nine cases of MPAO were retrieved from our files of which twenty six cases had available slides. Patient demographic, initial diagnostic impression, serum tumor marker, treatment, and follow-up data were collected along with pathologic parameters including laterality/size of ovarian metastases, pancreatic site/size of primary lesions, and metastatic burden.
Results: Patients' age ranged from 30-77 years (mean 58). Tumors were bilateral in 58.6% (17/29), unilateral to the right ovary in 27.6% (8/29), and unilateral to the left in 13.8% (4/29) of the cases. MPAO size (range 1.5-29, mean 9.0 cm) was generally larger than the pancreatic tumor (range 1.7-11.0, mean 5.6 cm). Primaries were located in distal pancreas in 48.3% (14/29), proximal pancreas in 27.6% (8/29), and mid/body in 24.8 % (7/29) of the cases. Extra-ovarian metastatic disease was widespread; peritoneal metastases were the most common, followed by omentum and liver. Initial clinical impression was that of a pancreatic primary in 51.7% (15/29) and ovarian primary in 44.8% (13/29) of cases. Serum tumor markers were not ordered in all cases: 83.3% (10/12) of CA 19.9, 73.3% (11/15) of CA 125, and 46.2% (6/13) of CEA results were elevated. Microscopically, the majority of MPAO had ovarian surface involvement (18/26) and desmoplasia (18/26). Infiltrative and nodular patterns of invasion were the most commonly encountered (10/26 and 9/26 respectively). MPAO had cytologic atypia ranging from mild to high grade with a predominance of moderate atypia (12/26). Surgery plus gemcitabine–based chemotherapy was the most common treatment. Median survival ranged from 1 day (necropsy diagnosis) to 58 months (median 11 months). 5 of 29 patients are alive with disease after a follow-up period ranging from 3 to 108 months.
Conclusions: 1. MPAO still represents a diagnostic dilemma from the clinical and pathologic standpoints. 2. Up to 41% of the cases can present as a unilateral ovarian mass. 3. MPAOs are usually larger in size than the pancreatic tumor. 4. Although we encountered a constellation of histologic features present in MPAO, there are cases with overlapping histologic features with primary ovarian tumors. In such cases clinicopathologic correlation is of utmost importance to ensure an accurate diagnosis.

930 Differential MGMT Promoter Methylation Profiling between Low Grade and High Grade Papillary Serous Ovarian Carcinoma

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Background: Expression of O^6-methylguanine-DNA methyl transferase **(MGMT),** a DNA repair protein, is regulated mainly by promoter methylation. Silencing of the MGMT gene through promoter methylation in brain cancer is associated with increased sensitivity to alkylating agents. It has also been shown that different genetic pathways exist between low grade papillary serous carcinoma (LG-PSC) and high grade papillary serous carcinoma (HG-PSC). However, differential epigenetic alteration, such as DNA methylation, between LG-PSC and HG-PSC has not been well characterized. We have compared the methylation status of MGMT between LG-PSC and HG-PSC and correlated MGMT methylation with clinicopathologic features.
Design: Sixty-three patients with ovarian PSC, including 40 cases of HG-PSC and 23 cases of LG-PSC, were included in this study. All cases, except one, were stage IIIc. The pathologic diagnosis was reviewed and clinicopathologic features were tabulated. Genomic DNA was extracted from paraffin embedded tissue. After bisulfite treatment, methylation of the MGMT gene was evaluated using pyrosequencing methylation assay.
Results: Methylation of MGMT was found in approximately 24% of 63 cases of ovarian PSC. The frequency of MGMT methylation was significantly different ($P<0.001$) between HG-PSC and LG-PSC. Methylation of MGMT promoter was seen in approximately 39% (15/40) of HG-PSC, but none of LG-PSC (0/23). In HG-PSC tumors, MGMT methylation status did not correlate with patients' age, bilaterality, residual disease after debulking, or sensitivity to chemotherapy.
Conclusions: Differential MGMT methylation profiling exists between LG-PSC and HG-PSC. No MGMT methylation was seen in LG-PSC compared to about 39% MGMT methylation in HG-PSC. Our study indicates that silencing of MGMT through methylation is involved in the carcinogenesis of HG-PSC. Our data further demonstrates that different epigenetic pathways exist between LG-PSC and HG-PSC.

931 Epigenetic and Genetic Alterations in Low Grade Papillary Serous Carcinoma of the Ovary

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Background: Different genetic pathways exist between low grade and high grade papillary serous carcinoma (PSC) of the ovary. Genetic mutations of BRAF and Ras in low grade PSC and p53 mutations in high grade PSC have been reported. Epigenetic alteration in low grade PSC, however, has not been well characterized. We studied methylation profiles of 5 tumor suppressor genes in low grade PSC and correlated the methylation profiling with mutations of BRAF and K-Ras.
Design: Twenty-three patients with low grade PSC were included. All cases, except one, were stage III. Pathologic diagnosis was reviewed and clinicopathologic features were tabulated. Genomic DNA was extracted from paraffin embedded tissue. BRAF v600 and K-Ras at codons 12/13 mutations were tested using pyrosequencing SNP assay. After bisulfite conversion of unmethylated cytosine to uridine, methylation status of each of the 5 genes (RASSF1a, p16, E-Cadherine, MGMT and FGFR2) was evaluated using pyrosequencing quantitative methylation assay. Student t-test and Chi-square tests were applied for statistical analysis.
Results: Approximately 48% (11/23) of low grade PSC harbored methylation of at least one of three genes, RASSF1a 35% (8/23), E-cad 13% (3/23) and p16 13% (3/23). Methylation of MGMT or FGFR2 was not detected in low grade PSC (0/23). Methylation of RASSF1a was mutually exclusive with methylation of p16, but was concurrent with methylation of E-Cadherine. Genetically, 56.5% (13/23) of low grade PSC tumors harbored mutations of either BRAF v600 (3/23) or K-Ras at codons 12/13 (10/23). BRAF and K-Ras mutations were mutually exclusive. BRAF mutation was also mutually exclusive with DNA methylation in all except one case. In contrast, the majority (75%) of cases harboring RASSF1a methylation had concurrent K-Ras mutations. Taken genetic mutations and DNA methylation together, about 74% of low grade PSC had either mutations for BRAF or K-Ras or methylation of at least one of 3 genes (RASSF1a, p16 and E-Cadherine).
Conclusions: Both genetic and epigenetic alterations are involved in tumorigenesis of low grade PSC of the ovary. Among molecular alterations, BRAF and K-Ras mutations and RASSF1a methylation are the most common events (82%,14/17). Our study indicates that alteration of RAS-RAF-MEK pathway may play the most important role in pathogenesis of low grade PSC.

932 Confirmation of the Germ Cell Origin of Ovarian Tumours Using a Standard Panel of Microsatellite Markers

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Background: Ovarian germ cell tumours are believed to arise from post meiotic germ cells and global lack of heterozygosity has been previously reported in 7/8 mature ovarian teratomas. Ovarian mucinous tumours are currently classified as surface-epithelial tumours, but have been suggested by some as arising out of a monodermal teratoma. The purpose of the study was to determine if any mucinous tumors could be proven to be of germ cell origin.
Design: Frozen tissue from 6 ovarian germ cell tumours (2 teratomas, 2 yolk sac tumours, 1 immature teratoma and 1 dysgerminoma), and 6 mucinous tumours (mucinous intraepithelial carcinomas and carcinomas) were included in the study. DNA was extracted, and genotyping performed using AmpFLSTR (Applied Biosystems), a standard PCR based forensic assay. The assay used includes 14 highly polymorphic short tandem repeats. FISH was performed on one case using a multiplexed 4 probe assay designed to detect aneusomy in breast cancer (Abbot Molecular).
Results: The dysgerminoma, 2/2 yolk sac tumours, 1/2 teratomas, and 2/2 mucinous tumours showed a heterozygous genetic profile (ie. diploid). One out of the two teratomas, and the immature teratoma were found to be monoalleic at all loci tested. FISH performed on these two cases demonstrated at least two copies of each of the four chromosomes assessed, suggesting these tumors arose from non-segregation during meiosis II or through a post-meitotic duplication of chromosomes. As occasional heterozygosity due to meiotic recombination at meiosis I would be expected in meiosis II errors the latter is more likely. Analysis of the other tumors is ongoing.
Conclusions: DNA genotyping using a standard microsatellite panel can elucidate the histogenesis of ovarian carcinomas. These results demonstrate that the majority of germ cell tumors are not of post-meiotic origin. One of the mature teratomas and an immature teratoma showed global lack of heterozygosity, suggesting at least some of these tumors arise from meiosis II or post meiotic errors. We were not able to prove germ cell origin for 2/2 mucinous tumors.

933 Metastatic Endocervical Adenocarcinoma in the Ovary: The MD Anderson Experience

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Background: The ovary is a common location for metastatic adenocarcinoma, particularly colorectal carcinoma. Metastasis from an endocervical adenocarcinoma (EAC), by comparison, is uncommon. Occasionally, such metastases can mimic a primary ovarian mucinous tumor and rarely precede the diagnosis of EAC. The distinction of these two entities has significant therapeutic and prognostic implications. In this study, we present the clinicopathologic features of 36 such cases.
Design: Thirty-six cases of metastatic endocervical adenocarcinoma (MtEC) were retrieved from the files of our institution over a 20 year period. Clinical information was retrieved from patients' (pts) charts. Clinical and pathologic features noted included: patient age, depth of invasion of the EAC, time elapse between diagnosis of endocervical and ovarian tumors, and histologic features of the MtEC.
Results: Pts ranged in age from 22 to 72 years (median 46). Presenting findings included abnormal vaginal bleeding or discharge (9 cases), abdominal pain (5 cases), increased abdominal girth (3 cases), pelvic mass on imaging studies (2 cases), and abnormal Pap smear (2 cases). In 2 pts, MtEC preceded the diagnosis of EAC by 18 and 21 months, respectively. In 25 pts, MtEC was detected at the time of curative surgery. Nine pts had a prior history of EAC, and surgery was performed to evaluate a new pelvic mass. In two of these patients, MtEC occurred 7 and 25 years, respectively. In 19 pts, EAC was deeply invasive into the outermost endocervical stroma. In 1 pt, invasive EAC was confined to the inner half of the stroma. In 3 pts, EAC was minimally invasive: 3/15 mm, 1/5 mm and 2/7 mm respectively. In the remaining cases, only biopsies were performed precluding an accurate assesment of invasive carcinoma. Histologic features of MtEC included: surface involvement, 14 cases; increased mitotic activity for level of cytologic atypia, 7 cases; infiltrative invasion, 6 cases; apoptosis, 4 cases, pseudomyxoma ovarii, 4 cases; tumor location in hilum, 3 cases; tumor in lymphatic spaces of ovary only, 2 cases; dirty necrosis, 1 case.
Conclusions: MtEC in the ovary can present a diagnostic challenge. In 5.5% of the series cases, MtEC was the first manifestation of disease and originally interpreted as a mucinous borderline tumor. Additionally, the absence of deep cervical stromal invasion should not preclude a diagnosis of MtEC as this can occur in the presence of superficially invasive EAC as observed in 3 of the study cases. Further study is required to determine whether ovaries should be left in situ in patients with EAC.

934 High Grade Endometrial Carcinoma: Serous & Grade 3 Endometrioid Carcinoma Have Different Immunophenotypes and Outcomes

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Background: High-grade endometrial carcinomas are a heterogeneous group of tumors and include grade 3 endometrioid, serous, and clear cell carcinomas. There are conflicting data about the prognosis of these endometrial carcinoma subtypes; this might be due to lack of reproducibility in classifying some tumors based on their cell type. The purpose of this study was to examine differences in immunophenotype and prognosis in a series of high grade endometrial carcinomas.

Design: We selected 180 endometrial carcinomas of pure serous, endometrioid or clear cell type. We chose the following immunohistochemical markers (ER, IMP3, p16, p53, PR, PTEN) as being significantly differentially expressed in pure endometrial carcinomas subtypes and validated their discriminatory value on this set of tumors. Tumors were stratified into four groups based on their cell type and grade: endometrioid tumors of grade 1 or 2, endometrioid tumors of grade 3, serous, and clear cell carcinomas.

Results: Univariate survival analysis revealed significant differences in outcome between the four groups ($p<0.0001$), with significantly longer disease specific survival for grade 1 or 2 versus grade 3 endometrioid carcinoma ($p=0.0001$), and grade 3 endometrioid versus serous carcinoma ($p=0.0003$). p16, PTEN and IMP3 expression was observed more frequently in serous carcinomas, compared to grade 3 endometrioid carcinomas ($p<0.0001$, $p=0.021$ and $p=0.031$, respectively). These three markers showed the highest sensitivity and specificity expressed as area under the curve with 0.85, 0.69 and 0.71, respectively. ER and p53 approached but did not reach significance for differential expression in grade 3 endometrioid versus serous carcinomas ($p=0.055$ and $p=0.068$, respectively). A combination of p16 and PTEN predicts grade 3 endometrioid versus serous type with a sensitivity of 90.0% and specificity of 96.8%.

Conclusions: p16 and PTEN can aid in the diagnosis of grade 3 endometrioid versus serous carcinoma of the endometrium, and are superior to ER and p53 for this purpose. Grade 3 endometrioid carcinomas have a significantly better prognosis than serous carcinomas of the endometrium.

935 Loss of PAX-2 Expression Is a Sensitive Marker for Neoplastic Endometrium

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Background: Immunohistochemical markers of to better define areas of neoplastic endometrium would be of diagnostic use. The *PAX-2* gene has been previously reported to be involved in estrogen and tamoxifen driven endometrial carcinogenesis, but there are limited studies on PAX-2 expression by IHC in endometrial hyperplasia.

Design: PTEN, PAX-2, BCL-2 and MLH-1 expression were evaluated in a subsample of endometrial biopsies from women enrolled in the Endometrial Hyperplasia Outcomes Cohort Study. Cases with an index biopsy consensus diagnosis of either complex or atypical hyperplasia with both neoplastic and adjacent normal endometrial tissue present were included. Women receiving high dose progestin were excluded. Antibody staining was scored by one of two study pathologists in both the lesional tissue and the adjacent normal tissue as 0%, 1-25%, 26-50%, 51-75%, or 76-100%. Separate analyses were conducted for individual markers using an exact McNemar's test of the paired binary data.

Results: We found a statistically significant difference in loss of PAX-2 expression between lesional and normal tissue in 76 cases of complex hyperplasia and 47 cases of atypical hyperplasia ($P<0.005$). Using $\geq$25% loss of staining as a threshold, 95% of complex and 96% of atypical hyperplasias had loss of expression of Pax-2 in neoplastic tissue, compared with 21% and 9% of the respective matched adjacent normal endometrium. 53% of cases of complex and 73% of atypical hyperplasias had >25% loss of PTEN expression, while 21% and 29% of adjacent normal endometrium had loss. Loss in the normal endometrium occurred more frequently in secretory endometrium for PTEN (7 of 11 normal secretory controls had loss), while PAX2 had less frequent loss in secretory endometrium (1 of 10 normal secretory had loss). There was not a statistically significant difference in the loss of MLH-1 expression between normal and complex or atypical hyperplasia. We found a statistically significant difference in BLC-2 loss of expression only among women with atypical hyperplasia. Of the four IHC markers, only PTEN had a significant difference in loss between those with complex and atypical hyperplasia (*$P=0.045$*).

Conclusions: PAX-2 may be more sensitive and specific than PTEN as a marker of neoplastic endometrium. However, PTEN may be diagnostically useful in the distinction between complex and atypical hyperplasia.

936 Increased Expression of Inflammatory Markers in Type I and Type II Ovarian Carcinomas

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Background: Recently, it has been proposed that epithelial ovarian cancers (EOC) are subdivided into 2 types: type I tumors are slow growing, genetically stable and are characterized by mutations in a number of different genes including KRAS, BRAF, PTEN, and beta-catenin. Type II tumors are rapidly growing and highly aggressive neoplasms. The objective of this study was to determine the expression of inflammatory markers based on this new classification.

Design: Using our institutional database, we identified 226 cases of ovarian carcinoma treated between 1992 and 2002 for which pathological specimens were available. Patients' information and tumor characteristics were collected from the database. Survival data were retrieved from the SEER and the institutional database. Cases were categorized as Type I or Type II based on histopathology review and the above criteria. Tissue Microarray sections were stained by immunohistochemistry for Glut-1, INOS, COX-1, COX-2, and NFK-B. Expression was scored high or low based on intensity and percentage of positive cells and compared in the two types of tumors. Statistical analysis and survival data were calculated using Chi-square, T test, and Kaplan-Meier method.

Results: Table 1 compares the two groups. 89 cases were Type I, and 137 Type II. Overexpression of COX-1, COX-2, iNOS and GLUT-1 were significantly more frequent in type II tumors. In fact, there was high expression of COX-1, COX2, GLUT-1 and iNOS in 38%, 76%, 33% and 76% of type 2 tumors, an overexpression rate that is significantly higher than that seen in type I tumors($P<0.05$). On the other hand, there was a trend toward lower NFK-B expression in type II tumors($p=0.053$). COX-2 was an independent prognostic factor; the median survival was 28 months for tumor with high expression vs. 57 months for the low expression tumors.

		Type I (n=89*) (%)	Type II (n=137*) (%)	P value
COX-1	Low	68(47)	76(53)	0.00
	High	8 (15)	46 (85)	
COX-2	Low	32 (52)	30 (48)	0.01
	High	46 (32)	96 (68)	
Glut-1	Low	72 (46)	84 (54)	0.00
	High	6 (12)	42 (88)	
iNOS	Low	32 (52)	30 (48)	0.01
	High	45 (32)	97 (68)	
NFK-B	Low	47 (34)	91 (66)	0.05
	High	31 (47)	35 (53)	

* Some cases were not available for evaluation

Conclusions: The new proposed subclassification of EOC seems to correlate with changes in the expression of various proteins of the inflammatory pathway. These changes may explain the different biologic behavior of the 2 tumor types and provide different targets for therapy based on tumor types.

937 Expression Levels of Nuf2 in Cervical Dysplasias and Cancers

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Background: Aneuploidy is a common feature of cervical cancer. Inhibition of p53 and pRb by high risk HPV types has been shown to induce centrosome duplication errors and resultant aneuploidy. More recently abnormal kinetochore function has also been suggested as a potential cause of aneuploidy. Recent publications have demonstrated over-expression of Nuf2 and KNTC2/HEC1 (2 components of the kinetochore) in lung cancer and have also indicated that nuf2 expression levels are affected by HPV E6 and E7.

Design: CaSki, C33A, HeLa and SiHa cervical cancer cell lines were cultured and RNA extracted. Normal cervical RNA was purchased from Biochain Inc. Gene expression profiling of all cell lines was performed using Applied Biosystems human genome survey microarrays and gene expression profiles were compared with expression profiles of normal cervix. Taqman PCR was then performed to validate a number of over-expressed genes, incuding $p16^{IN4A}$ and nuf2. Finally, immunohistochemical analyisis of nuf2 expression was performed in CIN and cervical cancer.

Results: Nuf2 was found to be 39 fold up-regulated in the cervical cancer cell lines compared to normal cervical tissue on gene expression profiling, while HEC1 was 35 fold up-regulated. Further evalation of Nuf2 by TaqMan PCR confirmed its over-expression. Immunohistochemical analysis of 90 normal cervix, CIN and cervical cancer specimens was performed. Nuf2 was not detected in normal cervical tissue or in the squamous cell cervical cancers specimens. CIN 1, 2 and 3 and cGIN cases showed varying intensities of cytoplasmic staining, while all 8 villoglandular cancers tested stained positive for nuf2.

Conclusions: As part of the kinetochore, nuf2 plays an important role in ensuring accurate chromosomal segregation during mitosis. In this study we demonstrate nuf2 gene over-expression in cervical cancer cell lines. The expression of nuf2 at protein level was found to be more variable. While the HeLa cervical cell line, villoglandular adenocarcinomas and pre-invasive cervical lesions stained positively with nuf2 antibody, the squamous cell cervical cancer specimens did not display any staining. We postulate that this may be due either to a structural change in nuf2 protein not detected by the antibody used or to the effect of a naturally ocurring siRNA that interferes with nuf2 protein production.

938 Gastrointestinal Mucin Expression in Glandular Epithelia of the Uterus

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Background: Aberrant expression of mucin (MUC) glycoproteins has been implicated in pathogenesis of various precancerous conditions and cancers. There is recent surge of interest to gastrointestinal (GI) phenotype of MUC expression in glandular lesions of the uterus. In this study we evaluated the utility of immunohistochemical (ICH) expression of MUC1 (panepithelial mucin), and GI mucins MUC2, MUC5AC, MUC6 (intestinal-type, gastric foveolar-type and pyloric gland-type mucins, respectively) in the differential diagnosis of glandular lesions of the uterus.

Design: IHC staining using monoclonal antibodies against MUC1, MUC2, MUC5AC, and MUC6 was performed on formalin-fixed paraffin-embedded archival tissue samples. These included: 4 normal endocervix, 5 tubal metaplasias, 5 microglandular hyperplasias, 9 endocervical polyps, 5 endometrial polyps, 6 endocervical adenocarcinomas (including 3 in situ adenocarcinomas), 5 normal endometrium, 4 endometrial hyperplasias (1 simple hyperplasia and 3 complex hyperplasias, including 1 with atypia), 9 endometrial adenocarcinomas. IHC staining was reported as negative, weakly positive, and strongly positive.

Results: The results are summarized in Table 1.

Table 1. MUC Expression in Glandular Epithelia of the Uterus

Diagnoses/# of cases	MUC1	MUC2	MUC5AC	MUC6
Normal endocervix, 4	4/4	0/4	3/4	0/4
Tubal metaplasia, 5	5/5	0/5	0/5	0/5
Microglandular hyperplasia, 5	5/5	2/5	5/5	3/5
Endocervical polyp, 9	8/9	4/9	9/9	9/9
Endocervical adenocarcinoma, 6	6/6†	0/6	6/6	5/6
Normal endometrium, 5	5/5	0/5	1/5	1/5
Endometrial polyp, 5	5/5	2/5	2/5	3/5
Endometrial hyperplasia, 4	4/4	1/4	4/4	3/4
Endometrial adenocarcinoma, 9	9/9‡	2/9	7/9	6/9

†Predominantly apical/membranous staining; ‡Predominatly cytoplasmic staining

MUC expression in endometrial and endocervical polyps was most erratic. Contrary to previous reports, MUC2 was expressed in benign glandular epithelia of the uterine cervix and corpus. Only MUC1 was expressed in tubal metaplasias. MUC1 expression in endometrial hyperplasias and carcinomas was predominantly apical/membranous, whereas in endocervical adenocarcinomas it was predominantly cytoplasmic.

Conclusions: GI MUC expression is not uncommon in the uterine cervix and corpus. Significant overlap in GI MUC expression limits its utility in the differential diagnosis of benign and malignant glandular lesions of the uterus.

939 Role of EZH2 in Ovarian Carcinogenesis

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Background: Despite efforts to improve outcome in patients with ovarian cancer, it remains the leading cause of death from gynecologic malignancy. Therefore, new targets for therapeutic development are needed. The aim of the study was to evaluate the significance of a novel transcriptional repressor, enhancer of *Zeste* 2 (EZH2), in patients with ovarian cancer via immunohistochemistry (IHC).

Design: The departmental archives were searched and 209 patients diagnosed with epithelial ovarian neoplasms (between 1992 and 2002) were included in the study. Cases were reviewed by a gynecologic pathologist and tissue micro-array sections were stained for Cyclooxygenase-1 (COX-1), Cyclooxygenase -2 (COX-2), EZH2, glucose transporter-1 (GLUT-1), VEGF-1 and VEGF-R by immunohistochemistry. The expression of these proteins was scored as low and high based on intensity and percentage of positive cells. Patients' characteristics and follow-up were obtained from the database of the division of gynecologic oncology. The stage (I-IV), grade (borderline, low grade and high grade) and histologic type (serous and non-serous) was also noted. Statistical analysis was performed using Pearson Chi square and Spearman correlation test. Survival analysis was done using Kaplan Meier curves and log rank test.

Results: The average age of the patients was 57.7 years (range 24-89 years), 145 had high stage (III and IV), 140 had high grade, and 164 had serous histology. High levels of EZH2 expression correlated with stage, serous histology and high tumor grade. Patients with high levels of EZH2 expression had significant ($p=0.028$) shorter progression free survival (PFS, 88 months) and overall survival (OS, 92 months) compared to low expression (PFS 157 months) and (OS 157 months). Significant positive correlation was observed between expression of EZH2 and the expressions of several proteins involved in angiogenesis and metastasis such as COX-1, COX-2, GLUT-1, iNOS and p53 ($r=0.41$,$p<0.001$).

Conclusions: EZH2 warrants further investigation as a potential marker for targeted therapy given its prognostic significance and association with COX-1, COX-2 and GLUT-1.

940 HPV Genotyping of Biopsy-Proven Low Grade Dysplasia of Vulva – Potential Implication for HPV Vaccination

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Background: Reports indicate around 8 million women in US have already received at least one of three-part HPV vaccine Gardasil® since 2006. Speculation is rife for the protective role of Gardasil® against HPV-induced vulvar dysplasia/squamous cell carcinoma.

Design: In this prospective pilot study over 1 year, women from a vulvar surveillance clinic in Memphis were recruited. Lesions with clinical suspicion of vulvar dysplasia were vigorously brushed prior to biopsy. Brushings were collected in PreserveCyte® fluid for HPV-typing through a PCR assay, using a family of primers designated PGMY09/11 that amplify highly conserved L1 region of the HPV genome, known to infect the genital tract. Biopsied tissue was routinely processed through formalin fixation and stained with Hematoxylin and Eosin. Histology and PCR results were then correlated.

Results: Thirty-one lesions from 25 women, ranging in age from 19-65 years, which were reported as VIN1/condyloma at histology, with adequate brush sampling for HPV genotyping, qualified for assessment. Presence of HPV was confirmed in 25/31 lesions (80.7%). In a majority (n=14, 45.2%) of lesions, single HPV-type was present. In 2 (6.5%) lesions, 2 different HPV-types were present and in 9 (29%) lesions, 3 or more HPV types were detected. HPV type-6 was present in 9 (29%) lesions- as single virus type in 4/9 lesions. HPV-11 was identified in only 1 lesion, present as a single virus type. HPV-16 was present in 2 lesions, with 2 or more other HPV-types. HPV-18 was not identified in any sample. Other types included HPV-61 (n=4), HPV-62 (n=3), HPV-83 (n=3), unknown types (n=3), HPV-53 (n=2) and HPV types-32, -33, -52, -54, -58, -66, -67, -72, -LVX160 in single lesions.

Conclusions: In this pilot study useful trends were observed. HPV was not isolated in all cases of low grade vulvar dysplasia (19.4% in this study). Low grade vulvar dysplasia generally harbored a single HPV type (47.6% in this study), though in a significant percentage of cases (29% in this study), 3 or more HPV types were present. HPV-6 was the most common virus type in low grade vulvar dysplasia but large number of low grade lesions (67.7% in this study) did not carry HPV types 6/11. HPV type 16/18 was infrequently identified in low grade vulvar dysplasia. These trends indicate that the currently available vaccine Gardasil® may not be fully protective against low grade vulvar dysplasia.

941 VHL Deficiency in the Female Genital Tract

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Background: von Hippel-Lindau (VHL) disease is an autosomal dominant neoplasia syndrome that results from a germline mutation in the VHL tumor suppressor gene (3p26-p25). Affected individuals are at risk for developing various tumors, benign and malignant, most notably renal cell carcinoma (clear cell type). Papillary cystadenomas of the broad ligament also known as adnexal papillary cystadenoma of probable mesonephric origin (APMO) have been rarely reported and are regarded as the female counterpart of the male epididymal cystadenoma. These lesions form in remnant mesonephric duct tissue, typically embedded in the broad ligament. Recognition of these lesions is critical not only for its association with VHL disease but also for its diagnostic confusion with metastatic renal cell carcinoma and other primary tumors.

Design: Tumor and precursor cells were microdissected and subjected to loss of heterozygosity (LOH) analysis. Control samples were obtained from the matched normal tissue on the same histological slide. The samples were analyzed for LOH with the microsatellite markers D3S1038 and D3S1110 flanking the VHL gene on chromosome 3p25. Additionally, the expression of hypoxia-inducible factors (HIF 1) and (HIF 2) was investigated by immunohistochemistry.

Results: We identified broad ligament cystadenomas with deficiency at VHL gene locus (3p26-p25). PCR based LOH analysis of microdissected tumor cells, revealed a loss of the wildtype copy of the VHL gene. Adjacent normal tissue microdissected from the same slide did not reveal LOH (negative control). As expected for VHL deficient cells, neoplastic epithelium expressed HIF 1 and HIF 2 as well as HIF downstream targets CAIX and GLUT-1.

Conclusions: Results of microdissection with LOH analysis confirm that VHL broad ligament cystadenomas are unique, VHL deficient lesions of the female genital tract that can be useful clinically as an indicator for initiating VHL patient screening workups. Furthermore, awareness and recognition of this unique lesion can prevent misdiagnosis as metastatic renal cell carcinoma.

942 ASC-H Reconciliation: Limitations of Histocytologic Correlation and Importance of Representative Biopsy

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Background: An interpretation of ASC-H on a cervical Pap test represents a differential that includes high grade squamous intraepithelial lesion (HGSIL), immature squamous metaplasia, cervicitis with regenerative changes and lower uterine cell sampling. Colposcopy and directed biopsy are necessary to exclude a high grade lesion. If the biopsy diagnosis does not correlate with cytology, review and reconciliation of the pathology is warranted. Reconciliation requires review of Pap and scrutinization of biopsy for changes, benign or neoplastic, that are representative of or could explain abnormal Pap cytology.

Design: 35 cases interpreted as ASC-H were evaluated retrospectively in conjunction with cervical biopsies. Selection criteria included cases of ASC-H on Pap (Surepath®), that also had biopsies performed concurrently or within prior 6 months. All cases were re-examined microscopically and reviewed to reconcile any discrepancies between Pap and biopsy. Data gathered included adequacy of biopsy, diagnostic classification, discrepancies and if reconciliation between Pap cytology and biopsy was possible. Biopsies were considered adequate only if they contained changes that could explain Pap findings.

Results: Of 35 ASC-H cases, 9 cases (26%) had high grade lesions (CIN II / CIN III) on biopsy that correlated with cytology. The remaining 26 cases (74%) required review for reconciliation of Pap-biopsy discrepancy. After review, 15 cases (58%) remained unreconciled because biopsies did not represent lesion sampled on Pap. In 50% of reviewed cases, we obtained additional diagnostic information such as presence of squamous metaplasia, ulcerative cervicitis, or focal high grade lesions. This additional diagnostic information enabled reconciliation of 11 reviewed cases (42%).

Conclusions: A majority of ASC-H cases could not be reconciled for lack of representative biopsies. Skillfully performed colposcopy is essential to obtaining representative biopsies. When Pap-biopsy discrepancy existed, review with correlation proved imperative, often yielding additional, valuable diagnostic information. Benign changes that could explain abnormal Pap cytology were often omitted from surgical pathology reports. Upon review, identification of benign changes or focal high grade lesions enabled reconciliation of Pap with biopsy.

943 Guanylyl Cyclase C Is a Specific Marker for Differentiating Primary and Metastatic Ovarian Mucinous Neoplasms

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Background: Distinguishing primary ovarian mucinous neoplasms from metastatic mucinous adenocarcinomas can be difficult. Guanylyl cyclase C (GCC) is a brush border membrane receptor for the endogenous peptides guanylin and uroguanylin, and the homologous diarrheagenic bacterial heat-stable enterotoxins that is selectively expressed by epithelial cells from the duodenum to the rectum, but not by normal epithelia of the stomach or esophagus, or normal extramucosal cells in humans. We evaluated GCC

expression in primary ovarian mucinous neoplasms and in primary gastrointestinal adenocarcinomas with ovarian metastases to determine whether GCC could be used for differentiating primary and metastatic ovarian mucinous neoplasms.
Design: A total of 48 ovarian tumors were studied: 27 primary ovarian mucinous neoplasms (7 cystadenomas, 10 borderline tumors, and 10 cystadenocarcinomas) and 21 metastatic mucinous adenocarcinomas (12 colorectal adenocarcinomas, 4 gastric signet-ring cell carcinomas, and 5 appendiceal mucinous tumors including 3 adenocarcinomas and 2 cystadenomas). Immunostains for GCC were performed using formalin-fixed paraffin-embedded tissue. Tumors were scored as follows: negative (no staining in any tumor cells); focal positivity (staining in <5 % of tumor cells); diffuse positivity (staining in > 5% of tumor cells). Only cells exhibiting distinct apical membrane staining, independently of the level of expression, were considered positive for GCC expression. In contrast, cytoplasmic staining was not considered positive staining.
Results: For primary ovarian mucinous neoplasms, 25 of 27 were negative for GCC. Diffuse positive staining for GCC (in both primary and metastatic tumors) was seen in 12/12 colorectal adenocarcinomas, 3/3 appendiceal mucinous adenocarcinomas, and 2/2 appendiceal mucinous cystadenomas. Of 4 cases of gastric adenocarcinoma with ovarian involvement, only 1 (primary tumor) exhibited focal GCC staining.
Conclusions: GCC may be a useful marker for differentiating primary and secondary ovarian mucinous neoplasms.

944 Is the Expression Pattern of BD ProEx™ C the Same as Ki67? A Comparative Analysis in Cervical Intraepithelial Lesions (CIN)

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Background: BD ProEx™ C (ProExC) is a reliable marker for high grade CIN that can be applied to tissue sections to confirm the diagnosis of high grade (HG) CIN and to triage cases of atypical squamous metaplasia. ProExC is a novel immunohistochemical assay that combines two antibodies directed against topoisomerase IIa and minichromosome maintenance protein 2, both of which play important roles in DNA replication during S phase. Ki67, another cell proliferation indicator expressed during all phases of the cell cycle except G0 has also been demonstrated to be aberrantly expressed in HG CIN lesions and is also used as a biomarker for HG CIN. This study was designed to determine if the expression patterns of ProExC and Ki67 are similar.
Design: 158 cases of CIN including 39 benign/reactive, 21 atypical squamous metaplasia (ASQM), 47 low grade CIN, and 51 HG CIN cases were retrieved from our files. All cases had p16, ki67 and ProExC immunostains. Women in the study ranged in age from 19 to 83 years (mean: 36 yrs; median 32 yrs). HE and immunostained slides were evaluated by two pathologists and consensus diagnoses were recorded. Percent of positively stained nuclei were recorded as 0-25, 25-50, 50-75 and 75-100%. Cases with discrepant results were analyzed.
Results: 54 (34%) cases showed discrepant results, the majority (46 cases, 29%) showing a one-step discordance whereas a minority (8 cases, 5%) showed a difference of two steps or more. One-step discordant staining was noted across all morphologic diagnoses (varying between 23-39% of cases in each of the diagnostic categories). Two-step discordant staining was noted most frequently in cases with ASQM (3 cases, 14%). A minority of LG (3 cases, 6%) and HG CIN (2 cases, 4%) also showed this difference. Using >50% positive staining as a cut-off for a positive result, 14 (9%)discordant cases were noted, of which 7 were positive with ProExC (4 of which were strongly positive for p16) and 7 were positive with Ki67, of which 4 were strongly positive for p16.
Conclusions: Although both ProEx C and Ki67 are markers of aberrant proliferation, expression patterns are different in 34% of CIN with significant differences in a minority (5%).

945 Simultaneous Expression of Epidermal Growth Factor Receptor (EGFR) and Cellular Apoptosis Susceptibility Protein (CAS) in Serous Ovarian Carcinoma: Relation with High Grade in a Two-Tier Grading System and Outcome

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Background: This study investigated the simultaneous expression of epidermal growth factor receptor (EGFR) and cellular apoptosis susceptibility protein (CAS) in a cohort of ovarian serous carcinomas with regard to grade in a two-tier grading system (Malpica A et al., Am J Surg Pathol 2004; 496-504) and outcome. The main ligands of EGFR are epidermal growth factor (EGF) and transforming growth factor-α (TGF-α). The CAS gene maps at 20q13.1, is related with TGF-α mediated apoptosis and plays a role at the G1 checkpoint of the cell cycle.
Design: Formalin-fixed, paraffin-embedded archival tissues of 41 ovarian serous carcinomas were stained with antibodies to EGFR and CAS by immunohistochemistry. At first, immunostaining of both factors was scored with regard to quantity and intensity. Subsequently, cases were classified into two different groups of negative or weak (group 1, scores 0 and 1+), and moderate or strong factor coexpression (group 2, scores 2+ and 3+). Grading was based on nuclear atypia and frequency of mitotic figures.
Results: Patients with high EGFR/CAS coexpression (scores 2+ and 3+) had frequently a high tumor grade. One out of 18 grade 1 tumors and 17 out of 23 grade 2 cancers were categorized in group 2 (P<0.0001, Fisher's exact test). A trend for poor outcome was noted in group 2 patients (P=0.0232, logrank test), with a median survival of 20.5 months.
Conclusions: This study indicates that evaluation of dual marker immunoexpression for EGFR and CAS in serous ovarian carcinomas provides further support for the recently proposed two-tier grading system and that multiple marker testing may be an adjunct in the identification of high-risk ovarian serous cancers.

946 SALL4 Is a Novel Sensitive and Specific Marker for Ovarian Primitive Germ Cell Tumors and Distinguishes Yolk Sac Tumor from Clear Cell Carcinoma

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Background: Ovarian yolk sac tumor (YST) is rare but highly malignant. Morphologically YST can closely mimic clear cell carcinoma (CCC) or vice versa. EMA, CK7, CD15, glypican-3 and AFP have been used to distinguish YST from OCC but they lack high sensitivity and/or specificity. The goal of this study is to investigate the diagnostic utility of a novel stem cell marker SALL4 in ovarian YST and other primitive GCTs and its utility to distinguish YST from CCC.
Design: Eighty three ovarian GCTs were retrieved including 24 YST, 18 dysgerminomas, 6 gonadoblastomas (GBs), 2 embryonal carcinomas (ECs), 21 teratomas (10 mature, 11 immature), 7 primary carcinoid tumors, 2 strumal carcinoid tumors, and 3 struma ovarii. To testify SALL4 specificity in ovarian tumors, we also stained 160 primary non-GCTs including 45 CCCs, 20 endometrioid carcinomas, 20 serous carcinomas (CAs) (16 high grade and 4 low grade) , 23 mucinous CAs, 2 transitional cell CAs, 2 hypercalcemic type small cell CAs, 10 Sertoli-leydig cell tumors, 8 steroid cell tumors, 10 adult granulosa cell tumors, 8 juvenile granulosa cell tumors, 6 fibrothecomas, and 6 benign Brenner tumors. Unstained slides generated from 1 to 2 paraffin embedded tissue blocks per case were stained with a SALL4 monoclonal antibody. Only nuclear staining was counted as positive. The staining intensity was scored as weak or strong. The percentage of tumor cells stained was scored semiquantitatively as: 0 (no tumor cell staining), 1+ (<=30% cells), 2+ (31-60%), 3+ (61-90%), and 4+ (>90%).
Results: All 24 YSTs, 18 dysgerminomas, and 2 ECs showed 4+ (>90% tumor cells) strong SALL4 staining. The neoplastic germ cells in GBs showed 4+ strong SALL4 staining in 6/6 cases but the intermingled sex-cord type cells were negative. Eight of 11 immature teratomas showed weak to strong 1+ staining in immature elements and some teratomatous glands. Other germ cell tumors were all negative for SALL4. Among the non-GCTs, only 3 CCCs showed 1+ (less than 1% tumor cells) weak SALL4 staining. The remaining non-GCTs all were negative for SALL4.
Conclusions: SALL4 is a novel sensitive and specific marker for ovarian YST, dysgerminomas, GB, and EC. SALL4 distinguishes yolk sac tumor from CCC.

947 P21 in Endometrial Carcinoma, Correlation with MSI, Pathologic and Clinical Data

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Background: Senescence precludes cells with DNA damage from proliferating. p21 is a CDK inhibitor that mediates p53 dependent senescence. Although p21 polymorphisms may impact the risk of endometrial carcinoma (EC) and p21 expression may correlate with pathological findings such as myometrial invasion, the role of this protein in EC is not clear. There is some evidence that p21 may be downstream of PMS2 in mice, potentially linking cell cycle regulation and senescence with DNA mismatch repair (MMR). We investigated the expression of p21 in human EC and its relationship with clinical outcome, histologic grade, myometrial invasion, p53, CCND1 and microsatellite instability (MSI).
Design: Immunohistochemistry (IHC) using p21, p53 and CCND1 monoclonal antibodies was performed on tissue microarrays including 368 EC. Only nuclear positivity was recorded for this IHC. MSI had been determined by a genotyping method as part of previous studies. Log-Rank tests were used to compare for overall and progression free survival . Chi-square tests were used to compare proportions.
Results: 133(36.1%) tumors had no detectable p21 immunoreactivity and only isolated (1-5%) p21 positive nuclei was seen in 185(50.3%). 41 (11.1%) tumors had 6-30% positive nuclei and 9 (2.4%) had >30%positive nuclei. Nuclear reactivity for p21 was strong in 44 (88%) of tumors with >6% positivity. Although p21 expression was associated with a higher proportion of high grade histology (p=0.014) and presence of myometrial invasion (p=0.026), no statistically significant difference between early and late stage cases and overall and progression free survival was detected. There was a significant association between p21 and CCND1 immunoreactivity (p<0.01) but p53 (p=0.109) and MSI (p=0.255) did not show statistical significance.
Conclusions: While p21 nuclear expression is detectable by IHC in a majority of EC (63.9%) it is more often present only as isolated, scattered nuclei. However, a significant minority (13.5%) of EC have >6% positive nuclei with strong p21 immunoreactivity. Expression of p21 in EC is associated with high grade histology and myometrial invasion but not with clinical outcome. p21 and CCND1 are coexpressed in a significant number of EC probably reflecting their roles in cell cycle regulation. We present evidence against a link between DNA MMR, as determined by MSI, and p21 related senescence in EC.

948 Low Density Lipoprotein Receptor-Related Protein (LRP-1) Expression in Endometrial Carcinomas

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Background: Low density lipoprotein receptor-related protein (LRP-1) is a multifunctional cell surface receptor with a wide variety of ligands that modulate key processes in cancer regulation. Little is known about LRP-1 activity in endometrial carcinomas.
Design: LRP-1 expression was analyzed in 110 endometrial carcinomas (85 endometrioid endometrial carcinomas [EEC], 13 non-endometrioid endometrial carcinomas [NEEC], and 12 mixed EEC-NEEC). mRNA analysis was performed by real time PCR and protein expression was evaluated on tissue arrays by immunohistochemistry. Results of LRP-1 expression were compared with various genetic alterations including MI, *PTEN, PIK3CA, p53, K-RAS, CTNNB1* alterations, with the clinicopathologic parameters, and

patients follow-up.
Results: LRP-1 immunostaining was detected in 27% (30/110) of cases. Histologic type and grade varied significantly according to the expression of LRP-1 (*P*=0.001 and *P*=0.000, respectively); LRP-1 immunoreaction was more frequent in NEECs (61.5%) and mixed carcinomas (50%) than in pure EECs (19%). Strong immunostaining was more commonly found in grade 3 (48%) than in grade 2 (12%) or grade 1 (11%) carcinomas. Almost all tumors with LRP-1 expression had deep myometrial invasion but the association did not reach statistical significance (*P*=0.081). There was correlation between *LRP-1* mRNA expression and immunohistochemical staining. mRNA expression was stronger in NEECs and mixed tumors than in EECs and normal tissues. *p53* alterations (strong immunoexpression or mutation) were more frequently found in carcinomas with LRP-1 expression than in carcinomas lacking it.
Conclusions: Expression of LRP-1 in NEECs is stronger than in EECs and could contribute to the adverse prognosis of the former tumors.

949 Multiple Gene Expression Analysis by Taqman Low-Density Array in High-Grade Endometrial Adenocarcinomas

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Background: Gene expression profiling is an important tool to evaluate genetic heterogeneity in carcinomas and to identify tumor-specific genes.
Design: mRNA level expression was analyzed in 22 endometrial carcinomas (9 EEC, 8 NEEC, and 5 mixed carcinomas EEC-NEEC). Gene expression analyses was performed at mRNA level by Taqman low-density array (TLDA). We were configured the TLDA card with 22 genes involved in PIK3/AKT signaling pathway and apoptosis, and two housekeeping genes, *GAPDH* and *ABL-1*. PCR amplification was performed using an Applied Biosystems 7900HT PCR system. Hierarchical cluster analysis was performed with *Cluster* program and visualized with the *TreeView* program from Stanford (*http://rana.lbl.gov/EisenSoftware.html*). The results of the mRNA expression were correlated with MI, *PTEN, PIK3CA, p53, K-RAS, CTNNB1* and with clinical, pathologic parameters, and patients' follow-up.
Results: Hierarchical cluster analysis revealed two subgroups of high grade endometrial carcinomas: one group of 15 tumors showed overexpression of genes involved in PIK3/AKT signaling pathway (*AKT1, AKT2, FAK, PAK1, GSK3B*, and *mTOR*), and apoptosis (*XIAP, p53, NFKB,* and *caspase 3*). The other group of 7 tumors showed lower expression of these markers. Interestingly, in the first group there was high frequency of exon 20 *PIK3CA* or *PTEN* mutations (10/15) whereas the second group had p53 alterations (6/7). Coexpression of *AKT1* and *AKT2* and correlation with exon 20 *PIK3CA* mutations were found. There was no correlation with histological type or clinicopathological features. However, all tumors were high-grade carcinomas.
Conclusions: Hierarchical cluster association in multiple gene expression analysis for the PIK3/AKT and apoptosis showed two groups of high-grade endometrial carcinomas: one correlated with exon 20 *PIK3CA* or *PTEN* mutations and the other with p53 alterations.

950 ALDH1 Expression in Ovarian Cancers: Correlation with Histologic Type and Clinical Prognosis

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Background: Aldehyde dehydrogenase 1 (ALDH1), a detoxifying enzyme responsible for the oxidation of intracellular aldehydes. ALDH may have a role in early differentiation of stem cells, through its role in oxidizing retinol to retinoic acid. It has been shown that ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome in breast cancer. The authors hypothesized that the expression of ALDH1 may be correlated with the clinical outcome of the patients with the ovarian cancer.
Design: Immunohistochemical staining of ALDH1 expression was analyzed in 442 primary ovarian carcinomas using tissue microarray. The association between the expression of the ALDH1 and the clinical factors including diagnosis, tumor grade, stage, ascities, and clinical response of the chemotherapy, as well the overall survival (OS) and disease free survival (DFS) was analyzed.
Results: Expression of ALDH1 was found in 49.3% of the samples. Fisher's exact test suggested that high expression of ALDH1 was significantly associated with endometroid adenocarcinoma ($P < 0.0001$), early-stage disease ($p = 0.006$), low grade (OR=10.54, p=0.0002), completely response of the chemotherapy ($p = 0.007$), and the low level of the serum CA125 ($P = 0.02$). A high percentage of ALDH1 expression was associated with a longer overall survival time in univariate analyses.
Conclusions: In contrast to breast cancer, ALDH1 was identified as an independent favorable prognostic factor in patients with ovarian carcinoma.

951 An Early Precursor to Pelvic Serous Carcinoma (p53 Signature): Site Specificity and Impact of Genetic Risk (BRCA)

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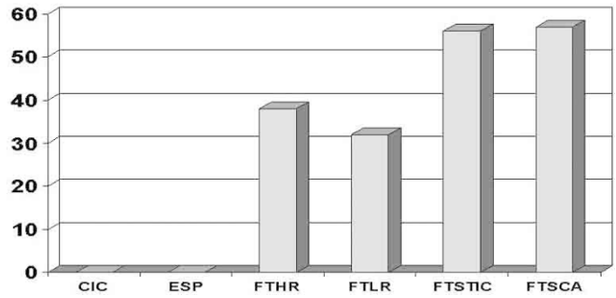
Background: The p53 signature, an entity in the distal fallopian tube, has been linked to early serous carcinoma and is proposed as an early phase of serous carcinogenesis. However, the distribution of the p53 signature in the pelvis has not been systematically determined.
Design: We analyzed cases with benign müllerian epithelium in the ovaries (CIC, 75), endosalpingiosis (ESP, 54), fallopian tubes from BRCA+ women (FTHR, 75), controls (FTLR, 41), women with serous tubal intraepithelial carcinoma (FTSTIC,17), and serous carcinoma (FTSCA, 33) for p53 signatures by p53 immunostaining.
Results: p53 signatures were identified in 0, 0, 38, 32, 53 and 57 percent of CIC, ESP, FTHR, FTLR, FTSTIC, and FTSCA, respectively. With rare exceptions, p53 signatures were identified only in epithelium of the salpinx proper and were significantly more common in fallopian tubes containing STIC or in association with pelvic serous carcinoma (see figure). Genetic risk (BRCA) does not influence frequency of p53 signatures.



Conclusions: p53 signatures are significantly associated with the fallopian tube ($p < 0.001$) and in that organ, with malignancy ($p = 0.037$). The evidence strongly suggests that the majority of serous carcinomas that are not directly associated with a pre-existing condition (such as endometriosis) arise either in the fimbria or in salpingeal epithelium that is in continuity with the fimbria (adhesions). The terms "fimbrial ovarian" or simply "pelvic" serous carcinoma are more accurate descriptors for this disease.

952 "Progression" from LSIL to HSIL: Interpretation and Significance

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Background: In practice and in research, progression from a low to high grade squamous intraepithelial lesion of the cervix is an important outcome variable that is vital to both diagnostic and therapeutic strategies. The goal of this study was to ascertain the validity of such an interpretation in a series of biopsies with an initial histological interpretation of LSIL by report, in the context of histologic re-review of initial and outcome pathology and immunostaining for p16.
Design: Consecutive cases of LSIL based on biopsy material were obtained from the archives. Follow-up cases were reviewed with attention given to those with a subsequent HSIL (CIN2 or 3) diagnosis based on biopsy, LEEP or cone material. In all such cases, the initial and subsequent pathology was re-reviewed, and diagnoses were confirmed or re-classified as LSIL or HSIL. A "numerical severity score" (NSS) was recorded for each case: LSIL (1-2), CIN2 (3-4) and CIN3 (5-6) with lower and higher values corresponding to the degree (low vs high) of histologic severity within each category.
Results: 264 consecutive biopsy cases of LSIL were reviewed. 29 (11%) were associated with a subsequent HSIL diagnosis, and material for review (both initial and subsequent cases) was available in 24 of these cases. SIL was confirmed in all initial biopsies with 22 confirmed LSILs; 2 cases were re-classified as HSIL. In the former group of 22 cases, the subsequent biopsy was originally diagnosed as CIN2 and CIN3 in 12 and 10 cases, respectively. Only 3 cases were confirmed as HSIL with high NSS (5- 6); the remainder were re-classified as LSIL, including 6/12 CIN2 cases with a NSS=2 (more severe LSIL) and 6/12 CIN2 cases with a NSS=1 (less severe LSIL). In 18/24 cases (75%), the initial biopsy and the subsequent specimen interpretation was within 1 NSS (e.g. LSIL(1) vs LSIL(2)); whereas, in 6/24 cases the difference in NSS was 2 points or greater (e.g. LSIL(2) vs HSIL(4)). P16 immunostains on 12 sets of cases showed identical immunoreactive staining patterns in 6, and disparate staining patterns in the remaining 6.
Conclusions: Based on this study, the most likely explanations for "progression" from LSIL to HSIL are, in increasing frequency, 1) actual progression, 2) under-diagnosis of HSIL on initial biopsy, 3) change in HPV type over time (based on p16 stain discrepancy), and 4) over-diagnosis of HSIL on subsequent biopsy/cone. The latter is accentuated when the outcome diagnosis is CIN2, underscoring the importance of histologic re-review before assuming that histologic "progression" has taken place.

953 Indication and Method of Frozen Section in Vaginal Radical Trachelectomy

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Background: Vaginal radical trachelectomy (VRT) is a fertility-sparing surgical technique used as an alternative to radical hysterectomy in early stage cervical cancer. Defining the method of per-operative evaluation of VRT has become imperative because if tumor is found within 5mm of the endocervical margin, additional surgical resection is required. However, there are no agreed-upon protocols on how to sample VRT for frozen section (FS). In a previously published study from our center, we concluded that a FS should be done only when a cancerous lesion is grossly visible and it could be omitted in normal-looking VRT or VRT with non-specific lesions. We recommended that only one sample should be taken and that a longitudinal sample is superior to a transversal sample because it allows a precise evaluation of the distance between the margin and the tumor. Since 2002, we performed FS on VRT according to these recommendations and the current study is a reappraisal of our performance.
Design: Cases of VRT accessioned between January 2002 and August 2007 were retrieved from the pathology archive. Cases were classified into three categories: normal-looking VRT (VRTn), VRT with non-specific lesions (VRTns) and VRT with grossly visible cancerous lesions (VRTg). The data collected was: presence of residual invasive disease, final distance to the margin on paraffin sections and adequacy of the FS to predict the final distance to the margin. The final distance to the margin was considered satisfactory when it measured ≥5mm.

Results: We identified 53 VRT performed in our center since 2002; 15 were classified as VRTn, 24 as VRTns and 14 as VRTg. Final margins were satisfactory on all 15 VRTn. Of the 24 VRTns, 2 cases for which no FS was performed had unsatisfactory final margins (<5mm). Of the 14 VRTg, 3 cases were inadequately evaluated by FS due to sampling error which led to unsatisfactory final margins. There was no residual invasive disease in 14/15 VRTn (93.3%), 20/24 VRTns (83.3%) and 1/14 VRTg (7.1%).
Conclusions: Our results confirm that FS can be omitted on VRTn. However, we now recommend that a FS be performed on all VRTns (2 cases with unsatisfactory margins). As for VRTg, a FS should always be performed. We recommend that more than one sample should be submitted to improve the adequacy of FS because additional sampling of the 3 VRTg with unsatisfactory margins would have likely assessed the margin accurately.

954 The Fibroblast Growth Factor Receptor-4 Arg388 Allele Is Associated with Higher Grade and Lymphovascular Space Invasion in Endometrioid Type of Endometrial Adenocarcinoma

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Background: Recent studies have shown that a single nucleotide polymorphism at codon 388 of fibroblast growth factor 4 (FGFR-4) is associated with worse prognosis in patients with breast, colorectal, and prostate cancer and with high grade soft tissue sarcomas.
Design: Tissue samples from 134 patients with endometrial adenocarcinoma were obtained. Genotyping of the FGFR 4 Gly388Arg polymorphism was determined by restriction fragment length polymorphism assay. The genotype was correlated with clinical and pathological markers of disease aggressiveness.
Results: We examined FGFR4 polymorphism status in 134 endometrial cancers of which, 28 were serous, 79 were endometrioid, 6 were clear cell, and 21 were mixed. Results were obtained in 131 cases and overall, 51 (39%) patients were wild type, 71 (54%) were heterozygous and 9 (7%) were homozygous for the polymorphism. When cases with the FGFR4 polymorphism (heterozygous and homozygous) versus wild type was anlaysed in the entire cohort, no association with cell type or outcome was found. Subtype-specific analysis revealed an association with higher grade, presence of lymphovascular space invasion and recurrence in the endometrioid subtype. No such association was seen in other cell types.
Conclusions: Homozygosity and heterozygosity for the fibroblast growth factor receptor-4 Arg388 allele is associated with higher grade and lymphovascular space invasion in endometrioid type of endometrial adenocarcinoma.

955 Endosalpingiosis in Ovarian Serous Tumors of Low Malignant Potential

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Background: It has been suggested that a certain proportion of serous tumors of low malignant potential (SLMP) may arise from endosalpingiosis, and that endosalpingiosis may be the progenitor lesion in a proportion of non-invasive peritoneal implants as well as in some SLMP proliferations in lymph nodes. The objective of this study was to examine the rate of endosalpingiosis in nodal and extranodal tissue ovarian SLMP (OSLMP) cases relative other types of Mullerian malignancies.
Design: Thirty cases of ovarian serous tumors of low malignant potential with at least two lymph node sites and two peritoneal sampling sites were randomly selected from our institutional database. Two separate control groups consisting of thirty cases of radical hysterectomy cases for cervical adenocarcinoma and thirty total hysterectomy cases for endometrial endometrioid carcinoma were retrieved and matched to the experimental group based on the year of tumor diagnosis. All cases in each control group contained at least two lymph node sites and two peritoneal sampling sites. The number of lymph node sites and peritoneal sampling sites was recorded for the study group and each of the control groups. The statistical comparison between the study group and each of the control groups was made using the two-tailed Fisher exact test.
Results: The average number of lymph node sites examined was 3.5 for OSLMP, 4.1 for cervical adenocarcinoma, and 4.2 for endometrial carcinoma. The average number of peritoneal sampling sites was 5.9 for OSLMP, 3.8 for cervical adenocarcinoma, and 3.7 for endometrial carcinoma. Nodal endosalpingiosis was present in 30% of OSLMP cases, compared with 0% in cervical adenocarcinoma (p=0.0026) and 3% in endometrial carcinoma (p=0.0056). Five of the thirty OSLMP cases also showed lymph node involvement by SLMP. Extralymphatic endosalpingiosis was identified in 40% of OSLMP cases compared with 13% in cervical adenocarcinoma (p=0.0011) and 3% in endometrial carcinoma (p=0.0391).
Conclusions: The incidence of endosalpingiosis in OSLMP in extranodal tissue and lymph nodes is significantly greater than that found in matched cases of both cervical adenocarcinoma and endometrial carcinoma. This is the first study to document the relative incidence of endosalpingiosis in OSLMP compared to other types of Mullerian malignancies and it raises the question of whether endosalpingiosis should be considered a risk factor for the development of SLMP tumors.

956 Role of GLUT1 as a Marker for Endometrial Malignancy

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Background: Among cases of endometrial hyperplasia, complex atypical hyperplasia carries the highest risk of being associated with or progressing to endometrioid carcinoma (20-30%). However, the diagnosis of "atypia" is fraught with considerable inter- and intraobserver variability. Also, up to 3% of cases without atypia may progress to carcinoma. The transmembranous glucose transporter-1 protein (GLUT1) is overexpressed in many carcinomas. We hypothesized that GLUT1 might be a marker for high risk of concomitant or subsequent endometrial carcinoma in biopsy material that shows hyperplasia but no morphologically diagnostic carcinoma. We correlated immunohistochemical GLUT1 expression in biopsies of hyperplastic endometrium with the presence of endometrial carcinoma in subsequent hysterectomy specimens.
Design: We analyzed 17 uteri removed for a diagnosis of endometrial hyperplasia based on a preceding biopsy. The uterus had endometrial carcinoma in 7 cases, while 10 cases showed either persistence or resolution of hyperplasia but no carcinoma. All biopsy material and selected hysterectomy sections were examined for GLUT1 immunoreactivity. Staining intensity was graded from weak (1+) to strong (3+), and the percentage of positive cells was estimated. Membranous GLUT1 expression in the biopsy was correlated with the presence or absence of carcinoma in the uterus. Fisher's Exact Test was used for statistical analysis.
Results: 5 of the 7 endometrial biopsies associated with carcinoma in the subsequent hysterectomy showed positivity and of these 4 had at least focal 2+ staining. Of the 10 biopsies not associated with carcinoma, only 2 had any staining which was weak and present in less than 10% of the specimen. The presence of any GLUT1 staining in the biopsy was associated with a high risk of carcinoma in the uterus, almost reaching statistical significance (p = 0.058). Moderate staining (>1+) in biopsies was statistically significant (p value = 0.0147).
Conclusions: GLUT1 was expressed in the majority of biopsies with endometrial hyperplasia whose subsequent hysterectomy specimen contained endometrial carcinoma. It was only infrequently expressed in hyperplastic biopsies when carcinoma was absent from the hysterectomy specimen. The presence of moderate staining in biopsies is a statistically significant predictor of carcinoma in subsequent hysterectomies. Our results suggest that GLUT1 may be a marker of high risk for concomitant or subsequent endometrial carcinoma in biopsies showing endometrial hyperplasia but no morphologically diagnostic carcinoma.

957 CpG Island Methylation within the TCF2 Promoter May Enable Epigenetic Modulation of HNF1-beta and Clear Cell Phenotype in Ovarian Clear Cell Carcinoma

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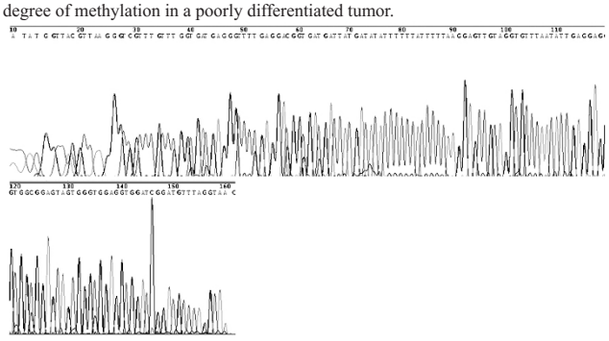
Background: Hepatocyte nuclear factor 1-beta (HNF1-beta), a hepatic and pancreatic specific transcription factor that is encoded by the TCF2 gene, is upregulated in ovarian clear cell carcinoma gene expression arrays. To determine whether HNF1-beta expression is lineage specific or more broadly expressed by other clear cell neoplasms, we developed a lexicon of HNF1-beta protein expression in a series of anatomically and histologically diverse clear cell tumors and evaluated potential epigenetic modulation of HNF1-beta expression.
Design: A monoclonal antibody directed against HNF1-beta (clone C-20, Santa Cruz, titer 1:2000) was evaluated on an anatomic and histologically diverse (n=929) set of tissue microarray and conventional tissue sections enriched for renal and gynecologic primaries with cytoplasmic clearing (n=261). Immunostaining was scored as negative, weak or strongly positive and the significance of expression was judged using the Fisher's exact test. Single-gene methylation analysis was performed to evaluate the CpG island in the promoter region of the TCF2 gene. DNA was extracted from a variety of ovarian carcinomas with a lymphocyte control. Following bisulfite modification, methylation specific PCR with primers to the promoter region of the TCF2 gene (HNF1- beta) was used to amplify a ~140 bp fragment which was analyzed by Sanger sequencing.
Results: HNF1-beta was identified in 74% (192/261) of tumors with clear cell change compared to 8.8% (59/668) of tumors without clear cell change (p<0.0001). Differential methylation was identified among subtypes of epithelial ovarian tumors. Serous histology showed a relative increase in CpG island methylation in the promoter of the TCF2 gene in comparison to clear cell, endometrioid and lymphocyte control with an intermediate degree of methylation in a poorly differentiated tumor.



Conclusions: Expression of HNF1-beta is associated with clear cell phenotype across anatomically and histologically diverse tumors. Promoter methylation may serve a permissive mechanism where absent or low level methylation is complicit in transcriptional regulation and potentially, phenotypic differentiation.

958 Ovarian Endometrioid and Clear Cell Carcinoma Arise Via Different Precursor Lesions and Have Better Prognosis When Associated with Endometriosis

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Background: Ovarian clear cell carcinoma (CCC) and endometrioid carcinoma (ECC) often develop in association with endometriosis, yet they are considered to have significantly different clinical behavior. To identify distinctive features that may play

a role in the differing biology of these tumors, we evaluated a series of CCC and ECC from the files of Stanford University.

Design: 60 CCC and 61 ECC were analyzed for the presence of associated ovarian/pelvic endometriosis, ovarian/pelvic adenofibroma, and synchronous uterine endometrial hyperplasia (U-EH) or endometrioid carcinoma (U-ECA). Tumors were classified on the basis of standard criteria; specifically, ECC required rounded gland contours and/or squamous differentiation. Results were correlated with patient age, FIGO stage, presence of bilateral ovarian disease, and follow up, when available.

Results: Endometriosis was significantly more common in association with ovarian CCC than ECC ($p<0.0001$), while synchronous endometrioid tumors were more common in association with ovarian ECC ($p<0.0001$). Bilateral ovarian involvement in CCC, when present, typically exhibited a metastatic pattern and only occurred in the setting of high stage disease. However, 18% of ECC were bilateral and 73% occurred in the setting of low stage disease (FIGO IB).

Feature	CCC-Endo	CCC-Adfib	CCC-Neither	ECC-Endo	ECC-Adfib	ECC-Neither
Age (mean)	50	55	56	49	56	50
Frequency	57%	15%	28%	16%	34%	50%
Bilateral	4%	0%	5%	20%	15%	16%
Stage I	47%	75%	25%	90%	100%	93%
Stage II-IV	53%	25%	75%	10%	0%	7%
U-EH	0%	0%	0%	0%	15%	10%
U-ECA	3%	12%	5%	33%	10%	45%
NED	66%	50%	44%	87%	89%	86%
AWD/DOD	33%	50%	56%	13%	11%	14%

Endo=endometriosis; Adfib=adenofibroma; U-EH=uterine endometrial hyperplasia; U-ECA=uterine ednometrioid carcinoma; NED=no evidence of disease; AWD=alive with disease; DOD=dead of disease

Conclusions: Of the observed clinicopathologic features, none contributed significantly to FIGO stage or survival in either CCC or ECC. CCC predominantly arise in association with ovarian/pelvic endometriosis, while ECC arise in the setting of synchronous endometrial carcinoma or adenofibroma. When strictly defined, most ECC are low stage and clinically benign. This data suggests a field defect in ovarian tumors with endometrioid histology, while no such defect appears to be operative in ovarian CCC.

959 Clinicopathologic and Immunohistochemical Analysis of 34 Uterine Sarcomas and 18 Benign Related Tumors

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Background: Uterine sarcomas account for only 3% of uterine cancers. Knowledge about their pathogenesis is limited and there is no consensus on prognostic parameters and optimal treatment. Most series have focused on specific histologic types, and few comparative studies including all major categories have appeared.

Design: Twenty leiomyosarcomas, 5 undifferentiated endometrial sarcomas (5 with pleomorphic and 2 with uniform nuclei), 12 endometrial stromal tumors, and 15 leiomyomas, were reviewed. Diagnostically equivocal categories were not included. Clinicopathologic data and follow-up were obtained. Tissue microarrays were immunostained for 16 selected proteins involved in cell proliferation, cell differentiation, and apoptosis. Hierarchical cluster analysis of the immunohistochemical results was performed.

Results: Most high-grade sarcomas (12 of 18 leiomyosarcomas and 4 of 5 undifferentiated endometrial sarcomas) with moderate to severe nuclear atypia, high mitotic index, and/or tumor cell necrosis, were associated with poor outcome, even if diagnosed at an early stage. In contrast, all patients with endometrial stromal sarcomas showing only mild nuclear atypia, low mitotic activity, and lacking necrosis, survived. Undifferentiated endometrial sarcomas with nuclear pleomorphism behaved more aggressively than tumors showing nuclear uniformity. Expression of Ki67, p53, p16, and Twist was stronger in high-grade sarcomas than in endometrial stromal sarcomas which, in contrast, immunoreacted for PR, bcl-2, and CD-10. However, 3 leiomyosarcomas associated with prolonged survival failed to react for Ki67, p53, p16, and Twist, and showed a strong immunoreaction for bcl-2. These tumors, which exhibited the morphologic features of malignancy, clustered with benign leiomyomas.

Conclusions: High-grade uterine sarcomas are associated with poor prognosis, even if surgically treated at early stage (stage I). Undifferentiated endometrial sarcomas, showing nuclear uniformity may represent an intermediate category equivalent to what was formerly called "high-grade endometrial stromal sarcomas". High-grade uterine sarcomas show stronger expression of Ki67, p53, p16, and Twist, than endometrial stromal sarcomas. Leiomyosarcomas that fail to react for Ki67, p53, p16, and Twist, and show a strong bcl-2 immunoreaction, may represent a subset of uterine sarcomas associated with specific molecular genetic changes and inhibition of apoptosis.

960 $p16^{INK4a}$ Staining Characteristics in Endometrioid Endometrial Adenocarcinoma; Information Useful When Establishing Cervical or Endometrial Origin

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Background: Distinguishing endometrioid adenocarcinomas arising from cervix from those arising from uterus is clinically important as therapeutic options vary with site-of-origin. Markers commonly applied in this scenario include ER, vimentin , CEA and more recently $p16^{INK4a}$. Tumours from the cervix typically exhibit moderate to strong p16 staining in the majority of tumour cells. Conversely, less than half of tumour cells from endometrial tumours stain, often with lower intensity. A detailed description of p16 staining across histologic grade and architectural pattern (glandular, solid and squamous) has, to our knowledge, not been reported. Of particular interest, since biopsies are often small, were the proportion of strongly staining glands and the size of foci exhibiting solid, intense staining, as these features may introduce diagnostic uncertainty.

Design: 33 randomly selected well, moderately, and poorly differentiated endometrioid endometrial carcinomas in hysterectomy specimens were screened and representative tissue blocks were identified (n=32, 32 and 29, respectively). Sections were stained with p16 antibody (16PO4). The intensities of p16 staining within glandular, solid and squamous components were scored. The percent of glands comprised exclusively of strong staining cells and the length of largest staining focus were also recorded.

Results: Overall, 43% of tumour cells did not stain for p16. Squamous areas had a larger proportion of cells staining with moderate or strong staining than did glandular areas (53% vs 39%, $p<0.05$). A trend toward stronger staining in high grade tumours ($p=0.085$, ns) was noted. High grade tumours more frequently had ≥10% of glands with exclusively strong staining cells (6/29) versus well (0/32) and moderately (2/32) differentiated tumours, and also more frequently had solid staining foci measuring >2mm (9/29 versus 0/32 and 3/32, respectively).

Conclusions: 1) 57% of tumour cells stained for p16 while 43% did not. 2) Albiet by a small degree, squamous areas stained for p16 more intensely than did glandular areas. 3) There was a non-significant trend of stronger p16 staining in high grade tumours. 4) When trying to distinguish the site of origin (cervical versus endometrial) on scanty biopsies / currettings, the presence of solid, strong foci of p16 staining or strong staining in ≥ 10% of glands may be consistent with an endometrial primary if other evidence is supportive.

961 The Morphologic Spectrum of Immunohistochemically Characterized Clear Cell Carcinoma of the Ovary: A Study of 83 Cases

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Background: Establishing a diagnosis of ovarian clear cell carcinoma (OCC) can be subject to significant interobserver variation. Accurately diagnosing this tumor is important because of its chemoresistance and reported association with hereditary nonpolyposis colon cancer (HNPCC). The spectrum of morphologic features of OCC has not been well described in a series composed of immunohistochemically characterized cases.

Design: Eighty-three cases were retrieved from the files of two institutions in order to analyze the architectural and cytological features of OCC. The cases were previously studied with immunohistochemistry; approximately 80% were positive for HNF-1beta, and negative for p53, ER, PR, and WT1. A comprehensive list of architectural and cytologic features was analyzed including, but not limited to, nuclear pleomorphism (>3x variation in size), cytoplasmic characteristics, background changes, and mitotic rate (mitoses per 10 HPF). Between 1 and 13 slides were available for review for each case.

Results: Background changes included endometriosis (19/83, 23%), adenofibroma (19/83, 23%), or neither (37/83, 54%). OCCs most commonly demonstrated a mixture of architectural patterns including papillary, tubulocystic, and solid (29/83, 35%). A papillary component was present at least focally in the majority of the cases (62/83, 75%). The papillary patterns included non-hierarchical branching (59/62, 95%), the presence of small and round papillae (57/62, 92%), and detached micropapillary clusters (22/62, 35%), but rarely stratification of more than 3 cells thick. Nuclear pleomorphism and macronucleoli, if present, were only rarely diffuse (7/83, 8%). Clear cytoplasm was frequently found at least focally (78/83, 94%) as was hobnailing (63/83, 76%). The mitotic rate ranged from 0-10 with an average of 2-3. Other features often identified in OCCs included hyaline globules (33%), targetoid cells (18%), psammoma bodies (18%), a lymphoplasmacytic infiltrate (17%), and nuclear pseudoinclusions (12%).

Conclusions: Most OCCs have at least a focal papillary component. Recognizing the shape of the non-hierarchically branched papillae, lack of epithelial stratification, coupled with frequent yet only focal nuclear pleomorphism and a low mitotic rate should enable reproducible separation from entities in the differential diagnosis.

962 p16 Immuno-Cytochemistry for the Triage of ASC-US and LSILPap Cytology Cases – Results from a Large Retrospective Study

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Background: Detection of over-expression of cell cycle regulator protein p16 in cervical cytology specimens as a surrogate marker for transforming HPV infections has been described in the literature. However, there is substantial variance regarding methods used for slide preparation, staining for p16, and slide interpretation. Typically, total numbers of cases are low, and biopsy follow-up data were only reported in a few studies. We therefore evaluated the performance characteristics of p16 immuno-staining in cervical cytology specimens categorized as ASC-US or LSIL to identify women with underlying CIN2+ in a large retrospective study.

Design: 810 LBC samples (ThinPrep, Cytyc) less than 3 years old and categorized as either ASC-US (n=385) or LSIL (n=425) on Pap cytology were retrieved from the archives of 5 cytopathology laboratories. Availability of histology follow-up data and access to corresponding paraffin tissue blocks was a selection criterion. Cytology slides were stained for p16 using the CINtec Cytology Kit (mtm) and reviewed by two cytopathologists and cytotechnologists. The presence of cells immuno-reactive for p16 and showing morphologic abnormalities generated a positive test result. hc2 HR HPV testing (Qiagen) was performed on all specimens. Sections from the original tissue blocks comprising the lesions showing the worst morphology were prepared and used to establish an expert gynecopathologists consensus diagnosis that served as Gold standard for study purposes.

Results: For p16 cytology, sensitivity of adjudicated cytopathologist results for the identification of women with underlying CIN2+ reached 95% for ASC-US and 96% for LSIL cases. Specificity rates for p16 cytology slide reviews by the cytopathologists

ranged from 66 to 71% for the ASC-US group, and from 47 to 53% for LSIL cases. For cytotechnologist reviews of the slides, sensitivity rates for CIN2+ were found at similar rates (ASC-US: 93%; LSIL: 92%), and specificity rates were 63% for ASC-US and 37% for LSIL cases, respectively. HPV testing resulted in sensitivity levels of 90% (ASC-US) and 96% (LSIL), and specificity rates of 38% (ASC-US) and 19% (LSIL).
Conclusions: Interpretation of p16 cytology slides has the potential to provide a comparable sensitivity, but significantly higher specificity for the identification of high-grade CIN as HPV testing in the triage of women with Pap cytology results categorized as ASC-US or LSIL.

963 Overexpression of EGFR Is a Poor Prognostic Indicator in Ovarian Cancer

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Background: Epidermal growth factor receptor (EGFR) is a surface-bound receptor tyrosine kinase of the ErbB receptor family, which plays an important role in the control of cellular proliferation, differentiation and survival. EGFR signaling is dysregulated in a variety of solid tumors, and targeted therapy against EGFR is now available. Studies of EGFR expression in ovarian carcinoma to date have had variable results. The goal of this project is to characterize the expression of EGFR in different histologic types of ovarian carcinoma and evaluate EGFR as a prognostic marker.
Design: Samples from women who had primary epithelial ovarian carcinoma and had undergone initial surgery at our institution between 1990 and 2001 were identified and treatment and follow-up information was updated to June 2005 through review of medical records. Parameters recorded for each case included histological type, grade, stage, extent of debulking surgery, clinical response to platinum-based therapy, disease free interval (DFI) and overall survival (OS). Tissue microarrays were constructed and using 1mm cores. The immunohistochemical staining for EGFR was performed. EGFR staining was scored as 0 (no staining), 1+ (<20% of cells staining or faint staining), 2+ (20%-50% of cells staining or incomplete strong membrane staining) and 3+ (>50% cells staining with complete membrane staining). Differences in proportions were evaluated by chi square analysis. Disease survival rates were calculated using the Kaplan-Meier method and were compared using the log-rank test.
Results: 297 cases were identified comprising 240 serous, 33 endometrioid, 17 clear cell and 7 mucinous adenocarcinomas. Serous carcinomas showed a higher proportion of cases with high EGFR expression compared to endometrioid ($p<0.05$) and clear cell carcinoma ($p<0.05$). Patients with no clinical response to platinum-based therapy had higher levels of EGFR relative to those with partial response ($p<0.05$). A higher proportion of patients who did not have disease relapse had a negative to low expression of EGFR compared to patients that relapsed ($p<0.05$) or had progressive disease ($p<0.05$). Finally, patients with negative to low EGFR expression levels had better OS and DFI than patients with moderate to high expression.
Conclusions: High EGFR expression is a negative prognostic marker in ovarian carcinoma. In addition, overexpression of EGFR has a high association with serous ovarian carcinoma. These findings suggest that EGFR may be an important therapeutic target in ovarian carcinoma, and particularly in serous carcinoma.

964 Lymph Node Involvement in Ovarian Serous Tumors of Low Malignant Potential

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Background: Lymph node involvement (LNI) in ovarian serous tumors of low malignant potential (OSLMP) has been reported in 21% to 42% of OSLMP. However, there is still limited experience with the clinical significance of LNI as well as with the variety of histologic patterns of LNI.
Design: 28 cases of OSLMP with LNI with available slides were retrieved from our files. A control group of 28 OSLMP with at least two sites of lymph node resection and no LNI was established by matching the year of diagnosis for each study case. Clinical follow-up was obtained from review of the medical records and from phone calls to the attending physicians. The pathologic features recorded included microinvasion and micropapillary/cribriform (MP/CP) pattern in the OSLMP and invasive and non invasive implants. The histologic features of LNI recorded were pattern (single cells, clusters, micropapillae, small papillae, papillae, glandular and intraglandular), location (subcapsular, medullary, parenchymal, lymphatics within the capsule) and endosalpingiosis. Statistical comparisons were made using the Fisher exact test.
Results: The mean patient age in the study group was 39.2 and 49.0 in the control group. Differences in microinvasion, MP/CP pattern, invasive and non invasive implants between the study and the control group were not statistically significant. However, OSLMP cases with LNI had 50% rate of endosalpingiosis compared to only 17% in the control group ($p=0.0227$). The average number of lymph node sites harvested was comparable between the study (3.81) and the control group (3.63). In 18% of cases in the study group LNI represented the only site of extraovarian disease. LNI displayed a combination of patterns in 82% of cases, with single cells being the most frequent pattern (64%) and occurring in the subcapsular location (83%). The intraglandular pattern was observed in 32% of the cases, and was the sole pattern in 33% of this subset. 89% of cases with the intraglandular pattern were associated with endosalpingiosis and 100% had a parenchymal location. Follow-up was available for 86% of the cases in both groups. The difference in overall survival was not statistically significant ($p=0.1085$).
Conclusions: Patients with OSLMP with LNI are on average 10 years younger and in 18% of cases LNI was the only indication of extraovarian disease, highlighting a need for staging in all OSLMP surgeries. The pattern of LNI seems to be single cell-related or endosalpingiosis-related. The pathogenetic and prognostic significance of these LNI patterns merits future investigation.

965 p16 Immunohistochemical Expression in Squamous Metaplasia of Endocervical Polyps

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Background: The immunohistochemical antibody directed against p16 is regarded as a surrogate marker for high-risk HPV infection and is used to differentiate between high-grade squamous intraepithelial lesions (HSIL) and their benign mimics. Endocervical polyps often have metaplastic squamous epithelium which may show reactive changes that can mimic HSIL. The aim of this study is to characterize the p16 immunoprofile of reactive squamous metaplasia in endocervical polyps.
Design: In-house cases of endocervical polyps accessioned between September 2007 and May 2008 were retrieved from the pathology archives. All slides were reviewed and cases with squamous epithelium were classified into two categories: polyps with reactive squamous metaplasia and polyps with mature, non-dysplastic squamous epithelium. The latter group served as a negative control. Sections were stained with $p16^{ink4a}$ and ki-67. Staining for p16 was recorded as positive when it showed strong and diffuse nuclear staining and/or cytoplasmic staining in at least the parabasal cells. Increased proliferation revealed by ki-67 immunostaining was evaluated in p16-positive cases, to exclude HSIL.
Results: We identified 164 consecutive cases of cervical biopsies or hysterectomies with benign endocervical polyps. 44 of the 164 polyps showed squamous epithelium and were included in the study. 19/44 were categorised as polyps with reactive squamous metaplasia and 31/44 as polyps with mature squamous epithelium (6 polyps showed both reactive and mature epithelium). P16 was positive in 8/19 (42.1%) polyps with reactive squamous metaplastic epithelium and in only 4/31 (12.9%) polyps with mature squamous epithelium**.** This difference was statistically significant using the chi-square test (p value $= 0.019$) and the Fisher exact test (p value $= 0.038$). None of the cases positive for p16 showed increased proliferation with ki-67. Furthermore, only one patient with a polyp showing reactive squamous metaplasia had a known history of HPV infection (low grade squamous intraepithelial lesion diagnosed 7 years earlier).
Conclusions: Our immunohistochemical results reveal that reactive squamous metaplasia of endocervical polyps can be positive for p16 in a significant proportion of the cases. This information is important to bear in mind in order not to overdiagnose HSIL in these cases. In cases positive for p16, staining with ki-67 is useful to exclude squamous intraepithelial lesion.

966 Prevalence and Genotype Identification of HPV Infection in a Population of Turkish Women

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Background: Infection with certain types of HPV is a very important risk factor for cervical carcinoma. Type specific distribution of HPV differs among regions around the world and epidemiological distribution of HPV in a given area is important for both developing health policies and success of vaccination programs. The aim of this study was to evaluate the prevalence of the different HPV genotypes in a population of Turkish women referring to gynecological outpatient clinics which will provide valuable information for developing future management and prevention policies.
Design: A total of 408 women (age range: 19-70) referred to gynecology outpatient clinics of a university hospital (Marmara University) and an affiliated private institution (Academic Hospital) were recruited from May 2008-August 2008. Split sampling was used for obtaining specimens for cytological examination. HPV typing were identified by PCR DNA microarray method which enables identification of 35 types. HPV typing and cytological examination were blinded.
Results: The overall prevalence of HPV was found to be 16 % (67of 408) with 75% being in the high risk group. Type 16 was the most frequent HPV type (23%) followed by types 53 (13%), 6(12%), 58 (10%) and 66 (10%). Multiple genotypes were found in 22%. 82% (55 of 67) of HPV positive women had normal cytology; 10% have LSIL; 4% and 3% have ASCUS and HSIL respectively. It was the first time screening for 33% of HPV positive women. The highest prevalence was observed in women aged 20-30 years (38%), followed by women aged 31-40 (32%) and over 40 (30%). Overall prevalence was similar among women referring to both institutions.
Conclusions: This study showed that HPV infections in our population has a wide age distribution and involve numerous types including those not present in the commercially available vaccine, even in patients with no evidence of cytological alterations in cervical cells. These findings will provide an important data for both epidemiological HPV profile and determining the future impact of vaccines.

967 Neuroendocrine Cells as a Component of Ovarian Brenner Tumors: The Source of Rare Ovarian Carcinoid Tumors Associated with These Neoplasms

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Background: Ovarian Brenner tumors (OBT) are a subtype of ovarian surface epithelial neoplasm and may be associated with other tumors within this group, especially mucinous tumors. The association of OBT and ovarian carcinoid is exceedingly rare and has raised the possibility of a germ cell origin for some cases of OBT. Up to 40% of OBTs have been reported to contain <10% of non proliferating argyrophylic cells by the Grimelius technique; however, no contemporary study has evaluated the presence of neuroendocrine cells in OBT. After encountering a case of carcinoid tumor arising in continuity with an OBT, we undertook this study to ascertain if neuroendocrine cells represent a normal component of OBT, providing a possible explanation for the presence of a carcinoid associated with OBT.
Design: Twenty-nine cases (index case included) of OBT were retrieved from the files of the Department of Pathology at our institution over a 15 year period. The presence or absence of an associated surface epithelial component was noted. Immunostudies utilizing the standard avidin-biotin technique were performed as follows: chromogranin

(CG), 20 cases; synaptophysin (SYN), 17 cases.
Results: Of the twenty-nine OBTs, thirteen had an associated surface epithelial tumor as follows: 9 mucinous cystadenomas , 1 mucinous tumor of low malignant potential , 2 serous cystadenomas, and 1 mixed serous/mucinous cystadenoma . None of the non index cases had an associated carcinoid tumor or evidence of neuroendocrine differentiation. The immunoperoxidase studies are summarized in Table 1.

Summary of Neuroendocrine Marker Expression in Ovarian Brenner Tumor

	Negative	≤ 10 cells	11-25 cells	26-50 cells	> 50 cells
Chromogranin (n=20)	6	4	4	3	3
Synaptophysin (n=17)	11	4	1	0	1

The index case had the highest number of CG + and SYN+ cells with most positive cells in the associated carcinoid tumor and in nests immediately adjacent to the carcinoid. Positive cells in the non index cases were present as isolated, single cells randomly distributed at the base of occasional OBT cell nests.
Conclusions: Non proliferative neuroendocrine cells may be a normal component of OBT. Rarely, this proliferation may result in small carcinoid tumors. Awareness of this phenomenon will allow the correct identification of these cases. The association of other tumor types with OBT is common, and carcinoid tumor may be included with this group. Carcinoid tumor, when associated with OBT, is likely primary in the ovary.

968 Histology and Pattern of Recurrence in Uterine Malignant Mixed Mullerian Tumor

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Background: Malignant Mixed Mullerian Tumor (MMMT) is an uncommon uterine malignancy associated with high rates of recurrence, particularly distant recurrence, and mortality. A contemporary study of a large case series of MMMT addressing the pattern and histology of recurrences with emphasis on the pathologic findings has not been done. In this study, we analyzed 67 such cases.
Design: One hundred fifty cases of MMMT from a 20 year period were retrieved from the files of the Department of Pathology at our institution. Sixty-seven (44.6%) cases with recurrent MMMT noted either in the pathology files or medical record were identified for review. Clinicopathologic features documented in the selected cases included: patient (pt) age, FIGO stage, time to first recurrence, recurrence histology and ratio of sarcoma (SA) to carcinoma (CA) in the primary tumor. All cases were reviewed by a gynecologic pathologist to confirm the H&E diagnosis.
Results: Pts' ages ranged from 39-82 years (median 63 years) with time to first recurrence ranging from 2-87 months (mos) (median 12 mos overall). By FIGO stage, the median time to recurrence was as follows: Stage 1 (32 pts), 10 mos; Stage 2 (14 pts), 14 mos; Stage III (14 pts), 15 mos; Stage IV (6 pts), 12 mos. Ninety-five sites of recurrence (43 pts with single recurrences; 24 pts with multiple recurrences) were noted: 38 local recurrences (27 vaginal, 11 pelvic) and 57 distant recurrences (20 lung/thorax, 14 omentum/peritoneum, 6 extra pelvic lymph node, 6 bone, 4 liver, 4 brain, 1 adrenal gland, 1 kidney, and 1 not specified). Forty-eight (50.5%) recurrences were composed entirely of CA, 10 (10.5%) of both CA and SA, and 6 (6.3%) of entirely SA. In 31 recurrences, histologic composition was not stated and slides were not available for review. Twelve of 48 (25%) recurrences composed entirely of CA had >50% SA in the primary tumor. Of the 6 recurrences composed entirely of SA (2 vaginal, 2 lung, 2 peritoneal), five (83%) had >50% SA in the primary tumor.
Conclusions: Nearly half of pts with MMMT experience recurrent disease, and of those, nearly 50% are FIGO stage I at diagnosis. While vaginal recurrence is most common, extrapelvic recurrence is a significant problem occurring in 44 pts (65.6%) in this series. The ratio of CA/SA does not necessarily predict the histology of the recurrence except in rare cases where the recurrence is composed entirely of SA. Biopsy of recurrent MMMT is essential to confirm the recurrence histology as this could direct or alter the chemotherapeutic regimen.

969 The Mitosis Marker Anti-Phosphohistone H3 (PHH3) Distinguishes Bizarre Leiomyoma from Leiomyosarcoma and Predicts Behavior in Uterine Smooth Muscle Tumors

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Background: In the uterus, distinction of bizarre leiomyoma from leiomyosarcoma can be challenging because bizarre nuclei may mimic mitoses. Immunohistochemical markers of cell proliferation or cell cycle activity, such as MIB-1, p53, and p16, are of limited value since their expression overlaps in these two entities. Antibodies to the serine-phosphorylated state of histone H3 (PHH3) are specific markers of cells in mitosis and have prognostic value in breast cancer and meningioma. In this study, we hypothesize that PHH3 can distinguish bizarre leiomyoma from leiomyosarcoma. We also evaluate the prognostic significance of PHH3 in a range of uterine smooth muscle tumors.
Design: Fifty cases of uterine smooth muscle tumors, including 11 bizarre leiomyomas, 12 leiomyosarcomas, 7 mitotically active leiomyomas, 15 cellular leiomyomas, and 2 tumors of uncertain malignant potential (STUMP) were evaluated. Slides containing the most atypical appearing nuclei were selected for PHH3 immunohistochemical staining (Upstate Cell Signaling, 1:1200). PHH3 score was defined as the average number of positive tumor cells in 10 high power fields (HPF); 3 separate counts of 10 HPF were performed to determine the average score. Staining was interpreted without knowledge of the pathologic diagnosis. Follow-up data was collected on all patients.
Results: The vast majority of bizarre nuclei in bizarre leiomyoma did not express PHH3. PHH3 score of bizarre leiomyoma (3.3/10 HPF) was significantly lower than the PHH3 score for leiomyosarcoma (34.1/10 HPF) ($p<0.001$). No significant difference in PHH3 score was observed between bizarre leiomyoma and typical leiomyoma (3.8/10 HPF), mitotically active leiomyoma (6.3/10 HPF), STUMP (9.2/10 HPF), or cellular leiomyoma (4.5/10 HPF). Average follow-up time was 36 months. None of the patients with bizarre leiomyoma or other benign diagnoses developed recurrence, spread, or death, whereas 6 of 12 patients with leiomyosarcoma had recurrence or spread and 3 patients died. Independent of the morphologic diagnosis, PHH3 score predicted outcome: using a threshold PHH3 score of 30 per 10 HPF, sensitivity and specificity for predicting recurrence were 75% and 92%, and for predicting death were 80% and 92%.
Conclusions: PHH3 distinguished bizarre nuclei from atypical mitoses in uterine smooth muscle tumors and reliably distinguished bizarre leiomyoma from leiomyosarcoma. Furthermore, high PHH3 score accurately predicted malignant behavior in uterine smooth muscle tumors, independent of morphologic classification.

970 Downregulation of miRNAs in Pluripotent Cancer Stem Cells Is Mirrored in Advanced Ovarian Serous Carcinoma

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Background: Cancer stem cells (CSCs) can self-renew, differentiate and regenerate tumours and have now been described in ovarian malignancy. Description of *de novo* tumourigenesis in SCID mice from a single CSC has fuelled the belief that CSCs drive primary tumourigenesis and that their persistence post-intervention contributes to metastasis and recurrence. We have previously reported independent identification of sets of microRNAs (miRNAs) specifically expressed during early differentiation of pluripotent Ntera2 CSCs. Despite their dissimilar origins, an overall comparison of CSC and tumour-specific data sets identified striking similarities with respect to downregulated miRNAs.
Design: Pluripotent Ntera2 cells were maintained in the self-renewal state, passaged via cell scrapping, and differentiation was facilitated by addition of 10mM retinoic acid (RA) for 3 days. Self-renewing and 3 days RA-differentiated samples were then harvested. Total RNA was extracted from Ntera2 cells and 6 fresh frozen serous carcinomas of high grade and advanced FIGO stage using mirVANA™ miRNA isolation kit. A novel Applied Biosystems 330 multiplex stemloop RT/PCR kit was used for miRNA gene expression profiling. Analysis of relative miRNA expression data was performed using the $2^{-\Delta\Delta Ct}$ method. miRNAs were subsequently analysed for clustering, predicted targets and pathway associations using the miRBase, miRGen and miRNApath web-based resources.
Results: Of 26 miRNAs upregulated in self-renewing CSCs, only one, miR-301, was similarly upregulated in tumour samples. However, of 56 miRNAs downregulated in self-renewing CSCs, 31 (~55%) were similarly downregulated in tumour samples. Seven of the top ten downregulated miRNAs were mirrored in tumour samples, two of which, miRs-509 and -188, were in the top ten downregulated miRs in tumour samples. These miRs target key developmental, malignancy and stemness signalling pathway genes.
Conclusions: Groups of miRs with roles in CSC self-renewal and differentiation function in CSCs alone and throughout tumourigenesis. State-specific miRs are likely to facilitate extensive self-renewal and differentiation to facilitate tumourigenesis while the constant repression of miR targets may facilitate key cancer processes such as avoidance of the immune system and internal growth regulation.

971 Evaluation of Lymphovascular Invasion in Endometrial Adenocarcinomas: Laparoscopic Versus Abdominal Hysterectomies

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Background: Total laparoscopic hysterectomy (TLH) is a minimally invasive procedure used to treat endometrial adenocarcinoma. Subsequent therapeutic decisions and prognosis are influenced by histologic findings, including the presence or absence of lymphovascular invasion (LVI). A recent report describing "vascular pseudo invasion" in a majority of TLH specimens with endometrial carcinoma has questioned how balloon tip intrauterine manipulators used in this procedure might affect the final pathology. To obtain a clearer understanding of the frequency of LVI following TLH, we compared pathologic findings in TLHs and total abdominal hysterectomies (TAHs) performed for uterine endometrioid adenocarcinoma.
Design: Reports from 58 TLHs performed with robotic assistance and 39 TAHs for grade 1 and 2 endometrioid endometrial adenocarcinomas were reviewed for stage, depth of invasion, LVI, and lymph node (LN) metastasis. Additionally, some cases were reviewed to determine if there are histologic findings that can reproducibly distinguish TLH from TAH.
Results: Nine of 58 (16%) TLHs and 4 of 39 (10%) TAHs showed LVI ($p>0.10$). TLHs+LVI ranged from FIGO stage 1A to 3C (with 44% ≥stage 1C), whereas TAHs+LVI ranged from FIGO stage 1C to 3C (100% ≥ stage 1C). Half (2/4) of the TAH+LVI cases had positive LNs; in contrast, LNs were only positive in 11% (1/9) of the TLH+LVI cases. When two pathologists blindly reviewed 10 cases with LVI, they accurately distinguished the TLH and TAH cases in 9 out of 10 and 7 out of 10 times, respectively, based on the presence of endomyometrial vertical clefts and inflammatory debris in vascular spaces, a likely result of increased intrauterine pressure.
Conclusions: The prevalence of LVI did not significantly differ between TLH and TAH cases in our series, but LVI in TLH cases was associated more frequently with lower stage/depth of invasion and percentage of LN metastases. These more favorable features in TLHs raise the possibility that LVI in some of these cases may in fact be artificial ("vascular pseudo invasion"). The presence of sensitive histologic findings (i.e. vertical clefts and intravascular inflammatory debris) in TLH cases should cause the pathologist to consider the possibility that LVI may be artifactual. Nevertheless, the clinical significance of "vascular pseudo invasion" remains unclear.

972 Chromosome In Situ Hybridization To Differentiate Early Partial Hydatidiform Mole from Hydropic Abortus: A Validation Study

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Background: The distinction between partial hydatidiform mole (PM) and hydropic abortus (HA) has important implications for patient management. Immunohistochemical

detection of p57kip2 protein has greatly facilitated the separation of early complete mole (CM) from HA, but no single technique has emerged that rapidly distinguishes early PM from HA. Flow cytometry, karyotype, and fluorescence in situ hybridization are expensive and require specialized equipment and expertise. Colorimetric chromosome in situ hybridization (CrISH) has recently been introduced as a possible alternative. The goal of the current study was to validate CrISH using a single chromosome 10 probe in triploid and diploid placentas.

Design: Fifty (50) hydatidiform moles and 25 hydropic abortuses characterized by flow cytometry were studied. De-paraffinized 5-micron sections were permiablized with Proteinase K and Triton X-100 and incubated with a biotin-labeled Chromosome 10 probe and visualized with 3,3'-diaminobenzidine and hydrogen peroxide according to standard protocols. The stained sections were independently evaluated by two observers (AKF and DWK). One hundred nuclei were scored for the number of signals. Gain in chromosome 10 was recorded when 15% or more of the nuclei of interest showed at least three (3) signals.

Results: Of the 50 hydatidiform moles, 22 were diagnosed as PM and 28 as CM. CrISH performed on the CM cases showed two signals in 25 cases and four signals in three. Twenty of 22 PMs (91%) contained three signals; two cases showed two signals. The concordance between CrISH and flow cytometry for the molar gestations was 96%. In the 25 cases of non-molar HA, CrISH studies revealed two (2) signals in 24 (96%). The remaining case had three signals. The overall agreement between the CrISH and flow cytometry was 96%.

Conclusions: CrISH using a single DNA probe is highly effective at differentiating between PM and CM and between PM and hydropic abortus. CrISH with a single DNA probe, used in combination with p57 immunohistochemistry, provides a simple, rapid, cost-effective means to exclude hydatidiform mole in the setting of HA.

973 Early Partial Hydatidiform Mole: A Clinicopathologic Study of 138 Cases

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Background: The widespread use of ultrasound in the diagnosis and management of intrauterine fetal death has results in hydatidiform moles being evacuated earlier than before.

Design: Clinicopathologic features, morphology, and DNA ploidy of early (≤12 weeks vs. late > 12 weeks) partial hydatidiform moles (PMs) were studied. A total of 160 cases of PMs were identified in the surgical pathology files (37 from 1981-90; 123 from 1991-2007). The diagnosis of molar pregnancy was based on the pathologic criteria of Szulman and Surti (Am J Obstet Gynecol 1978;132:20-27).

Results: The patients' ages ranged from 17 to 43 (mean: 30) years. Mean gestational ages were 9.5 weeks for early PMs and 14.9 weeks for late PMs. Early PM was more common in 1991-2007 (115/123, 93%) than in 1981-1990 (23/37, 62%). Pre-evacuation diagnosis of hydatidiform mole was achieved in only 5 early and 1 late PMs. There were no significant differences in histology between early and late PMs, except that villi were smaller in early PMs and there was extensive stromal fibrosis in late PMs. Although differentiating early PM from early complete mole was relatively straightforward, there were early PM cases that were histologically indistinguishable from hydropic spontaneous abortion. On ploidy analysis, 70 of 80 early and 19 of 20 late PMs were triploid, 5 early PMs were aneuploid, and 5 early and 1 late PMs were diploid. No significant histologic differences were seen between the triploid and nontriploid PMs. Ploidy heterogeneity among PMs was suggested. None of 65 patients with early PM and 1 of 11 late triploid PMs developed persistent gestational trophoblastic disease.

Conclusions: Early PM has become more prevalent than it was previously. There was no significant difference in histology except for smaller villi in early PMs between early and late PMs. The diagnosis of PM should be based on pathologic examination, since most PMs still elude clinical detection. Ultrasound examination may not be sensitive enough to detect early PMs that have not fully evolved. Practically, histologic differentiation between PM and hydropic abortion is extremely difficult in some placental tissues, and follow-up with the measurement of serum or urine hCG titers is required in these cases. DNA ploidy analysis is useful in the evaluation of problem cases. The risk of persistent disease seems to be very low in the case of early PM.

974 HPV-Negative Vulvar Intraepithelial Neoplasia (VIN) with Basaloid Features. An Unrecognized Variant of Simplex (Differentiated) VIN

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Background: Vulvar intraepithelial neoplasia (VIN) is currently classified into two distinct clinicopathological subtypes, classic (bowenoid-basaloid) and simplex (differentiated). Classic VIN, which is consistently associated with human papillomavirus (HPV) infection, has a predilection for relatively young women, is frequently associated with other HPV-positive tumors of the lower genital tract, and is tipicaly associated with invasive squamous cell carcinomas (invSCC) of basaloid or warty type. Simplex VIN, which is consistently negative for HPV, arises in elderly women, is frequently associated with squamous cell hyperplasia and lichen sclerosus, and is associated with invSCC of keratinizing type. Nevertheless, it is not known whether some VIN lesions may have unusual or mixed histological patterns.

Design: We studied the histological characteristics of the adjacent skin in a series of 250 invasive squamous cell carcinomas of the vulva (invSCC) negative for HPV by PCR using the SPF10 primers, focusing on VIN lesions. In all cases a immunohistochemical staining for p16 and p53 was performed.

Results: Simplex VIN was identified in 129 (51.6%) cases. In 9 cases (7%), VIN lesions showed a basaloid morphology, characterized by a diffuse replacement of the whole epidermis by the typical homogeneous population of small, "undifferentiated" keratinocytes with scanty cytoplasm extending throughout the entire thickness of the epidermis, showing no or only minimal maturation in superficial layers. All these lesions were negative for HPV. Immunohistochemically, all cases were negative for p16 and strongly positive for p53 with suprabasilar extension of p53 positive cells. Mean age of the patients was 73.7 years, and 8/9 patients were postmenopausal. None of the patients had synchronous or metachronous tumors of the lower genital tract. Squamous hyperplasia was present in the adjacent epidermis in 3 (33.3%) patients and lichen sclerosus was present in 2 patients (22.2%). Concurrent invSCC were of conventional keratinizing type in 8/9 cases.

Conclusions: (1) Simplex, HPV-negative VIN may occasionally have basaloid morphology. (2) Immunostaining for p16 and p53 protein may be helpful in the identification of these lesions and the differential diagnosis with classic, HPV-positive basaloid VIN.

975 A Comparison of Detection Rates between Conventional Pap Smears and the Liquid Based Pap Tests in Dysplasia Clinic

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Background: In the United States, there has been a trend of decreased use of conventional Pap smears with an increase in liquid based pap tests. The purpose of this study is to compare the detection rates of HSIL, LSIL and ASCUS/AGUS on conventional Pap smears versus liquid based Pap tests.

Design: The statistics of Pap tests from a dysplasia clinic population were compared from the years 1998-1999 (when only conventional Pap smears were performed), 2001-2002 (when the same high-risk clinic completely switched to ThinPrep Pap test); and 2006 (the clinic switched to SurePath Pap test and currently uses it exclusively). The Pap smears/tests were performed or supervised by the same gynecologists during the years studied and the same pathologists and cytotechnologists group evaluated all of the Paps manually. The Paps were categorized into six groups: Atypical Squamous Cells of Undetermined Significance/Atypical Glandular Cells of Undetermined Significance (ASCUS/AGUS), Low grade Squamous Intraepithelial Lesion (LSIL), High grade Squamous Intraepithelial Lesion (HSIL), Negative for Intraepithelial Lesion or Malignancy/Within Normal Limits (NILM/WNL), unsatisfactory and carcinoma.

Results:

Results of Conventionals, ThinPrep and SurePath Pap Tests

Categories:	Conventional (Total: 34,649)	ThinPrep (Total: 16,310)	SurePath (Total: 6,585)
ASCUS/AGUS	5%	8%	7%
LSIL	3%	7%	11%
HSIL	2%	4%	4%
NILM/WNL	>89%	81%	78%
UNSAT/CARCINOMA	<1%	<1%	<1%

The ASCUS/AGUS rates increased from 5% in conventional to 8% in ThinPrep and 7% in SurePath (two tailed P value is less than 0.0001 for the conventional versus liquid based, which is considered to be statistically significant). The LSIL rates increased from the conventionals (3%) to ThinPrep (7%) to SurePath (11%) (Two tailed P value is less than 0.0001 between conventional versus liquid based Pap tests). The detection rate of HSIL doubled from the conventionals (2%) to ThinPrep and SurePath (both 4%)(P value of less than 0.0001 for the conventional versus liquid based Pap tests). The unsatisfactory rates and carcinoma detection rates were similar, with all pap tests having less than 1% in either category.

Conclusions: The study shows that there was a statistically significant difference between the detection rates of abnormal Paps on conventional and liquid based Pap tests. There was only a statistical difference on LSIL detection rates between the two FDA approved liquid based systems in our high risk dysplasia clinic population.

976 Regulation of Toll-Like Receptors in Cancer Stemness: A Pro-Inflammatory Switch Model

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Background: Ovarian cancer, one of the most common cancers affecting women, is often detected in an advanced stage, resulting in a high mortality rate. Ovarian cancer has been shown to be associated with cancer stem cells (CSCs), the extensive self-renewal and differentiation of which is believed to drive primary tumourigenesis while contributing to metastasis and recurrence. As malignancies often arise at the site of chronic or prolonged inflammation, we have assessed whether inflammatory events may play a role in cancer stemness.

Design: Microarray data from early differentiation of teratocarcinoma derived CSCs revealed differential expression of a collection of inflammatory genes. Pro-inflammatory genes identified were validated over a two-week differentiation time course in Ntera2 (pluripotent) and 2102Ep (Nullipotent) teratocarcinoma CSCs using quantitative realtime PCR.

Results: Gene expression data indicates the presence of a two-pronged pro-inflammatory component of the switch of CSCs from self-renewal to differentiated growth, which involves alterations in expression of multiple genes. 1. MyD88-dependent TLR4 signaling is constitutively active in self-renewing CSCs and is downregulated upon differentiation resulting in decreases in pro-inflammatory cytokines NF-κB and TNFα. 2. Upon differentiation an alternative pro-inflammatory response may result in release of IFN- γ via Jak-STAT signaling. This mechanism likely results in a pro-malignancy microenvironment that facilitates tumourigenesis.

Conclusions: Teratocarcinoma CSCs employ pro-inflammatory signals during the switch from self-renewal to differentiated growth. This may provide a pro-malignancy microenvironment. Future work will focus on assessing the necessity of the mechanism towards the disruption of these genes to potentially remove stemness from CSCs.

977 Can Type Specific HPV Testing Predict Persistence/Progression (PP) of Cervical Intraepithelial Neoplasia (CIN)?

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Background: A diagnosis of CINI frequently results in low-yield expensive follow up for detection of high-grade CIN (HGCIN). There is a risk that persistent CINI lesions will progress to HGCIN but there is little information regarding whether HPV genotype analysis is predictive of progression. A cost effective method to predict persistence/progression (PP) based on the initial CINI in a small cervical biopsy (CBx) obtained at colposcopy could provide more individualized and cost effective follow up. A test utilizing Invader HPV reagents (Hologic, Inc, Bedford, MA) in a recently developed assay (Inv) uses three probe sets (A5/A6 detects HPV 51, 56, 66; A7 detects HPV 18, 39, 45, 59, 68; and A9 detects HPV 16,31, 33, 35, 52, 58) to identify high risk (HR) HPV DNA in cervical samplings. The A9 probe positive cases can be further analyzed to identify those that are HPV 16+. Our study was designed to assess the ability of Inv performed on CINI CBxs to predict PP and regressed (R) CIN.

Design: Thirty-nine cervical biopsies diagnosed as CINI were retrieved from our files and divided into two groups: PP and R. For purposes of this study, PP was defined as the presence of at least CINI for a minimum of six months. Patient age (17-62 yrs; mean 28.1; median 25) and length of follow up (6-43 mos; mean 14.3; median 11.5) were similar in both groups. CBxs from 10 additional women (5 CINIII and 5 negative) served as controls. After slides were reviewed and diagnoses confirmed, Inv and HPV16/18 typing were performed on formalin fixed paraffin embedded (FFPE) serial sections in accordance with manufacturer's recommendations. Eight (20%) of the CINI CBxs yielded insufficient DNA for Inv testing leaving 31 CINI CBxs (20 PP and 11 R) for study. HPV typing was correlated with follow up.

Results:

	HRHPV +	HPV16 +	HPV18 +	HRHPV+ non16/18
Persistent/Progressed CINI (20)	16 (80%)	3 (19%)	0 (0%)	13 (81%)
Regressed CINI (11)	6 (54%)	0 (0%)	0(0%)	6 (100%)
Negative Controls (5)	0 (0%)	0 (0%)	0(0%)	0 (0%)
CINIII Controls (5)	4 (80%)	3 (60%)	2 (40%)*	1 (20%)

*Both CBxs were + for HPV 16 and HPV 18

Conclusions: HPV typing can be performed on FFPE CBxs. All patients with CINI CBxs that were HPV 16+ had PP CIN. The utility of HPV 16 testing to predict PP in CINI lesions merits further study.

978 Endometrial Carcinomas in Women Age 40 Years and Younger

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Background: Endometrial carcinomas (ECs) in young women (≤ 40 yrs) are usually managed conservatively in selected patients. Whether oophorectomy with total hysterectomy is mandated for patients failing hormonal therapy is controversial. Recognition of features that might discourage conservative management and ovarian preservation are currently poorly characterized. We studied the presence of synchronous ovarian cancers and DNA mismatch repair (MMR) protein defects in this patient population.

Design: All ECs in women 40 years of age or younger were identified from review of institutional databases (1993-present) . All available slides were reviewed and DNA MMR immunohistochemistry (IHC) was performed using 4 markers (MLH1, PMS2, MSH2, MSH6) in cases with available blocks (n=56). Clinical information was obtained from electronic medical records.

Results: We identified 71 cases of ECs who underwent hysterectomy. ECs were predominantly endometrioid (65/71) and most were low grade (FIGO grade 1-2 = 83%). 8.5% were undifferentiated carcinomas (UCs). Most patients presented at early stage (stage I-II: 88%). A significant number of patients also had synchronous ovarian carcinomas (9/71 = 12%), predominantly endometrioid (7 of 9) while 2 were ovarian clear cell carcinomas (CCC). IHC for DNA MMR showed loss of at least one protein in 9 cases (16%) with slight predominance of MSH2/MSH6 abnormalities (5 of 9) compared to loss of MLH1/PMS2. The presence of defined histologic characteristics associated with microsatellite instability (MSI) (i.e LUS tumor, UCs, presence of tumor infiltrating lymphocytes) were noted in 8 of 9 tumors that showed loss of DNA MMR by IHC. None of the cases with synchronous ovarian and endometrial endometrioid carcinomas showed loss of DNA MMR. One of 2 ECs with synchronous CCC of ovary showed loss of MSH2/MSH6 while IHC for DNA MMR was not performed in the 2nd case. There were a total of 5 LUS tumors of which 4 showed loss of a DNA MMR protein.

Conclusions: Most ECs in young women are of endometrioid histology, well to moderately differentiated and present at early stage. However, UCs of the endometrium can also be seen in this age group. A significant proportion of these tumors showed loss of DNA-MMR by IHC. Synchronous endometrioid carcinomas of the ovary are frequent in this patient group, and this appears to be unrelated to DNA-MMR defects when both tumors are endometrioid. The presence of defined tumor characteristics, both gross and microscopic, appear to be powerful predictors of the possibility of MSI.

979 Significant Differences in Tumor Cell Type in Early Versus Advanced Stage Ovarian Carcinoma

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Background: Most patients with ovarian carcinoma present with advanced stage disease. It has been inferred that early detection can significantly impact on mortality due to ovarian cancer, as patients who present with early stage disease have dramatically better prognosis than patients with advanced stage tumors. While there are recognized differences between those tumors diagnosed at early versus advanced stage, there is a lack of data on stage distribution of ovarian carcinomas from large population-based cohorts, whose cell type assignment has been done, based on current diagnostic criteria.

Design: We reviewed cases of ovarian carcinoma referred to the British Columbia Cancer Agency over a 16 year period (1984-2000) to characterize differences in tumor cell type between early and advanced stage tumors. Slides were reviewed centrally and tumors assigned to one of six tumor cell types (clear cell, endometrioid, mucinous, low-grade serous, high-grade serous, carcinoma NOS), without knowledge of patient outcome.

Results: Tumor cell type distribution in early and advanced stages is presented in the Table.

Portion of Tumor Cell Types in Early and Advanced Stages

Stage	High-grade serous	Clear cell	Endometrioid	Mucinous	Low-grade serous	NOS	Stage
I/II	30.0%	26.2%	29.4%	8.5%	1.9%	4.0%	25%
III/IV	84.2%	4.9%	3.5%	1.1%	4.9%	1.4%	75%
All	70.0%	10.4%	10.3%	3.6%	3.5%	2.1%	100%

NOS - not otherwise specified

Conclusions: The distribution of tumor cell types differs in patients with early versus advanced stage ovarian carcinoma. This has broad implications, particularly for early detection and screening of ovarian carcinoma and studies on treatment of early stage ovarian carcinoma.

980 Expression of PTEN and MUC4 May Help Predict Disease Progression in Patients with Endometriosis

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Background: Molecular markers that may serve as risk factors for progression of cases with endometriosis to carcinoma have not been clearly delineated. In this study, we examined the expression of p53, PTEN and MUC4 in ovarian endometriosis associated and unassociated with ovarian borderline neoplasia/carcinoma to determine if differential expression of these genes may help in predicting disease progression.

Design: 231 consecutive cases of ovarian borderline neoplasia/carcinoma from 1996-2006 at our institution were examined for presence of concomitant endometriosis. Twenty nine cases of endometriosis without any history of ovarian neoplasia were randomly selected as controls. Expression of p53 and PTEN was studied using commercially available monoclonal antibodies. MUC4 expression was studied using a monoclonal antibody prepared in our laboratory. Immunostained slides were assessed using the H-score (summation of the product of staining intensity and proportion of cells staining) independently by two pathologists.

Results: Of the 231 cases studied 33 had concomitant endometriosis. Of these 33 cases, 13 cases of ovarian neoplasia (9 carcinoma, 4 borderline) with concomitant endometriosis had retrievable tissue for analysis. All 29 cases of ovarian endometriosis in patients without a history of ovarian neoplasia had retrievable tissue for study. None of the foci of endometriosis examined were morphologically atypical. The expression of PTEN (number of cases and H-score) was higher in the controls (62%) as compared to the cases (31% endometriosis; 8% neoplasia). More controls (79%) expressed MUC4 than cases (31% endometriosis; 46% neoplasia). Difference in expression of p53 within endometriosis was not significant between controls (20%) and cases (15%), although more tumors expressed p53 (69%).

Conclusions: Loss of PTEN and MUC4 expression likely play a role in the progression of endometriosis to neoplasia. These markers may help predict which cases of endometriosis will progress to neoplastic transformation.

981 Uterine Extramedullary Hematopoiesis: What Is the Clinical Significance?

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Background: Extramedullary hematopoiesis (EMH) represents abnormal development and growth of hematopoietic tissue outside the bone marrow. Recent studies have shown an association with myelofibrosis and myeloid metaplasia, chronic myeloproliferative disorders, or other hematologic malignancies in up to two thirds of cases. Only ten cases of uterine EMH (UEMH) have been previously reported; of these half had a concurrent or subsequently developed a clinically significant hematologic disorder. We studied a larger group of patients with UEMH to study the relationship with hematologic disorders.

Design: Cases diagnosed as UEMH between 1998 and 2007 were retrieved from our files (n=20). The slides were reviewed and categorized according to the type of specimen, uterine location, histopathologic diagnosis, number of EMH foci, and lineage composition. Clinicopathologic features recorded included age, history of an underlying hematologic disorder, baseline CBC, and subsequent work-up. Patients were followed from the time of initial diagnosis to the time of death or last follow-up.

Results: UEMH was confirmed in all 20 cases from 5 endometrial biopsies, 3 curettages and 12 hysterectomies. The mean number of EMH foci was 3.55 (range: 1-10). Eighteen cases were located in the fundus including 5 in endometrial polyps and 5 in leiomyomas. Two foci were located within the cervix. None of the patients had a concurrent gynecologic malignancy. The erythroid lineage was present in all foci; 35% also had myeloid precursors, and 20% megakaryocytes. The mean patient age was 44 years (range: 27-75 years). Twelve of 20 patients had underlying anemia (mean Hgb of 11 mg/dl, range: 5.5-15.7 mg/dl). No preexisting hematologic malignancy was identified in any of the patients. One patient had breast cancer with extensive bone metastases. Follow up information was available on 17 patients (mean 2.88 years; range 0.2-9 years). Only one patient had a bone marrow biopsy after the diagnosis of UEMH and the findings were consistent with iron deficiency anemia. None of the patients developed a significant hematologic disorder other than anemia during follow-up.

Conclusions: Based on our study, UEMH is often located in endometrial polyps or leiomyomas and frequently associated with chronic anemia. In comparison to existing literature suggesting a strong link between UEMH and hematopoietic disorders, our

findings suggest that UEMH is rarely associated with serious underlying hematologic conditions and therefore should not warrant extensive hematologic work up.

982 A Binary Grading System for Endometrial Carcinoma Compared with Existing Grading Systems

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Background: The FIGO grading system is the most widely accepted system for grading endometrial carcinoma, but has been critiqued for poor inter-observer reproducibility. Modified FIGO grading and binary grading systems were proposed in some studies, with encouraging improvement in reproducibility and prognostic power. This study compared prognostic power of a new binary grading system and other existing grading systems.

Design: Between 1995 and 2005, 254 patients underwent hysterectomy for endometrial carcinomas with matched survival information were included in this study. All H and E slides were evaluated by two gynecological pathologists. The morphologic criteria used for our new binary grading system were the presence of predominant solid or papillary architecture pattern, severe nuclear atypia, tumor necrosis and vascular invasion, which were prognostic predictors in univariate analysis ($P<0.05$). A high grade was assigned to tumors having at least two of these four features, while low grade tumors had at most one. All tumors were also assigned a FIGO grade (I, II, III) and another binary grade using the system described by Alkushi et al. in 2005. The Cox proportional hazards method was used to compare the new grading system and other existing grading system for prognostic significance, controlling for patient age, FIGO stage, and cell type.

Results: Applied to all tumor cell types, the new binary grading system and other tested grading system were independent predictors for survival with similar statistical power ($P<0.05$). The combination of FIGO grade I and II in the modified binary FIGO grading was an independent predictor but with decreased prognostic power (P value reduced from 0.0048 to 0.0289). When confining multivariate analysis to endometrioid carcinoma only, the new binary grading system showed enhanced predictive power for survival ($P = 0.0074$), and had more prognostic power than modified FIGO grading system (grades I and II vs.III, $P = 0.0289$). The traditional FIGO grading system retained its predictive power (P=0.0040), while Alkushi et al. grading system was not statistically significant for predicting survival (P=0.0978).

Conclusions: FIGO grading system demonstrates unequivocal prognostic value for patients with endometrial carcinoma, with additional advantage of being widely accepted by pathologists. The new binary grading system and modified binary FIGO grading systems may offer better reproducibility and ease of use while retaining independent prognostic power.

983 Testing Efficacies of p16, ProExC and HPV In-Situ Hybridization in Cervical Squamous Intraepithelial Lesions and Carcinoma

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Background: Testing of the biomarkers, p16 and ProExC (Topoisomerase II-a,/ minichromosome maintenance protein 2) and HPV DNA has been used in cervical tissue to distinguish high-grade cervical intraepithelial lesions (CIN2/3) from their mimics. However, studies of systemic evaluation and comparison of these markers are few. We compared efficacies of immunostaining of the biomarkers (p16, ProExC) and in-situ hybridization (ISH) of HPV DNA in cervical tissue with normal cervix, CINs and carcinoma.

Design: The cervical specimens included normal cervix (20), CIN1 (27), CIN2 (28), CIN3 (33) and carcinoma (29). Immunostains for p16 and ProExC and in situ hybridization for HPV DNA were performed. The testing results were compared with diagnostic classification and HPV genotyping results.

Results: Using diffuse staining pattern >1/2 thickness of epidermis, p16 and ProExC positivities (27-86%, 26-93%) and the concordance (50-79%) increased significantly with the severity of the cervical lesions. P16 and ProExC were significantly associated with oncogenic HPV types (P=0.1, P=0.35). For cervical lesions >CIN2, p16/ProExC showed the highest sensitivity (88%), followed by HPV ISH (82%), p16 (77%) and ProExC (65%). ProExC showed the highest specificity (96%) followed by p16 (84%), p16/ProExC (80%) and HPV ISH (62%). For cervical lesion >CIN3, p16/ProExC had the highest sensitivity (98%) followed by p16 (89%), ProExC (86%) and HPV ISH (82%). ProExC had the highest specificity (89%) followed p16 (70%), p16/ProExC (63%) and HPV ISH (45%). All the biomarkers were significantly associated with CIN2/3/carcinoma ($p<0.0001$).

Conclusions: Using both p16 and ProExC, the highest sensitivity can be reached in detecting CIN2 and CIN3. ProExC is the most specific marker in determining both CIN2 and CIN3.

984 Primary Ovarian Mucinous Tumors (OMT) Lack Loss of MLH-1 and MGMT and Are Characterized by KRAS Mutations and Aberrant p53 Expression When They Progress to Carcinoma

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Background: Primary OMTs show a morphologic spectrum from benign (BMT) to borderline (BOT) to carcinoma (MCA). Most of these tumors, especially in the BOT and MCA categories, show close morphological and some immunohistochemical overlap with tumors of the gastrointestinal tract. The goals of this study were to analyze the molecular events at each step of progression in primary OMTs and to determine whether microsatellite instability and aberrant methylation play a role in these tumors, similar to what has been reported in their gastrointestinal counterparts.

Design: Forty-four primary OMTs including 16 BMT, 18 BOT and 10 MCA (all gastrointestinal type) were retrieved and slides were reviewed. Immunohistochemistry for p53 (tumor suppressor gene), beta-catenin (Wnt signaling pathway), MLH-1 (microsatellite instability), and MGMT (O6 methylguanine methyltransferase; surrogate marker of gene promoter methylation),and mutational analysis for KRAS (by DNA sequencing of exon 1; codons 12,13) and BRAF (V600E mutation by PCR using allele specific primers) were performed in all cases. Confluent, strong p53 immunoexpression in discrete foci with > 50% of nuclei stained was considered positive. Immunostaining for beta-catenin was classified as membranous, cytoplasmic or nuclear and complete loss of nuclear staining for MLH-1 or MGMT was recorded.

Results: One/16 BMT, 3/18 BOT and 3/10 MCA showed aberrant p53 expression. Staining for beta-catenin was always membranous with no nuclear or cytoplasmic positivity. Intact nuclear staining for MLH -1 and MGMT was present in all cases. KRAS mutations (analysis successfully performed in 39/44 cases) were found in 0/16 BMT, 10/17 BOTs and 6/6 MCAs. No BRAF mutations were identified. Four tumors (2 BOT and 2 MCA) with KRAS mutations also showed aberrant p53 expression.

Conclusions: Our results indicate that despite morphological similarities with gastrointestinal tumors, primary OMT are genetically distinct in several aspects: 1) microsatellite instability, methylation abnormalities and aberrations of Wnt signaling pathway, and BRAF mutations are not prevalent, and 2) KRAS mutations are a frequent event occurring typically in BOTs and MCAs. Interestingly, this genetic profile (KRAS mutations and/or sporadic p53 overexpression) overlaps with that reported in the sequence of low-grade serous tumors of the ovary.

985 Characterization of Bone Tissue in the Ovary

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Background: The occurrence of bone in the ovary is uncommon. To date, there have been no series investigating this finding. The aim of this study was to characterize the various bone types and their associations as found in a large series of ovaries.

Design: Archival cases of oophorectomy in which bone was reported were studied. Patient age, associated ovarian pathology, unilateral or bilateral involvement, bone type (benign/malignant, woven/lamellar, spongy/cortical) and activity (osteoblastic/ osteoclastic) were documented and collated.

Results: We identified 25 cases from patients with a mean age of 38 years (range, 6 to 84 years). Bone was noted on gross examination in 16 (64%) cases. Benign osseous metaplasia was seen in 14 (56%) cases with either fibromas, endometriosis, fibrosis, follicular cysts, or a remote corpus albicans. Cortical-type bone was associated with 11 (44%) mature teratomas. Lamellar bone alone was seen in 17 (68%) cases, woven bone in 2 (8%) cases, and both types of bone identified in 5 (20%) cases. Osteosarcoma was present in 1 (4%) case of heterologous-type malignant mixed müllerian tumor. Associated cartilage (7 cases) and marrow (3 cases) were seen only in teratomas. Osteoblastic activity occurred in 9 (36%) cases, of which 8 were teratomas.

Conclusions: Bone may form in the ovaries of patients of all ages. In most cases heterotopic bone is of lamellar type. When present, bone is likely to be associated with either a metaplastic process (56%), mature teratoma (44%), or in rare cases a malignant mixed müllerian tumor (4%).

986 Endometrioid Endometrial Adenocarcinoma (EEA) in Elderly Women: A Clinico-Pathologic Study

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Background: EEA accounts for 80% of all endometrial malignancies, is usually diagnosed at an early stage and is associated with favorable prognosis. With increased life expectancy, it is likely that more women will be diagnosed with endometrial cancer at an older age. The clinico-pathologic features that affect adjuvant therapy and prognosis have not been well characterized in elderly women as a recognized subgroup. Typically, this subset of patients has more co-morbidities that potentially impact their treatment. In this study, we compared the outcomes and histopathologic prognosticators of EEA in women 70 or older to those of younger patients.

Design: Between 1999-2004, 825 patients were diagnosed with EEA in our hospital. Cases were divided into two groups: elderly women (age ≥70, n=242) and younger women (age<70, n=583). Follow up and pathologic data [tumor grade, stage, depth of myometrial invasion, cervical involvement, lymphovascular invasion (LVI), lymph node status] were retrieved. Chi square and Fisher exact test were used to compare differences for categorical variables and *t* test was used to examine differences between means.

Results: EAA in the elderly group was associated with lower proportion of grade 1 tumors (54.5% vs 63.1%; P=0.02), and higher proportions of deep myometrial invasion (41.3% vs 25.3%; P<0.0002), extra-uterine disease (26.8% vs 20.5%; P=0.04) and LVI (30.1% vs18.6%; P=0.0003). We found a trend toward higher rate of cervical involvement in women ≥ 70 (22.6% vs19.5%); however, this was not statistically significant (p=0.303). The likelihood of having surgical staging was comparable in both groups (90% vs 89.5%) but the proportion of positive lymph nodes was significantly higher in the elderly group (29.1% vs 9.8%; P=0.02). With a mean follow-up of 54 ± 25.5 months in elderly women and 65 ± 26.8 months in younger patients, the rate of local recurrence was significantly higher in former group (8.2% vs 3.9%; P=0.01). The difference in rates of distant recurrence was not significant. Time to recurrence was shorter in elderly patients (mean of 17.1 ± 1 m vs 20.72 ± 16 m).

Conclusions: In our experience, endometrial cancer in elderly women is characterized by increased proportion of FIGO grade 2 or 3 tumors and more advanced disease at the time of diagnosis. Moreover, elderly patients are at greater risk of local recurrence. These findings could affect future strategies in management of EAA in this subset of patients.

987 The Significance of Adenomyosis Involved by Tumor in Otherwise Low Stage Endometrioid Adenocarcinomas

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Background: Some features predictive of lymph node involvement by endometrioid adenocarcinoma include > 50% myometrial invasion and lymphovascular invasion. The significance of adenomyosis involved by endometrioid adenocarcinoma is uncertain and deciding whether tumor is confined to the adenomyosis or invades out of it can be challenging. Furthermore, whether this has any clinical implication is still debated. This study was conducted to evaluate the clinical significance of tumor involvement of adenomyosis in otherwise low-stage endometrioid adenocarcinomas.

Design: Hysterectomy specimens for endometriod adenocarcinoma with adenomyosis were reviewed. Cases with cervical involvement, serous histology, positive washings or deep myometrial invasion (T1c) were excluded, unless there was invasion out of adenomyosis that upstaged the patient. Cases were allocated into 4 groups: Group 1 (control group), adenomyosis uninvolved by carcinoma; Group 2, adenomyosis involved by carcinoma but encircled by endometrial stroma; Group 3, adenomyosis involved by carcinoma with incomplete peripheral endometrial stroma; Group 4: adenomyosis involved by carcinoma with invasion into the smooth muscle (desmoplastic response or jagged infiltration of the tumor). The location of the adenomyosis (inner or outer half of the myometrium) was noted. Positive lymph nodes, metastasis, or recurrence was considered a poor outcome. A disease free interval of at least 2 years was required for the good outcome designation.

Results: 71 patients with stage T1b or less endometrioid adenocarcinoma with adenomyosis were identified. The groups partitioned as follows: group 1, 36 cases; group 2, 25 cases; group 3, 6 cases, and group 4, 4 cases. The follow- up period ranged from 2-16 years (60% had greater than 7 years follow-up). The adenomyosis was in the outer half of the myometrium in 35 of the hysterectomies. Two patients had a vaginal recurrences, both from group 1.

Conclusions: In this series, adenomyosis involved by adenocarcinoma did not adversely affect the outcome in otherwise stage T1b or less endometrioid adenocarcinomas. Adenocarcinoma arising in adenomyosis and invading into myometrium that would potentially upstage the tumor was extremely rare (4/71 cases) and was not associated with a worse outcome. At the time of frozen section, adenomyosis involved by tumor should not upstage the patient, and seems to be equivalent to microinvasive disease.

988 Factors Affecting Treatment Response of Endometrial Hyperplasia

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Background: Endometrial hyperplasia (EH) may have variable response to progesterone treatment. This study compares the responders (R, spontaneous or post treatment non hyperplastic follow up biopsy) and nonresponders (NR) in terms of ploidy, s-phase fraction (SPF), estrogen (ER) and progesterone (PR) receptors, and PTEN and p53 immunostaining.

Design: 27 EH patients with initial and follow up biopsies were selected. The mean age was 53.2years (range 32-81). The initial biopsies showed 12 simple hyperplasias (SH, mean age50.6; range 34-65), 10 complex hyperplasias (CH, mean age 51.9, range 32-71) and 5 atypical hyperplasias (AH, mean age 60.0, range 45-81). 16 cases were treated with variable regimens of progesterone, 11 were untreated. All were rebiopsied average 15.4 weeks (range 3 days-15 months) later. All initial biopsies were analyzed for DNA ploidy and S-phase fractions (SPF). Immunoperoxidase stains for ER, PR, PTEN and p53 were quantitatively scored in both epithelial and stromal cells. Statistical analysis was done by student t-tests with significant p value set at 0.05 or lower.

Results: Total 19/27 cases showed spontaneous or treatment induced improvement (R) on follow up biopsies. Treated cases showed better rate of response (14/16, 87.5%), whereas only 5/11 (45%) untreated cases did so, The remaining 6 untreated NR cases included 3 that progressed to carcinomas on 2nd biopsy. Majority of NR cases were CH and AH (7/8). The R cases showed significantly stronger stromal ER (p=0.0366) and PR (p=0.0006) scores. Whereas significantly higher SPF (5.32 vs. 1.37; p=0.01) and PTEN scores (p=0.009) were seen in the AH cases compared to non-AH. All cases except one were diploid (26/27).

Conclusions: Majority of EH are diploid (26/27) and responsive to progestin treatment (14/16). We found stronger stromal ER and PR staining to be good predictors for spontaneous or treatment induced response. High SPF and PTEN stains separated the atypical from non-atypical hyperplasias.

989 Is HPV DNA Testing Useful for Women with LSIL Cytology?

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Background: Based primarily on ALTS data and ASCCP Guidelines, LSIL cytology is most often managed with colposcopic evaluation. Data on the efficacy of hrHPV triage and histologic follow-up of women with LSIL and HPV test results in different age groups remain limited.

Design: A computer-based search of Copath files of MWH, UPMC was carried out over a 34 month period (July, 2005 to April, 2008) to retrieve women reported to have LSIL who also were tested for hrHPV DNA. Pap tests were processed and prepared using an automated processor and imaged using the ThinPrep Imaging System. hrHPV DNA was detected by the HC2.

Results: 719 women with LSIL cytology and hrHPV DNA testing who had at least one subsequent cervical biopsy were included. The average follow-up period was 11 months (0 to 35 months) with a median 10.4 months. The interval between LSIL cytology and the initial histologic diagnosis of CIN 2/3 ranged from 1 to 29 months with a median 2.5 months. Either CIN 2/3 or CIN 1 were identified on histologic follow up in a significant greater proportion of LSIL women with positive hrHPV than in LSIL women with negative hrHPV (p=0.002 for CIN 2/3; p<0.001 for CIN 1). No CIN 2/3 was reported in women age 50 years or older with negative hrHPV test results. The percentage of CIN diagnosed on histology was not statistically significant between women with and without a TZ/ECS in their preceding LSIL cytology samples.

Table 1 Comparison of Histologic CIN between hrHPV Positive and Negative TPPT in LSIL Women Stratified by Ages

hrHPV Positive				hrHPV Negative		
Age	**F/U No**	**CIN 2/3**	**CIN 1**	**F/U No**	**CIN 2/3**	**CIN 1**
10-19	40	6 (15.0)	26 (65.0)	4	0 (0.0)	3 (75.0)
20-29	211	23 (10.9)	128 (60.7)	23	1 (4.3)	13 (56.5)
30-39	164	30 (18.3)	84 (51.2)	25	1 (4.0)	10 (40.0)
40-49	124	22* (17.7)	75 (60.5)	32	2 (6.3)	7 (21.9)
50-59	48	3 (6.3)	31 (64.6)	19	0 (0.0)	9 (47.4)
≥60	25	5 (20.0)	13 (52.0)	4	0 (0.0)	2 (50.0)
Total	612	89 (14.6)	357 (58.3)	107	4 (3.7)	44 (41.1)

* 1 case with CIN 3 and AIS

Conclusions: The absence of histologic CIN2/3 in women 50 years and older with hrHPV negative LSIL in this series supports that triage using hrHPV testing may be an efficient alternative to colposcopy in postmenopausal women with LSIL cytology in 2006 ASCCP consensus guidelines. hrHPV DNA results also help to stratify risk for underlying CIN2/3 in younger women and deserves further investigation along with other biomarkers for cervical carcinogenesis.

990 Immunohistochemical Expression of p16, MCM2, Topo IIα and MCM2/Topo IIα in Cervical Squamous Intraepithelial Lesions

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Background: The protein p16INK4a (p16) is a cell-cycle regulator that has shown to help in the detection of high-risk HPV infections. Minichromosome maintenance protein 2 (MCM2) is essential for eukaryotic DNA replication and drives the formation of pre-replicative complexes, which is the key first step during G1 phase. DNA Topoisomerase IIα (Topo IIα) is a nucleic enzyme that affects the topological structure of DNA by interacting with the double-helix DNA, thus playing an important role in DNA replication, transcription, recombination, condensation, and segregation. The objective of this study was to evaluate the IHC expression of p16, MCM2, Topo IIα, and MCM2/Topo IIα in different grades of malignancy of cervical squamous intraepithelial lesions.

Design: The expression p16 (clone 16P04), MCM2 (rabbit polyclonal), Topo IIα (clone 3F6) and a cocktail of MCM2/Topo IIα in 119 surgically resected FFPE tissues (43 normal, 40 LSIL and 36 HSIL's) was analyzed using IHC. Results were reported as follows: 0 when immunostaining was found only in the parabasal and basal cells, 1+ when 1/3 of the epithelium showed immunoreactivity, 2+ when 2/3 of the epithelial layer produced immunostainings and 3+ when positive reactions were seen throughout the cervical epithelium, including superficial cells. An independent sample T-test was used to compare the expression of the different markers in normal, LSIL and HSIL samples.

Results: p16, MCM2, Topo IIα, and MCM2/Topo IIα showed a statistically significant difference of expression among normal, LSIL and HSIL (p <0.001), with increased immunoexpression correlating with higher degree of malignancy for all markers. MCM2 and MCM2/Topo IIα were always expressed in higher quantities than p16 and Topo IIα in LSIL (p<0.001). The average immunopositivity for p16, MCM2, Topo IIα, and MCM2/Topo IIα in LSIL was 1.28, 1.71, 0.45 and 1.76, and in HSIL was 2.5, 2.4, 1.05 and 2.5, respectively. There was a 100% correlation in the expression and localization of IHC signals for p16, MCM2 and MCM2/Topo IIα in both LSIL and HSIL's.

Conclusions: The results of the current study show that expression patterns of MCM2 and MCM2/TOPO IIα correlate with p16, especially in HSIL. Their IHC expression is closely associated with progression of cervical squamous intraepithelial lesions, and they may be useful markers for assessing the staging of SIL's. MCM2/TOPO IIα increases the diagnostic sensitivity for both LSIL and HSIL's.

991 Chromosome Y-linked Testis Specific Protein Is Chracteristically Present in Gonadoblastoma

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Background: Gonadoblastoma (GB) is a rare gonadal neoplasm that usually occurs in intersex disorders including androgen insensitivy syndrome, mixed gonadal dysgenisis, Turner syndrome, and Turner syndrome mosaicism. The vast majority of GB develop in the setting of an abnormal karyotype with at least a portion of the Y chromosome present, a finding that led to the hypothesis of the existence of a gonadoblastoma locus (*GBY*) that is critical to the development of GB. The portion of the Y chromosome conserved in GB has been mapped to the centromeric portion of the short arm of the Y chromosome. While a number genes are present in this region, several factors have implicated the testis specific protein, Y-linked (*TSPY*) gene in the pathogenesis of GB. First, by immunohistochemistry *TSPY* has been noted to be over expressed in GB. Second, though the function of the TSPY protein is unknown, it appears to be involved in cell cycle regulation. TSPY has a high degree of homology with the cyclin B family of proteins that regulate the cell cycle. Third, *in vitro* experiments have suggested that expression of the TSPY may transition cells through the G(2)/M phase of the cell cycle.

Design: Six cases of GB were identified from our files dating back to 1989. A series of overlapping fluorescent in situ hybridization (FISH) probes designed to target approximately 450 kb of the *GBY* locus including *TSPY1* was developed from human BAC clones. A commercially available chromosome X CEP probe was used to identify the X chromosome. Dual color FISH using both probes simultaneously was used to evaluate cases for X and Y chromosomal DNA.

Results: FISH analysis demonstrated X and Y chromosomal DNA in five of six (84%) GB; in these five cases the adjacent noneoplastic ovary showed the same DNA pattern as the GB. In one of these cases an associated germ cell tumor showed the same DNA pattern as the GB. The GB that showed no Y chromosomal DNA arose in a Gravida 2 Para 2 woman with two X chromosomes; in this case the nonneoplastic ovary and an associated germ cell tumor showed the same DNA pattern as the GB.

Conclusions: FISH based testing for the *GBY* locus, specifically the *TSPY* gene, can aid in the detection of intersex disorders in women who develop GB. Our demonstration of the lack of an identifiable *GBY* locus in a fertile woman, specifically *TSPY* gene, suggests that this Y chromosomal region is not necessary for the development of GB.

992 Neuronal Elements within Gliomatosis Peritonei: Immunohistochemical Evidence of an Under-Recognized Feature

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Background: Gliomatosis peritonei (GP) is a rare condition in which small nodules of mature glial tissue are found throughout the peritoneal cavity, usually in a patient with concurrent or previous immature ovarian teratoma. Glial implants may be found with or without other teratomatous elements and they may occur within lymph nodes as well as on the peritoneum. While GP is considered to be a benign condition, it can be mistaken for peritoneal carcinomatosis and has been reported to undergo malignant transformation. Recent molecular studies have suggested that glial implants arise from peritoneal cells, presumably pluripotent Mullerian stem cells, rather than from the teratoma. Case reports of non-teratomatous glial implants in patients with a ventriculoperitoneal shunt support the theory that peritoneal cells may be induced to undergo glial metaplasia. Neuronal elements have only rarely been described in reported cases of glial implants and it is unclear whether this is a common finding. It is also unclear whether the presence of neuronal elements might differentiate teratomatous implants from metaplastic implants or provide prognostic information on the malignant potential of the implants. No studies to date have identified neurons with neuron-specific immunohistochemical stains.

Design: Seven cases of gliomatosis peritonei in 6 patients with concurrent or prior diagnosis of immature ovarian teratoma were compiled from our institution (two of the cases were separate surgical specimens from a single patient). Neuronal elements were not previously described within the glial implants in any of these cases. We performed immunohistochemical stains for glial fibrillary acidic protein (GFAP), Neu N, neurofilaments, and synaptophysin on slides with glial implants from the seven cases.

Results: Cells morphologically consistent with neurons were identified in six of the seven cases (representing 5 of the 6 patients) using three neuronal stains (Neu N, neurofilaments, and synaptophysin). The neurons generally had a mature appearance and appeared in clusters within a glial background. One case did not show positive neuronal staining but contained only small foci of GP.

Conclusions: Our findings suggest that neuronal elements are present in many cases of GP but are often overlooked. Further studies to determine whether neuronal elements have any histogenetic or prognostic significance are warranted.

993 Endocervical-Like (Mullerian-Type) Mucinous Borderline Tumors of the Ovary; Are They Mucinous Tumors?

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Background: Endocervical-like (mullerian-type) mucinous borderline tumor of the ovary (EMBT) is currently regarded as synonymous with the mixed-epithelial tumor of the mullerian type or with ovarian seromucinous tumor. It has many clinicopatholgic aspects similar to those of endometrioid tumors rather than to the intestinal type of ovarian mucinous borderline tumors because the former may have various epithelial metaplasia containing a mixture of mullerian-type epithelium, they can be more frequently bilateral, and more frequently the endometrioid tumor is associated with endometriosis as in EMBT. Because of these clinicopathologic findings, we have questioned the validity of the current classification system in which EMBTs are categorized as a subtype of ovarian mucinous tumor.

Design: To find better supporting evidence for categorizing the EMBTs either as endometrioid or mucinous tumors, we performed immunohistochemical stainings for estrogen receptor (ER), progesterone receptors (PR), PTEN, beta-catenin, cytokeratin (CK)7, and CK20 in 18 patients with EMBT, and mutational analysis for KRAS and exon 3 of PTEN using paraffin-embedded tissue sections in 16 cases and 9 cases, respectively.

Results: On the immunohistochemical stainings, all 18 cases showed ER and PR positivities and a consistent CK 7 (+)/CK 20 (-) pattern, both of which were not typical patterns of the mucinous borderline tumor, but closer to those of endometrioid tumor. Immunostaining for beta-catenin showed a cytoplasmic/membranous pattern in all cases without nuclear accumulation, which were different from endometrioid tumors. KRAS mutation at codon 12 were identified in 69% (11/16), and PTEN mutation in exon 3 was not found. It was difficult to interpret the immunohistochemical expression pattern for PTEN.

Conclusions: Although consistent the ER and PR positivities, CK 7 (+)/CK 20 (-) expression patterns and clinicopathologic findings in EMBTs were closer to those of endometrioid tumors, no further clue was identified for categorizing EMBT as an endometrioid tumor as they showed a high frequency of KRAS mutation, absences of PTEN mutation and nuclear accumulation of PTEN protein. However, an additional analysis for PTEN mutation in other area is going on and a meaningful result might wait us.

994 Uterine Smooth Muscle of Uncertain Malignant Potential: A Clincopathologic Analysis of 16 Cases from Multiple Centers

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Background: The current WHO classification states that any uterine smooth muscle tumor that cannot be histologically diagnosed as unequivocally benign or malignant should be termed smooth muscle tumor of uncertain malignant potential (STUMP). STUMPs represent a heterogeneous group of rare tumors that have been the subject of only a few published studies, some of which lacked clinicopathologic details and/or follow-up data. More recently, it has been suggested that immunohistochemical staining may be helpful in the diagnosis of STUMPs.

Design: The clinicopathologic features of 16 cases of STUMP that exhibited usual smooth muscle differentiation and diagnosed between 1992 and 2006 from 11 hospitals were studied and classified into 4 subgroups using terminology and criteria described by Stanford investigators (Bell SW et al, Am J Surg Pathol 1994;18:535-558). Immunohistochemical stains for p16, p53, ki-67, and estrogen and progesterone receptors were performed. The results were compared with those in the literature.

Results: Six tumors were "atypical leiomyoma with limited experience" (AL-LE), 7 "of low malignant potential, NOS", 2 "atypical leiomyoma, low risk of recurrence", and 1 "mitotically active leiomyoma, limited experience". Follow-up was 21 to 192 months (mean, 80.5). Only 2 tumors recurred, at 15 and 51 months respectively; both were AL-LEs (multifocal moderate to severe atypia, no tumor cell necrosis, and mitotic counts of 4 & 5 mfs /10HPFs, respectively). Both tumors had areas that were indistinguishable from benign leiomyoma; both had diffuse immunoreactivity for p16 and p53. Six other tumors that had focal staining for these markers all had a benign outcome. Both patients with recurrence were alive at last follow-up (at 36 and 74 months). All the other patients were alive and disease-free.

Conclusions: These results and other recent reports indicate that the combination of p16 and p53 stains may be useful in predicting the behavior of STUMPs. These findings also support previous observations that the occasional STUMPs that recur often behave as a low-grade malignancy. Patients with a STUMP should receive long-term surveillance as the median time to recurrence may be as long as 5 years.

995 Intraoperative Margin Assessment of the Radical Vaginal Trachelectomy Specimen

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Background: Radical vaginal trachelectomy (RVT) is a fertility preserving procedure for early cervical cancer where the cervix is excised at the level of the upper endocervix (EC) or lower uterine segment (LUS) along with a vaginal cuff and parametria. There is limited experience among pathologists regarding the intraoperative handling of this specimen. Our local protocol calls for examining the entire circumference of the EC/LUS margin through perpendicular serial sections. The aim of this work is to summarize our experience in the frozen section (FS) assessment of the EC/LUS margin.

Design: All surgeries from 1994 to 2007 were performed by one surgeon and the FS examination was carried out by a group of gynecologic pathologists. The peripheral soft tissue and the EC/LUS margins are inked with different colors. The proximal 1 cm segment is cut off the rest of the specimen and is opened to display its mucosa. It is then serially slices into 10-12 sections which are examined by FS. If the EC/LUS margin is positive or < 5 mm from the tumor, an additional segment of the LUS is resected.

Results: 132 patients were identified with complete pathology records. They ranged from 17 – 46 years old (median 31). Surgeries were performed for clinically Stages IA (n=39) and IB (n=93) tumors (63 adenocarcinoma, 59 squamous cell carcinoma, 7 adenosquamous and 3 others). In 78 cases (59.1%), no residual tumor was seen in the trachelectomy specimens as it was resected by the preceding LEEP or cone. The EC/LUS margin was reported as negative in 123, suspicious in 3 and positive in 6 cases. It was revised in 16 cases (6 positive, 2 suspicious and 8 negative but < 5 mm). Final margin assessment agreed with the FS diagnosis in 130 (98.5%) and showed interpretational overcall in 2 cases (1.5%); only one of which resulted in a revised margin. No false negative intraoperative assessment was found. After a follow up period of 1 – 149 months (average = 50), recurrence occurred in only 6 patients; none of which was related to the intraoperative reporting of the margin.

Conclusions: We describe our FS protocol and summarize our data. This protocol is reliable since none of the patients was under-treated. Examining the entire EC/LUS margin helps eliminate the chance for false negative results. Also, the perpendicular sections allow for the accurate measurement of the distance to tumor. The protocol needs to be considered by institutions that are beginning to perform this procedure.

996 IMP3 Staining of Serous Carcinomas and Endometrioid Carcinomas

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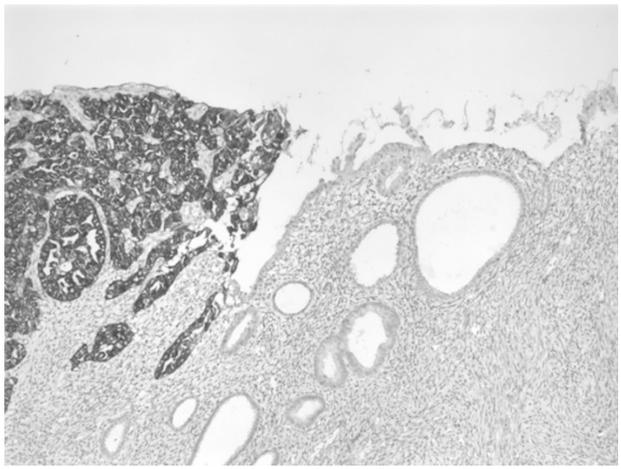
Background: Serous carcinoma (SC) or Endometrial type II carcinoma is an aggressive tumor. Because of difficulty in determining a morphologically evident precursor and lack of biological markers, a precursor lesion has not been identified yet. Insulin like growth factor mRNA-binding proteins (IMP1, 2, and 3), are oncofetal proteins expressed during embryogenesis and in malignant tumors. IMP3 expression has been studied in Serous Carcinoma (SC) and Endometrioid carcinoma (EC). The aim of our study is to detect IMP3 expression in precursor dysplastic epithelium of SC.

Design: We reviewed 99 cases of surgical resections from our surgical pathology archives which comprised of 16 SC, 57 EC, and 26 mixed carcinomas. H and E slides were selected with both tumor (glands and surface epithelium) and adjacent normal (glands and surface epithelium) tissue. Immunohistochemistry was performed on formalin fixed sections using monoclonal antibody to IMP3. Staining was scored on a scale of 0 to 3+ in the tumor and adjacent normal endometrium. Statistical significance was evaluated using the Chi-Square test with Yates' correction.

Results: Of the16 SC, 11 (78.6%) showed positive surface epithelial staining and 13 (81.2%) showed positive glandular staining.



Of the 57 EC, none showed epithelial but 9 (15.8%) showed glandular staining. SC showed more staining in the surface epithelium as compared to EC (p value=0.0001). In the mixed carcinomas, SC epithelial component showed more staining when compared to EC epithelial component (p value=0.0081). On comparing the staining in SC from both pure and mixed tumors, no difference was found (p value=0.5828). A significant difference was found on comparing the staining in EC from pure and mixed tumors (p value=0.0001). The staining character in EC of mixed tumors was focal and 1+ to 2+. Overall comparison between SC and EC of both pure and mixed forms showed a significant difference for staining (p value=0.0001).

Conclusions: Differential IMP3 staining in the surface epithelium of SC might aid in recognition of early changes of SC. Significant difference in staining for EC in pure and mixed tumors suggests a difference in biology and perhaps behavior. Further study to validate the use of IMP3 for detection of precursor lesions is being undertaken.

997 Carcinosarcoma of Uterus and Ovary: A Comparative Histological and Clinical Analysis

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Background: Carcinosarcoma of uterine corpus and ovary are high-grade malignancies of female genital tract. While both malignancies have similar morphological features, there are differences in the biology of these tumors, type and percentage of different components, modes of spread and patient's outcome. The object of this study is to compare these two groups of tumors in terms of different components, clinical stage and correlation of these pathological parameters with patients' outcome.

Design: The pathology database at University Health Network was searched and 42 cases of uterine carcinosarcoma (UC) and 6 cases of ovarian carcinosarcoma (OC) available were retrieved in the period of 2001-2008. Two Gynecologic pathologists reviewed the H& E slides of the cases. Types and percentage of different components as well as pathologic and clinical stage, status of lymph nodes, status of peritoneal washing and patient's outcome were documented.

Results: The median age for both groups of patient (UC) and (OC) was 69.5. All except one patient underwent surgery (TAH-BSO) and pelvic lymphadenectomies were done in 20 patients. The epithelial component in patients with (UC) made 53.3% of the tumor, which was serous type in 16 cases, endometrioid in 11, and mixed cell types in 15 patients and the mesenchymal element was mainly homologous (stromal sarcoma or leiomyosarcoma) in (28, 66.7%). In (OC), the epithelial component was 70% of the tumor with 3 of them being serous, one endometrioid and 2 mixed cell types and the mesenchymal element was homologous in 50% of cases. In patients with (UC), 20 were FIGO stage I, 3 stage II, 15 stage III and 4 patients stage IV. In patients with (OC), one was FIGO stage I, one stage II, 3 stage III and one stage IV. At the time of analysis, 9 patients were lost to follow-up (uterine group), six patients died of disease (26.2%), 10 alive without disease, 7 alive with disease and 7 in treatment. In (OC) 2 patients were dead of disease (33.3%), 1 is alive with disease and 3 are in treatment.

Conclusions: Serous carcinoma appeared to be the most common epithelial component in this series for both ovarian and uterine carcinosarcomas. The sarcomatous component is mainly homologous in (UC), with heterologous component being more frequent in (OC). It seems that OC shows less favorable outcome in comparison to uterine counterpart in higher stage of the disease. However a larger group of patients may be needed for a more accurate evaluation.

998 Morphometric Analysis of Normal Endocervical Glands with Emphasis on Applicability To Help Distinguish Adenocarcinoma In Situ from Early Invasion

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Background: Distinguishing cervical adenocarcinoma in-situ (AIS) from early invasive adenocarcinoma (EIA) of the cervix can be challenging because conventional criteria for invasion such as single cell infiltration or desmoplastic response are often lacking in a significant number of cases. Additional criteria proposed, but not uniformly agreed upon include 1) architecturally complex glands with confluent growth pattern; 2) cribriform growth pattern of malignant epithelium; 3) presence of small glands below the deep margin of normal glands and 4) presence of malignant appearing glands in close proximity to thick walled blood vessels (TV). If these criteria are to be relied upon to establish invasion, knowledge of the normal endocervical glandular epithelium relative to depth and association to large blood vessels is essential.

Design: We analyzed specific characteristics of normal endocervical glands with respect to their distribution and association to TV in the cervical stroma. Sections of the transformation zone and the lower endocervical canal from 29 women without glandular neoplasia were reviewed. The following parameters were recorded: 1) perpendicular depth of the deepest endocervical glands from the surface; 2) distance of the deepest endocervical gland from the nearest thick walled blood vessel (defined as blood vessel with a discernable muscle wall); 3) depth of this blood vessel from the surface and 4) the distance of other glands from its nearest thick walled vessel if less than the distance of the deepest gland from its vessel was also recorded.

Results: The age of the 29 patients ranged from 33 to 75 yrs (average 47 yrs). The deepest endocervical gland was recorded at a depth of 5.62 mm (range: 1.57-5.62 mm; average: 3.36 mm). The distance of the deepest gland from the nearest TV ranged from .08 to 4.5 mm (average: 0.94 mm), with the majority being less than 2.0 mm (27/29; 93%). In cases where an additional TV distance was recorded, the vessel was noted to be 0.06 to 0.7 mm away (average: 0.23 mm).

Conclusions: Our data suggest that normal endocervical glands can be observed at a considerable depth from the surface and provide a reference point to help distinguish AIS from EIA. Glands appearing to be involved by AIS deeper than 3.36 mm and certainly beyond 5.6 mm should strongly suggest the possibility of invasion. The close proximity of normal endocervical glands to TV suggests that glands lined by AIS type epithelium should not be construed as de facto evidence of early invasion.

999 pCHK2 Expression in High-Grade and Low-Grade Ovarian Serous Carcinomas

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Background: Check point kinase 2 (CHK2) plays an essential role in DNA damage response and it is phosphorylated by ataxia telangiectasia mutated (ATM)-dependent manner following DNA damage. Phosphorylated CHK2 (pCHK2) then activates several downstream pathways leading to cell cycle arrest through Cdc25C phosphatase. In human tissues, CHK2 is homogeneously expressed in renewing cell populations such as intestinal epithelium, and absent or at low level in static tissues such as muscle or brain. Given the fact that pCHK2 serves as a surrogate marker for DNA damage response, we conducted an immunohistochemistry study to compare expression of pCHK2 in high-grade and low-grade ovarian serous carcinomas.

Design: A phospho-specific antibody against the threonine 68 of CHK2 (Cell Signaling) was used for immunostaining on ovarian serous carcinomas arranged in tissue microarrays. Phosphorylation of CHK2 at threonine 68 is a prerequisite event for the full activation by ATM. A total of 239 high-grade serous carcinomas (including 148 primary and 91 recurrent tumors) and 28 low-grade serous carcinomas were analyzed. pCHK2 immunoreactivity was semi-quantitatively scored by two pathologists independently using a five-tier grading system (0 to 4+). Correlation of pCHK2 intensity and clinical data including grade (Chi square), disease-free interval and overall survival (K-M curves) was determined.

Results: pCHK2 immunoreactivity was exclusively localized in the nuclei of tumor cells. Using an arbitrary cutoff (2+/3+/4+ vs. 0/1+), 89 (37.2%) of 239 high-grade serous carcinomas, 4 (14.3%) of 28 low-grade serous carcinomas showed intense staining (2+/3+/4+) of pCHK2. Among high-grade carcinomas, primary and recurrent tumors had a similar frequency of pCHK2 expression. As compared to low-grade serous carcinomas, high-grade serous carcinomas demonstrated a statistically higher frequency of intense cases (p= 0.016, Chi-square). In contrast, ovarian surface epithelium, ovarian surface inclusion cyst and tumor stromal tissues show weak (1+) or undetectable levels of pCHK2 immunoreactivity. There was no statistical significance of pCHK2 expression and clinical outcome including disease-free survival and overall survival.

Conclusions: Our results show that high-grade carcinomas have a higher frequency of DNA damage response than low-grade serous carcinomas. This finding is consistent with the view that high-grade carcinomas are more genetically unstable than low-grade serous carcinomas.

1000 Role of Hofbauer Cells in Vasculogenesis of Chorionic Villi: Comparative Study in Normal Placentas, Complete Hydatidiform Moles, and Chorangioma

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Background: The circulatory function of the placenta appears at an early stage of placental development. The process of vasculogenesis in the chorionic villi involves the *de novo differentiation* of pluripotent mesenchymal cells to hemangiogenic stem cells (CD31 reactive) to angiogenic cell cords, and then the formation of mature blood vessels. Signals that regulate the vasculogenesis include complex processes with a number of factors involved, and the main regulator for angiogenesis is known to be the vascular endothelial growth factor (VEGF) family. It has recently been shown that Hofbauer cells (HCs) express high levels of VEGF mRNA, thereby indicating their involvement in vasculogenesis. However, we observed that CD31 reactive primitive stromal cells and angiogenic cell cords appeared prior to the appearance of HCs, thus raising the suspicion of their role in vasculogenesis.

Design: To investigate the role of HC in the vasculogenesis of chorionic villi, we compared the timing of the appearance and number of HCs in the villous stroma along with maturation of stromal blood vessels in 67 normal early placentas (weeks 4-11), 77 complete hydatidiform moles (CHMs, weeks 4-13), and 23 chorangiomas, using immunohistochemical stainings for CD68 (for Hofbauer cells) and CD31 (mature and immature blood vessels).

Results: In normal placentas, HCs began to appear during weeks 6-7, when angiogenic cell cords had been already formed. The number of HCs gradually increased after weeks

7-8 as the blood vessels matured and formed distinct lumen and hematopoietic cells. In 77 early CHMs in which the maturation of blood vessels was delayed or arrested, HCs were significantly decreased compared to normal placentas of the same gestational weeks; 45% (n=35) did not have HCs at all, 45% had few cells only in a few villi, and 9% (n=7) had them in multifocal villi (confined to 5-10 villi), but none of the CHMs had diffuse distribution of HCs as found in normal villi. The number of HCs within chorangioma was higher than in the adjacent normal villi, however, the amount seems to depend on the cellularity of chorangiomas; cellular and immature angiomatous types had more HCs compared to the mature angiomatous type.
Conclusions: HCs appear to be closely involved in the vasculogenesis of villous stroma, however, they may have a role in the vascular maturation rather than in the initiation of or commitment of pluripotent mesenchymal cells to the angiogenic cell cords.

1001 Mucinous Adenocarcinoma Involving the Ovary: Comparing Evaluation of the Classification Algorithms Using Tumor Size and Laterality

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Background: When intraoperative consultation of mucinous adenocarinoma involving the ovary, it would be useful to have additional approaching methods other then limited microscopic findings to determine the nature of the ovarian mucinous tumor, which is rarely encountered and notorious for difficulty in distinction of primary and metastatic tumor.
Design: The mucinous adenocarcinoma involving the ovary including 91 cases of metastatic mucinous adenocarcinomas and 20 cases of primary mucinous adenocarcinomas was evaluated with original algorithm (unilateral tumors ≥10cm are primary and the remaining are metastatic) and cut-off size modified algorithms.
Results: With the 10cm, 13cm and 15cm size cut-off, the algorithm correctly classified the primary or metastatic tumors in 82.0%, 86.5% and 88.3%, including 90%, 75% and 65% of primary tumors and 80.2%, 89.0% and 93.4% of metastatic tumors, respectively. Using Khunamornpong's modified algorithm (all bilateral tumors or unilateral tumors <10cm are metastatic, unilateral tumors ≥15cm are primary, and unilateral tumors with size between 10cm and 15cm are inderminate), 91.5% (86/94) were correctly classified and 15.3% (17/111) belonged to indeterminate.
Conclusions: The diagnostic algorithm using size and laterality, in addition to clinical history, preoperative image findings, operative findings, is a useful adjunct tool to differentiate metastatic mucinous adenocarcioma from primary mucinous adenocarcinoma of the ovary.

1002 Expression of HNF-1β in Ovarian Carcinomas with Clear Cells

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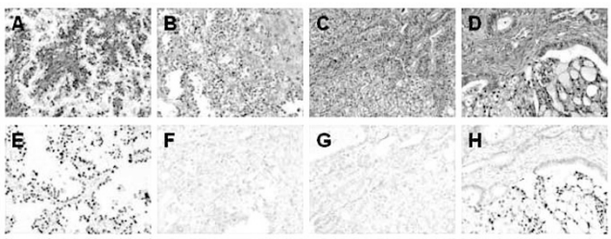
Background: Hepatocyte nuclear factor-1β (HNF-1β) is a transcription factor essential during the embryonic development of liver, kidney, pancreas and Mullerian duct. It was found to be overexpressed in ovarian clear cell carcinoma by cDNA microarray. The specific expression was confirmed by immunohistochemistry. However, the expression of HNF-1β in ovarian carcinomas with clear cells has not been addressed. To clarify if the expression of HNF-1β is specific for clear cell carcinoma but not in other ovarian carcinomas with clear cells, we studied the expression of HNF-1β in various ovarian carcinomas with clear cells.
Design: Archival HE slides of ovarian carcinomas diagnosed during 1996 to 2004 were reviewed. The study material included 40 ovarian clear cell carcinomas, 20 high grade serous carcinomas and 20 endometrioid carcinomas containing clear cells, and 13 ovarian carcinomas originally diagnosed as mixed endometrioid and clear cell carcinoma. The expression of HNF-1β was studied by immunohistochemistry on representative paraffin sections.
Results: HNF-1β was positive in all 40 clear cell carcinomas. The expression was usually diffuse and strong irrespective of the pattern and the amount of clear cytoplasm. In two tumors also containing areas of poorly differentiated carcinoma, the expression of HNF-1β was weak in the poorly differentiated area. Only one endometrioid carcinoma and two high grade serous carcinomas were positive for HNF-1β. The staining was weak and patchy in the two high grade serous carcinomas. In the 13 mixed endometrioid and clear cell carcinoma, HNF-1β was diffusely and strongly positive in the clear cell component in ten of the cases. The endometrioid components were negative in five and weakly positive in the other five.

Fig. 1 Ovarian clear cell carcinoma (A), high grade serous carcinoma (B), endometrioid carcinoma (C), mixed endometrioid and clear cell carcinoma (D) and corresponding HNF-1β stain (E, F, G, H)



In the three cases negative for HNF-1β, the clear cell components were arranged in solid or glandular pattern, randomly mixed with the typical endometrioid carcinoma. Correlating with the negativity of HNF-1β, they may represent endometrioid carcinomas with clear cell change.
Conclusions: Our results showed that the expression of HNF-1β in ovarian carcinoma is highly specific for clear cell carcinoma. Combining histomorphology and expression of HNF-1β, the diagnosis of ovarian carcinomas with clear cells can be made with higher accuracy.

1003 Diagnostic Reproducibility of Gastric-Type Endocervical Mucinous Adenocarcinoma: Validation of the Novel Diagnostic Criteria

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Background: Gastric-type endocervical adenocarcinoma is considered to be a distinct entity, which can be recognized based on morphology using the novel criteria as established by Kojima and Mikami et al. Because of its aggressive clinical course, it may be meaningful to detect the gastric-type adenocarcinoma and alert attending gynecologists for optimal management. This study aims to examine validity of the novel criteria.
Design: We selected 30 cases of endocervical adenocarcinoma, including gastric-type (n=15), usual-type (n=10), intestinal-type (n=2), endometrioid-type (n=1), clear cell-type (n=1), and serous-type (n=1), and prepared 1 to 6 electronic images to show representative features in each case. The gastric-type was defined as (1)mucinous adenocarcinoma with (2) abundant clear and/or pale eosinophilic cytoplasm, and (3) distinct cell border. In addition, a PowerPoint file was produced to understand the criteria, illustrating morphologic characteristics of the gastric-type. The image and instruction files were recorded on CD and mailed to 33 diagnostic pathologists. After the instruction, the attendees reviewed the images and divided cases into two categories, i.e., gastric-type and non-gastric-type. Non-gastric-type was further classified. The agreement with consensus diagnosis was estimated using kappa statistics.
Results: Regarding gastric vs non-gastric-type, the mean kappa value among reviewers was 0.66 (range: 0.33-0.93). There was no significant difference in rate of agreement with consensus diagnosis between usual- and gastric-type (65.0% vs 66.0%). The distribution of kappa was bimodal. The degree of agreement was correlated with neither experience after board certification nor subspecialty. Some cases of gastric-type were poorly recognized because cytoplasm was scant, and/or cells with clear cytoplasm resembled goblet cells.

Incidence of agreement with consensus diagnosis in each case

Histological type	Case #	Mean (%)	Range (%)
Gastric-type	15	66.0	3.6-96.4
Usual-type	10	65.0	28.6-89.3
Intestinal-type	2	82.1	78.6-85.7
Endometrioid-type	1	71.4	
Clear cell-type	1	64.3	
Serous-type	1	92.9	

Conclusions: The novel criteria for the gastric-type endocervical adenocarcinoma appears valid, and well-illustrated instruction is contributory for good reproducibility for the diagnosis.

1004 Invader HPV Test Reveals a Poorly-Recognized Deceiving Group of Cervical HSIL Associated with HPV Types-not-16/18

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Background: Cervical high-grade squamous intraepithelial lesions (HSIL) are easily diagnosed by established histologic criteria. However, we encountered problematic cases that are difficult to diagnose because classic histologic features are absent and metaplastic features are present. p16 & Ki67 stains proved HSIL in these difficult cases. To characterize them, we used the Third Wave Invader (Inv) test (a new human papillomavirus (HPV) screening assay applicable to tissue and amenable to rapid, sensitive & specific detection of 14 high to intermediate risk HPV types) and a panel of immunostains. Results of these difficult cases are compared to classic HSIL cases.
Design: We searched the last 12 months of our pathology files for: HSIL, CINII, CINIII, & p16. To identify cases of difficult HSIL simulating atypical metaplasia, we reviewed all surgical cases of HSIL that required p16 & Ki67 confirmation. Cases of interest were also stained with ProExC™. HPV screening & HPV 16/18 typing were performed by the Inv probe sets A5/A6 (HPV 51,56,66), A7 (HPV 18,39,45,59,68) & A9 (HPV 16,31,33,35,52,58) & Inv HPV 16/18 type specific assays.
Results: 10 cases of classic HSIL were easily diagnosed by hypercellularity, significant atypia, mitotic figures & diffuse staining by p16, Ki67 & ProExC™. Inv identified HPV16 (A9-positive/HPV16-positive) in 7 of 10 cases; the 3 others were A7-positive/not-HPV18 (1) & A9-positive/not-HPV16 (2). 8 cases of HSIL simulating metaplasia were identified. These showed only mild-moderate cellularity, lacked significant atypia, absent-rare mitotic figures but diffuse staining by p16, Ki67 & ProExC™. HPV DNA was detected in 5 of 8 cases: only 1 was A9-positive/HPV16-positive; 1 A5/A6-positive; 1 A7-positive/not-HPV18; & 2 A9-positive/not-HPV16. 3 remaining cases demonstrated sufficient DNA to be analyzed by Inv, but results were negative.
Conclusions: There is a poorly-recognized unusual group of cervical HSIL simulating atypical metaplasia that is easily confused by histology. Immunostains prove the high-grade nature of these lesions & Inv demonstrates association with HPV types other than 16/18 (ie. other HPV types detected by Inv). We are uncertain if this group represents a HSIL-variant or an early form of classic HSIL. In one case of classic HSIL, a focus of this deceiving HSIL was present, suggesting this group may represent an early form of classic HSIL. This study emphasizes that HPV screening should not be limited to HPV 16/18 alone since this deceiving form of HSIL appears to be caused by HPV-not-16/18.

1005 Reproducibility of Histological Diagnosis of Ovarian Clear Cell Adenocarcinoma

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Background: Ovarian clear cell adenocarcinoma (CCA) is relatively common in Japan compared to Western countries. It is often associated with endometriosis or an adenofibromatous component (AF), and one recent study suggested that the latter might be a factor for less aggressive behavior. Because its biological behavior including chemotherapy-resistance is different from ovarian cancer of other histological types, accurate pathological diagnosis is crucial for patient management. This study was carried out to examine reproducibility of the histological diagnosis of ovarian CCA. Frequencies of coexisting AF and endometriosis were also explored.

Design: Ninety-nine cases (age: 38-72 yrs, average 55.4 yrs; 50 FIGO stage I, 13 stage II, 30 stage III, 6 stage IV) of ovarian tumors submitted from 36 institutions for a Japanese Gynecologic Oncology Group (JGOG) clinical study with a referring institution's pathologist's diagnosis of CCA were reviewed by a panel of 4 gynecologic pathologists. Representative H-E slides were assessed independently by 3 of the panelists using WHO criteria before re-examination under a multihead-microscope by 4 of them. The panel diagnosis was based on diagnostic concordance by at least 3 of the 4 panelists.

Results: The panel diagnosis was CCA in 93 cases (94%). The rest included 2 cases of mixed carcinoma with minor component of CCA, and one each of clear cell borderline tumor, transitional cell carcinoma, endometrioid adenocarcinoma, and serous borderline tumor. Of 93 confirmed cases of CCA 13 (14%) were associated with AF and 15 (16%) with endometriosis. There was no association of either of these features with lower stage CCA.

Conclusions: Reproducibility of referring institutions' pathologists' diagnosis of ovarian CCA by a panel of gynecologic pathologists is excellent. Our results also suggest that the frequencies of AF and endometriosis associated with CCA are at least 14%, and 16%, respectively, and that these associated lesions do not correlate with tumor stage.

1006 Clinical Significance of Distinguishing Homozygous from Heterozygous Complete Hydatidiform Mole

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Background: Complete moles (CM) are abnormal conceptions composed entirely of paternal genetic material. About 80% are homozygous (HoCM) resulting from fertilization of an empty egg by one sperm with duplication of genetic material, while about 20% are heterozygous (HeCM) resulting from fertilization by two sperm. The literature suggests that HeCM may have a more aggressive course. We used multiplex PCR of short tandem repeats (STRs) at the CODIS loci to evaluate the accuracy of morphologic diagnosis of CM, to distinguish between HeCM and HoCM, and to investigate their clinical differences.

Design: Slides were reviewed from all consecutive, in-house cases of CM diagnosed on routine H&E at our institution between 1991 and 2007. DNA from maternal tissue (decidua) and villi was isolated by micro-dissecting unstained sections of formalin fixed paraffin embedded tissue. STR alleles at the CODIS loci, known to be highly polymorphic, were analyzed by multiplex PCR to distinguish HoCM from HeCM. Clinical data, including BhCG levels, adverse outcomes such as invasive mole and pulmonary metastases, and use of chemotherapy, were obtained from chart review.

Results: To date we have analyzed 40 cases, of which 35 had adequate DNA. In each case, molecular analysis of villous tissue showed a diploid karyotype with absence of maternal alleles, confirming the diagnosis of CM. Thirty-one cases (88.6%) were HoCM and 4 (11.4%) were HeCM. Mean peak BhCG levels were 523,499 Iu/L for HoCM and 322,193 Iu/L for HeCM. Complete follow up data were available for 17 patients with HoCM and 2 patients with HeCM. Mean time for BhCG to return to normal was 9.8 wks (HoCM) and 12 wks (HeCM). Seven of 17 (41.2%) HoCM required chemotherapy (mean duration: 9.9 weeks), compared with one of two (50%) HeCM (duration: 10 weeks). Three of 17 (17.6%) HoCM had invasive mole and/or pulmonary metastases and one of two (50%) HeCM had pulmonary metastases. No patients developed choriocarcinoma.

Conclusions: Multiplex PCR of STRs at the CODIS loci confirmed the morphologic diagnosis of CM, and differentiated HoCM from HeCM, in all cases analyzed. While peak BhCG levels were lower in HeCM than HoCM, mean time for BhCG to return to normal, use of chemotherapy, and presence of distant disease was greater in HeCM. These findings suggest that HeCM may behave in a more aggressive fashion, although analysis of more patients is needed.

1007 Reproducibility in Assessing Ovarian Carcinoma Cell Types – A Transcanadian Study

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Background: Reproducible histopathological cell type assignment for ovarian carcinoma becomes critical if different cell types are to be treated differently, as has been proposed. The purpose of this study was to test the concordance of cell type diagnosis between gynecological pathologists from across Canada.

Design: After an on site training session using a training set of 40 different ovarian carcinomas (available for review at http://spectrum.med.ubc.ca/pathology/ (username: ovcare, password: diamond then click on "Search all Digital Slides" and enter Folder name "ovcare"), six gynecological pathologists reviewed one representative slide of each case of the test set. The test set consisted of 40 cases from a population based review series were selected consecutively to ensure a representative distribution of cell types as follows 22 high-grade serous, 10 clear cell, 7 endometrioid, 5 mucinous, 4 low-grade serous. In a second round, pathologists received in tabular form the results of immunostains of the test set cases with 10 selected biomarkers, including WT1, ER, TP53 etc. and had the opportunity to change their diagnosis. Diagnosis of cell type had to be from one of the following six categories: high-grade serous, clear cell, endometrioid, mucinous, low-grade serous, unclassified. Concordance between pathologist pairs and overall concordance was calculated.

Results: The overall concordance between pathologists in assignment of cell type was 92.3% (range 85.0 – 97.5%), which was only slightly increased after consideration of immunohistochemistry staining data to 92.6% (range 87.5% - 97.5%).

Conclusions: Using a limited number of diagnostic categories, gynecological pathologists have a high concordance in diagnosis cell type, after a short training period. This is a large improvement over earlier studies of reproducibility of cell type diagnosis in ovarian carcinoma and has implications for clinical trials of subtype-specific treatments of ovarian carcinoma.

1008 Refining the Criteria for Adjuvant Therapy: Histological Cell Type Is a Significant Prognostic Indicator in Low Stage Ovarian Carcinoma

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Background: Adjuvant treatment is given to most patients with ovarian carcinoma. A challenge in the management of ovarian carcinoma is the identification of those patients with such a favourable prognosis that they do not stand to benefit from adjuvant therapy. We searched for clinicopathological parameters that identify such a subgroup in patients with stage I-II ovarian carcinomas.

Design: From a population based cohort of 2,555 woman with ovarian carcinomas referred to the British Columbia Cancer Agency between 1984 and 2000, we identified 685 cases of stage I-II ovarian carcinoma. From 95% of patients, histological slides were available for pathology review and 630 cases were confirmed to be ovarian carcinomas. 605 cases were assigned on pathology review to one of the five major subtypes of ovarian carcinoma (high-grade serous, low-grade serous, endometrioid, clear cell, mucinous). Univariate Kaplan-Meier analysis and multivariable Cox regression model was used to correlate clinicopathological factors with outcome.

Results: No single factor (stage, the presence of ascites, age, and subtype or histological grade) identified a patient subgroup with an excellent prognosis, i.e. 10 year disease specific survival rate of > 95%. Subtype yielded the most discriminative prognostic information (log rank $p < 0.0001$). Endometrioid subtype was associated with the best prognosis (10 year disease specific survival rate 84.9%), better than mucinous (78.7%), clear cell (70.1%), high-grade serous (56.6%) and low-grade serous (51.4%). Combing stage and tumor subtype, the subgroup of stage Ia/Ib endometrioid and mucinous carcinomas (n=77) was identified as having 95.4% 10 year disease specific survival. Most of these patients were not treated with adjuvant chemotherapy.

Conclusions: Our results show that tumor subtype has the potential to be used to guide recommendations regarding adjuvant therapy for patients with low-stage ovarian carcinoma.

1009 Expression of p16, p53 and EGFR in Squamous Cell Carcinoma (SCC) of the Vulva: A Study of 96 Cases

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Background: Two different pathogenetic pathways have been associated with vulvar SCC. In young women they are HPV/p16 related while in old women they originate in a background of simplex VIN and frequently express p53. Epidermal growth factor receptor overexpression (EGFR) has been linked to adverse outcome in vulvar SCC, however, the interplay between p53, p16 and EGFR has not been studied.

Design: Clinicopathologic features of 96 patients with SCC (age, smoking, grade, lymphovascular invasion (LVI), surgical margin, stage, recurrence, and survival) were recorded. Immunohistochemistry (IHC) for p16, p53 and EGFR, fluorescence *in situ* hybridization (FISH) (in EGFR IHC 3+ SCC), and statistical correlation were performed.

Results: Patients ranged in age from 40 to 99 (median 70) years. 5 tumors were grade 1 (5%), 44 grade 2 (47%), and 47 grade 3 (49%). 15 (16%) SCCs had LVI and surgical margin was positive in 17 (18%). 37 patients had stage I tumors while 31, 22 and 6 had stage II, III and IV SCCs and recurrences occurred in 31. Median survival was 7.1 years, 28 (29%) patients being alive with disease or dead of disease. 41% and 39% of SCCs had diffuse (>50%) and strong p16 and p53 staining with an inverse relationship ($p < 0.001$). p16+ SCCs were associated with lower grade ($p < 0.02$), smoking ($p < 0.001$), and young age ($p<0.001$), and p53+ tumors with higher grade ($p<0.02$) and advanced age ($p=0.05$). 3+ EGFR was seen in 18% of p16+ SCCs in contrast to 46% in p53+ SCCs ($p<0.01$). 8/24 EGFR+3 SCCs had EGFR amplification, all in p53+ SSCs ($p < 0.004$). By univariate analysis, p16 was associated with improved survival ($p<0.006$); advanced stage ($p<0.001$), EGFR gene amplification ($p<0.04$), and p53 diffuse staining ($p<0.03$) were associated with decreased survival. A Cox hazards model confirmed only EGFR amplification (HR 3.0) and p53 diffuse staining (HR 2) as robust risk factors.

Conclusions: p53 and p16 play distinct roles in the pathogenesis of vulvar SCC. High p16 expression is seen in lower grade tumors lacking EGFR mutations, and occur in younger women who smoke. The improved survival is directly related to young age. In contrast, p53+ tumors occur in older patients with higher grade tumors, EGFR amplification, and decreased survival. Age, stage, EGFR amplification, and p53 independently predict poor survival and may be useful to prospectively identify populations at risk.

1010 Primitive Neuroectodermal Tumors (PNETs) of the Female Genital Tract (FGT): A Morphologic, Immunohistochemical, and Molecular Study of 18 Cases

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Background: PNETs of FGT are very rare, ovary and uterus being the most common locations. Although these tumors have been traditionally classified as primitive neuroectodermal tumors and in the ovary frequently resemble neoplasms of the central nervous system (CNS), in other locations, distinction between CNS-PNET and peripheral Ewing/PNET is frequently difficult.

Design: The clinicopathologic features of eighteen PNETs involving ovary (9), uterus (8), and vulva (1) were studied. Immunohistochemistry for Fli-1, CD99, Synaptophysin, Chromogranin, CD56, Neuron Specific Enolase (NSE), Glial Fibrillary Acidic Protein (GFAP), Neurofilament (NF), S-100, Vimentin, and Cytokeratin (CK) was performed. A break-apart probe was used to evaluate any rearrangement of the EWSR1 gene by fluoresence *in situ* hybridization (FISH).

Results: Patients ranged in age from 14 to 66 (mean 37) years. Six patients had stage I tumors, while 1, 9, and 2 had stage II, III and IV tumors. Twelve neoplasms morphologically resembled CNS PNETs with variable degrees of neuronal differentiation, 3 of them also showing focal ependymal differentiation; one had classical features of medulloepithelioma and another showed features of mixed PNET and glioblastoma. In four cases, the morphologic features of CNS PNET were not recognized. Associated tumors included mature cystic teratoma (5), endometrioid endometrial carcinoma (2) and carcinosarcoma (1). Fli-1 was positive in 16/18 tumors (9 strong/diffuse), CD99 in 10/18 (5 strong/diffuse), Synaptophysin in 10/18, Chromogranin in 1/18, CD56 in 14/18, GFAP in 4/18, NSE in 8/18, S-100 in 8/18, Vimentin in 18/18, and CK in 4/18. Only 2/18 tumors showed EWSR1 gene rearrangement by FISH, both lacking morphological features of CNS PNETs.

Conclusions: Most PNETs of the FGT resemble histologically their CNS counterparts. Immunohistochemistry is not helpful in distinguishing peripheral from CNS PNET, particularly, Fli-1 and CD99 as they are expressed in both subsets. Tumors lacking any degree of CNS differentiation should undergo molecular testing as may represent peripheral PNET/Ewing. This distinction may be important for therapeutic and prognostic purposes.

1011 The Ovarian Stroma and Endometrial Carcinoma Subtype

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Background: Luteinized stromal cells are often observed in the ovaries of menopausal women and have been correlated with endometrial cancer in some studies. This study explored the relationship between luteinized ovarian stroma and endometrial cancer type.

Design: A consecutive series of endometrial cancers (EC) from women over 45 years of age was reviewed and sub-classified into endometrioid and serous categories. In each case ovarian sections were immunostained for calretinin (polyclonal, Biocare Medical, Walnut Creek, CA, U.S.A.) using a commercially available automated immunostainer (Bond Max, Vision Biosystems, Australia). Immunopositive polygonal stromal (**l**uteinized) **c**ells (**LC** score) and **s**pindle **c**ells (**SC** score) were semi-quantitively scored from 1 to 3. Hilus cell hyperplasia was also recorded. Data analyses were performed using SPSS 13.0 for Windows (SPSS, Chicago IL, U.S.A).

Results: 167 ovaries from 153 patients, including 97 with EC and 56 controls, were evaluated. Ovaries from patients with endometrioid carcinoma (n=67) exhibited a higher LC score, a higher SC score and a higher total score relative to serous carcinoma (n=30) (p=0.001). There was no statistically significant difference between total score of serous carcinoma patients and controls. There was a statistically significant relationship between LC and SC score and endometrioid tumor category (p=0.001 and 0.017 respectively). Mean max. dimension of ovaries from endometrioid carcinoma patients was higher (mean 2.48) than serous carcinoma (mean 2.20) and controls (mean 2.23), but the difference was not statistically significant.

Conclusions: The results of our study support a highly significant relationship between hormonally active ovarian stroma and endometrioid adenocarcinomas, in keeping with the role of estrogenic hormones in the development of this tumor type. Moreover, the similarity in ovarian findings between women with serous carcinoma and controls suggests that initiation of serous carcinogenesis is a function of local target (secretory) cell vulnerability to mutagenic factors rather than hormonal priming. This is reflected in transitions from benign to neoplastic that are devoid of alterations in the background glandular architecture. The relationship between LC/SC scores, carcinomas of mixed phenotype, and p53 staining is under study.

1012 Multiple Deep Level Sections of Tubal Fimbriae Blocks in Risk Reducing Salpingo-Oophorectomy from Women with BRCA Mutations: Do Levels Increase Detection of Occult Cancer?

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Background: Occult cancer can be found in a small subset of risk reducing salpingo-oophorectomies (RRSO) from women with BRCA mutations. Most occult cancer involves the tubal fimbriae and may be as small as 1 millimeter. Pathologic detection of occult cancer depends completely on the thoroughness of tissue examination. A special dissection protocol of embedding thinly sliced sections of the entire tube and ovary is required. We hypothesized that enhanced examination of tubal fimbriae via multiple deep level sections could uncover occult cancer not present on the original slides. The diagnostic value of automatically perfoming level sections in RRSO has not been formally tested.

Design: RRSO slides from 79 women with BRCA mutations were retrospectively studied. All cases were dissected by a special protocol to maximize visible tissue: tubes and ovaries were sectioned at 2-3 mm intervals and entirely embedded. Tubal fimbriae were sectioned parallel to the fimbriae length. For this study, we identified all blocks containing fimbriae and obtained 3 deeper level sections on each one for standard hematoxylin and eosin (H&E) staining. A single experienced histotechnologist performed all the levels, which were cut down to a depth of more than 75% of the remaining tissue block. Levels were reviewed without knowledge of the original diagnosis and without reviewing the original H&E slide.

Results: The original diagnoses identified 9 cases of occult carcinoma: 4 in tubal fimbriae; 1 in tubal isthmus; 2 in ovary; 2 in both tubal fimbriae and ovary. Size of tubal carcinomas ranged from 1 to 11 mm. All but one tubal serous carcinoma were non-invasive. Total number of blocks containing fimbriae ranged from 1 to 3 per adnexa. Review of level sections did not reveal any additional cases of occult cancer. Non-neoplastic findings in tubal fimbriae included tubal epithelial hyperplasia (15%), transitional cell metaplasia of fimbriae (32%), endometriosis (9%), paratubal cysts (62%), and serous micro-adenofibromas (1%).

Conclusions: In RRSO specimens from BRCA positive women that have been thinly dissected and totally embedded, performance of routine multiple deep level sections of tubal fimbriae tissue blocks does not improve detection of occult carcinoma.

1013 Correlates of Papillary Infarction and Microinvasion in Ovarian Atypical Proliferative (Borderline) Serous and Seromucinous Tumors (APST/APSMT)

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Background: Although papillary infarction in ovarian APST/APSMT is commonly observed, there are few data regarding its significance, including any association with microinvasion and peritoneal implants, which are also poorly understood. Recent reports suggest that microinvasion is found in 25-50% of these tumors, in contrast to reports of 10% stated in the older literature. This study characterizes the features associated with papillary infarcts and microinvasion to further understand these phenomena.

Design: From consecutive hospital-based (non-consultation) cases, 23 APST/APSMT in 19 patients (4 bilateral) were reviewed and evaluated for papillary infarcts, peritoneal implants, autoimplants, microinvasion (<5 mm), bilaterality, calcification, salpingoliths (tubal mucosal calcifications), and endometriosis.

Results: Tumors were sampled with a mean of 2.1 sections per cm of maximum tumor diameter. Infarcts were identified in 47% of patients, and microinvasion in 26%. Those with infarcts had a mean age of 50 years and a mean tumor size of 8 cm, versus 58 years and 6.4 cm for those without infarcts (not significant). All tumors with papillary infarcts were serous as compared to 40% of those without infarcts ($p = 0.0108$). Microinvasion was found in 44% of those with infarcts as compared to 10% of those without infarcts (p=0.14). Noninvasive peritoneal implants were found in 22% of those with infarcts as compared to 0% of those without infarcts (p=0.21). Other features evaluated showed no meaningful correlations with papillary infarction. Microinvasive tumors were significantly more likely to be bilateral (60% v. 7%, p=0.037) and more often had noninvasive peritoneal implants (40% v. 0%, p=0.0585). All tumors with microinvasion were serous as compared to 57% of those without microinvasion (p=0.13). Among patients with APST, 38% had microinvasion. Other features evaluated showed no meaningful correlations with microinvasion.

Conclusions: Papillary infarcts and microinvasion are more common in APST as compared to APSMT. Microinvasion in APST is more common than previously appreciated, and APSTs with microinvasion are significantly more likely to be bilateral than those without microinvasion. Findings suggesting a possible relationship between papillary infarction, microinvasion and noninvasive peritoneal implants warrant further study.

1014 Low Levels of DNA Damage Based on Phosphorylated CHK2 Immunoreactivity Support Ovarian Endometrioid and Clear Cell Carcinomas as Type I Tumors

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Background: DNA damage can occur in cancer cells in response to a variety of endogenous and tumor microenvironment cues. Such damage may elicit or enhance genetic instability in tumor cells. DNA damage response appears to be more frequently detected in high grade serous carcinomas (prototypical Type II tumors) than in low grade serous carcinomas (prototypical Type I tumors) of the ovary (*Jiang et al., abstract submitted to USCAP)*. It has been proposed that endometrioid and clear cell carcinomas of the ovary belong in the Type I category of ovarian tumors, based on their clinicopathologic features, and should therefore exhibit a low amount of DNA damage. We performed an immunohistochemical study using phosphorylated CHK2 (pCHK2) as the surrogate marker for DNA damage response to test this hypothesis.

Design: An antibody that reacts to phosphorylated CHK2 protein at the Threonine 68 residue was used for immunohistochemistry on paraffin sections from a total of 12 endometrioid carcinomas and 13 clear cell carcinomas. In addition, 28 low-grade serous carcinomas and 239 high-grade serous carcinomas of the ovary were tested, representing prototypical Type I and Type II tumors for comparison, respectively. pCHK2 immunoreactivity was semi-quantitatively scored by two pathologists independently using a five-tier grading system (0 to 4+).

Results: pCHK2 immunoreactivity was localized exclusively in the nuclei of tumor cells. Using an arbitrary cutoff (3+/4+ vs. 0/1+/2+), no endometrioid or clear cell carcinomas demonstrated intense immunoreactivity for pCHK2. Likewise, only 3 (10%) of 28 low grade serous tumors showed intense staining. When low grade serous, endometrioid

and clear cell carcinomas are grouped as Type I tumors, only 3 (5.7%) of 53 Type I tumors (p = 0.012, chi-square test) demonstrated intense pCHK2 immunoreactivity. In contrast, 48 (20%) of 239 Type II tumors showed intense pCHK2 immunoreactivity. Ovarian surface epithelium, ovarian surface inclusion cyst, and tumor stromal tissues showed weak (1+) or undetectable levels of pCHK2 immunoreactivity.
Conclusions: Endometrioid and clear cell carcinomas of the ovary exhibit low levels of DNA damage and instability. Therefore, from the standpoint of DNA damage response, they belong in the Type I group of ovarian tumors.

1015 ZNF217 Gene Amplification and PIK3CA Mutation Are Associated with Ovarian Clear Cell Carcinoma

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Background: Ovarian clear cell carcinomas (CCC) are histologically unique among different types of primary ovarian carcinomas but it is unknown if the molecular genetic signatures of CCC are different from other common types of ovarian cancer.
Design: A total of 89 ovarian CCC were studied for their molecular genetic alterations. We employed a high resolution (250K) single nucleotide polymorphism (SNP) array analysis on 12 CCC for which the tumor cells were affinity purified from fresh specimens to avoid potential stromal cell contamination and culture artifacts. The chromosomal instability (CIN) level in CCC was compared to high-grade (HG) and low-grade (HG) ovarian serous carcinomas. An additional 77 cases of CCC were used for mutation analysis of *KRAS*, *BRAF*, *PIK3CA*, and *CTNNB1 (b-catenin)*.
Results: The overall chromosomal instability level in CCC reflected by DNA copy number changes was similar to LG serous carcinomas but was significantly lower than HG serous carcinomas. Amplification of chr20q13 was detected in 5 of 12 cases based on analyzing SNP array data but rarely in HG and none in LG serous carcinomas. This 20q13 amplicon contains at least one candidate oncogene, *ZNF217*, which has been shown to participate in oncogenesis in several types of human cancer including ovarian carcinoma. Mutation analysis demonstrated somatic sequence mutations of *PIK3CA, KRAS, CTNNB1 (b-catenin)* and *BRAF* in 28 (31.5%), 7 (7.9%), 3 (3.4%) and 0 of 89 CCC cases, respectively.
Conclusions: Our current findings in combination with previously published data suggest that the molecular genetic changes in ovarian CCC are distinct among ovarian surface epithelial neoplasms. The table below shows the relative frequency of molecular genetic changes in different types of ovarian carcinomas.

	CIN level	TP53	PIK3CA mutation	KRAS mutation	BRAF mutation	CTNNB1 mutation	ZNF217 amplicon
CCC	*	*	***	*	-	*	***
LG	*	*	*	***	***	-	-
EMC	NK	*	**	-	-	**	NK
MUC	NK	NK	NK	***	-	-	NK
HG	***	***	*	-	-	-	*

EMC: ovarian endometrioid carcinoma; MUC: mucinous carcinoma; NK: not known, ***: frequently present; *: rarely present; -: not present based on published studies.

1016 Decreased Copy Number in Chr1p36 and 9p21 May Be the Key Molecular Events in the Transformation of Ovarian Serous Borderline Tumors to Low Grade Serous Carcinoma

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Background: In a new model of ovarian serous carcinogenesis based on clinicopathological and molecular studies (Am J Pathol, 164:1511, 2004) we proposed that low-grade (LG) micropapillary serous carcinomas develop from serous borderline tumors (SBT) in contrast to high grade (HG) serous carcinomas which develop from intraepithelial carcinomas arising in ovarian inclusion cysts or from the fallopian tube. Similarly, based on clinicopathologic studies we proposed that so called "invasive implants" which are almost invariably associated with noninvasive or invasive LG micropapillary serous carcinomas are in fact metastatic LG serous carcinomas. The distinction between SBTs and LG micropapillary serous carcinomas has been based entirely on morphologic findings. The elucidation of the underlying molecular mechanisms by which SBTs progress to LG carcinomas is not only of biologic interest but could also be important clinically.
Design: We assessed DNA copy number changes in 24 LG serous carcinomas and SBTs using high density SNP arrays. Affinity-purified tumor cells from fresh tumor tissues were used to enhance detection sensitivity, especially the small regions with low-fold copy number alterations.
Results: SNP array analysis demonstrated distinctly different levels of DNA copy number alterations in LG carcinomas as compared to SBTs. Hemizygous chr1p36 deletions was present in 58.3% of LG carcinoma but only in 8.3% of SBTs. Either hemizygous or homozygous deletions of chr9p21 were detected in 50% of LG carcinomas but none in SBT. The chr1p36 region contained at least two candidate tumor suppressors, CHD5 and miR-34a whereas chr9p21 harbored the well-established CDKN2A/B tumor suppressors (i.e., p16 and Arf).
Conclusions: Our data suggest that chromosomal instability as reflected by the levels of DNA copy number changes is significantly higher in LG carcinomas compared to SBTs. Tumor suppressors in both chr1p36 and chr9p21 deleted regions may be important in tumor progression from SBT to LG serous carcinoma. The deletions in both subchromosomal loci may serve as potential genetic markers in distinguishing florid SBTs from LG micropapillary serous carcinoma in the ovary and in the distinction of metastatic LG carcinoma ("invasive implants") from benign implants.

1017 Myxoid Leiomyomas of the Uterus: A Report of 14 Cases

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Background: Myxoid change has generally been considered a worrisome feature in smooth muscle tumors of the uterus since King et al, in 1982, described a series of six leiomyosarcomas with overall relatively bland cytologic features but assessed to be pathologically malignant largely on the basis of myometrial infiltration. That leiomyomas may be myxoid, as described herein, has received scant attention.
Design: All available cases of uterine leiomyoma with myxoid change submitted to a consultant for a second opinion were reviewed. Cases of predominantly edematous or hydropic change and cases of apoplectic leiomyoma with spotty myxoid change were excluded.
Results: Patients were 26-57 years of age (average 39). All fourteen tumors were solitary masses ranging from 1.2 to 12.5 cm (average 5.7 cm). Sectioned surfaces were described as tan-white to gray to yellow with at least focal mucoid to gelatinous appearance. Areas of hemorrhage were noted in three cases, with cysts in two, and gross necrosis in one. Microscopically, myxoid change encompassed 30-100% of each tumor. All tumor borders lacked definitive infiltration, although one case showed a focally irregular border in one of fourteen slides and in four myomectomy specimens the border was not well seen. Twelve tumors contained alternating myxoid and non-myxoid areas which occasionally mimicked infiltration of the myxoid neoplasm into surrounding myometrium. Cellularity was generally low but there was focal moderate cellularity in four cases. Overall, cytology was bland with only focal bizarre-type nuclear atypia in three cases. Eleven cases lacked necrosis, while the remaining three contained extensive infarct-type necrosis. Focal hemorrhage was present in seven cases. Ten tumors were mitotically inactive. In two cases, rare mitoses were found, with average counts of <1/10 hpf. In the remaining two cases, mitoses averaged <2/10 hpf but were focally up to 6/10 hpf.
Conclusions: Although myxoid change in leiomyomas is much less common than watery edema, so-called hydropic change, from which it should be distinguished, it may be seen. Accordingly, myxoid change alone should not prompt a diagnosis of malignancy without unequivocal destructive myometrial invasion or other conventional characteristics of malignancy. Alternating myxoid and non-myxoid areas may simulate infiltration and should be distinguished from true myometrial invasion at the periphery of the mass, requiring careful gross evaluation and block coding.

1018 Apoplectic Change in Uterine Leiomyomas: An Analysis of 70 Cases Highlighting Previously Underemphasized Aspects

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Background: Previous literature has drawn attention to a limited constellation of findings often seen in leiomyomas from patients who have received progestin therapy, termed "apoplectic" leiomyomas. These changes are stellate zones of hemorrhage with small foci of necrosis surrounded by hypercellular regions with increased mitotic rate. Our experience has indicated that additional frequent findings not emphasized to date may be a clue to the diagnosis and, if misinterpreted, may lead to an erroneous classification.
Design: All available cases of uterine leiomyoma with apoplectic change submitted to a consultant for a second opinion were reviewed.
Results: Patients ranged in age from 23-66 years (average 41). Of the 57 cases with available gross descriptions, 74% had "atypical" features; foci of hemorrhage (49%), discoloration (23%), softening (19%), cystic change (18%), and necrosis (18%). Microscopic findings of concern for the submitting pathologist were specified in 65 cases and included increased mitoses (66%), atypia (53%), hypercellularity (40%), necrosis (42%), pyknosis (11%), "epithelioid" morphology (11%), and mucinous/myxoid change (6%). Characteristic microscopic features seen in essentially all cases included multifocality, variable size, and stellate to oval configuration. Additional features frequently observed were zonal hypercellularity (81%), hemorrhage (81%), zonal necrosis (70%), individual cell necrosis/pyknosis (89%), hyalinization (81%), edema (56%), mucinous to myxoid matrix (30%), and cysts (30%). Mitotic activity in the smooth muscle at the periphery of the apoplectic regions was categorized as low (0-1/10 hpf) in 44%, moderate (2-5/10 hpf) in 30%, and brisk (>5/10 hpf) in 26%. Findings that were occasionally seen but not striking overall were slight nuclear atypia, vascular alterations, fibrin deposition, hemosiderin-laden or foamy macrophages, and tearing artifact. True epithelioid morphology was not identified.
Conclusions: Previously underemphasized findings common in apoplectic leiomyomas include lack of the characteristic hemorrhage and/or zonal hypercellularity, extensive regions of zonal necrosis, abundant pyknosis often mistaken for epithelioid morphology, central hyalinization or edema, focal mucinous to myxoid appearance (causing concern for myxoid leiomyosarcoma), cystic degeneration, and mitotic rate >5/10 hpf. Knowledge of this expanded spectrum of features is essential for recognizing variants of apoplectic change and avoiding a misdiagnosis of malignancy.

1019 Immunohistochemical Profiling of 312 Gynecologic and Soft Tissue Smooth Muscle Tumors

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Background: Leiomyosarcomas (LMS) are malignant mesenchymal tumors that display smooth muscle differentiation. The gynecologic tract and the soft tissue compartments are the two most common sites of occurrence. There are some indications that LMS of gynecologic and non-gynecologic origins may be different. More importantly, particularly for the gynecologic LMS, distinction between malignant and benign smooth muscle tumors on morphologic grounds alone can be difficult at times.
Design: Using tissue microarrays that included 263 cases of LMS (106 gynecologic) and 49 cases of uterine leiomyoma (ULM), we examined the ability of selected markers

to differentiate between malignant and benign gynecologic smooth muscle tumors (p16 and MIB-1) and between LMS of gynecologic and non-gynecologic origins (ER and WT1).

Results: Eighty-five percent of the gynecologic LMS and 2% of ULM displayed diffuse (≥50% of tumor cells) cytoplasmic and nuclear p16 staining. The majority of ULM showed focal (5-20%) p16 immunoreactivity ($p<0.01$). 81% of the gynecologic LMS and 0% of the ULM exhibited ≥10% MIB-1 proliferation index ($p<0.01$). A combination of the two markers (diffuse p16 and/or high proliferation index) yielded a detection sensitivity of 92% and a specificity of 99% for gynecologic LMS as compared to ULM. Non-gynecologic LMS showed similar p16 staining and MIB-1 proliferation index as gynecologic LMS. In differentiating between LMS of gynecologic and non-gynecologic origins, ER nuclear positivity is seen in 4% and 50% of the non-gynecologic and gynecologic LMS respectively ($p<0.01$), while WT1 nuclear/cytoplasmic positivity is seen in 51% and 55% of the non-gynecologic and gynecologic LMS, respectively. 100% and 90% of ULM were positive for ER and WT1, respectively.

Conclusions: The findings of diffuse p16 immunoreactivity and/or high MIB-1 proliferation index appear to be relatively sensitive and highly specific indicators of malignancy in gynecologic smooth muscle tumors. ER but not WT1 immuno-positivity can be used to support the gynecologic origin of a LMS.

1020 Does Basal Atypia in Early Cervical Intraepithelial Neoplasia Belong to High Grade Squamous Intraepithelial Lesion?

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Background: Basal atypia (BA), defined as a significant cellular atypia confined to lower layer of the cervical epithelium, was proposed as an important criterion to diagnose high grade squamous intraepithelial lesion (HSIL), however, in a CIN classification system the lesion with BA can be classified as a grade 1. Diagnosis of the lesion with BA is challenging because of the discrepancy between the two classification system. Recently, there were several manuscripts that ProExC could be a reliable marker for high grade CIN, and CIN 2 cases in which moderate to strong immunoexpression of p16 were observed simultaneously progressed to CIN 3.

Design: Early CIN (CIN 1 or 2) with BA (n=44, group 1) and CIN 1 or condyloma without BA (n=33, 16 exophytic and immature condyloma , 17 CIN 1, group 2) were retrieved during the period 2000-2008. Immunohistochemistry (p16 and ProExC) and HPV PCR were done and we analyzed expression pattern of biomarkers (p16 and ProExC) and compared those with HPV types to define the meaning of BA and to test the utility of biomarkers. Cases were scored positive for p16 if moderate to strong diffuse or focal staining was present.

Results: The group 1 showed immunopositivity for p16 in 91% and strong immunopositivity for ProExC in the lower and upper halves of the epithelium was observed in 98%. HPV PCR data were available only in 26 cases, but high risk HPV was identified in 100%. Immunopositivity for p16 and ProExC were 89% and 100%, respectively, in high risk HPV group. The group 2 showed immunopositivity for p16 in 42% and ProExC immunopositivity were seen mainly in lower half layer with scattered several positive cells in upper half of the epithelium. HPV PCR data were available only in 23 cases; low risk-HPV related lesions (n=8), high risk-HPV related lesions (n=15). The p16 immunopositivities in low risk-HPV and high risk-HPV related lesions were 0% and 87%, respectively.

Conclusions: High rate of p16 overexpression and ProExC expression pattern in the group 1 suggests that they belong to HSIL although the atypia is confined to the lower layer of the epithelium. Those two combined biomarkers were useful to detect and diagnose the lesion with BA as HSIL.

1021 The DNA Damage Response of Fallopian Tube Secretory Epithelial Cells Renders Them Susceptible to Carcinogenesis

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Background: A growing body of data point to the fallopian tube secretory epithelial cells (FTSECs) as the cell-of-origin of most of both hereditary and sporadic cases of serous pelvic carcinoma. The fallopian tube is lined with epithelium that is composed of two cell lineages: FTSECs and ciliated cells. We hypothesized that the FTSEC is susceptible to DNA damaging effects associated with ovulation and that repeated and prolonged genotoxic stress leads to the development of carcinoma through a multi-step process. Investigating the mechanisms underlying the increased susceptibility of FTSECs to DNA damage, relative to the ciliated cells required the development of unique *in vitro* tools for co-culturing the two sub-populations of cells from human tissue samples.

Design: We co-cultured primary human FT epithelial cells, including both FTSECs and ciliated cells, under unique conditions which preserve the specific differentiation markers ('*ex vivo* culture system'). We exposed the co-cultures to different modalities of low dose DNA damage, including ionizing radiation, chemotherapeutic drugs, and oxidative damage, and analyzed the response.

Results: The ex vivo co-culture system of primary human FT epitheial cells proved to be a reproducible phenocopy of the *in vivo* endosalpynx. FTSECs displayed a unique DNA damage response compared to the neighboring ciliated cells. It is characterized by accelerated activation of the cellular repair machinery along with ineffective resolution of DNA lesions over an extensive period of time. A similar pattern is seen *in situ* when DNA damage is induced in whole human FT fimbria specimens.

Conclusions: This study, for the first time, confirms that FTSECs are a unique population of epithelial cells with a distinctly different response to genotoxic stress relative to ciliated cells *in vitro*. This finding is in parallel with the observations *in vivo* that DNA damage and *TP53* mutations are specific for this cell type. Damaged FTSECs may persist in the endosalpynx under replication checkpoints, however repeated insults, induced by ovulation or other endogenous or exogenous factors, may eventually lead to acquisition of enough mutations to allow escape from the cell cycle arrest, resulting in malignant transformation.

1022 Novel Model Systems To Study the Fallopian Tube Secretory Epithelium – The Cell-of-Origin of Serous Pelvic Carcinoma

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Background: Serous carcinoma is the most aggressive sub-type of endothelial ovarian carcinoma. It has been traditionally attributed to the ovarian surface epithelium (OSE), or surface-derived intracortical Mullerian inclusions as the fields of origin. Recent studies suggest that the fallopian tube (FT) fimbria, rather than the OSE, may be the site of origin for over 50% of sporadic and hereditary serous carcinomas, with secondary involvement of the ovaries and peritoneum. An early precursor lesion, the 'p53 signature', characterized by DNA damage, *TP53* gene mutations and p53 protein accumulation has been described in normal-appearing secretory epithelial cells (FTSEC). With the emergence of the FTSEC as a putative cell-of-origin for high grade serous carcinomas, the development of *in vitro* and *in vivo* model systems is warranted to help propel further discovery.

Design: To address the series of events that culminates in transformation of the secretory cells, we isolated pure primary FTSECs and immortalize them in culture by overexpressing *hTERT*. Furthermore, we transformed several FTSEC lines by serial introduction of oncogenes, as previously described in other epithelial cell types, and established a xenograft model in mice.

Results: The immortalized FTSECs cell lines could be propagated for over 10 passages and be genetically manipulated. The transformed FTSECs acquired an aggressive morphology and an anchorage-independent growth capability *in vitro*, and, more importantly, formed tumors in immunodeficient mice.

Conclusions: FTSECs are a unique population of epithelial cells with an increased susceptibility to malignant transformation, as judged by its exclusive involvement in serous carcinoma. The FTSECs cell lines are an invaluable tool for the studying the effects of clinically-relevant defined genetic alterations (such as *TP53* and *BRCA1* and *2* mutations or the activation of the c-myc and PI3K/Akt pathways) both *in vitro* and *in vivo*. The artificially transformed FTSECs are the first of its kind proof-of-concept of the tumorigenic capacity of this cell lineage. These novel model systems, which will be illustrated, are essential for basic and translational research aimed at deciphering the underlying mechanism of serous carcinogenesis and developing targeted therapeutics and biomarkers for serous pelvic carcinoma.

1023 Involution of Latent Endometrial Precancers by Hormonal and Non Hormonal Mechanisms

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Background: Inactivation of the PTEN suppressor gene occurs in the majority of endometrial cancer cases. Somatic PTEN inactivation by deletion and/or mutation, the first detectible change of endometrial carcinogenesis, occurs at a high frequency in the endometrium of normal premenopausal women, though few of these progress to cancer. We hypothesized that the 50-60% reduced cancer risk of oral contraceptives (OCP) and intrauterine devices (IUD) occurs in part through their activity as negative selection factors for these subclinical mutated glands.

Design: 71 women with a history of oral contraceptive use and 80 with a history of IUD use were age matched with 191 and 119 controls, respectively. Endometrial biopsies were immunostained for PTEN and each scored for presence or absence of PTEN null glands (latent precancer).

Results: The frequency of latent precancers was significantly reduced in OCP (13%, OR 0.19, $p<0.001$) and IUD (18%, OR 0.42, $p=0.015$) exposed women compared to respective matched controls (43 and 34%). Presence or absence of endometritis did not significantly correlate with PTEN status within the IUD exposed group ($p=0.24$).

Conclusions: Normal appearing PTEN mutated endometrial glands, which are highly prevalent in the normal population, may be targets of endometrial cancer risk modulating exposures. Some exposures known to diminish endometrial cancer occurrences in epidemiologic outcome studies, including OCP and IUD use, are associated with a proportionate decline in the frequency of latent precancers. Involution of pre-existing endometrial latent precancers, as evaluated by PTEN analysis, may provide an accessible surrogate marker for long term endometrial cancer risk.

1024 Tissue DNA Genotyping of Hydatidiform Moles: Practical and Reliable Diagnosis

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Background: Hydatidiform moles are defined at the genetic level by their unique parental chromosome contributions, and genotyping analysis promises to offer ultimate confirmation and subclassification of these lesions. We report our experience of DNA genotyping in the routine workup of hydatidiform moles in the past 20 months.

Design: Consecutive cases of product of conception that had undergone DNA genotyping in the past 20 months were retrieved from our pathology files. All cases had initial tissue diagnoses and sufficient DNA materials were extracted from formalin-fixed paraffin embedded tissue sections of both chorionic villi and maternal gestational endometrium. DNA genotyping was performed using a multiplex PCR reaction targeting 15 tetrameric polymorphic loci of the human genome, and the PCR product was analyzed by capillary electrophoresis.

Results: A total of 118 products of conception were included in this study. All had initial diagnoses with variable certainties of molar pregnancy. DNA genotyping was successful in all, leading to the final identification of 36 cases of hydatidiform moles. Among 13 complete moles, 9 cases were monospermic and 4 cases were dispermic

at the genomic level. Twenty-three cases were confirmed by genotyping as triploid partial moles, all of which were dispermic and monogynic. Among nonmolar cases, 9 gestations were confirmed to have chromosomal trisomy syndromes: trisomy 16 (3 cases), trisomy 21(3 cases), trisomy 8 (1 case), trisomy 7 (1 case) and combined trisomy 8 and 21 (1 case). More complex chromosomal abnormalities were seen in 2 cases and 1 case was proven as digynic nonmolar triploidy. The remaining 65 cases were balanced biallelic gestations.

Conclusions: Replacing both p57 immunohistochemistry and DNA ploidy analysis, tissue DNA genotyping is a practical and highly accurate method for the confirmation and subclassification of hydatidiform moles and beyond. Our results further advocate a routine application of this "one-stop shopping " approach in the workup of hydatidiform moles.

1025 The von Hippel-Lindau Gene Product (pVHL) and Kidney Injury Molecule-1 (KIM-1) Are Useful Diagnostic Markers for Identifying Focal Clear Cell Carcinoma of the Uterus

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Background: Identification of even a small proportion of clear cell carcinoma (CCC) in uterine adenocarcinoma is important because focal CCC is considered a high-grade carcinoma, and the patient would receive more aggressive treatment. Differentiation of CCC from clear cell features (CCF) resulting from other conditions, such as squamous differentiation, secretory change, and mucinous change, can be challenging. No absolutely sensitive and specific tumor-associated markers are available to make this distinction. Our recent study demonstrated that pVHL was a relatively sensitive (90%) and specific marker for identifying CCC of both the uterus and ovary (Lin et al; AJCP 2008;129:592). In addition, KIM-1, a sensitive and specific marker for clear cell renal cell carcinoma, also showed some utility in the diagnosis of CCC of the ovary and uterus (Lin et al; AJSP 2007;31:371-381). In this study, we further investigate the usefulness of these two markers in identifying focal CCC of the uterus.

Design: A total of 64 paraffin blocks from 20 cases of uterine adenocarcinoma with focal CCF were included. Of the 20 cases, 17 cases were endometrioid carcinoma with focal CCF; and 3 cases were papillary serous carcinoma with focal CCF. Immunohistochemical stains with antibodies against pVHL and KIM-1 were performed as previously described. The staining intensity and distribution were recorded. The H&E-stained slides with clear cell features were marked and compared to the positively stained areas by either pVHL and/or KIM-1.

Results: A membranous/cytoplasmic staining for pVHL was observed in 11 of 20 cases (28 paraffin blocks). The pVHL-positive areas matched the H&E slides with clear cell features. In contrast, KIM-1 with membranous/cytoplasmic positivity was observed in 6 of 20 cases (11 paraffin blocks). The KIM-1-positive areas also matched the H&E slides with clear cell features. All KIM-1-positive cases were also positive for pVHL.

Conclusions: These data indicate that pVHL and KIM-1 may serve as diagnostic markers for identifying and confirming focal clear cell carcinoma presented in adenocarcinoma of the uterus, which in turn may minimize the over-diagnosis of other conditions such as squamous differentiation and secretory change as focal clear cell carcinoma. Further study in a large series with clinical follow-up is warranted to confirm this finding.

1026 The Distribution of HPV Types in Invasive Cervical Carcinoma. Analysis of 9760 Cases

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Background: Cervical cancer is caused by HPV infection, most frequently by high risk HPV types 16 and 18. However, the distribution of other less frequent types is not well described and may be important to predict the impact of first generation vaccines.

Design: The objective was to describe the HPV genotype distribution in invasive cervical cancer worldwide. Paraffin embedded cervical cancer cases were collected (from 1920 to 2005). HPV detection was done by SPF-10 broad-spectrum primers PCR subsequently followed by DEIA and genotyping by $LiPA_{25}$ (version 1). Samples were tested at HPV laboratories at ICO (Barcelona, Spain) and at DDL (The Netherlands).

Results: Of 13,239 cases included in the study, 11,171 have now been evaluated and 9,760 considered suitable for testing. Histologically, 89% were squamous cell carcinomas, 8% adenocarcinomas, 1.4% adenosquamous carcinomas and 1.6% other subtypes. HPV genotype data are available for 8,785 cases. The five most common types detected were HPV 16 (60.3%), HPV 18 (10.3%), HPV 45 (5.9%), HPV 31 (4.2%) and HPV 33 (4.0%). This distribution was consistent across continents with the exception of Asia where HPV 58 ranked 3rd and in Oceania where HPV 68/73 ranked 4th. Among adenocarcinomas, HPV 16 accounted for 47.8% (range by continent= 35.5%-53.2%) and HPV 18 for 29.0% (range by continent= 23.5%-36.9%). Presence of exclusively low risk types accounted for 0.44% of all cases. Multiple infections were detected in 4.8% of the samples.

Conclusions: HPV types 16, 18, 31, 33 and 45 were the most common types in cervical cancer cases around the world, accounting for 85% of all the cases. There were no statistically significant differences in the distribution of these types over time ($p>0.05$).

1027 Abnormal Myometrial Vasculature Explains Some Cases of Menorrhagia

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Background: The causes of menorrhagia are known in a limited number of cases. They include leiomyomas, endometrial polyps, and rarely bleeding diatheses. However, in most cases there is no anatomic explanation and because of profuse bleeding a hysterectomy is necessary. The goal of this study was to review this type of cases.

Design: We reviewed the H&E slides from 10 hysterectomies performed because of uncontrollable menorrhagia where an obvious anatomic cause was not found. These cases were age matched with 10 patients who underwent hysterectomies for reasons other than menorrhagia, i.e. cervical dysplasia or cancer, BRCA positivity, and uterine prolapse. From each case one section was selected based on the increased number of vessels in comparable amount of tissue. In order to avoid counting lymphatic vessels, only vessels with an obvious thick wall were counted. A group of vessels was defined as 4 or more vessels in an area without myometrial fibers between them. We looked at the number of vessels in the plexus arcuatus, total number of groups of vessels located between the endometrium and the plexus arcuatus, number of groups of vessels at one or less than one low power field (4x) from the endometrium, and number of vessels within vessels at one or less than one low power field (4x) from the endometrium in each patient and compared the results in both of the selected groups.

Results: There were no differences between the two groups in the number of vessels in the plexus arcuatus and the number groups of vessels located between the endometrium and the plexus arcuatus. However, there was a significant difference between the two groups in the number of groups of vessels and number of vessels in vessels located within one low power field from the endometrium. 70% of the patients in the group with menorrhagia had groups of vessels within 1 mm from the endometrium, while only 30% of the patients without menorrhagia had groups of vessels in this area. A similar proportion (70%) of patiens with menorrhagia had more than one vessel in vessel located within one low power field from the endometrium while only 30% of the patients without menorrhagia had more than one vessel in vessel in this area.

Conclusions: Abnormal myometrial vasculature immediately underlying the endometrium is probably the cause of menorrhagia in cases without an obvious anatomic cause. This would explain the lack of response to hormonal treatment in the unexplained cases of menorrhagia.

1028 Atypical Leiomyomas of the Uterus: A Clinicopathologic Study of 46 Cases

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Background: Atypical leiomyoma is a well-described smooth muscle neoplasm of the uterus. Only one study has addressed long-term clinical follow-up in a large series, and little is known about the adequacy of treatment by myomectomy.

Design: The surgical pathology archives were searched for consecutive cases of uterine atypical leiomyoma from 1992 to 2003. Glass slides were reviewed to confirm the diagnoses (Bell et al.) and patient age, treatment modality, and clinical follow-up data were recorded.

Results: 46 atypical leiomyomas with available glass slides and clinical follow-up were identified. 24 tumors exhibited diffuse, moderately to severely atypical cells, whereas 22 showed atypical cells in a more patchy distribution. 11 had ischemic-type necrosis. By highest count method, 34 cases had ≤1 MF/10 HPF, 11 showed 1-3 MF/10 HPF, and 1 was nearly entirely necrotic precluding mitotic assessment. Where adjacent non-neoplastic tissue was well-visualized, all had pushing margins (43 cases). The average tumor size was 6.8 cm (median 6.5 cm, range 0.7-14 cm). The average patient age was 42.5 years (median 42 years, range 21-72 yrs). 30 were treated with hysterectomy, and 16 with myomectomy. Average follow-up was 42 months (range 0.3-121.8 months). Of those treated with hysterectomy, 1 had recurrent atypical leiomyoma in the retroperitoneum at 87.5 months, 1 died of other causes, and all 28 others (93%) were free of disease. Of the myomectomy group, 2 had residual atypical leiomyoma in the subsequent hysterectomy specimen, and 1 underwent second myomectomy for atypical leiomyoma with 2 subsequent successful pregnancies. All 13 others (81%) were clinically free of disease.

Conclusions: Atypical leiomyoma has a low rate of extrauterine, intra-abdominal recurrence (2%) with no apparent risk for distant metastasis. Patients may be treated by myomectomy alone with successful pregnancy, but should be monitored for local intrauterine residual/recurrent disease (19% in this study).

1029 Evidence-Based Recommendations for Intra-Operative Pathologic Evaluation of Risk Reducing Salpingo-Oophorectomies from Women with BRCA Mutations

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Background: Occult cancer can be detected in up to 15% of risk-reducing salpingo-oophorectomies (RRSO) in women with BRCA mutations. Occult cancer is often microscopic. The role of intra-operative pathologic evaluation of RRSO remains undefined. While intra-operative cancer diagnosis allows for immediate surgical staging, there is a risk that a small cancer is cut away during frozen section (FS) preparation. Correlation of intra-operative gross and FS findings with those of the permanent slides has not been studied. We report this correlation in RRSO of 112 BRCA patients and we propose an algorithm for intra-operative evaluation.

Design: Intra-operative gross and FS findings in RRSO from 112 BRCA patients were retrospectively reviewed. Intra-operative decision to evaluate RRSO by gross exam or FS was made on an ad-hoc basis. A standardized algorithm was not used. For permanent sections, RRSO were sectioned at 2 to 3 mm intervals and entirely embedded. Tubal fimbriae were sectioned parallel to the fimbriae.

Results: Occult cancer was detected in permanent slides of 9/112 RRSO (5 in tube, 2 in ovary, 2 in tube and ovary), of which only 1 was diagnosed intraoperatively. Gross abnormalities were seen in 77/112 RRSO: ovarian cysts, 46%; paratubal cysts, 27%; ovarian nodules, 14%; tubal nodules, 7%. Intra-operative exam was performed in 48/112 RRSO; FS was performed in 19/48. Gross findings were seen in 8/9 occult cancers: ovarian cysts (n=3, up to 6 cm), paratubal cysts (n=2, up to 0.7 cm), ovarian nodule (n=2, 1.1 to 3 cm), tubal nodule (n=2, 0.9 to 1.2 cm). Among 9 occult cancers, FS was performed on 1 case with ovarian cysts, 1 with a paratubal cyst, and 1 with an ovarian nodule. The cyst FS were benign but permanent slides of fimbriae revealed occult carcinoma (0.15 to 0.2 cm). The ovarian nodule FS was malignant. Of 6 occult cancers without FS, all cases with nodules corresponded to cancer but none of the cysts did; in the latter, cancer was in fimbriae or ovary (0.1 to 0.2 cm). Among 82 RRSO with gross cysts, none were malignant. Among 24 RRSO with gross nodules, 4 (17%) were malignant.
Conclusions: Cancer in RRSO presents as a nodule or as a microscopic finding but not as a cyst. There is no role for frozen section of RRSO unless a nodule is visible. Otherwise, fixation, thin sectioning and total tissue embedding is the optimal method to detect occult cancer.

1030 Frequent Glypican-3 Overexpression in Ovarian Clear Cell Adenocarcinoma

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Background: Glypican-3 (GPC3) is a heparan sulfate proteoglycan that is overexpressed in hepatocellular carcinoma, malignant melanoma, and testicular yolk sac tumor (YST). GPC3 is also known as a tumor marker and potential target for immunotherapy. There have been relatively few studies regarding GPC3 expression in ovarian carcinoma, the results of which were inconsistent, especially with regard to ovarian clear cell adenocarcinoma (CCA).
Design: We used immunohistochemistry to evaluate GPC3 expression in 213 ovarian adenocarcinomas, including a large number of CCAs (n=94). In addition, GPC3 expression in ovarian surface epithelial inclusions, endometriosis, and other Müllerian duct derivatives, such as the fallopian tube epithelium, endometrial epithelium, and endocervical epithelium, were investigated. To determine the value of GPC3 immunostaining in distinguishing ovarian CCAs from YSTs, immunostaining was also performed in 6 YSTs.
Results: Among the four major histological subtypes of ovarian adenocarcinoma (clear cell, mucinous, endometrioid, and serous), GPC3 expression was detected in a significantly high proportion of CCAs (44%, P<0.0001). In contrast, positive immunoreactivity for GPC3 was rarely observed in other histological subtypes of ovarian adenocarcinoma: 4%, 5%, and 11% in mucinous, endometrioid, and serous adenocarcinomas, respectively. Ovarian surface epithelial inclusions and benign Müllerian duct derivatives were usually negative for GPC3. All 6 ovarian YSTs showed diffuse immunoreactivity for GPC3. In CCA cases, no correlation was observed between GPC3 expression and clinicopathological factors, such as tumor stage, lymph node metastasis, peritoneal dissemination, and mortality rate. However, when limited to stage III/IV CCA cases, GPC3 positivity was associated with poor overall survival (P=0.019).
Conclusions: We showed frequent GPC3 overexpression in ovarian CCAs. As ovarian CCA is known for its resistance to conventional chemotherapy, our results suggest that GPC3 may be a useful alternative target for immunotherapy in advanced stage CCA. For surgical pathologists, it is important to know that GPC3 immunohistochemistry is of limited value in discriminating between ovarian CCA and YST.

1031 Significant Overexpression of HMGAs in Serous Ovarian Carcinoma in Association with p53

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Background: HMGA1 and 2, high-mobility-group AT-hook proteins, are important regulators of cell growth, differentiation, apoptosis and transformation. Overexpression of HMGA1 and 2 has been reported in human ovarian cancer. Furthermore, HMGA2 overexpression was identified in early stage of ovarian cancer in animal models. Ovarian cancer is genetically and histologically heterogeneous. To further characterize whether HMGAs are the important molecules in association with the tumorigenesis of specific type ovarian cancer, and with p53 mutation, we initiated this study.
Design: A total of 60 cases with ovarian carcinoma were collected. Among them 30 were high grade papillary serous (PSC) and 30 endometrioid (EMC). Case matched normal tissues from fallopian tube and endometrium were used as controls. All PSC were FIGO grade 3. EMC was confirmed to be ovarian primary. Expression of HMGA1, HMGA2 and p53 were examined by immunohistochemistry (IHC). Semiquantitative immunoscores were scaled by immunointensity and percentage.
Results: There were significant differences of HMGA1 and 2 between high grade PSC and EMC in ovary. In PSC, overexpression of HMGA1 and HMGA2 was identified in 72% and 58%, respectively. In contrast, expression of HMGA1 and 2 was detected in only 44% and 17% in EMC. When we compared HMGA1 and 2 expressions with p53, a correlation of HMGA overexpression with p53 in PSC was found (r=0.54, r=0.38 respectively).

	HMGA1	HMGA1	HMGA2	HMGA2	p53	p53
	PSC	EMC	PSC	EMC	PSC	EMC
0	28%	56%	42%	83%	13%	72%
1+	16%	17%	15%	6%	22%	6%
2+	48%	28%	4%	6%	9%	6%
3+	8%	0%	38%	6%	57%	17%

Conclusions: This is the first study to examine HMGA expression in different histological types of ovarian cancer. We found overexpression of HMGAs is significantly higher in PSC than that in EMC. Particularly, overexpression of HMGAs is correlated with p53 overexpression. Findings further support the functional roles of HMGAs in p53 mediated tumorigenesis.

1032 HSD3B1 Is a Specific Trophoblast-Associated Marker Not Expressed in Various Normal and Neoplastic Tissues

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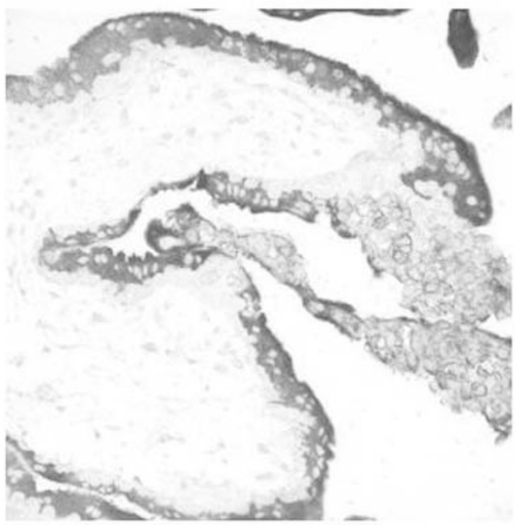
Background: HSD3B1 (hydroxyl-d-5-steroid dehydrogenase) is an enzyme involved in steroid hormone synthesis. It has been shown to be expressed in normal trophoblastic tissue, benign and neoplastic trophoblastic lesions. The expression of HSD3B1 was not detected in a large number of lung, breast and uterine carcinomas. To see if HSD3B1 is highly specific for trophoblasts, we examined the expression of HSD3B1 in a wide spectrum of normal tissues and carcinomas.
Design: Archival paraffin tissues of carcinomas and normal tissues from various sites were constructed onto tissue microarray (TMA). A total of 10 TMAs containing carcinomas from lung, breast, ovary, uterine cervix, liver, pancreas, stomach, and colon, and one TMA containing normal tissues from brain, GI tract, thyroid, pancreas, spleen, adrenal gland, liver, testis, ovary, endometrium and placenta were used in this study.

Type and number of tumors in this study

Tumor		Number
Hepatocellular carcinoma		54
Lung		
	squamous cell carcinoma	14
	adenocarcinoma	36
	bronchioloalveolar carcinoma	1
	lymphoepithelioma-like carcinoma	1
Renal cell carcinoma		45
Breast infiltrating ductal carcinoma		39
Ovary high grade serous carcinoma		52
Uterine cervix		
	sqaumous cell carcinoma	33
	adenocarcinoma	4
	adenosquamous carcinoma	1
Prostate adenocarcinoma		32
Colon adenocarcinoma		52
Stomach adenocarcinoma		47
Pancrease adenocarcinoma		29

Immunohistochemistry was carried out using a commercially available anti-HSD3B1 monoclonal antibody (1:500, Abnova, Taiwan).
Results: Positive staining was detected in the cytoplasm of intermediate trophoblasts and syncytiotrophoblasts in the placental tissue which served as positive control.

Fig. 1 HSD3B1 stain in normal placenta



Other normal tissues and carcinomas from various sites were all negative for HSD3B1.
Conclusions: As a trophoblastic marker, HSD3B1 is highly specific, and worked better than the commercially available HCG antibody. HSD3B1 can be used in the distinction of trophoblastic tumors from other commonly seen carcinomas and in the distinction of non-neoplastic trophoblastic lesions from other benign lesions.

1033 Ovarian Carcinosarcomas: A Histomorphologic and Molecular Study

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Background: Ovarian carcinosarcomas (MMMTs) are malignant tumors with aggressive behavior. Based on the proposed pathogenetic model of ovarian tumors, MMMTs are type II tumors with high frequency of p53 mutation. To examine whether all ovarian MMMTs belong to type II, we studied the histomorphologic and molecular features of 11 MMMTs.

Design: The HE slides were reviewed by two pathologists. The carcinomatous and sarcomatous areas were separately microdissected followed by DNA extraction. Genes frequently mutated in ovarian carcinomas were analysed by direct sequencing. Immunohistochemistry for p53 and β-catenin was also performed.
Results: The carcinomatous components included 5 high grade serous carcinomas, two grade 3 endometrioid carcinomas, two grade 1 endometrioid carcinomas, one clear cell carcinoma and one low grade serous carcinoma. Endocervical-like mucinous borderline tumor was present in one tumor with grade 3 endometrioid carcinoma. Choriocarcinoma was noted in one tumor with clear cell carcinoma. The sarcomatous components were heterologous in three tumors. Of the ten tumors with available DNA, p53 mutation was detected in 6 tumors. BRAF and PIK3CA mutations were found in the tumor with mucinous borderline tumor and grade 3 endometrioid carcinoma.

Table 1. Histomorphologic and molecular features of ovarian MMMTs in this study

Case	carcinoma	sarcoma	p53 stain	β-catenin stain	p53 mutation	BRAF mutation	PIK3CA mutation
1	LG serous	homologous	+ in sarcoma	-	-	-	-
2	HG serous	chondrosarcoma	+	-	135 TGC →TGG	-	-
3	MBT, gr 3 EM	homologous	+	-	272 GTG →GGG	593 GAT →GGT	545 GCA →GAA
4	HG serous	homologous	+	-	273 CGT →CAT	-	-
5	HG serous	homologous	+	-	-	-	-
6	HG serous	homologous	+	-	273 CGT →CAT	-	-
7	gr 1 EM	homologous	-	+	-	-	-
8	gr 1 EM	rhabdomyosarcoma	+	-	220 TAT →TGT	-	-
9	HG serous	homologous	+	-	245 GGC →GAC	-	-
10	clear cell	homologous	+ in sarcoma	-	-	-	-
11	gr 3 EM	chondrosarcoma	+	-	ND	ND	ND

ND: not done

The corresponding sarcomatous components showed the same mutation. No mutation of KRAS or β-catenin was detected. Nuclear staining of β-catenin was seen in one tumor containing grade 1 endometrioid carcinoma. Positive expression of p53 was present except 3 tumors containing clear cell carcinoma, grade 1 endometrioid carcinoma and low grade serous carcinoma.
Conclusions: Our results revealed high frequency of p53 mutation in ovarian MMMTs, compatible with the pathogenetic model. A few MMMTs may derive from type I tumors. Presence of choriocarcinoma within one tumor suggests that tumor stem cells may occasionally acquire the potential of trophoblastic differentiation.

1034 Immunohistochemical Expression of LYVE-1 Is a Prognostic Factor in Ovarian Carcinoma

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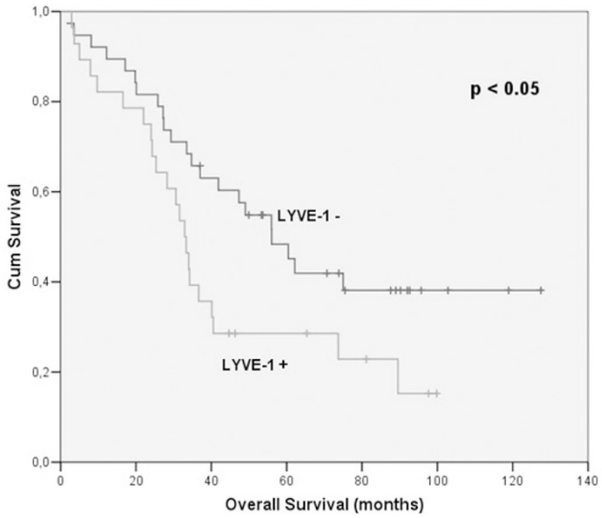
Background: Lymphangiogenesis and lymphatic density correlate with prognosis in several malignancies including melanoma, colorectal and lung cancer, but their significance in ovarian carcinoma (OC) is unclear. Expression of lymphatic markers in non-lymphatic cells has been reported but is not well understood.
Design: We studied 68 patients with OC treated at our Hospital between 1996 and 2003. All the patients were homogeneously treated by debulking surgery and a platinum/taxane-based chemotherapy. Immunohistochemistry was performed on tissue microarrays. We examined the expression of the vascular markers CD34, LYVE-1 and $D_2$40 in tumor cells (0/1) and the microvascular (MVD) and lymphovascular density (LVD) as detected respectively by CD34 and $D_2$40 positive vessels in tumor stroma (0-3). Expression values were correlated with clinicopathological variables, including progression-free (PFS) and overall survival (OS).
Results: Clinicopathological data and results of the study are summarized in Table 1. On univariate analysis, expression of LYVE-1 in tumor cells was significantly associated with worse OS (Figure 1). No association was found between MVD, LVD or the expression of CD34 and $D_2$40 in tumor cells with any clinicopathological variables.

Age (mean, range)	55.19 (21-82) yrs
Histology (n)	Serous (43), others (25)
Grade (n)	1 (5), 2 (26), 3 (37)
Stage (n)	I (2),II (5),III (51),IV (10)
PFS (mean, range)	19.58 (1.37-67.00) mo
OS (mean, range)	46.57 (1.57-127.47) mo
CD34 (n)	0 (67), 1 (1)
MVD [CD34+ vessels](n)	0 (5), 1 (32), 2 (23), 3 (3)
LYVE-1 expression (n)	0 (38), 1 (28)
D240 expression (n)	0 (35), 1 (31)
LVD [D240+ vessels] (n)	0 (16), 1 (18), 2 (22), 3 (10)



Conclusions: In our series, the expression of LYVE-1 on tumor cells significantly correlates with worse OS in OC. MVD and LVD do not have impact in PFS or OS. Further studies are needed to clarify the significance of the expression of lymphatic markers in tumor cells in OC. Grants: FIS PI07/0651 and Fundación Mutua Madrileña.

1035 Expression of D2-40, a Marker of Mesothelial and Lymphatic Endothelial Differentiation, in Adenomatoid Tumors: A Potential Diagnostic Pitfall in Distinguishing from Lymphatic Tumors

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Background: Adenomatoid tumors are benign tubulo-glandular proliferations of mesothelial immunophenotype (calretinin, WT-1 positive) that commonly involve the female and male genital tract. Though diagnosis is usually straightforward, the differential diagnosis may include Sertoli cell tumor, yolk sac tumor, adenocarcinoma, or lymphangioma/lymphangioleiomyoma, depending on the anatomic site. Because calretinin and WT-1 expression may overlap with some of these entities, we hypothesized that the novel mesothelial marker D2-40 may be of value in evaluating adenomatoid tumors. However, because D2-40 also reacts with lymphatic endothelium, we tested D2-40 expression in lymphatic tumors arising in similar anatomic sites to determine its specificity in this differential diagnosis.
Design: Immunohistochemical staining for D2-40 (Dako, 1:50), calretinin (Zymed, 1:200), and WT-1 (Dako, 1:200) was performed on 33 adenomatoid tumors (17 uterine, 6 fallopian tube, 9 paratesticular, 1 scrotal). Staining for each of these markers was also performed in 19 lymphangiomas (8 retroperitoneal, 5 omental/mesenteric, 2 mediastinal, 2 abdominal wall, 1 subcutaneous, 1 unknown origin), and 1 case of uterine lymphangioleiomyomatosis. Nuclear staining for calretinin or WT-1 was defined as positive. Cytoplasmic staining for D2-40 was defined as positive.
Results: Thirty-one of 33 (94%) adenomatoid tumors expressed D2-40 and calretinin, while 26 of 33 (79%) expressed WT-1. The staining pattern was strong and diffuse in the tumor cells for both D2-40 and calretinin, while staining for WT-1 was focal and weaker in intensity. Eighteen of 19 lymphangiomas showed diffuse and strong expression of D2-40 but no expression of calretinin or WT-1. The lymphatic component of the lymphangioleiomyoma case demonstrated strong D2-40 expression but no staining for calretinin or WT-1.
Conclusions: Adenomatoid tumors strongly expressed D2-40. This can be of value when the differential diagnosis includes tumors that also express WT-1 or calretinin. However, since D2-40 was also expressed in lymphatic tumors, caution is warranted in relying on this marker alone and a broad panel of mesothelial and lymphatic markers should be considered.

1036 Diagnostic Value of PAX2, a Urogenital Tract Transcription Factor, in Distinguishing Mesonephric Duct Proliferations of the Cervix from Endocervical Adenocarcinoma and Its Mimics

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Background: Proliferation of mesonephric duct remnants of the cervix can pose diagnostic difficulties with benign and malignant entities, notably minimal deviation endocervical adenocarcinoma. Markers of mesonephric differentiation such as CD10 and WT1 have not proved to be reliably helpful in this context. PAX2 is a transcription factor involved in urogenital tract development, particularly Wolffian duct derivatives, and is expressed in tumors of renal origin. Few studies of PAX2 in Mullerian derivatives exist and little is known about Wolffian duct remnants in the lower gynecologic tract. In this study we evaluated PAX2 in mesonephric duct remnants of the cervix and its diagnostic value in distinguishing such lesions from a spectrum of endocervical glandular metaplasias, hyperplasias and adenocarcinomas.
Design: PAX2 immunohistochemical staining (Zymed, 1:200) was performed on formalin-fixed, paraffin-embedded sections from the following entities: mesonephric duct remnants (hyperplastic and non-hyperplastic); mesonephric carcinoma; minimal deviation endocervical adenocarcinoma; conventional endocervical adenocarcinoma (in-situ and invasive); tubal/tuboendometrioid metaplasia; cervical endometriosis; deep

endocervical glands; and endocervical glandular hyperplasia. PAX2 nuclear expression was defined as positive.
Results: PAX2 was diffusely and strongly expressed in all cases of mesonephric duct remnants, both hyperplastic (20/20) and non-hyperplastic (4/4). PAX2 was expressed in all normal endocervix, 7/7 tubal/tuboendometrioid metaplasia, 12/13 cervical endometriosis, 3/3 endocervical glandular hyperplasia and 2/2 deep endocervical glands. Neither mesonephric carcinoma (0/1), minimal deviation endocervical adenocarcinoma (0/5), nor typical endocervical adenocarcinoma in situ (0/5) expressed PAX2. The mesonephric hyperplasia surrounding the mesonephric carcinoma strongly expressed PAX2 but the carcinoma did not. Patchy PAX2 was noted in 1/16 typical invasive endocervical adenocarcinomas and in 1/1 tuboendometrioid type endocervical adenocarcinoma.
Conclusions: Benign mesonephric duct proliferations and benign endocervical glandular hyperplasias and metaplasias expressed PAX2 whereas only rare endocervical adenocarcinomas showed patchy expression and mesonephric carcinoma did not. Loss of PAX2 expression in mesonephric and endocervical glandular lesions may be a diagnostic marker of malignancy.

1037 Diagnosis and Subclassification of Hydatidiform Moles Utilizing p57 Immunohistochemistry and Molecular Genotyping: Validation and Prospective Analysis with Development of an Algorithmic Approach

TG McConnell, K Murphy, M Hafez, RS Vang, BM Ronnett. Johns Hopkins Medical Institutions, Baltimore, MD.
Background: Distinction of hydatidiform moles (HM) from non-molar specimens and subclassification of HMs as complete (CHM), partial (PHM), or early CHM (eCHM) are important for clinical practice and investigational studies but diagnosis based solely on morphology suffers from poor interobserver reproducibility. Recent studies have demonstrated the utility of p57 immunostaining and molecular genotyping for improving diagnosis of HMs.
Design: A validation study of p57 immunostaining and genotyping with the AmpFISTR Profiler kit was performed on 24 archival specimens (7 CHM, 8 PHM, 9 non-molar). Prospective analysis of 42 consecutive cases (83% consultation) for which there was any consideration of a diagnosis of HM was conducted. After satisfactory experience with prospective cases, a modified diagnostic approach was adopted, with p57 immunostaining used with morphology to triage selection of cases for genotyping.
Results: Final diagnoses for the prospective cases were 24 CHMs (including 7 eCHMs), 7 PHMs, and 11 non-molar. P57 immunostaining was negative in all CHMs, with the exception of 1 molecularly confirmed CHM with diffuse p57 expression, and positive in all PHMs and non-molar specimens, with the exception of 3 molecularly confirmed PHMs with an equivocal extent of p57 expression. Molecular genotyping of 51 cases (24 validation, 27 prospective) yielded data consistent with p57 results in the 47 cases with unequivocal p57 expression patterns and was used to establish diagnoses for the 4 with aberrant or equivocal p57 results. All 17 genotyped CHMs demonstrated androgenetic diploidy, including the CHM with retained p57 expression; this case also demonstrated trisomy 11 (retained maternal allele accounting for aberrant p57 expression). The remaining 14 CHMs were diagnosed by morphology and negative p57 results alone. All 15 PHMs demonstrated diandric triploidy. All genotyped non-molar specimens demonstrated biparental diploidy.
Conclusions: This study validates p57 immunostaining as a prospectively applicable triage assay for diagnosis of CHMs based on morphology and a negative p57 result. Molecular genotyping is validated as a method to confirm a diagnosis of CHM by demonstrating androgenetic diploidy and to resolve p57-positive cases into diandric triploid PHMs, biparental diploid non-molar specimens, and the rare CHM with aberrant p57 expression.

1038 Expression of Platelet-Derived Growth Factor Receptor α (PDGF-Rα) and Vascular Endothelial Growth Factor (VEGF) in Intravenous Leiomyomatosis (IVL): An Immunohistochemical Study of 29 Cases

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Background: PDGF, a powerful mitogen to smooth muscle cells, and VEGF, a critical pro-angiogenic molecule, belong to the family of growth factors and have been shown to have a role in the pathogenesis of a variety of mesenchymal tumors. PDGF, VEGF, and their respective receptors have been implicated in the pathogenesis of leiomyomas but they have not been investigated in IVL of the uterus, an intravascular proliferation of benign smooth muscle cells of unknown etiology.
Design: We evaluated 29 cases of IVL (2 recurrences) in 27 women (mean age 48.2 +/- 10.8 years). Immunohistochemistry for PDGF-Rα and VEGF was performed in representative tissue sections. Intensity and extent of staining of tumor and myometrium were evaluated by a semiquantitative method as follows: 0 (no staining), 1 (weak), 2 (moderate), and 3 (strong); focal (25%) or diffuse (>25%). Data was analyzed by the Student t-test (bidirectional, $p<0.05$).
Results: With the exception of one case, the extent of PDGF-Rα and VEGF staining was diffuse in both IVL and myometrium. However, differences in intensity of staining were seen. Intensity of PDGF-Rα staining in IVL cases (mean score 2.33±0.62) was stronger than that of the background myometrium (1.33±0.44). Similarly, intensity of VEGF staining (mean score 2.36±0.61) was higher than that seen in the background myometrium (2.12±0.84). Differences in intensity were statistically significant for both PDGF-Rα ($p<0.001$) and VEGF ($p=0.04$).
Conclusions: Our findings suggest a potential role for PDGF-Rα and VEGF in the pathogenesis of IVL through proliferation and angiogenesis, perhaps allowing for lymphovascular invasion. Although estrogen and progesterone have been shown to increase expression of PDGF and VEGF leading to the use of anti-hormones in the treatment of IVL, the increased levels of PDGF-Rα and VEGF suggest that pharmacologic agents targeting tyrosine kinases downstream effectors of PDGF-Rα and VEGF may be useful in the treatment of these subset of patients.

1039 Liposarcoma Arising in Uterine Lipoleiomyoma: A Report of Three Cases

AG McDonald, PD Cin, A Ganguly, S Campbell, Y Imai, AE Rosenberg, E Oliva. Massachusetts General Hospital, Boston, MA; Brigham and Women's Hospital, Boston, MA; Kobe City General Hospital, Kobe, Japan.
Background: Primary sarcomas of the uterus are uncommon; leiomyosarcoma is the most frequent. Most uterine sarcomas arise de novo, with malignant transformation of a benign mesenchymal tumor being a very rare event and only reported in leiomyomas.
Design: The clinicopathologic features of 3 uterine liposarcomas arising in association with a lipoleiomyoma were studied. Immunohistochemistry for desmin, h-caldesmon, S-100 and MDM2, and fluorescence *in situ* hybridization (FISH) for the t(12;16)(q13;p11) were performed in all cases.
Results: Patients ranged in age from 49-70 (mean 59) years. All tumors were centered in the myometrium and ranged in size from 10 to 18.5 cm. They displayed a gelatinous cut surface with associated necrosis in two. On microscopic examination, the tumors had a well-circumscribed pushing margin. One tumor was uniformly hypocellular with prominent myxoid background and a striking delicate vascular network. Another showed alternating hypo- (myxoid) and hypercellular areas. The third tumor was uniformly hypercellular with a hyalinized background. In the myxoid areas, the cells were small and spindled with oval nuclei and inconspicuous nucleoli. In the hypercellular areas, the cells were pleomorphic with large, hyperchromatic nuclei. Mitotic activity ranged from 3 to 7/10 HPFs. Lipoblasts were present in all tumors but were more common in the hypercellular areas. Two tumors merged imperceptibly with a lipoleiomyoma (1 typical and 1 with bizarre nuclei), while the third tumor showed an infarcted area composed of mature fat admixed with hyalinized smooth muscle most consistent with an infarcted lipoleiomyoma. Tumors were classified as myxoid, mixed myxoid and pleomorphic, and pleomorphic liposarcoma. The benign and malignant adipose components were positive for S-100, while the benign smooth muscle component stained for desmin and h-caldesmon. MDM2 was positive in the two cases with a pleomorphic component. FISH analysis successfully completed in only one of three cases (pure pleomorphic liposarcoma) failed to show the t(12;16). Patients are alive and well 1, 2, and 20 years after surgery.
Conclusions: Primary liposarcomas of the uterus are extremely rare and most likely arise from malignant transformation of a lipoleiomyoma. These tumors should be added to the differential diagnosis of malignant mixed Mullerian (if pleomorphic) or myxoid mesenchymal tumors of the uterus.

1040 Mass-Forming Endometriosis Shows *HMGA1* and *HMGA2* Rearrangements

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Background: Endometriosis is a common gynecological disorder characterized by ectopic endometrium associated with pelvic pain and infertility. The pathogenesis is unclear and several genetic, endocrine, immune and environmental agents have been extensively studied as potential causative factors. Previous studies have demonstrated clonality in endometriotic cysts and indicated a wide range of cytogenetic and molecular genetic aberrations in endometriosis; however, consistent somatic genetic alterations have not been identified. Rarely, endometriosis presents as a mass lesion with an infiltrative pattern reminiscent of malignancy. Herein, we describe the cytogenetic and molecular genetic findings of a series of mass-forming endometriosis.
Design: One index case of pulmonary endometriosis underwent conventional cytogenetics analysis. Additional 16 cases of tumor-forming endometriosis were investigated by fluorescence in situ hybridization (FISH) for *HMGA1* and *HMGA2* loci. FISH was performed on paraffin-embedded thin tissue sections using custom-designed probes.
Results: The index patient presented with a lung nodule. Histopathologic examination revealed the presence of endometrial glands and stroma. Conventional cytogenetics analysis demonstrated the following karyotype: 46,XX, t(5;6)(q13;p21). Molecular cytogenetic analysis revealed *HMGA1* rearrangement. A second patient with a large abdominal mass during pregnancy that was composed of markedly decidualized endometrial stroma and endometrioid glands. FISH revealed an *HMGA1* rearrangement exclusively in the stromal component. Faced with these findings we evaluated additional cases of mass forming endometriosis for both *HMGA1* and *HMGA2* rearrangements. Of the 15 additional cases evaluated, 2 (13%) had *HMGA2* but not *HMGA1* rearrangements. Rearrangements were exclusively found in the stromal component.
Conclusions: Mass-forming endometriosis is an uncommon subset of endometriosis that harbors *HMGA1* or *HMGA2* rearrangements in up to 23% of cases (4 of 17). This finding supports the concept that endometriosis is clonal and that rearrangement of specific genes (*HMGA1/2)* likely contributes to neoplastic outgrowth. The role of HMGA1/2 in this process, and in the distinction of benign from more aggressive forms of endometriosis, bears further study.

1041 Intraoperative Frozen Section Examination of Endometrial Carcinoma: Do Diagnostic Discordances with Final Pathologic Diagnosis Impact the Operative Treatment Algorithm?

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Background: Intraoperative frozen section examination of hysterectomy specimens is routinely used to guide the decision of surgical staging in patients with endometrial carcinoma. The aim of this study is to assess the accuracy of frozen section diagnosis of

endometrial carcinoma when compared with permanent sections and how discordances can affect the operative treatment algorithm.
Design: The study comprised 422 consecutive patients undergoing hysterectomy for endometrial cancer at the Mayo Clinic between January of 2004 and December of 2006. The intraoperative frozen section report was compared with the final pathology report to access for discordances, including tumor type, size and grade, myometrial invasion and lymph node status.
Results: There were 340 endometrioid adenocarcinomas, 61 serous carcinomas, 12 clear cell carcinomas, 5 mixed carcinomas and 4 undifferentiated carcinomas. A median of 4 endomyometrial sections (range 2-15) were evaluated per case at the time of frozen section. In 14 (of 422) cases, the diagnosis was deferred to permanent sections to further characterize a poorly differentiated carcinoma or hyperplasia from adenocarcinoma. In 50 cases (11.8%) the pathology report was addended or amended. The most frequent reason for amendment (N = 19, 37%) were microscopic foci of metastatic carcinoma to lymph nodes that were not detected at frozen section. In 5 instances (10%) there was a change in tumor type. In 3 cases the frozen section diagnosis was serous carcinoma and permanent sections showed mixed serous and clear cell carcinoma. In 2 cases the tumor was initially classified as endometrioid FIGO grade 2 and 3, respectively and on permanent sections a serous component was identified. In 4 cases (8%) the process was initially categorized as endometrial hyperplasia and on the following day FIGO grade 1 endometrioid adenocarcinoma was recognized. In only one occasion the reason for amendment was the presence of superficial myometrial invasion. There was no change in depth of myometrial invasion or tumor dimension in any of the cases.
Conclusions: Despite the occurrence of minor discordances between the frozen section and final diagnoses, these changes did not usually affect the factors used in intraoperative decision-making. In only one case the change in tumor type and grade could have potentially affected the operative treatment algorithm.

1042 Epithelial-Mesenchymal Transition (EMT) in Non Endometrioid Endometrial Carcinomas

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Background: Epithelial-mesenchymal transition (EMT) is involved in the development of invasion and metastasis. Snail is a transcription factor that regulates the EMT program. We investigated the expression of EMT genes and proteins in non-endometrioid endometrial carcinomas (NEEC).
Design: The tumors investigated included: 7 serous, 3 clear cell, 2 undifferentiated, and 6 mixed endometrial carcinomas. Tissue-arrays for immunohistochemical analysis of Snail, Twist, E-cadherin N-cadherin, and vimentin were done. Expression of Snail, Twist, E-cadherin, Zeb-1, and HMGA2 was evaluated by real time RT-PCR. Ishikawa cells (IK) overexpressing a constitutive BRAF mutation (V600) were obtained and EMT markers were then analyzed. Clinicopathologic data were collected.
Results: Expression of Snail, Twist, Zeb-1, vimentin, and HMGA2, was found to be increased in NEEC compared with normal tissue ($p<0.05$). An inverse correlation between Snail, Twist and Zeb-1 upregulation and E-cadherin downregulation ($p<0.05$) was found. IK cells overexpressing BRAFV600 showed an induced mesenchymal phenotype with alterations in EMT proteins.
Conclusions: The mRNA and protein expression of EMT markers are increased in NEEC. IK cells overexpressing BRAFV600 showed EMT features that correlate with our observations in human tumor samples.

1043 Immunohistochemical (IHC) Characterization of Sex Cord Stromal Tumors

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Background: Sex cord stromal tumors (SCST) account for 8% of all primary ovarian tumors. Despite their rarity, SCST are often considered in the differential diagnosis of ovarian neoplasms and metastatic tumors since their morphologic features overlap with various epithelial and germ cell tumors. The aim of this study is to characterize the level and pattern of expression of an updated panel of immunohistochemical markers that will assist in their differential diagnosis. Special emphasis is given to the extent of EGFR expression since it represents a potential marker for targeted therapy with monoclonal antibodies.
Design: In-house ovarian and testicular SCST cases accessioned between 1999-2008 were retrieved from the archive of Sunnybrook Health Sciences Centre. Two gynecologic pathologists reviewed the cases using the WHO classification and studied the IHC profile using a panel of antibodies against: inhibin, calretinin, CD56, CD99, D2-40, LMWK (cam 5.2), CD10, Melan A, EMA and EGFR. Immunostaining was recorded semiquantitatively.
Results: We identified 67 SCST (63 ovarian and 4 testicular): 37 adult granulosa cell tumors, 9 fibrothecomas, 8 Sertoli-Leydig cell tumors, 4 sex cord stromal tumors with anular tubules, 3 sclerosing stromal tumors, 3 unclassified, 2 steroid cell tumors, and 1 gynandroblastoma. The following proportions of positive cases were demonstrated: inhibin 60/67 (88.5)%, calretinin 58/67 (86.5)%, CD56 56/67(83.5%), CD99 31/67 (46.2)% predominantly in <50% of the tumor, D2-40 28/67 (41.7%) (cytoplasmic granular pattern), LMWK 15/67 (22.3%) including 4 cases with positivity in >50% of the tumor, CD10 5/62 (7.4%), Melan A 5/62 (7.4%), and EMA 1/67 (1.1%), in <25% of the tumor. Interestingly, 74.6% of the cases expressed EGFR (membranous pattern), in 44 cases >50% of the tumor was positive.
Conclusions: Our IHC results (inhibin, calretinin, CD56, and CD99) confirm earlier reports of the sex cord lineage of these tumors. However, attention should be made when LMWK is used as part of a panel differentiating SCST from carcinomas since a significant proportion of SCST are positive. Our results provide a rationale for considering targeted therapy against EGFR in clinically aggressive tumors as it is expressed in a high proportion of cases.

1044 Accuracy of Intraoperative Consultation in Endometrial Cancers: Emphasis on Low Grade Low Stage Tumors

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Background: In gynecologic pathology, hysterectomy specimens are most commonly submitted for intraoperative consultation (IC) to confirm tumor type, grade and to determine depth of myometrial invasion and presence of cervical involvement. Cases with low grade and superficially invasive cancer may not require further surgical staging.
Design: To evaluate the diagnostic accuracy of IC of endometrial cancers, we retrospectively rewieved all radical hysterectomies that had IC at our instution from January 2005 to August 2008.
Results: Our study included one hundred twenty-eight radical hysteretomy cases with IC operated between January 2005 and August 2008. Fifty five of the cases (42.9%) had low grade (Grade 1 FIGO) and low stage (non invasive or superficially invasive-less than one third-) endometrioid type adenocarcinoma on IC.Thirty five of these early disease cases had surgical staging (63.6%). On final pathologic diagnosis, 13 cases (23.6%) were upgraded and upstaged; two of which didnot have surgical impact since IC was Ta and final diagnosis was superficially invasive tumor in both low grade cancers. There were no incidence of serous, clear cell carcinoma or carcinosarcoma in discrepant early disease cases. Nine of upstaged and upgraded tumors had surgical staging. In cases without surgical staging, 4 were either upgraded or upstaged. Seventy three cases had IC diagnosis of high grade and/or high stage tumors and all of them were surgically staged. In final diagnosis of these cases, 33 (45.2%) had either higher grade (including different tumor types like serous carcinoma) or increased stage.
Conclusions: Our findings indicate, at least a quarter of cases had discrepancy between IC and final diagnosis. However, significantly better correlation was detected between IC of early disease and final diagnosis than the IC of higher stage tumors ($p= 0.006$) Keeping in mind the limited correlation, IC is still useful in deciding the surgical treatment of endometrial cancers.

1045 Thyroid Transcription Factor-1 Expression in Benign Gynecologic Tissues

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Background: Thyroid transcription factor-1 (TTF-1) is a 38-kd nuclear protein, a member of the NKx2 family of homeodomain transcription factors. It is highly expressed in normal and neoplastic thyroid and lung tissues and considered as a reliable marker for lung adenocarcinoma and thyroid carcinoma. Recently, expression of TTF-1 has also been reported in ovarian, endometrial and endocervical epithelial neoplasms. Little was known for TTF-1 immunoreactivity in normal gynecologic tissues. In this study, TTF-1 expression in various non-neoplastic gynecologic tissues was investigated.
Design: 108 normal gynecologic tissues including 28 endometria (12 proliferative, 11 secretory, 5 inactive), 26 fallopian tubes, 28 cervixes (14 endocervical, 14 ectocervical), 14 myometria and 12 ovaries were studied. Tissues were routinely-processed surgical specimens from adult patients with non-neoplastic conditions. In addition, 4 normal fallopian tubes and 2 ovaries from 4 pediatric patients (aged from 3 months to 11 years) were evaluated. Monoclonal antibody (SPT24) was applied on Refine/Bond Max autostainer. Nuclear TTF-1 immunoreactivity was evaluated and recorded.
Results: Variable TTF-1 nuclear reactivity was identified in 25 of 26 (96%) fallopian tubes (positive percentage ranged 2-60%, median 25%), 15 of 28 (54%) endometria (1-10%, median 5%), and 6 of 14 (43%) endocervical samples (<5%). TTF-1 was also identified in 2 of 4 (50%) pediatric fallopian tubes with 5% and 20% of the tubal epithelium being positive, respectively. No TTF-1 expression was detected in ovarian tissue (neither epithelium nor stroma; neither adult nor pediatric samples), ectocervical squamous epithelium, myometrium, and all stromal tissue in endometrium, tube and cervix. TTF-1 reactivity was detected in both proliferative and secretory endometria but not in inactive endometria.
Conclusions: 1) In addition to gynecologic epithelial tumors, TTF-1 is frequently expressed in normal/non-neoplastic tubal (96%), endometrial (54%) and endocervical (43%) epithelia. 2) TTF-1 might have a role in the function and development of normal fallopian tube and endometrium as it is highly expressed in tubal epithelium of both adults and children, and in functional endometrium but not in inactive endometrium. 3) The high TTF-1 expression in tubal epithelium but not in normal ovarian surface epithelium suggests that some of the TTF-1 positive ovarian tumors might be related to the TTF-1 positive tubal epithelium.

1046 Detection of HPV-DNA by PCR-Based Method in Formalin Fixed, Paraffin Embedded Tissue of Rare Types of Endocervical Carcinoma

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Background: In addition to its role as a key risk factor for cervical squamous neoplasia, high risk HPV also appears to play a role in the pathogenesis of conventional endocervical adenocarcinomas of the mucinous and endometrioid cell types. Cervical serous, clear cell and small cell carcinomas differ from the conventional endocervical adenocarcinoma in their clinical characteristics. The data on the role of HPV in their pathogenesis are limited. In this study we examined the presence of high risk HPV-DNA in rare types of cervical carcinoma using polymerase chain reaction (PCR)-based test.
Design: In-house cervical serous, clear cell and small cell carcinoma cases accessioned between 2000-2008 were retrieved. The diagnosis of cervical serous or clear cell carcinoma was made only in the absence of concurrent or previous primary endometrial,

ovarian or peritoneal carcinoma. Small cell neuroendocrine carcinoma was diagnosed when it expressed at least one neuroendocrine marker; chromogranin A or synaptophysin. Cases were tested for HPV by PCR amplification of DNA extracted from deparaffinized sections using Roche AMPLICOR® HPV Amplification Detection and Control Kits (Roche Molecular Systems, CA, USA). The kit detects all 13 high-risk DNA genotypes HPV. The positive cut-off point for AMPLICOR® HPV Test was A450 = 0.2.

Results: We identified 4 serous carcinomas, 3 clear cell carcinomas, 1 mixed clear cell and serous and 5 small cell carcinomas that met our inclusion criteria. High risk HPV DNA tested positive in 3/4 serous carcinomas, 2/3 cervical clear cell carcinomas, all 5 cases of small cell carcinoma and the mixed cell type.

Conclusions: Our report is the first to document HPV status in a series of archival unusual types of adenocarcinoma of the uterine cervix. It suggests a robust association between high risk HPV and these rare subtypes. Despite their unique clinical setting and morphologic appearance, the majority of these tumors likely share a common HPV-mediated carcinogenic pathway. Our observation is particularly significant in cervical cancer prevention as we enter the HPV vaccination era.

1047 Interobserver Agreement for Endometrial Cancer Characteristics Evaluated on Biopsy Material

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Background: A shift towards a disease-based therapy applied according to patterns of failure predicted by pathologic determinants has recently led to considering a selective approach to lymphadenectomy for endometrial cancer in some North American Centers. Accordingly, lymph node dissection can be avoided in low risk patients (endometrioid histology, FIGO grade 1 and 2, <50% myoinvasion and <2 cm primary tumor diameter) whilst should be systematically applied in all non-low risk population. Therefore, it became critical to examine the agreement among pathologists regarding the cell type and grade of endometrial cancer evaluated on preoperative endometrial samples.

Design: This study assessed the diagnostic agreement by comparing the interpretations of 6 raters, with special expertise in gynecologic pathology. Pathologists independently assessed 105 consecutive endometrial biopsies originally reported as positive for endometrial cancer for cell type (endometrioid versus non-endometrioid), tumor grade (FIGO 3-tiered and 2-tiered), nuclear grade and risk category (low risk: endometrioid grade 1+2 and nuclear grade<3). Interrater agreement levels in each category were analyzed by the Fleiss' multiple-rater Kappa statistics with standard error (SE).

Results: Interrater agreement levels were substantial for identification of non-endometrioid histology (K=0.63; SE=0.025), high tumor grade (K=0.64; SE=0.025) and risk category (K=0.66; SE=0.025). The overall K was moderate for cell type (K=0.54; SE=0.021) due to disagreement on few cases that bordered on atypical complex hyperplasia. The overall agreement was fair for nuclear grade (K=0.21; SE=0.025) although the agreement on identification of nuclear grade 3 was moderate (K=0.52; SE=0.016).

Conclusions: There is agreement among pathologists in identifying high risk pathologic determinants on endometrial biopsies with cancer. This ascertainment is critical to substantiate the paradigm shift in surgical staging of patients with endometrial cancer.

1048 The Pathogenesis of Uterine and Extrauterine Carcinosarcoma Appear Similar – An Immunohistochemical Study of PTEN Expression

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Background: Carcinosarcomas are an intriguing group of mullerian-derived malignant tumors consisting of mixtures of both epithelial and mesenchymal components. These tumors arise primarily in the uterine corpus, however, also seen in extrauterine locations e.g., ovary, fallopian tube, and omentum. The current study compares the pattern of PTEN expression of uterine carcinosarcomas and extra-uterine carcinosarcomas.

Design: Thirty-seven high-grade mullerian carcinosarcoma cases were obtained from the archival files of Women & Infants. These included 24 uterine cases, and 13 extra-uterine (9 ovarian cases, 2 cervical cases, 2 pelvic cases, and 1 mesenteric) cases. Four μm sections were cut from each block and all slides were stained with antibodies to PTEN (Cascade Bioscience, Winchester, MA). The presence of the area(s) chosen for study was confirmed with H&E staining and positive and negative control slides were run with each batch of staining. Kruskal-Wallis test used for statistical analysis.

Results:

PTEN Expression in Uterine & Extra-Uterine Carcinosarcoma

	PTEN Null in both Carcinoma & Sarcoma Component	PTEN Expression Retained In both Carcinoma & Sarcoma Component	PTEN Expression Equivocal	PTEN Expression Discordent
uterine Carcinosarcoma (n=23)	20 (87%)	1 (4%)	1 (4%)	1 (4%)
Extra-Uterine Carcinosarcoma (n=14)	11 (78%)	1 (7%)	2 (14%)	0

p = 0.1102

PTEN Expression in Uterine vs. Ovarian Carcinosarcoma

	PTEN Null In both Carcinoma & Sarcoma Component	PTEN Expressed in both Carcinoma & Sarcoma Component	PTEN EXpression Equivocal	PTEN Expression Discordent
Uterine Carcinosarcoma (n=23)	20 (87%)	1 (4%)	1 (4%)	1 (4%)
Ovariasn Carcinosarcoma (n=9)	7 (78%)	0	2 (22%)	0

p=0.0657

Conclusions: 87% uterine and 78% extrauterine carcinosarcomas were PTEN NULL in both carcinomatous and sarcomatous components. Likewise, the majority of ovarian carcinosarcomas (78%) also exhibited a concordant PTEN NULL phenotype. Kruskal-Wallis test (a non-parametric ANOVA), showed that there was no significant difference in PTEN expression pattern between uterine and extra-uterine carcinosarcomas (p value = 0.1102) or uterine carcinosarcomas vs. ovarian carcinosarcomas (p= 0.0657). The high frequency of PTEN NULL concordance in uterine, extra-uterine and ovarian carcinosarcomas, suggests that the pathogenesis of uterine and extra-uterine carcinosarcomas is similar.

1049 Second Pathology Review of Hysterectomy Specimens for Endometrial Carcinoma: Notable Changes over a 10 Year Period

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Background: Although studies have shown that second pathology review can detect significant diagnostic discrepancies, there are very few studies focusing on hysterectomy specimens for endometrial carcinoma. Furthermore, there are no studies that have evaluated the changes in the pattern of discrepant diagnoses over a long period. This study compared the rate and contents of diagnostic discrepancies in two time period intervals 1996-1997 and 2006-2008, to determine if the different types of discrepancies have changed over a 10 year period.

Design: Cases of hysterectomy for endometrial carcinoma reviewed at a gynecologic referral center for the two-year periods from 1996-1997 (Group 1) and from 2006 to mid 2008 (Group 2) were studied. Each group consists of total 207 and 130 cases respectively. In both groups, the original and review pathology reports were reviewed and compared for the following pathologic information, (1) Tumor histologic classification, (2) Tumor grade, (3) Depth of myometrial invasion, (4) Cervical involvement, (5) Adnexal involvement, (6) Lymphovascular invasion (LVI), and (7) Others including endometrial hyperplasia vs carcinoma. Among discrepancies, major discrepancies were coded if it could lead to treatment alteration. The rate and contents of discrepancies were compared in these two groups.

Results:

Rate and Classification of Discrepancies

	Group 1 n=207	Group 2 n=130
Synoptic report in the original report	0 (0%)	126 (97%)
Agreement	77 (37%)	88 (68%)
Histologic classification	12 (6%)	2 (2%)
Tumor grade	97 (47%)	9 (7%)
Depth of myometrial invasion	32 (16%)	13 (10%)
Cervical involvement	12 (6%)	15 (12%)
Adnexal involvement	3 (1%)	1 (<1%)
LVI	11 (5%)	9 (7%)
Others	3 (1%)	1 (<11%)
Total	130 (63%)	42 (32%)

Rate and Classifications of Major Discrepancies

	Group1 n=207	Group2 n=130
Histologic classification	12	2
Tumor grade	20	1
Depth of myometrial invasion	20	8
Cervical involvement	12	14
Adnexal involvement	3	1
Others	3	1
Total	70 (37%)	27 (21%)

Conclusions: 1) Overall discrepancies have decreased over the past 10 years. 2) There are, however, still a significant number of major discrepancies, and assessment of cervical involvement remains as a major component. 3) Mandatory synoptic reporting appears to have contributed to some extent the decrease in overall discrepancy rates.

1050 Calretinin and B72.3 as Useful Markers in the Differential Diagnosis of Adenocarcinoma Versus Reactive Mesothelium in a Background of Adnexal Endometriosis

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Background: Florid reactive mesothelial hyperplasia can be seen in the ovary or fallopian tube of patients with endometriosis, and can simulate metastatic adenocarcinoma. The cytologic features alone may not be conclusive enough to render an unequivocal diagnosis. Immunohistochemistry can be a useful tool in differentiating between these two entities.

Design: Using our institutional database we identified six cases of ovarian endometriosis associated with florid reactive mesothelial hyperplasia within a period of 24 months. Five of the cases had a proliferation of small tubular structures on the ovarian surface that could have been misinterpreted as metastatic adenocarcinoma. One of the cases had a similar histologic appearance, but the tubular proliferation was in a fallopian tube. Immunostains for Calretinin and B72.3 were performed in all six cases.

Results: In all six cases, positive staining with Calretinin and negative staining with B72.3 confirmed the mesothelial origin of the tubular structures.

Conclusions: Ovarian endometriosis can be associated with florid reactive mesothelial hyperplasia in areas of adnexal endometriosis that can mimic metastatic low grade adenocarcinoma. Immunostains for Calretinin and B72.3 can be a helpful diagnostic adjunct.

1051 The Immunoexpressions of Biomarkers (p16, Ki67, and ProEx™C) Are Beneficial for the Differential Diagnosis of Transitional Cell Metaplasia from High Grade Cervical Intraepithelial Neoplasia of the Uterine Cervix in Perimenopausal and Postmenopausal Women

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Background: Transitional cell metaplasia (TCM), usually occuring in peri/postmenopausal women, is often misdiagnosed as high grade intraepithelial neoplasia (HGCIN) and thus can lead to unnecessary treatment. We have often encountered patients showing HGCIN associated with TCM in the background epithelium in the LEEP conization specimens and have thus difficulty in the determining the extent of their disease, especially when HGCIN showed grooved nuclei or TCM had nuclear atypia. To define the characteristics of TCM, we compared the histological findings and immunohistochemical expressions of p16 (CINtec^{R}), Ki67, and ProEx^{TM}C in various lesions, including TCM, HGCIN, senile atrophy, and normal exocervical mucosa in peri/postmenopausal women.

Design: Of 337 LEEP conization specimens from patients aged 50 or older during the period 2006-2007, TCM (n=29) with or without HGCIN, HGCIN (n=33), senile atrophy (n=23), and normal cervical epithelium (n=13) were retrieved, and compared their histological findings and the immunohistochemical expression patterns of p16 (CINtec^{R}), Ki67, and ProEx^{TM}C.

Results: As TCM showed occasional nuclear enlargement (41.4%), clear cytoplasm (75.9%), and frequent endocervical glandular involvement (48.2%) as seen in the cervical intraepithelial neoplasia, while HGCIN showed elongated grooved nuclei (48.5%) as seen in TCM, this often caused difficulty in arriving at the differential diagnosis. Ki-67 positive cells were mostly confined to the basal layer in all cases of TCM and senile atrophy, but occasionally only a few more Ki-67 positive cells were scattered in the upper layers of the TCM. P16 (CINtec^{R}), and ProEx^{TM}C expressions were absent in all cases of TCM and senile atrophy, in contrast to HGCIN. In the vicinity of HGCIN, thin epithelium composed of cells having mild to moderate nuclear atypia without mitoses showed immunopositivities for Ki-67, p16 (CINtec^{R}), and ProEx^{TM}C throughout the entire thickness, thus suggesting that atrophic HGCIN in peri/postmenopausal women may have lower levels of nuclear atypia and mitoses compared to those in younger women.

Conclusions: In our study, TCM and senile atrophy showed similar expression patterns of biomarkers that are distinguishable from HGCIN, thus suggesting that TCM may be a form of atrophy and that the biomerkers are very useful in the differential diagnosis in some difficult cases.

1052 Cross-Sectional Study of Ovarian Tumors among Hispanic Women Living in the United States

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Background: The prevalence of primary tumors of the ovary in the Hispanic female population living in the United States remains unexplored.

Design: The National Cancer Institute's Surveillance, Epidemiology, and End Results Program (SEER) was used to identify 4,159 Hispanic (Hisp.) and 43,867 non-Hispanic white (NHW) women diagnosed with a primary ovarian tumor from 1973 to 2004. Data derived from this registry was analyzed for differences in demographic characteristics and clinico-pathologic features.

Results: Hispanic women diagnosed with ovarian tumors were younger at presentation than NHW women (51 years vs. 59 years, P<0.001). They were also more likely to present with early stage (I/II) disease (OR=1.23, P<0.001). Epithelial tumors occurred in 94% of NHW women and 88% of Hispanic women (OR=0.49, P<0.001). When presenting with an epithelial neoplasm, Hispanic women were almost twice as likely as NHW women to have tumors of borderline malignant potential (17% vs. 10%, OR=1.98, P<0.001). Germ cell and sex-cord tumors were more prevalent in Hispanics than NHW women (8% vs. 3%, OR=3.48, P<0.001 and 2% vs. 1%, OR=1.44, P<0.001, respectively). Therapeutic surgical interventions was similar between the two groups (94% vs. 94%, P=0.353). Kaplan-Meier analysis showed increased overall survival for Hispanic women (mean 182 months vs. 145 months, P<0.001). Univariate Cox survival model revealed a 27% reduction in risk of dying from any cause among Hispanic women compared to NHW women diagnosed with a primary ovarian tumor (P<0.001).

Conclusions: Hispanic women diagnosed with primary ovarian tumors present at an earlier age and stage compared to non-Hispanic white women. Epithelial tumors are less prevalent among Hispanic women and are more likely to be of borderline malignant potential. Alternatively, germ cell and sex-cord tumors occur at a greater frequency in Hispanic women. These factors may account for increased survival among Hispanic cohorts. The basis for these differences need to be further studied. Collectively, the data presented here help define ovarian tumors among the Hispanic female population living in the United States.

1053 The Relationship of the Presence of Eosinophils with Chronic Endometritis

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Background: Chronic endometritis is pathologically diagnosed based on the presence of plasma cells in the endometrial stroma. However, many conditions can mimic or interfere with the search for plasma cells, including the plasmacytoid stroma cells or preducidual changes of stroma cells. Eosinophils are another type of chronic inflammatory cells, which can be easily identified with routine hematoxylin and eosin (H&E) stain by their characteristic eosinophilic granules. As far as we know, there have no published studies on the relationship between chronic endometritis and the presence of eosinophils in the endometrium. Therefore, the goal of this study is to investigate if eosinophils can be another marker for chronic endometritis.

Design: The H&E stained glass slides of consecutive endometrial biopsies in LSUHSC-Shreveport over a period of 2 months (06/01/2008 to 7/31/2008) were reviewed. The biopsies that revealed the presence of eosinophils were subjected to immunohistochemical staining with CD138, a marker for plasma cells.

Results: In all, 184 cases of endometrial biopsy were reviewed and 38 biopsies (20%) showed eosinophils. Among those 38 cases, 29 (70%) revealed presence of plasma cells (positive staining with CD138). Of these 29 cases, only one case was diagnosed as chronic endometritis. The average number of eosinophils in the biopsies with positive plasma cell staining was 3.96/40 HPF, while that with negative plasma cell staining was 1.78/40 HPF. However, there was no statistically significant correlation of the number of eosinophils with the number of plasma cells identified by immunostaining.

Conclusions: The presence of eosinophils in endometrial biopsy specimen suggests a need to search for plasma cells (with immunostaining if needed) for the diagnosis of chronic endometritis.

1054 Atypical Tubal Metaplasia in Endometrial Samplings Is Not Associated with Increased Risk of Developing Hyperplasia or Carcinoma: A Long Term Follow-Up Study of 63 Cases

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Background: Tubal metaplasia (TM) of the endometrium, characterized by ciliated cells with round vesicular nuclei and eosinophilic cytoplasm, is a well-recognized benign entity, often seen with dysfunctional uterine bleeding (DUB). In some cases, ciliated epithelia may exhibit worrisome cytologic features (ATM) and present a diagnostic dilemma regarding its clinical significance. The aim of our study is to evaluate the natural history of ATM and the risk of subsequent endometrial hyperplasia and neoplasia through a long term follow-up.

Design: We identified 63 cases of ATM within benign endometrial samplings including: 30 poorly active endometria; 16 atrophic endometria, 2 weakly proliferative endometria, 3 disordered proliferative endometria, 8 proliferative endometria with breakdown, and 4 endometrial polyps. No concomitant hyperplasia or malignancy was present. The criteria for cytologic atypia included: variably-sized and shaped enlarged nuclei; "smudgy" hyperchromatism and prominent nucleoli; variable amounts of eosinophilic granular cytoplasm, often abundant; and cuboidal and hobnail configurations. Follow-up information was available in all 63 cases. The controls consisted of 200 cases of benign endometrium from patients with DUB and no cytologic atypia. Fisher Exact Test was utilized for statistical analysis.

Results: The median age was 56 years (range 24-84) for ATM patients versus 54 years (range 27-85) for the controls. After a median follow-up period of 46 months (range 0.5-88), the ATM group developed 3 cases of simple hyperplasia (SH), one complex atypical hyperplasia (CAH) and one endometrioid carcinoma, the latter diagnosed 88 months after the initial biopsy showing ATM. The control group was followed by 7 cases of hyperplasia (4 simple and 3 complex), 1 atypical SH and 3 CAH (median follow-up 91 months).

	Hyperplasia without atypia	Atypical hyperplasia/carcinoma
ATM (63)	3 (4.8%)	2 (3.2%)
Control (200)	7 (3.5%)	4 (2%)
	p=0.44	p=0.44

Conclusions: Our study suggests that the entity of ATM *per se* is not a direct precursor to atypical endometrial hyperplasia or endometrioid carcinoma and its presence in endometrial specimens does not portend a greater risk for patients to develop those lesions as compared to the control population. Recognition of ATM in endometrial samplings is important and may prevent unwarranted aggressive clinical management.

1055 Strength and Weakness of Multi-level Sectioning in the Detection of HSIL: A Study Based on Inter-observer Variation and Imunohistochemical Markers

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Background: Distinguishing high-grade squamous intraepithelial lesions (HSIL) from their mimics can be difficult. Additional paraffin sections are commonly requested in order to improve diagnostic accuracy. The current study evaluated the performance of multi-level section analysis by multiple reviewers to differentiate HSIL from reactive or atrophic epithelium with atypia.

Design: 66 archived specimens (41 cervical biopsies, 13 cone products, 11 endocervical curettings, and 1 endocervical polyp) with up to 4 H&E stained slides (original plus levels, total of 234 slides) were independently reviewed by three pathologists and classified as squamous intra-epithelial lesion (SIL) or negative for SIL (NoSIL) for each level. Morphological identification of SIL in at least one of the levels by at least one of the reviewers, plus the positive result of at least two out of three immunohistochemical biomarkers (p16^{INK4a}, MIB-1 and ProEx^{TM}C) were employed to establish a final diagnosis. Morphologic diagnosis in each re-cut level was compared to the morphological/immunohistochemical final diagnosis. Kappa statistics and receiver operating characteristic analysis were used to assess agreement and test performance.

Results: Agreement on the diagnosis of SIL by H&E alone across all three pathologists was good to very good (kappa=0.41 to 0.58). The proportions of correctly diagnosed SIL and NoSIL respectively increased and decreased after the multi-level section analysis for all reviewers, as well as for the consensus morphological diagnosis (SIL: 77.5% for L1, 80.0% for L1+L2, 83.3% for L1+L2+L3 and 87.0% for L1+L2+L3+L4; NoSIL: 92.3% for L1, 84.6% for L1+L2, 81.0% for L1+L2+L3 and 77.8% for L1+L2+L3+L4). Diagnostic accuracy to detect either SIL or NoSIL for H&E diagnosis by level were 0.85

(95%CI 0.77-0.93) for L1, 0.82 (95%CI 0.73-0.92) for L1+L2, 0.82 (95%CI 0.71-0.93) for L1+L2+L3, 0.82 (95%CI 0.70-0.95) for L1+L2+L3+L4.
Conclusions: Whereas the use of multi-level sectioning improved SIL detection sensitivity, this occurred at the expense of specificity. Although the use of additional H&E slide sections can help identify otherwise undetected SIL cases, this also may lead to over treatment in some patients with SIL mimics.

1056 Differentiated Vulvar Intraepithelial Neoplasia Contains p53 Mutations and Is Genetically Linked to Vulvar Squamous Cell Carcinoma

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Background: Differentiated vulvar intraepithelial neoplasia (dVIN) is a unique precursor to vulvar squamous cell carcinoma (VSCC) that unlike classic VIN (cVIN) is typically HPV-negative and associated with nuclear *p53* staining. These features, in addition to allelic loss, imply a mode of pathogenesis involving somatic mutations. However, the genetic relationship of dVIN and VSCC and the role of *p53* mutations in this process have not been resolved.
Design: We selected 10 dVINs, 2 cVINs, and 6 associated VSCCs from our pathology files. Sections were stained for p53 and multiple epithelial sites representing normal control tissues (n = 10), dVIN (n=17), cVIN (n=2), and VSCC (n=6), were subjected to laser capture microdissection, and scored for *p53* mutations by sequence analysis of exons 2-11.
Results: In seven out of 10 cases at least one dVIN focus contained one or more *p53* mutations. Four were strongly *p53* immuno-positive, 3 were negative. All four dVIN associated carcinomas were *p53* mutation positive in contrast to 1 of 2 cVIN associated malignancies. In two of three dVIN/VSCC cases, both entities shared an identical *p53* mutation. However, disparate foci of dVIN often exhibited different mutations consistent with multiple neoplastic clones. One cVIN shared the same *p53* mutation with its corresponding VSCC.
Conclusions: This study shows, for the first time, that HPV-negative vulvar cancers evolve in association with *p53* mutations and that identical *p53* mutations can be identified in the precursor lesion (dVIN). *p53* immunostaining will identify some but not all of these lesions and will not invariably signal the presence of clones with *p53* mutations. The multiplicity of *p53* mutations in one or more epithelia from a single case is in keeping with the presence of multiple independent genetic events, some of which may lead to malignancy and which translate into the complex array of epithelial alterations associated with HPV-negative vulvar carcinomas.

1057 Endometrial Giant Cell Adenocarcinoma

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Background: Poorly differentiated endometrial adenocarcinomas of specific types includes lymphoepithelioma like carcinoma, hepatoid carcinoma, glassy cell carcinoma, carcinomas with trophoblastic differentiation and giant cell carcinoma. Typically these are admixed with areas of better differentiated adenocarcinoma. Since the original report of 6 cases of endometrial adenocarcinoma with a component of giant cell carcinoma, there have been no other published series on this entity.
Design: A search of endometrial cancer databases in two centres revealed a total of three cases of endometrial carcinoma with giant cells. The cases were reviewed and patient outcome obtained.
Results: In all three cases the giant cell component comprised the majority of the tumor but areas showing other more common patterns of endometrial adenocarcinoma were identified. In case one, this was minimal and was undifferentiated, case two showed areas of endometrioid carcinoma, grade 3 with sarcomatoid areas and case three showed areas of clear cell carcinoma with foci of oxyphil cell type. Case three also showed more spindled areas. The giant cells had abundant eosinophilic cytoplasm with irregular atypical nuclei. Emperipolesis was seen in two cases. Lymphovascular invasion was only seen in case 2 and in this case it was extensive. On immunohistochemical staining the giant cells were positive for EMA and cytokeratins but negative for muscle markers (smooth muscle actin and desmin) and beta HCG. Patient outcome is presented in the table.

Clinical features and follow up

	Age	Symptoms	FIGO Stage	Treatment	Follow-up
Patient 1	60	anemia	IIIA	TAH, BSO, pelvic and abdominal radiation	NED, 3 yrs 5 months
Patient 2	58	vaginal bleeding	1B	TAH, BSO, pelvic radiation	NED, 14 years
Patient 3	53	vaginal bleeding	IB	TAH, BSO, nodes, omentum; no adjuvant therapy	NED, 16 months

TAH, total abdominal hysterectomy; BSO bilateral salpingo-oophorectomy; NED no evidence of disease

Conclusions: Giant cell carcinoma of the endometrium is a rare entity. Prognosis is uncertain, based on the limited number of reported cases, but there are long term survivors, even with advanced stage disease. Differential diagnostic considerations include carcinosarcoma, undifferentiated sarcoma, poorly differentiated carcinoma and carcinoma with trophoblastic giant cells.

1058 The Circumferential Extent of Disease Should Be Reported in Cervical Adenocarcinoma In Situ (AIS) Excised by LEEP and Cone Biopsies

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Background: Watchful surveillance following treatment with loop electrosurgical excision procedure (LEEP) or cone biopsy is an option for AIS patients interested in fertility preservation. In this study, we investigated several histopathologic characteristics as possible markers for residual/recurrent disease after initial surgical resection.
Design: Cases with cervical AIS or glandular dysplasia diagnosed on LEEP/cone procedure and accessioned between 2000 and 2008 were retrieved from the Department of Anatomic Pathology at Sunnybrook Health Sciences Centre. Cases were reviewed and the association between putative markers such as margin status, extent of disease (% of circumference), and multifocality and residual/recurrent was examined using χ^2 or Fisher exact test. Data regarding subsequent excision or follow up pap smears were collected from the medical records.
Results: We identified 59 cases of AIS (35 cone biopsies and 24 LEEP). AIS was the most advanced lesion in 47/59, microinvasive adenocarcinoma was identified in 4/59, microinvasive squamous cell carcinoma in 3/59 cases and glandular dysplasia in 5/59 cases. Follow up was available in 48 cases. Subsequent resection (n=10) revealed 4 residual AIS, 1 invasive adenocarcinoma, 1 squamous cell carcinoma and 4 no residual dysplasia. Of the remaining 38 cases followed up conservatively, 2 had evidence of disease on cytology. The likelihood of achieving negative margins was significantly associated with cone biopsy versus LEEP (23/35 vs. 9/24 p=0.0326). Residual/recurrent disease was significantly associated with positive margins (p=0.0115) and extent of ≥50% circumferential involvement (p=0.0028) but not with multifocality (p=1).
Conclusions: The extent of circumferential disease is a significant histopathologic marker for residual/recurrent disease and needs to be reported in addition to margin status. The higher risk associated with conservative treatment in patients with ≥50% circumferential involvement needs to be conveyed to the gynecologists to help in their treatment decision making.

1059 Coordinate Expression of MUC4 and Palladin in Ovarian Epithelial Neoplasia: Role in Disease Progression

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Background: MUC4, a membrane glycoprotein, alters actin-cytoskeleton proteins and is involved in ovarian carcinoma cell motility. One such cytoskeleton protein, palladin, is involved in actin-cytoskeleton reorganization and cancer cell motility. Coordinate expression of MUC4 and palladin in ovarian benign, borderline and malignant epithelial tumors has not been previously published and is examined in this study.
Design: Formalin-fixed, paraffin-embedded ovarian tissue array samples (normal, benign, borderline and malignant) from serous, mucinous, endometrioid, transitional and clear cell tumors (n=38) were analyzed. Immunostaining was performed using anti-MUC4 (monoclonal, prepared in our laboratory) and anti-palladin (polyclonal, Protein Tech Group, Chicago) antibodies. The expression was assessed using the H-score (summation of the product of staining intensity and proportion of cells staining). The mean H-scores were compared between the different groups by applying the F-test and the Welch test (assuming unequal variances).
Results: The H-score for MUC4 expression was significantly higher in benign, borderline and malignant ovarian epithelial tumors as compared to normal ovarian epithelium ($p<0.001$). Further, MUC4 expression was higher in malignant tumors as compared to benign tumors ($p=0.03$). Palladin expression was significantly higher in benign ovarian tumors than in normal ovarian epithelium ($p<0.001$). The H-score for palladin expression was significantly higher in borderline and malignant ovarian tumors as compared to benign tumors ($p<0.001$). There was no correlation with histopathologic subtype.
Conclusions: Increased expresion of MUC4 in malignant ovarian epithelial tumors may suggest a role for this protein in disease progression and as a novel chemotherapeutic target for ovarian carcinoma. Progressive increase in expression of palladin in ovarian epithelial tumors (normal<benign<borderline<malignant) may suggest a role as a diagnostic marker for disease progression.

1060 Clinicopathologic Analysis of Tubal Intraepithelial Carcinoma (TIC) Associated with Pelvic (Non-Uterine) Gynecologic Carcinomas

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Background: TIC, which has been reported in association with 48% of pelvic serous carcinomas (Kindelberger, *Am J Surg Pathol* 2007), has been implicated as evidence of fallopian tube (FT) origin for carcinomas that would have been otherwise classified as primary ovarian or peritoneal. The current study addresses the frequency of identification of TIC, and thus potential FT origin, of pelvic (non-uterine) gynecologic carcinomas at our institution.
Design: A series of 94 consecutive cases of pelvic (non-uterine) carcinomas encountered from 1/2006-7/2008 was evaluated to identify those for which all resected FT tissue was submitted for histologic examination. The presence of TIC and its location and relationship to invasive carcinoma in the FTs and ovaries was assessed.
Results: Among 42 cases with complete microscopic examination of all FT tissue, carcinoma subtypes included 37 high-grade serous, 2 endometrioid, 1 clear cell, 1 poorly differentiated carcinoma, and 1 malignant mesodermal mixed tumor. TIC was identified in 12 cases (32%) of high-grade serous carcinoma but not among any of the other subtypes. Among these, all would have been classified as ovarian or peritoneal in origin per conventional criteria based on disease distribution (sufficient ovarian tumor or extensive peritoneal tumor). TIC was located in the fimbriated end in 11 cases. Both TIC and adjacent invasive carcinoma were present in the FT in 11 cases (10 unilateral,

1 bilateral); 1 case had only TIC without invasive carcinoma in the FT. The 11 unilateral TICs were associated with bilateral ovarian involvement by carcinoma in 9 and unilateral ovarian involvement in 2 (contralateral ovary absent in both). In 6 cases there was more than one focus of TIC in the same FT.
Conclusions: Despite the expected bias towards serous carcinoma in this series of pelvic carcinomas, TIC was not identified in association with other subtypes of carcinoma. This study, in conjunction with the one cited above, suggests that one-third to one-half of pelvic serous carcinomas are potentially of FT origin. Molecular studies are required to establish that TIC is the precursor lesion rather than intraepithelial spread from adjacent carcinoma of ovarian or peritoneal origin.

1061 Simultaneous Detection, Typing and Quantification of All 15 High Risk HPV Types in Adeno-Squamous Carcinoma of the Uterine Cervix – A Real Time PCR Based Study

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Background: Cervical adenosquamous carcinomas (ASqC) are a subtype of mixed glandular and squamous tumors with a genital HPV etiology. HPV 16 and 18 are regarded as the most common viral genotypes in cervical ASqC and have been reported in the following % proportions (HPV 16:18): 25:75; 33:33; 50:50 75:25. Multiple HPV types have been noted in pure cervical adenocarcinoma, but not in cervical ASqC. Type-specific detection of high risk HPV allows the detection of co-infection by multiple HPV serotypes as well as assessment of viral load per cell. We investigated the frequency of specific HPV serotypes in this largest series to date of cervical ASqC, and whether specific HPV serotypes and/or viral load per cell were associated with poor prognosis.
Design: 28 ASqC of the cervix were retrieved and a complex multiplex real time PCR test that simultaneously detects, types, and quantifies all 15 high risk HPV types was run. The test also detects and quantifies Beta-globin gene as internal control and also used to determine the normalized viral load (viral load/cell). A standard curve with the amplified products for each of the 15 high risk virus templates was plotted against the known standard concentration range of 10^0 to 10^7 to determine viral load. Prognosis-related histologic parameters of stromal invasion, VSI, LN and distant metastasis were correlated with the specific HPV serotypes and the viral copies.
Results:

Table 1: HPV Types in ASqC

Multiple HPV types	Triple HPV Types	HPV 73	HPV 31	HPV 33	HPV Other Than 16/18	HPV 16/18
9 (32%)	1 (3.5%)	3 (11%)	2 (7%)	2 (7%)	4 (14%)	24 (86%)

Poor prognosis histologic parameters e.g., +VSI, stromal invasion >50%, +LN, & vaginal/lung metastases were associated with i) 11 of 13 (84.6%) infected with HPV 16 ii) 6 of 13 (46.2%) cases of HPV 18, and iii) 4 of 4 cases of HPV 31, 45, 52, and 73 despite a low viral load. Viral loads range widely from 4 to 463906 copies per cell.
Conclusions: 32% of ASqC contain two or more high risk HPV types. 14.3% of cases are associated with NON-16/18 HPV types. Infection with 3 HPV serotypes is seen in 3.6% cases. Tumors with HPV 31, 45, 52 and 73 all have low (<500) viral loads but poor histologic prognostic parameters. HPV NON-18 is more frequently associated with a poor prognosis as compared to HPV 18. Viral load do not appear to correlate with poor prognosis histologic parameters.

1062 A 3-Tier Grading System and Mitotic Count Predict for Outcome in Ovarian Carcinoma Treated with Neoadjuvant Chemotherapy

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Background: Debulking surgery followed by adjuvant platinum based chemotherapy is the standard of care for advanced stage ovarian cancer. Preoperative chemotherapy may be offered to patients with non-debulkable disease, poor performance scores and co-morbidities, and retrospective analyses indicate no difference in survival for patients matched for age, stage, and grade. The histologic features associated with neoadjuvant chemotherapy are known, but correlation between tumor features at interval debulking and patient outcome has not been reported. The aim of this study is to identify histologic criteria in chemotherapy exposed tumor tissue that correlate with outcome, and possibly predict for acquired platinum resistance.
Design: The study population included 75 patients treated with preoperative chemotherapy followed by interval surgical debulking. Number of treatment cycles, debulking status, disease-free interval (RFS), and overall survival (OS) were recorded. Two pathologists reviewed at least one representative section of each tumour, blinded to clinical outcome. Histologic features assessed included Silverberg score/grade, mitotic count, nuclear, cytoplasmic, and stromal changes. Predictive value was determined by univariate Cox Proportional-Hazards regression.
Results: The mean age of patients at diagnosis was 60.3 (range 34 to 84 years). Optimal debulking was achieved in 48% of patients. High grade serous carcinoma was the predominant histologic type (n=71), with a Silverberg grade of 3 in 18.1% (n=13), and grade 2 in 75% (n=54). High Silverberg grade (OS p=0.02; RFS p = 0.0005) and high mitotic count (OS p=0.007; RFS p=0.0004) were significant predictors of both *lower* recurrence free and *lower* overall survival with univariate analysis. Younger patients at the time of diagnosis (p=0.006) and high architecture score (p=0.03) were significant for *lower* recurrence free survival. Debulking status and number of preoperative cycles were not significant predictors of outcome in this series (p > 0.05).
Conclusions: Results of this preliminary study indicate careful histologic evaluation of residual disease resected following neoadjuvant chemotherapy has predictive value, and should include the 3-tier, Silverberg grade and mitotic count.

1063 Placental Cell Islands, Origin and Significance in Abortions

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Background: Literature on placental cell islands is very limited. The origin of cell islands is unclear but has been proposed to arise as dettachments from the septae that contain intermediate trophoblasts. The significance and purpose of these cell islands is unknown.
Design: Archived H&E slides from 28 abortions including 13 spontaneous and 15 therapeutic, matched by gestational age were reviewed. Immunohistochemical stains for CD10, hPL, and hCG were performed on 5 cases from each group.
Results: Both groups had a similar number of placental cell islands with a median of 8. There was no significant difference in the number of placental cell islands according to the age of the patients or weeks of gestation between the two groups. However, there was a significant difference in the number of cell islands with more intercellular fibrinoid material than cells in the spontaneous abortion 11 of 13 (85%), than in the therapeutic abortion 1 of 15 (7%). In the therapeutic abortions, most of the cells in the islands did not have fibrinoid material around them. The morphologic features and their association with syncytiotrophoblasts identify these cells as cytotrophoblasts. The cells from the islands of spontaneous abortions had similar features to those in the islands from therapeutic abortions with the exception of association with more fibrinoid material. Immunohistochemistry showed that the cells in the islands from both groups had similar staining. In staining with CD 10, 30% of cells were positive, and with hCG all cells were negative. However, hPL showed a negative reaction in cells without associated fibrinoid material, but 30% of the cells were positive in islands with associated fibrionoid material.
Conclusions: 1. Placental cell islands do not derive from the columns of the septae, but from cytotrophoblastic cells. This could be the origin of the villous intermediate trophoblasts and might explain cases of intermediate trophoblast tumors following treated choriocarcinoma. 2. The accentuation of the fibrinoid material and positive reaction for hPL in the placental cell islands is associated with spontaneous abortions. 3. We can not determine if the development of fibrinoid area in placental islands is the cause or effect of the abortions; however, since the only case in the therapeutic abortions with more fibrinoid changes had hyperemesis gravidarum, they might be involved in the pathogenesis of abortions.

1064 Histopathological Work-Up and Interpretation of Sentinel Lymph Nodes Removed for Vulvar Squamous Cell Carcinoma

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Background: Identification / removal of sentinel lymph nodes (SLN) in vulvar squamous cell carcinoma (SCC) aims at the reduction of long-term morbidity from complete lymphadenectomies related to wound healing, inguinal seromas, lymph edema, infections and erysipel. Correct histopathological work-up of SLN is imperative, as patients with inguinal recurrence / disease have a high risk to die from systemic disease. Work-up and interpretation of SLN from vulvar SCC differs from that in other organs, in particular from breast cancer. Our experience with the SLN protocol of 49 patients with vulvar SCC during the last 8 years and the specific differences of this protocol with respect to other organ systems is presented.
Design: Only patients with pT1 or 2 vulvar SCC without clinically enlarged lymph nodes were included. SNL were identified with lymphoscintigraphy (Tc) and blue dye in a "same-day procedure" following essentially the sentinel lymph nodes protocol of the Groningen International Study on Sentinel lymph node precedure in vulvar cancer (GROINSS). A frozen section was perfomed on all removed SLN. After a negative frozen section, all SLNs were formalin-fixed, sectioned entirely at 325 µm intervals for 2 HE-stains and 1 unstained slide for immunohistochemistry per millimeter. In the absence of metastases on HE sections, ALL unstained slides are submitted for immunohistochemistry with antibody to cytokeratin (CK). **SCORING IS POSITIVE EVEN WHEN ONLY INDIVIDUAL CK-POSITIVE CELLS ARE IDENTIFIED.**
Results: 13 / 49 patients with SCC (34 pT2, 10 pT1b, 5 pT1a) had obvious metastases on HE stains. After CK-staining, the SLN of 28/35 patients remained negative and 4 patients revealed micro-metastases in the SLN . 4 patients showed only individual single CK-positive cells & debris in their SLN. In 1 of these 4 patients, the individual single cells were corectly identified but interpreted as "negative" in analogy to breast cancer SLN interpretation. This patient developed conglomerate metastases within 9 months. None of the patients with pT1a SCC had a metastasis. Except for 1 patient with a pT1b SCC, all metastases were from pT2 vulvar SCC. All patients with single cells and cytokeratin positive cell elements in the SLN had a well to moderately differentiated keratinizing SCC arsing in the background of lichen sclerosus.
Conclusions: With a careful and complete histopathological work-up of all removed SLN and a correct interpretation of the staining results, SLN dissection for vulvar SCC can be considered a safe procedure for patients with vulvar SCC.

1065 ProEx™C Is a Reliable Biomarker for High Grade Squamous Intraepithelial Lesion: A Comparative Study with p16 and MIB-1

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Background: In the histologic evaluation of possible high grade dysplasia, there are many diagnostic challenges, including atrophy, immature squamous metaplasia, and squamous metaplasia with dysplasia. To resolve these dilemmas, pathologists use immunohistochemical stains such as p16 and MIB-1 to aid their morphologic interpretation. However, in some cases the results of these stains are difficult to interpret. ProEx™ C, a new biomarker, detects the expression of topoisomerase IIA and minichromosome maintenance protein 2 (markers of an aberrant S-phase). The objective of this study was to evaluate ProEx™ C with MIB-1 and p16 in cases of cervical and vaginal dysplasia and its mimics.

Design: 44 cervical and vaginal biopsies and hysterectomy specimens including normal (2), HPV effect/low-grade dysplasia (8), high grade dysplasia (7), squamous metaplasia without high grade dysplasia (16), squamous metaplasia with high grade dysplasia (8), and atrophic (3) specimens were immunostained for p16, MIB-1 and ProEx™ C. The immunostains were interpreted without knowledge of the histologic diagnoses. The stains were interpreted as highlighting the lower one third or less of the thickness of the squamous epithelium (negative for high grade dysplasia), or as highlighting above the lower one third (positive for high grade dysplasia).

Results: The breakdown of positive high grade staining by case type is given below (SM=squamous metaplasia, LGD=low grade dysplasia, HGD=high grade dysplasia):

	p16	MIB-1	ProEx™C
Normal	0/2	0/2	0/2
HPV/LGD	0/8	0/8	0/8
HGD	6/7	7/7	7/7
SM without HGD	2/16	1/16	1/16
SM with HGD	6/8	8/8	8/8
Atrophy	0/3	0/3	0/3

The sensitivity and specificity for high grade dysplasia were:

	Sensitivity	Specificity
p16	80.0% (12/15)	93.1% (27/29)
MIB-1	100% (15/15)	96.5% (28/29)
ProEx™C	100% (15/15)	96.5% (28/29)

Conclusions: The results of our study show that MIB-1 and ProEx™ C have the highest sensitivity and specificity for separating difficult cases of high grade dysplasia from its mimics. Our study suggests that ProEx™ C is a comparable marker to MIB-1, and a more sensitive and specific marker for high grade dysplasia than p16.

1066 Anal Pap Smears Frequently Show Abnormalities in HIV-Positive Women

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Background: Over the past 20-30 years, the incidence of squamous cell cancer of the anus in the United States has increased by about 96% in men, and 39% in women. With cytology screening, anal squamous cell carcinoma may be one of the few preventable malignancies in HIV-positive individuals, who are at high risk of anal disease. This study evaluated prevalence of positive anal pap smears, and association with the cervicovaginal findings in HIV+ women.

Design: This retrospective study identified HIV+ women who had cervical or vaginal pap smears at an Infectious Disease Clinic between January 1, 2006 and June 30, 2007. Anal pap data was compared to cervicovaginal results.

Results: 200 anal pap smears were included in the study. 20 were unsatisfactory, leaving 180 for analysis. 35 out of 180(19.4%) of anal pap smears were abnormal. 65 out of 200 patients(32.5%) had abnormal cervical or vaginal pap smears. Of the 35 abnormal anal paps, 28 also had an abnormal cervicovaginal pap. 7 of the 35 had a normal cervical pap, so would have been missed if anal screening had been restricted to only patients with abnormal cervicovaginal cytology.

Conclusions: Anal pap smear abnormalities are common in HIV+ women, particularly those with an abnormal cervical or vaginal pap. For high risk populations, it is reasonable to perform anal pap screening.

1067 High Grade Serous and Endometrioid Carcinomas: A Convergence of Two Pathways in Pelvic Cancer Differentiation?

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Background: Pelvic epithelial cancer is histologically diverse and the distinction between high grade serous and endometrioid carcinomas can be difficult. Recent reports indicate that a significant proportion of serous tumors arise from the distal fallopian tube, supported by the presence of a serous tubal intraepithelial carcinoma (STIC) +/- dominant ovarian mass (DOM), whereas endometrioid tumors typically present as a DOM in the absence of any known fallopian tube findings. This study analyzes the relationship between pelvic serous and endometrioid tumors using p53 and WT-1 immunohistochemistry and DNA sequencing for *p53* mutations.

Design: Consecutive high grade pelvic serous and endometrioid carcinomas (grade 3) diagnosed in 2005-2007 were studied. The presence of a STIC and/or DOM in each case was determined and correlated with WT-1 and p53 expression by immunohistochemistry and the presence or absence of *p53* mutations. Immunostaining was considered positive if more than 50% of the tumor cells exhibited nuclear staining for WT-1 or p53. Genomic DNA from laser capture microdissected tumor cells and control tissue was isolated and the exons 2-11 of *p53* were amplified by polymerase chain reaction (PCR) and sequenced.

Results: 85 and 12 cases with a diagnosis of pure high grade serous or endometrioid carcinoma, respectively, were evaluated (fallopian tubes were entirely submitted for all cases). Of the 85 serous cancers, 3, 23, 27, and 32 cases were DOM+/STIC+, DOM+/STIC(-), DOM(-)/STIC+, and DOM(-)/STIC(-), respectively. 81/85 (95%) and 59/85 (69%) serous tumors were immunopositive for WT-1 and p53, respectively. Mutations in *p53* were seen in 78/85 (92%) cases. Of the endometrioid carcinomas, none were STIC+ ($p = 0.008$). 9/12 (75%) were DOM+ and 3/12 (25%) were DOM(-)($p = 0.004$). 9/12 (75%) were WT-1 positive ($p = 0.04$) and 7/12 (58%) were p53 positive ($p > 0.05$). Mutations in *p53* were detected in 9/12 (75%) cases ($p > 0.05$).

Conclusions: High grade serous and endometrioid carcinomas have distinct patterns of growth and potential sites of origin (including the distal fallopian tube) based on the parameters of a DOM and/or STIC. However, these two subtypes of Mullerian carcinoma are marginally different based on WT-1 expression, and cannot be readily distinguished by p53 immunostaining or mutational status.

1068 Immunohistochemical Panel To Differentiate Endometrial Papillary Serous Carcinoma from High Grade Endometrioid Carcinoma

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Background: Endometrial papillary serous carcinoma (EPS) can be difficult to differentiate histologically from high grade endometrial endometrioid carcinoma (EE). Recurrences have been found to be more frequent in EPS as opposed to high grade EE despite the same pre- and post-operative radio- and chemotherapy, indicating more aggressive tumor biology. To date, there is no well-defined immunohistochemical (IHC) panel to differentiate these two tumors.

Design: 12 cases of EE and 23 cases of EPS were retrieved from departmental archives and stained using Pax-2, WT-1 and p16 antibodies. PAX2, a homeobox gene, encodes a transcription factor expressed in intermediate mesoderm from which Wolffian and Müllerian ducts, and kidneys originate, whose expression was demonstrated in epithelial cells of the female genital tract, as well as ovarian serous carcinoma (OSC). Wilm's tumor gene (WT1) plays a role in early stages of development of kidneys and gonads, and its product is expressed in OSC. P16, a tumor suppressor gene involved in cervical carcinogenesis, is shown to be expressed in high grade carcinomas of Müllerian origin. A strong nuclear staining is considered positive for the former two anitibodes, with strong nuclear or nuclear and cytoplasmic staining for the latter. Statistical analysis via Fisher's Exact test is performed to compare proportions of antibody positivity in these two entities.

Results: See **Table-1**.

Table-1. Summary of IHC staining intensity in EPS and EE

	WT-1		Pax-2		p16	
Staining Intensity	Negative*	Positive*	Negative*	Positive*	Negative*	Positive*
EPS (n=23)	15	8	8	15	1	22
EE (n=12)	12	0	8	4	6	6

***Negative/Weak=Negative; Moderate/Strong=Positive**

Statistical analysis of individual markers and combined panel with p-values: WT-1: 8/23 (34.8%) in EPS to 0/12 (0%) in EE; *p-value = 0.02* (Sensitivity, specificity for EPS: 34.8%, 100%). Pax-2: 15/23 (65.2%) in EPS to 4/12 (33.3%) in EE; *p-value=0.08* (Sensitivity, specificity for EPS: 65.2%, 66.7%). p16: 22/23 (95.7%) in EPS to 6/12 (50%) in EE; *p-value=0.003* (Sensitivity, specificity for EPS: 95.7%, 50%); A combination of the three markers: 17/23 (73.9%) in EPS to 2/12 (16.7%) in EE; *p-value=0.002* (Sensitivity, specificity for EPS: 73.9%, 83.3%).

Conclusions: WT-1 is specific and p16 is fairly sensitive for EPS. Positive expression existing in two or more biomarkers is predictive of EPS; otherwise it is predicitive of EE.

1069 Is There an Association between the Micropapillary/Cribriform Pattern in Ovarian Serous Tumors of Low Malignant Potential and Microinvasion?

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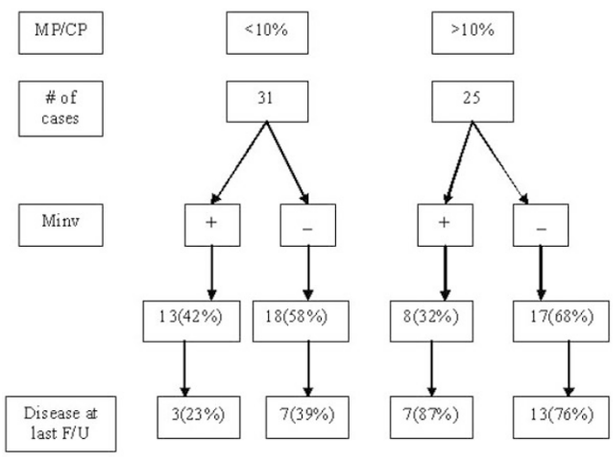
Background: Microinvasion (Minv) has been found to occur in 53% of advanced stage Ovarian Serous Tumors of Low Malignant Potential (SLMP), classic type. However this histological finding has not been specifically studied in SLMP with micropapillary or cribriform pattern (MP/CP). The purpose of this study is to determine the incidence, histological characteristics, and significance of MInv in cases of advanced stage OSLMP with a MP/CP.

Design: H&E slides from 56 cases of advanced stage SLMP with MP/CP were retrieved from the files of our department. Minv was recognized as foci of individual cells, clusters of cells or papilla within the stroma, surrounded by clefts without epithelial lining, measuring up to 3 mm in a single dimension or 10 mm^2. The following parameters were recorded: percentage of MP/CP in the SLMP (< 10% and ≥ 10%), and size and number of the focus/foci of Minv. Follow-up (F/U) was obtained from the patients' charts. Chi-square was used to determine statistical significance ($p<0.05$).

Results:



In addition, cases of SLMP with <10% MP/CP had foci of Minv that were smaller (<1mm) but more abundant (up to 22 foci), while cases with ≥10% MP/CP contain foci of Minv that were larger (≤2mm) but less numerous (up to 4 foci). In 18 (86%) of 21 cases, the Minv foci were not underlying MP/CP areas.

Conclusions: 1 – Minv is not associated with the presence or extent of MP/CP. 2 – Minv is not commonly identified in areas of MP/CP (14%). 3 – The frequency of Minv in cases with MP/CP (38%) is not statistically different from the frequency of Minv in previously reported cases of advanced stage SLMP, classic type (53%) (p=0.2). 4 – Independent of the amount of MP/CP, the frequency of Minv did not adversely affect the prognosis of the pts (p=0.7). 5- Most probably two very important functions of the cells in serous borderline tumors, proliferation and invasion, are independent and follow different pathways.

1070 Clinical Testing for Defective DNA Mismatch Repair in 335 Gynecologic Neoplasms

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Background: Endometrial and ovarian tumors are included in the spectrum of Hereditary Non- Polyposis Colorectal Cancer (HNPCC) neoplasms. Neoplastic lesions in HNPCC often have defective DNA mismatch repair (dMMR) which is defined by the presence of high level of microsatellite instability (MSI-H) or by loss of protein expression of one or more of the 4 main DNA mismatch repair enzymes (MLH1, MSH2, MSH6 and PMS2) as detected by immunohistochemistry (IHC). The aim of this study is to analyze gynecologic neoplasms submitted for HNPCC screening by MSI and/or IHC and compare the screening results with follow-up sequencing.

Design: The study comprised 335 consecutive gynecologic neoplasms (263 endometrial and 72 ovarian) tested from January 2002 to June 2008. The data collected included tumor type, grade, MSI and/or IHC testing and germline testing.

Results: Of the endometrial neoplasms, 77 of 256 (30%) were MSI-H, 16 of 256 (6.3%) were MSI-L and 163 (63.4%) were MSS. Of the ovarian neoplasms, 8 of 72 (11.1%) were MSI-H, 12 of 72 (16.7%) were MSI-L and 52 were MSS. By IHC, 76 of 252 (30.2%) endometrial neoplasms showed loss of protein expression, while 176 of 252 (69.8%) were normal. Over half of the endometrial neoplasms with dMMR were caused by MLH1 loss (42 cases). Loss of MSH2/MSH6 occurred in 21 cases, MSH6 alone in 9 and PMS2 alone in 4 cases. For the ovarian neoplasms tested by IHC, 9 of 67 (13.4%) had loss of protein expression while 58 of 67 (86.6%) were normal. Loss of MSH2/MSH6 occurred in 6 cases, 1 case showed loss of MLH1/PMS2 and 2 showed loss of MSH6 alone. Of those cases for which both MSI and IHC had been performed, 3 of 312 (1%) were discordant - MSI testing was MSS or MSI-L and the IHC showed loss of protein expression. Follow-up germline testing in 34 patients with endometrial neoplasms yielded 14 mutations (6 in *MSH2,* 4 in *MSH6,* 4 in *MLH1)*. Only 3 patients with ovarian neoplasms were sent for further testing and all 3 were found to have mutations (2 in *MSH2*, 1 in *MSH6*).

Conclusions: The rate of MSI-H was higher in endometrial than in ovarian tumors. The results show that occasional discordant cases are identified by performing both methods of DNA mismatch repair testing on gynecologic neoplasms. Of the patients with gynecologic neoplasms that underwent germline testing the majority (76%) were associated with *MSH2* and *MSH6* mutations.

1071 Immunophenotyping of Intestinal Type of Cervical Adenocarcinoma

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Background: Immunohistochemistry plays a critical role in identifying the primary site of metastatic tumors with unknown origin. Although CDX-2 is considered a specific and sensitive marker for colorectal carcinoma, some studies have shown its expression in other carcinomas with intestinal differentiation. There is limited data about its expression in cervical adenocarcinoma. In this study, we investigated the expression of CK7, CK20, CDX-2 and p16 in different types of cervical adenocarcinomas.

Design: Seventy specimens of cervical adenocarcinoma were retrieved from the archives of anatomic pathology, including 30 usual (endocervical), 26 intestinal, 6 endometrioid and 8 mixed types. All cases were stained for CK7, CK20, CDX-2 and p16. Staining was considered positive when it was cytoplasmic for CK7 and CK20, nuclear for CDX-2 and both nuclear and cytoplasmic for p16. Percentage of cells staining was recorded as follow: Negative (0-5%), weak 1+, 6-25%), moderate (2+, 26-50%), and strong (3+, >50%). Fifty cases of rectal adenocarcinoma were included as a control group.

Results: Adenocarcinoma in situ was seen in 51/70 (73%) of cases and their results were comparable to their invasive counterparts. CK7+/CK20+ immunophenotype was expressed in 10% of usual type, 46% of intestinal type, 38% of mixed type and none of endometrioid type. In rectal adenocarcinoma, CK7+/CK20+ immunophenotype was expressed in 10/50 (20%) of the cases; however, CK7 was focal (6-25%) in 6 of these cases. Three cases were negative for CK7/CK20 (6%).

	CK7	CK20	CDX-2	p16
Usual (30 cases)	30/30 (100%)	3/30 (10%)	6/30 (20%)	30/30 (100%)
Intestinal (26 cases)	26/26 (100%)	10/26 (46%)	21/26 (81%)	25/26 (96%)
Endometrioid (6 cases)	5/6 (83%)	0/6 (0%)	1/6 (17%)	5/6 (83%)
Mixed type	8/8 (100%)	3/8 (38%)	3/8 (38%)	8/8 (100%)
Rectal group (50 cases)	10/50 (20%)	47/50 (94%)	50/50 (100%)	7/50 (14%,Focal)

Conclusions: CDX-2 was expressed in the majority of endocervical adenocarcinoma, intestinal type. Therefore, CDX-2 is a marker for intestinal differentiation and should not be used as the sole basis for confirming the colorectum as the primary origin for metastatic cases. Although there is immunophenotypic overlap, diffuse staining for CK7 and p16 helps distinguish cervical from rectal adenocarcinomas. CK20+/CK7- immunophenotype is more specific in predicting the colorectal origin than CDX-2 expression alone.

1072 Lymphatic Microvessel Density (LMD) as a Prognostic Marker in Endocervical Adenocarcinoma

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Background: Lymphatic invasion and lymph node metastases serve as prognostic indicators for disease progression in endocervical adenocarcinoma and as a guide for therapeutic decisions. Most studies investigating lymphatic vessel density (LVD) in cervical carcinoma have dealt with squamous cell type. There is limited data evaluating the significance of LVD as a prognostic marker in cervical adenocarcinoma. In this study, we investigated intra- and peritumoral LVD, using the lymphatic marker D2-40, as a prognostic marker in endocervical adenocarcinoma.

Design: Surgical specimens from 45 consecutive patients with endocervical adenocarcinoma treated with total abdominal hysterectomy and surgical staging were reviewed. Selected tumor blocks were immunostained for D2-40 and CD31. Positively stained microvessels (MV) were counted in density vascular/lymphatic foci (hot spots) at x400 field in each specimen (=0.17 mm^2) by 2 pathologists. Results were expressed as the highest number of MV count identified within any single field and correlated with other prognostic parameters.

Results: Both CD31 MV and peritumoral D2-40 LVD showed significant correlation with depth of invasion, circumferential involvement, and angiolymphatic invasion (r=0.41 and 0.35; 0.39 and 0.52; 0.40 and 0.36; respectively, $P< 0.05$). Only peritumoral D2-40 LVD demonstrated a significant correlation with lymph node metastases (r=0.5, $P<0.01$). Intratumoral lymphatics were identified in 21/45 (47%) patients and their presence demonstrated a significant correlation with uterine involvement and angiolymphatic invasion (r=0.36, 0.38; respectively, $P< 0.05$). D2-40 detected angiolymphatic invasion in 14/45 (31%) cases, more than CD31 (10/45, 22%) and H&E (9/45, 20%). Angiolymphatic invasion detected with D2-40 showed a significant correlation with depth of invasion, lymph node metastases and parametrial involvement (r=0.41, 0.38, 0.33; respectively, $P< 0.01$).

Conclusions: Our study showed that both angiogenesis and lymphangiogenesis play an important role in the progression of endocervical adenocarcinoma. Peritumoral D2-40 LVD, in cervical adenocarcinoma, is significantly correlated with known prognostic parameters such as depth of invasion, circumferential involvement, angiolymphatic invasion and lymph node metastases. In addition, D2-40 detects more angiolymphatic invasion than commonly used endothelial marker (CD31) and routine H&E.

1073 Co-Localization of $p16^{INK4a}$ and MIB-1 Distinguishes High Grade Premalignant Lesions from Tuboendometrial Metaplasia in Cervical Mucosa

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Background: The histologic diagnosis of high grade premalignant squamous and glandular lesions of the uterine cervix is problematic in cases that have morphologic overlap with benign changes. $p16^{INK4a}$, a surrogate marker of HPV integration into the host genome, is overexpressed in CIN, AIS, carcinoma, and in tuboendometrial metaplasia (TEM), but not in benign proliferative lesions. MIB-1 is also overexpressed in CIN, AIS, carcinoma, and in some benign proliferative lesions, but not in TEM. Since TEM has a low proliferative rate, the current study was designed to test the hypothesis that the co-localization of $p16^{INK4a}$ and MIB-1, but not the localization of either marker in isolation, specifically detects clinically significant cervical lesions.

Design: Formalin-fixed cervical biopsy specimens, representative of 260 diagnostic regions, were subjected to two color immunohistochemical staining for $p16^{INK4a}$ (MTM labs AG) and MIB-1 (VectorLabs) using an EnVision polymer based method (Dako). The chromogen combination of DAB-brown for the detection of $p16^{INK4a}$ and alkaline phosphatase-blue for MIB-1 exhibited good color contrast in the distinction of these two antigens. Histologic regions were scored positive for either marker based on the detection of $p16^{INK4a}$ or MIB-1 in greater than 10% of the cells of interest.

Results: Positive test results with co-localization of $p16^{INK4a}$/MIB-1 were found in 20/40 cases of CIN I (n=40) and in all cases of CIN II/III (n=32), squamous cell carcinoma (n=11), AIS (n=10), and invasive adenocarcinoma (n=8). Co-localization of $p16^{INK4a}$/MIB-1 was also detected in 1/19 sections with TEM but was not detected in normal squamous mucosa (n=78), normal endocervical mucosa (n=72), or in microglandular hyperplasia (n=9). Sporadic staining for either $p16^{INK4a}$ or MIB-1 in isolation was detected in 14 sections of benign endocervical mucosa.

Conclusions: These findings demonstrate that the co-localization of $p16^{INK4a}$ and MIB-1 is more specific than either marker alone as a diagnostic adjunct for benign, premalignant, and malignant lesions of the cervical mucosa. Two color multiplex detection of $p16^{INK4a}$ and MIB-1 appears to overcome the problem of p16 expression in TEM as a potential source of false positive classification of test results. These observations highlight the utility of this approach for the classification of biopsy specimens and also suggest that a two-color immunocytochemical approach could be useful as a diagnostic adjunct for cervical cytology specimens.

1074 Adenomatoid Tumors of the Female and Male Genital Tracts: A Morphological and Immunohistochemical Study of 34 Cases

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Background: Although adenomatoid tumors (AT) of the female and male genital tracts are well-characterized as mesothelial in origin, a formal immunohistochemical (IHC) study comparing both traditional and newer mesothelial markers has not been previously reported. In addition, the morphologic differences of AT in male and female sites, if any, have not been formally studied.

Design: IHC analysis was performed on 34 AT from the female and male genital tracts with pankeratin (AE1/CAM5.2), WT-1, calretinin, CK5/6, D2-40, and caldesmon. Staining was semiquantitatively scored on one representative section per case as

negative (0, <5% cells stained), focally positive (1+, 5-10% cells stained), positive (2+, 10-50% cells stained), or diffusely positive (3+, >50% cells stained), and a mean extent calculated. Staining intensity was scored from 0 to 3+ and a mean intensity calculated. A variety of morphologic features previously described as characteristic of AT were also compared between tumors in the female versus male genital tracts.

Results: IHC results are summarized in Tables 1. All of the AT from both the male (n=7) and female (n=27) genital tract showed the thread-like bridging strand pattern. Lymphoid aggregates were seen in all 7 male AT, but in only 3 of 27 female AT (11%) ($p<0.0001$). The remaining morphologic features were variably present in AT with no clear sex predilection.

IHC of Adenomatoid tumors

	AE1/CAM5.2 (M/C)	WT-1 (N)	Calretinin (N)	CK5/6 (C)	D2-40 (M/N)	Caldesmon (N/C)
Female genital tract	27/27 (100%)	26/27 (96%)	27/27 (100%)	5/27 (19%)	27/27 (100%)	1/27 (4%)
	ME=3,MI=2.9	ME=2.1, MI=1.6	ME=2.9, MI=2.6	ME=0.3, MI=0.2	ME=2.9, MI=2.5	ME=0.1, MI=0.1
Male genital tract	7/7 (100%)	6/7 (86%)	7/7 (100%)	1/7 (14%)	7/7 (100%)	0/7 (0%)
	ME=3,MI=3	ME=2.4, MI=1.4	ME=3,MI=3	ME=0.1, MI=0.1	ME=2.7, MI=3	ME=0, MI=0

M=membranous, N=nuclear, C=cytoplasmic, ME=mean extent (0-3), MI=mean intensity (0-3)

Conclusions: While lymphoid aggregates are common in AT of the male genital tract, they are relatively infrequent in the female genital tract. Thread-like bridging strands are consistently present in all AT and remain useful in diagnosis. Of the putative mesothelial markers evaluated in this study, calretinin and D2-40 show a similar immunoprofile and have a higher sensitivity than WT-1 in AT. The low sensitivity of CK5/6 and caldesmon limit their utility in this setting.

1075 Immunohistochemical Features of Post-Radiation Recurrences of Endometrioid Carcinomas of the Endometrium

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Background: Endometrioid carcinoma of the endometrium (EC) is typically treated with surgery and radiotherapy. Post-radiation recurrences of EC are usually associated with increased risk of metastases. Even though the molecular alterations involved in post-radiation recurrences of EC have not been evaluated, alterations of genes responsible for the extrinsic and intrinsic apoptosis pathways, like p53 and NF-kb, have been involved in resistance to radiotherapy in other types of tumor.

Design: Ten post-radiation recurrences of EC were evaluated by immunohistochemistry (IHC) compared to a group of 95 primary EC. The IHC panel included estrogen (ER) and progesterone receptors (PR), p53, Ki 67, E-cadherin, beta-catenin, PTEN, HIF-alpha, CK2, FLIP, BAX, Bcl-xL, MLH-1, MSH-2, MSH-6, and members of the NF-kb family of genes.

Results: Overall, post-radiation recurrences showed decreased expression of ER and PR, and increased expression of p53 in comparison with primary EC. Interestingly, the vast majority of the recurrences exhibited beta-catenin nuclear expression, which is detected in 30% of the primary EC. Moreover, postradiation recurrences showed frequent nuclear expression for members of the NF-kb family of genes (c-Rel, p52).

Conclusions: The results suggest that alterations of genes involved in the control of apoptosis may play a role in resistance to radiation in EC. Moreover, nuclear expression of beta-catenin, which is regarded as a feature of indolent behaviour in EC is also frequent in post-radiation recurrences of EC, suggesting that beta-catenin can also play a role in resistance to radiation.

1076 Contrasts in Pathologic Presentation between Symptomatic and Asymptomatic BRCA Mutation-Associated Pelvic Cancer: One or Two Diseases?

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Background: Recent studies have presented evidence that over 80% of early pelvic carcinomas in women with inherited BRCA mutations (BRCA+), who undergo risk-reducing salpingo-oophorectomy, arise in the distal fallopian tube in the form of serous tubal intraepithelial carcinoma (STIC), in contrast to 30-50% of symptomatic malignancies. The purpose of this study was to compare the tumor distribution of pelvic carcinomas in symptomatic and asymptomatic BRCA+ women.

Design: Pathology from 19 symptomatic BRCA+ cancers and 11 early carcinomas discovered at prophylactic salpingo-oophorectomy were reviewed. The distribution of tumor involvement, including presence of a dominant tumor mass (2-fold greater disparity in ovarian longest diameter), and evidence of tubal mucosal involvement were recorded and compared. Symptomatic cases were evaluated by conventional processing and the asymptomatic cases by the SEE-FIM protocol (complete tubal exam).

Results: Of 19 symptomatic BRCA+ cases, 17 were high grade serous carcinomas. Eleven (65%) presented with a dominant ovarian mass; bilateral ovarian involvement was appreciated in 76%, and fimbrial mucosal involvement was recorded in 24%. Two were endometrioid carcinomas. Of 11 early tumors, eight were high grade serous carcinomas, all of which were associated with serous tubal intraepithelial carcinoma (STIC) in the distal fallopian tube, four with ovarian and peritoneal implants. One was associated with an intracystic intraepithelial carcinoma in one ovary. Three of the 11 early tumors were endometrioid carcinomas, two of which involved the mucosa of the fimbria, one with a surface ovarian implant. A third presented as a single surface implant of the ovary.

Conclusions: A high percentage of early serous carcinomas in BRCA+ women arise in the distal tube (82%). Despite this, advanced BRCA+ serous carcinomas present with a tumor distribution that is distinctly "ovarian" in appearance. Adoption of the SEE-FIM protocol is strongly recommended to confirm the origin of BRCA-associated symptomatic pelvic carcinomas and exclude other serous carcinogenic pathways in this group. Endometrioid carcinomas are not excluded from this population and most arise in the distal fallopian tube.

1077 Phosphorylated FKHR Is Associated with Serous Neoplasia of the Ovary but Not with Mucinous Tumors

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Background: The Phosphatidyl-inositol 3-kinase (PI3K)- p-Akt signal transduction pathway has been implicated in ovarian carcinogenesis by several studies based on in-vitro experiments and the genomic gains in the region of chromosome 3q26. Furthermore elements of this pathway are particularly interesting as potential therapeutic targets. Therefore, this study aims at correlating the expression and activation of individual components of this pathway with different histological types of ovarian epithelial neoplasia.

Design: 231 cases of epithelial ovarian neoplasms of different histological type and grade were analyzed by immunohistochemistry in a tissue microarray for expression of p110alpha PI3K), phospho-Akt, mTOR and pFKHR. Clinical follow up was available in 64 cases of advanced serous carcinoma (max. 114 months, median 30 months after time of operation).

Results: The most significant difference between the individual groups was found for pFKHR. This component was detected in at least moderate staining in 53% of all serous carcinomas (n=96), 43% of serous borderline tumors (n=37) and 25 % of serous adenomas (n=12) but only in 10% of mucinous carcinomas (n=10) and in none of the mucinous borderline tumors (n=14) or adenomas (n=13). All other components were expressed in varying amounts in borderlinetumors and carcinomas, but no specific pattern was observed. Furthermore, pFKHR was associated with a shorter overall survival in patients with advanced stage serous carcinoma (34 vs 58 months, p= 0,028).

Conclusions: Expression of phosphorylated FKHR is associated with development of serous neoplasia of the ovary both of low-grade and high grade neoplasms. It does not seem to be involved in mucinous neoplasia – which mostly relies on other signaltransduction pathways (eg. involving p42/44 MAPK). Expression of pFKHR also has a significant influence on overall survival of patients with advanced stage serous carcinoma of the ovary. It remains to examine the relevance of this parameter in lager independent patient collectives.

1078 Ovarian Low Grade Serous Neoplasms: Evaluation of Sampling Recommendations Based on Tumors Expected To Have Invasion (Those with Peritoneal Invasive Low Grade Serous Carcinoma (Invasive Implants))

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Background: Exclusion of invasion in atypical proliferative (borderline) serous tumors (APST) depends on sampling adequacy. Previous recommendations of 1 section per cm (SPC) of maximum tumor diameter, and 2 SPC for tumors $\geq$ 10 cm, have not been tested.

Design: 15 consecutive patients with 24 low grade serous neoplasms associated with invasive peritoneal carcinoma (9 bilateral) were evaluated for invasion on a section by section basis, with notation of which section per original submission order first showed invasion. Invasion < 5 mm was designated microinvasion, and $\geq$ 5mm, low grade carcinoma. 5 patients' tumors were sampled with <1 SPC, and 10, $\geq$1 SPC. Among the latter 10, 4 patients were sampled with $\geq$2 SPC.

Results: Invasion was identified in 11 of 15 patients (73%). At up to 1 SPC, 5 patients had APST, 5 had APST with microinvasion and 5 had low grade carcinoma. At up to 2 SPC, the diagnosis in two patients with APST (1 with microinvasion) was upgraded to carcinoma, and at >2 SPC, 1 additional APST with microinvasion was upgraded to carcinoma. Thus, increasing the sampling upgraded 3 of 10 patients with APST to carcinoma; only 8 of the latter 10 had been sampled at >1 SPC and therefore 3 of 8 (37.5%) APSTs were upgraded after increasing sampling to >1 SPC. All 3 tumors upgraded to low grade carcinoma had microinvasion in the earlier sections. 2 of 3 tumors upgraded to carcinoma were smaller than 10 cm. The mean sampling for tumors in which invasion was not found (n=6) was 0.8 SPC as compared to 1.9 SPC for tumors in which invasion was identified (n=18) ($P<0.05$, Student's t test).

Conclusions: 1 SPC is insufficient to adequately exclude invasion in APST. At least 2 SPC are needed, even for tumors smaller than 10 cm. Identification of microinvasion in APST is an important finding and should prompt submission of additional sections to exclude low grade carcinoma.

1079 Relationship of Underreporting of Placental Abnormalities to Clinical Diagnosis

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Background: A previous study of 100 cases of placental specimens showed a high rate of underdiagnosis of abnormalities in the final pathology report (Modern Pathology 1998;11:184A). This study sought to determine if the frequency of underreporting was influenced by the nature of the clinical diagnosis submitted with the specimen.

Design: The slides and diagnostic reports from a consecutive series of 100 placentas that were signed out by staff pathologists with no special training or experience in gynecologic pathology were examined retrospectively. Specimens had been submitted on the basis of specific clinical indications, and represented approximately 1/5th of all deliveries. For each case for which the clinical diagnosis indicated the potential for a pathologic abnormality that was evaluated in the study, the full report was reviewed

to determine whether or not the clinical diagnosis was confirmed in the original pathologic diagnoses.
Results: A clinical diagnosis was submitted with 98 cases, and study parameters were appropriate to identify confirmation of the clinical diagnosis in 89 cases. Of these, pathologic abnormality related to the clinical diagnosis was present in 70 cases (79%), but the clinical diagnosis was correctly confirmed by the original pathologic diagnoses in only 41 of the 70 cases (59%), as follows (clinical diagnosis [no. confirmed/no. present; %confirmed]): Chorioamnionitis [12/12; 100%]; premature rupture of the membranes or maternal fever [2/2; 100%]; maternal diabetes [1/1; 100%]; twin gestation [5/6; 83%]; meconium [3/5; 60%]; pre-eclampsia [4/8; 50%]; fetal distress [3/6; 50%]; fetal anomalies [1/2; 50%]; abruption [2/5; 40%]; gross placental abnormality, NOS [2/5; 40%]; pre/post term gestation [5/13; 38%]; intrauterine growth retardation [1/5; 20%]. A confirmatory diagnosis was less likely to be missed if the clinical diagnosis (such as chorioamnionitis or abruption) was explicitly associated with a distinct pathologic finding than if the clinical diagnosis (such as fetal distress or pre-eclampsia) implied a pathologic finding less directly (73% vs. 46%; chi-square, p=0.02).
Conclusions: The frequency of underdiagnosis of placental abnormalities was significantly affected by the nature of the clinical diagnosis. Underdiagnosis of placental abnormalities may be reduced if pathologists become more aware of potential pathologic findings that are only implicitly associated with a clinical diagnosis.

1080 Immunohistochemical Expression of Folate Receptor-alpha in Ovarian Epithelial Neoplasms Bears Clinical and Pathological Significance

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Background: Folate receptor-alpha (FRα) has the ability to internalize folic acid that has been conjugated to other molecules. Thus, it is being actively investigated as a target for folate-linked therapeutic agents for cancer treatment. Previous studies have shown increased FRα expression in some ovarian tumors, suggesting the potential utility of FR-targeted cancer therapy in these tumors. Furthermore, FRα expression has also been associated with tumor progression, histological grade and response to chemotherapy agents in ovarian tumors. The previous studies, however, have largely been performed using techniques such as cytofluorimetric analysis on frozen specimens. It remains to be determined whether immunohistochemistry (IHC), a simple tool applicable to paraffin-embedded tumor material, can selectively detect these clinically significant subsets of FRα -expressing ovarian tumors.
Design: IHC staining using mAb343 against FRα on tissue arrays containing 108 ovarian neoplasms was scored as low (0 or 1+) or high (2+) expression based on intensity, and correlated with clinicopathological characteristics. The 108 tumors included 71 papillary serous carcinomas, 13 endometrioid carcinomas, 8 mixed papillary serous and endometrioid carcinomas, 7 clear cell carcinomas, and 9 borderline epithelial tumors. Borderline tumors were not included in survival analysis.
Results: Of the 108 tumors, 65 (60%) showed high FRα expression, including 75% of all papillary serous carcinomas, 31% of all endometrioid carcinomas, 75% of all mixed papillary serous and endometrioid carcinomas, 14% of all clear cell tumors, and 11% of all borderline tumors. High expression was significantly associated with papillary serous histology, high grade, and high stage ($p<0.001$ for all variables). With a median follow-up of 64 months, high FRα expression significantly correlated with a worse 5-year-disease specific survival on univariate analysis (p=0.0015), along with tumor type, grade, and stage. On multivariate analysis, however, only tumor stage remained a significant survival predictor (p=0.0002).
Conclusions: IHC identified a subset of ovarian epithelial tumors with high FRα expression, providing a foundation for further assessment of IHC as a patient selection method in FR-targeted cancer therapy trials. Tumors with high FRα by IHC also tended to have higher histological grade, advanced stage, and worse clinical outcome, suggesting a potential prognostic utility of IHC detection of tumor FRα in these patients.

1081 Scoring with MUC2, MUC5, CK7, CK20, CDX2, CEA Immnohistochemical Staining Results Can Be Useful in Identifying Metastatic Colorectal Carcinoma Involving Ovary from Primary Ovarian Mucinous Adenocarcinoma

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Background: Colorectal adenocarcinoma, the most common tumor metastatic to the ovary, may closely mimic primary ovarian mucinous adenocarcinoma (POMA). The differential diagnosis is difficult but crucial to treatment and prognosis.
Design: We evaluated the immunohistochemical expression of cytokeratins7 (CK7), cytokeratins20 (CK20), MUC2, MUC5, CDX2 and CEA in 20 POMAs and 41 metastatic colorectal carcinomas involving ovaries (MCCOs).
Results: MCCOs were almost always negative for MUC5 (97.6%), often negative for CK7 (82.9%), positive for CDX2 (73.2%), diffuse positive for CK20 (65.9%), positive for MUC2 (51.2%) and diffuse positive for CEA (41.5%). MCCOs were contrasted to POMAs, which were almost always negative for MUC2 (100%) and negative or focal positive for CK20 (95%) and CEA (90%) and often positive for CK7 (85%) and MUC 5 (50%). When we considered MUC2 (+), MUC5 (-), CK7 (-), CK20 (diffuse +), CDX2 (+), CEA (diffuse +) as score 1, respectively, and added the scores (range 0~7), with the cut-off value 3, we can correctly classified 90% of POMAs and 91.7% of MCCOs.
Conclusions: Scoring with MUC2, MUC5, CK7, CK20, CDX2, CEA immnohistochemical staining results is a useful adjunctive diagnostic tool to differentiate MCCOs from POMAs, in addition to clinical history and gross and microscopic findings.

1082 Glandular Intraluminal Debris in Endometrioid Adenocarcinoma of the Endometrium Correlates with Tumor Grade and Stage: A Clinicopathologic Study of 115 Cases

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Background: We have observed that some endometrioid carcinomas of the endometrium (EMCA) show variable amounts of glandular intraluminal debris (GID). GID consists of organizing fibrinopurulent and cellular debris, with the latter comprised largely of necrotic neutrophils and histiocytes, superficially resembling the "dirty necrosis" in colon carcinomas. This study was aimed to investigate whether GID in EMCA would correlate with tumor grade and/or stage in hysterectomy specimens.
Design: Our institutional pathology archives between January 1 and December 31, 2003 were searched for cases of EMCA in specimens of total hysterectomy and bilateral salpingo-oophorectomy with staging. Upon review of the slides, we arbitrarily divided GID into two groups: i) minimal GID was defined as involving 0 to 4 carcinomatous glands, and ii) extensive GID as involving more than 4 carcinomatous glands. Both GID groups were correlated with tumor FIGO grade, depth of myometrial invasion, lower uterine segment (LUS) extension, cervical involvement, vascular invasion, lymph node metastasis, and pelvic washing result. Fisher Exact Test was used to compare each of the above parameters between the two GID groups.
Results: 82 of 115 cases (71.3%) fell into the minimal GID group, and 33 cases (28.7%) into the extensive GID group. Extensive GID was seen in 9 of 16 cases (56.3%) of FIGO grade III tumors and in 24 of 99 cases (24.2%) of FIGO grade I&II tumors ($p<0.05$). Within grade I&II lesions, extensive GID was identified in 22 of 71 tumors (31.0%) with myometrial invasion and in 2 of 28 tumors (7.1%) without myometrial invasion ($p<0.05$); it was seen in 14 of 37 cases (37.8%) with LUS extension and in 10 of 62 cases (16.1%) without LUS extension ($p<0.05$). Although extensive GID, as compared to minimal GID, was present more often in tumors with positive cervical involvement and positive pelvic washings, the difference did not reach statistical significance (p=0.075 and p=0.068, respectively). No difference was seen in vascular invasion or lymph node metastasis between the minimal and the extensive GID groups.
Conclusions: Extensive GID is seen more frequently in FIGO grade III EMCA than in grade I&II tumors. Within grade I&II EMCA, extensive GID is associated with myometrial invasion and LUS extension. Thus assessing GID in endometrial biopsy or curettage specimens may help predict the grade and extent of residual tumor in the uterus.

1083 Targeted Therapy with Tyrosine Kinase Inhibitors and the Frequency of EGFR and K-Ras Mutations in Endometrial Cancer

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Background: Protein kinase activation either by somatic mutations is a common mechanism of tumorigenesis. Somatic mutations involving epidermal growth factor receptor (*EGFR*) have been documented extensively in non-small cell lung cancer. Several studies have documented overexpression of *EGFR* in endometrial cancer (36%-87%) with conflicting reports of on their impact on prognosis. A recent phase II study of erlotinib in recurrent or metastatic endometrial cancer revealed an overall response rate of 12.5%. The efficacy of such tyrosine kinase inhibitors for EGFR is dependent on downstream *K-Ras* mutational status as demonstrated in colorectal carcinoma. To date to our knowledge no studies have focussed on the simultaneous detection of both *EGFR* and *K-Ras* mutations in endometrial carcinomas. We believe that identifying specific molecular mechanisms in endometrial carcinoma would lead to more optimized and individualized treatment options as are available at present for non-small cell lung cancer and colorectal cancer.
Design: We obtained ten cases of endometrial carcinoma from archival tissue. Mutation analysis was performed for the most common mutations occurring in *K-ras* codons 12 and 13 utilizing the LightMix^R kit from TIB MOLBIOL with melting curve analysis was performed on the Roche light cycler 1.2 instrument. Mutation analysis was also performed for the most common kinase domain mutations of the *EGFR* in exons 19 and 21. Exon 21 mutation (L858R) was identified by fluorescent single base primer extension (SNPStart, Beckman Coulter). Exon 19 deletions were detected using high resolution gel capillary electrophoresis.
Results: We detected four *K-Ras* mutations in the ten endometrial carcinoma samples examined. Although the sample studied was limited in size the findings are suggestive of a high rate of *K-ras* mutations (40%) in endometrial carcinoma. We found no *EGFR* kinase domain mutations in the samples similar to other studies.
Conclusions: The high frequency of *K-ras* mutations in endometrial carcinoma may be utilized to guide treatment planning with tyrosine kinase inhibitors. Therapy with tyrosine kinase inhibitors to *EGFR* kinase domain-as examined in a phase II study for endometrial cancer- would be dependent on the presence of wild-type versus mutated *K-ras*, as *K-Ras* is downstream of *EGFR*. Hence similar to colon cancer screening for *K-ras* mutations may help identify a sub-group of patients who will have a better response to tyrosine kinase inhibitors such as erlotinib.

1084 Cervical Stromal Invasion in Endometrioid Endometrial Carcinoma (EEC) Does Not Correlate with Overall Survival

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Background: EEC is the most common endometrial cancer, with stage being the most critical prognostic factor. Cervical involvement (CI), divided into IIA (epithelial involvement) and IIB (stromal invasion), overall is associated with decreased survival (70 vs 90%). However, the impact on prognosis of substages IIA vs IIB is unclear.
Design: 81 patients uniformly treated for stage II EEC were identified (1993-2003) in our institution. They were stratified into Group A (46) with available slides for review and Group B (35) with information obtained from the pathology report. In group A,

1 to 6 slides of cervix (mean 3) were reviewed. Tumors were classified as Stage IIA or IIB according to the most recent FIGO criteria. Stromal invasion (SI) in group A tumors was subclassified in 4 subgroups based on depth of invasion; A) ≤ 1 mm; B) > 1 mm and ≤ 3 mm; C) > 3 mm and ≤ 5 mm and D) > 5 mm. Other histopathologic parameters included grade, depth of myometrial invasion (MI), and lymphovascular invasion (LVI). Clinical data included age, type of surgery/ radiation, and survival. Statistical analysis was performed.

Results: Patients ranged in age from 33 – 91 (median 64) years. In group A, 11 patients had stage IIA and 35 stage IIB tumors. Depth of SI ranged from 0.1 to 1.2 (mean 0.34) cm (≤1 mm in 2, > 1 mm and ≤ 3 mm in 18, > 3 mm and ≤ 5 mm in 10 and > 5 mm in 5). In Group B, 15 patients had stage IIA and 20 stage IIB tumors with no further information regarding depth of SI. In group A, 12 EECs were Grade 1, 29 Grade 2 and 5 Grade 3. 31 tumors had < 50% MI while 15 had > 50% MI and LVI was present in 11. In Group B,13 tumors were Grade 1, 13 Grade 2, and 9 Grade 3. 21 had < 50% or no MI and 9 showed LVI. Median follow-up was 73 (range 5 – 210) months. Five and 10-year survival rates were 83% and 78% for patients with stage IIA and 71% and 65% for stage IIB EECs respectively. By univariate analysis, age, MI, LVI and type of treatment affected survival, while substaging or depth of SI did not. By multivariate analysis, only age (p=0.001), LVI (p =0.017), and type of treatment (p=0.022) were predictors of survival in stage II EECs.

Conclusions: This study showed that distinction between stage IIA and IIB or depth of SI do not affect survival in patients with EEC, suggesting that substaging should be eliminated.

1085 Lymphoepithelioma-Like Carcinomas in the Uterine Cervix and Endometrium: A Clinicopathologic Study of 6 Cases with Evaluation of Epstein-Barr Virus and Human Papilloma Virus Genomes

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Background: Lymphoepithelioma-like carcinomas (LELC) have been reported in many sites, including nasopharynx, salivary gland, thymus, stomach, breast, and uterine cervix. Lymphoepithelial carcinoma occurring in nasopharynx is known to be strongly associated with Ebstein-Barr virus (EBV) and the prognosis is favorable, but the causative relationship of oncogenic virus and the prognosis of LELC in other areas are poorly defined.

Design: To evaluate the pathogenetic significance of EBV and human papilloma virus (HPV) infections in LELC in the female genital tract, we performed in situ hybridization for EBV and genotyping DNA chip analysis for HPV in 6 cases of LELCs occurring in the uterine cervix and endometrium using paraffin embedded tumor tissue, which we have experience during twelve years.

Results: Two cases showed EBV genome and one had HPV genome of high risk type (type 16). FIGO stages were IB1 (n=2), IB2 (n=2), III (n=1), and IVB (n=1). Five tumors in the uterine cervix were composed of medullary carcinoma-like nests, and one endometrial carcinoma showed adenocarcinomatous differentiation, both of which were surrounded by dense lymphoplasmacytic infiltrates. One cervical cancer patient (FIGO stage IVB) died of disease, and four patients had no evidence of disease during the follow-up period (mean: 33months, 3-70 months).

Conclusions: LELC can be associated with either EBV or HPV infection, but their causative relationship was not clearly identified.

1086 Diagnostic Utility of Detecting 12p Alterations by Fluorescence In Situ Hybridization in Ovarian Germ Cell Tumors

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Background: Germ cell tumors (GCT) comprise nearly 20% of primary ovarian tumors and can be histologically heterogeneous, often displaying features overlapping with other primary ovarian tumors. Given that patient treatment and prognosis varies greatly depending on tumor type, an accurate diagnosis is critical. Alterations of chromosome 12 including duplication of 12p and formation of isochromosome 12p (iso(12p)) have been shown to be recurrent genetic features of testicular GCT, present in 60-80% of cases. Iso(12p) has also been detected in a subset of ovarian GCT. However, the diagnostic utility of detecting 12p alterations in differentiating ovarian GCT from other ovarian tumors has not been formally investigated.

Design: After IRB approval, formalin-fixed, paraffin-embedded tissue was collected from 20 primary ovarian GCT, 10 ovarian carcinomas, 7 sex cord stromal tumors, 4 ovarian large B-cell lymphomas, 20 testicular seminomas (positive control) and 25 normal ovaries (negative control). A novel dual-color FISH probe strategy was used to identify structural and/or numerical alterations of chromosome 12p. Three clones were used to build a 390 kb FISH probe mapping to 12p11.21. This homebrew probe was used in conjunction with a chromosome 12 centromeric probe (Abbott Molecular, Abbott Park, IL). This probe cocktail allowed interrogation of chromosome 12 centromere status relative to the status of the 12p arm. FISH analyses were conducted in a blinded fashion.

Results: In testicular seminomas, FISH showed iso(12p) in 11 cases, 12p gain in 4 cases, and polyploidy of chromosome 12 in 5 cases. Table 1 shows results of FISH for primary ovarian tumors. Iso(12p) was identified in 10 of 20 (50%) ovarian GCTs. No ovarian tumor showed gain of 12p. No chromosome 12 alterations were seen in normal ovaries.

	iso(12p)	Polyploidy Chr 12	Diploid Chr 12
B-cell lymphoma	0/4	1/4	3/4
Ovarian carcinoma, clear cell	2/4	1/4	1/4
Ovarian carcinoma, endometrioid	1/4	3/4	0/4
Ovarian carcinoma, mixed serous/ endometrioid	0/1	1/1	0/1
Ovarian carcinoma, mixed serous/ clear cell	1/1	0/1	0/1
Sex cord stromal tumor	0/7	1/7	6/7
Dysgerminoma	7/16	8/16	1/16
Mixed GCT	3/4	1/4	0/4

Conclusions: The present findings suggest that iso(12p) is a common finding in ovarian GCT and supportive of the diagnosis. However, demonstration of iso(12p) pattern by FISH is not a finding specific for GCT and can be demonstrated in other primary ovarian tumors, especially clear cell carcinoma.

1087 Undifferentiated Carcinomas of the Endomentrium and Ovary: A Clinicopathologic Correlation

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Background: Undifferentiated carcinomas (UCs) of the endometrium and ovary are rare and under diagnosed. They are important to recognize since they have been shown to pursue a more aggressive clinical course than usual high grade endometrioid carcinomas (EC). Given the scant clinical and pathologic data, we reviewed our experience with these rare tumors.

Design: We reviewed our pathology database between 2001-2008 to identify 29 cases of UCs. Clinical information was obtained from electronic medical records.

Results: The patient age ranged from 27-83 years (median 54). Many patients (pts) were of young age (24% <40 yrs, 17% 40-50 yrs, and 59% >50 yrs). Thirteen (45%) pts presented with stage I/II disease and 16 (55%) with advanced stage III/IV disease. Most UCs were of endometrial origin and 1 was ovarian. The lower uterine segment (LUS) was involved in 16/29 cases. Ten (35%) UCs were associated with a low grade EC, consistent with so-called de-differentiated carcinoma. Histologically, they evoked a broad differential diagnosis including FIGO grade 3 EC, neuroendocrine carcinoma, cervical carcinoma, lymphoma and sarcoma. The tumors consisted of sheets of ovoid cells with uniform, large vesicular nuclei and variable amount of necrosis. A few cases showed foci of nuclear pleomorphism. Many UCs also showed focal to large areas of rhabdoid cells in a background of myxoid stroma. BAF-47 (INI-1) stain was retained where performed. These tumors showed only focal EMA and keratin staining, and CK18 was the most frequently positive keratin stain. They were negative for neuroendocrine markers, smooth muscle markers and ER/PR. Mismatch repair (MMR) protein expression by IHC was evaluated in 12 cases (40%), and 8 (67%) were abnormal (6 with loss of MLH1/PMS2 and 2 with MSH2/MSH6 loss). Follow-up (F/U) information is available for 24 pts. Nine pts died of disease in 1 to 9 months, 6 are alive with disease (F/U of 1-45 mths), 9 pts have no evidence of disease at 18-85 mths.

Conclusions: UCs of the endometrium and ovary can occur in young women and should be diagnosed correctly as they can pursue a fulminant clinical course. They frequently occur in the LUS. Histologically, they have a broad differential diagnosis and can sometimes be associated with a low grade EC. Rhabdoid cells can be frequently seen in UCs and CK18 may be particularly helpful to demonstrate epithelial differentiation. These tumors frequently show loss of DNA-MMR proteins, particularly MLH1/PMS2.

1088 Can More Detailed Evaluation of Excision Margins Refine Cytological Follow up of Women Post LLETZ Excision of High-Grade Dysplasia?

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Background: The relationship between dysplastic changes in the cervical epithelium and progression to in-situ carcinoma and invasive carcinoma has been extensively studied. Removal of dysplastic epithelium via long loop excision of the transformation zone (LLETZ) in 95% of cases is curative. Between 18-37% of LLETZ specimens with dysplasia at the margins have been shown to have recurrent / residual disease. Previous small studies suggest the degree of dysplasia at margins could predict recurrence and allow risk-based stratification of follow-up (Cardosa-Favarato et al. Human Path Vol. 38; 5: 781-786). We tested this hypothesis in a large group of women post LLETZ with follow up histology and cytology.

Design: All LLETZ specimens containing high-grade dysplasia (CIN2 & CIN3) from the Mater Misercordiae University Hospital over a 12 year period (1995 – 2007) were reviewed. Follow up cytology and histology results were collected. All cases with a prior LLETZ, glandular intraepithelial neoplasia, invasive cervical carcinoma or subsequent hysterectomy were excluded. The cases were divided into two groups according to excision margin status; negative margins or positive margins. The groups were compared to assess if the LLETZ specimens' margin status had an impact on subsequent cytology or histology results. Positive follow-up results were defined as any grade of dysplasia in cytology or histology.

Results: 2321 women had LLETZs containing high-grade dysplasia over the 12-year period. 1534 (66.1%) had full histology and cytology follow up available. 820 (53.4%) of the LLETZ specimens had positive margins and 714 (46.6%) had negative margins. Grade of dysplasia at margins was available in 796 cases (97%). 170 (20%) of specimens with positive margins had positive follow up results compared with 105 (14.7%) of specimens with negative margins. Detailed results were as follows:

Follow Up Cytology & Histology Results

Margin Status	Positive Follow Up	Negative Follow Up
Positive Margin: CIN 3	77	293
Positive Margin: CIN 2	64	246
Positive Margin: CIN 1	22	94
Negative Margin	105	609

Conclusions: The presence of dysplasia at a LLETZ margin is associated with dysplasia on follow-up cytology and histology (P=0.0021), however the grade of dysplasia at the excision margin is not predictive of recurrent / residual dysplasia.

1089 Hyperplasia and Carcinoma, with or without Secretory Changes, in Secretory Endometrium: A Diagnostic Challenge

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Background: The diagnosis of endometrial hyperplasia or carcinoma in a background of secretory endometrium can be difficult. We attempt to establish the diagnostic criteria to be used in such cases.

Design: We examined 80 cases of endometrial hyperplasia, carcinoma and other conditions with glandular crowding arising in secretory endometrium, analyzed their morphologic features, assessed the volume percentage stroma (VPS) in each case and performed Ki67 immunostains on six cases. Thirteen cases each of secretory and gestational endometrium served as controls.

Results: The mean patient age was 45 years. The nonneoplastic diseases included simple hyperplasia without atypia (56%), endometrial polyps (12.5%) and chronic endometritis with glandular crowding (3%), while neoplastic diseases included complex hyperplasia without atypia (10%), atypical hyperplasia (12.5%) and endometrioid carcinoma (6%). The secretory changes were usually less advanced in the hyperplastic glands than in the background endometrium. The morphologic features that best distinguished hyperplasia or carcinoma from secretory endometrium included glandular crowding that stood out from the background; architectural disorder (the long axes of the glands pointing in different directions or parallel to the endometrial surface); dilated, irregular-shaped glands, including budding or branching glands and staghorn-shaped glands; stroma of a polyp; cribriform or confluent glands in cases of carcinoma; nuclear atypia in cases of atypical hyperplasia and carcinoma; and crowded nonsecretory glands. The VPS of neoplastic lesions was less than that of nonneoplastic ones (37% vs. 61%, $p<0.000001$) and that of secretory endometrium (37% vs. 68%, p=0.000006). Nonneoplastic lesions did not have more crowded glands than secretory endometrium (VPS 61% vs. 68%, p=0.11). Gestational endometrium had more crowded glands than nonneoplastic lesions (VPS 39% vs. 61%, p=0.000004), approximately equal VPS with complex hyperplasia (both close to 39%, p=0.53), and less crowded glands than endometrioid carcinoma (VPS 39% vs. 30%, p=0.0266). The Ki67 index was higher in hyperplasia and carcinoma than in the background secretory endometrium (mitoses per gland cross-section, 1.76 vs. 0.19, p=0.02).

Conclusions: Hyperplasia and carcinoma in secretory endometrium can be diagnosed based on increased glandular crowding, architectural irregularity, nuclear atypia and increased Ki67 index.

1090 Lymphoma-Like Lesions of the Lower Female Genital Tract: Morphology, Immunophenotype and Molecular Features

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Background: Lymphoma-like lesions (LLL) of the lower female genital tract were originally described by two of us in 1985. LLL are uncommon. They mainly affect women in their reproductive years. The microscopic features of these lesions differed from those of gynecologic lymphomas by having frequent surface erosion, polymorphous composition, intralesional acute inflammatory cells and plasma cells, and absence of a large mass, deep invasion or prominent sclerosis. All lesions studied by immunohistochemistry had polyclonal plasma cells. Follow-up of 6 months to 12 years was uneventful. With the advent of widespread molecular genetic testing, we have encountered cases with morphologic and immunophenotypic features of lymphoma-like lesions, but with evidence of clonal rearrangement of immunoglobulin heavy chain (*IGH*) genes. We examined the clinicopathologic features and outcome of these cases.

Design: LLL of the cervix (6 cases) and endometrium (2 cases) were retrieved from the consultation files of two of us. Hematoxylin and eosin-stained slides and immunohistochemical (IHC) studies for B and T-cell markers and immunoglobulin light chains were reviewed. The status of the *IGH* gene was analyzed with polymerase chain reaction (PCR) in 3 cases and is pending in the others. Clinical information was obtained from the referring physicians.

Results: Patients ranged in age from 18 to 54 (median 30) years. Six had abnormal PAP smears and 2 presented with vaginal bleeding. All lesions contained a dense, polymorphous inflammatory infiltrate, commonly associated with mucosal erosion. IHC showed a mixture of B and T cells without immunoglobulin light chain restriction. Three cases (all cervical) had a clonal *IGH* gene rearrangement by PCR. Two of the 3 patients had a coexisting high-grade squamous dysplasia and history of sexually transmitted disease. Staging studies in all 3 cases showed no clinical evidence of lymphoma. All 3 patients are alive and well after a follow-up of 1-13 months, with no clinical evidence of lymphoma.

Conclusions: We describe 8 patients with LLL of the lower female genital tract, 3 with clonal *IGH* gene rearrangement. The clinical and pathologic features of these cases suggest that a clonal *IGH* rearrangement in this setting is not sufficient for a diagnosis of lymphoma. Careful correlation of clinical, histologic, immunophenotypic and genetic features is required in these cases to avoid inappropriate treatment.

1091 Prognostic Significance of Endoglin (CD105) as Angiogenic Marker in Endometrial Carcinosarcoma

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Background: Carcinosarcoma of the uterus is a highly aggressive tumor with both malignant epithelial and mesenchymal components. Endoglin (CD105), a member of transforming growth factor beta1 receptor complex, has been shown to be a more useful marker than panendothelial markers such as CD31 in identifying tumor angiogenesis. We investigated microvessel density measured by endoglin as possible prognostic marker in uterine carcinosarcoma.

Design: Surgical specimens from 45 consecutive patients with uterine carcinosarcoma treated with total abdominal hysterectomy and surgical staging were reviewed. Selected tumor blocks with both carcinomatous and sarcomatous components were immunostained for endoglin and CD31. Positively stained microvessels (MV) were counted in densely vascular foci (hot spots) at x400 field in each specimen (=0.17 mm^2) by 2 pathologists. Microvessel counts in carcinomatous and sarcomatous components were reported separately. Results were expressed as the highest number of MV count identified within any single field and correlated with other prognostic parameters and survival.

Results: Endoglin identified MV in carcinomatous component in all cases (mean 25 ± 9) and sarcomatous component in 20/45 (44%, mean 5 ± 4). CD31 MV showed staining 44/45 in carcinomatous component (mean 41 ± 14) and 26/45 (58%) in sarcomatous component (mean 10 ± 7). Endoglin only stained proliferating MV in the tumour, while blood vessels in normal tissue were negative. Both CD31 and endoglin MV showed significant correlation with tumor stage, depth of myometrial invasion and cervical involvement (r=0.44 and 0.39; 0.40 and 0.43; 0.38 and 0.36; respectively, $P<0.05$). They also showed significant correlation with overall survival (log rank $P<0.05$). Endoglin MV showed significant correlation with involvement of lower uterine segment, presence of lymphovascular invasion and distance metastases (r=0.47, 0.44, 0.41; respectively).

Conclusions: Our results support that carcinomatous component plays the major role in the progression and behavior of uterine carcinosarcoma. By staining the proliferating MV, endoglin is a specific and sensitive marker for tumour angiogenesis. It has prognostic significance, with positive correlation with tumor stage, angiolymphatic invasion, lymph node and distant metastases and overall survival.

1092 Pseudolipomatosis in Hysteroscopically-Assessed Tissue (PHAT): Pathologic Features and Frequency

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Background: Pseudolipomatosis is an artifactual microscopic change in tissues that resembles fatty infiltration, most frequently seen in the GI tract. In this condition, fat-like spaces represent air or gas bubbles that enter the mucosa through microscopic ruptures secondary to gaseous insufflation. We recently encountered two cases exhibiting a similar appearance in tissues removed during hysteroscopic procedures in the gynecologic tract, a finding not previously described. We therefore performed a retrospective review to assess the frequency of pseudolipomatosis in hysteroscopically-assessed tissues (PHAT) and to define the morphologic features in a large series.

Design: We identified 300 consecutive hysteroscopic procedures performed at our institution from August 2006 to January 2008 using Current Procedural Terminology codes. Patient medical records were reviewed to collect pertinent clinical data. Slides from all cases were retrieved and systematically reviewed. All cases with features suggestive of PHAT were identified on an initial review. A subsequent consensus conference established the cases identified as PHAT.

Results: A total of 28 cases of PHAT, representing 9.3% of hysteroscopied patients, were identified. PHAT was found in 9 endometrial curettings or biopsy tissues, 8 endometrial or endocervical polyps, 8 uterine fibroids, 2 fallopian tubes, and 1 endocervical biopsy. Among the 28 cases, glycine was used as distention medium in 82%, dextran 70 in 3.5%, and saline in 3.5% (11% were not recorded). The type of distention media used and length of hysteroscopic procedure did not differ significantly between cases with and without PHAT. PHAT vacuoles varied in distribution from multiple crowded clusters to sparsely scattered and solitary. In several cases, vacuoles were found in vascular channels. Vacuoles were round or ovoid, unilocular, and generally uniform in size. Immunohistochemical staining for adipocyte and endothelial markers (S-100 and CD34 respectively), were completely negative.

Conclusions: We hypothesize that PHAT is dervied from the air that is almost invariably introduced into the uterus during media insufflation for hysteroscopy, creating a bubble under pressure. The air enters tissues either through lining microruptures or during the biopsy procedure. The type of distention medium and length of procedure do not appear to be independent predisposing factors. PHAT is a relatively common, easily overlooked finding in hysteroscopically-derived pathology specimens that may be mis-diagnosed when prominent.

1093 Combined p53 and p16 Expression Is Useful To Distinguish between Endometrial Serous and Endometrioid Carcinomas

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Background: p53 Immunohistochemistry is a widely used diagnostic test to distinguish between endometrial serous carcinoma (SC) and a subset of endometrioid carcinoma (EC), however, its diagnostic limitatiion remains a practical problem. Immunohistochemical overexpression of p16 has been also reported in SC. Although it has recently been suggested that an immunohistochemical panel including these two markers be used to overcome the limitations of using p53 alone, the diagnostic

value of such a panel has not been well studied. In this study, we assessed the immunohistochemical profile of SC and EC using a panel of five immunohistochemical markers including p53 and p16 to test the diagnostic value of a panel.

Design: Fifty two hysterectomies reported with SC and 50 with EC were retrieved from the surgical pathology file. Three gynecologic pathologists independently reviewed the HE slides. Equivocal cases or those with mixed histology were excluded; thus 32 SC and 46 EC FIGO 1 or 2 formed the study set. Immunohistochemical stains for estrogen receptor (ER), progesterone receptor (PR), p53, p16 and beta-catenin (Bcat) were performed on a representative section from each case. Two pathologists, masked to the review diagnosis, independently evaluated the immunohistochemistry (IHC) using qualitative and quantitative criteria. Any discrepancies among pathologists were resolved on consensus review.

Results:

Comparison of immunohistochemical expression

	SC (n=32)	EC (n=46)
ER	15 (47%)	38 (83%)
PR	13 (41%)	43 (94%)
p53	27 (84%)	3 (7%)
p16	26 (81%)	3 (7%)
Bcat	0 (0%)	13 (28%)
p53+ p16+ Bcat -	23 (72%)	1 (2%)
p53+ p16- Bcat -	4 (13%)	2 (4%)
p53- p16+ Bcat -	3 (9%)	2 (4%)
p53- p16- Bcat +	0 (0%)	13 (28%)
p53- p16- Bcat-	2 (6%)	28 (61%)

Accuracy of immunohistochemistry for SC and EC

Reference standard	IHC	Sensitivity	Specificity	+Likelihood Ratio
SC	p53+	84%	93%	12
SC	p53+p16+	75%	98%	38
EC	p53-	93%	84%	6
EC	p53-p16-	89%	94%	15

Conclusions: Double positive or double negative p53 and p16 profiles substantially increase the positive likelihood ratios for SC and EC, respectively, compared to p53 alone. As an individual marker, Bcat was exclusively seen in EC, but in only a minority of cases. Hormone receptors showed substantial overlap between SC and EC, and did not contribute to the tumor distinction.

1094 Ovarian Stroma and Adenofibroma in Fallopian Tubes of Women Undergoing BRCA Prophylactic Salpingo-Oophorectomy

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Background: The fallopian tube (FT), in addition to breast and ovary, is at very high risk for neoplastic transformation as a result of hereditary BRCA mutations. Recently, an increasing number of women with personal and/or family histories of BRCA mutation are undergoing prophylactic salpingo-oophorectomy (PSO). To search for carcinoma and its precursor lesions, these FTs are serially sectioned and entirely submitted for histological examination. We report the frequency of ovarian stroma (OS), tubal adenofibroma (TAF), and paratubal adenofibroma (PAF) in FT specimens from two groups of women whose BRCA status was determined.

Design: Ninety-two women underwent PSO of whom 73 were BRCA mutation carriers (MU) and 19 were BRCA wild type (WT) by genetic testing. Each FT was serially sectioned and submitted in its entirey for histological examination. H&E stained slides from 143 FTs (MU) and 36 FTs (WT) were reviewed by two gynecologic pathologists.

Results: In the MU group, OS was found in 38 (27%) and TAF in 3 (2%) of the 143 FTs. The OS ranged in size from <1 to 3 mm, involved the fimbria in 30 (79%) and was present in more than one focus in 8 (21%) of the affected FTs. The TAF ranged from 2 to 3 mm; most were at the fimbriated end, and all were unifocal. PAF from <1 to 8 mm were found in sections from 5 (3%) of the MU FTs. In the WT group, OS was found in 9 (25%) and TAF in 5 (14%) of the 36 FTs. The OS ranged in size from <1 to 3 mm, involved the fimbria in all (100%) and was present in more than one focus in 3 (33%) of the affected FTs. The TAF ranged from 1 to 3 mm, all were at the fimbriated end, and all were unifocal. PAF from 2 to 3 mm were found in sections from 2 (6%) of the WT FTs. TAFs were more frequent in the WT FTs than in the MU FTs (p=0.01). There was no significant difference in the frequency of OS or PAF between the two groups of women.

Conclusions: Foci of ovarian stroma and adenofibromas occur frequently in fallopian tubes irrespective of BRCA mutation status. Most likely limited sampling accounts for the paucity of OS and TAF previously reported in fallopian tubes. Characteristically these lesions are small (<3 mm) and localized to the fimbriated end. The relationship between OS, TAF, and PAF and other tubal lesions, and the co-incidence of TAF or PAF with ovarian adenofibromas are currently under study.

1095 Juxtatumoral Stromal Reactions in Uterine Endometrioid Adenocarcinoma and Their Association with Prognostic Factors

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Background: Uterine endometrioid adenocarcinoma is the most common invasive tumor of the female genital tract in the U.S. Tumor invading into myometrium frequently induces juxtatumoral stromal changes resulting in desmoplastic reaction or host inflammatory response. The aim of the study is to examine the association of different types of stromal reaction with known prognostic factors.

Design: A total of 103 consecutive cases of invasive uterine endometrioid adenocarcinoma were collected from the files at the authors' institution between 1/1/2004 and 12/31/2005. These tumors represented clinical stages IB, IC, II, III and IV (n=64, 11, 9, 15 and 4, respectively). Endometrioid adenocarcinomas with focal serous or clear cell differentiation were excluded. Tumor FIGO grade and lymphovascular invasion were noted. Desmoplastic reaction (n=25) was characterized by a new matrix formation due to activation of the juxtatumoral stromal cells. Inflammatory response (n=77) was further categorized as lymphocytic (mild, moderate and severe), plasmacytic and eosinophilic. No stromal response (n=20) represented the absence of both a desmoplastic reaction and an inflammatory response. Statistical analysis was carried out with Chi-square analysis and Fisher exact test.

Results: The presence of a desmoplastic reaction was associated with a higher FIGO grade ($p<0.01$) and lymphovascular invasion ($p<0.05$), as well as advanced clinical stage (stage IB vs. IC, $p<0.01$; stage I vs. II/III/IV, $p<0.05$). The intensity of the inflammatory lymphocytic response (no stromal reaction/mild vs. moderate/severe) was reversely associated with advanced tumor stage (I, II, III/IV; $p<0.05$), but not associated with tumor grade or lymphovascular invasion. In addition, no significant difference was seen between no stromal reaction and mild lymphocytic response. There was no correlation between plasmacytic or eosinphilic responses with the factors mentioned above.

Conclusions: A strong lymphocytic inflammatory stromal reponse was predominantly found in the uterine endometrioid adenocarcinomas with early clinical stages. In contrast, juxtatumoral desmoplastic reaction was mostly identified in moderately to poorly differentiated tumors with lymphovascular invasion and in advanced clinical stages. The presence of desmoplastic reaction in the stroma should prompt pathologist to a search for histologically unfavorable prognostic indicators such as lymphovascular invasion, cervical involvement and nodal metastasis.

1096 Personalized Treatment of Ovarian Cancer: The Role of the Pathologist

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Background: Personalized treatment of ovarian cancer is a new approach based on individual biological differences in the molecular characteristics of tumors. Immunostains identify receptors and proteins that may be therapeutic targets. Pathologists play a key role in selecting blocks on which to perform these stains. However, there are no established guidelines to select appropriate sections for immunostains.

Design: We randomly selected 10 high grade serous carcinomas (Cas) including nine ovarian and one fallopian tube Ca, each with > 2 metastatic foci. Three blocks from each of the primary Ca and metastases (Mets) from each case were stained with epidermal growth factor receptor (EGFR), estrogen receptor (ER) and progesterone receptor (PR). Stains were evaluated for percentage of positive cells and intensity of staining, on a scale from 1+ to 4+.

Results: There were no differences in the intensity of staining; however, there were significant differences in the percentage of positive cells. EGFR staining of primary Cas showed an average of 3 fold (maximum 7 fold) difference from area to area. EGFR staining of Mets showed four cases with relatively equal percentages of positivity as the primary, four cases with higher percentages of positive cells and two cases with lower percentages of positive cells. ER staining showed an average of 2 fold (maximum 5 fold) variation within primary Cas. Of Mets stained with ER, nine were similar to the higher staining areas in respective primary Cas and one had a lower percentage of cells staining positive than in the primary. Primary Cas stained with PR showed an average of 5 fold (maximum 10 fold) variation, with Mets showing a similar percentage of cells staining positive in nine cases and a higher percentage of cells staining positive in one case, when compared to the highest staining pattern observed in the primary.

Conclusions: 1) There are significant variations in immunohistochemical staining characteristics between different areas of Müllerian Cas 2) EGFR showed significant variations in staining patterns within the primary lesion as well as between primary and metastatic lesions. 3) ER and PR had similar staining in the primary and Mets when comparing areas with the highest staining patterns, but still had significant variations in different areas of the primary Ca. 4) We recommend selecting more than one section from the primary and metastatic site whenever possible to assess applicability of targeted therapy. Differences in staining patterns should also be taken into consideration when evaluating the results of treatments.

1097 Biomarkers Predicting Endometrial Cancer Stage: Identifying Patients Benefitting Most from Surgical Staging

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Background: On-going controversy exists regarding the surgical management of endometrial cancer patients. Identification of those who would benefit most from complete surgical staging is unresolved. Many patients with endometrial cancer are poor candidates for extended surgeries due to obesity, hypertension, and diabetes. Intra-operative frozen section is not universally available and is not always reliable, as up-grading and up-staging are relatively common upon pathological analysis of the final hysterectomy specimen. The goal of our study is to determine if a panel of molecular markers can potentially assist in the decision to perform complete surgical staging on women diagnosed with endometrial cancer. Given the relationship between estrogen exposure and endometrial cancer, especially low grade endometrial cancer, we hypothesized that such a biomarker panel will provide a clinically useful biomarker score to assist in the decision to perform complete surgical staging on women diagnosed with endometrial cancer.

Design: Microarray was performed in baseline and post-treatment endometrial biopsies from women taking estrogen-based HRT to identify genes regulated by estrogen. The expression six genes most strongly induced by estrogen (RALDH2, sFRP1, sFRP4, EIG121, IGF-I, and IGF-IR) were then quantified by qRT-PCR in 56 endometrioid-type endometrial carcinomas. Expression data was compared to clinico-pathologic characteristics, and an unsupervised cluster analysis was performed. Time to recurrence by cluster was analyzed using the Kaplan-Meier method. A receiver operating characteristic (ROC) curve was generated to determine the clinical utility of the panel to predict endometrial cancer stage.

Results: Unsupervised cluster analysis revealed two distinct groups based on estrogen-regulated gene expression. The low gene expression cluster had a recurrence rate 4.35 times higher than that of the high expression cluster. ROC analysis allowed for the prediction of endometrial cancer stage Ic or higher with a false negative rate of only 4.5% based on level of gene expression.
Conclusions: This biomarker panel was highly accurate in stratifying endometrial cancer patients into low risk (stage Ia or Ib) vs. high risk (stage Ic or higher) groups. The panel has a lower false negative rate than that reported in the literature for frozen section. The panel may therefore help to better identify the patients who would most benefit from extensive endometrial cancer staging.

1098 The Incidence of Fallopian Tube Primaries Reaches 15% of All Adnexal Tumors When Standardized Grossing Protocols Are Followed

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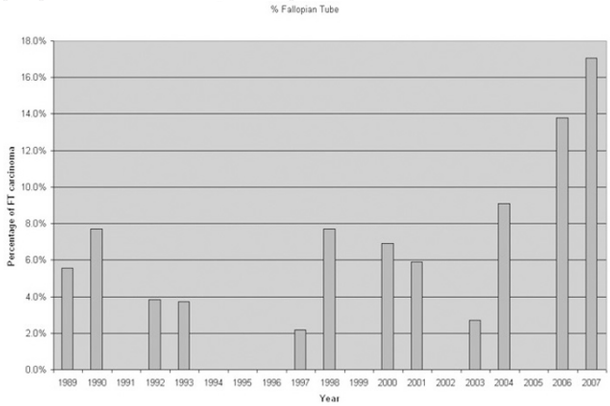
Background: Fallopian tube (FT) primaries have been regarded as uncommon, comprising ~3% of adnexal tumors in most large series. Risk reducing salpingio-oophorectomies are an increasing choice for pts with BRCA gene mutations. Large studies of these specimens have led to a better understanding of adnexal carcinogenesis and the recognition of occult primary FT tumors, both in BRCA(+) pts and sporadic adnexal neoplasia. The true incidence of FT primaries was previously underestimated, as complete submission of the FT has not been standard procedure in adnexal carcinoma specimens. In 2006 our institution implemented a procedure, based on a modification of the Brigham & Women's SEE-FIM protocol, for complete submission of ovaries and FTs. This procedure is applied to any prophylactic salpingio-oophorectomy and all staging oophorectomies for ovarian carcinoma. The objective of our study was to assess if this standardized grossing procedure has had an impact on the number of FT primaries identified.
Design: Index cases(cases with the primary diagnostic material at the parent institution) of FT and ovarian carcinomas of all histologic types were identified from the institutional registry of neoplastic disease during the period 1989-2007. All cases had been diagnosed or secondarily reviewed by a gynecologic pathologist, in nearly all cases the same pathologist, over the 18yr study period. The ratio of FT to all adnexal tumors was expressed as a percentage. For statistical analysis, data was aggregated into pre-protocol(1989-2005) and post-protocol(2006-7) periods 2x2 matrix and analyzed using X^2 table.
Results: 17 FT carcinomas were diagnosed from 1989-2005, and 11 cases from 2006-7. The pre-protocol incidence of FT carcinoma averaged approximately 3%, while the post-protocol incidence averaged 15%.($p<.001$).



Conclusions: Standardized and complete sampling of the FT, particularly the fimbria, has led to an increase in the diagnosis of FT carcinoma. The present study illustrates that the actual incidence of fallopian tube carcinoma is closer to 15% of all adnexal carcinomas, as opposed to the historical rate of 2-3%. When both structures are involved, criteria for assigning the primary organ have not been well delineated.

1099 Mucinous Change of the Fallopian Tube in Patients with Appendiceal or Ovarian Mucinous Tumors

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Background: The presence of mucinous epithelial cells in the fallopian tube of patients with appendiceal or ovarian mucinous tumors has been considered by most authors as metastasis and suggested by others as representing benign mucinous metaplasia. To study this unusual feature, we reviewed the fallopian tubes in patients diagnosed with mucinous tumors of the appendix or ovary.
Design: Fallopian tubes from patients who underwent an appendectomy and salpingo-oophorectomy for a mucinous neoplasm from 1994-2008 were retrospectively reviewed. This included 11 patients diagnosed with mucinous appendiceal tumors, 4 with non-mucinous appendiceal tumors and 43 with mucinous ovarian tumors. The presence of mucinous change in the fallopian tube was documented. Literature review was performed for additional reported cases. Four fallopian tubes, 2 associated with appendiceal and 2 with ovarian tumors, were stained with cytokeratin (CK) 7 and 20.
Results: Mucinous change was present in 2 of 11 patients diagnosed with appendiceal mucinous tumors (low malignant potential and well-differentiated mucinous carcinoma) and 2 of 43 with ovarian tumors (ovarian mucinous cystadenofibroma and endocervical mucinous borderline tumor). Mucinous change was not seen in fallopian tubes associated with non-mucinous appendiceal tumors. Literature review highlighted a patient with an appendiceal mucinous cystadenoma with mucinous change involving the fallopian tube, endometrium, and endocervix. All cases showed transition from benign tubal epithelium to the mucinous cells. The fallopian tube mucinous cells showed similar immunohistochemical profile to the associated tumor in one appendiceal tumor (CK7+, CK20+) and two ovarian tumor (CK7+, CK20-). However, in 1 appendiceal tumor the mucinous cells in the fallopian tube were CK7+ and CK20+ while the appendiceal tumor was CK7- and CK20+.
Conclusions: The mucinous change in the fallopian tubes is most likely metaplastic in nature as suggested by: 1) presence of mucinous changes in appendiceal tumors of only the mucinous type 2) mucinous changes seen in association with ovarian mucinous tumors 3) different immunophenotype between the fallopian tube and the appendiceal tumor seen in one case. Mucinous metaplasia of mullerian epithelium could be important in the pathogenesis of pseudomyxoma peritonei in patients with appendiceal or ovarian mucinous tumors.

1100 Activated Status of mTOR-HIF-1α-VEGF Pathway in Ovarian Clear Cell Adenocarcinoma

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Background: Malignant tumors are usually involved in a relatively hypoxic state, which induces overexpression of hypoxia-inducible factor-1 α (HIF-1α) to satisfactorily enable the tumor to survive. Recently, the mechanism of HIF-1α activation through the phosphoinositide-3 kinase (PI3K) signaling pathway was explored in detail. Inhibition of the mammalian target of rapamycin (mTOR) pathway including HIF-1α is expected to play a major role in suppression of tumor cell growth, having recently drawn much attention as an anti-cancer therapeutic strategy for malignant tumors. Clinical trials with treatment of mTOR inhibitors, represented by the analogues of rapamycin, have been performed in some malignant tumors as well as in vitro studies.
Design: Focusing on clear cell adenocarcinoma (CLA) of the ovary in comparison with serous adenocarcinoma (SEA), immunohistochemical expressions of mTOR, phosphorylated-mTOR (p-mTOR), HIF-1α, and vascular endothelial growth factor (VEGF) were examined in the surgically resected specimens of 29 SEAs and 49 CLAs. Using the cell lines of CLA (RMG-1 and W3uF), an experimental study was designed to clarify whether tumor suppression due to down-regulation of mTOR activity could represent a promising therapeutic strategy for CLA. After treatment of an analogue of rapamycin (everolimus), expressions of mTOR, p-mTOR, HIF-1α and VEGF were examined by western blotting. Then, a transplant xenograft model of RMG-1 was used to confirm the results of in vitro analysis.
Results: There were no significant differences in expressions of mTOR, HIF-1α and VEGF between SEA and CLA, but p-mTOR expression was more prominent in CLA than SEA. Although mTOR expression remained unchangeable in everolimus-treated cell lines, expressions of p-mTOR, HIF-1α and VEGF were shown to be sharply depressed. The same alterations were demonstrated in the xenograft model treated with everolimus. Many tumor cells were involved in necrosis with an inflammatory cell reaction. Irrespective of tumor degradation, no apparent decline of mTOR expression was observed, but p-mTOR reaction was shown to be nearly negative. HIF-1α and VEGF expressions fairly attenuated.
Conclusions: In comparison with SEA, CLA is characterized by activated mTOR status represented by marked expression of p-mTOR. This evidence is considered to support the mTOR-targeted therapeutic strategy for CLA.

1101 Correlation of Immunohistochemical Staining Patterns of p53 with Mutational Analysis in Ovarian Carcinomas

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Background: Immunohistochemical staining for p53 is used as a surrogate for mutational analysis in the diagnostic workup of ovarian carcinomas. Strong and diffuse immunostaining for p53 is generally interpreted as likely indicating a *TP53* gene mutation. The goal of this study was to confirm this impression and also to determine whether the low-level or absent expression correlates with mutation or wild type *TP53*..
Design: Thirty cases of ovarian carcinoma (25 high grade serous ovarian/peritoneal carcinomas, 2 low-grade serous carcinomas, 2 clear cell carcinomas, and 1 well-differentiated endometrioid carcinoma) were studied. These cases were analyzed for *TP53* mutation by nucleotide sequencing (exons 4-9) and also subjected to immunohistochemical analysis of p53 expression.
Results:

Immunohistochemical staining patterns and mutational analysis of p53 in ovarian carcinomas.

	Mutational analysis					
Staining Pattern (% positive cells)	Wild type	Nucleotide deletion	Nucleotide insertion	Nucleotide substitution	Nucleotide substitution with formation of Stop codon	Changes in intron region
Negative	-	3	1	-	2	1
‹ 10% †	3	1	-	-	-	-
10 - 50%	3	-	-	-	-	-
51 - 74%	-	-	-	3	-	1
75 - 100%‡	-	1	-	10	1	-
Total	6	5	1	13	3	2

†All cases demonstrated weak to moderate staining intensity; ‡ All cases demonstrated strong to moderate staining intensity

Conclusions: Complete absence of p53 expression is associated with a *TP53* mutation and is not characteristic of wild type *TP53*. Tumors in which less than 50% of cells are positive almost always contain wild type *TP53*. Tumors in which more than 50% of cells are positive have a *TP53* mutation.

1102 Histopathological Evaluation of Post Radio-Chemotherapy Response on Hysterectomy Specimens for Cervical Carcinoma

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Background: The examination of post radio-chemotherapy specimens represents a challenging issue. Cytotoxic therapies produce morphological changes that make the interpretation of these specimens extremely arduous. The evaluation of the residual disease is critical for the prognosis and planning of further treatments.

Design: The study group included 124 cervical cancers (mean age 53,8±12,3 years) treated with radio-chemotherapy followed by radical surgery. 95 patients had a pre-treatment biopsy: Squamous Carcinoma: 79 (89.1%); Adenocarcinoma: 10 (10.5%); Adenosquamous Carcinoma: 7 (5.3%); Other: 2 (2,1%). The cervix was sectioned clockwise, entirely embedded in 12-24 paraffin blocks and enterely examined. The local response to therapy was evaluated as follows: - pR0: Pathological Complete Response: absence of residual neoplastic cells. - pR1: Pathological Partial Response: presence of clusters of neoplastic cells <0.3 cm - pR2: Pathological No Response: presence of neoplastic mass ≥0.3 cm. All the patients were restaged according to AJCC staging manual and FIGO. FIGO stage 0-II were considered as Local Disease; FIGO stage III-IV as Disseminated Disease.

Results: Residual neoplastic cells showed: cytoplasmic eosinophilia, vacuolation and foamy degeneration. Mitotic activity was scanty. The stroma was fibrous containing inflammatory cells, fibrinous debris, cholesterol clefts and hemosiderin. We found 45 pR0 (36.3%); 41 pR1 (34.7%); 36 pR2 (29%); 108 patients had a Local Disease; 16 had a Disseminated Disease. Presence of neoplastic embolism represent a significant risk factor for Disseminated Disease (RR=7,1; risk increase: +671,4%; p<0,001). Tumor regression was seen in all cases with different extent; giant cells or microcalcalcifications did not yield significant in the regression groups (p>0,05). The pR2 residuals infiltrated significantly more than pR1 residuals (0.84 Vs 0.55 p<0,001) and more frequently involved the parametria (5 cases Vs 1 case). pR2 mean linear dimension was 1.6±0,9 cm. We did not find any difference between pR1 and pR2 in incidence of Disseminated Disease (3 cases Vs 8 cases p>0,05).

Conclusions: Neoadjuvant radio-chemotherapy strongly affects the neoplastic tissue with variable signs of regression. Neoplastic embolism is the most important histological feature to be reported. The depth of infiltration, the presence of giant cells or calcifications did not correlate with distant metastases. The residual cancer is often limited to few cells so embedding the whole cervix is suggested.

1103 Clinical Outcomes of Pregnant Women with Fetomaternal Hemorrhage Detected by Flow Cytometry

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Background: The purpose of this study was to evaluate the clinical outcomes of pregnant women with fetomaternal hemorrhage (FMH) detected by flow cytometry over the last four years at Jackson Memorial Hospital (JMH).

Design: We evaluated results from JMH using the flow cytometric method (FCM) to detect fetal red blood cells (RBCs) based on the anti-hemoglobin F (HbF) monoclonal antibody method, a positive test that is correspondent with FMH.

Results: During the last four years, 26 pregnant women had measurable fetal RBCs (>0.15%) in maternal blood among 267 blood samples sent for the test. Seventeen (17) of the 26 positive patients had intrauterine fetal demise (IUFD); 2 patients had motor vehicle accidents during the pregnancy when the tests were sent; 6 pregnant women showed clinical symptoms and signs of threatened preterm labor. Autopsies were performed on 11 of 17 IUFD fetuses. In 5 of the 11 autopsies, the cause of death was related to FMH. One fetus had multiple congenital anomalies, while in the remaining 5 cases, the cause of death was initially undetermined. Retrospectively, we reevaluated FMH in all 11 autopsy cases by calculating the fetal blood loss during the pregnancy and we found that FMH may be counted as the cause of all 5 undetermined autopsy cases; thus, FMH was overall associated with 10 of the 11 autopsies.

Conclusions: Cytofluorographic analysis of fetal RBC is a precise and quick method to detect FMH during pregnancy. There is high correlation between the presence of FMH and threatened preterm labor and IUFD. Our results suggest that obstetricians and pathologists can utilize flow cytometric measurement of fetal RBC as a powerful tool when considering the possibility of FMH as a cause of death of IUFD.

1104 A Panel of Immunohistochemical Markers To Distinguish Ovarian from Uterine Serous Papillary Carcinomas

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Background: Serous papillary carcinomas (SPC) have similar morphology whether they originated from ovary or uterus. When carcinomas are detected early, it is relatively easy to recognize the primary site. However, identification of the site of origin can be difficult once these tumors extend out of primary site or metastasize. Recognition of the site of origin influences the staging, management and prognosis of these malignancies. The purpose of this study is to identify a panel of markers to distinguish between ovarian and uterine primaries.

Design: Surgical specimens from 47 cases of SPC (33 uterine, 14 ovarian) were retrieved from the files. Hematoxylin&Eosin-stained slides were reviewed to confirm the diagnosis. Regardless of the histologic grade of the tumors, only sections with papillary growth pattern were selected for this study. Tissue sections were immunostained using antibodies for ER (1D5, Dako,1:10), p53 (Calbiochem, 1:250), WT1(Santa Cruz,1:200), IMP3(Dako,1:25), and P16 (Vision Biosystem,1:20). Cases were scored based on the percentage of positive cells: 0 (negative staining), 1 (focal, less than 50% of positive cells), and 2 (diffuse, more than 50% of positive cells). ER and p53 showed only nuclear staining. IMP3, WT1 and P16 showed strong cytoplasmic staining.

Results: Ovarian SPC expressed ER (92%), p53(92%), WT1 (100%), IMP3 (92%), and P16 (92%). Uterine SPC expressed ER (30%), p53(64%), WT1 (64%), IMP3 (85%), and P16 (76%). Ninety two percent of ovarian SPC had ER+WT1+ immunophenotype, whereas only 18% of the uterine SPC had the same immunophenotype. Seventy one percent of ovarian SPC had ER+ p53+ WT1+ IMP3+ P16+, whereas only 6% of the uterine SPC expressed this immunophenotype.

Table 1. Immunohistochemical Panel Used to Differentiate Ovarian vs. Uterine SPC

	ER+*	p53+	WT1+	IMP3+	P16+	ER+WT1+*	ALL markers +*
Ovarian SPC	92%	92%	100%	92%	92%	92%	71%
Uterine SPC	30%	64%	64%	85%	76%	18%	6%

* ($p < 0.001$)

Conclusions: A panel of ER+, WT1+, p53+, IMP3+ and P16 +immunohistochemical markers favors an ovarian origin and potentially may distinguish these tumors from metastatic uterine SPC (p<0.001). ER was expressed in most ovarian SPC whereas majority of uterine primaries were negative.

1105 Clinicopathologic Analysis of Ovarian Clear Cell Carcinoma: Comparison of Cases with and without Adenofibromatous Components and Implications for Pathogenesis

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Background: Ovarian clear cell carcinomas (OCCC) with an adenofibromatous (AF) background (CCC w/ AF) have been shown to have some clinicopathologic differences compared with CCC without AF components (CCC w/o AF). The purpose of this study was to assess the clinicopathologic similarities and differences in a large number of these 2 types of CCCs and determine whether there is evidence for 2 different lines of pathogenesis.

Design: OCCC from the AFIP were re-reviewed. 427 OCCC were included and subdivided into CCC w/ AF (n=141) and CCC w/o AF (n=286). CCC w/ AF included cases with a cytologically benign and/or malignant AF components. Cases were assessed for various clincopathologic features.

Results: CCC w/ AF more frequently had pure tubulocystic architectural patterns (27% vs. 7%, p<.001). CCC w/o AF more frequently had a predominantly cystic gross appearance (42% vs. 21%, p<.001), advanced stage disease (43% vs. 21% stage >I, p <0.001), destructive patterns of stromal invasion (94% vs. 39%, p<.001), pure solid architectural patterns (15% vs. 1%, p<.001), higher grade (43% vs. 14% grade 3, p<.001), and non-CCC carcinoma component (10% vs. 4%, p=.04). The frequency of endometriosis (E-osis) in the ipsilateral ovarian tumor in CCC w/ AF (11%) was lower than in CCC w/o AF (20%, p=.03), but the difference between both tumors for the frequency of E-osis in the contralateral normal ovary or in extra-ovarian sites (15% vs. 20%, respectively) was not significant. Both tumor groups showed no differences in patient age, tumor size, bilaterality, presence of tumor on the ovarian surface, and other histologic architectural patterns.

Conclusions: OCCC w/ AF and OCCC w/o AF have certain differences in their clinicopathologic profiles. CCC w/o AF exhibit features that generally correlate with more aggressive behavior, such as higher stage. The fact that E-osis was associated with a subset of CCC w/ AF and that the frequency of E-osis in CCC w/o AF was similar suggest that AF- and E-osis-associated pathways of pathogenesis of CCC are not independent of one another. Given the higher grade, higher stage, and more frequent destructive patterns of stromal invasion in CCC w/o AF, some CCC w/o AF may represent tumor progression of CCC w/ AF in which the AF component has been overgrown. This should be taken into consideration for future pathogenesis studies of CCC.

1106 The Oncofetal Protein IMP3 Is Differentially Expressed in Clear Cell Carcinoma of Ovary

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Background: Ovarian cancer is a lethal gynecological malignancy. Insulin-like growth factor II mRNA-binding protein 3 (IMP3) is an oncofetal protein that promotes tumor proliferation via an insulin-like growth factor II-dependent pathway and plays an important role in tumor invasion and metastasis. The expression of IMP3 has been reported in a variety of malignant tumors including those of the pancreas, lung, stomach, colon and some soft tissue sarcomas. Recently it has also been described as a marker for endometrial serous adenocarcinoma and cervical adenocarcinoma insitu. IMP3 was also found to be a prognostic biomarker for renal cell and urothelial carcinomas. This study aims at investigating the expression profile of IMP3 in ovarian carcinomas.

Design: The immunohistochemical expression of IMP3 (dilution 1:30; Cat. # M3626, Dako, Carpinteria, CA) was evaluated in 75 paraffin-embedded ovarian neoplasms including 7 benign cystadenomas, 15 borderline tumors, 17 serous carcinomas, 7 mucinous carcinomas, 15 endometrioid carcinomas and 14 clear cell carcinomas.

Results: Moderate to strong cytoplasmic IMP3 expression was found in 37 (70%) of 53 carcinoma cases. Carcinomas and borderline tumors displayed a significantly higher level of IMP3 expression than the benign cystadenomas (P<0.005) which showed negative staining. Interestingly, 78% (11/14) of clear cell carcinomas were immunoreactive to IMP3, which was significantly higher than in serous, mucinous and endometrioid carcinomas (P<0.05). No significant correlation between expression of IMP3 with tumor grade, clinical stage and patients' overall survival was found (P>0.05).

Conclusions: These results indicate that IMP3 may contribute to ovarian cancer progression. Moreover, IMP3 may be a useful diagnostic marker for clear cell differentiation when histologic typing is difficult in poorly differentiated tumors.

1107 Human Ovarian Surface Epithelial Cells Can Give Rise to Papillary Serous Carcinoma under the Influence of Specific Oncogenes and the Microenvironment

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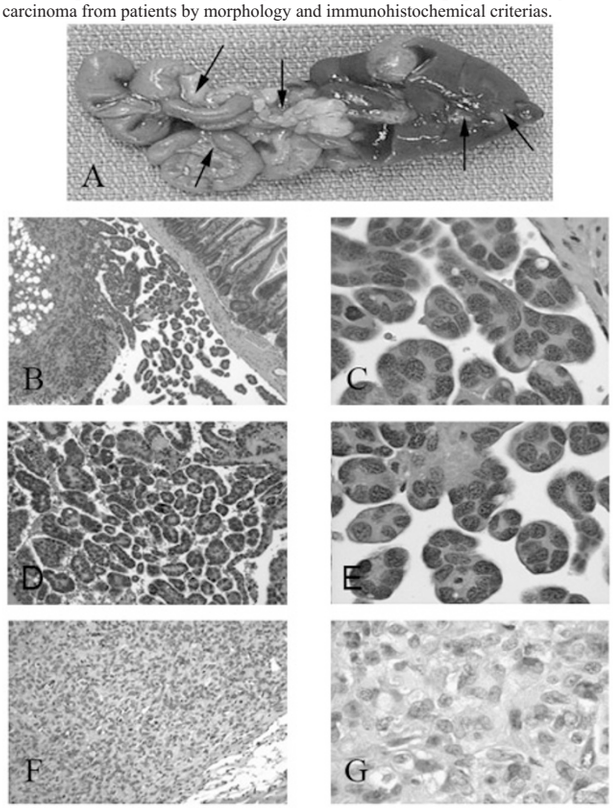
Background: Several possible cell origins have been proposed for human ovarian cancer including ovarian surface epithelial cells, fallopian tube epithelia, and rete ovarii. However, data supporting such conclusions are largely from morphologic and genetic correlative evidence, a causal relationship between the cell origin and ovarian cancer has not been established.

Design: In this study, we determined whether human ovarian surface epithelial cells could be the origin of ovarian cancer by introducing genetic elements into normal human ovarian epithelial cells and test whether such genetic modifications can induce the development of ovarian cancer.

Results: Enforced expression of HER-2/neu into two nontumorigenic human ovarian surface epithelial cells previously immortalized with SV40 T/t antigen and the catalytic subunit of telomerase resulted in anchorage independent growth of these cells on soft agar. HER-2/neu transfected cells injected sub-cutaneously developed carcinoma with morphology resembling undifferentiated carcinomas (Fig 1F-G). However, when these tumor cells was injected into the peritoneal cavity of nude mice, one of two lines developed papillary carcinoma similar to high grade papillary serous carcinoma (Fig. 1A-E). The tumor cells are strongly positive for cytokeratin, WT-1, p53, and CA125 staining. Thus, this genetically created papillary carcinoma resembled high grade papillary serous carcinoma from patients by morphology and immunohistochemical criterias.



Conclusions: These results provide first casual genetic evidence that ovarian surface epithelial cells can serve as the origin of high grade serous carcinoma and that the development of papillary serous carcinoma is also dependent on the tumor microenvironment. This newly created ovarian cancer model should greatly facilitate the study of pathogenesis of human ovarian cancer.

1108 HPV Genotypes and Cervical Carcinoma: Analysis of 163 Cases in a US Population

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Background: This study examines the distribution of human papillomavirus (HPV) genotypes and the relative association with different histologic cell types in 163 invasive cervical cancers in a US population.

Design: Cervical cytologic samples were genotyped using the LINEAR ARRAY® HPV Genotyping Test (Roche Diagnostics, Branchburg, NJ) that target the *L1* gene using PGMY09/PGMY11 consensus primers. High risk (HR) HPV genotypes included HPV16, 18, 31 and 45. Other carcinogenic HPV genotypes were classed as intermediate risk (IR). HR-negative cases using this system were secondarily tested using PCR with HPV type-specific primers for *E6* and *LCR* corresponding to HPV16, 18, 31, 33, 35, 39, 45, 52, 58 and 68.

Results: 153 histologically confirmed cancer cases were positive for one or more HR or IR HPV types using the PGMY09/PGMY11 primers. Of the 10 HPV-negative cases, 5 were subsequently shown to have HPV DNA using type-specific primers, including HPV16 (n=3), HPV18 (n=1) and HPV45 (n=1). After all analyses, 3.1 % remained HPV-negative.

HPV Genotypes in 163 Cervical Cancers

CELL TYPE	SQUAMOUS N (%)	ADENO CA N (%)	ADENO SQ N (%)	SMALL CELL N (%)	TOTAL N (%)
HPV16	84 (67.7)	7 (33.1)	3 (30.8)	0	94 (57.7)
HPV18	14 (11.2)	9 (42.9)	7 (61.5)	4 (100)	34 (20.9)
HPV16+18	5 (4.0)	1 (4.8)	1 (7.7)	0	7 (4.3)
HR, NOT 16,18	6 (4.8)	2 (9.5)	1 (7.7)	0	7 (4.3)
IR	13 (10.4)	0	1 (7.7)	0	14 (8.6)
NO HPV	3 (2.4)	2 (9.5)	0	0	5 (3.1)
TOTAL	125	21	13	4	163

Columns total 100%

The most frequent HPV genotypes associated with cancers other than HPV16 or 18 were HPV45 (4.3%) and HPV33 (2.4%). The association between HPV genotype and histologic cell type was highly significant ($p < .001$). HPV16 was closely associated with keratinizing squamous cancers while non-keratinizing cancers were more heterogeneous. IR genotypes were the highest risk genotypes in 8.6% of cancers.

Conclusions: This study documents the pattern of HPV genotypes in cervical cancers in a US population in the pre-vaccination era. HPV genotyping using PCR for *L1* can fail to identify HPV DNA in a small percentage of cancers, likely due to loss/modification of *L1* during integration. A small percentage of cancers remained HPV-negative after extensive HPV testing. Sampling variation may explain many of these cases.

1109 Prepartum Cervical Cytologic Changes Correlated with Abnormal Placental Changes and Preterm Delivery

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Background: The understanding of clinical implications of abnormal cytology screening results in the pregnant population is incomplete. The objective of this study was to determine clinical implications of abnormal cervical cytologic screening on the outcome of pregnancy.

Design: A 12-year review of cases from our institution yielded 2480 cases with data for comparison of the impact of abnormal cervical cytology on placental changes and pregnancy outcome in terms of prematurity.

Results: Analysis showed a statistically significant correlation between reactive, inflammatory, infectious, atypical, and dysplastic prepartum cytologic changes with abnormal placental diagnoses including but not limited to accelerated/delayed placental maturation, various placental infections and vasculopathies (Table 1). All but dysplastic cytologic changes showed a statistically significant association with preterm labor (Table 1). Statistically significant associations were also present between a positive HPV DNA assay and preterm labor and abnormal placental diagnoses.

Table 1. Associations between Pap findings, placental findings, and pregnancy outcome

Pap Dx	N=2480	Abn P	*p*	OR(95% CI)	Preterm	*p*	OR(95% CI)
NORMAL	715	175	-	-	116	-	-
AGUS	29	16	<0.001	3.80(1.79-8.05)	11	<0.01	3.16(1.45-6.86)
ASCUS	290	133	<0.001	2.61(1.96-3.48)	66	<0.01	1.52(1.08-2.14)
HSIL	103	44	<0.001	2.30(1.50-3.52)	24	0.051	1.57(0.95-2.58)
LSIL	161	69	<0.001	2.31(1.62-3.30)	32	0.208	1.28(0.83-1.98)
REACTIVE	1182	482	<0.001	2.12(1.73-2.61)	278	<0.001	1.59(1.25-2.02)
Trichomonas	187	96	<0.001	3.26(2.33-4.54)	63	<0.001	2.62(1.83-3.77)
Candida	476	193	<0.001	2.10(1.64-2.70)	100	<0.01	1.37(1.02-1.85)

Notes: Pap Dx, Pap diagnosis during pregnancy. Abn P, cases of placenta with pathological findings. Differences between each Pap Dx to NORMAL analyzed using Chi square test, two-tailed *p* values are shown. Statistical significance defined as $p<0.05$. OR, odd ratio. 95% CI, 95% confidence interval.

Conclusions: These findings indicate that prepartum dysplastic cervical changes do not affect the duration of gestation or correlate with abnormal placental changes, but that the presence of infectious agents and inflammatory atypia on prepartum cervical screening may serve as a risk marker for preterm labor and abnormal placental changes.

Head & Neck

1110 Utilization and Value of Frozen Section in the Diagnosis of Thyroid Cancer

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Background: In the era of FNA evaluation of thyroid nodules, the use of frozen section (FS) in the management of patients who require surgery is controversial. FS, including its complementary use of imprints, may be unnecessarily redundant. Since treatment for thyroid cancer is a total or near total thyroidectomy an intraoperative diagnosis of cancer will avoid a second procedure. FNA has been a valuable form of preoperative evaluation primarily because the diagnosis of papillary carcinoma relies on features of nuclear morphology. FNA is less valuable in separating true follicular neoplasms from colloid nodules and of no use in the diagnosis of follicular carcinoma. The value of FS in this context is debatable since the diagnosis of follicular carcinoma requires clear-cut vascular invasion, a rare event which is sampling dependent. This study examines the utilization and value of FS in establishing an intraoperative diagnosis of thyroid cancer.

Design: We reviewed 271 thyroid FNA reports and subsequent thyroidectomies from December, 2004 to July, 2007 comparing preoperative FNA, intraoperative FS and final pathologic diagnosis. Only patients with lobectomies were included.

Results: Of the total, 97 patients underwent surgery with FNA diagnoses suggestive of follicular neoplasm or positive of papillary carcinoma. Of these, 76 also had FS examinations with the following results: papillary carcinoma (14), follicular tumor (38), and colloid nodules (12). There were no FS diagnoses of follicular carcinoma. On permanent examination, there was a total of 24 confirmed cases of papillary carcinoma: 4 with an indeterminate FNA diagnosis but confirmed as papillary carcinoma by FS; 2 deferred FS, 6 not examined intraoperatively and 2 false negatives. Three eventual