Table 2. IHC for p63 and p40 in MPUC, LGUC or HGUC component of all cases studied and in tumors of different stages.

Marker	Positivity	MPUC (n=20)	HGUC (n=69)	LGUC (n=42)	<T2 (n=36)	≥T2 (n=62)	P values
p63	Positive Tumor Cells (%)	0.0 ± 0.0	37.1 ± 30.9	86.1 ± 6.0	64.2 ± 32.6	22.3 ± 29.6	P1 < 0.001, P2 < 0.001, P3 < 0.001, P4 < 0.001
p40	Positive Tumor Cells (%)	0.5 ± 1.5	51.4 ± 28.2	89.8 ± 5.8	68.3 ± 32.6	38.5 ± 33.6	P1 < 0.001, P2 < 0.001, P3 < 0.001, P4 < 0.001

Student T test. P1: MPUC vs. HGUC; P2: MPUC vs. LGUC; P3: HGUC vs. LGUC; P4: <T2 vs.≥T2

Conclusions: p63 expression is seen diffusely in all LGUCs, significantly decreased in HGUC and lost in MPUC. p40 expression is also decreased in HGUC and markedly decreased in MPUC. Based on these results, loss of p63 expression in a UC appears to be associated with adverse features—namely high grade, high stage, or MPUC. p40 is more frequently expressed in urothelial carcinoma irrespective of tumor grade, stage, or MPUC when compared to p63 and thus, may be a superior diagnostic marker than 4A4(p63). Further study including more cases and other UC variants are needed to substantiate these findings.

1611 Added Value of ERG to PIN Cocktail for Evaluation of Atypical Small Acinar Proliferations (ASAP) of Prostate

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Background: High correlation with prostate cancer (PCa) potentiates ERG as an additional marker to the PIN cocktail in determining ASAP versus PCa diagnosis. ERG is positive in 11-16% of atypical biopsies, but previous studies do not directly address the added diagnostic value of ERG to the PIN cocktail. This study determines the impact of ERG on the final diagnosis beyond assessment based on H&E and PIN cocktail (HE+PIN).

Design: Biopsies from 7/2010-5/2012 diagnosed as ASAP after HE+PIN (PINATYP excluded) were stained retrospectively with ERG(EPR3864). All cores with ASAP by H&E received from 6/2012-9/2012 were evaluated with PIN cocktail and ERG results. Subsequently, all ERG-positive cases from both arms were independently reviewed by 3 urologic pathologists to render diagnostic impression based on H&E only, HE+PIN, and HE+PIN+ERG evaluation. A negative ERG result is non-contributory.

Results: In the 107 retrospective cases, ERG was negative in 97. Of the 10(9%) ERG-positive cases, 3 cases by majority and 1 unanimously were upgraded from ASAP to PCa based on HE+PIN evaluation given absent basal cells and variably upregulated racemase. After HE+PIN+ERG, 1 case was upgraded to PCa based on ERG staining while 5 remained ASAP: 4 due to presence of rare basal cells despite increased racemase, 1 considered insufficient malignant histology by majority. In the prospective arm, ERG was positive in 3/24(13%). After HE+PIN, converted diagnoses were 13(54%) PCa and 5(21%) benign. One PCa was ERG-positive; benign cases were all ERG-negative. Of the remaining 7(29%) ASAP cases, 2 were positive for ERG: 1 was converted to PCa, the other remained ASAP due to weak inconsistent staining in suspicious glands and weak positivity in morphologically benign glands. In total, ERG was positive in 10%(13/131) of ASAPs; ERG impacted the diagnosis in 1.5%(2/131) of ASAPs.

Conclusions: The difficulty in achieving unanimous consensus diagnosis highlights the subjective aspect of HE+PIN interpretation and ASAP designation. ERG positivity adds to the malignant quality of atypical glands, but whether this contribution to histology and PIN exceeds the threshold for diagnosis of PCa varies. Regardless, overall ERG positivity in ASAP is low, and even within ERG-positive cases, the same conclusion was often achieved by just HE+PIN. ASAPs with at least rare basal cells were never designated as PCa, and it is unlikely ERG would change this. The limited added utility should be considered with costs and resources for its upfront use in routine evaluation of ASAP within standard practice.

Gynecologic & Obstetrics

1095 Mitotically Active Microglandular Hyperplasia of the Cervix: Absence of K-Ras Mutation and Implications for Differential Diagnosis

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Background: Microglandular hyperplasia (MGH) of the cervix is a benign lesion characterized by proliferation of tightly packed endocervical glands with relatively uniform nuclei and rare to absent mitoses. Endometrioid endometrial adenocarcinomas (EAC) with a microglandular pattern can closely mimic MGH of the cervix resulting in a significant diagnostic dilemma, especially when present in the endocervical curettage. Significant nuclear atypia and increased mitotic activity (>1 mitosis/10 high power field (HPF)) are generally considered to be in favor of EAC.

Design: The aim of this study was to review the morphologic features of MGH with special focus on mitotic activity and nuclear atypia in correlation with K-ras mutation analysis. A total of 380 cases of MGH were identified in our departmental archives between January 2005 and June 2012. Of those, 68 were identified in endocervical polyps, which were subjected to an initial screening to further select the final study population of 45 cases with extensive MGH arising in endocervical polyps. All available slides were reviewed by two gynecologic pathologists and the mitotic activity and degree of nuclear atypia were evaluated. Ten cases with increased mitotic activity (5 or more mitoses/ 10 HPF) were subjected to K-ras mutation analysis by single-strand conformation polymorphism. Clinical history and follow-up data were retrieved from the patients' electronic medical records. For a control group, we identified 10 cases of EAC with microglandular pattern, 7 of which were also subjected to K-ras mutation analysis.

Results: Fifteen of the 45 MGH cases (33.3%) had 5 or more mitoses/10 HPF. The highest mitotic count was 11/10 HPF, which was observed in 3 cases. Moderate cytologic atypia was seen in 13 MGH cases. Follow-up was available for 29 MGH patients (mean follow-up time 37 months), all of which were alive and well. K-ras mutation was absent in all 10 MGH cases tested. In the EAC group, 4 of the 7 cases tested positive for K-ras mutation.

Conclusions: MGH of the cervix may show significant mitotic activity (up to 11/10 HPF) in a relatively high proportion of cases without negatively affecting the clinical prognosis. Therefore, mitotic activity cannot be reliably used to differentiate between MGH and EAC in small biopsies. K-ras mutation analysis may be a helpful adjunct in difficult cases.

1096 Genetic Reclassification of Undifferentiated Endometrial Sarcoma: Clinical Relevance

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Background: Undifferentiated endometrial sarcoma (UES) is the current designation for tumors formerly considered as high-grade endometrial stromal sarcoma (ESS). Some UES exhibit uniform nuclear features (UES-U) whereas others exhibit nuclear pleomorphism (UES-P). We recently identified *YWHAE-FAM22* genetic rearrangement in tumors diagnosed as UES or high-grade ESS. *YWHAE-FAM22* tumors are clinically more aggressive than low-grade ESS with *JAZF1-SUZ12* genetic rearrangement, but it is yet unclear whether *YWHAE-FAM22* tumors differ in clinical course from UES lacking *YWHAE-FAM22*.

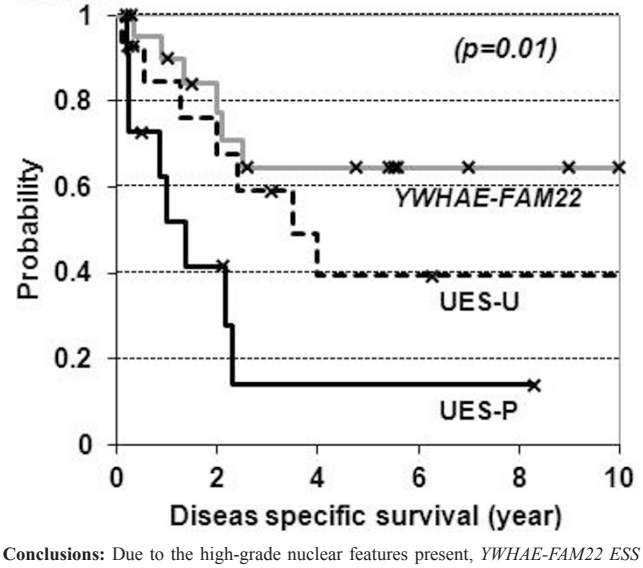
Design: We employed *YWHAE-FAM22* split-apart fluorescence *in situ* hybridization and RT-PCR assays to genetically classify 50 UES/high-grade ESS as *YWHAE-FAM22* ESS vs. *YWHAE-FAM22*-negative UES (UES-U and UES-P).

Results: Of the 50 tumors, 12 showed appreciable nuclear pleomorphism at 40x magnification (UES-P) and none of these had *YWHAE-FAM22* rearrangement. Among the 38 tumors with uniform nuclear features, 24 had *YWHAE-FAM22* rearrangement (*YWHAE-FAM22* ESS), including 12 with focal low-grade component that mimicked classic low-grade ESS. The remaining 14 cases with uniform nuclear features lacked apparent *YWHAE-FAM22* rearrangement (UES-U). The clinical features are shown in Table 1 with the result of Kaplan-Meier analysis shown in Figure 1.

Table 1

	YWHAE-FAM22	**UES-U**	**UES-P**
Age (average +/- SE)	46 +/- 3	55 +/-5	60 +/-3
FIGO (1988) stage			
1	6 (27%)	5 (42%)	5 (45%)
2-4	16 (73%)	7 (58%)	6 (55%)
Follow-up (with >1 year)			
Died of disease	6 (32%)	7 (58%)	8 (80%)
Alive with disease	8 (42%)	2 (17%)	0
Alive with no disease	5 (26%)	3 (25%)	2 (20%)
Adjuvant therapy			
Chemotherapy and/or radiation	12 (75%)	11 (84%)	10 (91%)
None	4 (25%)	2 (16%)	1 (9%)

Figure 1



Conclusions: Due to the high-grade nuclear features present, *YWHAE-FAM22 ESS* is frequently classified as UES. Our findings suggest that *YWHAE-FAM22* ESS is associated with a better prognosis than UES and it is therefore important to distinguish *YWHAE-FAM22* ESS from UES.

1097 Correlation and Prognostic Significance of Mismatch Repair (MMR) Protein and ARID1A Gene Expression in Endometrial Carcinomas (ECs)

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Background: DNA MMR system plays a crucial role in DNA repair during its synthesis. ARID1A gene encodes a protein, BAF250a, which is a component of SWI/SNF chromatin remodelling complex, involved in multiple cellular functions including DNA repair. Defects in MMR genes have been reported in 15-20% of sporadic ECs. Mutations and loss of expression of ARID1A gene have been observed in ~20-40% of ECs. In this study, we correlate MMR proteins loss with BAF250a negatibvity, and investigate their prognostic significance.

Design: A tissue microarray of 216 ECs were immunohistochemically stained for MMR markers (MLH1, MSH2, MSH6, and PMS2) and ARID1A gene product (BAF250a). The likelihood ratio (LR) and log rank test were used to correlate biomarkers expression and survival, respectively.

Results: Two hundred and eleven cases contained assessable tumour. Loss of any MMR proteins was observed in 69/211 (32.7%) ECs, with loss of MLH1 and PMS2 being the most common (table 1). BAF250a was negative in 60/211 (28.4%). ECs that retain MMR protein expression are more likely to be positive for BAF250a (100/194; 56.7%; LR, 33.6;p<0.0001). In univariate analysis, progression free survival (PFS) was worse in ECs with intact MMR proteins expression (Log rank, 5.7; p=0.0174), and in those positive for BAF250a (Log rank, 6.8; p= 0.0091). Moreover, ECs that show both BAF250a positivity and intact MMR expression have worse PFS than arid1- MMR lost EC (5Y PFS, 30% vs. 90%; log rank, 11.7; p=0.0084).

Conclusions: In univariate analysis, the retention of expression of MMR proteins and ARID1a gene product is associated with worse prognosis in endometrial carcinomas.

Loss of expression of MMR proteins and ARID1a gene product

Marker	number (%) of cases with loss
ARID1a	60/211 (28.4%)
Any MMR	69/211 (32.7%)
MLH1	51/211(24.2%)
MSH2	2/211 (4.7%)
MSH6	15/211 (7.6%)
PMS2	50/211 (23.7%)

1098 Comparative Prognostic Value of Histology, p16, p53, HPV PCR and HPV mRNA and DNA Chromogenic In-Situ Hybridization (CISH) in Vulvar Squamous Cell Carcinomas (VSCC)

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Background: The incidence of VSCC is increasing.Studies suggest the presence of two histologically and molecularly distinct subsets of VSCC;one contingent on and another independent of HPV infection. HPV E6/E7 proteins can result in degradation of p53,cell cycle deregulation and abnormal expression of cyclin dependant kinase inhibitor p16. The aim of this study was to find the optimal way to determine HPV status in VSCC.

Design: Sequential VSCC from patients treated at Princess Margaret Hospital from 2000-2008 were reviewed. Histology of the in-situ and invasive tumor was assessed, classifying cases based on the presence of histologic features of HPV infection and usual dysplasia (HPV histo vs non-HPV histo).A tissue microarray was constructed. p16 and p53 immunohistochemistry,HPV E6/E7 mRNA CISH(RNAscope,Advanced Cell Diagnostics), DNA CISH(Ventana Inform HPVIII),and PCR(Roche Linear Array) were performed. Clinical data were retrieved. Survival was analyzed using Kaplan Meier curves and log rank test.

Results: We identified 119 VSCC(median age71± 16 yrs). Using various methods, the proportion of HPV+ VSCC ranged from 15-37% (table 1). p16+ VSCC were 39% of cases. Compared to HPV PCR, the HPV detection rate was 75.7%(histology), 75.7%(p16), 62.5%(p53), 66.7%(RNA CISH), and 32.4%(DNA CISH). On univariate and multivariate analysis, DNA CISH+ VSCC had a better overall survival (OS) and progression-free survival (PFS), and HPV histo, p16+, and RNA CISH+ cancers had a better PFS(table 1-2). In the 46 patients treated with radiation, HPV histo, p16+, RNA CISH+, and DNA CISH+ were associated with better PFS (p=0.018, 0.021, 0.0038, and 0.04, respectively). p53 expression was not prognostic

Conclusions: The use of histology, p16 and HPV CISH as markers of HPV active infection can predict VSCC cases with improved PFS.

Table1: Univariate analysis of biomarkers in VSCC

Feature	n	OS		PFS	
		HR	p	HR	p
HPV histo	42/114 (36.8%)	0.84	0.63	0.48	0.011
p16+	45/116 (38.8%)	0.54	0.075	0.35	0.00013
p53 abnormal	70/110 (63.6%)	0.96	0.9	0.91	0.7
HPV DNA CISH+	17/112 (15.2%)	0.26	0.049	0.36	0.027
HPV RNA CISH+	34/115 (29.8%)	0.49	0.066	0.35	0.0011
HPV PCR+	40/118 (33.9%)	0.84	0.63	0.61	0.096

HR,hazard ratio; p,log rank p value

Table2: Multivariate analysis of biomarkers in VSCC

Feature	OS		PFS	
	HR	p	HR	p
HPV histo	0.65	0.25	0.54	0.044
p16+	0.59	0.14	0.46	0.015
p53 abnormal	0.84	0.58	0.8	0.4
HPV DNA CISH+	0.23	0.045	0.33	0.061
HPV RNA CISH+	0.59	0.19	0.47	0.04
HPV PCR+	0.71	0.33	0.64	0.19

HR,hazard ratio; p,log rank p value

1099 Correlation of K-Ras Mutation with Histological Subtypes of Endometrial Mucinous Lesions: Implications for Biological Progression and Molecular Diagnosis

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Background: Mucinous epithelial changes are frequently encountered in endometrial biopsies. Traditionally they have been classified into three morphologic categories (A, B and C) based on architectural complexity and cytological atypia to help predicting the risk of subsequent endometrial adenocarcinoma (EAC). K-ras mutations have been recently reported in atypical mucinous endometrial lesions and mucinous EAC. The aim of our study was to assess the prevalence and diagnostic and prognostic utility of K-ras mutation in the different morphological classes of mucinous endometrial changes.

Design: Forty endometrial biopsies with mucinous change were retrieved from our departmental archives. All available H&E slides were reviewed and the cases were categorized into simple mucinous change without cytological atypia (type A), complex mucinous epithelium or simple mucinous changes with cytological atypia (type B), markedly complex mucinous changes with cytological atypia (type C) or mucinous EAC. K-ras mutation analysis was performed by single strand conformation polymorphism. Clinical data and follow up were recorded.

Results: Thirty-eight cases with informative K-ras mutation status were included in the final analysis, including 2 type A lesions, 18 type B lesions and 11 type C lesions. Follow-up was available for 32 patients. All type A mucinous lesions were negative for K-ras mutation. K-ras mutation was present in 61.1% of type B mucinous lesions, in 45.5% of type C mucinous lesions, and in 85.7% of EAC. Additional details and follow-up information is presented in table 1.

Table 1. Results

Type of mucinous change	Number of cases (total n=38)	Number of K-ras positive cases	Number of cases with follow up (total n=32)		Follow up diagnosis	
			K-ras positive	K-ras negative	K-ras positive n (%)	K-ras negative n (%)
A	2	0	0	1	NA	1 (100%) IE
B	18	11 (61.1%)	9	5	5 (55.6%) CAH, 0 (0%) EAC	0 (0%) CAH, 2 (40%) EAC
C	11	5 (45.5%)	4	6	3 (75%) CAH, 1 (25%) EAC	3 (50%) CAH, 0 (0%) EAC
EAC	7	6 (85.7%)	6	1	6 (100%) EAC	1 (100%) EAC

CAH: Complex Atypical Hyperplasia, EAC: endometrial adenocarcinoma, IE: Inactive endometrium, NA: not applicable

Conclusions: Corroborating the existing data, the current study confirms high incidence of K-ras mutation in atypical mucinous epithelial lesions (type B and C) and mucinous carcinoma of the endometrium. Therefore, K-ras mutation may be a clinically useful molecular marker of early stage malignant transformation of mucinous lesions of the endometrium.

1100 Does Robotic Hysterectomy Increase the Incidence of Lymphovascular Space Invasion in Endometrial Cancer?

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Background: Lymphovascular space invasion (LVSI) is an independent risk factor for nodal disease and poor outcome in endometrial cancer. Uterine manipulators are a useful adjunct for robotic-assisted laparoscopic hysterectomy (RALH), but some surgeons avoid their use for fear of altering pathology or interpretation of LVSI. The aim of the study is to compare the incidence of LVSI in FIGO stages IA, IB and II endometrial cancer operated by laparotomy (TAH) vs. RALH.

Design: We retrospectively compared clinico-pathological data and tumor pathology from patients with endometrial cancer operated by laparotomy (TAH) vs. RALH. The data for two hospitals from May-2005 to July-2012 were reviewed for age, tumor histology, grade, FIGO stage, LVSI, depth of invasion, and tumor size. A ConMed V-Care® uterine manipulator was used in all robotic cases.

Results: 365 endometrial cancer cases (223 TAH, 142 RALH) with stages IA (115), IB (180) and II (70) diseases were reviewed. Histology types were endometrioid (68%), serous (9%), carcinosarcoma (5%) and others (18%). No significant difference in the age, grade, histology and myometrial invasion between TAH and RALH groups. LVSI was identified in 161 cases (44%) including 48 stage IA (41%), 77 IB (42%) and 36 stage II (52%). Overall, RALH group has a statistically significantly higher LVSI for stage IA (p=0.013) but not stage IB (p=0.65) or II (p=0.28) compared to the TAH group.

LVSI by stage

	TAH %		RALH %		p- Value
	Yes	No	Yes	No	
1	80	92	52	72	
IA	13	34	35	33	0.0129
IB	55	69	22	34	0.625
2	29 (56%)	23 (44%)	7 (38%)	11 (62%)	0.277
Total	97		64		

There was no difference in LVSI between the 2 procedures when only uterine serous tumors were analyzed.
Conclusions: RALH cases that utilized uterine manipulator show a significantly higher LVSI rate in stage IA but not in stage IB or II diseases. Further studies are recommended to review RALH conducted with no manipulator or other types of uterine manipulators.

1101 A Novel Classification System for Patients with Endocervical Adenocarcinoma (EAC): The Impact of Tumor Growth Pattern on Lymph Node Metastasis

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Background: Currently, the resection of lymph nodes (LN) in cases of EAC depends mainly on the depth of invasion (DOI) of the tumor. However, it is well known that many factors can prevent pathologists from accurately measuring the DOI. The recently described Silva method (SM) (Gyn Oncol 2012;125:S27-S28) has shown that classifying EAC by pattern correlates better with the presence or absence of LN metastasis than using DOI. The objective of this study was determine whether the presence of lymphovascular invasion (LVI), tumor size, grade of differentiation, DOI and the new method provide prognostic information.
Design: 411 cases collected from 14 international institutions were analyzed. The evaluation of stromal invasion was conducted according to the SM.
Pattern A: well-demarcated glands (regardless of DOI) no lymphovacular invasion is allowed
Pattern B: early stromal invasion arising from well-demarcated glands
Pattern C: diffuse, destructive invasion.
Results: Ages ranged from 20 to 86 years (mean 45.9). The patterns of growth were A in 20% tumors, B in 25% and C in 55%. All patients were treated with lymphadenectomy, of them 8 pattern B cases and 58 pattern C cases had LN metastasis. All pattern B and pattern C cases with LN metastasis had LVI. The mean DOI of cases with pattern A and B was the same (5.3mm) and the mean DOI of cases with pattern C was 10mm. 42% percent of pattern A cases were ≤10mm in size compared to 26% and 6% of the tumors with a growth pattern B and C, respectively.
Conclusions: Analysis of additional cases following our initial study further validates our observation of the clinical utility of the pattern-based classification. There were no lymph node metastasis in cases with a growth pattern A. All pattern B and C cases with LN metastasis had LVI. If superficial invasion is found without obvious LVI careful examination of the pathology is recommended, including obtaining deeper sections because in our series of 102 cases with pattern B, only those with LVI had LN metastasis (7.8%) as well as 26% of pattern C cases.

1102 Mutational Analysis of BRAF and KRAS in Atypical Proliferative (Borderline) Serous Tumors of the Ovary and Associated Peritoneal Implants from a Population-Based Cohort in Denmark

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Background: Atypical Proliferative (Borderline) Serous Tumors (APSTs) are a unique group of ovarian tumors characterized by the presence of "peritoneal implants". Peritoneal implants are subdivided in two morphological subtypes, non-invasive (NIIP) and invasive implants (IIP), the latter being considered as the most important prognostic marker. Whole genome exome sequencing analysis has revealed KRAS and BRAF as the main mutated genes in ovarian APSTs; however, the molecular nature of peritoneal implants remains largely unknown because of conflicting results in previous studies that focused on a small number of cases.
Design: The aim of this study is to compare KRAS/BRAF mutational status between APSTs and their associated peritoneal implants. 39 patients with advanced stage APSTs were identified from a nation-wide tumor registry in Denmark. For each patient the primary ovarian APST and one or more implants were selected to perform laser-microdissection and KRAS/BRAF mutational analysis, for a total of 92 tumoral lesions (39 APSTs, 43 NIIPs and 10 IIPs).
Results: *KRAS* and *BRAF* mutations were identified in 27 (69.3%) and 3 (7.7%) of 39 APSTs, respectively, while the remaining 9 APSTs (23%) were wild-type for both genes. KRAS and BRAF mutations were identified in 34 (64.1%) and 5 (9.4%) of 53 peritoneal implants, for total of 39/53 KRAS or BRAF mutated implants (73,6%), while the remaining 14 implants (26.4%) were wild-type for both genes. Comparison of the mutational status between the primary APST and the related peritoneal implant for each patient ("APST-implant pair") showed the same mutations in 34/37 APST-implant pairs associated with KRAS mutated primary APSTs and in 5/5 APST-implant pairs associated with BRAF mutated primary APSTs; no mutations were identified in 11/11 APST-implant pairs associated with KRAS and BRAF wild type primary APSTs. Overall the same mutational status of KRAS/BRAF was concordant in 50/53 APST-implant pairs (94,3%), independently of the type of implants (concordance was found in 40/43 APST-NIIP pairs (93%) and in 10/10 APST-IIP pairs (100%)).
Conclusions: We provide evidence that most peritoneal implants, regardless of their types, i.e., NIIPs vs. IIPs, harbor either KRAS or BRAF mutations. The demonstration of an identical mutational status of KRAS and BRAF between primary ovarian APSTs and related implants strongly supports the hypothesis that most non-invasive and invasive implants share a common origin with the associated ovarian tumor.

1103 BRAF Mutation-Associated Immunostaining Marker and Morphological Features in Peritoneal Implants and Atypical Proliferative (Borderline) Serous Tumors (APSTs)

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Background: Peritoneal implants associated with atypical proliferative (borderline) serous tumors (APSTs) represent a clinically and biologically intriguing group of lesions. While exome sequencing analysis has identified KRAS/BRAF mutations as the main genetic alterations in APSTs, the molecular nature of peritoneal implants remain largely elusive, mostly because their minute size and contamination of stromal cells challenge any molecular characterization. The VE1 antibody is a recently developed monoclonal antibody that recognizes the epitope generated by the V600E mutation in BRAF. In this study, we took advantage of VE1 immunoreactivity as a surrogate marker for BRAF V600E mutation to study the BRAF mutation status in those lesions that had sufficient DNA for mutation analysis.
Design: VE1 immunohistochemistry was performed in a group of 33 APSTs and 27 peritoneal implants. *BRAF* and *KRAS* mutational status was determined in all APSTs and in 19 peritoneal implants specimens which had enough genomic material to perform mutational analysis. Their mutation status was then correlated with the VE1 staining. In this study, we further identified the morphological features that were enriched in those specimens with *BRAF* V600E mutation.
Results: By analyzing 33 APSTs (including 20 cases with *BRAF* V600E) and 19 peritoneal implants (including 12 lesions with *BRAF* V600E), we were able to demonstrate 100% sensitivity and 100% specificity by applying VE1 immunoreactivity in detecting *BRAF* V600E. Moreover, we detected VE1 immunoreactivity in additional 7 implants that were too small to perform mutational analysis. By correlating molecular and morphological findings, we found certain morphological features in tumors that harbored *BRAF* V600E. Those features included 1) the presence of the "senescence-like" cells exhibiting round or polygonal shape with abundant eosinophilic cytoplasm and degenerating nuclei, 2) "budding" from the APST epithelial monolayer and 3) individual cells or poorly cohesive aggregates in the peritoneal implants.
Conclusions: Our data strongly suggest that VE1 immunoreactivity is the surrogate marker for *BRAF* V600E in APST and associated implants. The morphological features that are frequently observed in BRAF mutated samples are helpful in distinguishing APSTs and related implants with BRAFV600E mutations from those without. Both immunostaining and morphological features can be readily applied to study APSTs and their related implants in paraffin tissues including those that are minute in size.

1104 Tumor Progression from Ovarian Endometriotic Cyst to Endocervical-Like (Mullerian-Seromucinous) Tumors: Immunohistochemical Comparison of Endometriotic Cyst and the Neoplasm

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Background: Non-gastrointestinal, endocervical-like, Mullerian mucinous tumor of the ovary which has different clinicopathological and immunophenotypical characteristics from other mucinous ovarian tumors is characterized by higher frequency of bilaterality and hormone receptor reactivity. This tumor is also named "seromucinous neoplasm" due to its macroscopical and structural mimicry to ovarian serous tumors while histologically composed mostly of endocervical type mucinous epithelial cells, often admixed with various Mullerian-type epithelia. As its association with endometriosis is typical and endometriosis is thought to be a precursor of this neoplasm; we undertook an immunohistochemical analysis to compare the properties of endometriotic cyst with the neoplastic cells for aiding the classification, terminology and tumorigenesis.
Design: Our immunohistochemical study set consisted of 14 endocervical-like mucinous borderline tumors from 10 patients, out of which 10 tumors were in continuity with an endometriotic cyst. Immunohistochemical features of neoplastic cells were compared to endometriotic cyst for hormone receptors (ER, PgR), ARID1A, PTEN tumor suppressors, p21, WT-1, PAired –boX2 and 8 products which were major players in development, organogenesis and cell-lineage determination.
Results: Bilateral tumors showed similar immunophenotype and counted as one. Endometriotic epithelium and its non-neoplastic nature was confirmed by CD10, p53 and Ki67(MIB-1). WT-1, PAX2, PAX8, p21 and PgR were concordantly downregulated in endometriotic epithelium while strongly positive in tumor cells in 70% of cases. On the other hand, *ARID1A* and PTEN loss was observed concomittently in 1(10%) case where p21 was particularly positive in the endometriotic cyst and WT-1 was not expressed in tumor cells. ER did not show any particular difference between the tumor and the endometriotic cyst.
Conclusions: Our study suggest that endocervical-like (Mullerian-seromucinous) tumors have distinct immunophenotypical properties and it should be a unique additional tumor type arising in endometriosis.

1105 Low Stage Clear Cell Carcinoma of the Endometrium: A Review of Clinical Outcome and Survival

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Background: The clinical behavior and prognosis of endometrial clear cell carcinoma (ECC) is thought to be in between that of endometrioid and serous carcinomas (ESC). ECC is considered a type II endometrial carcinoma and like ESC even at low stage has a risk of recurrence and metastatic disease. However, the behavior and management

of Stage I ECC is unclear and there is only limited literature addressing this issue. The aim of this study was to evaluate the clinical outcome of Stage I ECC.
Design: All cases of ECC in which endometrial biopsy/currettings and hysterectomies were performed and slides available for pathologic review were retrieved from a 10-year time period of January 2002 to September 2012. All pathologic slides were reviewed by a gynecologic pathologist and only cases of Stage I pure ECC were included in this study. Clinical features were summarized including demographic information, treatment summary, and clinical follow-up.
Results: 13 cases of ECC that were Stage I (all cases IA) were identified. The depth of myometrial invasion was: 45%, 38%, 17%, <10% (2 cases), 0% (4 cases), no residual carcinoma (4 cases). None of the cases had lymphovascular invasion. In 11 patients the pelvic washings were negative, positive in one patient and not performed in one. The patient age range was 49-83 years of age with mean of 63 years. All thirteen patients were treated with total hysterectomy and bilateral salpingo-oophorectomy. Nine of these patients also got radiation and/or chemotherapy while three were under surveillance. The follow-up period ranged from one month to 8 years with a mean of approximately 36 months. Two patients developed ascites but cytology specimens were negative. To date, there are no local recurrences or distant metastases in this patient cohort.
Conclusions: In our study, Stage I ECC is uncommon with only 13 cases identified over a 10-year period. While this is a small data set and follow-up limited, the short term prognosis of ECC appears to be favorable. This also raises the question of utility of adjuvant therapy in Stage IA ECC. Larger prospective studies are recommended.

1106 Clinical Significance of Lymphovascular Space Invasion in Uterine Serous Carcinoma

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Background: Uterine serous carcinoma, although less common, is responsible for a larger proportion of uterine cancer related deaths. Vascular space invasion has been identified as a prognostic factor. The aim of our study was to analyze the clinical significance of extensive, low or absence of lymphovascular space invasion (LVSI) in uterine serous carcinoma (USC) patients.
Design: After IRB approval, 236 USC from the pathology databases of 4 large academic institutions were included. H&E slides (2-10 slides per case) were retrieved and reviewed by a gynecologic pathologist at each participating institution after reviewing 50 cases as a group to be consistent in the diagnosis. LVSI information was available for 151 patients. Patients were divided into three groups based on LVSI extent. Extensive LVSI (E-LVSI) was defined as ≥ 3 vessel involvement; Low LVSI (L-LVSI) was defined as less than 3 vessel involvement and the last group was absent LVSI (A-LVSI).
Results: Out of these 151 patients, 65 had A-LVSI, 55 had L-LVSI, and 31 had E-LVSI. Please see table for the association between LVSI and LN involvement, washing, myometrial invasion, cervical involvement, lower uterine segment involvement (LUS), and stage. Analyzing only stage I and stage II disease (n=68) for survival and recurrence, demonstrated a median survival of 177 months, 155 months and 34 months in the A-LVSI, L-LVSI and E-LVSI groups.

	A-LVSI (65)	L-LVSI (55)	E-LVSI (31)	
+ LN	15.4%	27.3 %	83.9%	*
+ Washing	18.8 %	24 %	37 %	NS
Myometrial Invasion	58 %	85 %	100%	*
Cervical Invasion	23 %	43 %	66 %	*
Lower Uterine Segment Invasion	34 %	55 %	77 %	*
Stage III & IV	31 %	40 %	78 %	*

* p< 0.05

Conclusions: In this large multicenter retrospective analysis, the extent LVSI was associated with multiple pathologic factors and had significant clinical implications in terms of survival and disease recurrence in early stage disease suggesting careful attention must be paid not only to the presence of LVSI but its extent in USC.

1107 Characteristics of Hydatidiform Moles: Analysis of p57 Expression and Molecular Genotyping Data

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Background: Recent studies have established that p57 immunostaining and molecular genotyping accurately subclassify molar specimens into complete hydatidiform mole (CHM) and partial hydatidiform mole (PHM) and distinguish these from non-molar specimens (NM). Detailed characteristics of a large series analyzed with these techniques have not been reported.
Design: All potentially molar specimens encountered in a large gynecologic pathology practice were analyzed with p57 immunostaining and genotyping. Initially, all specimens were subjected to both analyses; this was later modified to triage cases for genotyping based on p57 results (p57-negative cases diagnosed as CHMs without genotyping; all p57-positive cases genotyped).
Results: 519 cases were categorized as CHM (166), PHM (127), NM (208), and complex (18). Of 166 CHMs, 165 were p57-negative and 1 was p57-positive (due to retained maternal chromosome 11). 95 genotyped CHMs were androgenetic diploid; 81 (85%) were monospermic XX and 14 (15%) were dispermic (12 XY, 2 XX). 8 CHMs were invasive (6 monospermic XX, 2 dispermic XY), including 4 with atypical trophoblastic proliferations consistent with choriocarcinoma (3 monospermic XX, 1 dispermic XY); 2 non-invasive CHMs had atypical trophoblastic proliferations consistent with choriocarcinoma (1 monospermic XX, 1 dispermic XY). Of 127 PHMs, 124 were p57-positive and 2 were p57-negative (due to loss of maternal chromosome 11); 1 was non-reactive (due to degenerative changes). Of these, 122 were diandric triploid and 3 were triandric tetraploid; 2 were morphologically consistent with PHMs but with triploidy of undetemined origin (due to lack of decidual tissue for analysis). Of 122 diandric triploid PHMs, 118 (97%) were dispermic (67 XXY, 44 XXX, 7 XYY) and 4 (3%) were monospermic (3 XXX, 1 XYY); the 3 triandric tetraploid PHMs were at least dispermic (2 XXYY, 1 XXXY). Of 208 NMs, 204 were p57-positive and 1 was p57-negative; 4 were non-reactive. 205 were biparental diploid and 3 were digynic triploid; the p57-negative biparental diploid specimen had abnormal, enlarged villi but no trophoblastic hyperplasia, possibly an early form of Beckwith-Wiedemann syndrome. 18 cases were genotypically complex and included androgenetic/biparental mosaic specimens with discordant p57 expression (analyzed in another study).
Conclusions: CHMs are overwhelmingly p57-negative monospermic androgenetic diploid conceptions whereas PHMs are almost always p57-positive dispermic diandric triploid conceptions. Rare examples with aberrant p57 expression can be correctly classified by genotyping.

1108 Low Grade Squamous Intraepithelial Lesion/Cannot Exclude High Grade Squamous Intraepithelial Lesion (LSIL-H): A Unique Cytologic Abnormality

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Background: SIL of Indeterminate Grade was introduced in the 2001 Bethesda system. This term, or low grade squamous intraepithelial lesion, cannot exclude high grade squamous intraepithelial lesion (LSIL-H), has been used. Data about prevalence of hrHPV in women with LSIL-H and subsequent histopathology are limited.
Design: A search of CoPath files was performed to retrieve cases with a cytologic diagnosis of LSIL-H and concurrent hrHPV testing between 07/2005 and 12/2011. HrHPV testing and histologic follow-up results were recorded.
Results: 495 cases with a cytologic diagnosis of LSIL-H and concurrent hrHPV testing were identified. HrHPV was positive in 448 of 495 cases (90.5%). Patients' average age was 30 years old (range: 15-80, median: 27). Of 347 patients who had at least one subsequent histologic follow-up within an average period of 24 months (range: 6-66, median: 24), CIN2/3 was detected in 103 patients (29.7%) and CIN1 in 186 patients (53.6%). Of those 103 cases with a histologic follow-up of CIN 2/3, the median interval between LSIL-H and initial CIN2/3 diagnosis was about 3 months (range: 0.5-60). CIN2/3 was identified on histopathologic follow-up in a significantly greater proportion of women with hrHPV-positive LSIL-H than patients with hrHPV-negative LSIL-H ($p<0.05$). The hrHPV positivity data and histopathologic follow-up data are also compared to our published data in patients with LSIL, ASC-H, and HSIL from the same institution (Table 1).

Table 1. Comparison of Rates of HPV Positivity and CINs Between LSIL-H and Other Cytologic Abnormalities

Category	HPV Positive Rate	CIN2/3		CIN1	
		HPV +	HPV -	HPV +	HPV -
LSIL-H	90.5%	99/321(30.8%)	4/26 (15.4%)	172/321 (53.6%)	14/26 (53.8%)
HSIL	95.7%	197/273 (72.2%)	4/12 (33.3%)	52/273 (19.1%)	5/12 (41.7%)
ASC-H	54.3%	84/257 (32.7%)	3/248 (1.2%)	76/257 (29.2%)	32/248 (12.9%)
LSIL	80.2%	89/612 (14.5%)	4/107 (3.7%)	357/612 (58.3%)	44/107 (41.1%)

Conclusions: HrHPV and histopathologic CIN2/3 detection rates with LSIL-H (90.5% and 29.7%) were significantly lower than in patients with HSIL (95.7% and 71.2%) and significantly higher than in patients with LSIL (80.2% and 13.1%) and with ASC-H (54.3% and 17.2%). Whereas histopathologic CIN2/3 detection rates were similar in LSIL-H as in ASC-H for HPV positive cases (30.8% vs 32.7%), the rate is higher in LSIL-H than in ASC-H for HPV negative cases (15.4% vs 1.2%). Our results suggest LSIL-H is a unique category of cytologic abnormality associated with distinctive hrHPV and CIN2/3 detection rates.

1109 Mutation Analysis of *MED12* in Benign and Malignant Uterine Smooth Muscle Tumors

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Background: Progression from leiomyoma to leiomyosarcoma in the uterus has been suggested but molecular evidence is generally lacking. The distinction between atypical leiomyoma, cellular leiomyoma and low grade leiomyosarcoma can be challenging and requires identifying biomarkers for a differential diagnosis. Recent identification of frequent *MED12* mutations in uterine leiomyomas provides a valuable molecular marker for the study of uterine smooth muscle tumors. Although it is still unclear whether *MED12* mutations lead to leiomyoma development, high frequent *MED12* mutations in uterine leiomyomas may help us to reevaluate the molecular relationship between leiomyoma, its histologic variants and malignant counterparts—leiomyosarcomas.
Design: To examine the *MED12* mutations in different types of uterine smooth muscle tumors, we collected usual type leiomyomas (28 cases), cellular leiomyomas (18 cases), and leiomyosarcomas (32 cases). An additional four cases of leiomyosarcoma with leiomyoma-like areas were also collected for *MED12* mutation analysis. All DNA samples were extracted from formalin fixed and paraffin embedded tissue samples. *MED12* mutations were examined and analyzed by Applied Biosystem's DNA Analyzer and DNASTAR Lasergene 8 software.
Results: DNA for *MED12* mutation analysis from tumor and matched myometrium was collected and prepared in a total of 78 cases. All cases were revaluated histologically. Since cellular leiomyomas can mimic endometrial stromal nodules or low grade sarcomas, immunohistochemical (IHC) staining for CD10, SMA and desmin was performed in all cellular leiomyomas, which showed diffuse positivity for desmin, a Ki-67 index of <10% and no more than focal positivity for CD10. The leiomyoma-like areas were evaluated by p53, p16 and Ki-67, all of which demonstrated a different IHC pattern from their leiomyosarcoma counterparts. Over 60% of usual type leiomyomas were found to have *MED12* mutations, which is consistent with several independent studies.

MED12 mutation rates were very low in both cellular leiomyomas and leiomyosarcomas, accounting for 11.8% and 9.7%, respectively. In contrast to the *MED12* point mutations seen in leiomyomas, leiomyosarcomas demonstrate frameshift *MED12* mutations. No *MED12* mutations were identified in benign appearing leiomyoma-like areas in the additional leiomyosarcoma cases.

Conclusions: The low rate of *MED12* mutations in cellular leiomyomas and leiomyosarcomas suggests a different tumorigenic pathways from leiomyomas. Examination of *MED12* mutations may be valuable for a differential diagnosis of uterine smooth muscle tumors.

1110 Fallopian Tube Intraluminal Tumor Spread from Non-Invasive Precursor Lesions: A Novel Metastatic Route in Early Pelvic Carcinogenesis

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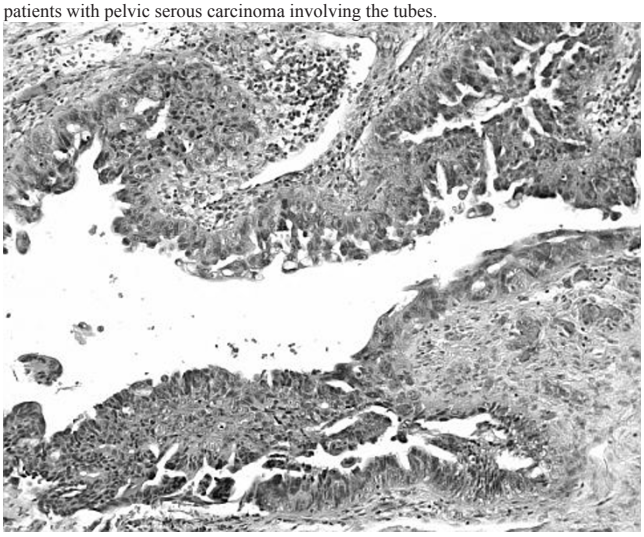
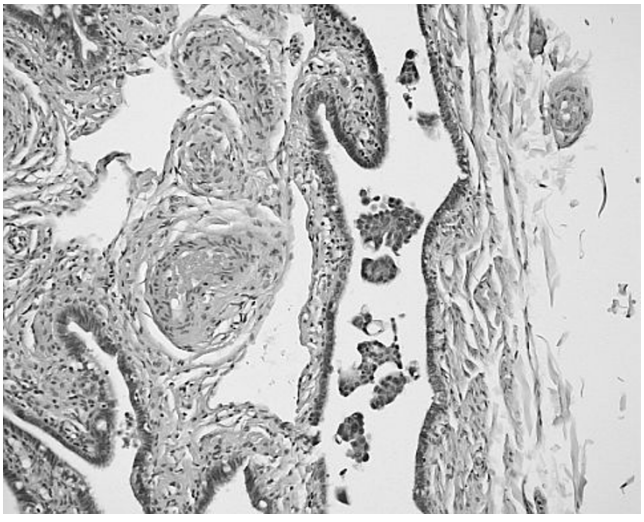
Background: Pelvic serous carcinoma is usually advanced stage at diagnosis, indicating that abdominal spread occurs early in carcinogenesis. Recent discovery of a precursor sequence in the Fallopian tube, culminating in serous tubal intraepithelial carcinoma (STIC) provides an opportunity to study early disease events. This study aims to explore novel metastatic routes in STICs.

Design: A BRCA1 mutation carrier (patient A) that presented with a STIC and tubal intraluminal shed tumor cells upon prophylactic bilateral salpingo-oophorectomy (PBSO) instigated scrutiny of an additional 23 women who underwent a PBSO and 40 patients with pelvic serous carcinoma involving the tubes.





Results: Complete serial sectioning of tubes and ovaries of patient A did not reveal invasive carcinoma, but subsequent staging surgery showed disseminated abdominal disease. STIC, intraluminal tumor cells and abdominal metastases displayed an identical immunohistochemical profile (p53+/WT1+/PAX8+/PAX2-) and TP53 mutation. In sixteen serous carcinoma patients (40%) tubal intraluminal tumor cells were found, compared to none in the PBSO group.

Conclusions: This is the first description of a STIC, which plausibly metastasized without the presence of invasion through intraluminal shedding of malignant surface epithelial cells in the tube and subsequently spread through the peritoneal cavity. These findings warrant a reconsideration of the malignant potential of STICs and indicate that intraluminal shedding could be a risk factor for early intraperitoneal metastasis. Though rare in the absence of invasive cancer, we show that shed intraluminal tumor cells in the Fallopian tubes from serous carcinoma cases are common and a likely route of abdominal spread.

1111 Diagnostic Clinical Application of Multi-Target Fluorescence *In Situ* Hybridization in Endometrial Biopsies: Prospective Study of 282 Cases

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Background: The diagnosis of endometrial hyperplasia, atypical hyperplasia, and well-differentiated endometrial adenocarcinoma is often challenging and prone to intra- and inter-observer variation. Genetic testing may allow more objective discrimination. We investigated the utility of chromosomal anomalies for the detection and diagnostic discrimination of endometrial abnormalities in biopsies using multi-target fluorescence in situ hybridization (FISH).

Design: Samples were prospectively collected by endometrial brush (Tao Brush®) or suction straw (Pipelle®) and processed by liquid-based cytological preparation protocol from 300 cases. A total of 18 cases were excluded owing to insufficient tissue (6%). Each of the remaining cases was hybridized using fluorescence-labeled DNA probes to chromosomes 1, 8, and 10. The FISH signals were enumerated in 100 cells per case, and the chromosomal anomalies were correlated with pathologic findings, including histologic diagnoses on matched endometrial tissue samples reviewed by at least two gynecologic pathologists. Given the small number (5) of cases of atypical hyperplasia, these were categorized as carcinoma for statistical analysis.

Results: Numeric chromosomal anomalies (positive FISH results) were found in 20% (6 of 30 cases) of benign endometrium, 27.8 % of hyperplasia without atypia (45 of 162), and 71.1 % (64 of 90) atypical hyperplasia/carcinoma specimens ($P < 0.001$)(WHO Classification). FISH anomalies had an overall sensitivity of 59.1% and specificity of 79.7% for the detection of atypical hyperplasia and/or endometrial carcinoma.

Conclusions: Multi-target FISH is useful for the differential diagnosis of hyperplasia, atypical hyperplasia, and endometrial adenocarcinoma, with moderate sensitivity and a high level of specificity. It is also a potential tool for the early detection of neoplastic cells in endometrial cytology specimens. Endometrial hyperplasia with FISH-detected chromosomal anomalies may represent a clinically significant subset of cases that warrants close clinical follow-up.

1112 Screening by Young Age and Family History of Colon Cancer Misses the Majority of Endometrial Cancer Patients with Lynch Syndrome

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Background: Current clinical screening guidelines to identify women with Lynch Syndrome rely heavily on young age at diagnosis of endometrial cancer (EC) or colorectal cancer and presence of family history of colon cancer. Such guidelines were codified by the Society of Gynecologic Oncologists (SGO) in 2007. These guidelines have not been tested in a population-based fashion.

Design: 408 consecutive, unselected EC cases were evaluated for immunohistochemical expression of DNA mismatch repair proteins. Tumors with loss of MSH2, MSH6 or PMS2 were designated as probable Lynch Syndrome (PLS). Tumors with loss of MLH1 and absence of *MLH1* promoter methylation were also designated PLS. Clinical data were collected from the electronic medical record.

Results: 43/408 (10.5%) EC tested had PLS. 29/43 (67.4%) EC cases with PLS did not meet SGO criteria. Table 1 summarizes known Lynch Syndrome risk factors for the 43 PLS patients. Those who met SGO Criteria were younger, more frequently had tumor rising in lower uterine segment, and more frequently had a family history of colon cancer. Note that the majority of EC cases with PLS in both groups did not have these historical risk factors.

Clinical Data for n=43 Endometrial Cancer Patients with Probable Lynch Syndrome

	Meets SGO Criteria (n=14)	Does Not Meet SGO Criteria (n=29)
Median age at Diagnosis	48.5	63.0
Average BMI	32.0	33.0
BMI less than 30	7/14 (50.0%)	10/29 (34.5%)
Family History Endometrial Cancer	2/14 (14.3%)	2/29 (6.9%)
Family History Colorectal Cancer	4/14 (28.6%)	4/29 (13.8%)
Lower Uterine Segment Tumor	3/14 (21.4%)	2/29 (6.9%)

Conclusions: When population-based screening is employed, 10.5% of EC patients have tissue testing findings consistent with Lynch Syndrome. This percentage is comparable to those in recent publications from the Netherlands and Ohio State. SGO Criteria correctly identified only 32.6% of these women, meaning that a large number of endometrial cancer patients miss the opportunity for further cancer risk assessment and heightened screening for colorectal cancer. As germline mutation testing of all endometrial cancer patients is not economically feasible, to capture the majority of women who have Lynch Syndrome, universal tumor testing (immunohistochemistry, *MLH1* methylation) of endometrial cancers should be employed.

1113 SOX2 Expression in Vulvar Squamous Epithelial Neoplastic Lesions: Relation with the Antiapoptotic Factor Survivin

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Background: SOX2 is a transcription factor that controls pluripotency in both embryonic and adult-tissue stem cells. Survivin is involved in the regulation of the cell cycle progression and a potent inhibitor of apoptosis. These factors are overexpressed in many cancers including squamous cell carcinomas and their precursor lesions, with a role of SOX2 as a cell lineage-survival oncogene in the latter tumors.

Design: SOX2 and survivin immunoexpression was evaluated in formalin-fixed, paraffin-embedded archival vulvar epithelia consisting of normal squamous vulvar epithelia (NE, n=25), high-grade classic vulvar intraepithelial neoplasia (HG-VIN, n=16), differentiated VIN (d-VIN, n=18) and vulvar invasive keratinizing squamous cell carcinoma (ISCC, n=33). Immunostaining for all factors was scored for quantity (score 0, 0% immunoreactive cells; score 1+, <5% immunoreactive cells; score 2+, 5% to 50% immunoreactive cells; score 3+, >50% immunoreactive cells) and intensity (scores 1+, 2+, 3+). These scores were added in the individual cases to achieve the final immunoscores. Final scores of 0 and 1+ were considered negative, scores 2 to 4 as moderately and scores 5 and 6 as strongly positive.

Results: Moderate and strong immunoexpression of SOX2 and survivin was noted in neoplastic lesions only. Immunoscores did not differ significantly in HG-VIN and ISCC (P=0.732, and 0.097, respectively) and in grades 1, 2 and 3 of ISCC, indicating a correlation of these factors. In d-VIN, SOX2 was upregulated as compared to survivin (P=0.003).

Conclusions: SOX2 and survivin are up-regulated and correlated in HG-VIN and ISCC compared to non-neoplastic squamous epithelia indicating relevance to vulvar carcinogenesis, consistent with the function of SOX2 as a cell lineage-survival oncogene and of survivin activation in the development of cellular longevity and resistance to apoptosis. A lack of a correlation in d-VIN points towards different marker biology in preinvasive intraepithelial lesions of different pathways.

1114 mTOR Over-Expression in Recurrent Low Grade Endometrioid Adenocarcinomas

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Background: Inhibitors of the mammalian target of rapamycin (mTOR) are of interest in the treatment of endometrial carcinoma (EMCA).The majority of EMCA present as early stage low grade tumors. Approximately 75% are cured by surgery with or without radiation therapy. When relapse occurs it frequently involves the vaginal cuff (local relapse) but may also present as pelvic or abdominal disease (regional relapse) or as distant metastasis. Previous studies have shown over-expression of mTOR in up to 7% of EMCA. The role of mTOR over-expression in recurrent disease has not been established. A biomarker which indicated risk of recurrence could be of clinical utility in guiding early adjuvant treatment (e.g. vaginal cuff radiation). Furthermore, if recurrent disease shows molecular progression, mTOR inhibitors may offer therapeutic benefit.

Design: A retrospective review of all endometrioid EMCA at our institution over a ten year span revealed 1685 cases. Of these, 944(56%) were grade (G)1, 517(31%) G2, and 224(13%) G3. 87 cases recurred: 27(31%) G1, 27(31%) G2, and 33(38%) G3. Archival paraffin embedded tissue of 24 G1 and 21 G2 with documented subsequent recurrence were examined by IHC for localization and over-expression of mTOR. Age and stage match controls and tissue from recurrent lesions (where available) were evaluated concurrently.

Results: Over-expression of mTOR was seen in 7/24 (29%) G1 tumors which subsequently recurred vs. 13/18 (72%) controls (*P*=0.01). Of the 10 recurrences tested, 5 showed mTOR over-expression. In 4 of these, the tumor was initially negative for mTOR.

mTOR over-expression in G1 and G2 recurrent endometrioid EMCA

	mTOR (+)	P value
G1 (n=24)	7 (29%)	0.01 (G1 vs G1 control); 0.005 (G1 vs. G2)
G1 Recurrence (n=10)	5 (50%)	
G1 Control (n=18)	13 (72%)	
G2 (n=21)	19 (90%)	0.63 (G2 vs. G2 control)
G2 Control (n=16)	13 (81%)	

Conclusions: mTOR over-expression in endometrioid EMCA is more prevalent than previously reported. Primary G1 EMCA which subsequently recur are less likely to show mTOR over-expression when compared to age, stage matched controls (*P*=0.01) and G2 EMCA (*P*=0.005) which subsequently recurs. However, tissue from recurrences shows mTOR over-expression 50% of the time and is seen in cases which were initially negative indicating molecular evolution. These results suggest possible utility of mTOR inhibitors in recurrent low grade EMCA.

1115 HER2 Testing in Endometrial Carcinoma: Four-Year Experience at a Large Tertiary Academic Center

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Background: HER2 overexpression and/or amplification have been previously reported in endometrial carcinoma (EC), suggesting that HER2 may be a promising therapeutic target. However, there is considerable variation in the reported rates, likely resulting from variability in testing methods, interpretation, and scoring criteria used. Currently there are no established guidelines for HER2 testing in EC.

Design: All EC cases between 2008 and 2012 with available HER2 status were retrieved from our departmental archives. H&E and immunohistochemical (IHC) slides were reviewed along with HER2 fluorescent in situ hybridization (FISH) images. HER2 IHC slides were systematically evaluated by two gynecologic pathologists and scores were assigned per the original FDA criteria and also per current breast scoring criteria. Clinical data were retrieved from the patients' medical records.

Results: A total of 112 EC - 85 pure serous carcinoma (SC), 23 mixed EC (with SC component) and 4 endometrioid EC - were included in the study. Thirty-eight cases (33.9%) were positive by HER2 IHC (3+) and/or FISH, 17 of which (44.7%) showed significant heterogeneity in IHC staining intensity. Significant IHC heterogeneity was also observed in 11 of 74 cases (14.8%) in the HER2 negative group. The % of cells with strong, complete membrane staining in cases with heterogeneity ranged between 5% and 80%. Incomplete, "U-shaped" staining pattern was frequently encountered (at least focally) in both the HER2 positive and negative groups. Five of the HER2 positive cases (13.1%) showed discrepant IHC scores when using the FDA versus the current breast scoring criteria. Discordant results between IHC and FISH were observed in 6 of 112 cases (5.3%).

Conclusions: This is the largest comprehensive study to date systematically evaluating HER2 overexpression and amplification in EC, using standardized scoring criteria. Overall, 33.9% of cases showed HER2 overexpression and/or amplification. Unlike in breast cancer, heterogeneous and incomplete ("U-shaped") HER2 expression occurs frequently in EC, potentially resulting in discrepant IHC scores. Our results underline the need for EC-specific HER2 scoring guidelines to reflect the unique biological features of these tumors. A clinical trial is currently underway evaluating trastuzumab in patients with HER2 positive SC, and ultimately the correlation of clinical response with HER2 results will help to standardize HER2 testing and scoring in EC.

1116 Clinicopathologic Characteristics of Intravenous Leiomyomatosis: An Experience of 12 Cases at a Single Medical Center

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Background: Intravenous leiomyomatosis (IVL) is a rare neoplasm of smooth muscle origin with the capacity for distant vascular and/or organ extension. The primary lesion is typically found in the uterus, with the most common extrauterine sites being the inferior vena cava, right heart chambers or pulmonary vasculature. Extrauterine involvement can result in direct mass effect or vascular obstruction with associated clinical sequelae.

Design: Twelve cases of IVL were identified in our pathology archives over a twenty-four year period (1988-2012). Patient medical records and gross pathological findings were reviewed. Histologic and related immunohistochemical slides (smooth muscle active, desmin, CD31 and CD34) were re-evaluated by two gynecologic pathologists to confirm the diagnosis using conventional diagnostic criteria.

Results: Clinicopathologic features are summarized in Table 1. Patient age at initial diagnosis ranged from 38 to 64 years (mean: 47.8 years), with a median age of 47 years. Three cases demonstrated extrauterine extension, one case was found to be extrauterine in origin (i.e. arising from the broad ligament) and one case was associated with disease recurrence.

Table 1. Clinical and pathologic findings.

Case	Age (y)	Presentation	Follow-up	Procedure	Size (cm)	Extrauterine Extension
1	52	Chest pain, syncope	AND	TAH-BSO; thrombectomy	1.0-18.0	Y
2	50	Abdominal distention/ pressure	AND, lost to F/U. Recurrence at 152 months.	Multiple mass excisions; initial - TAH	4.3-17.0	Y
3	49	Unknown	AND	TAH, thrombectomy	0.5	Y
4	41	FIB	AND	Supracervical hysterectomy	2.5	N
5	64	FIB	Dead, unknown cause	TAH-BSO	1.2	N
6	47	FIB, ovarian cyst	Lost to F/U	TAH-BSO	1.0	N
7	47	FIB, pelvic pain	AND	TAH-BSO	4.0	N
8	43	MEN	AND	TAH-BSO	4.3	N
9	42	FIB, MEN	AND	TAH	2.5	N
10	61	Uterine mass, bleeding	AND	TAH-BSO	2.0	N
11	40	FIB, MEN	AND	TAH	3.5	N
12	38	MEN, MET	Lost to F/U	TAH-BSO	3.5	N

Abbreviations: AND, alive with no evidence of disease; F/U, Follow-up; FIB, fibroid uterus; MEN, menorrhagia; MET, metrorrhagia; TAH-BSO, total abdominal hysterectomy-bilateral salpingo-oophorectomy.

Conclusions: Our study represents the fourth largest IVL cohort in medical literature to date, and the third largest from a single institution. IVL is a rare and potentially underdiagnosed neoplastic condition of smooth muscle origin that may result in significant morbidity when extrauterine vasculature or organs are involved. A high index of suspicion must be maintained. Prompt diagnosis combined with surgical excision has been shown to portend an increased survival rate and good overall prognosis in such cases.

1117 Differential Expression of Heart and Neural Crest Derivatives Expressed Transcript (HAND) 2 in Progesterone Treated Endometrium

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Background: Endometrioid (Type I) carcinomas (EMC) arise from an estrogen-dependent pathway. Progesterone inhibits estrogen-induced epithelial proliferation, is protective against endometrial hyperplasia and EMC, and is therapeutically used for these conditions. Expression of the progesterone induced heart and neural crest derivatives expressed transcript (HAND)2 has been shown to suppress production of fibroblast growth factors (FGFs), the mediators of the mitogenic effect of estrogen. In the presence of progesterone, HAND2 is upregulated and inhibits epithelial proliferation. We hypothesize that HAND2 mediated stromal epithelial-signaling plays a role in the pathogenesis of hyperplasia, EMC and in the therapeutic effect of progesterone.

Design: Archival paraffin embedded serial endometrial biopsies of 34 patients receiving progesterone were examined by IHC for expression of HAND2. Patients ranged in age from 20-66 years old. 16 patients received progesterone therapy via a hormone-releasing intrauterine device and 18 patients received intramuscular progesterone. Reasons for therapy included contraception (6/34), dysfunctional uterine bleeding (DUB) (9/34) and endometrial hyperplasia or EMC (19/34). HAND2 expression was scored as negative, weak, moderate or strong. Benign endometrium was used as a control.

Results: Of the patients with hyperplasia or EMC on initial biopsy, 32% progressed to or persisted with EMC, while the remaining 68% patients improved. HAND2 expression in the final specimen received from patients who progressed to EMC was negative in

5/6 of cases (83%), while HAND2 expression in patients who did not progress was either moderate or strong. Patients treated for DUB had strong HAND2 expression in 67% and moderate expression in 33%. All patients who received progesterone for contraception had moderate to strong HAND2 expression. Decidualization seen in response to progesterone effect always revealed moderate to strong HAND2 expression.
Conclusions: Despite the presence of exogenous progesterone in cases progressing to EMC, HAND2 was consistently not expressed. In contrast, HAND2 expression was maintained in cases responding to therapy, in areas of decidualization in response to progesterone, and in benign endometrium. These findings suggest that loss of HAND2 expression is an event in the progression of hyperplasia and EMC. The role of HAND2 in the stromal-epithelial signaling pathway may also explain why progesterone can be successfully used for the treatment of hyperplasia and selected EMC.

1118 High-Risk Human Papillomavirus Load and Biomarkers in Cervical Intraepithelial Neoplasia and Cervical Cancer

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Background: To elucidate the clinical significance of high-risk human papillomavirus (HPV) DNA load in predicting the presence and grade of uterine cervical intraepithelial neoplasia (CIN) and cervical cancer, and to investigate biomarkers for HPV-related oncogenesis.
Design: HPV DNA load test was performed in 343 women, liquid-based cervical cytology in 339 women, and colposcopic biopsy and immunohistochemistry for $p16^{INK4A}$ (p16), cyclin D1, p53, cyclooxygenase-2 (COX-2) and Ki-67 in 143 women.
Results: HPV DNA load, log10-transformed HPV DNA load and HPV infection status were correlated with the cytological and histological severity of cervical disease ($P <$ 0.05, respectively). Moreover, even in negative cytology group or 'atypical squamous cells of undetermined significance (ASCUS)' cytology group, the histological severity of cervical disease was associated with HPV DNA load, log10-transformed HPV DNA load and HPV infection status ($P< 0.05$, respectively). Besides, in the 'ASCUS' cytology group, 2.385 pg/ml of HPV DNA load appeared to be the cutoff value at which 'histological negative' or CIN I can be differentiated from 'histological high-grade' cervical diseases (AUC = 0.712, specificity = 0.721, sensitivity = 0.800), but not statistically significant ($P = 0.125$). The histological severity of cervical disease was positively associated with p16, COX-2, Ki-67 expression and HPV infection, but was negatively correlated with cyclin D1 expression ($P < 0.05$, respectively).
Conclusions: HPV DNA loads can be auxiliary data in predicting the presence and severity of CIN. However, in the ASCUS group, there is no absolute cutoff value of HPV load by which 'histological high-grade' cervical diseases can be predictable, although 2.385 pg/ml seems to be a tempting cutoff. The p16, COX-2 and Ki-67 proteins may be involved in HPV-associated cervical oncogenesis.

1119 Early Genomic Instability in Tubo-Ovarian Preneoplastic Lesions

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Background: Genetic instability plays an important contribution in ovarian carcinogenesis. We investigated the molecular mechanisms underlying the telomere shortening in early and pre-invasive stages of ovarian cancer, serous tubal intraepithelial carcinoma (STIC), precancerous serous tubal intraepithelial lesions (STIL) and ovarian dysplasia (OD).
Design: 51 STIL and OD from prophylactic salpingo-oophorectomies with BRCA1 mutation, 12 STICs, 43 high-grade serous ovarian carcinoma and 36 non cancerous controls were laser-capture microdissected from formalin-fixed paraffin-embedded sections, analyzed by comparative genomic hybridization (array CGH) and for telomere length (using quantitaive real-time polymerase chain reaction based on the Cawthon method). STIL, OD and STIC were defined by morphological scores and immunohistochemical expressions of p53, Ki67 and gammaH2AX.
Results: We found few subtle genomic alterations in dysplastic epithelium from STIL and OD in opposition to the more important genomic imbalances in STIC (most common regions with gain in chromosomes 19q, 16p, 12q, 10q and 11q and with loss in chromosomes 3q, 2q and 11q). The total number of genetic alterations was the highest in ovarian cancers. STIC had the shortest telomeres followed by STIL and OD ($p< 0.007$). Ovarian carcinoma had shorter telomeres than non cancerous controls ($p<0.01$) but statistically longer than STIC, STIL and OD. We found also a statistical correlation between gH2AX expression and telomere shortening ($p=0.0016$).
Conclusions: These findings suggest that genetic instability occurs in early stages of ovarian tumorogenesis. STICs and non invasive lesions are probably an important step in early serous ovarian neoplasia.

1120 Cellular Plasticity of Tubal and Ovarian Surface Epithelium: The Mixed Mesothelial-Mullerian Phenotype

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Background: There is accumulating evidence that the majority of high-grade serous carcinomas originate from the distal fallopian tube (FT), and several recent studies have suggested a similar model for low-grade serous neoplasms. There are however, serous carcinomas that bear striking resemblance, by morphology and immunoprofile, to epithelioid mesothelioma, suggesting that the cell of origin is capable of mesothelial differentiation. The purpose of this study is therefore to identify the process of transdifferentiation of ovarian surface epithelium (OSE) to Mullerian epithelium in salpingo-oophorectomy specimens.
Design: The distribution and intensity of immunostaining for Mullerian epithelial markers (E-cadherin, CA-125) and the mesothelial marker calretinin were analyzed in the distal FT and OSE in salpingo-oophorectomy specimens with epithelial inclusion cysts, serous cystadenoma, salpingitis or serous borderline tumour.
Results: In the OSE, the majority of flat/cuboidal cells exhibit a mesothelial phenotype (calretinin+/E-cad-/CA125-), with focal positivity for E-cadherin and CA-125. Columnar epithelium within cortical clefts and surface papillomatosis show a mixed mesothelial/Mullerian phenotype (calretinin+/E-cad+/CA125+). Cystic lesions, lined by ciliated or flat/cuboidal epithelium, may be composed of cells with Mullerian, mesothelial, or mixed immunoprofiles. Occasional calretinin+ cells are often present in the distal FT. Strong calretinin staining may be observed in the setting of salpingitis.

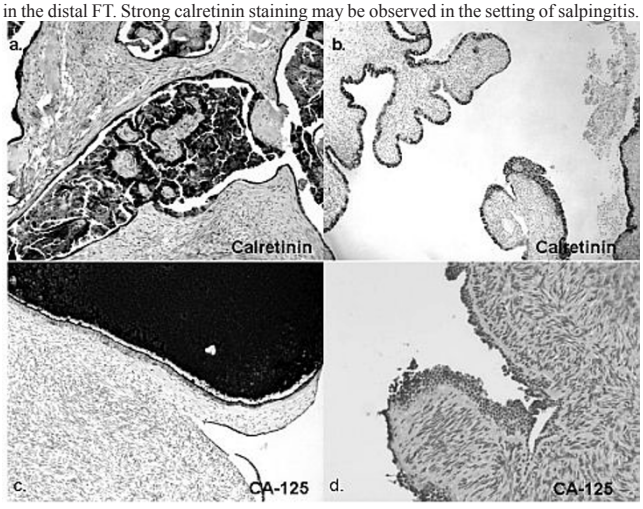

Figure 1: Strong calretinin positivity in serous borderline tumour, suggestive of origin from OSE (a); and in distal FT with acute salpingitis (b). CA-125 staining in cortical inclusion cyst lined by tubal-type epithelium and adjacent OSE (c), and in surface papillomatosis (d).
Conclusions: The OSE is capable of partial Mullerian epithelial transdifferentiation, whereas reactive FT epithelium can exhibit features of mesothelium, consistent with a transitional zone of differentiation at the tubal-ovarian junction. These preliminary results suggest that at least some serous ovarian tumors could originate via Mullerian metaplasia of the OSE.

1121 Non-Ascites Forming High Grade Serous Carcinoma of Pelvis Is Associated with a Distinct Immune Signature and Characterised by T and B Cell Infiltration

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Background: Studies have recently reported molecular subtypes of high grade serous carcinoma (HGSC) of the pelvis. Although these subgroups have been shown to have prognostic significance, histologic and clinical correlates of these subgroups are limited. We demonstrate on a limited number of cases that non-ascites forming HGSC of the pelvis is associated with the immune subgroup. In this study we further validate some of the differential biomarkers using tissue microarrays and correlate ascites status with intraepithelial B and T cell infiltration and tumor morphology.
Design: Whole genome gene expression profiling of primary tumor samples of 11 advanced stage high volume ascites HGSC and 9 advanced stage low volume ascites were compared. Significantly differentially expressed genes were mapped to the TCGA dataset. Validation of select differentially expressed biomarkers and assessment of T and B cell infiltration was performed on a tissue microarray containing 51 independent HGSC samples (25 high volume ascites and 26 low volume ascites). Blinded morphologic review of cases for pre-determined morphologic features [mitotic index, tumor infiltrating lymphocytes (TILS), necrosis, Silverberg grade] was performed.
Results: Of the fifty-two differentially expressed probes ($p<0.05$ with a 2-fold change cut-off), the low volume ascites group demonstrated enrichment of genes that regulate antigen presentation and T cell function, and this was shown to correspond to the immune subgroup. On TMA analysis, low volume ascites cases were significantly more likely to harbour intraepithelial T and B cells [CD3 ($p= 0.009$), CD8 ($p= 0.0003$) and CD20 ($p=0.04$)]. HLA-DA, CD 78 and TAP 2 were also significantly more highly expressed in this group. Our Initial results suggest that only TILS was shown to be significant on morphology review.
Conclusions: Low volume ascites HGSC of pelvis is characterised by enhanced immune response as demonstrated both with gene expression profiling, and with the presence of intraepithelial T and B cells and more intense expression of HLA-DA, CD78 and TAP2. Only TILS was found to be useful on morphology review. Additional morphologic studies using training and test set are being performed. Clinical correlates of immune response in HGSC are important as immunotherapy is considered.

1122 Interaction of Platelets with Ovarian Carcinoma Cells Leads to Induction of an Epithelial-Mesenchymal-Transition (EMT)-Associated Gene Expression Profile in Carcinoma Cells

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Background: While the relationship between malignancy and thrombosis has been recognised for centuries, the role of platelets in tumor growth and metastasis has been unclear. An association between malignancy and thrombocytosis has been noted in most solid tumor types, while the presence of induced thrombocytopenia in mouse models has been associated with a reduction in tumor metastasis. EMT represents the first step in the metastatic cascade in tumor cells, when they break free from their epithelial bindings and become primed for invasion. The expression of an EMT phenotype in carcinoma cells has been associated with increased cell survival, greater drug resistance and elevated metastatic potential. A recent study demonstrated that direct exposure to platelets in vitro caused induction of an EMT-associated gene-expression profile in two human breast cancer cell-lines. To date, this has not been demonstrated in any other subtype of human carcinoma. The aim of this study was to investigate whether direct interaction between ovarian carcinoma cells and platelets induced a similar EMT gene-expression signature.

Design: SKOV3 cells were seeded in 6-well plates and allowed to adhere overnight, followed by incubation with platelets.At the 24 hour time point, cells were harvested, RNA was extracted and gene expression analysis was performed using real time Taqman polymerase chain reaction (PCR) on a panel of EMT-associated markers.

Results: The adhesion of platelets to SKOV3 ovarian carcinoma cells resulted in the induction of an EMT phenotype. Gene expression analysis demonstrated changes in expression of EMT markers, including an increased expression of PAI-1 (Serpine 1), Snail, Slug and Vimentin, and reduced expression of epithelial cell markers E-cadherin and Claudin.

Conclusions: The direct interaction of platelets and ovarian carcinoma cells in vitro leads to induction of an EMT-like gene expression profile in ovarian carcinoma cells. This study demonstrates that this effect is not limited to breast carcinoma cells, and is likely to be a common feature of many forms of carcinoma. These results suggest a role for platelets in maximising the survival of metastatic carcinoma cells within the bloodstream, augmenting their ability to establish distant metastases.

1123 A Seven Year Survey of Serous Tubal Intraepithelial Carcinoma in Surgical Specimens from BRCA+ and Routine Salpingo-Oophorectomies Using the SEE-FIM Protocol

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Background: Serous tubal intraepithelial carcinoma (STIC) is considered an early phase of high-grade pelvic serous carcinoma (HGSC). Patients harboring mutations in the BRCA1 and BRCA2 genes (BRCA+) are at increased risk for HGSC and often opt for risk-reduction salpingo-oophorectomy (RRSO). Because STIC is clinically and radiographically undetectable, its true incidence in BRCA+ patients and in the general population is unknown. In 2005 we instituted the SEE-FIM protocol for analysis of BRCA+ tubes and a modified SEE-FIM protocol with exam of the entire fimbriae in all benign and malignant specimens for which an intact fallopian tube was received. The goal of this study was to arrive at a reasonable estimate of STIC in these two populations.

Design: 380 RRSOs performed on BRCA+ women along with approximately 2500 salpingo-oophorectomies (unilateral or bilateral) in women over age 50 performed for an indication other than BRCA+ or ovarian cancer were examined between 2005 and 2012. The latter encompassed only cases in which the entire fallopian fimbriae were received intact and included specimens received for benign conditions such as fibroids or pelvic pain, carcinomas of the endometrium or cervix. The records were reviewed to determine the number of STICs in each of the two populations.

Results: STICs were detected in 17 of 380 BRCA+ women for an incidence of 4.5%. The mean age was 56 (41-76). In women undergoing surgery for other indications, 6 cases of STIC and two of severe tubal atypia were discovered. Excluding the two atypias and three cases with early high-grade endometrial carcinoma, three of 2500 ((1:833 or .12%) fulfilled the criteria for an incidental STIC. Two were associted with low grade endometriod endometrial carcinomas and a third with a synchronous low grade mucinous tumor of the opposite ovary. The frequency of BRCA+ STIC was approximately 38 times that of incidental STICs.

Conclusions: In this study, BRCA+ women had a nearly 40 fold higher frequency of STIC relative to women undergoing surgery in whom a tubal carcinoma was not the indication. This analysis provides baseline data for estimating the likelihood of encountering a STIC in the general population and the frequency (1:833) approximates the prevalence of ovarian cancer in women over age 50. The rarity of this entity suggests a relatively brief period of clinical latency once carcinoma develops.

1124 MyD88 Is Central to the Process of Differentiation in Cancer Stem Cells Which May Explain Its Role in Chemoresistance of Ovarian Cancer

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Background: The prognosis of ovarian cancer is poor in part due to the high frequency of chemoresistance. Toll-like receptor-4 (TLR-4), its adaptor protein MyD88 and two MyD88-targeting microRNAs (miRs-21 and -146a) have been linked to ovarian cancer and cancer stem cells (CSCs), and the TLR-4/MyD88 pathway is believed to mediate some of this resistance and aggressive behaviour. The aim of this study was two-fold; to investigate the functional role of TLR-4/MyD88 in ovarian cancer and chemoresistance and to determine the role of TLR-4/MyD88 in a cancer stem cell model.

Design: Archival tissue samples, from 129 patients with EON and 50 with normal ovaries, were evaluated for TLR-4 and MyD88 by immunohistochemistry. Expression of microRNAs miR-21 and -146a was assessed in a subset of cancers in parallel with TLR-4/MyD88 gene expression, as well as in a series of chemosensitive and resistant ovarian cancer cell lines (n=9) using RT-PCR. Knockdown of TLR-4/MyD88 was carried out in SKOV-3 ovarian cancer cells. 2102Ep [nullipotent] and NTERA-2 [pluripotent] embryonal carcinoma cells were used as a model system to examine the influence of MyD88 on differentiation capacities in embryonal CSCs. In addition, differentiation capacity assays using retinoic acid [RA] were performed using the embryonal CSC model.

Results: TLR-4 expression was ubiquitous, whereas MyD88 was restricted to neoplastic cells, independent of tumour grade, associated with reduced patient survival, chemoresistance, and inversely linked to miR-21 and miR-146a. Knockdown of TLR-4 in SKOV-3 ovarian cells recovered chemosensitivity. Knockdown of MyD88 alone did not suggesting an intact/functioning TLR-4/MyD88 pathway is required for the acquisition of the chemoresistant phenotype. RA differentiation of pluripotent NTERA-2 cells demonstrated significant down-regulation of MyD88 protein expression. Forced RA differentiation in nullipotent 2012Ep cells did not show MyD88 down-regulation. Knockdown of MyD88 renders 2102Ep open to RA differentiation. Over-expression of MyD88 renders NTERA-2 cells nullipotent suggesting that MyD88 is centrally involved in differentiation mechanisms in embryonal CSCs. Thus MyD88 is necessary and sufficient for embryonal carcinoma nullipotency.

Conclusions: These findings demonstrate that expression of MyD88 is linked to ovarian malignancy and associated with significantly reduced patient survival, chemoresistance and altered microRNA levels. In addition, MyD88 is central to differentiation in a cancer stem cell model which may explain its role in chemoresistance in ovarian cancer.

1125 Endometrial Stromal Sarcomas with Sex Cord Differentiation Are Associated with PHF1 Rearrangement

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Background: Endometrial stromal tumors (ESTs) may pose diagnostic challenges particularly when they exhibit variant histologic appearances, involve extrauterine sites, or present as metastatic disease. In such cases, immunohistochemical markers as well as identification of specific nonrandom chromosomal rearrangements may be helpful. Over the last decade, fluorescence in situ hybridization (FISH) has been progressively incorporated as a diagnostic tool for the evaluation of ESTs. The purpose of this study was to review a series of these tumors and compare the results of FISH analysis with the clinicopathological characteristics.

Design: Three endometrial stromal nodules (ESN), 13 endometrial stromal sarcomas (ESS), and 7 undifferentiated endometrial sarcomas (UES) were reviewed. Three metastases from one of the ESS were also analyzed. Nine of these tumors (1 ESN, 8 ESSs, and 1 UES) exhibited unusual histological features, including smooth muscle (3), sex cord (7), epithelioid (1), fibromyxoid (1), and skeletal muscle (2) differentiation. A tissue microarray (TMA) was prepared and FISH analysis was performed using break-apart and fusion probes for JAZF1, SUZ12, EPC1, and PHF1 genes.

Results: FISH was successful in 22 cases and rearrangements involving JAZF1, SUZ12, EPC1, and PHF1 genes were detected in 10 of the 22 (45%) uterine tumors, including 2 of the 3 ESNs, and 8 of 12 ESSs. Genetic rearrangements were found neither in the three metastases of the ESS nor in any of the UES. Noteworthy, a correlation between sex cord differentiation and PHF1 rearrangement was encountered in endometrial stromal sarcomas (p=0.008).

Conclusions: In our series, all ESSs showing sex cords had PHF1 genetic rearrangement, suggesting that such rearrangement may induce sex cord differentiation.

1126 Evaluation of Wilm's Tumor-1 (WT-1) Immunohistochemistry in Synchronous Primary Adenocarcinomas of the Endometrium and Ovary

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Background: Primary ovarian papillary serous (OPS) carcinomas often demonstrate metastases to the uterus. Occasionally, specimens show tumor within the ovary as well as endometrium without evidence of metastases to the uterine serosa. Many consider these to be synchronous primary papillary serous (SPPS) carcinomas that developed independently. WT-1 immunohistochemistry has been shown to be positive in > 90% of primary OPS carcinomas but in less than 20% of cases of isolated primary endometrial papillary serous (EPS) carcinoma; endometrioid pattern carcinomas are consistently negative. These different immunoprofiles suggest separate biological mechanisms of development for OPS and EPS. To our knowledge, no study has evaluated a series of SPPS to determine if the ovarian and endometrial components demonstrate a similar or dissimilar WT-1 immunohistochemical profile.

Design: The following cases were collected from UAB archives: SPPS (N=7), synchronous primary endometrioid adencocarcinoma (N=11), and isolated EPS adenocarcinoma (N=12). WT-1 IHC was performed on representative tumor sites and the slides were evaluated on an intensity scale based on percentage of darkly staining tumor cells: 0, 1+ (<20%), 2+ (20-50%), 3+ (50-80%), 4+ (80-100%). Cases showing 2+ and greater staining were considered significantly positive.

Results: 57% of the SPPS cases (4/7) showed strong staining (3+ and 4+) in both the ovarian and endometrial tumor. Positive cases showed similar staining profiles while negative cases showed absence of expression in both tumors. WT-1 was positive in 17% (10/12) of the isolated EPS cases. All 11 synchronous endometrioid cases were negative for WT-1.

Conclusions: Our results for isolated EPS and synchronous endometrioid carcinomas are in concurrence with previous studies. A greater percentage of SPPS cases showing WT-1 expression and absence of heterogeneity between both carcinoma components suggest two possibilities: 1) metastasis of tumor between ovary and endometrium instead of two independent tumors and 2) SPPS tumors have a different biological mechanism of development due to negative WT-1 in the majority of isolated EPS while WT-1 showed expression within the endometrial tumor in a majority of SPPS cases. Additionally, both mechanisms may play a role. Distinguishing these possibilities is important for determining prognosis and appropriate therapy in these patients. Future studies involving a greater number of these cases as well as molecular evaluation are required to further evaluate these unusual tumors.

1127 Differential Vimentin Expression in Ovarian and Endometrial Endometrioid Adenocarcinomas: Diagnostic Utility in Distinguishing Double Primaries from Metastatic Tumors

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Background: When an endometrioid adenocarcinoma involves both the endometrium and the ovary, the challenge of diagnosing synchronous lesions, and distinguishing such tumors as metastasis from one organ to the other, is well known. While vimentin is known to be strongly positive in endometrioid adenocarcinoma of primary endometrial origin, its expression in ovarian counterpart has not been analyzed in detail. We assess the diagnostic value of vimentin expression in resolving these diagnostic scenarios.

Design: Immunohistochemistry with anti-vimentin antibody was performed on whole tissue sections from endometrioid adenocarcinoma 1) confined to endometrium (n=25), 2) metastatic from endometrium to distant sites (n=14), 3) involving endometrium and ovary and thought to be synchronous primaries (n=5), 4) metastatic from endometrium to ovary (n=7), 5) metastatic from ovary to endometrium (n=5), 6) metastatic to regional lymph nodes (n=10), and 7) tissue microarrays of 91 primary endometrial and 23 primary ovarian endometrioid carcinomas (pan-stage). Vimentin expression was semiquantitatively scored as negative, weak/moderately and strongly positive.

Results: Table 1 summarizes the results of vimentin expression. Vimentin was negative in 98% (38/39) of primary ovarian endometrioid carcinoma irrespective of whether or not they were associated with an endometrial tumor. In contrast, 84% of primary endometrial carcinomas were vimentin-positive. Five cases with double primary tumors were positive in endometrial tumors and negative in ovarian counterparts. The pattern of vimentin expression in the endometrioid tumors was maintained in their respective distant metastases. All normal endometrial glands strongly expressed vimentin. No correlation was identified between vimentin staining and tumor grade or clinical stage.

Table 1: Differential vimentin expression in ovarian and endometrial endometrioid adenocarcinomas

Vimentin (IHC)	Primary endometrial origin				Primary ovarian origin			
	Endometrium	Ovary	LN met	Distant met	Ovary	Endometrium	LN met	Distant met
Positive	107 (84%)	7 (100%)	4	11	1 (2%)	0	0	0
Negative	21 (16%)	0	2	0	38 (98%)	5 (100%)	4	3
Total	128	7	6	11	39	5	4	3

Conclusions: Ovary and endometrial endometrioid adenocarcinomas have different patterns of vimentin expression with high sensitivity (97%), specificity (84%) and negative predictive value (99%). As such, vimentin expression may be an excellent diagnostic tool in differentiating endometrioid adenocarcinomas of primary endometrial from primary ovarian origin, and for diagnosing double primary tumors.

1128 Analysis of EPCAM Expression in Lynch Syndrome Associated Neoplasia

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Background: Lynch syndrome is an inherited tumor predisposition syndrome due to defective expression of DNA mismatch repair enzymes, leading to microsatellite instability. Affected individuals have increased risk of developing colorectal, uterine, and sebaceous neoplasms. Mutations in *MLH1* and *MSH2* account for most cases, but deletions of the 3' end of *EPCAM*, located upstream of *MSH2*, have been implicated in approximately 20% of cases with MSH2 loss. Some studies have suggested significantly lower incidence of uterine neoplasia in this variant of Lynch syndrome. Loss of EPCAM protein expression has been observed in colorectal tumors associated with *EPCAM* deletion; however, no studies have characterized the expression of EPCAM protein in MSH2 deficient Lynch syndrome neoplasia other than colorectal tumors.

Design: A total of 288 colorectal, 481 uterine, and 56 sebaceous tumors were analyzed for MSH2, MSH6, MLH1, and PMS2 expression by immunohistochemistry. EPCAM immunohistochemistry was performed on tumors with loss of MSH2 as well tissue microarrays containing 251 colorectal and 62 uterine adenocarcinomas not associated with Lynch syndrome.

Results: 33 tumors from unique patients showed loss of MSH2 (7 sebaceous neoplasms, 17 uterine carcinomas, and 9 colorectal carinomas). EPCAM expression results for these tumors and Non-Lynch syndrome tumors are shown in Table 1. Tumors with membranous expression of EPCAM were scored as positive.

EPCAM Expression in MSH2 Negative Lynch Syndrome and Non-Lynch Syndrome Tumors

Tumors	EPCAM expression by IHC (%)
Non Lynch syndrome colorectal carcinoma	251/251 (100)
Non Lynch syndrome uterine carcinoma	62/62 (100)
MSH2 negative sebaceous neoplasms	0/7 (0)
MSH2 negative colorectal carcinoma	8/9 (89)
MSH2 negative uterine carcinoma	16/17 (94)

Conclusions: EPCAM is universally and strongly expressed in colorectal and uterine neoplasms not associated with Lynch syndrome. Loss of EPCAM expression is seen in 11% of MSH2 negative colorectal carcinoma and 6% of MSH2 negative uterine carcinoma. To our knowledge, this is the first demonstration of EPCAM loss in Lynch syndrome related uterine carcinoma. EPCAM immunohistochemistry may be useful in further classifying Lynch syndrome in patients with loss of MSH2 in colorectal and uterine neoplasms. EPCAM immunohistochemistry is not useful in workup of sebaceous neoplasms with MSH2 loss as these tumors are universally negative.

1129 Improving the Value of the Endometrial Biopsy: Molecular Diagnostics for Predicting Tumor Histotype and Stage

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Background: Lymphadenectomy for endometrial carcinoma (EC) extends operative time and causes morbidity, so it is best reserved for patients who would benefit most from the extended surgery. By distinguishing between endometrioid and non-endometrioid carcinomas, the endometrial biopsy (EMB) guides the extent of surgical staging. This distinction can be sometimes difficult histologically, especially with carcinomas with ambiguous features. Immunohistochemistry with p53 is of limited utility, as many high grade endometrioid tumors also over-express p53. Furthermore, for endometrioid carcinomas, routine microscopic examination of an EMB does not reliably yield information on risk of nodal disease. For breast and endometrial carcinomas, higher expression of estrogen-induced genes is associated with better prognosis. The purpose of this study was to determine if quantitative assessment of such genes in EMBs could provide "value added" to the pathologic evaluation of the EMB.

Design: 128 EC cases (105 endometrioid, 23 non-endometrioid) with formalin-fixed, paraffin-embedded (FFPE) pre-operative EMBs were identified. qRT-PCR was performed to quantify the expression of EIG121, RALDH2, sFRP4, IGF-I and IGF-IR. These genes are induced by estrogen in the endometrium, and previously published genomic work performed on frozen endometrial tissues identified EIG121 to be the single best molecular fingerprint to distinguish endometrioid from non-endometrioid carcinomas. Transcripts were correlated with tumor histotype and stage in the final hysterectomy surgical specimen.

Results: EIG121 and sFRP4 were significantly increased in endometrioid carcinomas compared with non-endometrioid tumors ($p<0.0001$). When considering the endometrioid tumors only, RALDH2 was significantly decreased ($p<0.0001$) in EMBs for which the corresponding hysterectomy had positive lymph nodes. All of the endometrioid tumors with lymph node metastases were FIGO grade 2 tumors. RALDH2, sFRP4 and IGF-I were significantly elevated ($p<0.0001$) in early stage (I and II) endometrioid tumors compared to their advanced stage (III and IV) counterparts.

Conclusions: Analysis of estrogen-induced genes can be applied to FFPE EMBs to provide important, clinically relevant data, such as tumor histotype and stage. These assays expand the clinical value of the EMB, particularly when histological information is limited, permitting up-front identification of patients with high risk tumors who require more aggressive clinical management.

1130 Utility of Phospho-Histone H3 in Problematic Uterine Smooth Muscle Tumors

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Background: Phospho-histone H3 (PHH3), a mitosis specific immunomarker, has been suggested as a robust way of evaluating mitotic index (MI) in neoplasms wherein MI is important in accurate classification. MI is a key histologic feature in classifying uterine smooth muscle tumors but there are several confounding factors. There is only one pilot study that suggested PHH3 as usefu in separating smooth muscle tumors of uncertain malignant potential (STUMP) from leiomyosarcoma (LMS). No studies have been done evaluating the PHH3 usefulness in other smooth muscle tumors such as cellular/mitotically-active and atypical/ bizarre leiomyomas (CL-BL) ocasionally difficult to separate from STUMP and LMS. The aim of this study was to evaluate the utility of PHH3 immunolabeling in a group of morphologically challenging smooth muscle tumors.

Design: 9 cases of CL-BL, 6 of STUMP and 5 of LMS (1 myxoid LMS) from 2000 to 2012, previously diagnosed using the presently accepted morphologic criteria, were retrieved from the archives of the Department of Pathology at Hartford Hospital. Representative H&E slides were reviewed, PHH3 immunohistochemical stains were performed and positivity scored by 2 pathologists in the hot spot areas of staining as # positive cells/ 10 HPF (PHH3 MI). The mean MI of each group (CL-BL, STUMP and LMS) was then calculated.

Results: See

Pathologic and immunophenotypic findings in the selected uterine smooth muscle tumors

	CL (n=9)	STUMP(n=6)	LMS (n=5)
MI	0-10 mitoses/10HPF (mean: 1.8)	0-10 mitoses/10HPF (mean: 4)	3-15 mitoses/10HPF (mean: 10)
Necrosis	Absent	Absent	Present in 4 out of 5 cases
Atypia	Mild (7) and moderate (2)	Moderate (3) and severe (3)	moderate (4) to severe (1)
PHH3 MI	0-11 mitoses/10HPF (mean: 4)	0-15 mitoses/10HPF (mean:6.5)	5-30 mitoses/10HPF (mean: 12)

Conclusions: There is excellent correlation between MI and PHH3 MI. The PHH3 immunoreactivity is easy to evaluate with mitotic figures readily identifiable making mitotic count more reliable, particularly in the CB-BL cases where degenerative atypia or apoptotic figures are prominent. These preliminary results suggest that a PHH3 MI standard could be established for categorization of problematic smooth muscle tumors. This could be useful in the gynecological pathology grey zone wherein accurate separation of CL-BL, STUMP and LMS remain challenging in routine practice as well as in low-grade LMS, myxoid and epitheliod smooth muscle tumors, wherein mitoses can be hard to find and diagnosis of malignancy difficult to establish.

1131 Papillary Serous Carcinoma of the Cervix – Two Diseases with Distinct Clinico-Pathologic Profiles?

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Background: Serous carcinoma of the cervix (SCx) is a rare variant of cervical adenocarcinoma with fewer than 50 cases reported. Original reports suggest a bimodal age distribution raising the possibility of separate etiopathogenesis. 13 additional cases are reported with clinico-pathologic details and TP53 mutational analysis.

Design: Clinicopathologic data were collected, immunohistochemistry (IHC) for p53, p16 and WT1and *TP53* mutational analysis from exons 4 to 8 were performed where possible.

Results: Three women were below 50; all are alive and well up to 20 years after diagnosis. Two had a co-existent non-serous component. In one case where additional studies yielded results, the serous component was p53 positive and demonstrated HRHPV by ISH. 10 women were postmenopausal (ages 54 –70 yrs), of whom 3 are dead of disease with intraabdominal metastases and two are alive with intraabdominal disease. All of these cases had pure serous carcinoma. Tumor was p53 and p16 positive in all cases. In 9 of 10 cases, the tumor was also WT1 positive. In the 5 relatively recent cases where the fallopian tubes were examined by the SEE-FIM protocol, serous tubal intraepithelial carcinoma (STIC) was identified. 2 of 3 cases demonstrated identical *TP53* missense mutations at both sites. The last case was WT1 negative at both sites, and *TP53* mutation was not detected.

Conclusions: So-called SCx may represent two distinct diseases. In young patients, SCx represents a vanishingly rare variant of cervical adenocarcinoma which does not necessarily have the often poor prognosis of traditional serous carcinomas. At least in some cases, it is HPV related. In older patients, at least some cases represent metastatic serous carcinoma from an occult tubal primary. These findings underscore the importance of using the SEE-FIM protocol for the examination of fallopian tubes in cases of possible cervical serous carcinomas.

1132 Incidental Gynecologic Malignancies in Morcellated Hysterectomies

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Background: Laparoscopic hysterectomy with morcellation (LHM) has become popular for being safe & less invasive; it has been shown to reduce postoperative hospital stay & shorten patient recovery. A decrease in morbidity, blood loss & recovery time has been noted compared to vaginal hysterectomy. Sparse cases of incidental gynecologic malignancies after LHM have been reported. The current study aimed to determine the frequency & types of incidental malignancies after LHM at a high volume institution.

Design: An electronic chart review was conducted searching all cases of LHM +/- cervices performed 01 January 2007 to 31 January 2012 at one institution. The incidence of gynecologic malignancies along with patient demographics & any preoperative investigation was noted.

Results: 352 cases of LHM were identified. There were three occult malignancies (0.9%), all with benign preoperative endometrial sampling. Case #1 was a 48 y/o with fibroids. Pathology after LHM revealed low grade endometrial stromal sarcoma with myometrial invasion & vascular space involvement. A focus of metastatic sarcoma was present in the right peri-adnexal soft tissue after subsequent staging. Case #2 was a 49 y/o with a longstanding history of menorrhagia s/p several D&Cs showing benign endometrial polyps. Pathology after LMH showed a grade 1 endometrioid adenocarcinoma, without myometrial invasion. Case #3 was a 47 y/o with menorrhagia and fibroids. Pathology after LHM showed a uterine tumor resembling ovarian sex cord tumor (UTROSCT) with an infiltrating growth pattern. There were four (1.1%) benign non-smooth muscle neoplasms (two adenomatoid tumors and two ovarian Brenner tumors). There were five (1.4%) atypical smooth muscle tumors, which posed a diagnostic dilemma because the border of the lesion and border of necrosis were disrupted due to the morcellation; the differential included smooth muscle tumors of unknown malignant potential (STUMP) and leiomyosarcoma.

Conclusions: Although LHM is a popular technique that is considered safe, there is a clinically important risk of occult malignancy. Proper pathologic evaluation, including staging, is limited when a malignant uterus is morcellated. In highly atypical smooth muscle lesions, diagnostic data maybe lost after LHM posing a diagnostic dilemma. This risk persists despite appropriate clinical preoperative work-up. Pathologists must be aware of the rate of malignancy (0.9%) in LHM specimens, approach these specimens grossly and microscopically with care, and educate clinicians.

1133 DPC4 Immunostaining in Mullerian Tumors with and without Mucinous Differentiation

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Background: Mucinous tumors in the ovary always raise a concern for a metastasis, and require a panel of immuno stains to determine site of origin. DPC4 immuno stain is always included in the diagnostic panel as the absence of staining for this antibody supports pancreatic origin of the tumor. While studies addressing DPC4 staining pattern in primary and metastatic mucinous tumors of pancreatic origin are well documented, knowledge of DPC4 in ovarian tumors or tumors of mullerian origin with or without mucinous differentiation is very limited. To address this issue, we stained tissue microarrays (TMAs) constructed from various mullerian and ovarian tumors with DPC4. In addition PAX8 was performed to ratify müllerian origin if DPC4 staining was negative.

Design: TMAs were constructed with three fold redundancy from various müllerian tumors with DPC4 results available on 37 endocervical adenocarcinomas, 55 endometrial and 113 ovarian tumors. An H score was used to evaluate both antibodies with negative interpretation for scores 0-10, and positive for 11-300.

Results:

DPC4 results

Tumor	Negative	Positive
EC-usual type CA	2 (11%)	17 (89%)
EC-variant CA	0 (0%)	18 (100%)
EM-endometrioid CA	1 (3%)	38 (97%)
EM-non endometrioid CA	1 (6%)	15 (94%)
OV-endometrioid CA	1 (8%)	11 (92%)
OV-high grade serous CA	0 (0%)	21 (100%)
OV-clear cell CA	4 (10%)	35 (90%)
OV-mucinous CA	0 (0%)	2 (100%)
OV-serous BT	0 (0%)	19 (100%)
OV-mucinous BT	0 (0%)	16 (100%)
OV-seromucinous BT	0 (0%)	4 (100%)

EC:endocervix EM:endometrial OV:Ovary CA:carcinoma BT:borderline tumors

Of the 2 DPC4 negative endocervical carcinomas, one was also negative of PAX 8. Of the 2 DPC4 negative endometrial carcinomas, one was endometrioid and another clear cell and both were positive for PAX8. Of the 5 DPC4 negative ovarian tumors, 3 (1 endometrioid and 2 clear cell) were negative for PAX 8 and 2 (both clear cell) positive. Negative results for PAX 8 staining were seen in 46% of endocervical, 15% of endometrial, and 24% of ovarian tumors.

Conclusions: DPC4 can be negative in müllerian tumors with or without mucinous differentiation. Our study expands the list of DPC4 negative tumors from pancreato-biliary tract, and colo-rectal carcinomas to include common mullerian tumors. Caution is advised in interpreting loss of DPC4 as *sine qua non* for pancreatic carcinoma. If there is clinical and histologic suspicion of metastatic tumor in the ovary, it is best to perform an expanded panel that includes site specific markers in addition to different cytokeratins.

1134 MicroRNAs Expression Varies with Stromal Signatures in Myoinvasive Endometrioid Endometrial Carcinomas

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Background: The tumor microenvironment reflects the interaction between cancer cells and host stromal cells including fibroblasts, endothelial cells, and inflammatory cells. We have previously described several robust stromal response patterns that vary across myoinvasive endometrioid carcinomas. The macrophage (CSF1) signature was associated with higher tumor grade, lymphovascular invasion, and *PIK3CA* mutations. MicroRNAs (miRNAs) are post-transcriptional regulators involved in several biological processes and their deregulation is implicated in cancer. MicroRNA expression patterns may be of value for prognostic determinations as well as for eventual therapeutic intervention. In this study, we explore whether miRNAs expression varies with stromal signatures in myoinvasive endometrioid carcinomas.

Design: We performed comprehensive analysis of miRNA expression profiles (GeneChip® miRNA Array 2.0 Affymetrix) of 6 normal endometria and 24 endometrioid carcinomas in which the stromal signatures had been previously studied both by immunohistochemistry and *in situ* hybridization. Then, we analyzed which miRNAs were differently expressed by the different stromal signatures.

Results: There were distinct miRNA expression profiles for each stromal response as demonstrated by the unsupervised hierarchical clustering. Some miRNAs were differentially expressed in each stromal response indicating their possible functional role in this subset of tumors.

Conclusions: Our data show that different stromal responses have distinct miRNA expression patterns. The identification of unique miRNA signatures in each tumor type may have important clinical applications. The finding of some miRNAs differentially expressed in each stromal signature suggests their implication in tumor microenviroment and progression.

1135 Sentinel Lymph Nodes and Vulvovaginal Melanoma: Comparison of Sentinel Lymph Node Protocols

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Background: Sentinel lymph node (SLN) biopsy is recommended for staging of patients (pts) with clinically node negative melanoma at any site; however, no universal pathology protocol for processing SLNs exists. Most centers utilize a combination of additional H&E levels (lev) and immunohistochemistry (IHC), but the number of

and optimal distance between levels remain subject to debate. This study presents a single institution experience with two different SLN protocols in cases of vulvovaginal melanoma (VVM).

Design: From 1995-2012 30 pts with VVM underwent SLN biopsy. Pt age, follow up, tumor size, tumor thickness, presence/absence of lymphovascular invasion, number/status of SLN and non SLN and size of SLN metastases were recorded. Ultrastaging (US) was performed on 26 pts with negative SLN on the initial H&E slide: H&E lev only, 3 pts; 1H&E lev (at 4-16μ) with IHC, 17 pts; superficial lev (at 4-16μ) plus 4 additional H&E lev (250μ) with IHC, 6 pts; not performed, 2 early pts. To determine whether wide interval (WI) H&E lev plus IHC was superior to one H&E lev plus IHC, 4 additional WI at 250μ were cut in all cases without WI. IHC with a PanMel stain was performed in cases lacking IHC.

Results: Pts' ages ranged from 17-85 yr (62, median) with 5-149 months follow up (29, median) available for 27 pts: alive, 11 pts; alive with disease, 2 pts; dead of disease, 13 pts; dead unknown cause, 1 pts. Tumors (.55 to 5.0 cm; median 1.6 cm) ranged from .43-12.0 mm (2.0 mm, median) thick. 110 SLN were identified (1-11 SLN/pt). 10 pts (30%) had 15 positive (+)SLN. Initial H&E section detected 6 (+)SLNs. US detected 9 (+)SLN; one (+)SLN was seen on the second WI H&E slide. Metastasis size ranged from 2 cells to 14.0 mm. In 4/15 (27%) SLN, metastases were detected by IHC only. 17 pts had 99 non SLN identified, and 2 pts had (+) nonSLN. There were no false negative SLN. Table 1 contains clinicopathologic features of the 30 pts.

Clinicopathologic Features of Pts with SLN

Feature	Positive SLN (n=10)	Negative SLN (n=20)
Median Age (yr)	56.5	65.5
Follow up	6/10 pts DOD	7/20 pts DOD
Tumor thickness (mean, mm)	3.25 ±3.35	3.17 ±3.17
Lymphovascular invasion present	5/10	10/20

Conclusions: An US protocol of 1 H&E lev and IHC improved detection of (+)SLN over one H&E slide (routine processing) alone (6/110, 5.45% vs 14/110, 12.72%). Additional WI H&E lev further increased detection of (+)SLN (15/110, 13.63%). Further study is required to determine whether the modest gain in (+)SLN detection of more comprehensive SLN protocols justifies increased time, labor and cost with respect to patient outcome.

1136 Predicting Behavior in Ovarian Clear Cell Carcinoma: Are We There yet?

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Background: Ovarian clear cell carcinoma (OCCC) is typically not graded although is considered a high grade malignancy. Attempts have been made to link various histopathologic parameters such as endometriosis and the presence of adenofibromatous component to outcome. Recently, some investigators have proposed a grading system based on the architectural pattern of the tumor. In this study, we evaluate the performance of this grading system as well as other histopathologic features as predictors of outcome in a consecutive series of OCCC.

Design: A search of the pathology database from 2002-2007 yielded 68 cases of OCCC with follow up. Mixed epithelial carcinomas containing OCCC were excluded. The following parameters were recorded: pt age; stage; tumor size; presence/absence of endometriosis in the tumor or elsewhere; presence absence of adenofibromatous component; %solid component; presence/absence of single cell invasion; number of mitoses (4 sets of 10 high power fields counted with highest mitotic index (MI) selected); patient outcome.

Results: Pt ages ranged from 29-68yr (median 51). Tumor stage was known in 66 pts: I, 28; II, 16; III, 22. 5-135 mo (median 50) follow up was available: dead of disease, 38pts; alive with disease, 9pts; no evidence of disease at last follow up, 21pts. 15/21pts did not experience recurrence. Percent solid component ranged from 0-80% for all tumors with a median of 10 for pts with and without recurrence. Remaining pathologic features of pts who died of disease or recurred and those who did not recur are presented in Table 1.

Clinicopathologic Features of Ovarian Clear Cell Carcinoma

	Stage I	Tumor size (median cm)	Endometriosis Present	Adenofibroma Present	>10% Solid Component	Single Cells Present	MI
All Cases (n=68)	28 (41%)	12	37 (54%)	9	30 (44%)	19 (28%)	5
Dead of Disease or Recurred (n=53)	18 (34%)	13.5	27 (51%)	7	23 (43%)	18 (34%)	5
No Evidence of Disease/ No Recurrence (n=15)	10 (66%)	10	10 (66%)	2	7 (47%)	1 (6.6%)	4

Conclusions: Low tumor stage is associated with fewer recurrences. There may be an association between improved outcome and presence of endometriosis either in the tumor or elsewhere. Presence of >10% solid component does not appear to differ between tumors that recurred and those that did not. MI was similar between recurrent and non recurrent OCCC. Single cell pattern of invasion was seen less frequently in pts with OCCC who did not recur and could be of value to predict outcome. Additional studies are required to determine whether this finding is significant and reproducible.

1137 Coordinate Patterns of ER, PR and WT1 Expression Are Useful in the Distinction of Ovarian from Endometrial Serous Carcinomas and Significantly Outperform Individual Markers

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Background: The pathologic distinction of ovarian serous carcinoma (OSC) from endometrial serous carcinomas (ESC) is important for accurate tumor staging, epidemiological data extracted therefrom, the reporting of probable primary sites in biopsies or cytologic preparations, and occasionally, chemotherapeutic choices. The purpose of this study is to assess whether composite or coordinate protein expression patterns of estrogen receptor (ER), progesterone receptor (PR) and Wilms tumor suppressor gene (WT1) can significantly distinguish between ESC and OSC.

Design: Imunohistochemical analyses were performed on whole tissue sections from 22 uterus-confined (FIGO stage I/II) ESC and on a TMA of 140 high-grade ovarian serous carcinomas of various stages, using antibodies to ER, PR, and WT1.

Results: ER, PR and WT1 expression was present in 37%, 49% and 81% of OSC respectively, but these markers were also expressed in 18%, 27% & 36% of ESC. The ER+/PR+/WT1+ coordinate profile was identified in 33.6% of OSC but in 0% of ESC (p=0.0006), resulting in a calculated sensitivity and specificity of this profile for OSC of 33.6% and 100% respectively. By contrast, the ER-/PR-/WT1- profile was identified in 41% of ESC but in only 6.4% of OSC (p=0.0001) resulting in a calculated sensitivity and specificity of this profile for ESC of 50% and 94% respectively,

Coordinate Immunophenotypic Patterns

Immunophenotypes	ESC. No of cases (%)	OSC. No of cases (%)	p value (Fisher's Exact test)
ER+/PR+/WT1+	0(0)	47 (33.6)	0.0006
ER+/PR+/WT1-	4(18)	3(2)	0.0070
ER+/PR-/WT1-	0(0)	0(0)	1
ER-/PR-/WT1-	9(41)	2(1.4)	0.0001
ER-/PR-/WT1+	7(32)	62(44)	0.36
ER-/PR+/WT1+	1(4.5)	15(11)	0.69
ER-/PR+/WT1-	1(4.5)	2(1.4)	0.36

Conclusions: Each of the 3 markers was significantly more frequently expressed in OSC than ESC, which affirms the differential localization of these antigens in OSC and ESC, their shared morphologic profile notwithstanding. However, none of them should be used in isolation for distinguishing OSC from ESC, as they each lack discriminatory power when so utilized due to their suboptimal specificity. Furthermore, the use of single markers has a substantial probability of generating erroneous results regarding the site of origin. In the differential diagnosis between OSC and ESC, positivity for all 3 markers strongly favors an extrauterine origin. Negativity for all 3 markers, although supportive of an endometrial origin, is not entirely conclusive in either direction. Composite profiles, in general, have a high specificity but low sensitivity in this differential diagnosis.

1138 The Clinicopathologic Significance of p53 and BAF250a *(ARID1A)* Expression in Clear Cell Carcinoma of the Endometrium

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Background: There is a dearth of information on the molecular events that underlie endometrial clear cell carcinogenesis, and validated biomarkers that are predictive of patient outcome have not been identified. Preclinical studies suggest functional interactions between the BAF250a and p53 proteins (encoded by the tumor suppressor genes *ARID1A* and *TP53* respectively). The significance of p53 and BAF250a expression in clear cell carcinomas of the endometrium (CCC) is assessed herein.

Design: Immunohistochemical analyses for BAF250a and p53 were performed on a group of 50 CCC cases that had previously been subjected to a rigorous review process for diagnostic accuracy, and results were correlated with clinicopathologic parameters. Cases were classified as positive if ≥50% of tumor cells displayed intense immunoreactivity or if the case displayed a null phenotype.

Results: 17 (34%) of 50 cases were p53[+]; the remaining 33 cases had a p53 wild-type (p53-wt) immunophenotype. Of the 10 relapses/recurrences in the entire dataset, 80% were in the p53[+] group (p=0.003). On univariate analyses, 1) the median overall survival (OS) for the p53-wt patients (82.7 mo) was longer than the p53[+] patients (63.1 mo), a difference that approached, but did not attain statistical significance (p=0.07), 2) the median progression-free survival (PFS) for the p53[+] group (56.1 mo) was significantly lower than the p53-wt group (88.2 mo), p=0.01; 3) p53 status was not significantly associated with clinicopathologic factors, including stage distribution and morphologic patterns. On multivariate analyses, p53 expression was not associated with reduced OS or PFS. 10 of the 50 cases were BAF250a-negative, and there was no significant correlation between p53 and BAF250a expression (r = -0.03). The p53+/BAF250a-, p53+/ BAF250a+, p53-/ BAF250a+, and p53-/ BAF250a- composite immunophenotypes were identified in 8%, 26%, 54% and 12% of cases respectively, but neither loss of BAF250a expression nor composite profiles were associated with reduced OS or PFS.

Conclusions: A significant subset of CCC express p53, and these cases are not definable by their morphologic features. p53 expression may be a negative prognostic factor, and warrants additional studies. Loss of BAF250a expression has no prognostic significance in CCC.

1139 Prognostic Factors in Clear Cell Carcinoma of the Endometrium: A Clinicopathologic Analysis of 50 Rigorously Classified Cases

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Background: Clear cell carcinoma of the endometrium (CCC) is an uncommon histotype whose analyses have generally been hampered by its rarity and issues of interobserver diagnostic variability. In this study, we analyzed the clinicopathologic

features of 50 CCCs that were assembled from multiple institutions and which we considered to be morphologically unambiguous after a rigorous, multi-layered review process for diagnostic accuracy.
Design: A wide variety of morphologic and other clinicopathologic features were assessed for any potential relationships with with patient outcomes.
Results: The median patient age was 67 years. FIGO stage distibution was as follows: stage I (n=19), stage II (n=8), stage III (n=14), stage IV (n=9). The 5-year progression-free survival (PFS) for the entire cohort was 61%, and was 88%, 75%, 22% and 28.6% for stages I to IV respectively. On univariate analyses, age >65 years, advanced FIGO stage, and the presence of any lymph node metastases were associated with reduced PFS (p=0.02, 0.002, and 0.002 respectively). There was a trend to reduced PFS for patients with Yamamoto architectural grade C tumors (at least 10% of the tumor composed of solid masses or individual infiltrating tumor cells, but this was not statistically significant (p=0.09). On multivariate analyses, the only variable associated with reduced PFS was patient age >65 years. The 5-year overall survival (OS) for the entire cohort was 78%, and was 94%, 87.5%, 66.7%, and 42.8% for stages I to IV respectively. On univariate analyses, the following factors were associated with reduced OS: age >65 years (p=0.04), advanced FIGO stage (p=0.003), distant metastases (p=0.003), myometrial invasion >30% (p=0.01), a mitotic index of greater than 4 (p=0.014), and Yamamoto architectural grade C (p=0.02). On multivariate analyses, only age >65 years and advanced FIGO stage were associated with reduced OS (p=0.023 and 0.022 respectively).
Conclusions: Age and stage are the principal prognostic factors in CCC. However, our findings suggest that there may be prognostically relevant subsets of CCC that are definable by their pathologic features. Our analysis of this group of morphologically unambiguous CCC indicates that patient outcomes are more favorable than has previously been reported for this histotype.

1140 Mucinous Ovarian Tumors in Childhood – An Institutional Experience

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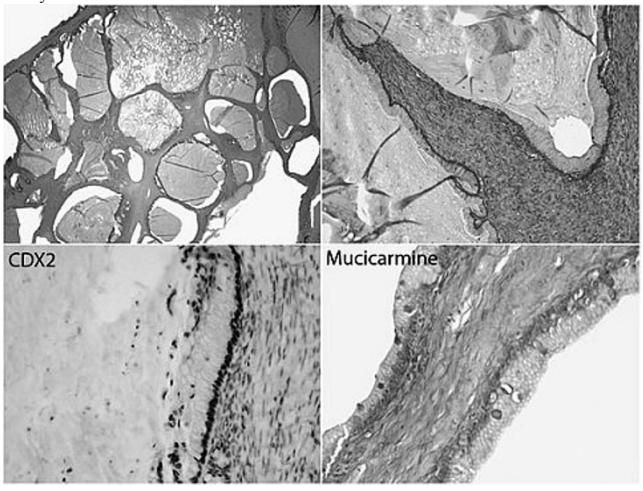
Background: Mucinous tumors of the ovary are primarily diagnosed in middle aged and elder woman and they are very rare in first two decades of life and exceedingly rare in premenarchal girls.
Design: We reviewed our institutional pathology files in the past 20 years and retrieved 15 cases of primary mucinous tumors of the ovary.
Results: Their clinical pathological profiles are as follows: Age range was 6-19 years, average 12.1 years; 4 patients were definitely premenarchal at the age of presentation, 6 of them better be grouped as peri-menarchal and rest of the 5 patients were post-menarchal adolescents. The tumor size ranged from 3.5 to 32 cm, average 14.3 cm The majority (9 cases) of the case were multiloculate but significant portions were unilocular and bilocular (3 cases each) 8 cases happened in the right side, 6 cases left and 1 case bilateral. The majority of the cases were mucinous cystadenoma (10 cases); 3 cases were mixed mucinous and serous cystadenoma and 2 cases qualified for borderline mucinous tumors. All of them presented as stage I tumor and behaved in benign fashion on follow-ups. There was no malignant cases in our study except one case of metastatic mucinous adenocarcinoma from colon origin which was excluded from the total number. Of histology subtypes, majority of them were lined by endocervical type epithelium and minority of them were lined by intestinal type endothelium. Depending on the cutoff criteria, certain proportions of cases showed mixed types lining epithelium. All the clinical suspicious cases were tested with serum tumor markers and were negative. The composite picture shows an 18.5 cm multiloculated mucinous cystadenoma from a 13 year old.



Conclusions: Mucinous tumors in childhood share the some same clinical characteristics as they are in the adult population- large size, expansile and mostly multilocular growth pattern. However, in the pediatric population, all of the cases followed behaved in a benign fashion including the ones with borderline features. Mucinous tumors of ovary can certainly happen in premenarchal girls and this group was perhaps under-reported in the medical literature. Common tumor markers (alpha-fetoprotein, CA-125 and beta HCG) are useful for preoperative predication.

1141 Immunohistochemistry Helps To Discriminate between Symplastic Leiomyoma and Leiomyosarcoma of the Uterus

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Background: Symplastic (bizarre) leiomyomas of the uterus can mimic leiomyosarcoma due to its wide range of histomorphologic changes such as high cellularity, intermediate mitotic count (up to 8/10hpf) and bizarre atypical cells. When tumor necrosis is absent, discrimination between symplastic leiomyomas and leiomyosarcomas can be difficult. Discrimination between these two entities with immunohistochemical staining has been described with discordant results.
Design: A tissue microarray of 70 cases including 34 usual leiomyomas, 11 cellular leiomyomas, 9 symplastic leiomyomas and 16 leiomyosarcomas diagnosed between 1991 and 2008 was constructed. In addition to H&E and toluidine blue staining, immunohistochemistry for 14 different markers was performed, namely MCT, c-kit, LCA, MIB-1, CD10, p16, p53, bcl2, ER, PgRA, SMA, Actin, Desmin and Myoglobin. The percentage of positive stained cells was assessed and staining intensity was scored as weak (1), moderate (2) and strong (3).
Results: None of the markers separately could clearly distinguish between leiomyosarcomas and symplastic leiomyomas. Expression of PgRA and Desmin was lower by trend in leiomyosarcomas compared to the three other groups, whereas expression of p16 was higher in leiomysarcomas. The combination of a PgRA >15% and p16 expression in <50% of the neoplastic cells or the combination of PgRA in <15% and Desmin expression in > 90% can help to distinguishe between symplastic leiomyomas and leiomyosarcomas (p<0.001).
Conclusions: Tumour necrosis and a high mitotic count are still the best indicators for leiomyosarcoma compared to symplastic leiomyoma. If these indicators are absent, combination of PgRA, Desmin and p16 expression might help to distinguish between leiomyosarcoma and symplastic leiomyoma of the uterus.

1142 Extrauterine Adenosarcoma: A Clinicopathologic Study of 24 Cases

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Background: Extrauterine adenosarcoma (EuAds) is a rare neoplasm occurring mostly in the ovaries and pelvis of women over a wide age range. In this study, we present the clinicopathologic features of 24 such cases seen in our institution over a period of 26 years.
Design: 24 cases of EuAds from 1985 to 2010 were retrieved from our files. Cases with questionable origin in the uterus were excluded. The following parameters were recorded: patients'(pts) age, clinical presentation, history of adenomyosis or endometriosis, initial diagnosis, site of involvement, gross appearance, tumor size, the presence of sarcomatous overgrowth (SO), treatment, and outcome.
Results: Pts ages ranged from 42-79 yrs (median, 51.5).The most common clinical signs/symptoms were: abdominal pain or fullness(37.5%), vaginal bleeding (18.7%), and leg edema(18.7%). 3 cases were incidentally detected (18.7%). History of adenomyosis or endometriosis was present in 35% and 61.9% of the cases, respectively. EuAds involved the following sites: pelvis(11 cases), ovaries(7 cases) uterine ligaments and adjacent soft tissue(2 cases), vagina(2 cases), lumbar plexus (1 case) and colon(1 case).Tumors ranged from 3-23.5 cm(mean 11.9 cm). SO was detected in 10/24 cases (41.6%). 2 cases with SO had heterologous elements: rhabdomyosarcoma, 1 case; rh abdomyosarcoma+chondrosarcoma, 1 case. 21 cases were received in consultation with a diagnosis other than EuAds in 10 (42%) cases:malignant mixed Mullerian tumor(4), endometriosis(2), adenomyoma(1), atypical mesenchymal component of a borderline tumor(1), malignant tumor of Mullerian origin(1) and high grade sarcoma(1). Primary treatment included: surgery/chemotherapy(9/24); surgery only(6/24); surgery/chemoradiation(2/24); surgery/radiotherapy(1/24). 13 pts (54.2%)had known recurrence 3 to 58 mos following diagnosis: pelvis(9/13), omentum(2/13), retroperitoneum(2/13). 1 pt never cleared her disease. 2 pts had metastasis: liver, 1 and lung, 1. Recurrence was unknown in 10 pts.Survival data was available for 22 pts: 9 pts died (DOD) 3 to 185 months after initial diagnosis. Of these 5 had tumor with SO (DOD 3-51 months) and 4 had no SO(DOD 26-185 months).
Conclusions: EuAds is rare and mostly seen in women in the 5th and 6th decades. EuAds is often associated with adenomyosis/endometriosis and is most commonly seen in the pelvis followed by the ovaries. EuAds can represent a diagnostic challenge as seen in 42% of our cases. SO is seen in almost half of the cases. EuAds tends to recur more frequently than their uterine counterpart due to the lack of anatomic barriers for the spread of disease.

1143 Isolation and Interrogation of Ovarian Cancer Stem Cells

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Background: Mice tumourgenicity studies identify cancer stem cells (CSCs) as the founding cells of tumours. CSCs have also been linked to chemoresistance, metastasis and disease relapse. Therapeutically targeting CSCs could remove the tumours malignant potential and circumvent chemoresistance, relapse and metastasis. Disease progression of Ovarian Cancer correlates with the predictions of the CSC hypothesis. For this reason ovarian malignancy was chosen as a system in which to study CSCs, with the intention of understanding the variation of CSC markers reported in the literature and to further understanding of the various aspects of ovarian malignancy.
Design: Six models of various stages of ovarian malignancy and one model of non-malignant ovarian surface epithelium were screened for the presence of CSCs and somatic stem cells respectively. Three flow cytometry based CSC screens were implemented; ALDEFLUOR™[/underline], Hoechst Side-Population and Cell Surface Protein Assays (CD44, CD117, CD133, CXCR4). Cells of interest were isolated via

Fluorescence-Assisted Cell Sorting. Putative CSCs (pCSCs) were validated in NOD. SCID Mouse Tumourgenicity Assays and Single Cell Asymmetric Division Assays (AsyD Assay).
Results: Each screening technique identified pCSCs in one or more model systems. There was poor overlap of the cells marked by each of the markers. A pair of cisplatin sensitive and cisplatin adapted cell lines have been sorted into their pCSC and non-pCSC sub-populations, based on ALDEFLUOR™ (ALDH) positivity. It was found that both ALDH+ and ALDH- cells were able to efficiently form tumours in NOD.SCID mice. All ALDH+ clones in the AsyD Assay made both ALDH+ and ALDH- cells. Some of the ALDH- (NegA) clones made both ALDH+ and ALDH-. Other ALDH- (NegB) clones made only ALDH- cells. ALDH+ and ALDH_NegA are able to asymmetrically divide, ALDH_NegB cells are not.
Conclusions: The various techniques for isolation of CSCs do not mark the same cells within an ovarian cancer context. This may reflect different stages/histologies of ovarian disease. There may be additional novel markers for Ovarian CSCs, surplus to the markers described above. Mouse Tumourgenicity Assays combined with AsyD Assays and microarray analysis has the potential identify such novel CSC markers in any malignancy. Mice experiments are currently under way to validate the ALDH_NegB clones as non-tumourigenic non-CSCs. Microarray analysis is currently under way to identify the differences between ALDH+ ALDH_NegA and ALDH_NegB cell types.

1144 Development of the Fetal Uterus and Comparison to the Post-Menarchal Uterus

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Background: The in-utero phase of uterine development is not fully understood. While it is known that the uterus develops from fusion of the Mullerian ducts, the morphologic evolution post-fusion is not well established. Beyond simple scientific curiosity, understanding the development of various components of the fetal uterus could potentially enhance our understanding of pathologic disorders.
Design: A total of 41 fetal uteri from autopsies performed at our institution between 2004 and 2010 were included in the study. The fetuses ranged in age from 19 to 37 weeks. Each uterus with attached adnexa was sectioned along their long axis to display the full thickness of the uterine wall. The thickness of the endometrium (surface epithelium and stroma) was compared to the myometrial thickness. Various immunohistochemical stains were performed on the uteri to confirm stromal and myometrial thickness and identify potential proteins/pathways involved in uterine development.
Results: Up to week 20, the endometrium, composed of purely stroma and a single layer of surface epithelium, and the myometrium had essentially the same thickness. Past 20 weeks the epithelium had shallow invaginations into the stroma, but true glands were not formed. During this time the stroma remained the same thickness as the myometrium. In cases over 30 weeks, the ratio of endometrium to myometrium decreased from 1:1 to 1:4. CD10 was consistently expressed in the uterine stroma while desmin and smooth muscle actin were expressed in the myometrium. Estrogen receptor (ER) was expressed predominantly in the stroma while the progesterone receptor (PR) was expressed in the epithelium and myometrium. As seen in the post-menarchal uterus, the epithelium was CK7 positive and CK20 negative.
Conclusions: Up to week 30 of gestation, fetal uteri have an endometrial/myometrial ratio of 1 (E/M=1) compared to a ratio of nearly 1/10 in the adult uterus. The adult endometrium has surface epithelium, glands and stroma, while fetal endometrium is composed of a single layer of epithelium overlying pure stroma. As the absolute thickness of both the stroma and myometrium is greater in the adult uterus, a differential rate of growth is probably responsible for the transformation in the E/M ratio under the influence of cyclic hormones.

1145 Immunohistochemistry for the Imprint Gene Products TSSC3 To Facilitate Differential Diagnosis of Trophoblastic Diseases

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Background: Differentiation between trophoblastic diseases, complete mole (CM), partial mole (PM), invasive mole (IM), hydropic abortion (HA), placental site trophoblastic tumor (PSTT), epithelioid trophoblastic tumor (ET), and choriocarcinoma (CC) is very important for patient management. Maternally expressed genes are critical for embryonic development and paternally expressed genes are critical for placental development.
Design: TSSC3 is one of the paternally imprinted, maternally expressed genes. TSSC3 is the most markedly suppressed among 13 transcribed genes from a maternal allele. It is said to be related to trophoblastic differentiation. In order to determine whether immunohistochemistry of TSSC3 can be used as a tool for the differential diagnosis of trophoblastic diseases, 40 CMs (including one putative CM case, 3 cases of CM/normal twins and one case with invasive mole), 23 PMs, 18 Has, 8 PSTTs, 2 ETTs, and 7 CCs were stained by immunohistochemistry for TSSC3.
Results: All PMs and HAs were positive for TSSC3. There was strong TSSC3 staining of the cytoplasm or nuclei in the villous cytotrophoblasts. All CMs except one putative case were completely negative in the villous cytotrophoblasts. It was expressed in cytotrophoblasts in normal villi of 3 cases of CM/normal twins. One putative CM case, in which some villi showed histologically typical CM features and other villi showed edema with stromal cell hyperplasia and the absence of trophoblastic hyperplasia, exhibited a biphasic pattern, typical CM-type negative staining in CM villi, and positive staining in cytotrophoblasts in non-CM areas. Some villi exhibited a mosaic pattern. Placental site intermediate trophoblasts were rarely positive for TSSC3 in limited cases. A small number of tumor cells in 5 of 8 PSTTs and 1 of 2 ETTs were positive for TSSC3. Syncytiotrophoblasts were focally positive for TSSC3 in 2 of 7 CCs.
Conclusions: Immunohistochemistry for TSSC3 serves as a practical and reliable diagnostic marker for the discrimination of CM from PM, especially for CM/normal twin cases, and CM from HA. TSSC3 is not useful for the differential diagnosis among PSTT, ET, and CC. One putative CM case may be due to androgenic/biparental chimera or a mosaic, although molecular cytogenetic analysis is necessary.

1146 Differential Diagnosis between Early Complete and Partial Mole Using TSSC3 and P57 (Kip2) Antibodies: Correlation with DNA Ploidy

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Background: The differentiation of complete mole (CM), aberrant androgenetic conceptus, from partial mole (PM) and hydropic abortion (HA) in early gestation is very important for patient management. TSSC3 and p57 (Kip2) (p57) are products of a paternally imprinted, maternally expressed gene.
Design: Ten diploid voluntary artificial abortions (ABs), 20 diploid HAs, 20 triploid PMs, and 44 diploid CMs (including 4 persistent diseases), all of which were in the first trimester, were evaluated by immunohistochemistry of formalin-fixed tissues using monoclonal antibodies against p57 and TSSC3. p57 and TSSC3 are coded by different genes. DNA ploidy in all cases was analyzed by flow cytometry using formalin-fixed, paraffin-embedded block sections.
Results: In all diploid ABs, nuclear p57 was strongly expressed in cytotrophoblasts, villous and extravillous intermediate trophoblasts, villous stromal cells, and decidual stromal cells, but was absent in syncytiotrophoblasts. TSSC3 was only expressed in cytotrophoblasts. In diploid CMs, p57 expression in cytotrophoblasts and villous stromal cells was either absent (37 cases) or very low (7 cases). Villous intermediate trophoblasts stained for p57 in 12 cases of CM. TSSC3 was completely negative in these trophoblasts and villous stromal cells in all CMs. On the other hand, all diploid HAs and all triploid PMs showed TSCC3 levels comparable to the findings observed in diploid ABs. 16 diploid HAs and 19 triploid PMs expressed p57 positivity in cytotrophoblasts and villous stromal cells. The remaining 4 diploid HAs and one triploid PM with the lack of p57 staining exhibited typical histologic features of HA or PM. Decidual stromal cells provided a reliable internal control for p57, but there was no internal control for TSSC3.
Conclusions: The findings support the hypothesis that misexpression of p57 and TSSC3 is involved in the abnormal development of androgenetic CMs. The immunohistochemical results were highly concordant with the DNA ploidy status. Immunohistochemical analysis is a useful tool for the differential diagnosis of CMs versus PMs and HAs, and TSSC3 is more sensitive than p57. Since the immunohistochemical diagnosis of CM is based on a negative result, the absence of staining, the use of both markers (p57 and TSSC3) together could increase the level of confidence when making this prognostically important distinction.

1147 Secretion of Specific Toll-Like Receptor Signaling Proteins in the Response of Embryonal Carcinoma Cancer Stem Cells to Differentiation, Hypoxia and Chemotherapy

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Background: We have previously reported that Toll-Like Receptor 4 (Tlr4) and Myeloid-Differentiation Primary Response Gene 88 (MyD88) are putative indicators of overall outcome in ovarian cancer and involved in differentiation-, hypoxia- and chemo-resistance in human embryonal carcinoma (hEC) cancer stem cells (CSCs). The Tlr4-MyD88 receptor-modulator complex of TLR signaling is known to regulate the profile of pro-inflammatory proteins secreted by the cell. In this study, these secreted proteins were profiled.
Design: Conditioned media from pluripotent NTera2 and nullipotent 2102Ep hEC CSCs grown in differentiation, hypoxia and chemotherapy conditions was assessed for the expression of secreted proteins. Proteins secreted were identified through 'Quantikine' array analysis (Ray Biotech).
Results: Populations of secreted proteins were identified for each treatment. Differential expression of Tlr4-MyD88 correlated to specific populations of secreted proteins. These populations differed, even when similar Tlr4-MyD88 expression patters were observed. This profile was similarly specific for cells in which MyD88 had been knocked down. Of note was the altered expression of receptor proteins
Conclusions: Tlr4-MyD88 signaling plays a role in ovarian tumorigenesis. It has been shown that proteins secreted by cells in various cancer-related conditions are specific to the treatment as well as the expression pattern of Tlr4-MyD88. Overall, the data present a model whereby receptor proteins are secreted by malignant cells to, perhaps, act as biochemical sponges to prevent ligands reaching receptors on the cell surface.

1148 Inhibition of Human Embryonal Carcinoma Cancer Stem Cells by Co-Culture with Their Differentiated Progeny

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Background: Cancer stem cells (CSCs) and the differentiated cells they produce co-exist in tumors. However, our understanding of their interaction is sparse. Addressing this, undifferentiated and differentiated cells were co-cultured and their interaction assessed.
Design: Pluripotent NTera2 human Embryonal Carcinoma (hEC) CSCs were terminally differentiated via retinoic acid. Undifferentiated and differentiated cells were co-cultured at ratios of 1:1 and 1:9. Proteins secreted in conditioned media were identified trough 'Quantikine' array analysis (Ray Biotech).
Results: 1:1 co-culture of undifferentiated and differentiated cells had little effect on the proliferation or viability of either cell type. In contrast, 1:9 co-culture inhibited the proliferation of undifferentiated hEC cells. Inhibition was more pronounced when hEC cells were differentiated for longer (1 week vs. 2 weeks). Conditioned media from differentiated cells was found to be sufficient for partial inhibition. Quantikine array analysis identified several proteins, including receptors, secreted by differentiated cells, which may be responsible for this phenomenon.

Conclusions: Proliferation of undifferentiated CSCs can be inhibited by products secreted by their differentiated progeny. Inhibition requires an excess of differentiated cells and is more pronounced when with longer differentiated cells. Data suggests that proteins secreted by differentiated cells may a useful for therapeutic inhibition of CSC proliferation.

1149 Practical Value of Systematic and Complete Examination of Fallopian Tubes in Unselected Women Undergoing Salpingectomy for Benign Indications: Results of a Prospective Study

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Background: Recent studies implicate the fallopian tube (FT) as one of the earliest sites of cancer development in women with hereditary risk for pelvic serous carcinoma. Clinically occult serous tubal intraepithelial carcinoma (STIC) can be found in about 10% of risk reducing salpingo-oophorectomy (RRSO) specimens in BRCA mutation carriers, if a systematic and complete pathologic evaluation is performed. This study prospectively tested a protocol of systematic and complete evaluation of FTs from unselected women undergoing GYN surgery for benign indications. The aims were to define the incidence of occult tubal carcinoma in this patient population and to quantify the additional labor and burden from diagnostic work up of problematic findings using this protocol.

Design: All cases where the entire FTs were examined for benign indications from 2009 to 2012 were included. Lesions that met morphologic and immunohistochemicaL criteria for STIC or invasive tubal carcinoma (ITC) were considered malignant. Lesions that were morphologically atypical but fell short of STIC were coded as atypical for this study.

Results: A total of 396 patients with benign surgical indications were evaluated by this protocol. Primary surgical indications included cystic pelvic/adnexal mass (clinical/radiologic impression of benign, benign diagnosis confirmed by pathology) (263), fibroids (64), hormonal suppression for breast carcinoma (17), prolapse (16), pelvic pain (11), endometriosis (10), abnormal bleeding (8),hydrosalpinx (3), torsion (3), hemoperitoneum (1). The average number of tissue blocks per fallopian tube was 4. A total of 2 cases of STIC (0.5%) and 0 cases of ITC were identified. Both STICs were located in the fimbriae. 3 cases had morphological atypia prompting further workup. Patients with STIC were 46 and 83 years old who underwent surgery for ovarian dermoid cyst and serous cystadenoma respectively.

Conclusions: Systematic microscopic examination of the fallopian tubes in patients presenting for benign indications may identify rare early cancers, all of which are found in the fimbriae in our study. This comes with a cost of additional labor and the cost of detecting lesions of uncertain significance. Inclusion of the non-fimbriated tube in this protocol does not appear to be of value. These results may contribute to the discussion of whether this practice is justified as a routine screening tool in unselected women.

1150 Metabolomic Profile of Endometriod Adenocarcinoma of the Endometrium

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Background: Cancer cells need to change their metabolism for proliferation. Metabolomics have been used to identify individual metabolites which could be end points in molecular pathways affected in cancer cells.

Design: Fresh frozen tissue corresponding to normal endometrium in different phases of the menstrual cycle (25 cases) and endometrioid carcinoma, from surface (22 cases) and from myometrial invasive front (24 cases) were used for the metabolomics analysis. After metabolites extraction, samples were subjected to liquid chromatography coupled to mass spectrometry (HPLC-ESI-QTOF MS/MS) and analysed by both univariate and multivariate statistics.

Results: Multivariate analysis, used for pattern recognition, revealed differences between the three groups (normal endometrium, carcinoma from surface and carcinoma from myometrial invasive front). When tumoral *vs.* non tumoral samples were compared (T-Student, $p<0.05$), 54 differential molecules were found, three of them related to lipid metabolism. Interestingly, 112 different molecules (paired T-Student, $p<0.05$) were found between surface and myometrial invasive front samples involving changes in purine and tyrosine metabolism, among others.

Conclusions: The present results demonstrate different metabolomic pattern between normal endometrium and endometriod carcinoma, but also in endometrioid carcinoma with different levels of invasion. Such information is important to understand the molecular pathways involved in endometrioid carcinoma of the endometrium development and progression.

1151 PAX8 in Conjunction with p16 and ER Aids in the Distinction between Mesonephric Proliferations and Adenocarcinomas of Cervix

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Background: Mesonephric remnants, usually located deep in the lateral cervical wall, may become hyperplastic resulting in a florid proliferation. These can be misinterpreted as malignant and confused with endocervical adenocarcinomas. Recent data has shown PAX2 to be diffusely expressed in mesonephric remnants/hyperplasias and rarely in endocervical adenocarcinomas. PAX8 is a related transcription protein that is expressed in tissues of mullerian and wolffian origin. In this study, we have investigated the utility of an immunohistochemical panel comprising of PAX8, ER and p16 in the differential diagnosis between mesonephric proliferations and cervical adenocarcinomas.

Design: A database search was conducted for cases of mesonephric remnants/hyperplasia/carcinoma of cervix and invasive cervical adenocarcinomas from January 2001 to December 2011. The corresponding slides and paraffin blocks were retrieved. Immunohistochemical stains for PAX8, ER and p16 were performed with adequate controls using the avidin-biotin peroxidase technique on the most representative tissue. The staining pattern was recorded as positive (diffuse, focal or patchy) or negative with the intensity of staining as weak, moderate or strong.

Results: The search yielded 27 cases of mesonephric proliferations of cervix (15 mesonephric remnants, 11 mesonephric hyperplasias and 1 mesonephric carcinoma) and 16 cases of cervical adenocarcinomas (15 usual type and 1 adenoma malignum). Immunohistochemically, all the mesonephric proliferations displayed a consistent staining pattern - diffusely and strongly positive for PAX8, negative for ER and patchy and weak staining for p16. The cervical adenocarcinomas exhibited a variable staining pattern.

Table 1. Immunostain profile for cervical adenocarcinomas

Diagnosis	PAX8		ER		p16	
	Pattern	Number of cases	Pattern	Number of cases	Pattern	Number of cases
Usual type	Negative	2	Negative	6	Strong (diffuse)	15
	Moderate (difffuse)	7	Moderate (diffuse)	5		
	Strong (diffuse)	4	Weak (focal)	4		
	Weak (diffuse)	2				
Adenoma malignum	Strong (diffuse)	1	Negative	1	Weak (patchy)	1

Conclusions: The mesonephric proliferations, regardless of being benign or malignant, showed a consistent immunostaining pattern with PAX8, p16 and ER. In contrast, the usual type of endocervical adenocarcinomas showed a uniform strong and diffuse staining pattern with p16, but stained heterogeneously with PAX8 and ER. Our study suggests that a panel of immunostains composed of PAX8, p16 and ER is useful in the distinction between mesonephric proliferations and cervical adenocarcinomas.

1152 What Predicts Recurrence in Low-Stage Endometrial Endometrioid Carcinoma? A Clinicopathological Review of 159 Cases

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Background: Even though most patients with low-stage endometrial endometrioid carcinomas (EEC) have favorable prognosis, some develop recurrent disease. We reviewed a series of low-stage EEC, with the aim of identifying additional prognostic parameters in patients with poor outcome.

Design: All low-stage EEC diagnosed between 2000 to 2010 were reviewed to evaluate: tumor grade, depth and pattern of myoinvasion {infiltrating glands (IG), pushing front (PF), microcystic elongated and fragmented (MELF) or adenoma malignum-like (AM)}, lymphovascular invasion (LVI), cervical and lower uterine segment involvement (LUSI). Clinical follow-up data was obtained from the electronic patient records.

Results: 159 cases of low-stage EEC were identified (127 grade 1, 24 grade 2 and 8 grade 3). 156 patients underwent THBSO and 3 had hysterectomy. 101 and 88 patients also had pelvic lymphadenectomy and peritoneal washings performed, respectively. Tumor size ranged from 0.4 to 10 (mean 4.1) cm. 123 cases (77.4%) were stage 1A, 25 (15.7%) stage 1B and 11 (6.9%) stage 2. 74 and 31 tumors showed < and > 50% myoinvasion. The invasive pattern consisted of IG (73; 69.5%), PF (21; 20.0%), MELF (9; 8.6%) and AM (2; 1.9%). 47 tumors had LUSI and 15 demonstrated cervical involvement (4 glandular, 2 stromal and 9 with glandular and stromal involvement). LVI was identified in 24 tumors. 74/127 (58.3%) grade 1, 23/24 (95.8%) grade 2 and all 8 (100%) grade 3 tumors showed myoinvasion. Higher FIGO grade (2 and 3) tumors were associated with cervical stromal involvement ($p=0.012$) but no association between grade and pattern of myoinvasion or LUSI was identified. The average length of follow-up was 59.2 (range 1-147) months. 150 (94.3%) patients were alive and well, 1 was alive at 8 months but developed supraclavicular lymph node metastasis, 2 died of disease (1 at 31 months with vault, lung and abdominal lymph nodes metastases and another at 25 months with hip, pelvic and inguinal nodes and costophrenic sulcus metastases), and 6 died of other causes. All patients with recurrence had myoinvasive disease with IG invasion {2/3 stage 1A (grade 1 and 2), 1/3 Stage 1B (grade 3)} and LUSI and 2 had LVI. The patient with a grade 1, stage 1A tumor also has cervical glandular involvement.

Conclusions: Patients with low-stage EEC generally have excellent prognosis. Tumors with higher FIGO grade, LUSI, LVI, and an IG pattern of invasion were associated with disease recurrence.

1153 Quantitative Ki-67 Index as an Ancillary Tool in the Differential Diagnosis of Endometrial Lesions with Secretory Change

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Background: "Secretory change" or "secretory metaplasia" in various endometrial lesions, including hyperplasia and carcinoma, is characterized by vacuolization simulating normal secretory endometrium. Normal secretory endometrium (SEM) can display considerable crowding, therefore, secretory change in hyperplasia can be difficult to distinguish from SEM with glandular crowding. Proliferative activity drops precipitously in the early secretory phase; this led us to hypothesize that in contrast to SEM, hyperplastic lesions with secretory change would show significantly greater proliferation. The aim of this study was to evaluate Ki-67 labeling to confirm this hypothesis.

Design: 52 endometrial lesions with secretory features, along with 24 controls were stained for H&E and Ki-67. Three fields with hot spots of Ki-67 were photographed at 200X with each field comprising 4-22 glands and a total of ~[/underline]500-1000 cells. 200-300 cells per field were manually counted, Ki-67 positive cells were recorded and reported as a percentage (GTG); a second pathologist (AJB) performed an independent, review of the H&E slides of the same pre-selected fields as well as another estimate of hot spots within entirely imaged slides.

Results: There was an incremental increase in the Ki-67 proliferation index for endometrial lesions with secretory change that paralleled the progression of hyperplasia to endometrial carcinoma. There was no significant change in Ki-67 index between secretory endometrium and focal glandular crowding, but a significant increase compared to hyperplasia with or without cytologic atypia, and endometrial carcinoma. Similar findings were observed by two independent H&E estimates.

Conclusions: Ki-67 labeling in SEM was close to zero and increased incrementally in hyperplasia, atypical hyperplasia and endometrial carcinoma. The difference in between SEM and hyperplasia was significant ($p \leq 0.01$) supporting the use of this technique in differentiating these lesions from hyperplasia.

Ki-67 positivity:	Interval Endometrium (pos control)	Secretory Endometrium (neg control)	Focal Glandular Crowding	Hyperplasia w/o atypia	Hyperplasia with atypia	Endometrial Carcinoma
Manual Count (Gold Standard)	55±21%	1.4±1.3%	2.3± 1.3% NS	18±7.9% **	31±10% **	57±21% NS
Estimate (pre-selected)	70±28%	0.8±1.9%	1.4± 2.4% NS	15±16 % **	36±26% NS	86±14% **
Estimate (whole slides)	64±24%	0.6±0.7%	0.9± 0.7% NS	11±12 % **	16±7.2% NS	72±35% **
Age (±SD)	41±4.6 yo	42±5.8 yo	42± 5.0yo	49±15yo	49±13yo	58±12yo
# cases (n)	12	12	8	24	10	10

**p<0.01 vs SEM AND both bordering diagnostic categories within the spectrum

1154 Myxoid Smooth Tumors of the Uterus: Can Histology Predict Behavior?

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Background: Myxoid smooth muscle tumors (MSMT) of the uterus are diagnostically problematic. Due to their rarity, their criteria for malignant behavior are not well defined with overlapping histologic features between clinically benign and malignant tumors. This clinicopathologic study represents the largest series of such cases to date.

Design: A 20 year search (1992-2012) of our pathology data base yielded 35 MSMT. 15 cases were excluded based on presence of conventional leiomyosarcoma (LMS) or containing <50% myxoid change. H&E slides were reviewed in all cases. The following parameters were recorded: patient (pt) age, stage of disease, tumor size, tumor border, % myxoid component, degree of cellularity, presence of coagulative tumor cell necrosis (CTCN), presence of marked atypia, mitotic index (MI), treatment and outcome.

Results: Pts ages ranged from 28 to 64 yrs (median 48) with 9 to 213 mos follow up (median 66) available for 16 pts. Stage of disease was known for 16 pts: Stage I, 13; Stage II, 3. 6 pts had a diagnosis of smooth muscle tumor of uncertain malignant potential (STUMP), and 14 pts had a diagnosis of LMS. 3 tumors had at least focally increased cellularity. 15 tumors had bland spindle cells, and 4 had epithelioid cells. Remaining pathologic features of cases with follow up by outcome are presented in Table 1;pathologic features by tumor diagnosis are presented in Table 2.

Features of Myxoid Smooth Muscle Tumors by Outcome

	Tumor Size (cm, median)	Infiltrative Tumor Border Present	% Myxoid (Median)	LVI Present	CTCN Present	Severe Atypia Present	MI (median)
No Evidence of Disease (n=13)	7	5	80	3	5	4	4
Recurrent/Dead of Disease (n=3)	10	2	90	1	3	0	9.5

Pathologic Features of Myxoid Smooth Muscle Tumors By Diagnosis

	Tumor Size (cm, median)	Infiltrative Border Present	% Myxoid (Median)	LVI Present	CTCN Present	Severe Atypia Present	MI (median)
STUMP (n=6)	4	1	77.5	1	1	0	2
LMS (n=14)	8.9	8	90	3	9	5	6

9 pts received treatment after surgery: chemotherapy, 6;radiation, 3. 3 pts recurred at these sites: pelvic 3; abdomen 1; lung, 1. No STUMPS recurred; 3/14 (21%) LMS recurred and died of disease.

Conclusions: MSMT are uncommon and occur over a wide age range. Recurrent and nonrecurrent MSMT have overlapping histologic features. Of recurrent MSMT, all had CTCN and two had an infiltrative border. Although there is a trend to increased MI in recurrent MSMT, one case had 1 mitosis. MI may not be a reliable predictor of malignant potential. Once MSMT with foci of conventional LMS are excluded, atypia may not be a reliable indicator of malignancy. Compared to conventional LMS, MSMT have a better outcome.

1155 Claudin-18 and MUC5AC Immunohistochemistry Is Useful for Distinction between Primary Ovarian Mucinous Adenocarcinoma and Metastatic Colorectal Carcinoma Involving the Ovary

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Background: Among ovarian epithelial carcinomas, mucinous adenocarcinomas pose the greatest difficulty in terms of differentiation between primary and metastatic disease. Distinguishing these two lesions is often challenging, not only at the time of intraoperative assessment when requested for surgical management, but also at the time of final pathological diagnosis. The current study was undertaken to investigate the expression of claudin-18 (CLDN18, a tight-junction protein specifically expressed in the gastric epithelium) in both primary ovarian intestinal type mucinous adenocarcinoma (IMCa) and metastatic colorectal carcinoma (CRC) involving the ovary. We also investigated the expression of CK7, CK20, CDX2, MUC2, MUC5AC, and ER to identify a panel of markers useful for differential diagnosis between primary ovarian IMCa and metastatic cancer of colorectal origin.

Design: A total of 33 cases of ovarian adenocarcinoma, including primary IMCa (n = 17) and metastatic CRC (n = 16), were retrieved from the archive of the Department of Pathology of the University of Tokyo Hospital. Tumors were classified based on a review of hematoxylin and eosin–stained slides. A representative whole-tissue section was selected in all cases, and immunohistochemistry for CLDN18, MUC2, MUC5AC, CK7, CK20, CDX2, and ER was performed. Immunohistochemical staining was performed according to standard techniques on a Ventana Benchmark XT autostainer (Ventana Medical Systems Inc., Tucson, AZ).

Results: CLDN18, MUC5AC, and CK7 were expressed specifically in primary ovarian IMCa. Metastatic CRCs, in contrast, were almost always negative for these markers. Expression of CDX2, MUC2, and CK20 was more frequently detected in metastatic CRC than in primary IMCa. Both primary IMCa and metastatic CRC were almost always negative for ER.

Table1

	Primary IMCa	Metastatic CRC	P
CK7	17/17 (100%)	0/16 (0%)	<0.0001
CK20	9/17 (53%)	14/16 (87%)	0.057
CDX2	10/17 (59%)	16/16 (100%)	0.0072
MUC2	2/17 (12%)	9/16 (56%)	0.0104
MUC5AC	10/17 (59%)	0/16 (0%)	0.0003
CLDN18	14/17 (82%)	1/16 (6%)	<0.0001
ER	1/17 (6%)	0/16(0%)	>0.99999

Conclusions: Our findings suggest that immunohistochemistry for CLDN18, MUC5AC, CK7, and CDX2 is a useful adjunctive diagnostic tool for differentiation of primary ovarian IMCa and metastatic colorectal cancer involving the ovary. Furthermore, expression of gastric markers (CLDN18 and MUC5AC) along with intestinal markers (CK20 and CDX2) suggest a gastrointestinal phenotype of primary ovarian IMCa.

1156 Morphologic Features Suggestive of Endometriosis in Nondiagnostic Peritoneal Biopsies

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Background: Endometriosis is a common disorder that causes significant morbidity from dysmenorrhea, pelvic pain, and subfertility. Establishment of a definitive diagnosis has important therapeutic implications; however, only 50% of laparoscopic biopsies of suspicious areas provide a diagnosis of endometriosis. Histologic criteria for diagnosis require the presence of endometrial glands or endometrial-type stroma. We hypothesize that other frequently identified, but nondiagnostic, histologic features of endometriosis suggest its presence in patients with nondiagnostic peritoneal biopsies.

Design: We performed a retrospective clinicopathologic study of morphologic features that may be suggestive of endometriosis by comparing peritoneal biopsies diagnosed as negative for endometriosis (n = 44) from pathologically confirmed endometriosis cases (endometriosis was seen in other biopsies obtained at the same time) with negative peritoneal biopsies (n = 84) from early stage gynecologic cancer cases without spread to the pelvis. Statistical analysis employed Fisher's exact test.

Results: Foamy macrophages and clustered small vessels were the only morphologic features significantly increased in biopsies from endometriosis patients over negative controls ($p = 0.005$ and $p = 0.0334$, respectively). Presence of foamy macrophages had a positive predictive value of 71.4% and likelihood ratio of 4.773 for a diagnosis of endometriosis. Other features (hemosiderin, hemosiderin-laden macrophages, chronic inflammation, fibrosis, elastosis, myxoid change, and smooth muscle metaplasia) did not differ between the two groups

Morphologic Features of Endometriosis in Nondiagnostic and Negative Peritoneal Biopsies

	NONDIAGNOSTIC BIOPSIES (N=44)		NEGATIVE CONTROLS (N=84)		
	N	%	N	%	TWO-SIDED P VALUE
HEMOSIDERIN	7	15.91	9	10.71	0.4108
HEMOSIDERIN-LADEN MACROPHAGES	2	4.55	3	3.57	1.0000
FOAMY MACROPHAGES	10	22.73	4	4.76	0.0050
CHRONIC INFLAMMATION	18	40.91	42	50.00	0.3561
FIBROSIS	13	29.55	23	27.38	0.8375
ELASTOSIS	3	6.82	1	1.19	0.1170
MYXOID CHANGE	3	6.82	3	3.57	0.4130
SMOOTH MUSCLE METAPLASIA	10	22.73	13	15.48	0.3384
MESOTHELIAL HYPERPLASIA	2	4.55	5	5.95	1.0000
CALCIFICATION	1	2.27	0	0.00	0.3438
CLUSTERED SMALL VESSELS	22	50.00	25	30.95	0.0334

Conclusions: Identification of select morphologic features on routine H&E stains can increase the diagnostic yield of peritoneal biopsies for suspected endometriosis.

1157 The Cervical Squamocolumnar Junction Is Not the Same as the "Transformation Zone": Evidence for Two Related but Divergent Populations with Different Cancer Risks

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Background: A fundamental precept in cervical carcinogenesis is the existence of a target cell of origin that is uniquely vulnerable to carcinogenic HPV infection and

capable of multiple tumor phenotypes. A recent study has proposed a cuboidal *non-squamous* cell of origin that resides in the squamocolumnar (SC) junction.
Design: Using expression arrays derived from SC junction cells, antibodies to SC junction-specific gene products were applied to mouse and human embryo and adult cervical specimens in order to identify, characterize and determine the dynamics of SC junction cell development. Remodeled cervical epithelia (epithelial metaplasia, microglandular hyperplasia), low and high grade SILs were also analyzed to ascertain their relationship to SC junctional cell population.
Results: In embryonic and adult cervices, cuboidal embryonic/SC junction cells were tightly linked to subjacent metaplastic basal/reserve cells, the latter expanding from beneath embryonic or SC junction cells to form transformation zone/metaplastic epithelium. This basal pattern of transdifferentiation was termed reverse or top-down differentiation and was associated with a progressive loss of the SC junction immunophenotype and acquisition of squamous markers. In contrast to most LSILs, HSILs displayed an expression of SC junction markers on the apical surface, suggesting an initial infection of the SC junction cells by HPV followed by loss of the SC junction immunophenotype during transdifferentiation to the expanded basal- oriented metaplastic progeny. HPV DNA *in situ* hybridization and p16ink4 staining verified HPV infection in the superficial SC junction cells.
Conclusions: This study indicates that most HSILs are strongly linked to direct infection of SC junction cells followed by transdifferentiation, whereas LSILs are a consequence of the infection of less vulnerable metaplastic cells in the transformation zone that have already completed their exit from the SC junction cell compartment. This geographically-dictated, population-specific (SC junction) vulnerability raises the distinct possibility that cervical cancer risk in a given individual is a function of not only carcinogenic HPV infection but also of infected cell biology.

1158 Squamocolumnar Junction-Specific Markers Define Biologically and Clinically Distinct Subsets of Low-Grade Cervical Intraepithelial Neoplasia

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Background: A fundamental dilemma in the management of early cervical neoplasia is the pathologic distinction of low (LSIL) from high (HSIL) grade squamous intraepithelial lesions. In a recent report, a putative cell of origin in the squamocolumnar (SC) junction was identified; SC junction-specific biomarkers were expressed in high frequency in HSIL, whereas LSILs included both SC junction positive and negative (metaplastic) subsets. In this study, we compared the characteristics of SC junction positive and negative LSILs.
Design: 203 cases of SIL were independently reviewed by two experienced pathologists and classified as LSIL or HSIL using published criteria. Concordant LSILs (agreement by both observers) were subdivided into SC junction positive and negative using SC junction-specific antibodies. Agreement with the original diagnosis (pathology report), HPV type, p16 staining pattern and outcome were compared.
Results: 71 and 37 SC junction (-) and SC junction (+) LSILs were identified. Respectively, 0 (0%) and 17 (46%) were originally classified as HSIL, 42 (59.2%) and 35 (94.5%) displayed a diffuse basal or full-thickness p16ink4 staining suggesting an infection with a high-risk HPVs. This was confirmed by PCR amplification with HPV type-specific primers. Of 48 and 31 SC junction (-) and SC junction (+) cases with followups averaging 34 and 28 months, HSIL was documented by consensus agreement of a biopsy or smear in 0 (0%) and 7 (23%) respectively. Interestingly, six SC junction (-) LSILs had succeeded a cone biopsy for HSIL, suggesting that ablation of the prior SC junction influenced the type of recurrent SIL.
Conclusions: LSILs arising in the SC junction are significantly more likely to: 1) be classified as HSIL on pathology reports, 2) contain high risk HPVs, 3) and have a consensus outcome diagnosis of HSIL. Although outcome data will be influenced by the original diagnosis, together these findings are compelling evidence for the existence of two categories of SIL with low grade nuclear features based on putative SC junction origin. A model for precursor classification, including LSIL, HSIL and "QSIL" - problematic SC junction marker-positive (L)SILs - will be discussed.

1159 Can We Successfully Subtype High Grade Endometrial Carcinomas: The Role of Morphology and Immunohistochemistry Revisited

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Background: Endometrial cancer is a common malignancy of the gynecological system and has been classified into 2 groups:Type 1 are estrogen-related,low-grade endometrioid tumors;type 2 are aggressive,high-grade non-endometrioid tumors.The objective of this study was to investigate the usefulness of Ret finger protein(RFP);which we have recently reported to be a marker of serous differentiation and to evaluate the utility of commonly used immunomarkers(ER,PR,p53,p16,Wilms tumor 1(WT-1))for the differentiation of serous endometrial adenocarcinomas from grade 3 endometrioid endometrial carcinomas.
Design: Tissue microarrays constructed from 124 primary endometrial carcinoma cases(24 grade 1 endometrioid,61 grade 3 endometrioid,39 serous) from 3 institutions were evaluated independently by five experienced gynecopathologists.The scoring of immunohistochemical expression was done with compositional method (intensity X percentage of staining) for all the markers. P16 staining was also evaluated in a 2 tier system where diffuse staining in almost 100% of tumor cells was separately noted. Sensitivity,specificity,area under the ROC curve,Kappa statistic and the Kruskal Wallis tests were done for statistical analysis.
Results: Kappa values for interobserver concordance were 0.737 and 0.727 for endometrioid and serous carcinomas respectively($p < 0.001$).Diffuse p16 staining and p53 emerged as statistically the most sensitive(74% and 74% respectively)and specific(88% and 92% respectively)markers for the differentiation of grade 3 serous from grade 3 endometrioid subtype($p < 0.001$).ER,PR,WT-1 and RFP did not reach statistical significance for subtyping of grade 3 tumors whereas RFP score was found to be significantly increased in grade 3 endometrial carcinomas compared to grade 1 counterparts($p<0.001$).
Conclusions: Grade 3 endometrioid endometrial carcinomas are often lumped together with serous carcinomas as both are high risk tumors.However many studies have shown that they differ with respect to age,stage,and outcome such as extranodal metastasis.In this cohort of high grade endometrioid and serous carcinomas,we were able to reach a strong interobserver concordance and showed that p53 and diffuse p16 staining as the most sensitive and specific immunomarkers for differentiation.In addition we confirmed the selective expression of RFP in high grade endometrial carcinomas compared to grade 1 endometrial cancer.

1160 Histotype-Genotype Correlation in 36 High-Grade Endometrial Carcinomas

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Background: Serous carcinomas and endometrioid/clear cell carcinomas of the endometrium are genetically distinct tumor types with differing prognoses. The distinction between these tumor types can be difficult, particularly in high-grade cases.
Design: 36 high-grade endometrial carcinomas (J Path 2012;228:20-30) were included; 23 endometrioid/clear cell genotype (*PTEN* and *ARID1A* mutation either one without *TP53* and *PPP2R1A* mutation) and 13 serous genotype (*TP53* and/or *PPP2R1A* mutation without *ARID1A* or *PTEN* mutation). 8 pathologists reviewed representative online slides and rendered diagnoses before and after receiving p53, p16 and ER immunoprofiles. Kappa statistics for histotype-genotype concordance were calculated for each pathologist.
Results: The average kappa values for histotype-genotype concordance was 0.56 (range: 0.31-0.67) based on morphology alone, and improved to 0.68 (range: 0.54-0.81) after immunoprofile consideration ($p=0.009$). Genotype incompatible diagnoses were rendered by at least 2 pathologists in 12 of 36 cases (33%) (3 with 2/8, 2 with 3/8, 2 with 4/8, 1 with 5/8, 3 with 6/8 and 1 with 8/8). Scenarios prone to genotype-incompatible diagnoses are shown in Table 1.

Endometrioid/clear cell genotype that mimic serous	Solid growth with high nuclear grade and high MI	***p53 not helpful;*** *Focal endometrioid glandular pattern (at periphery), extensive comedonecrosis and squamoid areas suggest endometrioid*
	Papillary pattern with cell budding and intermediate nuclear grade	*Low MI favors endometrioid and/or clear cell;* ***p53 recommended****: normal result suggests endometrioid and/or clear cell carcinoma*
Serous genotype that mimic endometrioid/clear cell	Papillary pattern with uniform nuclei	*Diffusely elevated MI favors serous;* ***p53 recommended****: abnormal result (either diffuse or completely absent) suggests serous*
	Papillary pattern with endometrioid-like glandular pattern	*Diffuse high nuclear grade or diffusely elevated MI favors serous;* ***p53 recommended****: abnormal result suggests serous*

MI: mitotic index, cut-off of 10 MF/10 HPF
Conclusions: While the majority of morphologic diagnoses are genotype-concordant, genotype-incompatible diagnoses are made in a significant subset of cases. p53 immunohistochemistry improved histotype-genoype concordance.

1161 Foam Cells on Endometrial Biopsies/Curettings – An Indication for Additional Sampling or Not?

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Background: Endometrial stromal foam cells are often noted in association with atypical hyperplasias, endometrioid adenocarcinomas and stromal neoplasms. Though these cells resemble histiocytes, they reveal ultrastructural features of endometrial stromal cells. In this study, we examine whether the finding of foam cells in benign endometrium, anovulatory enometrium or in hyperplasias without atypia warrants additional sampling of the endometrium or not.
Design: A retrospective database search was conducted for "foam cells" in endometrial biopsy/curettings specimens accessioned at our institution from January 1990 to August 2012. For cases with benign endometrium, anovulatory pattern endometrium and endometrial hyperplasia without atypia, clinicopathologic data including histologic follow-up was recorded.
Results: In total, 65 cases were retrieved; 14 cases with complex hyperplasia with atypia and 2 cases with endometrioid FIGO Grade 1 adenocarcinoma at initial diagnosis were excluded from further analysis. For the remaining 49 cases - mean age was 52.8 years (range 32-89 years), diagnoses included disordered proliferative endometrium (10), simple hyperplasia without atypia (8), complex hyperplasia without atypia (12), proliferative endometrium (7), endometrial polyp (4), benign superficial endometrium (7) and abnormal secretory phase endometrium (1). Stromal breakdown was present in 23 (46.9%) cases and morular squamous metaplasia in 3 (6.1%) cases. Six patients underwent treatment with progestins. Of 23 cases with histologic follow-up (follow-up interval of 1-98.5 months, mean 16.5 months), only 1 patient developed complex hyperplasia with atypia (index biopsy with proliferative endometrium, interval from

index biopsy of 8.2 years). None of the patients were found to have carcinoma on follow-up. Of the rest of 26 patients, clinical follow-up information was available for 12 and was unremarkable.

Conclusions: Our study suggests that foam cells may not be harbingers of endometrial carcinoma or atypical hyperplasia and may simply be a manifestation of chronic bleeding and stromal breakdown. Their presence should prompt a careful scrutiny by the pathologist but may not necessitate additional endometrial sampling.

1162 HPV Testing and Cytologic/Histopathologic Follow-Up Results after Excisional Treatment for High Grade Cervical Intraepithelial Neoplasia

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Background: Excision of the transformation zone under colposcopic guidance is standard treatment for CIN2/3. This large study assessed factors impacting risk of residual or recurrent disease after excisional treatment for CIN2/3.

Design: A search of the CoPath file was carried out to retrieve cases with histopathologic diagnoses of CIN2/3 during a period of 54 months. Surgical pathology reports, follow-up hrHPV testing, cytologic, and histopathologic results were recorded.

Results: 1453 patients with histopathologic CIN2/3 diagnosis treated by conization were identified and 432 patients without follow-up were excluded. 988 patients with cytologic and/or histopathologic follow-up results were included with an average age of 31.8 years (range: 16 - 80; median: 28) and average follow-up period of 35.5 months (range: 1-87, median: 34). CIN2/3/HSIL detection rate was 6.8% (67/988). The interval between conization and initial follow-up CIN 2/3/HSIL diagnoses was approximately 20 months (range: 1- 57, median: 13). Residual/recurrent disease was associated with significantly higher CIN grade, positive margins after conization, or hrHPV positive results, and was not related to age (<30 yr vs >=30 yr) (Table 1). 514 women had hrHPV testing during the follow-up period and 32.3% had positive hrHPV results. CIN2/3 was detected in only 1.4% (5/348) of patients with follow-up hrHPV-negative results, compared to 15.1% (25/166) of patients with HPV-positive results. 45 of 67 (67.2%) cases with recurrence of CIN2/3 were present within 2 years after the conization.

Conclusions: In a large study of 1453 patients with CIN2/3 treated by conization, follow-up hrHPV testing was confirmed as very helpful in identifying patients with residual/recurrent CIN2/3, especially in patients with negative conization margins. Cytology and HPV cotesting is preferred for follow-up of women after CIN2/3 excisions.

Risk factors associated with recurrent CIN lesions in women with CIN2/3 after the conization

	Total Patients	CIN2/3/HSIL	CIN1/LSIL
Histopathology	219	64 (29.2%)	109 (49.8%)
Cytology only	769	3 (0.4%)	56 (7.3%)
Margin positive	231	34 (14.7%)	49 (21.2%)
Margin negative	757	33 (4.4%)	116 (15.3%)
Original dx:CIN2	677	36 (5.3%)	118 (17.4%)
Original dx:CIN3	311	31 (10.0%)	47 (15.1%)
<30 y	531	32 (6.0%)	89 (16.8%)
>=30 y	457	35 (7.7%)	76 (16.6%)
HPV positive*	166	25 (15.1%)	66 (39.8%)
HPV negative only	348	5 (1.4%)	40 (11.5%)

*at least one positive HPV test.

1163 Clinical Significance of Positive P16/Ki-67 Dual-Stained Cytology in Negative Papanicolaou Cytology

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Background: Dual immunocytochemistry for p16/Ki-67 has been reported as a useful biomarker to identify cells of underlying high-grade cervical intraepithelial neoplasia in the Papanicolaou (Pap) cytology. Positive p16/Ki-67 dual-stained cytology was rarely found in the category of negative cytology. The objective of this study was to investigate the clinical significance of positive p16/Ki-67 dual-stained cytology in women with Pap negative.

Design: Cytology p16/Ki-67 dual-staining test was performed on 691 liquid-based residual samples from a cohort of women with ASC-US/ LSIL and co-testing human papillomavirus (HPV) positive. There were 21 Pap negative smears showing positive p16/Ki-67 dual-stained cytology during periodic follow-up survey from 18 women. All 21 Pap negative smears were reviewed: cytology interpretations of two smears were revised as ASC-US on one original smear and on the other repeated smear.

Results: Positivity of p16/Ki-67 dual stained cytology was well correlated to cytology interpretation: 5.3% in Pap negative, 17.5% in ASC-US, 75.7% in ASC-H, 60.0% in LSIL and 100.0% in HSIL. Sensitivity (100.0%) for the detection of HSIL or specificity (95.2%) for Pap negative was higher than those of HPV test. Twelve of 18 women with positives dual-stained cytology in Pap negatives showed persistent infections by same HPV types (16, 16/52, 30, 31, 35/39, 51, 52, 56, 58, 68, 90). The duration of the persistent infection of same HPV type was 6 to 22 months (mean, 15month). Three of 12 women having persistent HPV infections were also disclosed negative cytology with negative dual-stained cytology. In these cytology, the duration of the persistent infection was short (mean, 7months). The others 6 women revealed 2 false positives for dual-stained cytology, and 4 variable infections in HPV type or positivity.

Conclusions: p16/Ki-67 dual stained cytology could provide high both sensitivity and specificity for the detection HSIL in Pap cytology. Positive p16/Ki-67 dual-stained cytology in Pap negative was highly associated with the persistent infections by same HPV type. Therefore, in cases of positive dual-stained cells morphologically showing benign atypical features, further follow-up would be necessary.

1164 Expression of Embryonic Markers in Endometrial Epitheliium: A Unique Topography with Implications for Tumorigenesis

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Background: We have previously shown that Krt7 is expressed in embryonic mullerian epithelium, identifiesthe squamocolumnar junction (SCJ), cancers and precursor lesions in the cervix. This study investigated expression of Krt7 in the uterus during development, reproductive life and in neoplasia. The goal was to determine if a parallel existed in the endometrium and if expression patterns correlated with tumor type.

Design: Sections of human and murine uteri in early development were stained with Krt7. Cases were evaluatedfor the distribution of Krt7 expression and its topographic relationship to the surface epithelium and subjacent endometrial glands. In addition endometrial samples including polyps, 13 grade 1-2 and two grade 3 endometrioid endometrial adenocarcinomas and six uterine serous carcinomas were stained.

Results: During development, Krt7 in both the human (20 weeks) and mouse (6 days post natal) intensely stained the surface epithelial cells lining the uterine cavity. With the onset of subjacent gland formation, Krt7 expression diminished, in parallel with the reduction in staining seen in the endocervical epithelium away from the squamocolumnar junction. During reproductive life, staining was retained on the surface. 5/6 serous carcinomas and one grade 3 endometrioid carcinoma were strongly Kr7+. In contrast, all 13 grade 1 or 2 endometrioid carcinomas were either negative or showed heterogenous staining.

Conclusions: The uterine surface lining is immunophenotypically distinct from the endometrial glands and shares identity with embryonic epithelium in keeping with a role as epithelial progenitor. The emergence of Krt7- glands from this epithelium during development, and persistence of these glands into adulthood underscore the differences between the two epithelial compartments and suggest that the surface epithelium harbors the stem cells required for regeneration. Differences in Krt7 expression in uterine serous and endometrioid carcinomas imply that these tumors may arise from different cell types; a concept reinforced by the superficial nature of early serous carcinomas and the fact that endometrioid neoplasms are predominately gland forming. The potential for these markers to augment current endometrial tumor classifications based on site or cell of origin merits further investigation.

1165 Immunohistochemical Analysis of HMB-45, MelanA and CathepsinK in a Series of 35 Uterine Leiomyosarcoma

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Background: Morphologic overlap between uterine leiomyosarcoma (uLMS) and perivascular epithelioid cell tumor (PEComa) may occur, particularly in cases of uLMS with epithelioid morphology. HMB-45 positivity can be seen in both uLMS and PEComa, so this marker is not considered discriminatory. As other markers frequently expressed in PEComa have not been systematically analyzed in uLMS, we chose to study expression of a panel of melanocytic markers and correlate the immunoprofile with tumor morphology.

Design: 35 uLMS (25 spindled, 5 myxoid, 4 epithelioid, and 1 spindled and epithelioid; consultation and in-house archives 1997-2012) were assessed for features described in PEComa, including abundant eosinophilic granular cytoplasm, multinucleated giant tumor cells, melanoma-like macronucleoli, prominent nuclear pseudo-inclusions, and typical vasculature. Based on H&E morphology, the tumors were classified into those having classic (uLMS-C), or those with either PEComa-like (uLMS-P), or lymphangioleiomyomatosis-like (uLMS-LAM) features. In addition, 3 uterine STUMP with PEComa like features were also evaluated (STUMP-P). Cases were stained for HMB-45, MelanA, and CathepsinK. Staining was scored as follows: rare cells (<1%), focal (1-10%), patchy (11-50%), or diffuse (>50%).

Results: Of the 35 cases, 15 (43%) were classified as uLMS-C, 18 (51%) as uLMS-P, and 2 (6%) as uLMS-LAM. All uLMS-C were negative for HMB-45 and MelanA, and 8/11 were Cathepsin K + (3 patchy, 5 focal). Of 18 uLMS-P, 3 were HMB-45 + (focal); however, no uLMS-P showed staining with MelanA. CathepsinK was + in 15/17 uLMS-P (2 diffuse, 8 patchy, 5 focal). Of uLMS-LAM, 1/2, 0/2 and 2/2 were + for HMB-45, MelanA, and CathepsinK (1 diffuse, 1 patchy) respectively. 1 of 3 STUMP-P was + for HMB-45 (rare cells), none for MelanA, and 1 for CathepsinK (patchy).

Conclusions: Uterine smooth muscle tumors (uSMT) with PEComa-like morphology are more likely to be positive, albeit focally, for HMB-45 than those with classic morphology. CathepsinK is more likely to show patchy to diffuse positivity in this subset as well. None of the uterine smooth muscle tumors, regardless of morphology, were positive for MelanA, which may be useful diagnostically. Nevertheless, the overlap in morphology and immunoprofile suggests a closer relationship between uterine PEComa and uSMT which may represent a morphologic continuum. Whether uSMT with PEComa-like features share a common pathogenesis and would be responsive to mTOR inhibitors merits further study.

1166 VCAM1 Expression Correlated with Tumorigenesis, Chemoresistance and Poor Prognosis in High Grade Serous Ovarian Cancer

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Background: High expression of vascular cell adhesion molecule 1 (VCAM1) has been shown to be associated with several cancers although its function in cancer development is largely undefined. Our previous studied demonstrated that VCAM1 expression is increased in RAS-transformed ovarian epithelial cells. The purpose of this study is to investigate its role in ovarian cancer using RAS-transformed ovarian

epithelial cells and ovarian cancer cell lines and correlate its expression with clinical pathologic parameters in a cohort of ovarian cancer patients.
Design: We examined the expression of VCAM1 by immunohistochemical staining in 251 high grade serous carcinoma using ovarian cancer tissue microarray. The expression of VCAM1 was silenced in RAS-transformed ovarian epithelial cell lines and three high grade ovarian cancer cell lines (OVCA433, OVCA429, and SKOV3) using retrovirus-mediated silencing. Cell proliferation, colony formation, and migration, and chemosensitivity assay were analyzed in *vitro* and effect on tumor growth was analyzed in nude mice.
Results: High VCAM1 expression in cancer cells was related with response to treat with surgery and chemical drugs ($P = 0.025$) and elder age at diagnosis ($P = 0.008$). Cox regression univariate and multivariable analysis showed that VCAM1 expression in tumor cells was an independent prognostic factor. Ovarian cancer cells with VCAM1 overexpression, compared with corresponding control cells, had increased cell proliferation, colony formation, migration, chemoresistance, and enhanced growth of xenograft tumors in mice, especially the tumor cells maintained with fibroblast cells with high VCAM1 expression.
Conclusions: VCAM1 plays an important role in promoting tumor growth of ovarian cancer. It may be used as a prognostic factor and novel therapeutic target for ovarian cancer.

1167 Endometrial Carcinomas with DNA Mismatch Repair Abnormalities: Analysis of 75 Cases

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Background: Endometrial carcinoma (EC) can be associated with defective DNA mismatch repair (MMR) in ~[/underline]20% of patients. The morphologic differences in tumors with loss of MLH1/PMS2 versus MSH2/MSH6 by immunohistochemistry (IHC) have not been well described.
Design: All ECs with abnormal IHC for MMR with slides available (2006-present) were reviewed. A total of 75 patients were identified. All available slides were reviewed. Clinical history was obtained from electronic medical records.
Results: The clinicopathologic characteristics of each group are described.

Clinicopathologic characteristics of the 2 groups of patients

Variable		MLH1/PMS2 loss (n=42)	MSH2/MSH6 loss (n=33)
Age		Mean 57 (27-78)	Mean 52 (34-69)
Histologic subtype	Endometrioid	26 (63%)	23 (76%)
	Clear cell carcinoma	0	2 (6%)
	Un/dedifferentiated	11 (27%)	1 (3%)
	MMT	1 (2%)	1 (3%), focal
	Mixed endometrioid/clear cell/ undifferentiated	1 (2%)	2 (6%)
	Mixed endometrioid/clear cell	1 (2%)	2 (6%)
	Mixed endometrioid/mucinous	0	1 (3%)
FIGO grade (for endometrioid)	I	4 (15%)	8 (35%)
	II	16 (62%)	11 (48%)
	III	6 (23%)	4 (17%)
FIGO stage	I	25 (60%)	16 (48%)
	II	5 (12%)	2 (6%)
	III/IV	11 (28%)	15 (46%)
Lymphovascular invasion		24 (57%)	15 (45%)
Myometrial invasion		35 (83%)	27 (82%)
MELF inasion		29/35 (83%)	16/27 (59%)
Heterogeneity		26 (62%)	15 (45%)
Mucinous differentiation		29 (69%)	20 (61%)
Intratumoral lymphocytes		38 (90%)	30 (91%)
Peritumoral lymphocytes		34 (81%)	25 (76%)
LUS involvement		14 (33%)	9 (27%)
Synchronous ovarian carcinoma		1 (endometrioid)	1(endometrioid);1 MMT(endometrioid/clear cell/rhabdomyosarcoma); 1(mucinous/endometrioid/ undifferentiated);1(clear cell)
Atrophic endometrium		11 (26%)	7 (21%)
Genetic testing		1 (MLH1 mutation); 2(PMS2 mutation); 7 (MLH1 hypermethylation); 2 negative	2 (MSH2 mutation); 4 (MSH6 mutation);2 (MSI-high); 3 negative

Conclusions: Patients with EC and MSH2/MSH6 defects by IHC tend to present at a higher stage, compared to patients with MLH1/PMS2 loss. Un/dedifferentiated carcinomas are more likely to demonstrate MLH1/PMS2 loss, while tumors with a clear cell component are more likely to show MSH2/MSH6 loss. MELF (microcystic, elongated, fragmented) pattern of myometrial invasion is more frequent in patients with MLH1/PMS2 loss. Finally, synchronous ovarian carcinoma tends to occur more frequently in MSH2/MSH6 loss and show a variety of histologies.

1168 MELF Pattern of Invasion: A Frequent Finding in Endometrial Carcinoma with DNA Mismatch Repair Abnormalities

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Background: MELF is a recently described pattern of myometrial invasion by endometrial carcinoma (EC), characterized by microcystic, elongated, and fragmented glands surrounded by inflamed fibromyxoid stroma, and can be seen in approximately 15% of ECs. MELF is associated with the presence of lymphovascular invasion (LVI) and mucinous differentiation. The aim of this study was to assess the frequency of MELF invasion in ECs that show abnormal immunohistochemical (IHC) staining for the DNA mismatch repair (MMR) proteins.
Design: All ECs with abnormal IHC for MMR with slides available (2006-present) were reviewed. A total of 74 patients were identified. All available slides were reviewed. Clinical history was obtained from electronic medical records.
Results: Of the 74 EC patients with abnormal IHC-MMR, myometrial invasion was found in 62 (84%), of which 45 (73%) had MELF pattern of myometrial invasion. Patients with DNA MMR defects and MELF pattern had a mean age of 55 years (range, 27-76). Of these 45 patients, MSH2/MSH6 loss was found in 16 (36%), while MLH1/PMS2 loss was identified in 29 cases (64%). Among all patients with MLH1/PMS2 abnormalities, 29/42 (83%) showed MELF while 16 of 26 (62%) cases with MSH2/MSH6 abnormalities had MELF. The majority of tumors (33/45) were endometrioid type; 5 were undifferentiated/dedifferentiated; 6 showed mixed histology; and one was a carcinosarcoma (MMMT). Of the endometrioid tumors, 7 (21%) were FIGO grade 1; 19 (58%) were grade 2; and 7 were (21%) grade 3I.Thirty-one cases (69%) had LVI. Mucinous and squamous differentiation was found in 31 (69%) and 27 cases (60%), respectively. The majority had increased tumor infiltrating lymphocyte and peri-tumoral lymphocytes. Clinically, 23 (51%) of the patients presented at FIGO stage I; 5 (11%) at FIGO stage II; while the remaining (38%) were FIGO stages III and IV.
Conclusions: MELF pattern of myometrial invasion is frequently seen in myoinvasive EC with DNA MMR abnormalities. The rates of MELF in EC with abnormal IHC-MMR appear to be much higher than those reported in the literature for unselcetd ECs, suggesting an association between the two. Although previously described as occurring in predominantly low-grade tumors, in our series, MELF occurred in a significant number of tumors with high-grade histology.

1169 Frozen Section Biopsies of Uterine Smooth Muscle Tumors: A Multicenter Clinicopathologic Study of 86 Cases Emphasizing Diagnostic Pitfalls

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Background: Frozen section (FS) biopsies for uterine smooth muscle tumors (U-SMT) are infrequently performed and detailed studying under this scenario is rarely done.
Design: Clinicopathologic features of 86 U-SMTs sent for FS between 1997 and 2012 from 5 hospitals were reviewed.
Results: Patients' age was 31 to 90 years (median, 44.5). Commonest preoperative finding was a mass (n=61, 71%) with 10 suspected ovarian tumors. Commonest reasons for request was extrauterine involvement with or without adhesions to other organs (n=43, 50%) or an atypical intraoperative appearance (n=28, 33%). Lesions were 0.5 to 40 cm (median, 15), mostly subserosal (n=46, 53%) or pedunculated (n=20, 23%). There were 82 leiomyomas including variants; 4 were leiomyosarcomas. In 87% (n=75) the FS concurred with paraffin diagnosis. The false positive and false negative rates were 1.2% (n=1) and 0%, respectively. Deferral rate was 10.5% (n=9). The false-positive case was due to misinterpretation of stromal hyalinization as tumor cell necrosis (TCN). That tumor also had atypia but no mitosis. This was diagnosed as malignant and the patient had hysterectomy and full staging. Another case with stromal hyalinization resulted in compartmentalization of tumor cells was misinterpreted as an epithelial tumor. However, extent of operation was unaltered. Accurate FS diagnosis of malignancy was made in all 4 leiomyosarcomas. Reasons for deferral were hypercellularity, epithelioid differentiation, necrosis of an uncertain type, atypia, myxoid change, unusual growth pattern or combinations thereof. Difficult tissue-cutting in 2 was due to adipose tissue and calcification. The sensitivity and specificity were 100% and 98.6%, respectively. The positive and negative predictive values were 80% and 100%, respectively.
Conclusions: FS of U-SMT is reasonably accurate. Intraoperative appearances are not specific. Alterations of stroma accounted for most cases of errors or deferrals; specifically, stromal hyalinization may potentially mimic TCN and this latter feature is unreliable in FS. Diagnosis of leiomyosarcoma is possible even if the tumor has only diffuse atypia and high mitotic rate. Malignancy should always be diagnosed with caution as it may lead to unnecessary overtreatment.

1170 Vaginal Squamous Cell Carcinoma and Intraepithelial Neoplasia Do Not Express Selected Junctional Markers Expressed in Cervical Carcinoma

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Background: Vaginal squamous cell carcinoma (SCC) is rare, accounting for only 1 % to 2% of all gynecologic malignancies. Similar to cervical squamous cell carcinoma, vaginal SCC and its precursor (vaginal intraepithelial neoplasia), has a strong association with human papillomavirus (HPV) infection and lesions contain a wider range of HPV types. Although the estimates of persistent oncogenic HPV infections are similar in cervical and vaginal lesions the incidence rates of cervical cancers are much higher. Recently a discrete population of cervical squamocolumnar junction (SCJ) cells was identified that have a specific morphology and gene expression profile. These SCJ cells were found to be immunoreactive with junction-specific antibodies including keratin 7 (Krt7). The aim of this study was to determine if vaginal squamous cell carcinomas and their precursor lesions develop from SC junction cells.
Design: 28 vaginal biopsies, including 6 VAIN1 (LSIL), 14 VAIN 2/3 (HSIL) and 8 invasive squamous cell carcinomas were studied. All cases were evaluated for Krt7 and p16 expression by immunohistochemistry. Staining for p16 was classified as patchy or horizontally diffuse. History of cervical intraepithelial carcinoma and follow-up information were obtained from patients medical records.

Results: Immunohistochemical staining with Krt 7 was positive in 0/6 (0%) of VAIN1 cases, 2/14 (14%) of VAIN 2/3 cases and 1/8 (12%) of invasive squamous cell carcinomas. The incidence of concurrent or prior cervical squamous intraepithelial lesions was similar to previous reports. All cases of VAIN1 were negative for p16. One case of VAIN2/3 had patchy p16 staining; the remaining cases of VAIN2/3 had horizontally diffuse p16 staining.
Conclusions: This study confirms that most vaginal SILs and invasive squamous cell carcinomas do not arise from SCJ cells. This supports the more traditional mechanism of HPV lesion formation in this site, being disruption of the basal epithelium and infection of basal keratinocytes. It also explains the marked differences in lesion incidence between the cervix and vagina and underscores the fact that the vagina is not protected from SIL following removal of the cervix. Whether occasional SCJ positive SILs or carcinomas arise by extension of prior cervical lesions, or develop from residual embryonic SCJ cells is unknown.

1171 Predictive Value of Squamo-Columnar Junction Markers and p16ink4 in Multi-Observer Classification of Cervical Precursor Lesions

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Background: Currently p16ink4 (p16) staining is recommended for SILs bordering on CIN2, a positive result warranting a diagnosis of HSIL (CIN2). Recent studies have shown that most HSILs express squamo-columnar junction (SCJ) markers (krt7,ARG2) and that two types of LSILs exist; one SCJ marker (-) and associated with a very low risk of HSIL on followup, and one SCJ marker (+), at greater likelihood to be both diagnostically problematic and manifest with an HSIL outcome. This study compared the blind histologic assessment of SILs by three different pathologists against a standard set by an experienced pathologist, SCJ biomarkers and p16 staining pattern. The goal was to address theoretical strengths and weaknesses of these biomarkers.
Design: 158 SILs were classified as LSIL (101) or HSIL (57) and immunostained for Krt7 and p16. Positive p16 staining occupied more than one third of the epithelial thickness and was continuous throughout the lesion. Lesions were subdivided into three categories; SCJ+/p16+ HSIL (57), SCJ+/p16+ LSiL(20), and SCJ- LSIL (81), signifying a descending order of "risk" for HSIL outcome. Each of three observers reviewed the biopsies and scored them as LSIL, HSIL and indeterminate. The results for each observer was compared to the standard. Theoretically, underdiagnosis of SCJ+HSILs increased the risk of recurrence; overdiagnosis of SCJ-, p16+ LSILs increased the risk of unneccesary cone biopsy.
Results: For all observers, an LSIL diagnosis correlated with descending order of risk category (p<.001). Table 1 summarizes the percentage of cases classified as HSIL or indeterminate in each group by each observer.

Observer (O) Diagnosis of HSIL/Indeterminate

Reference	No	O1	O2	O3
SCJ+ HSIL	57	93%	91%	91%
SCJ+ LSIL	20	30%	65%	45%
SCJ- LSIL	81	16%	35%	32%
SCJ-LSIL P16+	45	17%	49%	46%
SCJ-LSIL P16	36	14%	17%	14%

From 30-65% of SCJ+/p16+ LSILs were interpreted as HSIL/indeterminate, consistent with considerable diagnostic disagreement for this group. However, 16-35% of SCJ- LSILs were also classified as HSIL /indeterminate, increasing to 17-49% for p16+ lesions.
Conclusions: A high percentage of SCJ- LSILs, lesions with a low risk of HSIL outcome, are either classified as HSIL or would be if p16 staining as currently defined was relied on to adjudicate an indeterminate diagnosis. This is compounded by the lack of evidence showing that p16+/SCJ- LSILs carry a significant risk of HSIL outcome. Further study of SCJ markers as an adjunct in segregating lower risk (L) SILs is warranted.

1172 Digital Image Analysis Shows Associations between Vitamin D Receptor, Age, and Tumor Type in Endometrial Carcinoma

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Background: The vitamin D receptor (VDR) influences many processes from calcium homeostasis to cellular proliferation and maturation. Ligand dependent and independent pathways have been described that involve nucleo-cytoplasmic translocations of VDR. Some of these may be at play in common malignancies such as colon and breast carcinomas. Additionally, recent research indicates an interplay between vitamin D, insulin resistance, and adiposity, the latter of which is an established risk factor for endometrial carcinoma (EC).
Design: Immunohistochemistry (IHC) with a commercially available VDR antibody (Santa Cruz Biotechnology) was performed on sections of a tissue microarray (TMA) of 381 EC. Digital slide images were produced with the Aperio ScanScope XT (Vista, CA). Tissue Studio 3.5 software by Definiens (Munich, Germany) was used to analyze component pixels, leading to quantification of the intensity and completeness of IHC stain in epithelial tissue only. A percentage for high intensity VDR staining was assigned to each core. Clinical data was extracted from electronic medical records and the high-intensity VDR staining was compared to age, tumor type, tumor stage, body mass index (BMI), and presence or absence of four IHC stains for mismatch repair (MMR) proteins (MLH1, PMS2, MSH2, MSH6).
Results: IHC displayed cytoplasmic EC staining almost exclusively. EC from women <50 years of age had a significantly higher VDR than those of patients ≥50 years of age (36.38% +/- 22.24 vs 26.97% +/-18.15), p=0.009, Wilcoxon Rank Sum Test. The Spearman's Correlation Coefficient between age and EC VDR was also significant at -0.115, p=0.024, showing a slight decrease in VDR as age increased. Additionally, FIGO grade 1 and 2 EC showed significantly higher VDR than higher grade tumors (29.29% +/- 18.68 vs 23.84% +/- 19.01), p=0.007. VDR did not correlate with BMI, stage, or MMR expression.
Conclusions: VDR localizes to the cytoplasm of neoplastic cells in EC where it may be polyubiquitinated and degraded. EC VDR expression is slightly but significantly higher in EC of younger women and in lower grade tumors. These findings indicate a possible role for VDR and its ligands and coregulators in EC. While these associations are unlikely to have immediate clinical, prognostic, or treatment implications for EC patients, further investigation is clearly warranted.

1173 Body Mass Index Associations Including Mismatch Repair Protein Expression in 1061 Endometrial Carcinomas

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Background: Obesity is a risk factor for endometrial carcinoma (EC). Body mass index (BMI) may be used to estimate the estrogen milieu that supports many EC, given adipose tissue production of estrone. Recent research indicates that estrogen may play a role in mismatch repair protein (MMR) expression maintenance in benign endometria and EC. While other clinicopathological features have consistently correlated with obesity, no clear association between BMI and MMR has emerged from recent series of EC. MMR immunohistochemistry (IHC) done in all hysterectomies with EC at our institution allows thorough analysis of MMR associations, now with more than double the cases we have previously analyzed.
Design: MLH1, PMS2, MSH2 and MSH6 on 1061 consecutive hysterectomy specimens with EC were classified as present or absent. Clinical databases were used to extract BMI data at the time of surgery. Relationships between MMR IHC, BMI, age, stage, and tumor type were explored.
Results: Women <50 years constituted 14% of the cases, had a significantly higher BMI (n=149, median BMI=39.2, range 17.8-89.7) than women ≥50 years (n=912, median BMI=34.9, range 14.2-82.4), p<0.001, and 135 (90.6%) of their tumors were type 1. Regardless of age, when separated by tumor type, more type 1 patients were obese (BMI ≥30) than type 2 patients, p<0.001. Additionally, type 1 tumors had a significantly lower stage than did type 2 tumors, p<0.001. MMR protein loss occurred at the same rate between type 1 and type 2 tumors, but obese patients had a lower MMR protein loss overall than under, normal, or overweight patients, p=0.004. In the <50 age group, BMI was significantly lower in patients who lost MSH2 or MSH6 individually, compared to those who retained expression, p=0.002 and 0.004 respectively. Similarly, obese patients <50 years old had a lower rate of MSH2 or MSH6 loss, p<0.001 and p=0.003. Women with loss of MLH1 (n=176) and/or PMS2 (n=191) were older than women with both proteins present (both p<0.001), and women with absent MSH2 (n=21) and/or MSH6 (n=50) were younger than women with both proteins present, p=0.007 and p<0.001, respectively.
Conclusions: BMI showed multiple significant associations. Higher BMI was seen in premenopausal women and in type 1 tumors. Overall, a higher BMI correlated with normal MMR supporting a possible role for estrogens in the maintenance of DNA repair in EC. These findings indicate particular BMI significance on MSH2 and MSH6 expression in obese women <50 years with EC.

1174 PTEN and P16 Expression Highlights Atypia and Malignant Transformation in Smooth Muscle Tumors of the Uterus

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Background: Tumor supressor genes prevent uncontrolled cell proliferation and provide genetic stability through regulation of cell cycle and promotion of apoptosis. Alteration of tumor supressor gene function either by deletion, mutation or an epigenetic event leads to tumor development. Immmunohistochemical expression of tumor suppressor genes can help in detection of malignant transformation in various tumors. We analyzed the differences in immunohistochemical expression of PTEN and p16 in 74 smooth muscle tumors of the uterus, including leiomyomas, atypical leiomyomas and leiomyosarcomas.
Design: 40 leiomyosarcomas, 12 atypical leiomyomas and 22 leiomyomas from the files of the Department of Pathology, Magee Womens Hospital of UPMC were reviewed and representative sections were immunostained with PTEN and p16 antibodies. Immunohistochemical staining was graded semi-quantitatively by considering the percentage and the intensity of the staining of tumor cells. The H score was obtained by applying the following criteria: H score = 1 X % of weakly (1+) staining cells + 2 X % of moderately (2+) staining cells + 3 X % of strongly (3+) staining cells. The H score ranged from 0 (no immunoreactivity) to 300 (highest immunoreactivity).
Results:

H Scores of PTEN and P16 in LM, ALM and LMS

Tumor/Marker	Range	Mean	Median
LM/PTEN	10-220	104	95
LM/P16	0-120	30	15
ALM/PTEN	150-300	210	250
ALM/P16	30-280	183	190
LMS/PTEN	30-300	200	215
LMS/P16	0-300	235	280

LM; leiomyoma; ALM; Atypical Leiomyoma; LMS; Leiomyosarcoma

Atypical leiomyomas and leiomyosarcomas show no statistically significant difference in staining pattern for PTEN and p16 (p value= 0.387 for PTEN and p value=0.153 for p16, ANOVA analysis, Tukey's HSD Test). Furthermore, the immunostaining in atypical leiomyomas was more intense in areas with severe atypia compare to less atypical zones. However, there are significant differences in expression of both markers between leiomyomas and atypical leiomyomas (p value <0.0001, Tukey's HSD test) and leiomyomas and leiomyosarcomas (p value <0.0001, Tukey's HSD test).

Conclusions: PTEN and p16 may be used as adjunct diagnostic markers in evaluation of atypia in smooth muscle tumors. Especially in scant specimens like curettage or myomectomy strong positivity for PTEN and p16 may determine the need for follow-up and possible hysterectomy.

1175 Ease of Application of Diagnostic Criteria for Premalignant Endometrial Lesions

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Background: Two classification systems are currently used to evaluate premalignant endometrial lesions: the World Health Organization (WHO) criteria and the endometrial intraepithelial neoplasia system (EIN). The WHO system is more widely used, but the EIN system has made recent gains in acceptance. The goal of this study was to determine the ease of use and reproducibility of the 2 systems among naive users.

Design: Twenty-two 3rd and 4th year medical school students, with no prior training in either system, reviewed an online, 20 minute, educational module on both systems' key histologic components and were assigned to take a quiz (48 questions) testing diagnostic criteria including: percent glands; percent stroma; gland to stroma ratio; presence of gland crowding; atypia; cytologic demarcation; and WHO or EIN diagnosis. The answers of both groups were compared with the consensus diagnosis of two gynecologic pathology trained pathologists.

Results: The results are listed in Table 1.

Gland Crowding (EIN)	85% concordance
Gland : Stroma >1 (WHO)	68% concordance
Cytologic Demarcation (EIN)	68% concordance
Atypia (WHO)	52% concordance
EIN Diagnosis	85% concordance
WHO Diagnosis	36% concordance
Percent Glands	Mean standard deviation = 0.128
Percent Stroma	Mean standard deviation = 0.107

Conclusions: These findings suggest that the application of EIN criteria is more reproducible than WHO criteria among naive users and that percent stroma is easier to evaluate than percent glands. Assuming both schemes provide similar prognostic predictive value, EIN is easier to learn and likely to provide more consistent results.

1176 The Müllerian Marker PAX-8 Is Expressed in Peritoneal Mesothelial Proliferations in Women with and without Gynecologic Malignancies

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Background: PAX-8 is a transcription factor critical to Müllerian tract embryogenesis and is highly expressed in ovarian and uterine epithelial neoplasms. Although PAX-8 expression has not been reported in pleural malignant mesotheliomas, some cases of well-differentiated peritoneal mesotheliomas and a small minority of peritoneal malignant mesotheliomas have been reported as PAX-8+; however, the number of cases in these studies was quite small. We recently identified expression of PAX-8 in reactive peritoneal mesothelium in women and therefore wished to determine the extent of PAX-8 expression in reactive and neoplastic mesothelial lesions in the peritoneum of women with and without gynecologic malignancies (GM).

Design: A series of peritoneal resection/excision specimens from women which includes 8 cases of mesothelial hyperplasia (MH) associated with reactive disease, 18 cases of MH associated with GM, 1 well-differentiated papillary mesothelioma (WDPM), and 7 adenomatoid tumors (AT) was obtained. Immunohistochemistry (IHC) studies were performed with a panel of monoclonal antibodies to the Müllerian marker PAX-8 (BC12, Biocare), estrogen receptor (SP1, LabVision), glycoprotein markers BerEp4 (DAKO) and MOC-31 (DAKO), Müllerian/mesothelial marker WT-1 (6F-H2, DAKO) and mesothelial marker calretinin (5A5, Novocastra). The following scoring criteria were employed: 0 = negative, <1% = rare cell, 1-25% = focal, 26-75% = variable, and >75% = uniform.

Results: AT: 0/7 PAX-8+, 5/7 ER+, 0/7 BerEp4+, 0/7 MOC-31+
WDPM: 1/1 PAX-8+, 1/1 ER+, 0/1 BerEp4+, 1/1 MOC-31+
MH (not associated GM): 3/8 PAX-8+, 0/8 ER+, 0/8 BerEp4+, 2/8 MOC-31+
MH (associated GM): 10/18 PAX-8+, 9/18 ER+, 3/18 BerEp4+, 6/18 MOC-31+
All of the mesothelial lesions were + with WT-1/calretinin.

Conclusions: The Müllerian marker PAX-8 is expressed in the peritoneum of women in a significant number of reactive mesothelial proliferations associated with and without GM (56% and 43%, respectively), 1/1 case of WDPM, and is not identified in AT. These findings demonstrate that PAX-8 may not be a reliable marker in discriminating mesothelial proliferations from Müllerian neoplasms, given the considerable degree of immunophenotypic overlap. PAX-8 should only be used judiciously in female patients with a panel of appropriate IHC when attempting to define the cell lineage of epithelioid proliferations in peritoneal fluids or peritoneal biopsies/resections.

1177 PAX8 in Smooth Muscle Tumors of Mullerian Versus Non-Mullerian Origin

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Background: Uterine smooth muscle tumors (SMT) and SMT of the retroperitoneum, mesentery, viscera or deep somatic soft tissue cannot be distinguished by routine light microscopy. Distinction of uterine smooth muscle tumors from non-uterine SMT is essential because of different criteria to classify malignant potential. Estrogen receptor (ER) immunohistochemistry (IHC) is of limited value due to low sensitivity and specificity for uterine SMT. PAX8, a transcription factor that regulates development of the Mullerian system, is expressed in normal Mullerian epithelium and in many Mullerian carcinomas. However, its expression in normal human myometrium and uterine SMT is unknown; the rare non-uterine SMT that have been studied do not express PAX8. Therefore, we evaluated the role of PAX8 in distinguishing uterine from non-uterine SMT and the concordance between PAX8 and ER in these tumors.

Design: We analyzed the expression of PAX8 and ER in uterine leiomyosarcoma (LMS, n=55, 14 primary and 41 metastatic), non-uterine LMS (n=23, retroperitoneal, mesenteric, pulmonary, inferior vena cava, gastric and soft tissue; 18 in women, 5 in men), uterine leiomyomas (n=12), parasitic leiomyomas of uterine origin (n=12) and visceral/gastrointestinal leiomyomas (n=3) by IHC on tissue microarrays. Stains were interpreted independently by the three authors who were blinded to the diagnoses. Tumors were scored for nuclear expression (negative/positive) and intensity of expression (0-3).

Results: PAX8 was expressed in 28 of 55 (51%) of uterine LMS, 12 of 23 (52%) of non-uterine LMS (including 3/5 occuring in men). Howewver, PAX8 was not expressed in any leiomyoma (0/27), regardless of origin. ER was expressed in 31 of 55 uterine LMS (56%) and 4 of 23 non-uterine LMS (17%, none in men). There was no correlation between PAX8 and ER expression in either uterine or non-uterine LMS, and the combination of PAX8 and ER expression was not significantly different between uterine and non-uterine LMS.

Conclusions: PAX8, either alone or in combination with ER, does not help discriminate between uterine and non-uterine origin of benign SMT or LMS. PAX8 expression in both uterine and non-uterine LMS, but not benign SMT, raises the possibility that this transcription factor may play a role in the pathogenesis of malignant tumors, irrespective of Mullerian origin.

1178 The Utility of Vaginal Pap Test in Patients with Endometrial Carcinoma Post Hysterectomy

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Background: Endometrial cancer is the most common gynecologic malignancy in developed countries and surgery is the standard treatment. New screening guidelines does not emphasize if vaginal screening test is needed for patients with endometrial carcinoma after hysterectomy. Our aim was to evaluate the utility of the vaginal Pap test for women with endometrial carcinoma post hysterectomy.

Design: A retrospective review of all cases of endometrial carcinoma who underwent hysterectomy at our institution over a period of 17 months was performed to look at the clinical data, histological diagnosis at the time of surgery, cytological and histological follow-up results.

Results: A total of 227 patients underwent hysterectomy for endometrial carcinomas and the hystrectomy specimens included 120 (53%) endometrioid carcinoma, 26 (11%) serous carcinoma, 7 (3%) clear cell carcinoma, 3 (1%) malignant mixed mulerian tumor and 5 (2%) other types of carcinomas. The mean age of the patients was 62.3 years (range 27-91 years) and, 161 (71%) patients had vaginal histological and/or cytological results in our database during the follow-up period. The mean follow-up period was 43 months (range: 3 to 80 months) and the number of follow up Pap smears visits averaged 6.4 times (range: 1 to 16 times). The follow-up histological and cytological results were summarized in Table 1.

Table 1. Summary of follow-up cytological & histological results

Follow-up Histo-Cytological Results	(Total cases)	Carcinoma	VAIN2/3 & HSIL	VAIN1/LSIL	Atypical Squamous lesion	Negative
Histology	17	2	0	3	0	12
Cytology Only	144	0	0	1	18	125
Total Cases	161 (100%)	2 (1%)	0	4 (2%)	18 (11%)	137 (85%)

Two cases (1%) were detected with vaginal adenocarcinoma (recurrent endometrial carcinoma) at 5 months and 26 months post treatment by both vaginal cytology and biopsy. Four (2.5%) cases developed vaginal intraepithelial neoplasia 1(VAIN1) or low grade squamous intraepithelial (LSIL) lesions and no vaginal intraepithelial neoplasia 2/3 (VAIN 2/3) or high grade squamous intraepithelial lesion (HSIL) were identified during the follow-up period.

Conclusions: Vaginal cuff cytology for women with endometrial carcinoma after hysterectomy may play two functions including surveillance of endometrial carcinoma recurrences and the screening of vaginal dysplasia or cancer. The majority of recurrences occur within three years after hysterectomy. No high grade dysplasia was identified during the period of 4 years. We suggest that these women should not be regularly screened after three years of hysterectomy. More larger case series studies are needed.

1179 Clinicopathological Characteristics of Ciliated Change of Endocervical Gland: A Mimic of "Endocervical Glandular Dysplasia"

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Background: A variety of benign endocervical glandular lesions or changes can be misinterpreted as "endocervical glandular dysplasia", of which ciliated change (CC) is considered to be a representative because of nuclear stratification. However, clinicopathologic characteristics of CC have not been well described.

Design: A total of 455 cases were retrieved from the file to review HE slides of uterine cervix without significant lesions, were included in the study. Clinical records were reviewed, and presence or absence of CC and coexisting tuboendometrioid metaplasia, and if exists its distribution, were evaluated. In addition, nuclear features with columnar epithelium with CC were evaluated in selected 100 cases and compared with 6 cases of adenocarcinoma in situ (AIS). Specifically, degree of nuclear stratification and heterogeneity in nuclear size and nuclear staining pattern were scored with a 3-tier scale. immunohistochemistry for p16, vimentin, and Ki-67 was performed to characterize CC.

Results: Among the 455 cases, 383 (84.2%) showed CC, of which 228 cases (59.5%) showed diffuse and 155 (40.5%) showed focal distribution. CCs were identified equally in transformation zone and endocervical canal. Presence or absence of CC

was not correlated with age, menstruation status, and tuboendometrioid metaplasia. Reproductive history was available in 441 cases. CCs were significantly more common in nulligravida women (99/106, 93.4%) compared with multi gravida women (273/335, 81.5%) (p=0.003). Significant nuclear stratification (score 1, n=5; score 2, n=41; score 3, n=54) and mild to moderate nuclear enlargement and heterogeneity in nuclear size (score 1, n=25; score 2, n=64; score 3, n=11) were common in CC, but hyperchromatism was minimal (score 1, n=69; score 2, n=31; score 3, n=0) as seen in cases of AIS (score 1 and 2, n=0; score 3, n=6). Mitotic figures were only rarely seen. Immunohistochemically, CCs were delineated as thin rod- or pyramid-shaped cells tapering from apical side with cytoplasmic staining for p16 and vimentin, intervened by non-ciliated cells, resulting in "keyboard appearance". The Ki-67 labeling index was less than 5% (mean, 1.5; range, 0.1-4.2).

Conclusions: The CC is commonly seen in uterine cervix, and has a relation with reproductive history. Occasionally it shows significant nuclear stratification and enlargement, and thus can be interpreted as "glandular dysplasia". However, hyperchromatism is minimal, mitosis is rare, and Ki-67 index is low. Vimentin and p16 staining reveals "keyboard appearance".

1180 Upregulation of miR-141 Potentially Limits Expression of JAG1 in Epithelial Ovarian Neoplasia

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Background: Ovarian cancer is the fifth most common cancer in women and has the highest mortality amongst gynecological cancers. This is largely due to the fact that ovarian cancer is difficult to detect at early stages. Each histological subtype of epithelial ovarian cancer has a distinct set of molecular characteristics making it difficult to pinpoint one set of molecular biomarkers that maybe used as diagnostic aids. MicroRNAs are small non-coding molecules that have been associated with the virtually all cancers. The miR-200 family which includes miR-141 has been linked to the Notch Signaling pathway, of which Jagged1, the protein product of JAG1 is part. JAG1 has been shown to have decreased expression in ovarian neoplasia due to a variety of reasons including changes in WNT signaling and knockdown of Notch3.

Design: FFPE blocks from normal ovary, benign and malignant mucinous and serous ovarian neoplasia (n=327) dating from 2005-2009 were retrieved from the archives of St. James Hospital and were microscopically reviewed for confirmation of diagnosis. 10μm sections were cut, H&E stained and laser capture microdissected to collect homogenous cell populations for subsequent analysis. RNA was subsequently extracted and reverse transcribed to cDNA. TaqMan-PCR was carried out on the cDNA, targeting miR-141 and JAG1. n=294 microRNA samples and n=250 gene samples were successfully amplified.

Results: miR-141 expression was consistently upregulated in disease cohorts relative to normal ovarian epithelium, whilst JAG1 was down-regulated across cohorts relative to normal ovarian epithelium. The two exceptions were a slight down-regulation of miR-141 in mixed benign ovarian neoplasias, and a slight upregulation of JAG1 in benign serous neoplasias.

Conclusions: miR-141 displays a reciprocal expression profile with JAG1. This suggests a regulatory role for miR-141 in ovarian cancer disease progression linking it with JAG1. miR-141 may be a potential therapeutic target for si-RNA intervention ameliorating aberrant Wnt signalling and restoring cellular equilibrium.

1181 Molecular Characterization of Undifferentiated Carcinomas That Are Associated with Low-Grade Endometrioid Carcinoma

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Background: Uterine and ovarian undifferentiated carcinomas (UC) are often associated with low-grade endometrioid carcinomas (EMC), and are morphologically characterized by a solid growth pattern, and a lack of appreciable features of differentiation. UCs are highly malignant. The molecular pathogenesis that leads to disease aggressiveness remains largely unknown. This study aims to better understand the molecular features of UCs by comparing the molecular alterations in several genes between the UC and the EMC component.

Design: A total of 20 UCs from the uterus (18) and the ovary (2) were collected. Among them, 12 cases contained both UC and EMC components. Immunohistochemistry (IHC) staining was used to determine the expression pattern of ARID1A, β-catenin and PTEN in all specimens. Furthermore, mutation analysis was performed in 11 cases for the following genes that are mutated in endometrioid carcinoma: *CTNNB1*, *FBXW7*, *KRAS*, *PIK3CA*, *PPP2R1A* and *TP53*.

Results: Concordance of IHC pattern for ARID1A, β-catenin and PTEN and gene mutations were recorded in the 12 pairs of samples containing both UC and EMC components, except in three cases of which *CTNNB1* mutations and/or nuclear β-catenin staining were detected in the UC but not in the associated EMC. Overall, the most common molecular genetic alterations in UC were mutations in *PIK3CA* (55%), *CTNNB1* (36%), *TP53* (27%) and *PPP2R1A* (18%). No mutations were identified in *FBXW7*, and *KRAS*, as well as no loss of ARID1A immunoreactivity, in all cases analyzed. As compared to uterine endometrioid carcinoma without concurrent UC, retained ARID1A expression was significantly associated with endometrial carcinomas containing the UC ($p< 0.001$).

Conclusions: The above results support a clonal relationship between EMCs and their associated UCs. In some cases, activation of β-catenin may contribute to tumor progression from EMC to UC. Retention of ARID1A expression in all cases with UC suggests a unique molecular mechanism in the development of UC distinct from the classical tumor progression from low-grade to high-grade endometrioid carcinoma, as the latter is frequently associated with loss of ARID1A expression.

1182 *CCNE1* Amplification May Precede Centrosome Number Abnormality in Progression from Serous Tubal Intraepithelial Carcinoma to High-Grade Ovarian Serous Carcinoma

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Background: Genomic instability is the hallmark of most cancer cells, and has profound biological significance in tumor development. Overexpression of cyclin E, often due to amplification of the encoding gene *CCNE1*, facilitates genomic instability. Moreover, centrosome - the primary microtubule-organizing center in human cells - plays a critical role in chromosome segregation, and abnormalities of centrosome number result in genomic instability. Therefore, increased centrosome number (also called centrosome amplification) has been used as a surrogate marker of chromosomal instability. In this study we asked whether *CCNE1* and centrosome amplification occur early during tumor progression from serous tubal intraepithelial carcinoma (STIC) to ovarian high-grade serous carcinoma (HGSC).

Design: A total of 37 STIC and 43 HGSC were investigated for *CCNE1* copy number alterations using a fluorescence in situ hybridization (FISH) assay. *CCNE1* amplification and high polysomy were considered FISH positive (+). A double-color immunofluorescence for γ-tubulin and α-tubulin was performed to simultaneously visualize centrosomes and microtubules, respectively.

Results: We found that 8 (22%) of 37 STICs were *CCNE1* FISH +, of which 2 had high polysomy and 6 amplification. Interestingly, one out of 3 STICs not associated with HGSC showed *CCNE1* high polysomy. 12 (28%) of 43 HGSCs were *CCNE1* FISH +, including 2 high polysomy and 10 amplification. In this series, 30 STICs were associated with HGSC, of which 11 were bifocal. We found a significant concordance in *CCNE1* copy number between STIC and HGSC from the same patient (p-value <0.001). There was no significant difference in the percentage of *CCNE1* FISH + cases between STIC and HGSC (p=0.613, Chi square). On the other hand, centrosome amplification was recorded in only 3 (14%) of 21 STICs, but in 8 (47%) of 17 HGSCs. There was a significant increase in the percentage of cells with centrosome amplification in HGSC as compared to STIC (p= 0.0006, Wilcoxon rank test).

Conclusions: Our findings suggest that *CCNE1* copy number gain occurs early in tumor progression while centrosome amplification may likely represent a later molecular event. Therefore, these two markers associated with genomic instability are involved at different stages of tumor progression in HGSC carcinogenesis.

1183 Frequent *CCNE1* Amplification in Uterine Serous Carcinoma and Endometrial Intraepithelial Carcinoma

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Background: Uterine serous carcinoma (USC) accounts for only 10% of all uterine cancers, but is the leading cause of uterus corpus cancer-related death. The pathogenesis of this aggressive neoplasm has been largely unknown until recently, when we performed a comprehensive genome-wide analysis of USC, including whole-exome sequencing and gene copy number array. Our study revealed that *TP53*, *PIK3CA*, *FBXW7*, *PPP2R1A* are the most commonly mutated genes, and *CCNE1*, encoding for cyclin E, is one of the most frequently amplified genes in USC. In the current study we applied fluorescence in situ hybridization (FISH) to evaluate *CCNE1* copy number at a single cell resolution in USC and concurrent endometrial intraepithelial carcinoma (EIC), and correlated the molecular alterations with clinicopathological features.

Design: A total of 27 USCs and 20 concurrent EICs were collected. All the available slides were reviewed and clinicopathological features recorded. Two-color FISH assay was used to measure the gene copy number of *CCNE1* in USC and EIC. The copy number was classified into six FISH strata. *CCNE1* amplification was defined as the presence of loose or tight *CCNE1* cluster or *CCNE1* to centromeric probe ratio ≥ 2 in more than 20% of the analyzed cells. All the molecular characteristics and clinicopathological features were correlated.

Results: We found that 12 (44%) of 27 USCs and 9 (45%) of 20 EICs showed *CCNE1* amplification. Overall, we found concordance in *CCNE1* copy number in concurrent EIC-USC pairs (p-value <0.001). Three USCs showed intra-tumoral heterogeneity in term of *CCNE1* copy number alteration, because there were tumor areas with focal *CCNE1* amplification in a background of tumor without amplification. No evidence of correlation was observed between *CCNE1* copy number and clinicopathological features, including age, race, clinical stage, overall survival, angiolymphatic invasion, as well as mutations in *TP53*, *PPP2R1A*, *PIK3CA* and *FBXW7*.

Conclusions: We demonstrated that amplification of *CCNE1* is one of the most common molecular genetic changes in USC. *CCNE1* amplification in many EICs suggests that this genetic event occurs early during tumor progression. Further studies are required to better delineate the clinical and biological impact of *CCNE1* amplification on overall survival and therapy response in USC patients.

1184 P16 Immunoreactivity in CIN1 Predicts Malignant Progression to High-Grade Dysplasia

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Background: P16 immunohistochemistry is widely used to facilitate accurate diagnosis of high grade cervical dysplasia (CIN2-3). It has also been noted that a subgroup of CIN1 lesions shows focal p16 immunoreactivity. However, the clinical significance of focal p16 expression in CIN1 is largely unknown. To investigate whether focal p16 expression in CIN1 confers risk of progression to high-grade dysplasia, we compared long-term clinical outcomes of women with p16-negative CIN1 and p16-positive CIN1. We systematically studied follow-up data including HPV testing, cytology, colposcopy-guided biopsy, LEEP, and conization in 243 patients initially diagnosed with CIN1.

Design: A cohort of 243 consecutive patients diagnosed with CIN1 on biopsy was retrieved from our hospital archive and reviewed. P16 immunostaining (MTM antibody, Ventana, Arizona) was performed on all cases. Results of p16 staining patterns were recorded as negative, focal, or diffuse. Follow-up information, including HPV testing, cytology, biopsy, LEEP, and conization diagnoses, were reviewed and tabulated for up to 36 months.
Results: A total of 243 women with CIN1 lesions were included in the study. Based on p16 immunoreactivity, CIN1 was divided into two groups: p16-negative CIN1 (123, 50.6%) and p16-positive CIN1 120 (49.4%). Cervical biopsy, LEEP, and/or conization were performed on 116 patients. The remaining 127 patients who were clinically considered to be low risk were followed subsequently with HPV testing and/or cervical cytology. HPV infection persisting for at least 12 months was seen in 66 (27%) patients, of whom 29 (43.9%) had p16-negative CIN1 and 37 (56.1%) had p16-positive CIN1. CIN1 persisting for at least 12 months was seen in 27 patients, including 13 with p16-negative CIN1 and 14 with p16-positive CIN1. Twenty-six (10.7%) patients progressed to CIN2-3. Among those, 25 (96.2%) had p16-positive CIN1 and one (3.8%) had p16-negative CIN1 ($P<0.0001$). Patients with p16-positive CIN1 progressed to CIN2-3 at a significantly higher rate than those with p16-negative CIN1 (21% vs. 1%, $p<0.0001$).
Conclusions: Our study shows that patients with p16-positive CIN1 are significantly more likely to progress to high-grade dysplasia than those with p16-negative CIN1. Also, of the patients with CIN1 who progressed to high-grade dysplasia, the overwhelming majority (96%) had p16-positive lesions on biopsy. These data suggest that focal p16 staining in CIN1 may represent an early event of malignant transformation.

1185 Diagnostic and Prognostic Potential of HE4 in Ovarian Cancer

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Background: Ovarian cancer is the fifth most common cancer in women and the most frequent cause of gynaecologic malignancy-related mortality in women. Clinical outcome and survival may be improved if the disease is identified in the early stages. Recently a novel biomarker Human epididymis protein 4 (HE4) has been demonstrated to be a sensitive and specific serum biomarker for ovarian cancer that is elevated less frequently by benign conditions that occur in premenopausal women. The aim of the study was to evaluate the utility of HE4 as a diagnostic and prognostic marker of ovarian cancer in premenopausal and postmenopausal women.
Design: *Diagnostic arm:* Serum was collected from 386 women of various histological subtypes prior to surgery for invasive, borderline and benign ovarian disease. *Prognostic arm:* Serum was collected from 30 women 4 to 10 days after surgery for ovarian cancer and benign ovarian disease. Another set of serum samples were collected from 9 women undergoing chemotherapy for ovarian cancer. These samples were taken prior to commencement of chemotherapy, half way through the course of treatment and post chemotherapy. All chemo samples recruited in the study were of the same histology (serous papillary adenocarcinoma stage 3 and grade 3). HE4 EIA protocol and CanAg CA125 EIA protocols were carried out according to manufacturer's instructions (FUJIREBIO Diagnostics). Risk of Ovarian Malignancy Algorithm (ROMA), which combines the result of HE4 and CA125, was calculated for premenopausal and postmenopausal women. Non-parametric tests (Kruskal-Wallis Test and Mann-Whitney test) were used for statistical analysis.
Results: The combination of HE4 and CA125 in the ROMA index increased the sensitivity and specificity of detecting ovarian cancer than either marker alone. HE4 alone is more specific than CA125. Significant decreases in HE4 were observed in the majority of patients in the post operative setting and throughout chemotherapy. However, in a minority of cases HE4 values did not decrease and these women went on to develop progressive/chemoresistant disease.
Conclusions: The study shows that HE4 increases the specificity of ovarian cancer detection and in combination with CA125 the overall sensitivity and specificity of detecting ovarian cancer is improved. HE4 also holds promise as a prognostic marker in the postoperative setting and for monitoring response to chemotherapy.

1186 Laminin ß1 and ß2 Immunostaining in Ovarian Carcinoma

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Background: Laminins are a family of trimeric extracellular matrix glycoproteins that contain α, β, and γ chains and form the major component of basement membranes (BM). Each chain type has several isoforms. Two of the β chain variants are β1 and β2. Alterations in BM composition and/or assembly play a role in diverse processes that include cell integrity, adhesion, migration, and metastasis. Recent studies have shown that disruption of angiogenesis via laminin-targeted therapy may be possible. Laminin has been less studied in epithelial tumor cells.
Design: Formalin fixed paraffin embedded sections of 20 primary ovarian carcinomas (7 high grade serous, 5 mixed serous/undifferentiated, 2 clear cell, 2 mixed mullerian, 2 endometrioid, 1 clear cell/serous, 1 undifferentiated) were stained with Laminin β1 antibody (clone 3G133, abcam) and Laminin β2 antibody (clone C4, Santa Cruz Biotechnology). Intensity (0 negative, 1+ weak, 2+ moderate, 3+ strong) and extent (percentage) of staining were assessed in tumor-associated blood vessels (TA-BV) and tumor basement membrane (TBM). The presence of cytoplasmic staining in tumor cells was also recorded.
Results: TA-BV in 18 (90%) of the 20 cases stained for both β1 and β2; TA-BV in 1 serous and in 1 mixed serous/undifferentiated carcinoma were negative for β1 and β2, respectively. Extent of TA-BV staining ranged from <10% to 90% and intensity ranged from 1+ to 3+. TBM in 18 (90%) cases stained for β2; 1 endometrioid and 1 mixed mullerian carcinoma were negative. In 16 (80%) cases, TBM staining (extent & intensity) for β2>β1; in 3 cases β1 and β2 staining were equal. In only 1 case (endometrioid) was TBM β1>β2 staining. β1 staining was absent in TBM of 11 cases, including all 7 high grade serous and 4 of 5 mixed serous/undifferentiated carcinomas. Three cases showed cytoplasmic staining for β1 (2 clear cell, 1 undifferentiated). None of the carcinomas showed cytoplasmic staining for β2.
Conclusions: Our preliminary findings suggest that:
-Laminin β1 and β2 are both expressed in TA-BV of diverse histologic types of ovarian carcinoma, representing potentials for laminin-targeted therapy;
-Laminin β1 is not expressed in TBM of high grade serous but is expressed in other ovarian carcinomas, possibly reflecting differences in tumorogenesis and/or tumor spread;
-Laminin β2 is expressed in TBM of most ovarian carcinomas irrespective of histologic type whereas β1 expression seems to be histologic type dependent. In epithelial tumor cells, Laminin β2 expression appears confined to TBM whereas β1 expression is detected in both TBM and cytoplasm of clear cell carcinoma.

1187 Collateral Sensitivity to Cisplatin in KB-8-5-11 Is Confluence Dependant

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Background: KB-8-5-11 are a multi-drug resistant cell line derived from KB-3-1 cervical carcinoma cells by selection with colchicine. KB-8-5-11 are resistant to colchicine and taxol due to their over-expression of P-glycoprotein. Some cancer cell lines resistant to platinum-based drugs are sensitive to taxanes. It is thought that this collateral sensitivity is also present in the reverse scenario, where cell lines, which are resistant to taxanes, could be sensitive to platinums.
Design: Five day cytotoxicity assays were carried out in 96-well plates using the MTT assay. A comparative 3-day growth assay was carried out were cells were seeded at low-cell density ($5x10^4$/dish) and high-cell density ($2x10^5$/dish). The low-cell density represents a scale up of the 96-well assay to the 10cm dish. Untreated cells plated at the high-cell density achieve confluence at the end of the 3-day incubation. Both low and high cell density plates were drugged with 50ng/ml of cisplatin and growth was determined by cell counts. Annexin/PI apoptosis assays and cell cycle analysis was performed by FACS.
Results: KB-8-5-11 was 35.76 ± 5.4 fold resistant to taxol ($p=0.03$) compared to KB-3-1. KB-8-5-11 were 1.30 ± 0.35 fold sensitive to cisplatin ($p=0.02$), compared to KB-3-1. KB-8-5-11 displayed a 1.19 fold sensitivity to cisplatin relative to the control. KB-8-5-11 had a % growth of 67.7% ± 14% compared to the control, KB-3-1, which had a % growth of 80.65%± 13% ($p=0.03$), a difference in growth of 12.95%. This sensitivity was reversed when the cells were plated at high density were KB-8-5-11 had a % growth of 78.34% ± 0.10 compared to the control, KB-3-1, which had a % growth of 78.37% ±0.14, a difference in growth of 0.03%. The KB-8-5-11 cells are more apoptotic and experience more cell death than KB-3-1 cells exposed to 200ng/ml cisplatin, 6.7% apoptosis compared to 3.31% apoptosis in KB-3-1. KB-8-5-11 cells also experience a higher proportion of cells in an S phase cell cycle arrest (74.72% compared to the parent cells at 60.80%). Whole genome gene expression analysis will also be carried out to determine any changes in gene expression between KB-8-5-11 and their parental cell line KB-3-1 which could be associated with collateral sensitivity to cisplatin at low-cell density.
Conclusions: Cisplatin sensitivity in KB-8-5-11 is associated with increased apoptosis and cell cycle arrest compared to KB-3-1. This study will help further the understanding of the genes and pathways that play a role in cisplatin sensitivity in vitro. This may yield bio-markers suitable for identifying platinum sensitivity in the clinical treatment of cancer.

1188 miroRNAs Involved in Development and Progression of Ovarian Serous Tumor

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Background: To define the role of microRNAs (miRNAs) in development and progression of ovarian serous tumor.
Design: The possible candidate miRNAs were selected from miRNA Chip study and verified using RT-PCR in 10 normal, 33 benign (SAD), 24 borderline (SBT), and 90 malignant tumor (SCA) tissues. The expressions of miRNAs were also evaluated with known clinicopathological parameters and patient's survival.
Results: The development of serous tumor from normal ovarian tissue was associated with the increase of miR-200b and miR-200c, but the decrease of miR-483, miR-125a and miR-146b. In SBT, miR-21, miR-210, miR-34a, miR-200b, and miR-200c were up-regulated than in SAD, whereas miR-483 and miR-30d were down-regulated. With progression from SBT to low grade SCA, miR-483 was up-regulated, whereas miR-34a was down-regulated in progression from low grade to high grade SCA. The up-regulation of miR-210, miR-483, miR-146b, miR-303, miR200b and miR-200c were associated with high FIGO and Silverberg grades, and clinical stage, whereas miR-34a was inversely correlated with high grade and high p53 expression. Up-regulation of miR-483 was associated with poor overall patient's survival.
Conclusions: Different miRNAs seem to play the role in development and progression of ovarian serous tumor and have prognostic significance.

1189 Precursor Lesions and Prognostic Factors in Primary Peritoneal Serous Carcinoma

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Background: Primary peritoneal serous carcinoma (PPSC) is uncommon and carcinogenetic and prognostic factors are incompletely described. Most pelvic high-grade serous carcinomas appear to originate in the fimbriated end of the fallopian tube as a serous tubal intraepithelial carcinoma (STIC) and later spread to involve the ovary and/or peritoneum. STIC may evolve from a p53 signature also found in the fimbriated end of the tube, and is termed a latent precursor since there is no morphological alteration of the epithelium. Some PPSC may also originate in the fallopian tube via the p53 signature-STIC-carcinoma pathway.

Design: Charts of 22 women with PPSC were reviewed for clinical and pathology data. Glass slides were reviewed for areas of PPSC, ovarian surface epithelium (OSE), ovarian cortical inclusion cysts (OCICs), normal tubal epithelium, tubal epithelial atypia (TEA), and serous tubal intraepithelial carcinoma (STIC). p53 and p16 immunohistochemical expression in these areas was compared for statistical associations and PPSC outcome was correlated with p53 and p16 expression and clinico-pathological variables.

Results: Mean age at presentation was 60 years. Most tumors were high grade, FIGO stage IIIc and treated by surgery and/or chemotherapy. Tubal pathology of STIC, TEA and/or p53 signatures occurred in 45.5% of tubes. p53 and p16 expression amongst PPSC and tubes were high and higher than OSE and OCICs. Differences in expression of p53 and p16 between all lesions were significant except for the p53 comparison of PPSC and fallopian tubes. Tubal pathology was more frequent in tubes with p53 overexpression ($p<0.001$), but did not associate with PPSC p53 or p16 expression. p53 and p16 expression amongst the OSE, and OCICs did not associate with PPSC expression. The median overall survival was 53 months and low grade tumors had a better prognosis ($p=0.02$).

Conclusions: A precursor role for p53 but not p16 related tubal pathology in the development of some PPSC was supported whereas a role for either antibody amongst OSE and OCICs was not. Tumor grade was the only significant prognostic factor.

1190 Well-Differentiated and Non-Invasive Diffuse Periteonal Mesotheliomas Treated with HIPEC: Histologic Spectrum

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Background: Well differentiated mesotheliomas include multicystic mesothelioma (MCM) and well-differentiated papillary mesothelioma (WDPM). Although many of these tumors are localized, some present with diffuse disease. Diffuse malignant mesothelioma of the epithelioid type may in some cases lack histologic evidence of invasion. We compared the histologic and clinical features of these three entities from a single institutional series of peritoneal mesothelioma.

Design: We retrospectively studied histologic specimens of 66 diffuse peritoneal mesotheliomas treated with hyperthermic intraperitoneal chemotherapy (HIPEC) after tumor debulking. Invasion was defined as absence of tumor within fat and tumor confined to surface projections on the serosa. MCM and WDPM were defined as previously reported.

Results: There were 15 well-differentiated and non-invasive mesotheliomas (23%): 3 WDPM, 4 MCM, and 8 non-invasive epithelioid mesotheliomas (NIEM). Five of 8 NIEM were in men, all 3 WDPM were in women, and 3 of 4 MCM were in women. The multicystic tumors occured in younger patients (32 ± 12 years) than the WDPM (55 ± 13 years) and NIEM (57 ± 11 years, $p=.01$). None of the patients with WDPM or MCM had prior surgery, compared to 5 of 8 NIEM. Two patients (one with WDPM and one with NIEM) had prior history of pneumonectomy for pleural mesothelioma. The mean peritoneal cancer index was identical in the WDPM and NIEM (16 ± 4), and slightly higher in MCM (23 ± 3, $p = 0.3$). Histologically, WDPM had surface non-invasive papillary projections lined by flattened cuboidal cells without nucleoli or pleomorphism; many papillae were vascular, and there were no or rare detached clusters. One WDPM had areas of infiltrating adenomatoid tumor, which was bland without mitotic activity or atypia. MCM was defined as possessing cysts lined by flattened epithelium without atypia, at least 2 mm in diameter, in a portion of the tumor. These tumors possessed a cellular stroma with stellate and spindled cells which was difficult to separate from invasion; one invaded into fat, with a prominent adenomatoid appearance. All of the multicystic tumors demonstrated persistence or recurrence (mean at 3 years), and one of the WDPM (15 years); none of the NIEM showed recurrence, but follow-up was short (mean 3.5 years).

Conclusions: Low-grade peritoneal mesotheliomas may have significant PCI and clinically mimic typical invasive malignant mesothelioma. These tumors can be be separated into three distinct histopathologic groups. In this small group with limited follow-up, MCM appears to be the most aggressive of the three.

1191 Biomarker Based Ovarian Carcinoma Typing: A Retrospective Analysis of the OTTA Consortium

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Background: The five major histological types of ovarian carcinoma are high-grade serous (HGSC), clear cell (CCC), endometrioid (EC), mucinous (MC), and low-grade serous (LGSC). Histological type is associated with outcome and is predictive of therapeutic response. The aim of this study was to evaluate the agreement of histological type with a 10 marker immunohistochemical (IHC) calculator of ovarian subtype probability (COSP version 2, COSPv2 based on Kalloger et al. Mod Pathol 2011:24:512) with the original diagnosis and to determine if reclassification impacts outcome.

Design: The Ovarian Tumor Tissue Analysis (OTTA) consortium is dedicated to tissue based ovarian cancer research. The final test set (N=524) was obtained from three OTTA studies: Mayo clinic, UKOPS, and HOPE studies. The following three type assessments were compared: original diagnosis, COSPv2, and a WT1 assisted review of the tissue microarray (TMA) cores (core review). Consensus type was defined as agreement between all three assessments. Statistical analysis was done with JMP version 10.0 (SAS).

Results: Table 1 depicts agreement between the three methods. For the 26% of cases in which COSPv2 disagreed with the original diagnosis, the core review and COSPv2 agreed in 13%, the core review with the original diagnosis in 7%, and in 6% of cases there was no agreement between the three methods. A consensus type was established in 66% of cases. Assuming consensus type represents the most accurate method of typing, nearly 50% of EC were overcalled in the original diagnosis. Reclassification of EC by consensus type resulted in a higher 5-year survival (57% to 93%), reduction in grade 3 EC (50% to 22%), and decreased WT1 expression (40% to 3%). Only consensus type was an independent predictor of outcome when adjusted for age and stage.

Table 1: Concordance rates in % and kappa agreement statistics for the three type assessments.

	Original Diganosis	COSPv2	Core Review
Original Diagnosis	X	74%	75%
COSPv2	0.502	X	83%
Core Review	0.504	0.629	X

Conclusions: Biomarker expression assessed by IHC is a feasible tool to screen large retrospective cohorts on TMA in order to achieve accurate histological typing. The outcome of reclassified EC is more compatible with the expected outcome, which supports reclassification of EC.

1192 Frequent Immunohistochemical Expression of KIT in *YWHAE-FAM22* Endometrial Stromal Sarcoma

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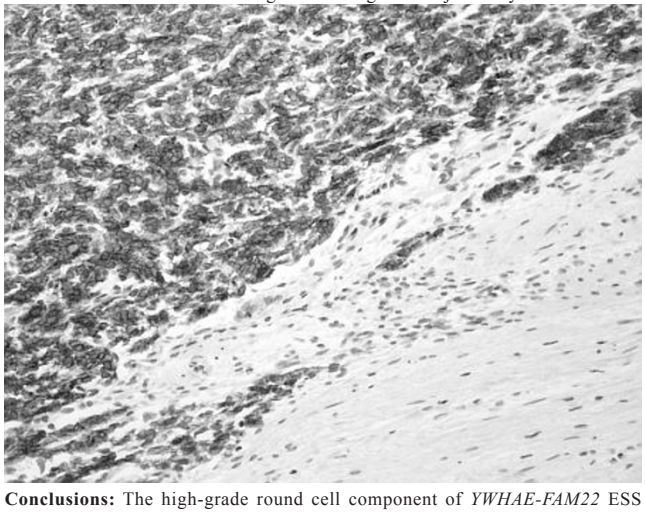
Background: Endometrial stroma sarcoma (ESS) with *YWHAE-FAM22* genetic rearrangement is clinically more aggressive than ESS with *JAZF1-SUZ12* genetic rearrangement and frequently shows extrauterine tumor extension at presentation. In addition to the high-grade round to epithelioid cell component that is invariably present, a subset of *YWHAE-FAM22* ESS also contains an admixed low-grade fibrous spindle cell component. Its appearance in the round/epithelioid cell area can resemble that of gastrointestinal stromal tumor (GIST). The aim of this study is to characterize the immunohistochemical expression of two commonly used diagnostic markers for GIST - KIT and DOG1 in *YWHAE-FAM22* ESS.

Design: The immunohistochemical expression of KIT (Dako, A4502) and DOG1 (Spring Bioscience, SP31) was examined using whole tissue sections of 12 *YWHAE-FAM22* ESS. Membranous and/or cytoplasmic staining was evaluated, and the staining intensity was assessed (negative, weak, moderate and strong) relative to KIT and DOG1 positive GIST tumor.

Results: Of the 12 *YWHAE-FAM22* ESS, 5 showed only high-grade round cell component, 2 showed only low-grade fibrous spindle cell component and 5 showed a mixture of the two components in the slides evaluated. The high-grade round cell component displayed moderate to strong membranous/cytoplasmic KIT staining in 10 of 10 cases (Figure 1). The low-grade spindle cell component showed weak cytoplasmic KIT staining in 2 of 7 cases and negative KIT staining in 5 of 7 cases. DOG1 staining was negative in all 12 cases examined, irrespective the components present.

Figure 1: An *YWHAE-FAM22* ESS showing positive KIT staining in the high-grade round cell area of the tumor and negative staining in the adjacent myometrium.



Conclusions: The high-grade round cell component of *YWHAE-FAM22* ESS consistently expresses KIT, with an intensity that is comparable to that seen in GIST. This represents a potential diagnostic pitfall in the evaluation of extrauterine *YWHAE-FAM22* ESS tumor mass, particularly in situations where its uterine origin is not apparent.

1193 Cervical Polyps: Is Histologic Evaluation Necessary?

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Background: Multiple clinical studies have shown that cervical polyps are essentially benign, with a prevalence of malignancy of 0.1%; for this reason, several studies have recommended that polypectomy is not indicated in all cases. However, histologically examined cervical polyps may contain unexpected cervical intraepithelial neoplasia (CIN), endometriosis, metaplasia and primary malignancies. The purpose of this study was to examine a consecutive series of clinically identified polyps, determine the incidence of clinically significant pathologic findings, and correlate them with atypical pap test results if available.

Design: 369 consecutive endocervical polyps (identified by clinical terminology of "cervical polyp" in pathology reports) from the past 12 years were reviewed. A histologic evaluation of the polyps was performed, followed by a chart review to identify clinical presentation and the immediately prior or concurrent pap test results.

Results: Our dysplastic/malignant findings and benign/reactive findings are listed in tables below. The incidence of each finding is the number of involved cases compared to all of the polyps evaluated (369 cases), except for the pap test percentages, which are compared to the patients with pap tests (228 cases).

Dysplastic \ Malignant Findings	Incidence
ASCUS	29 (12.7%)
AGC	13 (5.7%)
CIN 1	6 (1.6%)
CIN 2/3	2 (0.5%)
Adenosarcoma	2 (0.5%)
Atypical Polyp / Possible Adenosarcoma	2 (0.5%)
Endometrioid Adenocarcinoma	1 (0.3%)
Adenocarcinoma in-situ	1 (0.3%)

Benign \ Reactive Findings	Incidence
Inflammation	356 (96.5%)
Thick walled vessels	361 (97.8%)
Reactive epithelium	336 (91.1%)
Squamous metaplasia	145 (39.3%)
Tubal\endometrioid metaplasia	79 (21.4%)
Microglandular hyperplasia	67 (18.2%)
Endometriosis	18 (4.9%)
Atypical Stromal cells	29 (7.9%)
Stromal Mitoses	5 (1.3%)
Granulation\Ulceration	53 (14.1%)

Conclusions: We demonstrate a higher rate of clinically significant findings in cervical polyps (14 of 369 cases, 3.8 %) than previously reported in clinical studies. In addition, College of American Pathologist guidelines state that ThinPrep testing has a mean expected reporting rate of 4.9% for ASCUS and 0.2% for AGC. The increased incidence of ASCUS (12.7%) and AGC (5.7%) in the pap test of our study patients is most likely related to the associated reactive and inflammatory changes present in the polyps, as none of the patients with abnormal paps had squamous intraepithelial lesions on biopsy. In contrast to recent clinical literature, our results suggest that removal of all cervical polyps and subsequent histologic review is warranted.

1194 Endometrial Clear Cell Carcinoma: Incidence and Clinicopathologic Features

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Background: The clinicopathologic features of endometrial clear cell carcinoma (CCC) are poorly described, in part due to the poor reproducibility of the diagnosis amongst experienced gynecologic pathologists.

Design: All cases of EC with "clear cell" in the diagnostic line were identified in archival files from 2001-0212. Four gynecologic pathologists (median post-training years: 17) independently classified the cases as pure CCC, CCC mimic favor endometrioid (ME), CCC mimic favor serous (MS), or unknown (U). IHC for ER, p53, p16, HNF1-β, and WT1 was performed on all cases.

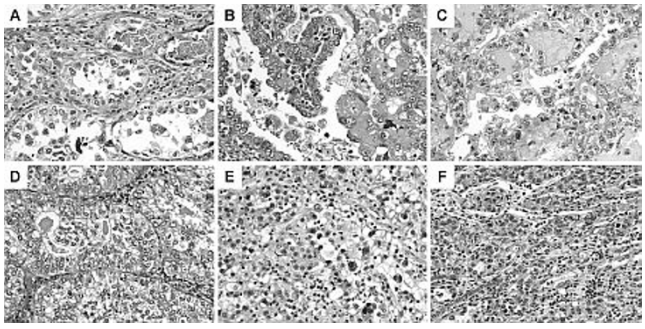
Results: There were a total of 852 endometrial carcinomas and 15 with "clear cell" in the diagnostic line. All had tumor cells with clear cytoplasm. The 4 gynecologic pathologists' diagnoses and IHC results are in Table 1.

Table 1

1	2	3	4	ER	p53	p16	HNF
CCC	ME	ME	U	-	-, 5%	-	-
ME	ME	ME	ME	3+, >95%	3+, 90%	-	-
CCC	CCC	CCC	CCC	-	-, 0%	2+, 10%	1+, 10%
ME	ME	ME	ME	3+, 90%	-, 0%	2+, <1%	-
U	U	U	U	3+, 60%	3+, 80%	2+, 80%	-
U	U	U	U	-	3+, 80%	2+, 80%	-
ME	ME	U	ME	3+, 50%	-, 0%	2+, 90%	-
ME	ME	U	ME	-	3+ 90%	-	1+, 80%
U	U	CCC	MS	-	3+, >95%	2-3+, 80%	1+, 70%
U	U	CCC	CCC	3+, 80%	-, <5%	-	2+, 90%
CCC	CCC	CCC	CCC	-	3+, 90%	2-3+, 50%	1+, 80%
U	U	U	ME	-	-, 0%	-	1+, 20%
U	U	U	ME	2+, 60%	3+, 30%	2-3+, 50%	2+, >95%
CCC	CCC	CCC	CCC	2-3+, 90%	-, <5%	1+, 50%	2+, 90%
U	U	CCC	CCC	1+, 5%	1+, 10%	1+, 5%	1+, 50%

1-4 refer to GP

There were only 3 confirmed CCC: each had hyalinized stroma, non-stratified epithelium, papillary or glandular architecture, abundant clear cytoplasm, and enlarged, centrally located, angulated nuclei. Figure 1 depicts the 3 CCC (A-C); 1 ME (D) and 1 U agreed upon by all 4 gynecologic pathologists (E) and 1 discrepant case (1 CCC, 2 ME, 1 U, panel F). The specificity of HNF1-β for CCC was 58%, with positive predictive value (PPV) of 0.46%. All were WT1-negative.



Conclusions: Endometrial CCC is extremely rare. The IHC phenotype of CCC appears to be ER-negative (2/3), WT1-negative (3/3), p53-negative (2/3), p16-patchy (3/3), and HNF1-β -positive (3/3), but data are limited. HNF1-β is not specific, and has a very low PPV (0.46%).

1195 PAX8 Expression Is Useful To Distinguish Dedifferentiated Endometrioid Adenocarcinoma from Grade III Endometrioid Adenocarcinoma

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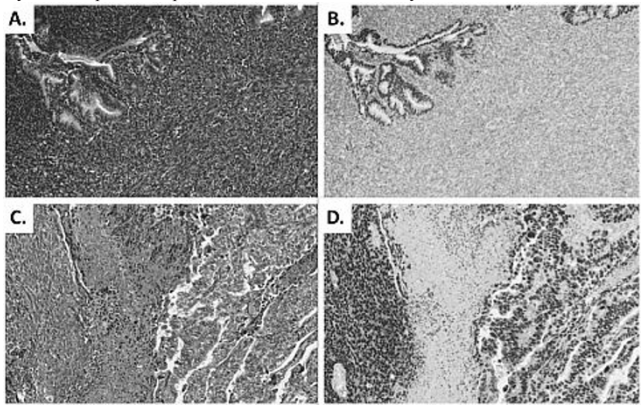
Background: Dedifferentiated endometrioid adenocarcinoma (DEAC) of the uterus or ovary is characterized by the coexistence of low grade endometrioid adenocarcinoma (FIGO grade I/II) and an undifferentiated carcinoma (UC) with solid sheet of medium-sized monotonous epithelial cells. This admixed carcinoma has not been widely recognized because the solid areas of UC have usually been misdiagnosed as solid form of FIGO grade III endometrioid adenocarcinoma**.** These tumors have been shown to be clinically aggressive; therefore, accurate diagnosis is necessary for proper patient management.

Design: DEAC cases were retrieved from our pathology database. All H&E slides were reviewed by two pathologists to confirm the diagnosis and exclude other entities. Immunohistochemical stains were assessed and clinical information was obtained.

Results: 11 DEACs including 7 endometrial and 4 ovarian origins were identified. All patients presented at advanced stages with 72% of cases showing vascular invasion, 64% showing lymph node metastases and 27% showing distal metastases. The UC components were composed of diffuse sheets/solid nests of medium-sized epithelial cells with scant to moderate cytoplasm, uniform vesicular nuclei and inconspicuous nucleoli. All cases showed diffuse and strong nuclear PAX8 staining in FIGO I/II endometrioid component and no PAX8 staining in UC components (Figure 1A/1B). In control group of 11 FIGO grade III endometrioid adenocarcinomas, PAX8 was positive in both solid and glandular areas (Figure 1C/1D). UC components of DEACs are variably positive for cytokeratin AE1/3 (88%), CAM5.2 (86%) and hormone receptors (ER or PR, 66%). All patients received chemoradiation therapy after surgery. Follow-up data were available for 8 patients and revealed all patients had either recurrent or metastatic diseases within 3 years except for one patient with free of tumor for 3 years.



Conclusions: The recognition of DEAC with low-grade endometrioid adenocarcinoma is extremely important due to its aggressive behavior. Our study results indicated that the unique PAX8 staining pattern in DEAC can be very helpful to distinguish DEAC from FIGO grade III endometrioid adenocarcinoma.

1196 Stathmin Expression in Tumors from Various Organs – A Useful Biomarker for Confirming Endocervical Adenocarcinoma and Cervical Squamous Intraepithelial Lesions

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Background: Stathmin is a cytosolic microtubule destabilizing oncoprotein. The expression of stathmin in carcinomas from human organs has not been explored. We investigated the expression of stathmin in carcinomas and normal tissues from various organs.

Design: Immunohistochemical evaluation of stathmin (Epitomics; EP247) expression on 1,095 cases of carcinomas on tissue mircroarray sections and 154 biopsy specimens (Tables 1 and 2) of endocervical adenocarcinoma in situ (AIS) and invasive endocervical

adenocarcinoma (IECA), high- and low-grade squamous intraepithelial lesions (HGSIL and LGSIL), benign squamous mucosa (BSM) and benign endocervical mucosa (BECG) was performed. The results (other than squamous dysplasia) were recorded as negative, 1+ to 4+. The stathmin expression on BSM, LGSIL and HGSIL was recorded as: 1+ (basal 2-3 layers stained); 2+ (bottom 2/3 stained); or 3+ (full thickness stained). Cytoplasmic staining with or without nuclear staining were regarded as positive.

Results: The positive staining results of carcinomas from various organs were as follows: germ cell tumors (64 of 64, 100%), urothelial CA (47 of 47, 100%), lung ADC (45 of 61, 74%), lung SCC (47 of 47, 100%), colonic ADC (62 of 64, 97%), breast CA (116 of 127, 91%), endometrial CA (131 of 131, 100%), ovarian serous CA (40 of 41, 98%), and other carcinomas (182 of 513, 35.5%). The stain in the normal tissues adjacent to these carcinomas was only focally and weakly positive. The results for cervical and endocervical lesions are summarized in Tables 1 and 2.

Table 1. Summary of immunostaining results on BECG, AIS and IECA

Diagnosis	Negative	1+	2+	3+	4+	Total positive cases (%)
BECG (N=24)	22	2	0	0	0	9%
AIS (N=11)	0	0	0	1	10	100%
IECA (N=31)	0	3	2	0	26	100%

Table 2. Summary of immunostaining results on BSM, LGSIL and HGSIL

Diagnosis	Negative	1+	2+	3+	Total positive cases (2+ and 3+)
BSM (N=20)	0	20	0	0	0
LGSIL (N=34)	0	9	21	4	25 (74%)
HGSIL (N=17)	0	0	4	30	34 (100%)

Conclusions: These data demonstrate that stathmin 1) is useful marker in differentiating benign endocervical glands from endocervical adenocarcinoma and in confirming the diagnosis of HGSIL and the majority of LGSIL; and 2) is a potential useful marker for confirming the diagnosis of urothelial carcinoma, endometrial carcinoma, ovarian serous carcinoma and lung squamous cell carcinoma, which is currently under investigation.

1197 Uterine Leiomyomata with Bizarre Nuclei: Expression of p53 and p16^{ink4} Is Common; However, Mutations of *TP53* Are Rare and May Be Associated with Hereditary Leiomyomatosis and Renal Cell Carcinoma Syndrome

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Background: Leiomyoma with bizarre nuclei (BL) can be confused with atypical leiomyoma or smooth muscle tumor of uncertain malignant potential, making predictions of biologic behavior imprecise. Thresholds for mitotic count and/or Ki67 expression to distinguish these neoplasms vary in different studies. Recent studies have proposed p53 and p16^{INK4} expression analysis may be useful in predicting behavior. Although expression of p53 and p16 has been reported in BL, to our knowledge, no study identifying *TP53* mutations in BL with follow-up data has been published. In this study, we examined BL [including those arising in the setting of hereditary leiomyomatosis and renal cell carcinoma syndrome (HLRCC)] for expression of p53, p16^{INK4} and Ki67, and *TP53* mutation(s).

Design: Consecutive cases diagnosed as BL (n=11) with <2 mitoses/10 hpf (from 11 patients) with follow-up data were utilized. Representative sections were immunostained for p53, p16^{INK4} and Ki67. Expression of p53 and p16^{INK4} was assessed using the H-score [summation of the product of staining intensity (0-3) and proportion of cells staining (0-1) with a score range of 0-3]. Ki-67 expression was determined as a percentage of nuclear staining. *TP53* mutation analysis on exons 5-8 was performed on microdissected lesional tissue using Sanger sequencing.

Results: The 11 cases (age range: 27-54 years) had a follow-up period of 3-96 months. No recurrences were noted. One case had HLRCC. The Ki67 index ranged from 0-1%. p53 expression was strong and diffuse (H-score: 2.25-3) in 4 cases and moderate-weak (H-score:1.2 and 1.6) in 2 cases. p16^{INK4} was diffusely and strongly expressed in 4 cases (H-score: 2.4-2.85) and moderate-weak in 2 cases (H-score: 1.2 and 1.4). 4 cases had expression of both markers; however, only one case had strong and diffuse expression of both. The case with HLRCC had strong expression of p53 and moderate-weak expression of p16^{INK4}. *TP53* mutation analysis revealed only one case (with HLRCC) with a missense mutation (Arg273Cys) and a base pair deletion [amino acid 288, del(A)] leading to a frameshift mutation on exon 8.

Conclusions: Expression of p53 and/or p16^{INK4} is frequent (60%) in BL. However, mutations of *TP53* are rare (9%). The presence of a *TP53* mutation does not seem to impact the behavior. Identification of *TP53* mutation(s) in BL may suggest an association with HLRCC and follow up with renal imaging studies.

1198 Mismatch Repair Status in Recurrent Low Grade Endometrioid Adenocarcinomas

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Background: The majority of EMCA present as early stage low grade tumors. Approximately 75% are cured by surgery with or without radiation therapy. When relapse occurs it frequently involves the vaginal cuff (local relapse) but may also present as pelvic or abdominal disease (regional relapse) or as distant metastasis. While the molecular mechanisms at play in the development of EMCA have received much attention, the role of these biomarkers in recurrent disease has not been established. The loss of mismatch repair (MMR) proteins occurs in 7-40% of EMCA. We hypothesize that MMR protein loss may play a role in EMCA recurrence in low grade lesions.

Design: A retrospective review of all endometrioid EMCA at our institution over a ten year span revealed 1685 cases. Of these, 944(56%) were grade (G) 1. A total of 87 cases recurred; 27(31%) were G1. Sixteen recurred at the vaginal cuff; 5 had regional relapse; and 6 had distant metastasis. Archival paraffin embedded tissue of 6 primary G1 EMCA and their respective vaginal cuff recurrences were examined by IHC for loss of expression of MMR proteins, MLH1, MSH2, MSH6 and PMS2.

Results: Of the 6 primary cases 2 cases (30%) showed loss of MMR proteins MLH1 and PMS2. The recurrent lesions in both cases showed the same molecular alteration with loss of MLH1 and PMS2. Statuses of MSH2 and MSH6 were not altered in both primary and recurrent lesions. Time to recurrence was on average 13.5 months (m) (range 12-15 m) in the cases with loss of MMR and an average of 17.5 m (range 12-21 m) in those with retained expression (P=0.1415).

Conclusions: Recurrence of G1 EMCA is uncommon; 2.9% of G1 EMCA recurred in this 10 year series. Two out of six cases we evaluated showed loss of MMR proteins in the primary tumor and in the recurrence. For these cases recurrence occurred 4 months earlier on average as compared to tumors with retained MMR. Further studies will be needed to elucidate the role of MMR defects in recurrent G1 EMCAs.

1199 Population Study of Uterine Leiomyosarcoma Incidence, Mortality and Survival

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Background: Uterine leiomyosarcoma (U-LMS) is the most common uterine sarcoma. Overall crude survival is 15-30%. The current 2003 WHO classification is derived from the 1994 Stanford study that correlated histologic features with patient outcomes. Most survival estimates for U-LMS are based on single institution studies, with few population-based studies. Stage predicts for survival; other factors have been inconsistent prognosticators. We estimated trends in incidence and mortality indices for patients diagnosed with U-LMS in Canada from 1992 to 2005.

Design: We searched the Canadian Cancer Registry with ICD-10 codes C54 (uterine corpus) and C55 (uterus), and then included only cases with ICD-O-3 codes 8890/3 (U-LMS), 8891/3 (epithelioid LMS) and 8896/3 (myxoid LMS). A flexible parametric model was used to estimate relative survival (RS) (observed survival rate among cancer patients divided by expected survival rate in the general population), by age group (<50, 50-59, 60-69, 70 years and older) in women diagnosed with LMS between 1992 and 2005. Crude probability of death was modeled to identify proportionate contributions of death due to LMS and death due to other causes.

Results: There were 682 women with LMS, with median age at diagnosis of 52.0 years, and 382 deaths. The number of cases of LMS decreased 1992 to 2005. Excess mortality rate showed a sharp peak in the first year after diagnosis for all age groups. RS decreased significantly with increasing age. RS progressively worsened with year of diagnosis, in that women with LMS diagnosed in more recent years had greater risk of mortality than women diagnosed in 1992. Crude probability of death showed that while most deaths were due to LMS in all age groups, a greater proportion was due to other causes as age increased.

Conclusions: This is the first study that reports incidence rates and compares mortality indices for U-LMS across Canada based on patient age and year of diagnosis. The peak in excess mortality in the first year after diagnosis is likely due to deaths in patients who present in advanced stage. Decreased RS with age warrants further investigation to determine if factors such as presentation in more advanced stage or less access to supportive care in the elderly may be factors. Refinement in histologic criteria are likely responsible for the apparent decrease in U-LMS through the years which may leave tumors with worse prognosis in the U-LMS category and explain the apparent worsening of RS in recent years.

1200 Loss of ARID1A in Uterine Endometroid Carcinoma Is Associated with Microsatellite Instability

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Background: ARID1A, a tumor suppressor gene participating in chromatin remodeling, has been found to be mutated in several types of human cancer. Loss of expression of ARID1A, presumably due to inactivating mutation, has been found in 26% of low-grade uterine endometrioid carcinoma (EM CA) and up to 39% to 45% in high-grade endometrioid carcinoma. However, the association with microsatellite instability (MSI) is unknown in uterine endometrioid carcinoma.

Design: Totally 23 grade 1, 7 grade 2 and 22 grade 3 uterine EM CA were included in the study. The expression of ARID1A was investigated by immunohistochemistry. Complete loss was defined as absence of ARID1A immunoreactivity in all tumor cells, whereas clonal loss was defined as lack of staining in a discrete tumor area(s) in the background of ARID1A positive tumor cells. MSI test was performed using 5 mononucleotide microsatellite loci and analyzed by capillary electrophoresis. MSI-low cases had instability in 1 microsatellite, and MSI-high cases had instability in 2 or more microsatellites.

Results: Complete and clonal loss of ARID1A was found in 5(22%) and 7(30%) of grade 1 EM CA, 2(29%) and 3(43%) of grade 2 EM CA, and 12(55%) and 1(5%) of grade 3 EM CA respectively. MSI-H was present in 7 (30%) grade 1, 4(57%) grade 2, and 10(45%) grade 3 EM CA. Correlating with ARID1A expression, MSI-H was present in 6(50%) grade 1, 4(80%) of grade 2 and 8(62%) of grade 3 tumors with complete or clonal loss of ARID1A, and in 1(9%) grade 1, 0(0%) grade 2 and 2(22%) of grade 3 tumors with retained ARID1A ($p<0.0001$). Three grade 3 tumors with concurrent grade 1 components had MSI test in both low-grade and high-grade components. Both components had the same MSI status in all three tumors.

Conclusions: Our study confirmed the previous finding that loss of ARID1A is more frequent in high-grade EM CA, with more tumors having complete loss than clonal loss compared to low-grade EM CA. MSI is also more prevalent in high-grade EM

CA than in low-grade EM CA, and is significantly associated with tumors with loss of ARID1A expression. Loss of ARID1A and MSI may cooperate and contribute to tumor progression.

1201 PAX-8 and ER in Mucinous Tumors in the Ovary: Are They Useful in Distinguishing Primary from Metastasis?

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Background: PAX-8 is expressed in tumors of the Mullerian system, kidney, and thyroid. Studies have shown that PAX-8 can be useful in differentiating primary ovarian mucinous tumors (OV-MT) from metastatic tumors (mets) of gastrointestinal and pancreatic origin. However, the expression of this marker in OV-MT has been reported to be variable, ranging from 8.3% to 65%. Also, ER expression in OV-MT has been reported to vary from 0% to 70%. In this study, we present our experience with the expression of these markers in OV-MT and in mets.

Design: 13 mucinous cystadenomas (Mcyst), 12 mucinous tumors of low malignant potential, intestinal type (MLMPi), 2 mucinous tumors of low malignant potential, endocervical type (MLMPe), 8 primary ovarian mucinous carcinomas (MCa) and 4 cases of mets (1 appendiceal, 1 pancreatic and 2 colonic carcinomas) to the ovary were retrieved from our files. PAX-8 and ER were performed. Nuclear staining was assessed semiquantitatively: 0 (negative), 1+ (<5%), 2+ (6-25%), 3+ (26-50%), 4+ (51-75%), and 5+ (>76%).

Results: PAX-8 was positive in 11/13 Mcyst (85 %), 10/12 MLMPi (83 %), 2/2 MLMPe (100 %) and 5/8 MCa (63 %). In Mcyst, 3 cases (23 %) were 1+, 1 (8 %) was 2+, 3 (23 %) were 3+, 1 (8 %) was 4+, and 3 (23 %) were 5+. In MLMPi, 1 case (8 %) was 1+, 3 (25 %) were 2+, 2 (17 %) were 4+, and 4 (33 %) were 5+. In MLMPe, 2 cases were 5+ (100%). In MCa, 1 case (13 %) was 1+, 1 (13 %) was 2+, and 3 (38 %) were 5+. Mets were negative for PAX-8. ER was positive in 2/13 Mcyst (16 %), 1/12 MLMPi (8 %), 2/2 MLMPe (100%), and 3/6 MCa (50 %). In Mcyst, 1 case (8 %) was 2+, and 1(8 %) was 5+. In MLMPi, 1 case (8 %) was 1+. In MLMPe, 2 cases (100 %) were 5+. In MCa, 2 cases (33 %) were 2+, and 1 (17 %) was 5+. Mets were negative for ER.

Table-1: Expression of PAX-8 in Mucinous Neoplasms of the Ovary

PAX-8	0	1+	2+	3+	4+	5+
Mcyst (n=13)	2	3	1	3	1	3
MLMPi(n=12)	2	1	3	-	2	4
MLMPe(n=2)	-	-	-	-	-	2
MCa (n=8)	3	1	1	-	-	3

Table-2: Expression of ER in Mucinous Neoplasms of the Ovary

ER	0	1+	2+	3+	4+	5+
Mcyst (n=13)	11	-	1	-	-	1
MLMPi(n=12)	11	1	-	-	-	-
MLMPe(n=2)	-	-	-	-	-	2
MCa (n=6*)	3	-	2	-	-	1

*ER was not performed in 2 of 8 cases of MCa.

Conclusions: In this study, PAX-8 was expressed in 28 of 35 (80 %) OV-MT and negative in the mets. Mcyst and MLMP show much higher PAX-8 expression when compared to MCa. ER is positive in only 8 of 33 (24 %) OV-MT and this precludes its utility as a distinguishing marker between OV-MT and mets. In a mucinous tumor in the ovary, PAX-8 expression strongly favors an ovarian origin.

1202 Microinvasion in Ovarian Serous Tumor of Low Malignant Potential – Does It Matter?

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Background: The significance of microinvasion (MI) in ovarian serous tumors of low malignant potential (OV-SLMP) is controversial. Early reports indicated that OV-SLMP with MI were of low stage with favorable outcome and associated with pregnancy. Subsequent studies have shown that MI in OV-SLMP was associated with higher stage disease, disease progression and/ or death due to disease. The aim of this study is to present our experience with cases of MI in typical OV-SLMP.

Design: 31 cases of OV-SLMP with MI and follow-up were retrieved from our files over a period of 16 years (1995-2010). Cases with micropapillary/cribriform pattern (MP/CP) were excluded. The following parameters were recorded: patients' (pts) age, pregnancy status, tumor size and laterality, ovarian surface involvement, focal vs multifocal MI, non-invasive vs invasive implants, stage of disease and outcome.

Results: Pts' age ranged from 18-82 yrs (median 47yrs). 2 pts were pregnant. Tumor size ranged from 2.5 cm to 25 cm (median 8.5cm); laterality was: left (6), right (9) and bilateral (16). Ovarian surface involvement was present in 10 cases and absent in 19 cases. In 2 cases, this information was not available. In 6 cases, MI was multifocal (MF), whereas in 25 cases, focal MI was seen. Cases were staged as follows: Stage I (11), Stage II (1), Stage III (12). No staging was performed in 7 cases. 7 patients had non-invasive implants (NI-IM), 6 had invasive implants (I-IM). Follow-up ranged from 6-261 months (median 58 months). 26 patients (84 %) were alive with no evidence of disease, 2 were alive with evidence of disease, 3 died of unrelated causes. One patient progressed to low grade serous carcinoma in 4 years, however she died of urothelial carcinoma.The 2 pregnant patients were alive with no evidence of disease at 27 and 106 months.

Conclusions: OV-SLMP with MI appears to be associated with bilaterality, ovarian surface involvement and advanced stage disease. Focal versus MF MI does not appear to correlate with clinical outcome. Only 2 pregnant patients were present in our study and had favorable outcomes as previously reported. An unexpected finding noted in our study was a higher incidence (46%) of invasive implants in these cases. Additional studies will be necessary to determine if this latter finding is truly associated with OV-SLMP with MI.

1203 Novel Hypoxia-Associated Markers of Chemoresistance in Ovarian Cancer

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Background: Ovarian cancer is the fifth leading cause of cancer in women and has poor long-term survival, in part, due to chemoresistance. Tumour hypoxia is associated with chemoresistance in ovarian cancer. However, relatively little is known about the genes activated in ovarian cancer which cause chemoresistance due to hypoxia. This study aimed to firstly identify genes whose expression is associated with hypoxia-induced chemoresistance, and secondly select hypoxia-associated biomarkers and evaluate their expression in ovarian tumours.

Design: Cisplatin-sensitive (A2780) and cisplatin-resistant (A2780cis) ovarian cancer cell lines were exposed to combinations of hypoxia and/or cisplatin as part of a matrix designed to reflect clinically relevant scenarios. RNA was extracted and interrogated on Affymetrix Human Gene arrays. Differential gene expression was analysed for cells exposed to hypoxia and/or treated with cisplatin. Potential markers of chemoresistance were selected for evaluation in a cohort of ovarian tumour samples by RT-PCR.

Results: A wide range of genes associated with chemoresistance were differentially expressed in cells exposed to hypoxia and/or cisplatin. Selected genes [ANGPTL4, HER3 and HIF-1α] were chosen for further validation in a cohort of ovarian tumour samples. High expression of ANGPTL4 trended towards reduced progression-free and overall survival. High expression of HER3 trended to increased progression-free but reduced overall survival, while high expression of HIF-1α trended towards reduced progression-free and increased overall survival.

Conclusions: In conclusion, this study has further characterized the relationship between hypoxia and chemoresistance in an ovarian cancer model. We have also identified many potential biomarkers of hypoxia and platinum resistance and provided initial validation of a subset of these markers in ovarian cancer tissues.

1204 Differential Expression of SIRT1 and PGC1a in Ovarian Clear Cell Carcinomas vs Endometrial Carcinomas and Renal Clear Cell Carcinomas

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Background: Agonists of the fatty acid metabolism regulator PPARγ have been shown to inhibit growth of ovarian cancer cell lines. PPARγ has also been demonstrated in endometrial carcinomas (EC) as a potential therapeutic target. The deacetylase SIRT1 is required for PGC-1α coactivation of PPARγ in liver cells. However, the presence and potential roles of PPARγ regulators in gynecological cancers has not been elucidated. We sought to determine the expression of PGC-1α and SIRT1 in ovarian clear cell carcinomas (OC) and EC. Renal clear cell carcinomas (RC) were used for comparison given their morphological similarity to the ovarian tumors and known expression of PPARγ.

Design: Immunohistochemistry (IHC) using SIRT1 (Novus, 1:200) and PGC-1α (Bethyl, 1:100) polyclonal primary antibodies was performed on sections of tissue microarrays (TMA) including 375 EC, 39 OC and 28 RC. TMA's were scored by conventional light microscopy. Positive reactivity was defined as intense nuclear staining in >50% of neoplastic cells. Chi-square tests were used to compare proportions.

Results: 56% of OC were SIRT1-positive compared to only 14% and 4% of RC and EC respectively ($p<0.001$), see table. With the exception of three OC all SIRT1-positive tumors were negative for PGC-1α, as were most tumors overall.

SIRT1 and PGC1α Immunoreactivity

	OC	RC	EC
SIRT1+	22/39 (56%)	4/28 (14%)	15/375 (4%)
PGC1α- that were SIRT1+	17/20 (85%)	4/4 (100%)	15/15 (100%)
PGC1α+	4/37 (11%)	0/28 (0%)	3/375 (0.8%)
SIRT1+ that were PGC1α+	3/4 (75%)	N/A	0/3 (0%)

Conclusions: SIRT1 was detected in 56% OC, a significantly higher proportion when compared to EC (another Müllerian carcinoma) and RC (another carcinoma with clear cell morphology), $p<0.001$ for both. We speculate that PPARγ regulation by SIRT1 could be of significance in OC. Negative IHC for PGC-1α, may indicate other roles for SIRT1 in many OC. Alternatively, SIRT1 deacetylation of three lysine residues located within the immunogen of the PGC-1α antibody used has to be explored. The relevance of these findings is underscored by the recent development of PPARγ agonists.

1205 Prognostic Impact of Sarcomatous Component in Uterine Carcinosarcoma

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Background: Uterine carcinosarcomas are rare and highly aggressive tumors. Prognostic factors influencing clinical outcome remains conteroversional. However, stage at the time of presentation is the most important prognostic factor. We assessed the impact of the sarcomatous component (SC), estimated as the percentage of tumor volume on clinical stage at the time of initial diagnosis.

Design: All cases of carcinosarcoma between 2006 to current were retrieved. We found 21 cases. Stage was determined using the clinical staging system of the International Federation of Gynecology and Obstetrics (FIGO). All tumor slides were reviewed. The volume of the SC was estimated based on the number of the 10x power fields of the tumor slides showing SC then calculating the mean of all tumor slides from each case. These findings were correlated with the disease stage.

Results: In our assessment of the SC percentage, four cases showed less than 10%, four cases had 10-30%, five cases had 30-60% and eight cases showed more than 60% SC. Ten (48%,10/21) cases presented with advanced stage with extrauterine extension or lymph node involvement. Nine (90%, 9/10) of these showed 30% or more SC. In one

additional case with > 60% SC, the patient presented with stage II and had positive pelvic washings. All four patients with <10% SC presented at stage IA or IB. The carcinoma component was composed of endometrioid in eight cases, mixed serous / clear cell and endometrioid in five cases, serous/clear cell in six cases and two dedifferentiated carcinoma. The distribution of SC percentage, carcinomatous component, and staging are illustrated in.

Table 1

percentage of SC	Stage and carcinomatous component					
10%	IA (SE&EN)	IA (EN)	IB (EN)	IA (EN)		
10-30%	II (EN)	IIIB (SE)	IB (SE&EN)	IA (CC)		
30-60%	IIIB (EN)	IA (SE&EN)	IIIC2 (SE)	IIIC2 (EN)	IVB (CC&EN)	
>60%	IA (CC&EN)	IIIA (DD)	IA (SE)	II(PW+)*(EN)	IIIB (DD)	IVB(x3) (SE, EN, CC)

SE: Serous carcinoma, CC: clear cell carcinoma, EN: endometrioid carcinoma, DD: dedifferentiated carcinoma *PW+: positive pelvic washing

Conclusions: The tumor stage and histologic grade of the carcinomatous component are among the most important prognostic factors cited in the literature. This study shows that the percentage of the SC is a useful prognostic indicator. According to our data almost 70% of the patients with 30% or more SC presented in an advanced stage, on the other hand, six out of eight patients (75%) with less than 30% SC were stage I. These findings demonstrate the importance of reporting SC percentage as a useful prognostic indicator, and suggest that more than 30% SC is associated with an unfavorable outcome.

1206 Comparative Analysis of Low Stage Ovarian Carcinomas: Detailed Morphologic Assessment of Stage I and II High Grade Serous Carcinoma as Compared to Other Low Stage Ovarian Carcinoma Subtypes

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Background: Recent studies have suggested that many high grade serous carcinomas (HGSCs) that present as ovarian carcinomas may derive from microscopic precursors in the fallopian tube but the majority present at high-stage, complicating detection of the origin of disease. Studying features of low-stage disease may help to provide information relevant to this issue. We anticipate that most non-high grade serous carcinomas will demonstrate features consistent with derivation from precursors located in or transplanted to the ovary and that many will be distributed in a pattern suggesting secondary involvement.

Design: We examined 76 patients with low stage (FIGO I/ II) ovarian carcinoma who underwent primary surgical management at our institution from 1980 to 2000. H&E slides were reviewed and histologic type assigned using *Gilks, Soslow et al* criteria. Ovarian mucinous, endometrioid, clear cell and HGSCs were included and primary versus metastatic origin was assessed based on the presence or absence of bilaterality, multifocality, surface involvement, size, and pattern of invasion.

Results: Twenty-two cases of HGSC, 30 cases of endometrioid, 11 cases of mucinous and 13 cases of clear cell adenocarcinoma were evaluated and the 54 non-serous carcinoma (NSC) subtypes were grouped for the purposes of comparison. Surface involvement was identified in 14 cases (63%) of HGSCs compared to 7 cases (13%) from the non-serous group. Bilaterality was identified in 12 cases (55%) of HGSCs versus 4 cases (7%) of NSCs. The mean size of the HGSCs was 8.95 cm compared to 13.8 cm for the NSCs. Twelve (55%) cases of HGSC were multifocal compared to 4 cases (7%) of NSC. A significant difference in growth pattern was also identified. Eight cases (36%) of HGSCs showed an infiltrative growth pattern whereas only 12 cases (22%) of NSCs; showing instead a more expansile pattern. Of note, a subset of HGSCs did emerge that fit a more primary pattern. Four cases (18%) of unilateral, unifocal HGSCs without surface involvement and a mean size of 13 cm were identified.

Conclusions: Stage IA HGSC is exceedingly rare. This along with higher rates of "metastatic" features as compared to the non-serous subtypes support the hypothesis that most derive from extraovarian precursors. We also identified a small subset of HGSCs that exhibited a "primary" growth pattern, which lends credence to the hypothesis that some HGSCs arise within ovarian inclusion cysts.

1207 Vulvar Squamous Cell Carcinoma: A Study of 71 Tumors with Emphasis on Characterization and Distinction of Tumors Associated with HPV-/Classic VIN from Non-HPV-/Differentiated VIN-Associated Tumors

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Background: Vulvar squamous cell carcinoma (SCC) develops via human papillomavirus (HPV)-associated and non-HPV-associated pathways. The more common, non-HPV-associated keratinizing SCC typically occurs in older women and arises in a background of differentiated vulvar intraepithelial nesoplasia (dVIN) and lichen sclerosus (LS). The HPV-associated warty/basaloid form of SCC occurs in younger women and arises in a background of classic VIN (cVIN). In this study, we investigated the morphologic spectrum of vulvar SCC and its associated lesions with respect to pathogenesis.

Design: Patients diagnosed with invasive vulvar SCC between 1978 and 1998 were identified from the MSKCC institutional pathology database. Histopathologic features were reviewed, and their associations with HPV changes and VIN type were analyzed.

Results: Seventy-one patients with invasive vulvar SCC were identified. The median tumor size and depth were 13mm and 3.0mm, respectively. The tumor grade was 1, 2 and 3 in 22 (31%), 27 (38%) and 22 (31%) cases, respectively. Variable amounts of keratin were present in 62 tumors, 50 of which contained keratin pearls. HPV-related change and associated lichen sclerosus were present in 29 (41%) and 25 (35%) cases, respectively, with both being present in 7 tumors. Associated VIN was present in 67/70 (96%) tumors, and was of differentiated, classic, mixed, or indeterminate types in 38 (54%), 19 (27%), 4 (6%), and 6 (9%) tumors, respectively. Several features were significantly associated with HPV changes and VIN type:

Table 1. Significant associations with HPV changes

	HPV-associated tumors	Non-HPV-associated tumors	p-value
Median size	7mm	18mm	0.005
Median depth of invasion	1.7mm	6.3mm	<0.0001
Histologic grade 1	52%	17%	0.007
Perineural invasion	7%	45%	0.0005
Pushing borders	38%	2%	0.0002

Table 2. Significant associations with VIN type

	dVIN-associated tumors	cVIN-associated tumors	p-value
Median size	18mm	5.5mm	0.02
Median depth of invasion	5mm	1.4mm	0.0007
Moderate-marked keratin	74%	37%	0.005
Keratin pearls	84%	42%	0.002
Perineural invasion	39%	11%	0.03
Infiltrating borders	97%	61%	0.001
Associated HPV changes	15%	95%	<0.0001
Associated lichen sclerosus	55%	5%	0.0002

Conclusions: Vulvar SCCs associated with HPV/cVIN changes have distinct pathologic features that distinguish them from those associated with dVIN and lacking HPV changes. Confirmatory HPV testing by immunohistochemistry and molecular detection would be of interest and is in progress.

1208 Evidence That High-Grade Serous Cancers and Their Precursors Arise from a PAX2-Negative Progenitor Cell in the Oviduct

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Background: Both a precursor (p53 signature) and early cancer (serous tubal intraepithelial carcinoma or STIC) have been linked to high-grade pelvic serous cancers (HGSC), both by p53 mutations and physical proximity in the fimbria. All strongly express PAX8, implying an origin in secretory-type cells of the tube. A number of other genes are up and down regulated in HGSC, including PAX2, which like PAX8, localizes to secretory cells. Absence or near loss of PAX2 expression (PAX2-) is seen in over 75% of HGSC. We hypothesized that absence of PAX2, rather than occurring during the development of precursors, might actually identify a population of precursor progenitor cells in the normal oviduct.

Design: We immunostained and compared oviductal epithelia stained with PAX8, a generic marker for secretory cells, and PAX2, to determine if PAX2 negative secretory cells existed. We next characterized a repository of precursors, including p53 signatures, atypias, STICs and other secretory cell outgrowths (SCOUTs) for loss of PAX2.

Results: Analysis of immunostains revealed discrete PAX2- cells in the oviducts, accounting for less than 10% of the resident secretory cell population. Immunostained sections of p53 signatures and STICs were typically PAX2-. PAX2- cell groups ranged from a few cells to over 30 (SCOUTs). Of the PAX2- SCOUTs, both pure secretory and mixed secretory and ciliated phenotypes were seen.

Conclusions: This report describes, for the first time, a specific candidate cell of origin for HGSC in the oviduct that is the PAX2- secretory cell. Based on their relative frequency, and proportion of precursors that are PAX2-, PAX2- cells appear to be particularly sensitive to clonal expansion. This population of PAX2- cells thus provides a unique substrate for studying cell-specific vulnerabilties in serous carcinogenesis as well as understanding the molecular underpinnings of the varied differentiation paths that seem to ensue during clonal expansion of this unique cell type.

1209 BAF250a (ARID1A) Expression in Ovarian Clear Cell Adenocarcinoma with an Adenofibromatous Component

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Background: Ovarian clear cell adenocarcinoma (CCA) is frequently associated with endometriosis (EMosis), and less often with an adenofibromatous component (AF). Recent studies have suggested that mutation of the ARID1A gene and loss of the corresponding protein BAF250a as a frequent event in CCA. Approximately half of CCA have an ARID1A mutation resulting in loss of BAF250a expression in immunohistochemistry. Since an ARID1A mutation has been also found in EMosis adjacent to CCA but not in distant EMosis, it may be an early event in neoplastic transformation. This study was conducted to clarify BAF250a expression in AF-related CCA and to compare with that in EMosis-related CCA.

Design: Ninety-three cases of CCA surgically treated between 2000 and 2012 were included in the study. All H-E slides of the ovarian tumors were reviewed for the presence of AF and EMosis associated with carcinoma. Immunohistochemical staining for BAF250a was performed to exam its expression in carcinoma, and in AF and/or EMosis concurrent with CCA. Nuclear immunoreactivity of more than 90% of the cells was considered positive.

Results: Of 93 cases, 17 were associated with AF alone, 45 with EMosis alone, 18 with both AF and EMosis, and 13 with neither AF nor EMosis. BAF250a was positive in AF and EMosis in all 80 cases with these components. BAF250a was negative in carcinoma in 53/93 (57%): 6/17 (35%) of AF alone, 30/45(67%) of EMosis alone, 8/18 (44%) of AF+EMosis, and 9/13(69%) of both negative CCA. Loss of BAF250a expression was significantly less frequent in AF alone CCA compared to that of EMosis alone CCA (p=0.026, Chi-Square test, Pearson).

Conclusions: Our results suggest that genetic background of carcinogenesis is different between AF-related and EMosis-related CCA in terms of an ARID1A mutation.

1210 Additional Insights on Ovarian Low Grade Serous Carcinoma: A Study of Consecutive Cases with Primary Surgery Performed at a Single Institution

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Background: Low grade serous carcinoma(LGSC) is a subtype of ovarian carcinoma (OC) with distinct pathogenesis, pathologic features and clinical behavior. Although several studies have covered different aspects of this entity, no study has evaluated this disease in patients (pts) who have received their primary surgery at a single institution. In this study we present our experience with a cohort of such cases.

Design: To determine incidence of LGSC, all cases of OC from 1995-2010 were recorded. From 2005-2010, all LGSC and high grade serous carcinomas (HGSC) were also extracted to determine survival differences. Pt age, stage and outcome were recorded. All available H&E slides were reviewed for presence/absence of serous borderline tumor (SBT), presence/absence of micropapillary/cribiform pattern (MP/CP), architectural pattern in the invasive component, presence/absence of desmoplasia or fibrosis.

Results: Of 471 OC from 1995-2005, 22 LGSC were identified (4.7%). In addition 11 LGSC were collected from 2005-2010. Mean age for LGSC was 52 yrs (range:19-70;17.5% <40) and 62 yrs for HGSC (range:38-90;1.6% <40). LGSCs were staged as follows:Stage I (2), Stage III (23), Stage IV (8). 28 LGSC had concurrent SBT, usually MP/CP. Of cases with SBT, SBT usually comprised > 50% of the tumor. The architectural pattern of invasion included small stroma poor papillae (92.3%), cribiform nests (73.1%), elongated papillae (34.6%), medium sized papillae with fibroconnective tissue (FCT) core (34.8%), large papillae with FCT core (19.2%), solid nests (15.4%), single cells (7.7%), desmoplasia (46.2%) and fibrosis (65.4%).Follow up from 13 mos to 195 mos (mean 67.2) was available for 30/33 LGSC:dead of disease, 18 pts (60%);dead of other cause, 1 pt (3.3%);alive with disease, 5 pts (16.7%);no evidence of disease, 6 pts (20%). For 185 HGSC with follow up (7 days to 169 mos, mean 57):dead of disease 132 pts (71.4%):dead of other cause, 3 pts(1.6%);alive with disease, 21 pts(11.3%);no evidence of disease, 29 pts (15.7%).

Conclusions: LGSC is a rare tumor accounting for 4.7% of all primary OC at a single institution. LGSC can present over a wide age range, and is more likely to occur at a younger age than pts with HGSC. Most of the cases of LGSC are associated with a SBT, particularly with MP/CP pattern. Most of the cases with associated SBT with MP/CP pattern had an invasive component histologically similar to what was seen intracystically. The majority of pts with LGSC present at advanced stage disease, and have a survival advantage over pts diagnosed with HGSC.

1211 Microsatellite Instability Influences Expression of Endometrial Carcinoma Biomarkers

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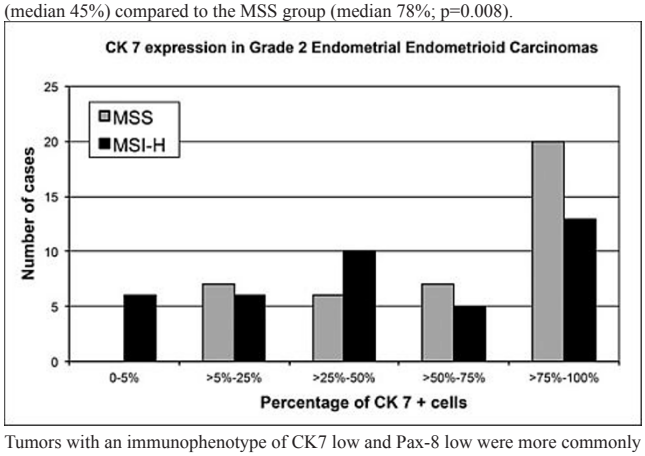
Background: Endometrial carcinomas can have a wide range of biomarker expression, even when tumor histotype and grade are constant. The basis for such variability is not known. The purpose of this study was to examine the effect of microsatellite instability (MSI) on the immunohistochemical expression of clinically relevant biomarkers.

Design: 408 consecutive, unselected endometrial carcinomas were evaluated for MSI via immunohistochemical testing of DNA mismatch repair proteins and *MLH1* methylation. Loss of immunohistochemical expression of MLH1, MSH2, MSH6, or PMS2 was defined as MSI-high; positive expression was defined as MS-stable. Immunohistochemistry for ER, PR, CK7, CK20 and Pax-8 was evaluated in 80 grade 2 endometrioid carcinomas from this set (40 MSI-high, 40 MS-stable). Cases were matched for histotype, grade, and age. These biomarkers were chosen because they are used in the diagnostic work-up of endometrial biopsies or tumors of unknown primary origin. Percent tumor cells positive for each biomarker was recorded.

Results: Percent tumor cells CK7+ was significantly lower in the MSI-high group (median 45%) compared to the MSS group (median 78%; p=0.008).



Tumors with an immunophenotype of CK7 low and Pax-8 low were more commonly MSI-high (42.5%) compared to MS-stable (20%). The combined immunophenotype CK7 high, ER high and PR high was more common in the MS-stable group (42.5% of all cases) compared to MSI-high (20%).

CK7/ER/PR Immunoprofiles

Immunoprofile	MS-stable	MSI-high
CK 7 high/ER high/PR high	42.5%	20%
CK 7 low/ER high/PR high	25%	37.5%
CK 7 high/ER low/PR low	12.5%	7.5%
CK 7 low/ER low/PR low	0%	7.5%

CK20 was minimally expressed in both groups and was thus not affected by MSI.

Conclusions: MSI influences the expression of clinically important biomarkers for endometrial cancer. These results need to be considered when considering carcinomas of unknown primary origin. Given the decreased number of CK7 high/ER high/PR high tumors in the MSI-high group, mismatch repair defects may contribute to the variability of responsiveness of endometriod adenocarcinomas to hormonal manipulation.

1212 Cervical Conisation for High Grade Dysplasia (CIN III) in 38 HPV Vaccinated Women

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Background: To the best of our knowledge fenomenom of high grade dysplasia in HPV vaccinated women has not been systematically investigated so far. The biggest prospective multicentric study PATRICIA studying more than 9000 vaccinated young women yielded only 3 cases. We present single institution experience describing 38 cases of high grade dysplasia in cervical cone-biopsy of HPV vaccinated women.

Design: Cross-reference of laboratory's database for the words "HPV vaccination" and "conisation" yielded 38 patients. Histology slides were reviewed and tested for HPV infection using a set of 3 different PCR methods and ISH. All available PAP-smear results of each patient were analyzed. They were not available in 6 cases.

Results: 8 (21%) women were of the age 16 to 20 years when vaccinated. Only 1 woman was less than 18 years old. 25 (78%) women had abnormal PAP-smears before the vaccination was finished. Of those 36% had abnormal PAP-smears only in the year of vaccination. Only 1 (3%) woman had negative PAP-smears prior to vaccination (proved negative even after review). Spectrum and distribution of HPV types detected in conisation tissue did not differ from normal Czech population, except for higher rate of HPV type 35. In the subgroup of women younger than 20 years HPV types 31, 51, 52 were detected more often than expected.

Conclusions: High grade dysplasia of cervix appears mostly (78%) in women with abnormal PAP-smears prior to vaccination. 97% of cases developed in women over 18 years of age at the time of vaccination. There was a statistically significant increase of high grade dysplasia associated with HPV 35. In the subgroup of women younger than 20 years there was ascending trend of cases associated with HPV 31, 51, 52. We propose establishment of National Dysplasia After Vaccination registry to monitor this fenomenon.

1213 DNA Double Strand Break Repair Protein Expression Is Significantly Reduced in Tubal Secretory Cell Outgrowths

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Background: Secretory cell outgrowths (SCOUTs) are suspected to be early precursors of tubal serous carcinoma. Previous studies have shown that some SCOUTs, as well as more advanced lesions such as serous tubal intraepithelial carcinoma (STIC), have increased levels of DNA double strand breaks (DNA DSB) as detected by increased staining of gamma-H2AX. DNA repair proteins in the MRN complex (MRE11, RAD50, NBS1) and 53BP1 are essential in the initial steps of DNA DSB repair. Our goal was to determine if expression of DNA DSB repair proteins is altered in SCOUTs.

Design: Archival fallopian tube blocks from patients with BRCA mutations, serous carcinoma with identified STIC, and benign hysterectomies (5 cases each) were used. Immunohistochemistry was performed using antibodies against MRE11, RAD50, NBS1, 53BP1, and PAX2. Absence of nuclear PAX2 staining in 30 or more secretory cells identified SCOUTs and allowed for localization of the other antibodies to the SCOUT. The nuclear intensity of antibody staining and percentage of epithelial cells (MRN proteins and 53BP1) was scored as nil (0), low (1+), medium (2+), and high (3+) and no staining (0), <25% (1), <50% (2), and >50 (3), respectively. The product of the two scores gave a single value from 0 to 9 and accounted for both the number of cells staining as well as the overall staining intensity. Results were analyzed by fisher exact test with $p<0.05$ considered significant.

Results: SCOUTs were identified in 3 of 5 cases from BRCA mutation carriers, 2 of 5 cases with serous carcinoma/STIC, and 1 of 5 from benign hysterectomies. SCOUTs showed markedly diminished staining of MRE11, RAD50, NBS1, and 53BP1. The combined staining score was significantly reduced in SCOUTS compared to the background tubal epithelium (2.8 +/-2.08 compared to 7.0 +/-1.0 for MRE11; 2.5 +/-1.9 compared to 8.5+/-0.4 for RAD50; 1.2+/-1.5 compared to 7.0+/-1.0 for NBS1; and 4.2+/-1.7 compared to 7.8+/-0.5 for 53BP1). The decreased staining in SCOUTs was statistically significant ($p<0.05$).

Conclusions: The markedly decreased to absent staining of MRE11, RAD50, NBS1, and 53BP1 in SCOUTs suggests a significant defect early in the DNA DSB repair sequence, which strengthens the postulate that SCOUTs are neoplastic precursors. These findings suggest that the increased DNA DSB indicated by increased gamma-H2AX expression in fallopian tube lesions may, in part, be due to aberrant DNA DSB repair. Further studies are needed to determine if there is single gene defect causing a breakdown in DNA DBS repair or a global defect in DNA repair mechanisms.

1214 Histopathologic Changes in Progesterone-Treated Endometrial Hyperplasia and Carcinoma

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Background: Endometrial carcinoma is the most common gynecological malignancy. For pre-menopausal patients with complex atypical hyperplasia and well-differentiated endometrial adenocarcinoma, there are alternative therapies to definitive hysterectomy. Previous studies have described the morphologic changes associated with progesterone therapy. This study endeavors to further characterize treatment effect to aid in the evaluation of post-therapy endometrial samples.

Design: 70 cases of endometrial proliferation (including simple hyperplasia, complex hyperplasia, complex atypical hyperplasia, and endometrioid adenocarcinoma) with samples from before and after progesterone therapy were identified from 1/1/2001 to 9/1/2012. The reports from these cases were used to identify treatment-related morphologic findings. We then examined seven cases (three with material before and after treatment and four with only post-therapy material) for the presence of these morphologic findings.

Results: Of the 70 cases, 57 retained a complex architecture and 28 retained significant nuclear atypia. The most common forms of metaplasia identified included morular/squamous (23/70, 33%), mucinous (19/70, 27%), eosinophilic (13/70, 19%), and secretory-like (11/70, 16%). A small number demonstrated cribriform (12/70, 17%) and papillary (8/70, 11%) architecture in the post-treatment biopsies. 4 of the 69 cases showed solid architecture. The treatment regimens included oral progestogens (38/70, 54%), progesterone IUD (7/70, 10%), and combination oral and IUD therapy (6/70, 9%). Of the seven cases with post-treatment biopsies, 5 demonstrated complex architecture. One case retained the pre-treatment nuclear atypia. Interestingly, mucinous metaplasia was seen in 5 of 7 cases, 3 of which had pre-treatment biopsies without mucinous change. Almost all of the cases showed eosinophilic metaplasia (6 of 7). Tubal and morular metaplasia were also noted.

Conclusions: We confirm that metaplasias and resolution of atypia are associated with progesterone-treated hyperplasia and carcinoma. In our study, mucinous metaplasia was associated with therapy, as it was present in post-treatment biopsies but not in the pre-treatment biopsies examined. Secretory-like change was also noted in some cases following treatment. Although it is important to consider an underlying malignant process, such as a low grade mucinous carcinoma or a clear cell carcinoma, it should be recognized that these cytologic changes are common following progesterone therapy. Recognition of the spectrum of changes can assist in the evaluation of progesterone-treated endometrium.

1215 Metastatic Adenocarcinoma from Pancreas Involving the Ovary; Histopathologic Analysis of 11 Cases

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Background: Metastatic carcinoma involving ovary from pancreas (MOCPA) is rare, but one of the most frequently misdiagnosed neoplasm as a primary benign, borderline and malignant mucinous neoplasm of the ovary. Histopathologic features are among the most important clue for the diagnosis because immunohistochemical profiles between primary and metastatic carcinomas are considerably overlapping, and the ovarian mass is often a first manifestation of the disease. However, there are only a few literature describing detailed histologic features.

Design: In an attempt to find helpful findings for differential diagnosis, we reviewed histopathologic features and immunohistochemical profiles for cytokeratin 7(CK7), cytokeratin 20(CK20), CDX-2, Villin, DPC-4, PAX-2, and PAX-8 of 11 cases of MOCPA that were diagnosed at Asan Medical Center, Seoul, Korea, during 19 year-period and recognized a few hitherto undescribed histologic findings.

Results: At lower magnification, there were four major histologic patterns including 1) cystadenoma-like, 2) microcystic serous cystadenoma-like, 3) primary mucinous borderline tumor-like, and 4) infiltrative and desmoplastic patterns. Two or more histologic patterns were admixed in most cases. At higher magnification, single layered epithelium in the cystadenoma-like pattern was lined by cytologically malignant cells, showing cytologic-architectural dissociation and having significantly increased Ki-67 labeling index (17-38%, mean 29%), whereas in primary mucinous tumors the degrees of architectural complexity and cytologic atypia were usually parallel and Ki-67 labeling index is low (less than 6%) in the cystadenoma. Immunohistochemical expressions were variable; CK7 (100%), CK20 (75%), CDX-2 (71%), villin (100%), loss of DPC-4 (63%), and PAX-2 (0%) and PAX-8 (0%). Cystadenoma-like and microcystic serous cystadenoma-like features have not been described in metastatic carcinoma to the ovary from other organs. Primary mucinous borderline tumor-like pattern showed signficantly increased number of goblet cells compared to primary mucinous borderline tumors. Since all extraovarian tumor foci within the same tumor showed usual infiltrative patterns regardless of intraovarian histologic patterns, the peculiar intraovarian histology appears to be related to the specific ovarian environment.

Conclusions: Awareness of major histopathologic and cytologic-architectural patterns can be useful for the recognition of metastatic carcinoma of pancreatic origin and differentiation from primary mucinous neoplasm, especially during the frozen sections.

1216 Primary Cervical Carcinoma with Secondary Adnexal and Uterine Corpus Involvement: Detailed Morphologic Analysis of 14 Cases

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Background: Cervical adenocarcinomas (AdCa) and squamous cell carcinomas (SCC) uncommonly metastasize to adnexa or involve uterine corpus. Metastatic cervical AdCa to ovaries may be difficult to distinguish from primary ovarian endometrioid or mucinous tumors. We analyzed the morphology of adnexal and/or uterine corpus involvement by cervical AdCa and SCC.

Design: Patients with cervical SCC and human papillomavirus-associated AdCa who underwent surgery at MSKCC from 1997-2012 were identified. The following features were recorded in cases involving adnexa or uterine corpus: clinically visible lesion; depth of invasion; lymphovascular invasion (LVI); pattern of corpus and/or adnexal involvement (colonizing pre-existing epithelium, nodular vs infiltrating, bilaterality, presence of surface ovarian nodules and size of ovaries).

Results: Of 538 patients with invasive cervical cancer, 14 involved the adnexa and/or uterine corpus (SCC= 8; AdCa= 6). 11 had visible masses (6 SCC, 5 AdCa) and all had deep cervical invasion with LVI. 10 involved the corpus (6 SCC, 4 AdCa) all colonizing endometrial epithelium and 8 invading the myometrium, mostly deep/diffuse. 5 of 8 SCC had ovarian metastases (ovarian size from 2cm to 8cm): bilateral with surface involvement (n=1), unilateral with nodular and/or diffuse parenchymal tumors (n=4). Five had fallopian tube (FT) involvement (4 also involving ovary, 1 bilateral) with nodules of SCC around the FT (n=3) and SCC colonizing FT epithelium (n=2). Two of 6 AdCA had unilateral ovarian metastases (ovarian sizes 2.6cm and 4.0cm). One with a nodule of AdCa on the surface, and the other with papillary and cribriform glands with confluent growth, simulating primary ovarian endometrioid carcinoma. 4 had FT involvement (including those with ovarian metastasis), all with AdCa colonizing normal FT mucosa mimicking primary endometrioid adenocarcinoma and even serous tubal intraepithelial carcinoma. They contained elongated nuclei, apical mitoses and apoptotic bodies, typical of usual endocervical AdCa.

Conclusions: Adnexal involvement by cervical carcinoma is rare. Most of our cases had concurrent endometrial and deep myometrial extension of the cervical carcinoma. Both SCC and AdCa may colonize tubal and endometrial mucosa, and adenocarcinoma in particular may mimic an ovarian primary. Bilaterality is not a common feature of metastatic endocervical adenocarcinoma. Awareness of these features and clinico-pathologic correlation is important for accurate diagnosis.

1217 Morphometric Analysis in the Distinction between Invasive and In Situ Endocervical Adenocarcinoma

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Background: Morphologic distinction between in situ (ACIS) and invasive (INVA) endocervical adenocarcinoma, while having important clinical implications, can be challenging. As diagnostic digital pathology gains momentum, we aimed to identify quantifiable histologic features by digital image analysis that can differentiate between ACIS and INVA, which may be used as an adjunct diagnostic tool.

Design: Scanned images of 7 cases of ACIS and 7 cases of INVA in cone or hysterectomy specimens were included. Representative glands (2-4) were selected from each case and analyzed using Mercator Version 1.0 (ExploraNova, La Rochelle, France). The system detects structures based on optical density and analyzes them as closed objects. We collected morphometric variables in glands and periglandular stroma. Non-parametric Wilcoxon Mann Whitney test was used to evaluate the difference between both groups.

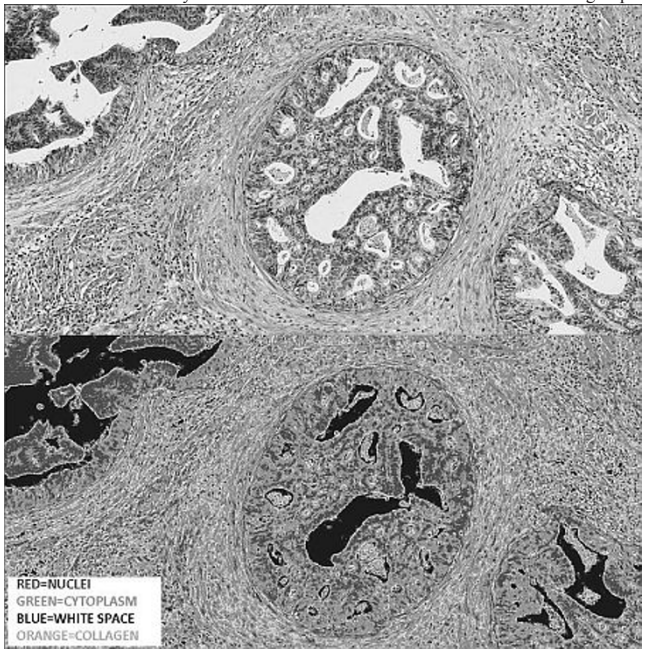

Table 1. Morphometric variables in ACIS and INVA

	VARIABLE	FORMULA	P value
GLANDS	Nuclear density	NC/TSA	0.33
	Mean nuclear form factor (MNFF)	SNF/NC	0.18
	% Nuclear surface area	SNA/TSA*100	0.75
STROMA	Nuclear density (ND)	NC/TSA	0.85
	Mean nuclear form factor (MNFF)	SNF/NC	0.009
	% Nuclear surface area	SNA/TSA*100	0.48
	% Collagen surface area	SCoA/TSA*100	0.02

NC = Nuclear count, TSA = Total Region Surface Area, SNF=Sum of nuclear form factors, SNA = Sum of all nuclear areas, SCoA = Sum of all collagen areas. All areas in μm2.

Results: Parameters related to nuclear density, as well as nuclear size and cytoplasmic size in both glands and stroma showed no significant difference. However, analysis of the periglandular stroma revealed that INVA had a greater mean nuclear form factor (0.372 vs 0.208, p=0.009) and lower % of collagen per region (2.82 vs 5.04, p=0.02) than ACIS.

Conclusions: Glandular cell density and cell size are similar between INVA and ACIS. However, features of the periglandular stroma, such as the nuclear contour of stromal elements (eg. fibroblasts) and the amount of collagen appear to be significantly different. This findings correlate with the standard qualitative criteria conventionally used, and underscore the potential of measurable variables using image analysis as an adjunct diagnostic tool.

1218 Targeted Development of a Specific Biomarker of Endometrial Stromal Cell Differentiation Using Bioinformatics: The IFITM-1 Model

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Background: Distinction of endometrial stromal tumors from smooth muscle tumors can be difficult. Although immunohistochemistry (IHC) is helpful, care must be taken as the only established stromal differentiation marker, CD10, is expressed diffusely in 20% of leiomyosarcomas. Consequently, we took a bioinformatics approach to identify a new marker by searching the Human Protein Atlas, a database of protein IHC expression profiles.

Design: The search for a new endometrial stromal cell differentiation biomarker began by downloading the raw data file from proteinatlas.org, and importing it into a Microsoft Access database. Our method used Structured Query Language to filter IHC results for those with "strong" staining in endometrial stroma AND "low" staining in endometrial glands and myometrium. Performance of the top candidate antibody was then evaluated with tissue sections from a variety of pathological diagnoses having or related to stromal cell differentiation [Table 1]. IHC was scored in terms of intensity (negative=0 to strong=3) and distribution (absent=0 to diffuse=3).

Results: 790,019 normal tissue-antibody test pairs were filtered by our purely informatic-based method to identify 10 unique candidates. From these 10, interferon induced transmembrane protein 1 (IFITM1) was selected as the top candidate by visually inspecting images of endomyometrium published on the Protein Atlas website. Both the intensity of normal stromal cell staining in proliferative endometrium and the absence of staining in myometrial cells were considered in this evaluation. The selected antibody was then tested against our expanded tissue collection. Table 1 summarizes our IHC results.

Table 1: IFITM1 IHC

Diagnosis	N	Average Intensity	Average Distribution
Endometrial stroma tumor of any type	22	2.36	2.09
Endometrial stromal sarcoma, low grade	13	2.54	2.31
Proliferative endometrium	22	2.91	2.86
Inactive endometrium	19	3.00	2.79
Adenomyosis	11	2.91	2.73
Leiomyoma, usual type	14	0.64	0.36
Cellular leiomyoma	16	0.88	0.63

Conclusions: Database mining proved to be a rapid, productive method of biomarker discovery. IFITM1 is a specific marker of endometrial stromal differentiation from proliferative endometrium to metastatic stromal sarcoma. Like CD10, IFITM1 is moderate-strongly and diffusely expressed in stromal cells. IFITM1 expression was found in about 25% of cellular leiomyomas, but typically with limited staining intensity and in a patchy distribution. This new marker should be a valuable addition to the IHC panel used in the diagnosis of cellular mesenchymal uterine tumors.

1219 Features Predictive of Negative Excision Findings in Patients with Biopsy Diagnosis of HSIL (CIN II-III)

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Background: Patients with cervical high grade dysplasia (HGD) usually undergo a cone/LEEP for excision. However, frequently the excision shows only low grade dysplasia (LGD) or no dysplasia. There is a small but possible chance of complications with these excision procedures, including cervical insufficiency, stenosis, bleeding or infection. We aimed to examine additional morphologic features in cervical biopsies with HGD, that may predict lack of HGD in excisions. This may potentially allow a more conservative approach in these patients.

Design: 51 consecutive cone biopsies/LEEPs from 2010 were selected from files after IRB approval. Previous cervical biopsies and ECCs which led to these excisions were reviewed for the following features: Number of biopsies with HGD, grade, proportion of biopsies with HGD, ECC with HGD, Cytology findings, Proportion of squamous epithelium with HGD, negative biopsy edges, presence of endocervix/exocervix, suboptimal epithelial orientation on biopsy, severe inflammation, condyloma with marked nuclear atypia and patients age.

Results: Out of the 51 patients, 38 had undergone excision procedure directly indicated by HGD on colposcopic biopsy. The rest had undergone the procedure due to cytology or other indications. Out of 38 patients, 25 (66%; positive excision group) showed HGD on excision while 13 (34%; negative excision group) showed LGD or negative excision. 23.8% (5/21) of biopsies with >10% epithelial involvement with HGD showed negative excision. In contrast, 47% (8/17) of biopsies with <10% epithelial involvement showed negative excision. Using Fisher's exact test, one sided p value is 0.12. Also, in the negative excision group, 46% (6/13) cases had the highest biopsy CIN grade of 2. In the positive excision group, only 28% (7/25) cases had highest biopsy CIN grade of 2. Other examined criteria, such as presence of severe inflammation, marked nuclear atypia, patient age, presence of transformation zone and involvement of biopsy edges showed roughly equal distribution between both groups. The average patient age in either group was 37.3 years.

Conclusions: The percentage involvement of squamous epithelium, with a cutoff of 10%, and the highest CIN grade on biopsy show trends for predicting presence or absence of high grade dysplasia on Cone/LEEP excision. A larger study set may possibly establish the significance of these trends. It is worthwhile to convey the percentage involvement and grade of dysplasia on a 3-tier system in cervical biopsy reports, so that the gynecologist can weigh the risks or benefits of an excisional procedure.

1220 Clinical Value of p16 Biomarker in Low Grade Squamous Intraepithelial Lesions

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Background: The biomarker p16 has been investigated extensively for utility in squamous intraepithelial lesions. However, studies on the clinical value and appropriateness of p16 immunohistochemistry (IHC) in low grade squamous intraepithelial lesion (LSIL) are limited and remain unclear. Recently, the Lower Anogenital Squamous Terminology (LAST) Project issued limited recommendations on the use of p16 IHC as biomarker in human papilloma virus (HPV)-associated lesions. The objective of this study is to evaluate the utility of p16 as a potential biomarker in predicting the clinical risk behavior in LSIL cervical biopsies.

Design: An institutional retrospective review of all cervical biopsies from 2008-2010 by Pathology Laboratory Information System identified 66 cases with a histologic diagnosis of LSIL. Of these, 37 (56%) cases with greater than one year follow-up data (pap smears, high-risk HPV status, biopsies and excisions) were included in the study. Upon re-review by a gynecologic pathologist, 20 confirmed LSIL cases were stained for p16 IHC using the EnVision+ system (Dako Cytomation, Glostrup, Denmark). Positive p16 staining was defined as strong diffuse staining in at least the basal 1/3 of the epithelium. Staining patterns and clinical follow-up outcome were analyzed.

Results: In all 20 LSIL cases, HPV+ was detected in 65% (n=13), p16+ was identified in 50% (n=10), and HPV+ p16+ was seen in 35% (n=7) of cases. For the p16+ LSILs, clinical regression and persistence occurred in 90% (n=9) and 10% (n=1) of cases, respectively. In LSILs that are HPV+ p16+, 86% (n=6) regressed while 14% (n=1) persisted. In addition, HPV+ p16- LSILs showed 67% (n=4) regression, 17% (n=1) persistence, and 17% (n=1) progression. Interestingly, p16 was negative in two LSILs with clinical follow-up of cervical intraepithelial neoplasia (CIN) 2 and 3.

Conclusions: This study demonstrates that p16 IHC is a poor predictor of clinical outcome and supports the LAST recommendation against the use of p16 IHC in morphologic LSILs. In addition, our data show that half of LSILs have strong diffuse positive staining for p16. As LAST recommends the usage of p16 IHC when entertaining the interpretation of CIN2, the possibility of over-interpreting LSIL with strong diffuse positive p16 as CIN2 may lead to subsequent over-treatment of lesions that are likely to regress.

1221 PSMA Is Highly Expressed in Gynecologic Tumor Neovasculature, but Lacks Expression in Normal Tissues

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Background: Prostate-specific membrane antigen (PSMA) has been reported to be uniquely expressed in neovasculature of malignant tumors but not in the endothelium of normal tissues. The goal of the study was to examine whether neovasculature of cervical, endometrial, ovarian and vulvar carcinoma expresses PSMA.

Design: The study cases included normal cervix (n=12), cervical squamous cell carcinoma (n=19), cervical adenocarcinoma (n=8), endometrioid adenocarcinoma of endometrium (n=23), vulvar squamous cell carcinoma (n=20), ovarian serous adenocarcinoma (n=24). Immunostaining was performed using anti-CD31 (clone 1A10; Novocastra) or anti-PSMA antibody (clone 3E6; Dako). The staining was assessed in the tumor and in the surrounding normal tissues. The staining with PSMA antibodies was scored for intensity (0, 1+, 2+, 3+) and semiquantitatively for the percentage of positive intratumoral capillaries with the following brackets: less than 5%, 6% to 25%, 26% to 50%, 51% to 75%, and 76% to 100% positive. CD-31 staining was used as a reference 100% positive control.

Results: PSMA staining positivity was found exclusively in the tumor vasculature and was not seen in neither normal cervix nor in peritumoral normal tissues. The average percentage of tumor capillaries positive for PSMA was 50.6% in cervical cancer, 72.1% in endometrial cancer, 63.8% in ovarian cancers, and 16.1% in vulvar cancer. The stainig intensity of PSMA in tumor vasculature was 2+ to 3+ for cervical, endometrial and ovarian cancer, and 0 to 1+ for vulvar cancer. CD-31 staining was used as a reference positive control, and as expected, was positive in all normal tissues as well as in tumor capillaries.

Conclusions: Prostate-specific membrane antigen is highly and specifically expressed in the neo-vasculature of gynecologic cancers rendering it a potential therapeutic vascular target.

1222 V600E BRAF Mutation in Vulvovaginal Melanoma Is Rare: A Study of Anti-Human BRAF V600E Monoclonal Antibody in 24 Cases

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Background: BRAF mutation may be present in up to 60% of melanoma, and its detection has therapeutic significance. The gold standard detection method is DNA sequencing (DNAs), which is labor intensive. In mucosal melanoma (MM), the reported incidence of BRAF mutation from all mucosal sites is low (10-15%). This study presents the findings of BRAF mutational analysis in 24 patients (pts) with vulvovaginal melanoma (VVM) and evaluates a new antibody directed against the most common BRAF mutation, V600E, to determine whether it could be used in place of DNAs particularly in tumors with a low incidence of BRAF mutation.

Design: Twenty-four VVM (8 vulvar; 16 vaginal) melanomas had BRAF mutation analysis by a PCR-based primer extension assay screening for the following mutational hot spots: G464, G466 (bases 1 and 2), G469, D594, L597R, V600 (all 3 bases) and K601 (bases 1 and 3). All VVM were then stained with anit-human BRAF V600E monoclonal antibody using the Bond Max stainer (clone VE1; 1:50, Spring Bioscience, Pleasanton, CA). Immunohistochemistry on nonMM previously tested by DNA sequencing with (n=21) and without (n=4) a V600E BRAF mutation served as a control.

Results: 21 pts with VVM had no BRAF mutation, and 3 pts (12.5%) had detectable BRAF mutation by DNAs: V600E, 1 pt; V560 1 pt; L576, 1 pt. Staining with anti-BRAF V600E antibody was positive in the 1 pt (4%) with a V600E mutation by DNAs. There was no staining observed in the 2 pts with variant BRAF mutation. No cases of VVM negative by DNAs had staining by immunohistochemistry (IHC). 20 of 21 cases (95%) with V600E mutation detected by DNAs had staining with the V600E antibody. 1 of 4 cases of non MM with no BRAF mutation by DNAs had staining by IHC. In this case, the amount of tumor in the sample was low; the possibility of tumor amount below the limit of detection could not be excluded.

Conclusions: The incidence of BRAF mutation in VVM is low (12.5%) and within the reported range of BRAF mutation detection in MM from all sites. BRAF antibody directed against the V600E clone is sensitive (95.5%) and specific (96.3%) in this small series. The antibody does not appear to detect variant BRAF mutations. Based on the results of this small study, IHC for BRAF antibody may be a cost effective alternative to DNAs particularly in tumors with a low incidence of BRAF. Further study on a larger scale is required to determine whether BRAF IHC could be used in place of molecular testing in all cases of melanoma.

1223 Complement Deposition in the Decidual Vasculature of Placentas

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Background: Pregnancy complications have an enormous impact on the health of the mother and the neonate. Histologic examination of the placenta of these patients often reveals characteristic findings that indicate chronic placental malperfusion, particularly decidual vasculopathy. However, the etiology of the vasculopathy as of yet cannot be readily determined. Pregnancy can be thought of as a model of immune regulation. Complement is an important element of the immune system that currently has a role in evaluation of antibody mediated rejection in transplant. This study aims to characterize the deposition of complement in the decidual vasculature (where activation and resulting thrombosis are the most likely to result in fetal harm) in a variety of pathologic pregnancies. We hypothesize that patients with pregnancy complications will have a greater degree of complement deposition in the decidual vasculature than normal controls.

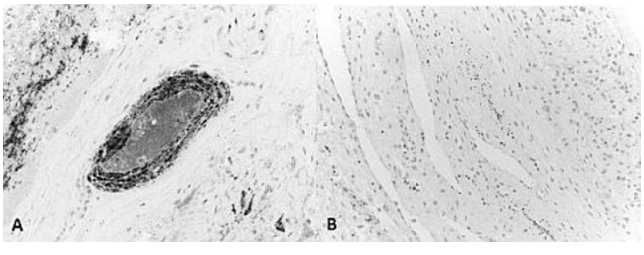
Design: Paraffin embedded membranes from placentas delivered from 2009-2012 were collected to form a cohort of 26 women with normal and complicated pregnancies. The tissue was immunohistochemically stained with antibodies to C3d and C4d; clinical information was collected from the patients' charts. Degree of vascular staining was evaluated using the H-score method for percentage and intensity by a single blinded observer.

Results: Placentas from pregnancies complicated by intrauterine fetal demise or pre-eclampsia had a significantly higher H-score than normal controls, however the difference did not reach statistical significance in early spontaneous abortion and intrauterine growth restriction due to small sample size. Table 1 includes mean H-scores for each category examined, while Figure 1A demonstrates positive vascular C4d staining and 1B negative staining.

Table 1

	n	C3d	C4d	Total H-score	p
Normal	6	45.8	35.7	81.5	-
SAB	4	61.3	68	129.3	0.23
IUFD	6	68.3	83.3	151.7	0.05*
Pre-eclampsia	6	90	70	160	0.03*
IUGR	4	37.8	65	102.8	0.35

*Statistically significant



Conclusions: This study supports that the complement system may have a role in the pathogenesis of chronic placental malperfusion. Further study is needed to confirm the association between complement dysregulation and pregnancy complications. To our knowledge this is the only study to examine complement deposition in the decidual vasculature.

1224 Uterine Smooth Muscle Tumors with Features Suggesting Hereditary Leiomyomatosis and Renal Carcinoma (HLRCC) Syndrome: Detailed Morphologic Analysis and Correlation with 2SC Imnohistochemistry

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Background: Hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome, an autosomal dominant disorder with affected patients harboring a germline mutation in the fumarate hydratase gene (FH-M), confers a predisposition for skin and uterine leiomyomas and aggressive renal cell carcinomas. Women with HLRCC usually present with multiple fibroids at a young age. Morphologic/immunohistochemical (IHC) correlations were studied with an antibody against S-(2 succino) cysteine (2SC). High levels of modified cysteine residues have been reported in FH-deficient cells and HLRCC tumors, but not in normal tissues or a wide range of non-HLRCC tumors.

Design: Uterine smooth muscle tumors (USMTs) from unselected patients were prospectively analyzed for features suggesting HLRCC (HLRCC-F: prominent eosinophilic macronucleoli with perinucleolar halos) and genetic testing performed as a result in 3 cases. A detailed morphological analysis was undertaken and 2SC IHC was performed with controls from a tissue microarray (TMA) that included leiomyomas (19), leiomyosarcomas (29), and endometrial stromal tumors (15).

Results: 8 tumors had HLRCC-F and 2 tested patients had germline FH-M. Patients' ages ranged from 20-50y. 4 had multiple USMTs. Solitary tumors ranged in size from 7-13 cm. All cases had increased cellularity, pericytomatous vasculature and highly fibrillary cytoplasm with pink globules. Mitotic index was 3-4/10HPF. Nuclear atypia was severe in 2 cases. 5 cases had multinucleated cells and 2 had focal pseudoinclusions. Nuclei were round and vesicular, sometimes imparting the impression of an epithelioid neoplasm. All cases had inclusion-like nucleoli with perinuclear halos (7 diffuse, 1 focal). 3 cases had a neurilemmoma-like pattern. All tumors displayed diffuse granular cytoplasmic labeling with 2SC. Cytoplasmic globules and adjacent myometrium were negative. Of the TMA controls, only 2 tumors were IHC positive and both showed focal HLRCC-F.

Conclusions: 2SC positive, morphologically distinctive USMTs, some in confirmed FH germline mutation carriers, contrast with 2SC negative USMTs without HLRCC-F. This suggests that HLRCC-F is reliably associated with FH abnormalities, including FH-M characteristic of HLRCC. An HLRCC diagnosis should be considered for young women with USMTs that display characteristic morphology so that affected patients and family members can be referred for genetic testing.

1225 Predictors of Absence of Cervical Intraepithelial Neoplasia in the Conization Specimen

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Background: Conization is the standard treatment in patients with high-grade squamous intraepithelial lesions (H-SIL, CIN2-3) and also in a subset of patients with low-grade squamous intraepithelial lesions (LSIL, CIN1). Several studies have shown that in over 10% of women who undergo conization for CIN, no lesion is observed in the surgical specimen. We aimed to determine whether these patients could be identified before conization using clinical, virological and/or cyto-histological characteristics, to avoid unnecessary treatment.

Design: Of 687 women with CIN treated by conization in the Hospital Clinic of Barcelona between 2008 and 2011, all patients (n=110, 16%) showing no lesion in the surgical specimen were included as the study group. Patients with glandular abnormalities or previous conization were excluded. The control group included a series of randomly selected women with CIN confirmed in the cone specimen (n=220). Pre-conization clinical, cytological and histological characteristics as well as high-risk human papillomavirus (hr-HPV) status determined by Hybrid Capture 2 were analyzed as possible predictors of absence of lesion.

Results: A negative pre-conization hr-HPV test or a low viral load (< 10 relative light units[RLU]) significantly increased the probability of absence of CIN in the conization specimen (75.0%, and 52% respectively) compared with patients with a viral load higher than 10 RLU, (28.9%, p<0.001). This association was confirmed in the multivariate analysis (p<0.001). No significant differences were observed in terms of percentage of persistent/recurrent disease after conization between patients from the study and control groups.In contrast, the risk of developing persistent/recurrent disease after treatment was significantly lower in patients with negative result or a low viral load (16.1% CIN1, 0% CIN2-3), than in patients with a high viral load (27.6% CIN1, 4.1% CIN2-3, p=0.031).

Conclusions: Women with negative pre-conization hr-HPV test results or a low viral load have a high probability of having no lesion in the conization specimen. These patients should be excluded from immediate surgical excision and considered for follow-up.

1226 Risk Factors in Patients with Low-Grade Endometrial Adenocarcinoma and Lung Recurrence Versus Recurrence at Other Sites. A Multi-Institutional Study

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Background: There is no significant data analyzing risk factors for low-grade endometrial tumors that recur in the lung vs other sites.

Design: In this multi-institutional study, we analyzed data from 79 patients with FIGO grade 1 and 2 endometrial adenocarcinomas with extrauterine recurrence (excluding vagina) and 187 age matched controls with negative lymph nodes and no treatment that did not recur; with similar follow-up time (44 and 59 months, respectively).

Results: Twenty patients had lung recurrence and 59 patients had recurrence in other sites at initial recurrence (Table 1). Tumors that recurred in lung showed a significantly greater depth of myometrial invasion, compared to other recurrences ($p<0.05$) and controls ($p<0.0001$), respectively. Cervical involvement was more frequent in cases with lung recurrence than in other sites ($p< 0.05$); moreover, cervical stromal involvement was a highly significant risk factor ($p<0.003$) for lung recurrence. All analyzed features, except necrosis, were significantly different in all recurrent tumors compared to controls ($p<0.001$).

	Gross	Size	Necro-sis	Myoinvasion	MELF	Desmo-plasia	Cervical invasion
Lung (20)	Flat 8/Exo 11/Unk 1	4.9	9	61.2% (12 >50%; 3>33%)	10 (25.3%)	17	13 (10 stroma)
Other rec (59)	Flat 20/Exo 36/Unk 3	5.1	34	44.1% (24>50%; 8>33%)	31 (18.2%)	38	23 (13 stroma)
Controls (187)	Flat 49/Exo 138	3.3	73	26.9% (41>50%; 22>33%)	61 (10.2%)	80	15 (11 stroma)

Exo: exophytic; Unk: unknown; Myoinvasion depth: average (number cases over 50%; cases 33- 50%); MELF: microcystic, elongated and/or fragmented myoinvasive pattern: number of cases with MELF (average of MELF % at invasive front of tumor)

Conclusions: 1. Low grade tumors that recur in the lung have significantly greater myometrial invasion, invade the cervical stroma and show desmoplasia more often than tumors that recur at other sites.

2. Differences in the site of recurrence might be related to the type (veins vs. lymphatics) or location of involved vessels; veins are usually deeper in the myometrium and the route of dissemination from cervical vascular invasion may be different.

1227 Risk Factors for Recurrence in Sites Other Than Vagina in Patients with Low Grade Endometrial Adenocarcinoma. A Multi-Institutional Study

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Background: There is no significant data analyzing risk factors in low grade endometrial tumors that would predict site of recurrence.

Design: We compiled data from 1991-2011 for patients with FIGO grade 1 and 2 endometrial adenocarcinomas, including 79 patients with extrauterine recurrence (follow-up 44 months), 38 patients with vaginal recurrence alone (follow-up 57 months), and 187 age matched control patients that had negative lymph nodes (LN), no adjuvant treatment, and did not recur (follow-up 59 months). We further stratified the recurrence group (those with metastatic LN, negative LN and those without resected LN; pelvic/abdominal recurrence (excluding vaginal) and those with recurrence at distant sites.

Results: Overall, 56 recurrent cases (71%) had lymphovascular invasion (LVI) and 41 (73%) of these recurred at distant sites. Of 187 controls, only 26% had LVI ($p<0.0001$), similar to 21% LVI in vaginal recurrence cases ($p<0.0001$). Recurrent cases with LN metastasis at the time of hysterectomy showed LVI in 96% and 78% recurred at distant sites. While 45% of cases with negative LN lacked LVI and 64% of these recurred in the pelvic/abdominal region. Additional data is summarized in Table 1. Tumor size, myoinvasion depth, cervical involvement and cervical stromal involvement were statistically different between recurrences (excluding vaginal) and controls ($p<0.0001$) while the presence of microcystic, elongated and/or fragmented (MELF) pattern of invasion was statistically different ($p<0.03$).

	Size	Myoinvasion	MELF	Cervical stroma
Distant (54)	4.9	51.6% (26>50%; 8>33%)	20.2%	17
Pelvic (25)	5.3	41.4% (10>50%; 3>33%)	17.8%	5
Controls (187)	3.3	26.9% (41>50%;22>33%)	10.2%	11

MELF: average MELF % at invasive front of tumor; Myoinvasion depth: average (cases >50%; cases 33-50%)

Conclusions: 1. LVI is a significant risk factor for recurrences in low grade endometrial adenocarcinoma, but not significant for vaginal recurrence.

2. Risk factors other than LVI do not appear to be useful in predicting the site of recurrence, pelvic/abdominal or distant.

3. Recurrences at LN or distant sites were significantly associated with LVI, indicating lymphvascular dissemination.

4. However, pelvic/abdominal recurrences were not associated with LVI, especially in cases with negative LN, suggesting a different dissemination route other than lymphvascular, possibly transtubal.

5. Low grade tumors that are smaller, show less myoinvasion (less than 33%), and no cervical involvement are significantly less commonly associated with recurrence.

1228 Comprehensive Genomic Profiling of Epithelial Ovarian Cancer (OC) by Next Generation Sequencing (NGS) Reveals New Routes to Targeted Therapies

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Background: The recent introduction of NGS to clinical samples has enabled the discovery of novel and unanticipated genomic-derived drug targets of therapy for patients with relapsed OC.

Design: NGS was performed on hybridization-captured, adaptor ligation based libraries using DNA extracted from 4 formalin-fixed paraffin embedded sections cut at 10 microns from 31 pre-treatment OC that had relapsed after primary surgery and platinin-based chemotherapy. The exons of 182 cancer-related genes were fully sequenced using the Illumina HiSeq 2000 (Illumina Inc. San Diego, CA) to at an average sequencing depth of 943X and evaluated for genomic alterations (GA) including point mutations (mut), insertions, deletions, copy number alterations (amp), and gene fusions/translocations. Actionable GA were defined as impacting anti-cancer drugs on the market or in registered clinical trials (CT).

Results: The study included 22 (71%) papillary serous (PS), 5 (16%) endometrioid (EC), 3 (10%) clear cell (CC), and 1 (3%) undifferentiated carcinomas. One (3%) tumor was FIGO grade 1, 5 (16%) grade II and 25 (81%) grade III. Twenty-two (71%) OC were Stage III and 9 (29%) Stage IV at diagnosis. NGS found a total of 89 GA in the OC series with an average of 2.9 GA per tumor. 25 GA (0.8 per tumor) were potentially associated with clinical benefit of targeted therapies and 0 GA associated with targeted therapy resistance. The GA generated potential entry into a total of 150 CT (average 4.8 per tumor). The most common GA were *TP53* mut (65%); *myc* amp (19%), *KRAS* mut/amp (19%) and *BRCA1* mut (13%) There were no *HER2* amp, but one tumor featured a *HER2* mut. 4/5 (80%) of *ARID1A* mut occurred in non-PS tumors. 2/3 (67%) of CC featured *cMET* amp validated by both FISH and IHC.

Conclusions: NGS of conventional therapy resistant OC uncovers an unexpectedly high frequency of GA that could influence therapy selection for the disease. Deep sequencing of genomic DNA can provide a broad cancer-related gene survey at a depth of coverage that provides sensitive detection for all classes of GA, and when applied to OC patients can reveal actionable GA that inform treatment decisions.

1229 The Use of Progesterone Receptor (PR) and p53 in Assessing Uterine Smooth Muscle Tumors of Uncertain Malignant Potential (STUMP)

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Background: Uterine smooth muscle tumors (USMT) are extremely common, of which the overwhelming majority are benign leiomyomas (BLM) which pose little diagnostic difficulty. Similarly, as leiomyosarcomas (LMS) typically exhibit frankly malignant features including significant nuclear pleomorphism, tumor necrosis and brisk mitotic activity, the diagnosis is usually not overly challenging. However, a small subset of USMT cannot be diagnosed unequivocally as BLM or LMS, thus are classified as smooth muscle tumors of uncertain malignant potential (STUMP). Yet, the diagnosis of STUMP is largely subjective, with no generally accepted criteria. Because of this, attempts to further characterize USMT, specifically STUMP, are warranted. A recent paper by Hewedi, et al showed promise in this regard. The authors used a simple immunohistochemical (IHC) panel (PR and p53) and showed that all LMS show low PR expression and high p53 expression while all non-LMS (STUMP and BLM) stain oppositely (that is, low p53 and high PR).

Design: We sought to assess the usefulness of this panel (p53 and PR) in our cohort of USMT. A total of 27 USMT cases, including 5 BLM, 10 STUMP and 12 LMS, were included. Both were scored on a scale from 0-3 (0=negative, 3=strong, diffuse). We plan to correlate this with clinical follow up data (IRB approved; work in progress).

Results: While PR and p53 did separate many tumors into LMS or non-LMS (BLM/STUMP), this was far from 100%. Based on expected findings (as outlined above - Hewedi, et al), only 17 of 27 tumors behaved exactly as expected.

Combined PR and p53 Profile

Tumor Type	High PR/Low p53	Low PR/High p53	High PR/High p53	Low PR/Low p53
Non-LMS (BLM/STUMP)	13	1	0	1
LMS	4	4	1	3

The clinical follow up in these cases will be key, and preliminary data reveal that outcomes generally correlate well with the H&E diagnosis. For example, even the LMS cases that stained unexpectedly (that is, high PR and low p53) had poor outcomes. Classification of LMS based solely on p53 and PR (classified as LMS if either p53 was 2 or 3 or PR was 0 or 1) gives a sensitivity of 0.667, a specificity of 0.867 and an accuracy of 0.778.

Classification of USMT based on p53 and PR

Sensitivity	Specificity	PPV	NPV	Accuracy
0.667	0.867	0.80	0.765	0.778

Conclusions: While H&E assessment remains the most important tool in diagnosing USMT, PR and p53 may serve as a useful adjunct in the proper classification of equivocal lesions, specifically STUMP. However, relying too heavily on IHC could be problematic. Correlation of these markers with clinical outcomes in these patients is ongoing.

1230 Malignant Melanoma of Lower Female Genital Tract: A Histomorphological Review and Genotyping Analysis, Including BRAF, NRAS and C-KIT Mutations

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Background: Though rare, melanoma is the second most common malignancy of vulva and vagina. The prognosis, even when localized at presentation, is poor. BRAF and NRAS mutations are reported in approximately 50% and 15% of cutaneous melanomas respectively. The presence of these mutations predicts aggressive tumor behavior. Clinical trials of the BRAF kinase inhibitor have shown response rates of more than 50% in metastatic melanoma harboring this mutation however the frequency of the mutations is not the same in different sites. Other studies suggest that C-KIT mutations are more common in vulvar melanomas however molecular characteristics of vulvar and vaginal melanomas have not yet been extensively explored.

Design: 33 cases of vulvar or vaginal melanoma were retrieved between 2002- 2012. The hematoxylin & eosin stained slides and clinical records were reviewed to record the size of the tumor, histological pattern, depth of invasion, tumor thickness, ulceration status, lymphovascular invasion and clinical outcome. Representative blocks were selected for genotyping studies to detect mutations in BRAF, NRAS, C-KIT as well as 45 other genes. Genotyping studies were performed either using a specific PCR based ARMS assay, Sanger sequencing or the Illumina TSACP Cancer panel on the Miseq platform.

Results: We identified 24 vulvar and 9 vaginal melanomas with mean age of 60 years (range 17-83). Tumor size varied from 0.4 to 6.2 cm. Breslow thickness ranged from 0.75 to 29 mm (mean: 10.9 mm). Ulceration was seen in 16 of 25 cases. The follow up period was 6 mo to 10 years. 10 patients were dead, 2 lost to follow up and 21 were alive at the time of the study. Preliminary data on 16 cases identified a BRAF mutation in 12.5% (2 of 16 cases- 1 by ARMS and 1 by Miseq), C-KIT mutation in 12.5% (2 of 16 cases- 1 by Sanger sequencing and 1 by Miseq; the case identified using Miseq had 2 C-KIT mutations) and NRAS mutation in 33% (2 of 6 cases- only Miseq). We detected a D594V BRAF mutation using the Miseq platform in one case which hadn't been detected by the ARMS PCR designed specifically for the V600E mutation.

Conclusions: Preliminary data identified BRAF mutations with less frequency as compared to non-genital cutaneous melanomas however NRAS mutations were detected in one third of our cases. More studies and with larger numbers of cases are necessary in order to increase the understanding of the pathological features and the molecular biology of melanoma of lower female genital tract. Such data may guide more therapeutic innovations.

1231 Trefoil Factor 3 (TFF3) Is an Independent Biomarker for Better Prognosis in Endometrial Endometrioid Adenocarcinoma but Not in Uterine Serous Carcinoma

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Background: Previously, using the Illumina Human HT-12v3.0 gene expression array we found the trefoil factor family 3 (*TFF3*) gene to be differentially expressed in high grade endometrioid adenocarcinoma (EAC) of the endometrium in comparison to uterine serous carcinoma (USC). We subsequently confirmed and validated this using quantitative RT-PCR. TFF3 is one of 3 members of the trefoil factor family of proteins that typically contribute to a barrier coating the surface of epithelial cells. However, their role in cancer biology is still unclear. The aims of this study were to evaluate the expression and prognostic value of TFF3 in endometrial cancer (EC).

Design: A tissue microarray of 328 EC cases was developed and stained with TFF3 monoclonal antibody. To test the association of TFF3 expression with histological and clinical parameters (age, tumor subtype, myometrial invasion, FIGO grade and stage, lymph node status, lymphovascular invasion (LVI), and disease status at last visit) Fisher's exact test was performed. Univariate and multivariate Cox regression analysis were used to determine the association between TFF3 and overall survival (OS) and recurrence free survival (RFS).

Results: Type I carcinoma accounted for 74% of cases and type II 26%, 66.5% were stage I +II and 33.5% were stage III+IV. TFF3 was positive in 60% of all cases. In univariate Cox regression analysis, TFF3 was associated with tumor grade ($p < 0.0001$), tumor stage (p 0.0008), LVI (p 0.018), tumor subtype ($p<0.0001$) and disease status (p 0.0097). The 5 year OS was 55.8% for $TFF3^-$ EAC cases and 83.3% for $TFF3^+$cases ($p=0.04$). The 5 year RFS was 62.3% for $TFF3^-$ EAC cases and 85.4% for $TFF3^+$ (p 0.053). Furthermore, multivariate Cox regression analysis revealed TFF3 to be an independent predictive factor for RFS and OS in EAC but it had no value in USC.

Conclusions: 1- $TFF3^+$ was associated with EAC subtype and with early stage disease. 2-TFF3 was an independent marker for better prognosis in EAC and it could be of use in patient management, i.e. patients with tumors expressing TFF3 may require less surveillance and follow-up. 3- We speculate that TFF3 may play a role as a tumor suppressor gene in EAC. Our results are novel and more mechanistic studies of TFF3 in the endometrial cancer are needed.

1232 Chronic Vestibulitis Is Associated with Interleukin-4 Polymorphisms Linked to Interstitial Cystitis and Atopy

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Background: Chronic vestibulitis, also known as provoked localized vulvodynia (PLV), occurs in the rim of mucosa between the labia minora and hymen. This common disease affects approximately 15% of women during their lifetime and is the leading cause of painful intercourse. The cause is unknown, but neurogenic inflammation appears to play a key role. Neurogenic inflammation is also a shared feature with interstitial cystitis, which is present in 20% of women with PLV. Many investigators suspect a genetic basis for interstitial cystitis. However, testing for a genetic predisposition for PLV is only in the early stages of discovery. We hypothesized that PLV would be associated with the IL-4 promoter (rs22432250) and intron 2 (rs2227284) variants commonly seen in patients with interstitial cystitis and atopy.

Design: Retrospective analysis of 212 clinically confirmed cases of PLV diagnosed from 2002-2012 at Oregon Health & Science University. Subjects were classified into primary PLV (pain with first introital touch) and secondary PLV (*de novo* pain usually after childbirth or menopause). Clinical charts were reviewed to confirm diagnoses and screen for a history of interstitial cystitis and allergies. Since patient race may significantly affect allele frequencies, only data from non-hispanic Caucasian women were included for genetic analysis (205/212). Subject DNA was available for 195 cases (91 primary and 104 secondary). IL-4 genotypes were determined using real-time PCR-based Taqman Allelic Discrimination (ABI). Data were analyzed by X^2 analysis.

Results: Secondary PLV was associated with both IL-4 variants ($p<0.01$) known to increase IL-4 activity and lead to elevated serum IgE levels. The allele frequency of the T-allele at -589 (promoter) was 0.20 (Caucasian controls 0.14); the allele frequency of the T-allele at 3017 (intron 2) was 0.31 (controls 0.25). IL-4 allele frequencies in primary PLV were not significantly different than controls. The prevalence of interstitial cystitis was increased in both primary and secondary PLV (32%, 27%, respectively) compared with controls (6%). The odds ratio for atopy in secondary PLV was 2.34 [1.3-4.4] ($p<0.01$); it was not increased in primary PLV.

Conclusions: We show for the first time that IL-4 genetic variants associated with interstitial cystitis and atopy may also play a role in secondary PLV. We have recently demonstrated that primary and secondary PLV may have different underlying causes. Our data support this working hypothesis and suggest drugs targeting the IL-4 pathway may provide a potential treatment.

1233 Perivascular Epithelioid Cell Tumor (PEComa) of the Gynecologic Tract: A Clinicopathologic Analysis of 16 Cases Including *TFE3* Rearrangement Analysis in a Subset

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Background: PEComa is a family of tumors characterized by co-expression of melanocytic and muscle markers. It is often misclassified because it is rare, under-appreciated and has morphologic overlap with epithelioid smooth muscle tumors. This study, the largest to date, characterized the clinicopathologic features of PEComa arising in the female genital tract.

Design: 16 cases of gynecologic PEComa were identified in our institutional and consultation files. All were analyzed for numerous histologic and immunohistochemical features. Staining in >1% of cells was considered positive for all antibodies except TFE3 (>25% staining required). FISH for TFE3 rearrangement was performed in 3 cases. Clinical history and follow up were obtained.

Results: 13 cases were primary of the uterus, 2 of the adnexa and 1 of the vagina. 43.5% of submitting diagnoses were leiomyosarcoma or poorly differentiated uterine sarcoma. Tumors ranged from 0.3 to 15.5 (mean 7.6) cm. 3 patients DOD, 5 are AWD and 8 are AWOD (2 mo-13 yrs follow up, mean 31.2 mo). All patients with adverse outcome had histologic features defining malignancy in non-gynecologic PEComa.

Histopathologic Features

Tumor Interface (multiple patterns observed)	14 infiltrative, 10 pushing, 3 endometrial stromal sarcoma-like
Nested Growth	13/16 (81%)
Stromal Hyalinization	9/16 (56%)
Tumor Necrosis	8/16 (50%)
LV Invasion	5/16 (31%)
Cell Morphology	9 predominantly epithelioid (56%), 7 predominantly spindled (44%)
Dyscohesive Cells	14/16 (88%)
Cytoplasmic Quality	15 clear and eosinophilic (94%), 1 clear vacuolated (6%)
Melanoma-like Nucleoli	10/16 (63%)
Intranuclear Pseudoinclusions	6/16 (38%)
Mitotic Index (per 10 hpf)	Range 0-84 (mean 29)
Mitotic Index (per 50 hpf)	Range 0-395 (mean 136)

IHC Features

HMB45	16/16 (100%)
MelanA	14/16 (88%)
MiTF	10/11 (91%)
Cathepsin K	12/12 (100%)
TFE3*	5/12 (42%)
SMA	14/15 (93%)
Desmin	14/14 (100%)
h-Caldesmon	11/12 (92%)

*Three cases with TFE3 immunoreactivity were negative by FISH for TFE3 gene rearrangement.

Conclusions: Gynecologic and non-gynecologic PEComa share distinct morphologic and immunophenotypic findings. Established histologic features of malignancy appear to apply to gynecologic tumors. Although TFE3 expression was frequent, it did not correlate with gene rearrangement. Gynecologic PEComa is under-recognized and often misclassified; therefore, awareness of its appearance and immunohistochemical profile will aid in its separation from uterine smooth muscle tumors, which is essential as PEComa may respond to mTOR inhibition therapy.

1234 *MED12* Mutations in Uterine and Extrauterine Smooth Muscle Tumors

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Background: A recent report of exome sequencing in uterine leiomyomas showed that a majority (70%) harbor mutations in the *mediator complex subunit 12* gene (*MED12*). These mutations occur almost exclusively in exon 2, and have been substantiated by several subsequent studies. While other, recurrent genetic abnormalities have previously been identified in leiomyomas (del 7q, trisomy 12, rearrangements of 12q14), these occur with a lower frequency. *MED12* exon 2 mutations represent the most common genetic abnormalities of uterine leiomyoma to date. We sought to determine the incidence of *MED12* exon 2 mutations in intra-and extrauterine smooth muscle tumors.

Design: We identified cases of leiomyomas, leiomyosarcomas, and smooth muscle tumors of uncertain malignant potential (STUMPs) in intra- and extrauterine sites resected from 1994-2012. Cellular areas of tumor were identified by histological examination, and formalin-fixed tissue was micro-punched from corresponding paraffin-embedded blocks. DNA was extracted and PCR amplified using primers specific for *MED12* exon 2. Amplified DNA was then bi-directionally Sanger sequenced and the resulting traces analyzed for sequence variation.

Results: We identified *MED12* exon 2 mutations in 9/18 (50%) classical uterine leiomyomas and 0/5 cellular, atypical or symplastic subtypes. *MED12* exon 2 mutations were identified in 2/20 (10%) extra-uterine leiomyoma/leiomyomatosis, 5/20 uterine leiomyosarcomas (25%), 2/24 (8%) extra-uterine leiomyosarcomas and 1/8 (12%) intra- and extrauterine STUMPS. Mutations were predominantly single-base pair substitutions, most often affecting codon 44, and rare deletions.

Conclusions: Our results show that exon 2 *MED12* mutations are common in classical uterine leiomyomas, but are uncommon in subtypes of leiomyomas (0%). Similarly, *MED12* mutations are less common in extrauterine leiomyomata (10%); intra- and extrauterine STUMPS (12.5%); uterine leiomyosarcoma (25%); and extrauterine leiomyosarcoma (8%). These findings demonstrate that *MED12* exon 2 mutations are present in various tumors of smooth muscle derivation, albeit at a lower frequency than classical leiomyomas. Further, molecular testing for *MED12* mutations may be a useful adjunct to confirm the diagnosis of uterine and extra uterine smooth muscle tumors.

1235 Is Radical Hysterectomy Necessary in Early Cervical Cancer?

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Background: The purpose of this study was to estimate the prevalence of tumor spread to the parametrium, vagina and uterine corpus in radical hysterectomy specimens and define a subgroup of patients with low-risk of extra-cervical involvement, who may benefit from less radical surgery.

Design: We retrospectively reviewed 96 patients with stage IA1-IIA cervical cancer who had undergone radical hysterectomy and pelvic lymphadenectomy.

Results: Tumor spread beyond the uterine cervix was evident in 45 (47%) patients. Thirteen (13%) of the 96 patients had parametrial tumor spread, 12 (13%) had vaginal tumor extension, and 23 (24%) had uterine corpus involvement. Tumor size >2 cm, stromal invasion to a depth of ≥8 mm, lymph vascular space invasion (LVSI), high histological tumor grade, and advanced tumor stage were significantly associated with extra-cervical invasion. In multivariate analysis LVSI, tumor size >2 cm and stromal invasion of ≥8 mm were found to be associated with extra-cervical invasion.

Variables associated with extra-cervical invasion (Multiple Logistic Regression Model, n=94, C-statistic=0.890)

	Odds ratio	Lower	Upper	P value
Lymph vascular space invasion (vs. no invasion)	12.5	3.8	40.4	<0.001
Tumor size > 2 cm (vs.≤ 2 cm)	5.7	1.6	19.9	0.006
Stromal invasion (per 5 mm)	1.9	1.05	3.6	0.033

95% Confidence Interval

Twenty-five patients had stromal invasion of <8 mm and no LVSI, of which only 1 (4%) had extra-cervical involvement. On the contrary, extra-cervical involvement was evident in 44 patients (63%) among those who had stromal invasion of ≥8 mm and/or LVSI (p<0.001). Among women with LVSI extra-cervical tumor spread was seen with any tumor size and any depth of stromal invasion. All patients with stromal invasion to a depth of >15 mm had extra-cervical invasion.

Conclusions: Patients with tumor size <2 cm, depth of invasion of <8 mm and no LVSI could be considered for less radical surgery.

1236 Positive Margin Status in Uterine Cervix Cone Specimens Is Associated with Persistent/Recurrent High-Grade Dysplasia

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Background: The frequency of positive cone margins and its significance in cervical intraepithelial neoplasia (CIN) are under controversy. The purpose of the current study was to identify factors associated with positive cone margin status and to evaluate its clinical significance in high-grade CIN.

Design: Medical records of women who underwent loop electrosurgical excision procedure at Soroka University Medical Center (January 2001 - July 2011) were reviewed retrospectively. Patient age, extent of dysplasia, endocervical glands involvement, positive margin status, type of margin involved, degree of margin involvement, and post-cone endocervical curettage results were evaluated as possible factors associated with persistent/recurrent disease.

Results: Three hundred seventy six women were included in the study. Cone margin involvement was observed in 33% (endocervical – 22%, ectocervical -8%, both margins – 3%). Factors significantly associated with cone margin involvement were older age (>35 years), widespread dysplasia in the cone specimen (≥4 sections) (p<0.001 for each) and endocervical glands involvement (p=0.003). Fifty patients (13%) had persistent/recurrent disease. Involvement of the cone margins (focal: HR=17, p<0.001; extensive: HR=28, p<0.001) and older age (HR = 1.18 for every five additional years, p=0.03) were associated with persistent/recurrent disease.

Conclusions: We conclude that women older than 35 years with widespread high-grade dysplasia in the cone specimen and involvement of endocervical glands are more likely to have positive cone margins. Positive cone margins, particularly when extensively involved, as well as increased patient age are associated with persistent/recurrent disease. These factors should be considered when planning further management.

1237 Utility of p16 Immunohistochemistry in Evaluating Negative Cervical Biopsies Following High-Risk Pap Test Results

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Background: The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions (LAST) recommends p16 immunohistochemical staining as an adjunct to morphologic assessment of cervical biopsies interpreted as negative from patients that are at high risk for missed high-grade disease (defined as a prior cytologic interpretation of HSIL, ASC-H, ASC-US/HPV-16+, or AGC-NOS) (Darragh, 2012). However, to our knowledge no studies have specifically evaluated the utility of performing p16 on negative cervical biopsies and endocervical curettage specimens following high-risk Pap test results.

Design: A search of Pap test cases from 7/1/2002-12/31/2009 for the term "HSIL" yielded 907 cases with diagnoses of ASC-H, HSIL, and "LSIL cannot exclude HSIL" (AGC-NOS and Atypical Endocervical Cells were secondary diagnoses). 478 cases had tissue follow-up. Cases were excluded if the biopsy was diagnosed as atypical, LSIL, HSIL, or insufficient, or if the time to follow up was >1 year. Immunostain for p16 (CINtec) was performed on 113 cases (91 ASC-H, 22 HSIL) from 106 patients (age 39.6+/-13.1 years) and scored as positive (diffuse strong) or negative. For the positive cases, the corresponding H&E section was re-examined and HPV in situ hybridization (ISH) (Ventana HPV III probe) was performed; follow-up data was also obtained.

Results: Strong p16 staining was seen in 7/106 patients (6.6%) corresponding to missed diagnoses of LSIL (2 cases), HSIL (CIN2, 1 case), and SIL-indeterminate grade (3 cases). One case exhibited staining of bland metaplastic cells undermining endocervical glands; follow-up biopsies showed HSIL. Of 21 patients with a prior HSIL Pap test, 2 (9.5%) had p16(+) biopsies that were also HPV ISH(+). Of 85 patients with prior ASC-H, 2 (2.4%) had p16(+) biopsies and HSIL on follow-up.

p16(+) Cases

CASE	AGE (YRS)	PRIOR PAP	HPV ISH	REVISED BIOPSY DIAGNOSIS	FOLLOW-UP
1	45	HSIL	+	SIL-indeterminate grade	HSIL
2	34	ASC-H	-	SIL-indeterminate grade	HSIL
3	39	ASC-H	-	SIL-indeterminate grade	Negative
4	25	HSIL	+	HSIL (CIN2)	HSIL
5	39	ASC-H	-	LSIL	Negative
6	33	ASC-H	-	LSIL	Negative
7	43	ASC-H	-	No change	HSIL

Conclusions: p16 immunostain does increase the detection rate of SIL in benign appearing cervical biopsies from patients with a prior high-risk Pap test result. The benefit is most apparent in cases with a prior Pap test diagnosis of HSIL where p16(+) cases correlated with positive HPV ISH and HSIL on follow-up. Fewer significant lesions were detected when evaluating cases with a prior Pap test diagnosis of ASC-H and less than 50% of p16(+) cases had HSIL on follow-up.

1238 Morphologic Predictors of Early Complete Mole: A Comparison of Histology, p57 and Ploidy

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Background: Complete hydatidiform mole is followed by persistent gestational trophoblastic disease in up to 20% cases and about 50% of choriocarcinomas arise in women with a previous history of complete mole. The risk of such sequelae is <5% following a partial mole which makes it critical to distinguish between the two. With use of effective chemotherapy and highly sensitive b-HCG assays; these patients have a high chance of cure. However with high resolution ultrasound; molar pregnancies are being diagnosed earlier which poses a diagnostic challenge as these do not have the classic histology that is seen in later gestation. Aim of this study was to identify key histological features associated with early complete mole vs other morphologically abnormal gestations.

Design: We retrospectively evaluated 82 first trimester curettage specimens which were previously classified into complete (n=20) and partial or nonmolar hydropic gestation (n=62) based on the results of p57 immunostain and DNA ploidy study by either flow-cytometry or FISH on paraffin embedded tissue. Each specimen was evaluated without prior knowledge of final diagnosis for following features: uniform villous population; short bulbous polypoid terminal villi; trophoblast inclusions; circumferential trophoblastic proliferation; trophoblast atypia; stromal hypercellularity, cisterns, basophilia, karyorrhexis, canalicular network and atypia; absence of fetal nucleated red cells in the villous capillaries and detached sheets of trophoblasts. Results were analysed for sensitivity (SN) and specificity (SP).

Results: SN and SP results for each feature were as follows: uniform villous population (SN, 90%; SP, 70%); short polypoid terminal villi (95%; 32%); trophoblast inclusions (45%; 61%); circumferential trophoblastic proliferation (100%; 11%); trophoblast atypia (100%; 24%); stromal hypercellularity (75%; 55%); cisterns (90%; 42%); stromal basophilia (65%; 73%) stromal karyorrhexis (95%; 82%); stromal atypia (60%; 69%); absence of fetal nucleated red cells (100%; 34%); stromal canalicular network (100%; 26%); detached sheets of trophoblasts (75%; 60%).

Conclusions: Stromal karyorrhexis, uniform villous population, detached sheets of trophoblasts and stromal hypercellularity were strongly associated with complete mole with highest SN and SP. None of the complete moles had nucleated fetal red cells within the villous capillaries. Stromal cisterns, basophilia and atypia demonstrated a good SP. Using a set of these histological criteria will definitely allow an improved diagnostic yield in detecting early complete mole.

1239 p53, WT1, and PAX8 Immunohistochemical Profiles in Ovarian Malignant Mesodermal Mixed Tumors

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Background: Ovarian malignant mesodermal mixed tumors (MMMT) are rare but aggressive malignancies. Their immunohistochemical profiles and molecular characteristics are poorly characterized.

Design: 20 cases of MMMT (18 primary resections and 2 completion resections) from 2000-2012 were identified through our pathology database. All available slides were reviewed by two gynecologic pathologists. 1-2 representative slides with both carcinomatous (CC) and sarcomatous (SC) components were selected from each case. Nuclear stainings of three markers were scored semi-quantitatively and separately for CC and SC as follows: p53: null(n) (0%), wild(w) (1-75%), positive(p) (>75%); WT1: 0(0-5%), 1(6-50%), 2(>50%); PAX8: 0(0%), 1(1-25%), 2(26-50%), 3(>50%).

Results: The mean patient age was 65. 18/20 (90%) cases presented with stage III disease. 7/20 (35%) had neoadjuvant therapy. The CC in 16/19 cases (84.2%) were serous, 2/19 (10.5%) cases were endometrioid, 1/19 (5.3%) case was mixed serous and endometrioid. One completion oophorectomy had no CC remaining. The SC of 14/20 (70%) cases contained heterologous elements (7 chondrosarcoma, 6 rhabdomyosarcoma, and 1 with both elements). 6/20 (30%) cases had only homologous elements. Most cases showed concordant over-expression or null phenotype for p53 in both components (Table 1). One case with endometrioid CC showed wild-type p53 in CC, but overexpression in SC. The case with no residual CC was p53 wild-type in SC. 12/16 (75%) cases with serous CC demonstrated diffuse (>50%) WT1 staining. The cases of mixed and endometrioid CC were negative for WT1. None of the SC had >10% WT1 nuclear staining. However, 19/20 (95.0%) of the SC had strong cytoplasmic WT1. Diffuse (>50%) PAX8 was seen in CC in 78.9% of the cases, including 2 endometrioid type, but not in SC. Staining profiles between cases with heterologous and homologous elements were similar.

Table 1: Immunohistochemical profile of 20 ovarian MMMT cases

COMPONENT	p53	WT1	PAX8
CARCINOMA (n=19)	n= 8 (42.1%)	0= 5 (26.3%)	0 = 2 (10.5%)
	w= 1 (5.3%)	1= 2 (10.5%)	1 = 1 (5.3%)
	p = 10 (52.6%)	2 = 12 (63.1%)	2 = 1 (5.3%)
			3 = 15 (78.9%)
SARCOMA (n=20)	n = 8 (40.0%)	0 = 16 (80.0%)	0 = 11 (55.0%)
	w = 1 (5.0%)	1 = 4 (20.0%)	1 = 7 (35.0%)
	p = 11 (55.0%)	2 = 0 (0.0%)	2 = 2 (10.0%)
			3 = 0 (0.0%)

Conclusions: The majority of CC display histologic and immunohistologic features similar to high-grade ovarian serous carcinoma. In most cases the SC shows concordant p53 staining with the CC but the WT1 and PAX8 staining are altered. This may suggest a common origin of carcinogenesis with high-grade serous carcinoma with divergent differentiation into sarcomatous elements.

1240 Placental Pathology in IUGR Infants: An 8 Year Overview

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Background: Infants with intrauterine growth restriction (IUGR) are at an increased risk of perinatal disease, including death. Placental disease is an important cause of IUGR, and gross and microscopic examination of the placenta is critical in explaining such cases. IUGR is variably defined as less than the 10th, 5th or 3rd centiles: use of the smaller centiles means that fewer constitutionally small but normal infants are likely to be included.

Design: Reports of placentas of infants with a birth weight <2 Standard Deviations from the mean (approx. 3rd centile) born between January 1st 2004 and December 31st 2011 were evaluated. Exclusion criteria were multiple gestation, congenital anomaly and a gestational age <24 weeks. Cases with autopsies were included. The primary pathology was determined in each case and assigned a category 1-8 as given in the table below. Where two or more pathologic findings were present, they were ranked as primary or secondary pathology in terms of severity of disease. Data for acute pathologies eg. acute chorioamnionitis was not included.

Results: There were 69,439 deliveries over the study period. 461 IUGR cases were identified. 21 were excluded as above. No placentas were available in 44 cases, leaving a study group of 396 cases. In 380 cases a full macroscopic examination with optimal sampling was available, in 16 a delivery ward sample was reviewed.

Placental Pathology by Category

Group	Finding	1°Pathology(%)	2°Pathology
1	Shallow implantation/ ischaemia	148 (37.4)	15
2	Fetal Thrombotic Vasculopathy	29 (7.3)	26
3a	Villitis, low grade	31 (7.8)	16
3b	Villitis, high grade	38 (9.6)	12
4	Increased Perivillous fibrin w/without intervillositis	20 (5.1)	6
5	Delayed maturation	62 (15.6)	13
6	Small Normal	23 (5.8)	
7	Normal	40 (10.1)	
8	Other	5 (1.3)	
Total		396	88

Table 1.

Conclusions: With an active triage system 90% of placentas of interest were reported. Pathology potentially causing or contributing to IUGR was identified in 88% of cases. Significant dual pathology was identified in 88 (22%) of cases. Placental examination provides key information in understanding IUGR.

1241 Reproducibility and Cancer Risk Stratification of Endometrial Samplings Utilizing World Health Organization and Endometrial Intraepithelial Neoplasia Classifications

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Background: Endometrial carcinoma is the most common malignant tumor in the female genital tract. Two diagnostic schemas are currently used for identifying premalignant disease. The more widely practiced World Health Organization (WHO) classification is categorized into typical hyperplasias (simple or complex hyperplasia without atypia) and atypical hyperplasias (simple or complex hyperplasia with atypia). The more recent Endometrial Intraepithelial Neoplasia (EIN) criteria include: 1) area of glands exceeds that of stroma, 2) nuclear and/or cytoplasmic features of epithelial cells differ between architecturally abnormal glands and normal background glands, 3) maximum linear dimension exceeds 1 mm, and 4) exclusion of mimics/cancer. This study examines the diagnostic reproducibility and cancer risk stratification under both systems.

Design: An institutional retrospective review of all endometrial samplings from 2008 to 2010 by the Pathology Laboratory Information System identified 40 cases with a diagnosis of hyperplasia by the WHO classification with clinical follow-up. A randomized blinded re-review of all cases was performed by two gynecologic pathologists, each was asked to categorize individual cases under the WHO (benign, simple hyperplasia with or without atypia, and complex hyperplasia with or without atypia) and EIN (benign, gland crowding, and EIN) classifications on separate occasions (1 month apart). Inter-observer agreement and risk stratification were analyzed.

Results: Clinical follow-up revealed endometrial carcinoma in 28% (n=11) of these study cases. The inter-observer agreement for EIN and WHO diagnostic schemas was 73% and 58%, respectively. The sensitivity of predicting cancer risk was 95.5% for EIN and 77.3% for WHO, and the negative predictive value was 97.7% for EIN vs. 90.9% for WHO. Under the EIN criteria, the only case that had a cancer outcome without an EIN diagnosis was categorized as gland crowding.

Conclusions: This study demonstrates that the EIN criteria have greater inter-observer reproducibility, higher sensitivity and better negative predictive value when compared to the WHO classification. In addition, it highlights that the rare cases sub-diagnostic of EIN, while having low risk of cancer occurrence, require appropriate clinical follow-up.

1242 Mechanisms of Expression and Regulation of SOX2 and Its Targets in Two Embryonal Carcinoma Cell Lines

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Background: SOX2 is a conserved pluripotency-associated transcription factor considered to be crucial for embryonal stem cell (ESC) and embryonal carcinoma cell (ECC) maintenance. In addition to its ability to autoregulate its expression, it closely regulates the expression of a wide range of genes important to 'stemness' and is also activated in various types of cancer stem cells (CSCs). MicroRNA (miRNA) are short, non-coding RNAs of approximately 23 nucleotides in length. Their most important documented capability is the regulation gene expression post-transcriptionally by preventing the translation of specific target mRNAs.

Design: We silenced SOX2 in two human ECC lines, NTera-2 and 2102Ep, which resulted in the initiation of the differentiation program of these cell lines. After three days, whole-genome and whole-miRNAome expression was measured.

Results: Hundreds of genes were differentially deregulated in both cell lines, revealing pathways directly and indirectly regulated by SOX2. Besides a large overlap in differential gene expression of NTera-2 and 2102Ep cells, large discrepancies were also found, including in the expression of core pluripotency markers, POU5F1/OCT4 and NANOG. This could possibly indicate a difference in roles SOX2 plays in these cells. Compared to previous data from a third, widely used ECC line, NCCIT, 2102Ep showed a higher overlap with this cell line than NTera-2. Whole-miRNAome analysis revealed tens of differentially regulated miRNAs in both cell lines. Overlap was found but important discrepancies between NTera-2 and 2102Ep were also recorded, with the most notable difference being the downregulation of the C19MC polycistronic miRNA cluster in 2102Ep but not in NTera-2. This cluster has been associated with cancer and also with the differentiation program of ESCs. Our data indicates that members of this cluster could target various parts of the Wnt-signalling pathways, providing a possible novel mechanism through which SOX2 regulates cancer stemness and embryonic development.

Conclusions: Our results showed that SOX2 directly and indirectly regulates hundreds of genes and tens of miRNAs in ECCs, but has distinctive roles in different ECC types. The C19MC polycistron stood out from the results and could have novel implications in the regulation of Wnt signalling. Further analysis of this data and future research could reveal what these precise roles are.

1243 Application of FNCLCC Grading to Uterine Smooth Muscle Neoplasms: Is STUMP Analogous to Low Grade Soft Tissue Leiomyosarcoma?

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Background: Unlike soft tissue, uterine leiomyosarcoma (LMS) is not consistently graded. In the soft tissue, minimal mitotic activity, cytological atypia, and/or tumour

necrosis are generally indicative of malignancy. The aforementioned criteria are used in grading and staging of sarcomas, including LMS. In contrast, the International Federation of Gynecology and Obstetrics does not incorporate grading into tumor staging criteria. We investigated the prognostic value of grading uterine LMS and smooth muscle tumours of uncertain malignancy potential (STUMP) using the Federation Nationale des Centres de Lutte Contre le Cancer (FNCLCC) criteria. We also hypothesize that uterine STUMPs will have similar grade (G) as low grade LMS of soft tissue.

Design: 63 cases of LMS and 15 cases of STUMP were retrospectively reviewed by 2 gynecologic and one soft tissue pathologist. The cases were scored based on pleomorphism, mitotic activity and necrosis by FNCLCC criteria. Follow-up and survival data was collected and a Kaplan Meier curve was plotted with stratification by grade. Univariate regression was also performed to correlate grade with survival.

Results: Of LMS's, 2 were G1 (3%), 16 were G2 (25%), and 45 were G3 (71%). For the STUMPs, 13 were G1 (87%), and 2 were G2 (13%). No deaths were reported for STUMP patients, though 1 patient with a grade 1 lesion had metastases. The 5-year overall survival was 100% for G1 lesions (including both STUMPS and LMS), 86% for G2 and 18% for G3. For LMS alone, G3 lesions fared worse than G2 with 5-year survival of 18% vs. 85% (hazard ratio 4.01, p=0.02).

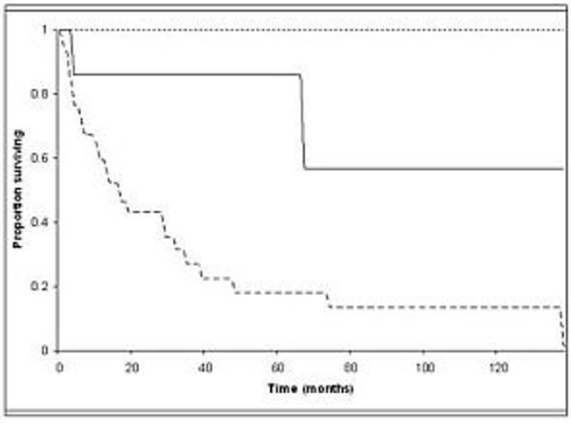

Fig 1. Overall survival rate by grade. The dotted line represents grade 1, solid grade 2, and dashed grade 3.

Conclusions: The FNCLCC criteria can be used to stratify uterine smooth muscle lesions with multiple concerning features. In this context, most uterine STUMPs correspond to a grade of 1 by FNCLCC criteria. While no deaths were reported in this category, metastasis did occur. This is similar to outcomes reported in literature for low grade non-uterine LMS. Conversely, almost all uterine LMS were grade 2 or 3; a difference in survival was noted between G 2 and 3 LMS, suggesting prognostic value in reporting FNCLCC grade.

1244 Expression of the Stem Cell Associated Transcription Factor SOX2 in Squamous Cell Carcinoma of the Vulva in Comparison to p16 and Ki-67

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Background: The high mobility group transcription factor SOX2 is essential for preservation of embryonic stem cell pluripotency and self-renewal of tissue specific adult stem cells. SOX2 has recently been identified as a novel major oncogene that plays a role in squamous cell carcinomas (SCCs). SOX2 has been shown to be recurrently amplified and activated in SCCs of the lung, esophagus, and oral cavity. Approximately 95% of vulvar malignancies are SCCs. Two distinct pathways of carcinogenesis in the vulva have been proposed: one related to infection with high-risk human papilloma virus (HPV), and the other independent of HPV, related to chronic inflammatory and granulomatous disorders of the vulva. The role of SOX2 expression in vulvar SCC is unknown. In this study, we compared the expression of SOX2 in vulvar SCC to the expression of the HPV-surrogate p16 and the proliferation marker Ki-67.

Design: A tissue microarray of invasive vulvar SCCs was constructed with tissue cores from paraffin embedded material of 24 patients. Normal vulva tissue was used as control. The tissue microarrays were examined using immunohistochemistry for the expression and localization of SOX2, p16, and Ki-67. Expression of SOX2 and p16 was scored by intensity (weak, moderate, strong). Ki-67 was scored by expression in percentage of cells (low: 0-29%; medium: 30-59%; high: 60-100%).

Results: In normal vulva, moderate SOX2 expression was confined to the basal one-third cell layers of the epithelium. Moderate to strong diffuse SOX2 expression was present in 15 of 17 cases (88%) of invasive SCC of the vulva. The two cases that were negative for SOX2 expression did express p16 with moderate and high intensity. Eleven of 17 (65%) cases of invasive SCC expressed p16 with moderate or strong intensity. The 6 cases that were devoid of p16 staining did however express SOX2. The proliferation marker Ki-67 demonstrated a high proliferation rate (>60%) in all cases (100%). Seven cases of vulvar SCC were lost during processing of the tissue microarray and could not be evaluated.

Conclusions: These findings suggest that, like SCC in the lung, reactivation of SOX2 is involved in the progression of vulvar SCC. In concordance with the currently proposed model of two distinct pathways of vulvar carcinogenesis, 35% of vulvar SCC cases in our study did not express the HPV-surrogate p16 and seem to be unrelated to an HPV-dependent pathway. All of these cases however did express SOX2, as did the majority of cases that also expressed p16, suggesting that SOX2 plays a role in both pathways.

1245 The Stem Cell Associated Transcription Factor SOX2 as a Diagnostic Marker of Cervical Neoplasia in Comparison to p16 and Ki-67

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Background: SOX2, a high mobility group transcription factor, is essential for preservation of embryonic stem cell pluripotency and self-renewal of tissue specific adult stem cells. *SOX2* has been described as a novel major oncogene that plays a role in squamous cell carcinomas (SCCs), including cervical carcinomas. A cancer stem-like population from primary cervical carcinomas was recently identified and characterized via RT-PCR, showing the expression of adult stemness-related genes, including *SOX2*. In this study, we evaluated the expression and diagnostic utility of SOX2 in cervical intraepithelial neoplasia (CIN) and invasive carcinoma, compared to the more commonly used proliferation marker Ki-67, and the HPV-surrogate p16.

Design: Tissue microarrays were constructed from paraffin embedded tissue of 63 cases. Included were cases of CIN I (n=19), CIN II (n=14), CIN III (n=8), invasive SCC of the cervix (n=18), and normal cervix as control (n=4). The tissue microarrays were examined using immunohistochemistry for the expression and localization of SOX2, p16, and Ki-67. Expression of SOX2 and p16 was scored by distribution pattern (basal 1/3, basal 2/3, full thickness) and intensity (weak, moderate, strong). Expression of Ki-67 was scored by localization pattern (basal 1/3, basal 2/3, full thickness) and percentage of cells (low: 0-29%; medium: 30-59%; high: 60-100%).

Results: In normal cervix, SOX2 and Ki-67 expression were confined to the basal 1/3 of the epithelium, and p16 was negative.

Cervical dysplasia and carcinoma

	CIN I	CIN II	CIN III	SCC
SOX2 neg	2/19	0/14	0/8	1/18
SOX2 1/3	14/19	1/14	0/8	NA
SOX2 2/3	3/19	12/14	6/8	NA
SOX2 full	0/19	1/14	2/8	17/18 (moderate, strong)
p16 neg	12/19	1/14	0/8	2/18
p16 1/3	5/19	1/14	0/8	NA
p16 2/3	1/19	6/14	0/8	NA
p16 full	1/19	4/14	8/8	16/18
p16 upper 1/3	0/19	2/14	0/8	NA
Ki-67 1/3	17/19	4/14	0/8	NA
Ki-67 2/3	2/19	7/14	2/8	NA
Ki-67 full	0/19	3/14	6/8	18/18 (moderate, high)

1/3 = basal 1/3; 2/3 = basal 2/3; full = full thickness; NA = not applicable

Conclusions: These findings support SOX2 as a diagnostic marker for the identification and classification of low and high-grade cervical dysplasia, comparable to p16 and Ki-67. SOX2 seems to be more sensitive to detect low-grade dysplasia than p16. In CIN I, p16 was negative in 63% of cases, compared to 11% being devoid of SOX2 expression. A clear advantage of SOX2 is its easily interpretable nuclear staining in contrast to the diffuse expression of p16. Similar to p16 and Ki-67, SOX2 is not a suitable marker to distinguish high-grade dysplasia from invasion.

1246 Endometrial Carcinoma with Mucinous Differentiation and *Kras* G12D Mutation Is Associated with Lymph Node Metastasis and Vaginal Recurrence

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Background: Oncogenic mutation of *Kras* gene is critical for the development of multiple malignancies. Our previous studies showed endometrial carcinomas with significantly mucinous differentiation are associated with higher frequency of *Kras* mutations. A recent publication suggested that mucinous histology is a risk factor for nodal metastases. The current study investigated the correlation between mucinous histology and/or *Kras* mutations to lymph node metastasis and vaginal recurrence.

Design: Pathology archives were examined for cases of ECMD; 28 cases were identified and 16 endometrioid carcinoma without mucinous differentiation (EC) were chosen as controls. Data abstraction revealed 21 cases with lymph node (LN) examination and 4 vaginal recurrences. Cases with LN examination or vaginal recurrence were analyzed by PCR for *Kras* codon 12 and 13.

Results: Of the 21 cases with LN examination, 14 had ECMD and 7 EC. 5 were positive for metastasis, all in cases of ECMD (5/14, 35%). Ten cases had *Kras* mutations detected (8 (57%) ECMD and 2 (29%) EC). There was overlap of LN metastasis and *Kras* mutation in three (60%) of the 5 ECMD cases with LN metastasis. All harbored the G12D *Kras* mutation. G12D is significantly associated with LN metastasis compared to wild type and/or other types of *Kras* mutation ($P<0.05$ and $P<0.001$, respectively).

Table 1. Lymph node positivity and Kras mutation status in ECMD and controls.

	ECMD	EC
Kras mutation	8/14	2/7
LN+	5/14	0/7

Four cases of vaginal recurrence were identified, including 3 (3/28, 11%) ECMD and 1 (1/16, 6%) EC. Among these 4 cases, *Kras* mutations were detected in 2 of the 3 (2/3, 67%) ECMD with one G12V and one G12D, and one (1/1, 100%) EC with G13D.

Table 2. Vaginal recurrence and Kras mutation status in ECMD and controls.

	ECMD	EC
Vaginal recurrence	3/28	1/16
Kras mutation	2/3	1/1

Conclusions: *Kras* G12D mutation is associated with higher frequency of LN metastasis. Our result suggests that *Kras* G12D may be associated with more aggressive biological behavior. The fact that all LN metastasis cases are associated with significant mucinous

differentiation supports recent finding of mucinous morphology associated with more LN metastasis. Recurrence of low grade EC is uncommon, however, we found that 11% of ECMD had vaginal recurrence and, of these, 67% harbored a KRAS mutation.

1247 miR-146a Expression in Cervical Squamous Cell Carcinoma

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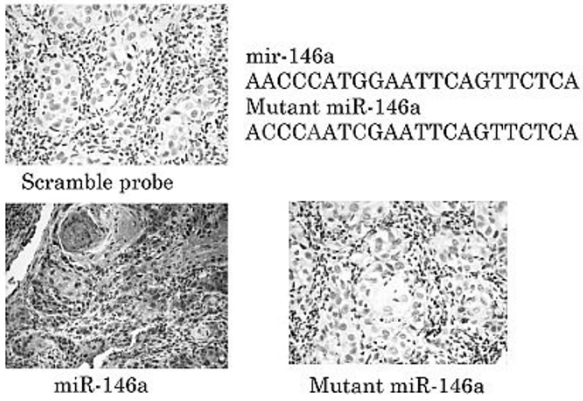
Background: MicroRNA (miRNA) are short single stranded RNA molecules that act as post-transcriptional regulators by binding to complementary sequences on target messenger RNA transcripts. miR-146a, initially characterized as playing an important role in innate immune response to microbial infection, has been implicated in recent studies in control of cell growth, differentiation and survival. Lower miR-146a expression has been associated with more extensive lymph node metastasis and venous invasion in gastric and breast carcinoma. However, the expression of miR-146a in cervical squamous cell carcinoma (SCC-Cx) and its role in prognosis is not fully established. It has been reported that miR-218 levels in patients with high-risk CIN were lower than in those with low-risk CIN. In this study, we have examined the expression of miR-146a and miR-218 in SCC-Cx.

Design: Tissue microarrays were constructed from 60 cases of SCC-Cx and 10 normal Cx biopsies. FAM-labeled scramble probe (BioGenex, PR032), miR-146a (BioGenex, HM146A), mutant miR-146a (BioGenex, HM146AM), miR-218 (BioGenex, HM218) and One-step ISH Detection Kit (BioGenex, DF400) were used in this study. Briefly, following dewaxing and rehydration, FFPE tissue slides were heated in Nucleic Acid Retrieval Solution I (NAR-I, Biogenex) for 10 min at 92C. After incubation with 40 nM of microRNA probe for 60 min at 50 C, the signal was amplified with anti-fluorescein antibody and poly-HRP detection system.

Results: The expression of miR-146a was up-regulated in 40/60 (67%) of SSC-Cx cases (see figure) and 20/60 (33%) SCC-Cx cases exhibited basal level of expression. Most cases with increased miR-146 expression had moderately to poorly differentiated morphology. miR-218 was up-regulated in 14/60 (23.3%), basal levels were seen in 34/60 (56.7%), and was down-regulated in 12/60 (20%) of cervical cancer samples.



Conclusions: SCC-Cx cases showed upregulation of miR-146a in the majority of cases and showed a trend towards increased expression in moderately to poorly differentiated SCC-Cx. miR-218 expression was decreased in most SSC-Cx cases. Larger studies to examine the prognostic implications of these two markers are warranted.

1248 Serous Tubal Intraepithelial Carcinoma: Unusual Variants Encountered in a 7 Year Experience

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Background: Tubal intraepithelial carcinoma (TIC) is a recognized predecessor of metastatic pelvic serous carcinoma and has been studied in earnest since 2005. The majority of reported cases consist of high grade serous intraepithelial carcinomas; however, variants are emerging with the use of more comprehensive protocols for analysis of tubes (SEE-FIM protocol) and study of tubes from women in various risk groups. This study reviewed all TICs coded in a large hospital practice and consultation files between 2005 and 2012, to identify unusual presentations or histologies.

Design: The pathology reports from in-house and consultation cases were reviewed for either unexpected presentations of TIC or variant histologies.

Results: 150 in-house diagnosie of TIC and 75 consultations for tubal atypias or TICs were reviewed. Three discrete categories were identified. The first was serous TICs found incidentally in women with uterine cancer (7); four were associated with endometrioid adenocarcinomas and three with early serous carcinomas diagnosed by cervical cytology. All four cases associated with endometrioid carcinoma exhibited evidence of altered p53 expression consistent with a separate primary tumor. All four were confined to the fallopian tube at the time of discovery. The second was TICs showing prominent secretory or mucinous differentiation (4). In two there was a coexisting ovarian tumor with the same immunophenotype and strong immunostaining for p53. These also tended to be low stage at the time of discovery. The third was rare early serous carcinomas with apparent altered p53 expression but a very low (less than 10%) proliferative index and a visibly lower grade tumor cell cytology, with focal ciliated differentiation in some cases. All four were discovered incidentally and were not associated with metastases.

Conclusions: The three scenarios described from this institutional and consultation experience underscore a potentially wider phenotypic range of tubal intraepithelial carcinomas and suggest that some of these unusual tumor subsets are more likely to be localized. The possibility that a unique subset of women are vulnerable to both endometrial and tubal adenocarcinomas bears further study as does the possibility that rare tumor variants with intermediate (grade 2) histology exist, with a different cell of origin and natural history.

1249 Expression of p16 in Low-Grade Squamous Intraepithelial Lesions (LSIL/CIN 1): Correlation of Immunohistochemical Staining Patterns with Analysis of Human Papillomavirus (HPV) Types

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Background: Immunohistochemical analysis of p16 expression is currently recommended to distinguish precancer (high-grade squamous intraepithelial lesion, [HSIL/CIN2 and CIN3]) and mimics of HSIL (immature squamous metaplasia, atrophy, reparative epithelial changes), with diffuse strong staining supporting a diagnosis of HSIL. P16 is also recommended when a diagnosis of HSIL/CIN 2 is being considered, with downgrading to LSIL/CIN 1 when negative/patchy and upgrading to HSIL/CIN 2 when diffuse. However, a subset of adjudicated LSIL is known to have diffuse p16 expression but the data regarding HPV typing of these lesions is lacking. This study correlates p16 expression patterns in LSILs with HPV types.

Design: One hundred ninety-nine cervical specimens were diagnosed as LSIL by 2 pathologists. Immunohistochemical staining for p16 was scored as positive (diffuse/strong band-like staining in at least the lower third of the epithelium) or negative (either absent or focal/patchy staining throughout the entire lesion). HPV typing was performed on the same tissue block.

Results: 117 (59%) LSILs were positive for p16, 48 (24%) were negative, and 34 (17%) demonstrated a mixed pattern of staining with discrete foci of positive and negative/patchy expression within the lesion. Multiple HPV types were detected in 136 (69%) cases. In one case that had no HPV detected, p16 was negative. Data are summarized in Table 1.

Table 1. Patterns of p16 expression and distribution of HPV types in LSILs

HPV types (n)	P16 expression pattern		
	Positive (%)	Negative (%)	Mixed pattern (%)
HRHPV only (107)	74 (69%)	18 (17%)*	15 (14%)
LRHPV only (11)	0 (0%)	11 (100%)	0 (0%)
HRHPV and LRHPV (47)	24 (51%)	14 (30%)	9 (19%)
HRHPV and HPV of uncertain risk (29)	17 (59%)	2 (7%)	10 (34%)
LRHPV and HPV of uncertain risk (1)	0 (0%)	1 (100%)	0 (0%)
HPV of uncertain risk only (3)	2 (67%)	1 (33%)	0 (0%)
Total (198)	117 (59%)	47 (24%)	34 (17%)

HRHPV - high-risk HPV; LRHPV - low-risk HPV; *The HRHPV types detected in this group included most commonly HPV 51, 52, 66, 68, 82; occasionally HPV 35, 39; rarely HPV 16, 18 (one case each)

Conclusions: Diffuse/strong p16 expression is observed in a significant proportion of LSILs (59%). While positive p16 expression in LSIL is strongly correlated with the presence of HRHPV (98%), a subset of p16-negative LSILs (17%) also contains HRHPV types only. However, the latter group commonly contains HRHPV types other than 16 and 18. Detailed HPV typing using in situ hybridization is required for type attribution in specimens with multiple HPV types.

1250 Distinct *GATA1* Point Mutations in Identical Twin Boys with Down Syndrome and Transient Abnormal Myelopoiesis from a Triplet Pregnancy

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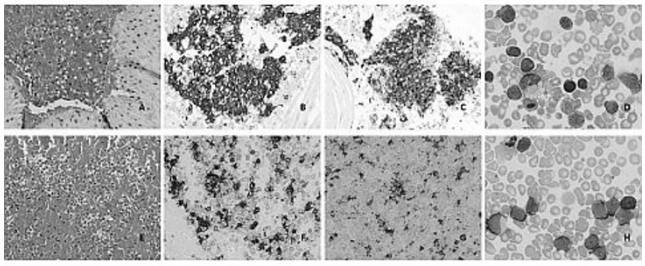
Background: Transient abnormal myelopoiesis (TAM) is a unique clonal hematologic abnormality manifested by a proliferation of myeloid blasts in peripheral blood (PB) and bone marrow (BM) in newborns with Down syndrome (DS), which is indistinguishable from acute megakaryoblastic leukemia. The hematopoietic cells in TAM are characterized by mutations in the key megakaryocyte-erythroid transcription factor, *GATA1*. The identification and characterization of DS newborns with TAM from a multiple pregnancy has not been previously described. We evaluated the clinical and pathological features of 34-week gestational age identical twin boys with DS and TAM from a triplet pregnancy.

Design: PB smears, BM aspirate smears (Triplets A and B), and placentas were evaluated. Flow cytometric immunophenotyping of PB (Triplets A and B) and immunohistochemical (IHC) stains for all placentas were performed. Karyotype was performed on triplets A, B, and C. Molecular analysis of short tandem repeat (STR) DNA profiling and DNA sequencing of *GATA1* were performed on triplets A and B.

Results: 1) Triplets A and B had a 47,XY,+21 karyotype while Triplet C had a 46,XX karyotype. **2)** Triplets A and B were monozygous twins by STR DNA profiling analysis. **3)** Triplet A had a 49C>T *GATA1* point mutation while Triplet B had a distinct 37G>T point mutation. Both point mutations result in a premature stop codon. **4)** Triplets A and B showed blasts with megakaryocytic differentiation in both the postnatal PB and placentas (Figure below) by flow and IHC staining, while Triplet C had no blasts in either the PB or placenta.

	Birth weight (g)	WBC (10^3)	PB blasts (%)	BM blasts (%)	GATA1	
					cDNA	Protein
Triplet A	2100	203	45	33	c. 49C>T	p.Q17X
Triplet B	1860	249	95	42	c. 37G>T	p.E13X

A-D: Triplet A. E-H: Triplet B. A&E: Umbilical cord, H&E; B&F: CD42b; C&G: CD61; D&H: PB.



Conclusions: This is the first report of identical DS twins developing TAM *in utero*. The identification of distinct *GATA1* point mutations in each twin indicates that these somatic mutations resulting in TAM developed independently rather than as a result of twin-twin transfusion.

1251 Primary Squamous Cell Carcinomas of Upper Genital Tract Show Immunonegativities for P16^{INK4a} and Absence of Human Papilloma Virus DNA

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Background: Squamous cell carcinoma (SCC) involving upper genital tract, including endometrium, fallopian tube and ovary, is exceedingly rare. Possible pathogenetic mechanisms are; 1) *de novo* carcinogenesis, 2) extensive squamous metaplasia with malignant transformation into squamous cell carcinoma, 3) endometrioid adenocarcinoma with predominant squamous differentiation, and 4) mucosal spread from cervical squamous cell carcinoma. Thus, before making a diagnosis of primary SCC arising in the upper genital tract, mucosally spred cervical SCC should be excluded by thorough examination of the uterine cervix. In the diagnosis on the endometrial biopsy or curettage samples, the decision of primary or metastatic SCC can be very difficult.
Design: To examine the pathogenetic role of HPV and its utility in the differential diagnosis, we compared the immunoexpression for P16^{INK4a} between the primary SCC of the upper genital tract and mucosally spred cervical SCC, and examined human papilloma virus (HPV) DNA using DNA chip. Nine cases of SCC involving upper genital tract including endometrium, fallopian tube or ovary were divided into the mucosal extension of cervical SCC (n=5), and the primary SCC arising in the upper genital tract (n=4) depending on the presence or absence of cervical SCC after thorough histological examination. Metastatic carcinoma, endometrioid adenocarcinoma and SCC arising in ovarian teratoma were carefully excluded.
Results: All SCC of cervical origin (n=5) showed strong expression of P16^{INK4a}, and three of them showed high risk HPV DNA, while primary SCC of primary endometrium and ovary were all negative for p16^{INK4a} and HPV DNA.
Conclusions: Primary SCC of upper genital tract appear to have a different pathogenetic mechanism from that of cervical SCC, and immunonegativity for P16^{INK4a} is a useful adjunct in the decision of primary or metastatic SCC of upper genital tract.

1252 Ovarian Borderline Tumors: Features Predictive of Progression

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Background: Although the majority of ovarian borderline tumors (OBT) are clinically benign, predicting their progression remains a challenge. Tumor morphology and the extent of disease may help predict progression. To evaluate the impact of various features on progression, we reviewed a group of staged ovarian borderline tumors in our files.
Design: We identified 249 patients diagnosed with OBTs over a 22 year period (1983-2005). A subset of patients with positive implants, lymph nodes, and/or cytology were selected for slide and chart review. Various features (bilaterality, microinvasion, micropapillary features and extraovarian disease) were recorded and correlated with outcome and compared to the outcome of borderline tumors lacking these features.
Results: Of 249 identified patients, 72 (age range: 15 to 84 years) were selected for review. Among the 72 OBTs, 55 were serous, 12 mucinous and 5 seromucinous. Features evaluated included extraovarian implants (n=47, 9/47 invasive), nodal involvement (n=15), microinvasion (n=12), micropapillary/cribriform pattern (n=18), appendiceal mucinous lesion/pseudomyxoma peritonei (n=10) and/or positive cytology (n=39). Sixty of the 72 patients had follow-up (1 to 22 years). Eleven women died 5 to 22 years after diagnosis and 12 had recurrences requiring surgery. Of the 19 women who died and/or had a recurrence, 4 had an appendiceal tumor or pseudomyxoma peritonei at presentation, 2 microinvasive tumor, 6 micropapillary/cribriform lesions, 4 invasive implants, 8 non-invasive implants only, and 4 nodal implants. The two patients who died with disease both had micropapillary features in addition to either microinvasion, invasive implants and/or nodal involvement. In contrast, none of the OBTs lacking these features developed recurrences or proved fatal at last follow-up (1-21 years).
Conclusions: The presence of micropapillary pattern, microinvasion, invasive implants and lymph node involvement, particularly when they co-exist, is predictive of recurrence and progression. Adjuvant therapy could be useful for lesions with invasive implants or combined features to reduce chances of progression.

1253 Neoadjuvant Radio-Chemotherapy for Uterine Cervix Carcinoma: A Regression Grading System as a New Prognostic Parameter

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Background: Concomitant radio chemotherapy represents an effective option for cervical cancer. Surgical treatment following radio-chemotherapy is still controversial. Therefore we analyzed the effectiveness of this procedure on a series of surgical specimens collected in our institution. Pathological findings and a tumor regression grading system (pR) are presented.
Design: Archival slides from the Surgical Pathology Unit of Agostino Gemelli Hospital – Catholic University were revised for the period 1996-2012 in search of Uterine Cervical Carcinoma cases after neoajuvant therapy. We identified 249 consecutive, unselected patients, which underwent radical hysterectomy with lymph nodes removal and peritoneal sampling. All the surgical specimens were routinely processed. In all the cases the whole cervix was sectioned clockwise and examined on multiple histological slides. The following tumor regression grading is proposed
- pR0: Complete Pathological Response: no invasive residual neoplastic cells are observed
- pR1: Partial Pathological Response: clusters of invasive neoplastic cells <0,3 cm of diameter
- pR2: No Pathological Response: neoplastic masses >0,3 cm.
All the cases were restaged according to pTNM and FIGO staging system.
Results: All the specimens showed variable but relevant signs of regression: 101 patients resulted pR0 (40,6%); 73 patients resulted pR1 (29,3%); 75 patients resulted pR2 (30,1%). In pR0 cases the previous tumour volume was replaced by fibrotic tissue; foamy macrophages and erosive cervicitis were more often seen (p<0,001). In pR1 cases neoplastic residual is significantly associated with regressive and reactive phenomena such as multinucleated cell and calcium deposits (p=0,009). pR2 residuals significantly infiltrate more than pR1, involving a larger percentage of the cervical wall (p<0,001), more frequently shows signs of neoplastic embolism (p=0,009), have metastatic lymph nodes (p<0,001) and positive surgical margins; all these features resulted in a higher FIGO staging. For all these reasons pR2 patient had an adverse prognosis with a worse Overall and Disease-Free Survival (p<0,001).
Conclusions: Histopathological examination of cervical carcinoma following neoadjuvant treatment represents a challenging issue: when present the residual can be scarce and deeply alterated. The proposed regression grading system could have a relevant role in characterizing prognosis of these patients and planning further treatments.

1254 Expression of p16 in CIN2-3 Lesions with Negative HPV Status

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Background: Knowledge of the HPV status often influences pathologists' decision in rendering diagnosis of cervical dysplasia. A subset of CIN2-3 lesions is found in those women with negative HPV test. P16 as a surrogate marker for HPV integation has been applied to facilitate accurate diagnosis of high grade dysplasia. To determine if there is any difference of p16 immunostaining pattern between HPV-positive and HPV-negative CIN2-3 lesions, we compared p16 immunohistochemistry in 76 cases of high grade cervical dysplasia with either HPV-negative or HPV-positive status.
Design: Thirty-six women with histopathologic diagnoses of CIN2-3 and negative HPV status were identified from our hospital archives. All women had at least one HPV testing on cervical smear collections using HCII (Qiagen) within 6 months of initial biopsy. Forty cases of CIN2-3 lesions with confirmed positive HPV status were included as control. Immunohistochemical staining was performed using p16 antibody from Vantana. P16 immunostaining patterns were evaluated as negative, focal, and diffuse pattern. Patients' clinicopathology information were reviewed and correlated with p16 immunostain.
Results: Of 76 patients diagnosed with CIN2-3, 40 patients had positive HPV and 36 patients had negative HPV status. P16 immunoreactivity was seen in 75 (98.7%) of all CIN2-3 cases, including 40 (100%) cases of HPV-positive cases and 35 (97.2%) of HPV-negative cases. Diffuse staining pattern was seen in all 40 cases of HPV-positive CIN2-3 lesions. Among cases with negative HPV status, p16 immunostaining revealed 28 (77.8%) cases with diffuse staining pattern, 7 (19.4%) cases with focal staining pattern and 1 (2.8%) case with negative p16 staining. On retrospective review, 4/7 cases with focal pattern would have been re-classified as CIN1 or condyloma and all these 4 cases had subsequent negative LEEP. The remaining 3/7 cases with focal pattern had focal CIN2 in a background of CIN1. CIN2-3 lesions were found in all subsequent LEEP specimen. The only case with negative p16 staining showed immature squamous metaplasia with a negative LEEP on follow up.
Conclusions: Expression of p16 was seen in all cases with CIN2-3 lesions, regardless of HPV status. Diffuse p16 staining pattern is the hallmark for high grade dysplasia. Our data suggest that p16 immunostain is a more sensitive tool in facilitating accurate diagnosis of CIN2-3. In our experience, when dealing with borderline and challenging cases, the pathologists are advised to be careful in rendering the diagnosis of high grade in the presence of negative or focal p16 staining pattern.

1255 Strong P16 Expression Associates with Invasion of the Vulvar Extramammary Paget's Disease

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Background: Vulvar extramammary Paget's disease (VEMPD) is a rare vulvar neoplasm which can be histopathologically mistaken as vulvar intraepithelial neoplasia (VIN). P16, as a surrogate biomarker for HPV-related squamous dysplasia, has been applied to facilitate differential diagnoses between classic VIN and other vulvar

lesions such as VEMPD. However, the expression pattern of p16 in VEMPD has not been reported. We studied the expression of p16 protein immunohistochemically in 40 cases of VEMPD.
Design: A cohort of 40 cases of VEMPD was searched and retrieved from our hospital archive. Clinicopathologic data was reviewed and tabulated. P16 immunostaining was performed on recuts of all cases. P16 immunostaining pattern was categorized as negative, focal or continuous. Focal pattern is defined as patch and discontinuous staining in less than 30% of cells with weak staining intensity. Continuous pattern is defined as diffuse staining of >90% cells of Pagetoid tumor cells with strong staining intensity.
Results: p16 expression was seen in 36/40 (90%) of VEMPD. Four cases (10%) of VEMPD showed negative p16 staining. Focal p16 staining pattern was observed in 20 (50%) cases. Continuous staining pattern was seen in 16 (40%) cases. VEMPD with dermal invasion, ranging from microinvasion to large nodular mass, was identified in 5 (12.5%) cases. Interestingly, all 5 VEMPD cases with invasion had continuous staining pattern. About 31% (5/16) of VEMPD with continuous p16 staining pattern associated with invasion. In contrast, none of the 24 VEMPD cases with either negative p16 or focal p16 staining pattern was associated with dermal invasion.
Conclusions: We have found that p16 protein is expressed in a vast majority (90%) of VEMPD. Strong and continuous p16 immunostaining pattern is associated with invasive VEMPD. Given overexpression of p16 seen in both VEMPD and usual/classic VIN, p16 immunostaining is not useful in differentiating VEMPD from usual/classic VIN. Our data indicates that p16 tumor suppressor gene is likely involved in the pathogenesis of VEMPD and a continuous pattern of p16 immunostaining may be linked to invasion.

1256 Does Ovarian Hyperthecosis Contribute to the Genesis of Endometrial Polyps, Endometrial Hyperplasia, and Endometrioid Carcinoma in Postmenopausal Women? A Clinicopathologic Study of 238 Cases

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Background: Hyperestrinism due to anovulatory cycles is associated with endometrial polyps (ENPO), hyperplasia (ENHY), and endometrioid adenocarcinoma (EMCA) in pre- and perimenopausal patients. ENPO, ENHY, and EMCA also occur in postmenopausal (PMP) patients but the cause is less clear. Hyperthecosis (HT), the presence of luteinized theca cells in the (non-follicular) ovarian stromal parenchyma, may occur to varying degrees in PMP women. HT largely produces androgens that are converted to estrone in the peripheral adipose tissues. Our aim was to determine if there was a correlation between the presence of ovarian HT and the occurrence of ENPO, ENHY, and EMCA in PMP women.
Design: We selected PMP EMCA specimens with both uterus and ovaries removed in 2011 and matched with a benign control group from 2006 to 2011. EMCA cases were subdivided into FIGO G1, FIGO G2, and FIGO G3. Benign control group was subdivided into endometrial atrophy, ENPO, and ENHY. Archival H&E slides were reviewed to identify the presence of HT. The patient's age was also recorded. We used Chi Square test to compare the frequency of HT and Variance of Analysis to compare the patient's age among different groups.
Results: Our study consisted of 238 PMP women: 108 with an EMCA diagnosis and 130 with a benign diagnosis. Within the benign cases, 71 (54.6%) had atrophic endometrium, 32 (24.6%) ENPO, and 27 (20.8%) ENHY. Among the EMCA cases, 48 (44.4%) were FIGO G1, 46 (42.6%) FIGO G2, and 14 (13.0%) FIGO G3. The frequencies of HT in patients with ENPO (46.9%), ENHY (55.6%), FIGO G1 (43.7%), FIGO G2 (54.3%), and FIGO G3 EMCA (57.1%) were each significantly higher than that in patients with atrophic endometrium (23.9%), supporting an association of HT with ENPO, ENHY, and EMCA in PMP women. No significant difference was seen in patient's age among different groups.
Conclusions: Our study indicates that HT with its resultant risk factor of hyperestrinism may contribute to the pathogenesis of ENPO, ENHY, and EMCA in PMP patients. Although some workers postulate that FIGO G3 EMCA may have a different histogenesis from its lower grade counterparts, our study suggests that EMCA of all FIGO grades may share the common risk factor of hyperestrinism.

1257 Vulvar Bartholin Gland Lesions: A Clinicopathologic Study of 111 Cases

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Background: Bartholin gland is the major vulvar vestibular gland that is normally impalpable and gradually involutes after the age of 30 years. It is histologically composed of simple tubuloalveolar glands with mucin producing alveoli, reminiscent to Cowper s glands in men and minor salivary glands, drain into a central ducts mostly lined by transitional epithelium. Other than Bartholin cyst, very few benign lesions are reported in the literature. This study reviewed the clinicopathologic features of 111 cases of Bartholin gland lesion.
Design: Pathology reports and all available slides of 111 cases of vulvar Bartholin gland lesions from a single Institution (1990-2011) were reviewed. Clinicopathologic features including age, presenting sign or symptoms, extent of surgery, lining epithelium, inflammation, and histopathologic changes of Bartholin gland were evaluated.
Results: Of the 111 patients, the mean age was 41.4 years (range 18-82). The most common presenting symptom was cystic or mass lesion. Surgery ranged from simple biopsy to resection. A cyst was present in 101 cases (91%) (intact 79, ruptured 22). A lining epithelium was identified in 93 of 111 (83.8%) cases. Acute inflammation or abscess was identified in 43 of 111 (38.7%) of the cases. The most common cystic lining epithelium was transitional (33.3%), mixed transitional and squamous (33.3%), squamous (23.7%), or mucinous (9.7%). Bartholin mucous glands were identified in 46 (41%) of cases, with 9 cases of hyperplasia and 1 case of adenoma. In addition, one case of myoepithelioma, adenoid cystic carcinoma, invasive squamous cell carcinoma, and severe squamous dysplasia extending to the Bartholin gland was identified.
Conclusions: Bartholin gland lesion commonly presents as cyst or mass in middle aged women (average of 41.4 years). The most common cystic epithelial linings are squamous, transitional or mixed type. Acute inflammation or abscess is a frequent associated finding in Bartholin cyst, which may be an important histogenetic factor for the cyst formation. Besides Bartholin cyst, other pathologic processes including rare adenoid cystic carcinoma and squamous carcinoma also occur.

Head & Neck

1258 Comparison of p63 and p40 (ΔNp63 Isoform) as Basal and Myoepithelial Markers in Salivary Gland Tumors

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Background: Immunohistochemical staining for p63, a p53 homologue, is frequently used in the clinical setting to confirm squamous, basal or myoepithelial differentiation in a variety of organ sites. There are two main isoforms of p63: TAp63 and ΔNp63, both of which are recognized by the most commonly used antibody to p63, clone 4A4. Recent studies in lung tumors have called into question the specificity of this p63 antibody for squamous differentiation and have demonstrated that an antibody directed towards only ΔNp63 isoforms (p40) has better performance in this respect. We herein performed a similar survey in salivary gland tumors to compare the performance of both p63 and p40 antibodies as markers of basal and myoepithelial differentiation.
Design: Nineteen salivary gland tumors, 15 with adjacent normal salivary tissue, were stained with p63 (BC4A4, prediluted, Biocare) and p40 (5-17, 1:1000, Calbiochem). Tumors were broadly categorized as ductal (n=7; 1 canalicular adenoma, 1 salivary duct carcinoma ex pleomorphic adenoma, 1 mammary analogue secretory carcinoma, and 4 adenocarcinomas NOS), biphasic (n=7; 1 adenoid cystic carcinoma, 2 myoepithelial carcinomas ex pleomorphic adenoma, 1 epithelial-myoepithelial carcinoma, 1 pleomorphic adenoma; 1 basal cell adenoma, and 1 basal cell adenocarcinoma), composed of ductal and myoepithelial/basal elements, purely myoepithelial (n=4; 1 myoepithelioma and 3 myoepithelial carcinomas) and one mucoepidermoid carcinoma (presumed excretory duct phenotype). Nuclear staining was considered positive.
Results: Both p63 and p40 stained the myoepithelial cells surrounding acini and intercalated ducts as well as the basal cells of the striated and larger excretory ducts. All biphasic and myoepithelial tumors showed a similar distribution of reactivity and intensity in the basal and myoepithelial cells for both p63 and p40. In the mucoepidermoid carcinoma, p63 and p40 highlighted epidermoid and intermediate cells in a similar distribution and intensity. However, 3/7 tumors regarded as ductal tumors showed focal p63 positivity, but no p40 expression. These consisted of a minimally invasive adenocarcinoma ex pleomorphic adenoma, one adenocarcinoma NOS, and one canalicular adenoma.
Conclusions: Overall, p40 has a similar distribution to p63 in both normal salivary gland and salivary gland neoplasms. However, p40 does appear to be more specific with respect to a subset of ductal tumors of the salivary gland.

1259 Reduced Expression of the Putative Tumour Suppressor Spinophilin Is an Adverse Prognostic Factor in Squamous Cell Carcinoma of the Head and Neck

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Background: Spinophilin (SPN), a multifunctional intracellular scaffold protein, has been involved in carcinogenesis of lung and other types of cancer. To date, there exist no data about the role of SPN in squamous cell carcinoma of the head and neck (SCCHNs).
Design: In the present study, we evaluated SPN expression in SCCHN tumor tissue by immunohistochemistry in 85 patients who underwent a curative tumor resection. The SPN expression was correlated with clinico-pathological characteristics and multivariate Cox proportional models were used to define its prognostic relevance.
Results: Immunoreactivity for SPN was reduced in 40 (47%) tumors and 9 (10.5%) cases showed complete loss of SPN. Kaplan Meier curve analysis demonstrated that reduced SPN expression is associated with poor survival (p=0.022, log-rank test). Multivariate COX regression analysis confirmed clinico-pathological parameters as independent prognostic factors of survival.
Conclusions: A reduced expression of SPN is frequently found in SCCHNs, which indicate an important role of SPN in the pathogenesis of SCCHNs. Based on our results SPN may represent a novel prognostic factor for predicting patient's outcome in SCCHNs.

1260 Human Papillomavirus Detection and Genotyping in Squamous Cell Carcinomas of the Tongue

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Background: Carcinoma of the tongue is the predominant cancer type of the oral cavity. Human papillomavirus (HPV) has been detected in 99% of carcinomas from the uterine cervix and in many cases of head and neck carcinomas. The carcinogenic influence of genital HPVs in head and neck carcinoma development is controversial. We designed a study that analyzed the presence of HPV in squamous cell carcinomas (SCC) of the