and miR-1257 were significantly overexpressed in sunitinib responders compared with non-responders and the fold change was 2.1, 2.3, 1.9, 1.9, 1.5 and 1.8, respectively (p values all <0.05). MiRNAs miR-9, miR-138, miR-9*, miR-376a*, miR-144* and miR-223* were significantly down-regulated in sunitinib responders, and the fold change was 0.20, 0.23, 0.21, 0.53, 0.49 and 0.44, respectively (p values all <0.036).

Conclusions: By whole genome miRNA screening, 12 miRNAs were found to have differential expression patterns between CCRCC that responded to sunitinib treatment and non-responders. Several of these miRNAs were reported in the literature to affect the maturation of immune regulatory bone marrow derived dendritic cells (miR-223) and hypoxia inducible pathways (miR-138). These findings suggest that miRNAs could be informative biomarkers for predicting response to tyrosine kinase inhibitors in patients with mCCRCC.

1080 Immunohistochemical Expression of HER2 in Urothelial Carcinoma of the Bladder (UC): Comparison of the Breast Cancer (BC) and Gastric Cancer (GC) HER2 Scoring Systems

B Zhu, X Lin, S Rohan, M Zhong, R Goyal, E Gersbach, X Yang. Northwestern University, Chicago.

Background: Immunohistochemistry (IHC) for HER2 expression is important for determining prognosis and treatment in several types of cancer. HER2 protein expression has been described in UC, ranging from 5 to 98%. The criteria for scoring HER2 IHC expression in BC and GC are well established but differ significantly. In this study, we compare HER2 IHC expression using the GC and BC scoring systems in UC of various grades and micropapillary UC (MPUC) of different stages.

Design: 20 MPUC, 57 high grade UC (HGUC) and 21 low grade UC (LGUC) were evaluated. IHC for HER2 was performed on paraffin-embedded tissues and expression was evaluated using the BC and GC scoring systems. The BC system requires at least 30% tumor cells with complete membrane staining, and is scored as 0 (absent staining), 1+ (faint intensity), 2+ (moderate intensity), and 3+ (strong intensity). The GC system requires at least 10% tumor cells with basolateral staining, and is scored as 0 (absent staining), 1+ (faint intensity), 2+ (moderate intensity), and 3+ (strong intensity).

Results: See tables.

Table1. HER2 expression in MPUC, HGUC and LGUC

Subtypes	BC System		GC System	
	Mean±SD	T test	Mean±SD	T test
MPUC (n=20)	1.65±0.81	*0.668, **0.049	2.15±1.09	*0.069, **0.002
HGUC (n=57)	1.54±1.25	^0.091	1.60±1.27	^0.063
LGUC (n=21)	1.05±1.07		1.05±1.07	

*: MPUC vs. HGUC. **: MPUC vs LGUC. ^: HGUC vs. LGUC

Table2. HER2 expression in different stages of MPUC

Stages	BC System		GC System	
	Mean±SD	T test	Mean±SD	T test
T1 (n=4)	0.75±0.5	*0.059,**0.005	1.00±0.81	*0.16, **0.003
T2 (n=8)	1.63±0.93	^0.183	1.88±1.13	^0.025
T3 (n=8)	2.13±0.35		3.00±0.00	

*:T1 vs. T2 **: T1 vs. T3. ^: T2 vs. T3

Conclusions: 1. Regardless of the scoring system used, there was a trend towards higher expression of HER2 in HGUC compared to LGUC, and in MPUC compared to both HGUC and LGUC. In addition, overexpression of HER2 is seen in higher stage MPUC. These data suggest that HER2 overexpression may be prognostically significant. 2. The GC scoring system appears to correlate better with tumor stage grade than the BC system. The true prognostic and therapeutic relevance of this finding, however, will be dependent upon further studies comparing IHC scoring systems in conjunction with gene amplification studies and the potential clinical trial of HER2-target therapy.

1081 Decreased p63 Expression Is Common in Micropapillary Urothelial Carcinoma (MPUC) and High Grade Urothelial Carcinoma (HGUC)

B Zhu, X Lin, S Rohan, M Zhong, R Goyal, E Gersbach, X Yang. Northwestern University, Chicago.

Background: In normal human tissues strong nuclear p63 protein expression is present in the basal layer of stratified squamous and transitional epithelia. Immunohistochemistry (IHC) for p63 is frequently used in clinical practice to aid in diagnosing urothelial carcinoma (UC). However, loss of p63 expression has been described in high stage UC. Micropapillary urothelial carcinoma (MPUC) is a variant of bladder cancer with an aggressive behavior that frequently presents at an advanced stage. The aim of our study was to investigate p63 IHC in MPUC in comparison to conventional UC of different grades and stages.

Design: IHC for p63 expression was performed on paraffin embedded tissue sections of 23 cases of HGUC, 20 cases of low grade urothelial carcinoma (LGUC) and 20 cases of MPUC. IHC staining was scored semiquantitatively as follows: 0 (no reactivity); 1+ (< 10% cells labeling); 2+ (10 – 50% cells labeling); 3+ (50 – 75% positive); 4+ (75– 90% positive); and 5+ (> 90% positive). Only nuclear labeling was considered positive.

Results: p63 expression was identified in non-neoplastic surface urothelium in all MPUC cases, however, the MPUC tumor cells were negative for p63 (Table 1). 22% of high stage (pT2 or pT3) HGUC were also negative for p63. Additionally, 22% of pT2 or pT3 HGUC showed only 1+ labeling. In contrast, 60% of low stage (pTis or pT1) HGUC exhibited more than 3+ labeling. Finally, 70% of LGUC exhibited 5+ (more than 90% tumor cells) labeling for p63.

Table1. P63 staining in MPUC and HGUC

P63 staining	MPUC (n = 20)	Conventional UC (n = 23)
positive	0	19
negative	20	4

Table 2. P63 staining in HGUC

% of tumor cells	pTis (n = 2)	pT1 (n = 3)	pT2 (n = 15)	pT3 (n = 3)	Total (n = 23)	percentage
negative	0	0	4	0	4	17%
< 10% positive	0	1	3	1	5	21%
10-50% positive	0	1	3	1	5	21%
50-70% positive	0	1	3	0	4	17%
75-90% positive	1	0	1	1	3	13%
> 90% positive	1	0	1	0	2	9%

Conclusions: Our study indicates that p63 IHC is negative in the majority of MPUC cases. Additionally, 44% of high stage (≥ pT2) HGUC exhibited no more than focal (<10% of cells) labeling for p63. All of our LGUCs were positive for p63. Our findings suggest that decreased p63 expression in UC is more common in tumors with aggressive features. Future studies evaluating the utility of p63 as a prognostic marker in UC are warranted.

1082 Micropapillary Urothelial Carcinoma: A Clinicopathological Study of the Experience of One Academic Center

B Zhu, X Lin, S Rohan, M Zhong, R Goyal, E Gersbach, X Yang. Northwestern University, Chicago.

Background: Micropapillary variant of urothelial carcinoma (MPUC) is a rare UC variant. MPUC have been reported in the urinary bladder, ureter and renal pelvis. Its prognosis and treatment is controversial in the literature. Some studies showed the overall prognosis for MPUC is poor and suggest the early treatment with cystectomy. Whereas one study demonstrated that there is no considerable difference between micropapillary pattern positive and negative groups according to the progression rates in non-muscle-invasive and muscle-invasive groups. In this study, we tried to study the clinicopathological correlation of MPUC, particularly comparison to conventional urothelial carcinoma with same grades and same stages.

Design: 23 MPUC, 135 low grade (LG) and 92 high grade (HG) UC are retrieved. The tumor stage, lymphatic invasion and metastasis including lymph node and distant organs are evaluated. Clinical follow-up is up to 8 years.

Results: See tables.

Table 1. The tumor stage of UC

	Non-invasive	Stage T1	Stage T2	Stage ≥ T3	Median
MPUC (n=23)	1 (4%)	6 (26%)	8 (35%)	8 (35%)	T2/≥ T3
HGUC (n=89)	21 (23%)	25 (27%)	27 (29%)	16 (17%)	T2
LGUC (n=135)	132 (98%)	3 (2%)	0 (0%)	0 (0%)	Non-invasive
Total (N = 247)	155 (62%)	33 (13%)	35 (14%)	24 (10%)	Non-invasive

Table 2. Lymphovascular invasion and distant metastasis of UC

		Lmphovascular Invasion	P Value	Distant Metastasis	P Value
Total	MPUC (n=23)	14 (61%)	< 0.001	12 (52%)	< 0.001
	HGUC (n=89)	8 (9%)		6 (7%)	
T1	MPUC (n=6)	2 (33%)	< 0.001	1 (17%)	< 0.001
	HGUC (n=25)	0 (0%)		0 (0%)	
T2	MPUC (n=8)	5 (63%)	< 0.001	6 (75%)	< 0.001
	HGUC (n=27)	1 (4%)		3 (11%)	
≥ T3	MPUC (n=8)	8 (100%)	0.023	6 (75%)	0.004
	HGUC (n=16)	7 (44%)		3 (19%)	

Fisher exact test.

Conclusions: Micropapillary urothelial carcinoma tends to invade deeper (higher stage), and associated with significantly higher incidence of lymphovascular invasion and distant metastasis than conventional high grade urothelial carcinoma, indicating that this variant should be recognized and reported to facilitate studies to understand its molecular mechanism and develop better treatment.

Gynecologic & Obstetrics

1083 Adequacy of Lymphadenectomy in Endometrial Cancer: A Threshold Change Is Needed

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Background: Adequate pelvic lymph node (PLN) dissection has been arbitrarily determined, by some authors, as yielding >10 LNs and paraaortic (PALN) dissection as yielding >5LNs. Others have suggested that thorough PLN and PALN dissection should yield a median of 35 and 17 LNs respectively. Recent studies from a large institution have shown that the median yield is 11 for PLN and 6 for PALN in completely submitted tissue. The aim of this study is to assess if this was consistent among a cohort of cases in which LNs and adipose tissue were entirely submitted for histologic evaluation.

Design: We reviewed endometrial carcinomas (EC) from 2007 to present, in which pelvic and/or para-aortic lymph node sampling was performed, including the submission of remaining adipose tissue in entirety, if present. The histologic subtype, FIGO grade and pathologic stage were evaluated for all cases. Total number of LNs in entirely submitted cases as well as presence of additional LNs when the remaining adipose tissue was submitted was recorded. The presence of metastasis if any, in LNs in remaining adipose tissue was determined.

Results: 45 EC with entirely submitted LNs were identified. The patients' age ranged from 34-83 yrs (median 64yrs) and tumor size from 1.0 -8.0cm (median 7.25cm). 32 were endometrioid carcinomas, and 13 were non-endometrioid carcinomas (2 serous carcinomas, 9 mixed carcinomas, 1 clear cell carcinoma and 1 sarcomatoid carcinoma). 29 were FIGO grade 1-2 and 16 were grade 3. Pathologic stage was as follows: 31 pT1, 8 pT2, and 6 pT3. Total number of PLN ranged from 0-22 (median 5) and PALN 1-11 (median 4). Remainder of adipose tissue was submitted in 1-9 cassettes (median 2). Additional LNs were identified 21/45 (47%) cases in which remainder of adipose tissue

was submitted and numbers ranged from 1-13 (median 3) for PLN and 1-7 (median 2) for PALN. Size of additional LNs ranged from 0.1-1.3 cm (median 0.3 cm). No metastatic carcinoma was identified in any of the additional LNs.
Conclusions: The median number of LNs obtained after complete submission of LNs and remainder of adipose tissue is 5 for PLN and 4 for PALN. Additional LNs were identified in less than half the cases and were subcentimeter in all but one case. The one case in which a large LN was not grossly identified was likely prosector dependent. No metastatic disease was identified in additional LNs detected in the remainder of adipose tissue. Given that these results are from 2 different institutions the expectation for "adequate" pelvic and paraaortic LN dissection must be re-examined.

1084 Prevalence of Lynch Syndrome among Unselected Endometrial Cancer Patients

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Background: Endometrial cancer (EC) is the most common cancer in women with Lynch syndrome (LS). The purpose of this study is to determine the frequency of Lynch syndrome among unselected incident cases of endometrial cancer.
Design: A total number of 178 consecutively and newly diagnosed EC patients were included in this study. Recruited patients were diagnosed and treated at the University Hospital of Alicante between years 2004-2009. All tumors were tested for microsatellite instability (MSI), immunohistochemistry of MLH1, MSH2, MSH6 and PMS2 (MisMatch Repair proteins) and *BRAF V600E* mutation. Methylation analysis of *MLH1* gene promoter was performed in cases with loss of MLH1 expression. Germline mutation analysis of MMR genes was performed in all cases with molecular or immunohistochemistry alteration.
Results: Loss of MMR protein expression and/or MSI was evidenced in 70 cases (39.3%). Thirty four of these cases showed *MLH1* methytation and were considered as sporadic tumors. Therefore, the remaining 36 cases were seen as suspected LS (10 *MLH1* no methylated, 5 loss of *MSH2/MSH6*, 9 loss of *MSH6*, 1 loss of *PMS2* and 10 MSI without loss of protein expression). Discordances between IHC and MSI were found in 25 cases (35.7%). None of the cases had *BRAF* mutation. Germline mutation analysis was completed in 17 out of 36 suspected cases. Patogenic germline mutation was found in 7 patients (1 in *MLH1*, 3 in *MSH2*, 2 in *MSH6* and 1 in *PMS2*).
Conclusions: Our results show that at least 3.9% (7/178) of endometrial cancer patients have LS and the predicted frequency might be over 8% in Spanish population. Early LS diagnosis followed by intense cancer surveillance and/or prophylactic surgery can prevent morbidity and mortality from LS cancers. Further efforts focused on the increase of LS diagnosis are compulsory.

1085 STAT3 and the Immune Response in CIN and Invasive Squamous Cell Carcinoma

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Background: The STAT3 pathway has been shown to be activated in HPV-associated pathology of the uterine cervix. Because STAT3 is transcriptionallyinvolved in the activation of T regulatory cells and myeloid derived suppressor cells, we hypothesized that it could reduce both antiviral and antitumoral immune surveillances in cervical intraepithelial neoplasia (CIN) and invasive squamous cell carcinoma (SCC).
Design: The study population comprised 6 invasive SCC of the uterine cervix, 10 CIN cases, and non-neoplastic contiguous squamous mucosa from 7 cases. Immunohistochemical staining was performed for: signal transducer and activator of transcription (STAT) 3, phosphorylated on tyrosine 705; CD8, cytotoxic T lymphocytes; FoxP3, T regulatory cells. The expression levels of p-STAT3 in the epithelial cells were graded on a 0 to 3 + scale. Numerical counts of the CD8+, FoxP3+ and nuclear p-STAT3 + lymphocytes were carried out in the intratumoral and stromal compartments. Statistical analysis of the data involved ANOVA.
Results: p-STAT3 was overexpressed in the nuclei of the CIN and SCC cells at 1 to 3+ in from 60 to 100% of tumoral nuclei vis-a'-vis the non-neoplastic mucosal epithelium (1+ involving primarily the basal cell layer). This coincided with the statistically significant increase ($p<0.05$) in the numbers of CD8+, FoxP3+ and p-STAT3 + lymphocytes in the intratumoral epithelium and stroma of the CIN and SCC cases. These are illustrated below:

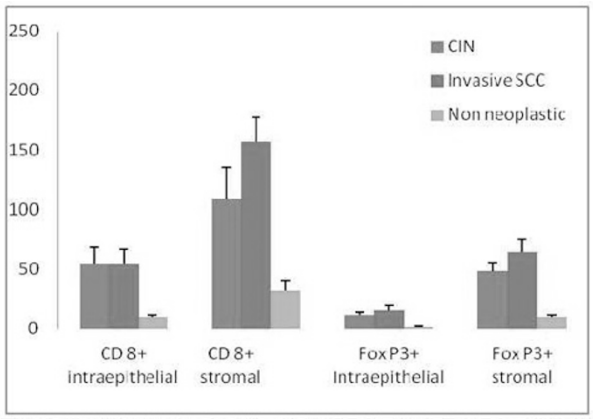

Fig 1 illustrates the increase in CD8+ and FoxP3+ cells in the CIN and SCC vis-à-vis the non-neoplastic mucosa.

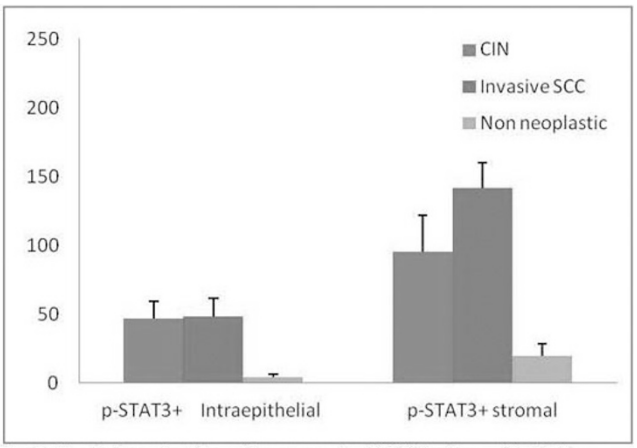

Fig 2 illustrates significant increase ($p<0.05$) in the p-STAT3 + lymphocytes in the CIN and SCC cases compared to non-neoplastic mucosa

Conclusions: This study confirms the overexpression and constitutive activation of the STAT3 pathway in CIN and SCC of the uterine cervix with a concomitant increase in the T regulatory (FoxP3+) and p-STAT3 + lymphocytes (the latter to include myeloid derived suppressor cells). This coincides with the scientific literature in supporting a role for the STAT3 pathway in downregulating host immune surveillance in CIN and SCC and identifies the STAT3 pathway as a therapeutic target in this disease.

1086 ARID1A Gene Expression in High-Grade Endometrial Carcinomas

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Background: ARID1A is a putative tumour suppressor gene in endometriosis-associated ovarian carcinomas. Its protein product, BAF250a, is a key component of the multi-protein SWI/SNF chromatin remodelling complex, related to multiple cellular functions including DNA repair. Loss of BAF250a expression has recently been described in 96 of 332 endometrial carcinomas of different types. In this study, we analyze expression of BAF250a in high-grade endometrial carcinomas, and correlate it with patients' clinical parameters.
Design: Annotated double-cored tissue microarrays of 216 primary endometrial carcinoma cases were immunohistochemically stained for ARID1A gene product using BAF250a mouse clone 3H2 antibody (Abgent, San Diego, CA, USA). Statistical analyses were performed using IBM SPSS Statistics v.19.0.0.
Results: Tumour was present in 210 of 216 stained cases, of which 61 cases (29.01%) showed loss of BAF250a expression, including 47.42% high-grade endometrioid carcinomas (n=46/97), 10.3% serous carcinomas (n=10/97), and 31.3% uterine clear cell carcinomas (n=5/16). Mean age of patients with BAF250a expression loss was slightly lower than that of patients with retained protein expression (62.36 ±11.68 vs. 68.64±9.88 years; $p<0.001$). In addition, lymph node metastasis was more likely to occur in high-grade endometrioid endometrial carcinomas with BAF250a loss than those with retained expression (33.3% vs. 9.7%, respectively; p=0.027, OR=.214, 95%CI [0.051-0.9]). There was no significant correlation between BAF250a expression loss and the tumour T-stage.
Conclusions: This study confirms the loss of expression of ARID1A gene product in endometrial carcinomas in comparable proportion to what is previously reported. In addition, we show a potential correlation of gene expression loss and development of lymph node metastasis in high-grade endometrioid endometrial carcinomas.

1087 Loss of Ciliated Cells Correlates with Tumor Progression in Ovarian Low-Grade Serous Carcinoma

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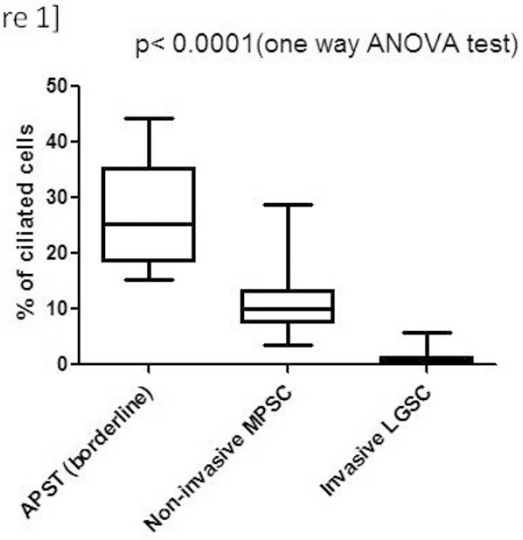
Background: Morphological and molecular studies have demonstrated that ovarian invasive LGSC develops from a non-invasive micropapillary serous carcinoma (MPSC), which develops in turn from an atypical proliferative (borderline) serous tumor (APST). Identification of morphological and molecular features associated with tumor progression in LGSC is fundamental in understanding the pathogenesis of this disease.

Design: Because ciliated cells are an integral component of APSTs, we hypothesized that the percentage of ciliated cells in different stages of tumor progression, i.e., APST, MPSC and LGSC may be reflective of this process. To facilitate the identification of ciliated cells, we performed immunohistochemistry to identify acetylated alpha-tubulin as ciliated cells are characterized by the presence of this protein. Paraffin tissues from a total of 45 serous ovarian tumors including 16 APSTs, 17 MPSCs and 12 LGSCs were analyzed and the percentage (%) of cells positive for acetylated alpha-tubulin determined.

Results: There was a progressive decrease of the percentage of acetylated alpha-tubulin positive ciliated cells from APST to MPSC then to LGSC. The average percentage of ciliated cells was 26.3 % in APSTs, 11.1% in MPSCs and 1.2% in LGSCs ($p< 0.0001$) (Figure 1). The four tumors that had concurrent APST and MPSC areas showed a decrease in the percentage of ciliated cells in the MPSC component (11.7%) as compared to the associated APST (28.0 %).

Conclusions: The findings in this study demonstrate a progressive loss of ciliated cells in the progression from APST to LGSC, and suggest that expansion of the non-ciliated cell population may be a prerequisite for malignant transformation. Additional studies are needed to investigate whether the percentage of immunohistochemically defined ciliated cells can be used as an adjunct in the differential diagnosis of different types of low-grade serous tumors.

[Figure 1]



1088 The Presence of Basement Membrane Does Not Distinguish In Situ from Invasive Adenocarcinoma of the Endocervix

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Background: Identifying areas of invasion in adenocarcinoma of the cervix can be difficult and it is crucial for deciding the type of surgery and additional treatment. Recently it has been proposed that laminin, a basement membrane glycoprotein composed of alpha, beta, and gamma heterotrimeric chains, might be able to distinguish in situ from invasive cases.

Design: 20 endocervical adenocarcinomas including areas of normal glands, in situ component (10 cases) and obvious invasive tumor (10 cases) were included in the study. 3 of the obvious invasive adenocarcinomas were gastric type which are known to be aggressive tumors. These 3 cases had lymph node mets which were included in the study. Anti-Laminin-5 (γ2 chain) (Millipore, clone D4B5), was applied on 4 micron thick sections from paraffin embedded tissue using the Ventana Benchmark Ultra instrument. Pretreatment was performed using Ventana Protease for 12 minutes at room temperature. Anti-Laminin-5 (1:100) was applied and stained with Ventana Ultraview DAB detection kit.

Results: 1) Normal Glands: The basement membrane of the normal epithelia was clearly stained by laminin-5 where the glands were separated; however, in areas with groups of glands laminin-5 was not continuous between glands.

2) Adenocarcinoma in situ: In adenocarcinoma in situ laminin-5 was clearly seen around single glands but was often not detected when several glands converged forming a tight group.

3) Invasive adenocarcinoma: In obvious invasive tumor some single glands also showed adjacent laminin staining, even in deep areas of the cervix and in lymph node mets.

Other factors that diminished the staining of laminin-5 were tangential sectioning of glands and inflammatory infiltrate between glands. In addition, 5 cases demonstrated a positive reaction in the cytoplasm of tumor cells which impeded the interpretation of basement membrane staining.

Conclusions: 1) Laminin-5 immunostain is positive around normal glands.

2) Laminin-5 can be difficult to identify in cases of tangential section, inflammatory infiltrate and when tumor cells show a positive reaction in the cytoplasm.

3) In adenocarcinoma in situ, laminin-5 is positive around single glands but it becomes difficult to see when glands converge to an area forming a group.

4) In invasive and metastatic adenocarcinoma single glands can show adjacent laminin staining demonstrating invasive and metastatic glands can produce basement membrane.

1089 Evidence Supporting Endometriosis as a Precursor of Ovarian Clear Cell and Endometrioid Carcinoma Based on Expression of ARID1A

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Background: *ARID1A* is a recently identified tumor suppressor gene involved in chromatin remodeling. Somatic inactivating mutations and loss of expression of ARID1A are most frequently detected in ovarian clear cell and endometrioid carcinomas and uterine endometrioid carcinomas.

Design: Since endometriosis is thought to be a precursor of ovarian endometrioid and clear cell carcinomas, we undertook an immunohistochemical analysis using an antibody against the ARID1A protein, comparing its expression in ovarian clear cell carcinomas (n=25), well-differentiated endometrioid carcinomas (n=16) and mixed clear cell and endometrioid carcinomas (n=4) and the associated endometriotic cysts (total 45 cases).

Results: ARID1A loss occurred in 30 (67%) of the total 45 ovarian endometriotic cysts with concurrent ovarian carcinomas. In 12 of the 45 cases, ARID1A immunoreactivity was retained in both the endometriotic cyst and the concurrent carcinoma and thus they were not informative. Of the 33 informative cases, there were 21 clear cell carcinomas, 10 endometrioid carcinomas and two mixed clear cell and endometrioid carcinomas. Among these cases ARID1A loss was demonstrated in both the endometriotic cyst and the associated carcinoma in 19/21 (90%) clear cell carcinomas, 9/10 (90%) endometrioid carcinomas and in 2/2 (100%) mixed clear cell and endometrioid carcinomas; in contrast, it was retained in the endometriotic cyst and lost in the carcinoma in the remaining cases. None of the cases demonstrated ARID1A loss in the endometriotic cyst but ARID1A retention in the associated carcinoma. Thus, loss of ARID1A staining occurred in both the endometriotic cyst and the carcinoma in 30 (91%) of 33 informative cases.

Conclusions: Our findings support the role of endometriosis as a precursor of clear cell and endometrioid carcinoma. It appears that loss of ARID1A expression (presumably due to a mutation) is an early molecular event in the development of the majority of ovarian clear cell and endometrioid carcinomas, most occurring before malignant transformation from pre-existing endometriosis and some in the transition from endometriosis to carcinoma.

1090 Tumor Size as a Prognostic Factor in Uterine Serous Carcinoma: A Large Multi-Institutional Study

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Background: Uterine serous carcinomas (USC), while constituting 10% of all endometrial carcinomas, are responsible for a high mortality rate in these patients. Tumor size although often used by our colleagues is not a well defined prognostic variable. The goal of our study is to evaluate tumor size as a prognostic parameter and its association with other clinicopathologic parameters.

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 gynecologic pathologists at each participating institution after reviewing 50 cases together to be consistent in the diagnosis. Clinical and pathologic variables including tumor size (≤ 2 versus > 2 cm), myometrial invasion (MI), lymphovascular invasion (LVI), lymph node status and FIGO stage, recurrence, outcome and survival were recorded. Pearson Chi-Square tests were used to assess potential associations and survival data were generated using the Kaplan Meier method.

Results: Patients' mean age was 67.2 years (median: 67.00 years, range 47-82 years). Survival ranged from 0-184 months with a mean and median of 42.8 and 29.9 months respectively. 155 of 236 tumors were > 2 cm while 81 were ≤ 2cm in size. Significant correlation was seen between tumor size and LVI ($p<.001$) and with FIGO stage ($p<.001$). Analyzing Stage I, although not significant, there was a trend for tumors ≤2 cm to correlate with stage IA (44/90) compared to stage IB (4/16) ($p=0.06$). Finally, a higher recurrence rate was observed in patients with tumors > 2 cm ($p=0.04$). However, no association was found with lymph node status and overall survival.

Correlation of tumor size with prognostic factors

	TUMOR SIZE ≤ 2 CM	TUMOR SIZE > 2 CM	p value
LVI	26/130 (20%)	104/130 (80%)	<.001
LN positive	13/51 (25.5%)	38/51 (74.5%)	.140
MI	46/181 (25.4%)	135/181 (74.6%)	<.001
Recurrence	15/61 (24.6%)	46/61 (75.4%)	.043
Stage I	48 (64%)	58 (38.6%)	<.001
Stage II	6 (8%)	13 (8.7%)	<.001
Stage III & IV	21 (28%)	79 (52.7%)	<.001

*Cases with incomplete data were excluded from the analyses

Conclusions: Tumor size showed significant association with prognostic variables such as LVI, MI and recurrence in USC. Based on this preliminary data, larger studies would be useful in testing the validity of this variable as an independent prognostic indicator for these tumors.

1091 The Detection of Endometrial Carcinoma Using Fluorescence In Situ Hybridization (FISH) and Routine Cytology on Endometrial Brushing Specimens

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Background: Endometrial carcinoma is the most common gynecologic malignancy in the USA; however, there is currently no screening test. A fluorescence in situ hybridization (FISH) probe set has been developed for the detection of endometrial carcinoma using endometrial brushings. The study aims were to establish thresholds for FISH positivity and to evaluate the performance of FISH and routine cytology for the detection of malignancy.

Design: Tao brush samplers (Cook OB/GYN, Spencer, IN) were utilized to collect cells from 97 hysterectomy specimens. A ThinPrep (Hologic, Bedford, MA) slide was prepared for cytology and categorized as nondiagnostic, negative, atypical, or positive for malignancy by consensus of 2 pathologists. A second slide was hybridized with FISH probes directed to 1q25, 8p11, 8q24, and 20q13. Receiver operator curves were generated to determine thresholds for FISH positivity. The hysterectomy histologic result was the gold standard.

Results: Histology, cytology and FISH results are shown in Table 1. Optimal FISH cut-off values were ≥4 cells with polysomy, ≥14 cells with gain of 1q25, and ≥10 cells with gain of 8p11, 8q24, or 20q13. The sensitivity and specificity of a positive cytology result were 57% and 100%, respectively, while including an atypical cytology diagnosis resulted in significantly (P<0.0001) increased sensitivity (95%) with decreased specificity (67%). The sensitivity of FISH was significantly higher than positive routine cytology (84% vs. 57%; P<0.0003) with similar specificity (95% vs. 100%).

Table 1: Comparison of Cytology and FISH Results by Histologic Category

	Cytology Positive (%)	Cytology Atypical + Positive (%)	FISH (%)	Cytology Positive + FISH (%)
Benign (n=39)	0 (0)	13 (33)	2 (5)	2 (5)
Complex Hyperplasia (n=3)	0 (0)	3 (100)	1 (33)	1 (33)
Grade 1 Endometrioid Carcinoma (EMC) (n=26)	10 (38)	23 (88)	20 (77)	22 (85)
Grade 2 EMC (n=12)	8 (67)	12 (100)	12 (100)	12 (100)
Grade 3 EMC (n=11)	10 (91)	11 (100)	10 (91)	10 (91)
Non-endometrioid Carcinoma (n=6)	5 (83)	6 (100)	6 (100)	6 (100)
Overall Sensitivity*	57%	95%	84%	88%
Specificity	100%	67%	95%	95%

EMC = Endometrioid Carcinoma, *Endometrial polyp and simple hyperplasia considered benign and complex hyperplasia categorized with carcinoma

Conclusions: This study suggests that FISH and routine cytology can detect endometrial carcinoma using Tao brush samplers. Additional studies are needed to validate the FISH thresholds determined in the current study and to further define a testing algorithm, such as routine cytology as a screening mechanism with reflex to FISH.

1092 The Prognostic Significance and Outcome of Patients with Grade 1 Stage 1 Endometrioid Endometrial Adenocarcinoma Involving the Lower Uterine Segment

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Background: It has been postulated that due to its proximity to the cervix involvement of the lower uterine segment by endometrial adenocarcinoma is a poor prognostic indicator of patient outcome. This study investigates whether lower uterine segment involvement (LUSI) is associated with poor prognostic histopathological features and whether it correlates with disease recurrence or decreased patient survival in women with Grade 1 Stage 1 disease.

Design: After receiving ethics approval from our institution, we studied 164 consecutive Grade 1, Stage 1 endometrial endometrioid adenocarcinomas from 2001 to 2010. Some cases included patients who underwent lymph node dissection. Our cohort was divided into two groups based on the presence or absence of LUSI and were compared with regard to prognostic factors using the Pearson χ^2 test and disease-free survival or overall survival using Cox proportional hazard models and Kaplan-Meier product-limit curves.

Results: Seventy-two cases (44%) of all Grade 1 Stage 1 cases were positive for LUSI. The average follow-up time for the LUSI and non-LUSI groups was 35 and 27 months, and the rate of nodal dissection was 24% and 30% respectively. The average age of women positive for LUSI was 58 years, and 61 years for non-LUSI. There was a significant association between LUSI and lymph-vascular invasion (p=0.04), however no significant association with deep myometrial invasion (p=0.19), and involvement of adenomyosis by adenocarcinoma (p=0.38). Both univariate (HR = 1.70; 95% CI 0.30, 9.63; p = 0.547) and multivariable analysis (HR = 1.44; 95% CI 0.23, 9.12; p = 0.698) showed that patients with and without LUSI had similar rates of disease free or overall survival.

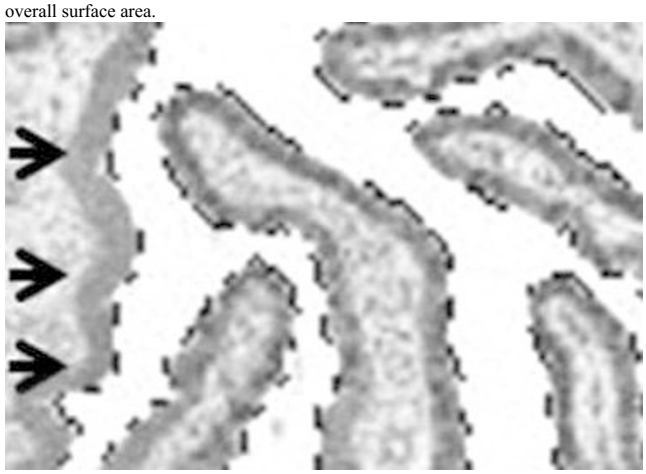
Conclusions: Lower uterine segment involvement of Grade 1, Stage 1 endometrial endometrioid adenocarcinomas is associated with higher lymph-vascular tumor invasion but not with higher recurrence rates or poorer overall patient survival. A larger cohort of patients may be necessary to conclude whether the poor prognostic histological parameters and patients outcomes are associated with the presence of LUSI.

1093 Precise Precursor Frequency Assessment by Digital Quantification of Oviductal Epithelium

JG Bijron, G Ning, AR Laury, CM Quick, NM Monte, FD McKeon, W Xian, E King, CP Crum. Brigham and Women's Hospital, Boston, MA; Harvard Medical School, Boston, MA; UCLA Medical Center, Los Angeles, CA; University of Arkansas Medical Center, Little Rock, AR; Genome Institute, A*STAR, Singapore.

Background: Presumed clonal expansions of secretory-type cells in fallopian tube epithelium include so-called secretory cell outgrowths (SCOUTs) that may be p53 positive (p53 signatures) or contain wild type p53. These entities have been associated with loss of PAX2 expression and, using tissue sections as a denominator, estimated to be more common in older women and in women with pelvic serous cancer. However, precise assessment requires determining frequency as a function of the surface area in the oviduct, which varies considerably from section to section. We have created a high throughput, low cost method for quantifying total epithelial length in microns using virtual slide images.

Design: Fallopian tube sections were selected from 35 patients with serous ovarian cancer and 35 controls. Each cross section was digitalised using an iScan brightfield scanner (BioImagene) and a scale bar was added. The images were uploaded in Adobe® Photoshop® CS3 Extended. Subsequently, pixel length was translated into microns and epithelial length measured using primarily the built in Magic Wand tool (Figure 1). PAX2-null SCOUTs were counted (arrows) and expressed as a function of overall surface area.



Results: The mean SCOUT incidence per micron was $3.88E^{-06}$ in cancer associated tubes and $1.28E^{-06}$ in control tubes (p=0.007). SCOUT incidence was significantly correlated with age, when divided in 4 age groups (36-45, 46-55, 56-65 and 66-7) the incidence increased with age in both cancer and control tubes (p=0.01).

Conclusions: The described method has verified higher SCOUT frequency in cancer-associated fallopian tubes and increases with advancing age. The described method of assessment can be used for measuring total epithelial length in a variety of tissues and is suited for both H&E and immunohistochemical staining with sufficiently high contrast. The total epithelial length can be used to more accurately assess and compare surface areas at risk and assign greater meaning to the numbers of lesions detected.

1094 PAX2-Null Secretory Cell Outgrowths (SCOUTs) in the Fallopian Tube Comprise Two Distinct Subgroups

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Background: The fallopian tube hosts precursor and precursor-like entities known as secretory cell outgrowths (SCOUTs). These SCOUTs are typically PAX2-null and have a higher frequency as a function of older age and are increased in women with serous carcinoma. Although they are described as secretory in nature, we have identified additional patterns of differentiation in SCOUTs. This report describes a study in which SCOUTs were catalogued and classified according immunophenotype.

Design: Fallopian tube sections from 23 patients were stained with PAX2 and the presence of PAX2-null SCOUTs determined. Sections containing PAX2-null SCOUTs were then immunostained with markers for both secretory (PAX8) and ciliated (tubulin) cell differentiation. Attention was paid to histopathology suggesting either differentiation pathway and immunophenotype. In addition, SCOUTs were immunostained with a set of three markers that we have been recently associated with SCOUTs, including β-catenin, LEF1 and ALDH1.

Results: Sixty-eight PAX2-null SCOUTs were identified. Of these 45 (66%) showed the presence of ciliated cells within an otherwise predominantly secretory population. Secondly, 25 (36.7%) were positive for the selected markers. Two groups of SCOUTs emerged based on differentiation and could be further subdivided according to the biomarker panel; Type I were devoid of ciliation and included Type IA (markers+/ciliation-) and Type IB (markers-/ciliation-). Type II contained ciliation and included Type IIA (markers+/ciliation+) and type IIB (markers-/ciliation+). Type II showed mixed ciliated and secretory differentiation with patterns of columnar cell shape (type IIa) and stratified, papillary shaped epithelium (type IIb). Type II SCOUTs tended to be larger, often showing a mild papillary architecture.

Conclusions: We have found two subsets of SCOUTs that exhibit both differences in differentiation and gene expression. The fact that these oviductal cell expansions differ in their capacity to generate ciliated differentiation raises the distinct possibility that they

signify perturbations of two different differentiation pathways and perhaps different cells of origin. Because genes altered in this process are often perturbed in serous cancers we propose that progressive functional gene alterations in oviductal epithelium occur with age and may reflect an accumulation of risk-associated molecular events germane to not only the oviduct but in other Mullerian epithelium.

1095 Development of Novel Endometrial Cancer Molecular Diagnostics: Assessment of a qRT-PCR Biomarker Panel of Estrogen-Induced Genes Using Formalin-Fixed, Paraffin-Embedded Tissues

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Background: Two important clinical problems for endometrial cancer provide opportunity for use of novel molecular diagnostics. One, there is on-going controversy as to which endometrial cancer patients benefit from surgical staging. Intra-operative frozen section is not universally available and is unreliable in a subset of cases. Two, the pathological distinction between endometrioid and non-endometrioid carcinoma can be difficult. p53 immunohistochemistry is not always helpful in this distinction. Using frozen tissues, we previously identified genes that are induced by estrogen in the endometrium. Estrogen exposure is associated with low grade, early stage endometrioid carcinoma. We modified the qRT-PCR assays to be applied to formalin-fixed, paraffin-embedded (FFPE) tissues. We hypothesized that assessment of this gene panel could be potentially useful for these 2 clinical problems.

Design: qRT-PCR for the estrogen-induced genes EIG121, RALDH2, sFRP4, IGF-IR, and IGF-I was performed using microdissected FFPE endometrial carcinomas derived from hysterectomies (endometrioid grade 1, n=31; grade 2, n=33; grade 3, n=35; non-endometrioid, n=72). The non-endometrioid group was composed of carcinosarcoma (n=25), pure serous carcinoma (n=4), mixed endometrioid/serous carcinoma (n=31), and undifferentiated carcinoma (n=12).

Results: EIG121 and IGF-I transcripts were significantly increased in endometrioid tumors compared to non-endometrioid. EIG121, RALDH2, and sFRP4 were significantly higher in endometrioid tumors compared to mixed endometrioid-serous tumors, implying that the mixed tumors are biologically more like pure serous carcinoma. EIG121, RALDH2, and IGF-I were significantly greater in non-myoinvasive endometrial carcinomas compared to all other stages. EIG121 and RALDH2 were significantly higher in early stage disease (Stages I and II) compared to tumors with spread outside the uterus (Stages III and IV).

Conclusions: All genes in the panel could be quantified using FFPE tissues. The panel of EIG121, IGF-I, RALDH2, and sFRP4 showed the most promise in distinguishing endometrioid from non-endometrioid histologies and early stage from late stage disease. The ultimate goal is to perform these same quantitative assays in FFPE endometrial biopsies. This will potentially allow pathologists to predict tumor stage from a routinely acquired endometrial biopsy.

1096 Differential Expression of Heart and Neural Crest Derivatives Expressed Transcript (HAND) 2 in Benign and Neoplastic Endometrium

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Background: Approximately 80% of endometrial carcinomas arise through an estrogen dependant pathway (type I carcinomas). In normal endometrium, progesterone inhibits estrogen-mediated proliferation, prevents hyperplasia, and is required for successful pregnancy implantation. Recently, expression of the progesterone induced helix-loop-helix transcription factor (Hand) 2 has been shown to suppress production of fibroblast growth factors (FGFs), the mediators of mitogenic effect of estrogen. In Hand2 knockout mice, continued expression of FGFs lead to persistent stimulation of estrogen-depended pathways and resulted in failed implantation. We hypothesize that Hand2 mediated stomal-epithelial communication also plays a role in the pathogenesis of hyperplasia and endometrial carcinoma.

Design: Archival paraffin embedded material of 62 hysterectomy specimens with a diagnosis of simple and complex hyperplasia, endometrioid, serous, mixed and clear cell carcinoma as well as carcinosarcoma were examined by IHC for expression and localization of Hand2, ER, PR, and Ki-67. Staining was graded for intensity in the stroma and epithelial component. Normal endometrium was used as control.

Results: In secretory and proliferative endometrium, Hand2 exhibits strong nuclear staining in the stroma only; whereas ER and PR show strong expression in glands and stroma. In simple and complex hyperplasia, Hand2 reveals decreased to absent expression adjacent to the abnormal glands. In endometrial carcinomas (type I and II), Hand2 is consistently absent in the stroma surrounding the carcinoma glands. Areas of normal endometrium present in some carcinoma cases showed an abrupt transition with normal stromal expression of Hand2. Comparative analysis of serial sections analyzed for stromal ER, PR, and Ki67 expression revealed consistent downregulation when compared to normal endometrium.

Conclusions: Our study suggests that HAND2 has a function in type I and II uterine carcinoma development due to dysregulated stomal-epithelial signalling. As Hand2 expression is progesterone induced, unbalanced levels of estrogen and progesterone could cause the observed absence of Hand2 adjacent to neoplastic epithelial glands. Loss of Hand2 expression may subsequently lead to lack of suppression of the FGFs, the mediators of the mitogenic effect of estrogen, and support neoplasia. Hand 2 may also explain why progesterone can be successfully used for the treatment of hyperplasia and selected endometrial adenocarcinomas.

1097 Microsatellite Instability and *K-Ras* Mutation Analysis in Tamoxifen-Associated High Grade Endometrial Cancer

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Background: Tamoxifen (TAM), a selective estrogen receptor modulator, is widely used in breast carcinoma therapy. The effects of TAM on the endometrium have been attributed to partial agonist activity of estrogen receptors; however, TAM patients are also at increased risk for developing type II endometrial carcinomas. While the mechanism of TAM induced tumorigenesis is unknown, several genetic alterations have been identified in endometrial carcinoma. *K-ras* mutations result in constitutively active signal transduction pathways and are seen in up to 30% of endometrial carcinomas. Microsatellite instability (MSI), an indicator of the functionality of DNA mismatch repair, is found in up to 40% of endometrial carcinoma. We performed *k-ras* mutation and MSI analysis on high grade endometrial tumors, associated with a history of TAM use, and compared them to a matched control group.

Design: Tumor tissue from 13 high grade endometrial carcinomas (malignant mixed mullerian tumor (MMMT)=7, serous=5, grade III endometrioid=1) arising in patients previously treated with TAM for breast cancer (mean duration of TAM treatment 6 years, range 2-12 years), and 13 controls, matched for age, tumor type, and stage, were microdissected and genomic DNA extracted. *K-ras* mutation analysis was performed via melting curve analysis. MSI was evaluated using the fluorescent multiplex PCR-based MSI Analysis System with seven consensus markers.

Results: Mutation of *k-ras* was found in 8/13 (62%) cases with a history of antecedent TAM use. Of these, MMMTs comprised the majority with five cases (72% of TAM-MMMTs). *K-ras* mutation was also found in three serous carcinomas (60% TAM-serous carcinomas). No *k-ras* mutation was found in the endometrioid carcinoma. In the control group, k-ras mutations were seen in 5/12 (42%) cases. These included four MMMTs (57% of control-MMMTs) and one serous carcinoma (25% of control serous carcinomas). One control serous carcinoma could not be analyzed.

MSI analysis of the cases with history of TAM use revealed no MSI-H tumor and one MSI-L serous carcinoma (7.7%). In the control group, one MSI-H MMMT (7.7%) and one MSI-L serous carcinoma (7.7%) were found.

Conclusions: TAM-associated high grade endometrial carcinomas revealed more *k-ras* mutations than the control group. Additionally, *k-ras* mutations segregated with the histologic subtype and were more frequently found in MMMTs. This applied to both the TAM and control group. No significant differences in MSI were observed between the TAM-associated and the sporadic tumors.

1098 Diagnosis of Partial Hydatidiform Mole: Histological Reassessment in Correlation with DNA Genotyping

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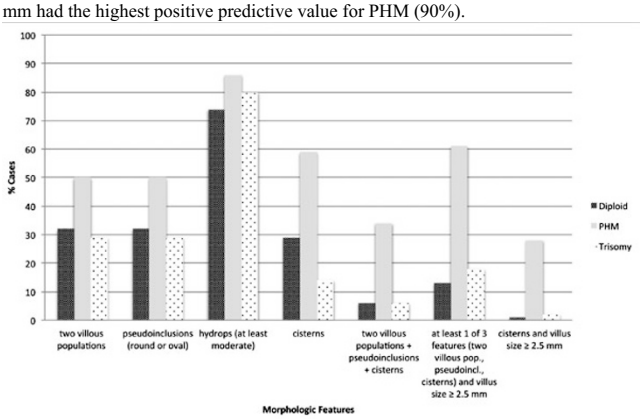
Background: Routine histological diagnosis of partial hydatidiform mole (PHM) continues to be challenging. DNA genotyping has recently become available to precisely separate PHM from its mimics, allowing an opportunity to reassess the histological criteria for the workup of PHM. We undertook a comprehensive reevaluation of histological parameters of PHM in correlation with DNA genotyping results.

Design: A total of 143 early abortion specimens (< 14 weeks gestational age) were included in the study. All cases have been subjected to short-tandem-repeat (STR) genotyping. Of the 143 cases, 60 were diagnosed as PHM, 52 had various chromosomal trisomies, and 31 cases were non-molar diploid gestations by genotyping. All available hematoxylin and eosin (H&E) stained slides were reviewed independently by two gynecologic pathologists blinded to the genotyping results, and the morphologic variables were evaluated in detail. Cases with major discrepancies in interpretation were re-reviewed by the two specialty pathologists together.

Results: Of the morphologic parameters assessed, the following emerged with diagnostic significance for PHM: villus size, presence of two villous populations, round or oval pseudoinclusions, at least moderate villous hydrops, cistern formation and trophoblastic hyperplasia. The average villus size of PHMs measured 3.2 mm, compared with 2.2 mm in trisomies and diploid abortions. The most sensitive (although non-specific) morphologic feature for PHM is villous hydrops (86%), or presence of at least one of the following three parameters: two villous populations, round or oval pseudoinclusions and cisterns (84%). The presence of cisterns and villous size equal to or larger than 2.5 mm had the highest positive predictive value for PHM (90%).



Conclusions: Confirming previous studies, significant histological overlaps exist among PHM, hydropic abortions and chromosomal trisomy syndromes. The presence of any one of the following histological findings should prompt DNA genotyping workup to

rule out PHM: round or oval pseudoincludions, cistern formation, two populations of villi and villous size of 2.5 mm or larger. The presence of both cisterns and villous size of 2.5 mm or larger has a 90% positive predictive value for PHM.

1099 Lynch Syndrome Screening Tests in Uterine Cancer Patients >50 Years Depends on Clinical and Tumor Morphology Criteria: Evidence Against Universal Testing

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Background: Controversy exists about which uterine cancer patients should undergo Lynch syndrome (LS) screening testing by mismatch repair (MMR) immunohistochemistry (IHC). Bethesda Guidelines (BG), designed for colon cancer patients, use age <50 and personal/family cancer pedigree as criteria but sensitivity is low for uterine cancer. Tumor morphology suggestive of MMR deficiency (TM-MMR) may also serve as screening criteria (tumor infiltrating or peritumoral lymphocytes, undifferentiated histology, lower uterine segment origin, concurrent ovarian tumor). Optimal integration of age, cancer pedigree and TM-MMR into an algorithm to determine who should get MMR IHC remains to be defined. Our institution has prospectively used BG and TM-MMR to trigger MMR IHC. In this algorithm uterine cancer patients without BG or TM-MMR do not get tested; such untested patients are all age >50. In this study, we retrospectively asked whether the protocol missed any abnormal MMR cases among untested patients, with the aim of determining whether universal testing should be implemented.

Design: We retrospectively performed MMR IHC on all untested uterine cancer patients age >50 from mid 2007 to mid 2011. These patients lacked BG criteria and lacked TM-MMR. IHC for MLH1, MSH2, MSH6 and PMS2 was performed using triplicate tumor samples in a tissue microarray; all abnormal results were validated by repeat IHC on whole slide sections from individual tumor blocks. MLH1 promoter methylation was evaluated for cases of MLH1 loss. Results were compared to those from the prospectively tested uterine cancer patients >50 years who had BG criteria and/or TM-MMR.

Results: Among 182 uterine cancers (89% pure endometrioid type) in patients >50 years, lacking BG and TM-MMR, only 5 (2.7%) had abnormal MMR: 4 had loss of MLH1/PMS2 (3 of 3 tested for MLH1 promoter methylation were positive) and 1 had loss of MSH6. In comparison, abnormal MMR was detected in 47% (35/74) of patients >50 years with TM-MMR and in 20% (4/21) of patients with BG criteria but lacking TM-MMR. Germline testing in 5 patients with abnormal MMR in the prospective cohort identified 1 patient with a PMS2 mutation.

Conclusions: In uterine cancer patients over 50 years, LS screening by 4 protein MMR IHC is of questionable value unless the patient fulfills Bethesda Guideline criteria or the cancer exhibits TM-MMR. The role of universal MSH6 screening in older patients deserves further study.

1100 Radial Margins Status Impact in Squamous Cervical Cancer Recurrence: A Considerable Prognostic Factor

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Background: Cervical carcinoma is the third most common tumor in females and "early stage" disease is predominantly treated by surgery.

Morphological parameters are known to be associated with prognosis. Our aim was to study radial margin status in patients(pts) with cervical cancer treated by radical hysterectomy (RH).

Design: We evaluated cervical carcinomas treated by RH at our institution between 2002-2008. From the 251 cases reviewed, we retrieved all squamous cell carcinomas (SCC) and excluded 152 cases—with previous ablative cone biopsy(119); other histological type(30) and other primary location(3).

The following clinicopathological parameters were evaluated: tumor size(TS), lymph node metastases(LNM), vascular invasion(VI), thickness of invasion of cervical wall(ICW), distance to radial(RM) and vaginal(VM) margins and follow-up(FU) data. Statistical analysis was done using Fisher exact test.

Results: 99 cases with SCC were evaluated. The FU period average was 45months(1-109). At the end of FU 81pts were alive without disease; disease recurrence (DR) occurred in 10pts having 5 died of disease and 5 alive with disease. The remaining pts were lost for FU(6) or died of other cause(2). The results of the clinicopathological parameters are presented in table 1.

Clinicopathological parameters

Parameters		with DR	without DR	p value
TS	≤40mm	6	83	
(n=99)	>40mm	4	6	0.0085
LNM	+ve	6	21	
(n=99)	-ve	4	68	0.0233
VI	+ve	7	52	
(n=99)	-ve	3	37	n sign
ICW	≤66%	1	29	
(n=90)*	>66%	7	53	n sign
RM	≤1mm	7	26	
(n=99)	>1mm	3	63	0.0146
VM	≤10mm	3	15	
(n=99)	>10mm	7	74	n sign

* not available in exophytic tumors; +ve: positive; -ve: negative n sign - not significant

DR was statistically correlated with TS>4cm(p=0.008), LNM(p=0.023) and RM≤1mm(p=0.014). No correlation was found regarding VI, ICW and VM and disease recurrence. TS and LNM were not statistically associated between themselves but RM was significantly correlated with TS(p=0.014), LNM(p=0.007) and ICW(p=0.0001). VI was associated with the presence of LNM and deep ICW (p=0.0009 and p=0.0001, respectively).

Conclusions: In our series of cervical SCC, the tumor size, lymph node metastases and distance to radial margin were significantly associated with disease recurrence. Distance to radial margin is a useful marker of disease recurrence in radical hysterectomies, however this parameter was significantly associated with TS and LN status.

1101 Levels and P16 Are Valuable Adjuncts in the Evaluation of Cervical Cone or Loop Electrosurgical Excision Procedure (LEEP) Specimens Initially Considered Negative for Dysplasia

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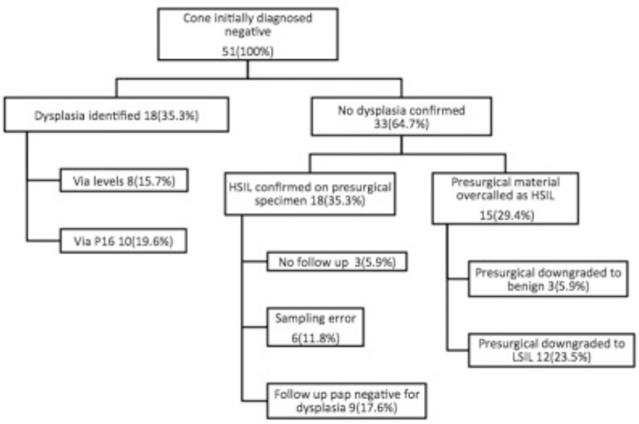
Background: At University of California San Diego Medical Center an average of 19% of the cervical cone biopsies are negative for dysplasia or malignancy. In order to identify sources of misdiagnosis and error, we evaluated the cause and outcomes of these cervical conization specimens.

Design: Fifty one cone biopsies with an original negative diagnosis were selected at UCSDMC. These specimens and the presurgical Pap smears, biopsies and endocervical curettages (ECC) were reviewed by up to 6 surgical pathologists in order to obtain a consensus diagnosis. Deeper levels and then p16 immunostains were performed on all the cone cases and select biopsy sections.

Results: Please see figure 1.



Conclusions: Our results suggest four categories of cases.

MISSED DYSPLASIA IN CONE SPECIMEN: In 35.3% of cases the original cone diagnosis was overturned to positive (SIL, LSIL or HSIL).

SAMPLING ERROR: For cases with confirmed pre-surgical diagnosis of HSIL but negative cone specimen, 6 of the original 51 cases (11.8%) were found to have dysplasia or even invasive squamous cell carcinoma on subsequent follow-up, suggesting that the conization sampling may have been incomplete due to factors that complicated the surgical procedure.

PRESURGICAL SPECIMEN OVERCALLED AS HIGH GRADE SQUAMOUS INTRAEPITHELIAL LESION (HSIL): In 29.4%, conization was not clinically indicated, due to an overcall of HSIL on the preceding Pap smear, cervical biopsy, or ECC.

PRESURGICAL MATERIAL CONFIRMED AS HSIL, WITH NEGATIVE CONE AND NEGATIVE FOLLOWUP: In 17.6% of cases, no errors were detected in either pre-surgical or conization diagnosis and the patient had negative subsequent follow-up. The implications of this category are unclear, but could result from either incomplete tissue examination, regression of the lesion, or possibly complete excision of small foci of HSIL by biopsy.

Our results, taken as a whole, emphasize the necessity for close clinical follow up, liberal use of p16 immunohistochemistry, examination of deeper level sections, review of prior material, and consensus group review to reduce errors in the pathologic workup of cervical dysplasia.

1102 Early Invasive Cervical Adenocarcinoma: Is Radical Treatment Indicated?

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Background: While it is accepted that microinvasive cervical squamous cell carcinomas can be treated conservatively, there is little published prognostic data and little agreement on the appropriate management of FIGO 1A1 and 1A2 cervical adenocarcinomas. This study focuses on the management and outcome of 55 patients with FIGO 1A1 or 1A2 cervical adenocarcinoma.

Design: The pathology database of a tertiary care hospital was searched for cases of early invasive cervical adenocarcinoma accessioned from 1985 to June 2009. A central slide review and chart review were performed. Cases with a villoglandular, serous, clear cell or neuroendocrine component were excluded. Data was previously published on 32 cases and these are included with additional follow-up data.

Results: 55 patients with early invasive cervical adenocarcinoma were identified. 50 patients (91%) had FIGO 1A1 tumors and 5 patients (9%) had FIGO 1A2 tumors. 46 (84%) of the patients had radical surgery consisting of radical hysterectomy with pelvic lymph node dissection (PLND) (44 patients) or radical trachelectomy with PLND (2 patients). 9 patients (16%) received conservative surgery consisting of simple hysterectomy (4 patients) or cervical conization (5 patients). No lymphovascular space invasion was identified, final margins were clear in all cases and no lymph node metastases were identified in the patients who underwent PLND. Follow up information was available for 54 of 55 patients with a mean follow of 7 years. 21 of 46 patients (46%) who underwent radical surgery experienced complications -- 5 cases of lymphedema (of which one case resolved), 1 case of severe peripheral neuropathy, and 16 cases of transient bladder or sexual dysfunction. In the conservatively managed patients, 1 patient (11%) experienced a post conization infection. No recurrences have been reported to date.
Conclusions: This is the largest reported case series of FIGO 1A1 and 1A2 cervical adenocarcinoma. No lymph node metastases nor recurrences were identified in this cohort of 55 patients, yet 5 of 46 patients (11%) who underwent radical surgery experienced significant long term complications. Conservative surgery should be considered in this low risk patient population.

1103 STMN1 Expression Is Associated with FIGO Grade and Presence of Cervical Involvement in Uterine Endometrioid Carcinoma

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Background: Endometrial carcinoma is the most common cancer of the female genital tract in the US. Currently, there are few molecular prognostic markers. Our previous research has shown that while PTEN and PI3KCA mutations are common in uterine endometrioid carcinoma (UEC), they do not correlate with FIGO grade. Stathmin (STMN1) regulates microtubule dynamics in cell proliferation. Its expression is associated with activation of the phosphatidylinositol 3-kinase (PI3K) pathway, and its expression has been shown to correlate with grade and other clinicopathologic features in UEC. In this study, we evaluated UEC for expression of STMN1 as a possible prognostic marker.
Design: A tissue microarray was constructed using tumor tissue from 68 cases of UEC grade 1, 66 cases of UEC grade II, and 20 cases of UEC grade III. Samples of proliferative and secretory endometrium were also included in the analysis. STMN1 expression was detected using standard immunohistochemical staining with polyclonal STMN1 antibody (#3352, Cell Signaling). STMN1 expression was scored by extent of staining (0=absent, 1=<10%, 2=10-50%, 3=>50%). Institutional IRB approval was obtained for this study.
Results: The average STMN1 staining score for grade I tumors was 0.6 (σ=0.13), grade II was 1.01 (σ=0.16), and grade III was 2.36 (σ=0.25). Tumors that invaded the cervix had an average STMN1 score of 1.5 (σ=0.13) while tumors without cervical involvement had an average STMN1 expression of 0.9 (σ=0.13), p=.025. There was no statistically significant correlation between STMN1 staining and other histological/clinical tumor attributes including depth of invasion, lymph node metastasis, or mucinous tumor phenotype.
Conclusions: In this study, we demonstrate that STMN1 expression is significantly different between FIGO grades in UEC with increased mean levels of expression in higher FIGO grades. Additionally, tumors with cervical involvement have statistically significant levels of STMN1 staining. The association between STMN1 expression and FIGO grade suggests that alterations in the PI3K pathway downstream of PI3K activation could correlate to increasingly aggressive tumor phenotype. Given the association between STMN1 expression and cervical invasion, STMN1 may serve as a molecular marker of direct local extension rather than distant metastatic spread. Future directions include examining STMN1 expression as a predictive marker of response to microtubule inhibitors in endometrial carcinoma.

1104 mTOR and STMN1 Expression Is Associated with Type I Endometrial Carcinoma

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Background: Endometrial carcinoma is the most common cancer of the female genital tract in the US. Endometrial carcinoma is broadly classified as Type 1 (low grade carcinoma with endometrioid histology with good prognosis) and Type 2 (high grade carcinoma with serous or clear cell histology with aggressive behavior), which also have different molecular genetic profiles. Our previous research has shown that Type 1 cancers have gene mutations in both PTEN and PIK3CA, however Type 2 cancers are associated with only PIK3CA gene mutations. Mammalian target of rapamycin (mTOR) and stathmin (STMN1) expression are associated with activation of the phosphoinositide 3-kinase (PI3K) pathway. In this study, we evaluated Type 1 and Type 2 endometrial carcinomas for expression of mTOR and STMN1 for use as possible prognostic markers.
Design: A tissue microarray was constructed using tumor tissue from 153 cases of Type 1 carcinoma (FIGO 1=68, FIGO 2=65, FIGO 3=20) and 34 cases of Type 2 carcinoma (serous type). Samples of proliferative and secretory endometrium were also included in the analysis. mTOR and STMN1 expression were detected using standard immunohistochemical staining with rabbit monoclonal mTOR antibody (clone 49F9, Cell Signaling) and polyclonal STMN1 antibody (#3352, Cell Signaling). Expression was scored by extent of staining (0=absent, 1=<10%, 2=10-50%, 3=>50%). Institutional IRB approval was obtained for this study.
Results: The average mTOR staining score for Type 1 tumors was 2.1(σ=0.17), while the average mTOR score for Type 2 tumors was 1.7(σ=0.10). The average STMN1 staining score for Type 1 FIGO 3 tumors was 2.4 (σ=0.3), while the average STMN1 score for Type 2 tumors was 0.94 (σ=0.24). This difference in staining score between Type 1 and Type 2 endometrial tumors was statistically significant with $p < 0.05$. Neither STMN1 nor mTOR staining scores were significantly different when samples were evaluated for other tumor characteristics including depth of invasion or lymph node involvement.
Conclusions: Type 1 endometrial carcinoma revealed higher mTOR and STMN1 staining scores than Type 2. In STMN1 staining, this difference is only seen in Type 1 FIGO grade 3 tumors; however, the association between mTOR staining and Type 1 carcinoma was independent of FIGO grade. The increased mTOR and STMN1 expression may be a reflection of underlying PTEN mutations. Further studies are necessary to address this possibility. mTOR and STMN1 may be useful molecular markers in distinguishing Type 1 versus Type 2 endometrial carcinoma regardless of other confounding factors.

1105 Do Mitotic Index and Tumor Cell Necrosis Predict Patient Outcome in Low-Grade Endometrial Stromal Sarcomas? A Study of 33 Patients

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Background: Endometrial stromal sarcomas (ESSs) were historically divided into low- and high-grade based on mitotic index (MI), with brisk MI (>10/10 HPF) being considered a poor prognostic factor. Since then, there have been conflicting studies regarding the prognostic value of MI in these tumors. The presence of tumor cell necrosis (TCN) in ESSs has also been recently reported to correlate with aggressive behavior in tumors confined to the uterus. The aim of this study is to evaluate the utility of MI and TCN in primary uterine low-grade ESSs in predicting patient outcome.
Design: We reviewed all available slides of primary uterine low-grade ESS from 33 patients with clinical follow-up from 3 institutions. MI, as determined by number of mitoses/10 HPF averaged over 100 HPF, and presence or absence of TCN and infarct-type necrosis (ITN) were recorded. Progression-free and overall survival probability based on MI, TCN, and ITN was estimated by the Kaplan-Meier method.
Results: Twenty-one patients presented with stage I tumors, while 3, 4, and 5 patients had stage II, III, and IV disease, respectively. MI ranged from <1 to 22, <1 to 5, <1 to 26, and <1 to 5 per 10 HPF for stage I, II, III, and IV tumors, respectively. Sixteen tumors, including 10 stage I ESSs, showed MI <1/10 HPF, and in this group, 3 patients (stages I, II, and IV) experienced one or more relapses, and 2 died of disease (stages I and II). Of the 17 tumors with MI ≥1/10 HPF, 6 patients relapsed (4 stage I with MI ranging from 4 to 22/10 HPF, 1 stage III, 1 stage IV), and 1 died of disease (stage I with 22 mitoses/10 HPF). ITN was present in 12 ESS (9 stage I), and 5 of them also showed juxtaposed focal TCN. Among patients with tumors exhibiting TCN, only 2 had relapses (stage II and IV, both with MI <1/10 HPF) of whom 1 died of disease. Among patients with tumors showing either ITN or TCN, only 3 relapsed of whom 2 died of disease. Progression-free and overall survival data based on MI, TCN, and TCN or ITN were not statistically significant for tumors confined to the uterus or the entire cohort.
Conclusions: Although the number of patients is limited in this study, MI, TCN, and ITN do not appear to be predictive of tumor progression or outcome in patients with low-grade ESS, including those with stage I tumors.

1106 microRNA Expression Profiling of Low-Grade Endometrial Stromal Sarcomas and Undifferentiated Endometrial Sarcomas

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Background: microRNAs (miRNAs) are small, non-coding transcripts that regulate gene expression and may contribute to tumor development, progression, and metastasis. The aim of this study is to analyze miRNA signatures in endometrial stromal sarcomas (ESSs) with and without known gene rearrangements and undifferentiated endometrial sarcomas (UESs).
Design: Real-time PCR was used to acquire genome-wide miRNA expression profiles of 9 and 10 ESSs with and without rearrangements of JAZF1, SUZ12, EPC1, or PHF1 genes, respectively, and 4 UESs. miRNA signatures were compared between all ESSs and UESs and between ESSs with and without gene rearrangement.
Results: Similar miRNA expression profiles were observed in ESSs with and without gene rearrangement. However, 3 miRNAs (miR-203, -31, and -383) were upregulated and 6 (miR-224, -331-5p, -411, -486, -671-3p, and -197) were downregulated in tumors harboring known gene rearrangements. Fifteen miRNAs (miR-139-5p, -455-5p, -22, -10b, -183, -34a, -337-5p, -452, -187, -224, -187, -224, -886-5p, -379, -126, -193a-5p, and -744) were downregulated and 3 (miR-489, -518b, and -10a) were upregulated in UESs compared to ESSs. Analysis of miRNA-target gene pathways showed that several miRNAs altered in UESs are involved in WNT, VEGFR, and EGFR signaling pathways.
Conclusions: ESSs with and without gene rearrangement have similar miRNA expression profiles. WNT, VEGFR, and EGFR signaling pathways may be implicated in UES tumorigenesis, suggesting that UESs and low-grade ESSs may be related as both share WNT and EGFR signaling pathways.

1107 Does HPV RNA Chromogenic *In Situ* Hybridization (CISH) Discriminate between Low and High Grade Cervical Squamous Intraepithelial Lesions (SIL)?

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Background: We previously demonstrated that HPV DNA *in situ* hybridization signal patterns distinguish CIN1 from CIN2/3 lesions. A diffuse intranuclear signal represents episomal virus, whilst a punctate signal represents integrated HPV DNA. This study explores a potential grading discriminatory role of a novel HPV RNA CISH assay in cervical SILs.

Design: 40 formalin-fixed, paraffin-embedded cervical biopsies were reviewed (KMC, KC) and the following diagnoses confirmed: 20 CIN1, 10 CIN2, and 10 CIN3. Samples were screened by RNAscope® CISH for high-risk HPV *E6/E7* (18 types). CISH for ubiquitin C RNA served as a positive control. The RNA probe for *Bacillus subtilis* gene dapB served as a negative control.

Results: Tissue was exhausted in 4 lesions. HPV E6/E7 RNA was detected in 100% of the CIN1 and CIN2/3 lesions. Positive staining was defined as granular cytoplasmic and/or nuclear brown staining stronger than the background signal in non-lesional tissues. Two distinct staining patterns emerged: 15/18 CIN1, 8/9 CIN2 and 2/9 CIN3 lesions showed abundant finely granular nuclear and cytoplasmic signals in the lower half to two-thirds of the epithelium, with a distinctive segregation of diffuse strong nuclear staining in the superficial third to one-half of the epithelium (type I signal pattern). One CIN2 and 7/9 of the CIN3 lesions showed abundant finely granular nuclear and cytoplasmic signals throughout the entire epithelium, with focal randomly distributed diffuse nuclear staining (type II signal pattern). The remaining 3/18 CIN1 lesions showed granular nuclear and cytoplasmic signals in the lower half of the epithelium.

Conclusions: The CIN1/2 lesions (type I pattern) confirmed the *productive* (episomal) cycle with low level viral replication (granular signal) in the lower third to one-half of the SIL, with viral genome amplification (diffuse signal) in the upper half to two-thirds supporting viral assembly and packaging of episomes. In contrast, the majority of CIN3 cells (type II pattern) poorly supported viral gene amplification, were scarce and varied in their location and distribution, consistent with the *proliferative/transformative* (integrated) cycle. The unexpected finding of the majority of CIN2 lesions aggregating with the CIN1 RNA CISH pattern raises biological questions regarding potential progression (regression vs persistence), prognostic, and management implications for CIN2.

1108 Follow-Up Outcomes in a Large Patient Cohort with HPV-Negative ASC-H Computer-Imaged Liquid-Based Cytology (LBC) Results

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Background: Adjunctive HPV testing with co-testing-based risk stratification has become increasingly common. Current guidelines recommend a colposcopy referral for women with ASC-H cytology regardless of HPV status. To the best of our knowledge, no large follow-up data has been published in women with ASC-H cytology and negative HPV test results.

Design: Patients with ASC-H ThinPrep LBC and negative HC2 HPV test results were retrieved from our database between June 2005 and December 2010. Cytology specimens were screened with computer-assistance by the ThinPrep Imaging System (TIS). Cytologic, histopathologic, and repeat HPV test follow-up results were analyzed.

Results: 1049 negative hrHPV and ASC-H LBC cases were identified. Patients without repeat cytologic testing or histopathologic follow-up results in our database were excluded from the study leaving 912 patients with an average age of 37.4 years (17-89). The average follow-up period was 29 months (1-73). Histopathologic and cytologic follow-up results are shown in Table 1. Eighteen (2.0%) patients subsequently developed CIN2/3 or cytologic HSIL. 208 (22.8%) patients developed histopathologic CIN1 or cytologic LSIL.

The average time to diagnosis for CIN2/3 cases was 10 months (range 1-40). 384 (42%) patients had a Pap test as initial follow-up with an average interval of 10 months (1-57). 470 (51.5%) patients had repeat hrHPV testing of which 45 (9.6%) had hrHPV-positive results (Table 2). An initial repeat hrHPV test was positive in 35 of 470 (7.4%) of patients. The average follow-up interval to an initial repeat hrHPV test was 16 months (1-64) and 22 months (1-62) to an initial repeat positive hrHPV test. The average number of repeat hrHPV tests was 1.5 (1-6 range).

Summary of Histologic and/or cytologic Follow-up Results

Follow-up Method(s)	Total Cases	CIN2/3/HSIL#	CIN1/LSIL
Histology	570	17(3.0)	194(34)
Cytology only	342	1(0.3)	14(4.1)
Total	912	18(2.0)	208(22.8)

Repeat hrHPV testing results

HPV testing	Cases (%)
Positive only	26 (5.5)
Negative only	425 (90.4)
Both positive and negative	19 (4.1)
Total	470 (100)

Conclusions: Our data, the largest study size to date, shows a very low incidence of CIN2/3 among women in this subgroup. Females with ASC-H Pap tests and negative hrHPV testing may be more efficiently managed by regular Pap and hrHPV testing rather than universal colposcopy. Additional long term follow-up studies are needed on patients with hrHPV testing and divergent cytology results utilizing new methods of cytology screening.

1109 YWHAE Rearrangement Identified by FISH in a Series of Undifferentiated Endometrial Stromal Sarcomas: Genetic and Pathological Correlations

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Background: Endometrial Stromal tumors (EST) of the uterus represent less than 10% of all uterine mesenchymal tumors. Undifferentiated Stromal Sarcomas (U-ESS) are aggressive malignant tumors with moderate to marked cytological atypia and little resemblance to proliferative-phase endometrial stroma. Unlike ESS, U-ESS doesn't generally harbor a JAZF1/JJAZ1 chromosomal rearrangement. Recently, a rearrangement of (WYHAE) on 17p13 with FAM 22 A-B on chromosome (Chr) arm 10q has been described in U-ESS. The aim of our study was to evaluate the frequency of WYHAE rearrangement and to look for a correlation with morphology.

Design: We collected 29 cases of U-ESS from our Department diagnosed between 1995 and 2011. At the morphological level, we distinguished the U-ESS Uniform (U-ESS-U) and pleiomorphic (U-ESS-P) types according to Kurihara S et al criteria. An immunohistochemical study including CD10, H-caldesmon and desmin was performed. *FISH analysis* using *break-apart* probes targeting the WYHAE gene on chr17p13 and JAZF1gene on chr7p15 was performed on paraffin-embedded tissue sections. In-house FISH probes were selected and validated on chromosome spreads.

Results: Mean age at diagnosis was 58.6 years (31 to 93). 22/29 (76%) tumors expressed CD10 marker. Only 2/29(7%) and 2/29(7%) cases showed a weak and heterogeneous positivity for desmin and H-caldesmon, respectively. The tumors expressed estrogen and progesterone receptors in 6/27 (22%) and 8/28 (28%) respectively. FISH was interpretable in 22/29 cases. 9/22 (41%) cases showed a WYHAE chromosomal rearrangement of which 8/9 (89%) showed a uniform morphology (U-ESS-U): 2 with fibromyxoid features, 3 with spindle morphology and 1 with spindle and epithelioid appearance. Only one case (1/9) (11%) showed a pleomorphic morphology (U-ESS-P). No chromosomal rearrangement for JAZF1-JJAZ was detected. Three hybridization profiles were observed: 1 chromosomal rearrangement for YWHAE and 1 normal Chr 17; 1 chromosomal rearrangement for YWHAE and the loss of other Chr 17 and 1 chromosomal rearrangement for YWHAE and a gain of the non-rearranged Chr 17. Never less these tumors showed a very unstable chromosomal profile with loss or gain of part of chromosomes.

Conclusions: YWHAE t(10;17) rearrangement was found in 41% U-ESS in our series and correlated with the uniform, spindle and fibro-myxoid variant.

1110 Small Cell Carcinoma Hypercalcemic Type: Consistent Clinico-Pathologic Features and Lack of Molecular Markers

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Background: Small cell carcinoma hypercalcemic type (SCC-HT) is a unique ovarian cancer of unknown histogenesis with highly consistent clinicopathologic features and aggressive behaviour. Consistent immunohistochemical markers, other than weak expression of cytokeratins and a strong vimentin immunoreaction, are lacking. We tried to identify genetic alterations in 21 of these rare neoplasms.

Design: Twenty cases were seen in consultation over a 19-years period and one was from the hospital files. Twenty juvenile granulosa cell tumors (JGCT) were used as controls. A tissue microarray (TMA) was stained with AE1/AE3, vimentin, p53, WT1, and FOXL2. We also investigated mutation of the *TP53* gene (exons 5-8) and the *KRAS* gene (exon 2, codons 12 and 13) by direct sequencing.

Results: The mean age of the patients was 22 years (range 10-39) and the tumor size 15 cm (range 7-20 cm). Nine cases were confined to the ovary (stage I) and 12 had widespread tumor (stage III). Necrosis was found in 14 cases and the average mitotic rate was 15 (range: 4-27 MF x 10HPF). Twelve patients died of their disease within 2 years, and 5 were alive with disease 1-2 postoperatively. Three patients were alive and well at 2, 4, and 6 years. Tumor stage was a strong prognostic factor. All 21 tumors were focally immunoreactive for cytokeratins AE1/AE3, and 14 tumors exhibited diffuse immunoreaction for vimentin. Tumor cells showed low to moderate immunohistochemical expression of p53 in a mean of 36.25% of cells (range 5-68%). Eighteen cases exhibited WT1 positivity, 9 with 3+ intensity. FOXL2 immunostaining was expressed in 14 of 19 JGCTs, but in none of the SCC-HTs. Neither p53 nor K-RAS mutations were found in any case.

Conclusions: Although the diagnosis of SCC-HT was easily made by H&E stained sections, there was a striking lack of reliable molecular markers. Focal positivity with AE1/AE3 and diffuse WT1 immunoreaction suggests an epithelial origin. In difficult cases, negative staining with FOXL2 may aid in the differential diagnosis with JGCT. p53 immunoreaction was not supported by p53 gene mutation. The absence of both, p53 and K-RAS mutations, suggest that SCC-HT follow alternative carcinogenic pathways.

1111 PAX8 Immunohistochemical (IHC) Expression in Endocervical Glandular Lesions

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Background: Glandular lesions of the endocervix can be diagnostically challenging and occasionally the differential diagnosis includes both endocervical (ECA) and endometrial adenocarcinomas (EmCA). PAX8 expression has been recently described in the normal endocervix, but there is limited literature evaluating its expression in benign and malignant endocervical lesions, particularly in the context of currently utilized IHC markers such as p16, Ki-67 and CEA. We wanted to evaluate the potential utility of PAX8 to this IHC panel.

Design: We searched our pathology files for benign cervical lesions including: microglandular hyperplasia (MGH), endocervical laminar hyperplasia (ELH), tuboendometrioid metaplasia (TEM), cervical endometriosis (CEM), and tunnel clusters (TC). Premalignant and malignant cohort included: endocervical adenocarcinoma in situ (EC AIS), invasive endocervical adenocarcinoma (ECA), and mucinous/ MGH variants of endometrial adenocarcinoma (EmCA). An IHC panel of CEA, Ki-67, p16 and PAX8 was performed on all cases. Immunoreactivity was scored on degree of positivity (S0 – no staining, S1- up to 10% cells staining, S2- between 10-50% cells staining, S3- >50% cells staining) and intensity (I1-mild, I2-moderate, I3-strong).

Results:

Table 1

Diagnosis/# of cases	CEA	Ki-67	p16	PAX8
TC (5)	S0/I0	S0/I0	S1/I2	S3/I2
CEM (4)	S0/I0	S1/I2	S1/I3	S3/I3
ELH (2)	S0/I0	S1/I1	S1/I1	S3/I2
MGH (4)	S0/I0	S0/I0	S1/I2	S2/I2
TEM (2)	S0/I0	S0/I0	S1/I3	S3/I2

Table 2

Diagnosis/# of cases	CEA	Ki-67	p16	PAX8
ECA (3)	S2/I3	S3/I2	S3/I2	S1/I1
EC AIS (4)	S2/I2	S2/I2-3	S3/I2	S2/I1
EmCA (5)	S0/I1	S2/I2-3	S3/I3	S3/I1-2 (4)*

* 1 case of EmCA was negative for PAX8

Conclusions: 1. PAX8 shows diffuse positivity with at least moderate intensity in a spectrum of benign endocervical glandular lesions.
2. PAX8 intensity and extent of staining progressively decreased from benign lesions to EC AIS to ECA with ECA showing only focal/ rare staining with mild intensity. This alteration raises possible role of PAX8 in ECA.
3. Variants of EmCA (mucinous/ MGH) show diffuse PAX8 positivity with variable intensity.
4. PAX8 IHC could be added to a panel of other IHC markers in distinguishing benign endocervical lesions from ECA and extent of PAX8 staining could help in separation of ECA from EmCA.

1112 Gene Expression Signatures Differentiate Uterine Leiomyosarcoma and Endometrial Stromal Sarcoma

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Background: Leiomyosarcoma (LMS) and endometrial stromal sarcoma (ESS) are the two most common uterine sarcomas, but both are rare cancers. The aim of the present study was to compare the global gene expression patterns of LMS and ESS, in order to expand and improve the panel of biomarkers currently available for their diagnosis, as well as to define type-specific biological targets.

Design: Gene expression profiles of 7 ESS and 13 LMS were analyzed using the HumanRef-8 BeadChip from Illumina. Differentially expressed candidate genes were validated using quantitative real-time PCR and immunohistochemistry.

Results: Unsupervised hierarchical clustering using all 54,675 genes in the array separated ESS from LMS samples. We identified 549 unique probes that were significantly differentially expressed in the two cancers by greater than 2-fold with 1% FDR cutoff using one-way ANOVA with Benjamini-Hochberg correction, of which 336 and 213 were overexpressed in ESS and LMS, respectively. Genes overexpressed in ESS included *SLC7A10, IGDCC3, EFNB3, CCND2, ECEL1, ITM2A, NPW, PLAG1* and *GCGR*. Genes overexpressed in LMS included *CDKN2A, FABP3, TAGLN, JPH2, GEM, NAV2, RAB23*. The top 100 differentiators for each entity included the *MYLK* and *CALD1* genes, coding for myosin light chain and caldesmon, respectively, both overexpressed in LMS, but not the genes coding for CD10, desmin or actin. Results for selected genes were validated by quantitative real-time PCR and immunohistochemistry.

Conclusions: We present the first study in which gene expression profiling was shown to distinguish between ESS and LMS. The molecular signatures unique to each of these cancers may aid in expanding the diagnostic battery for their differentiation, and may provide a molecular basis for prognostic studies and therapeutic target discovery.

1113 PARP-Inhibitor Olaparib in the Treatment of Ovarian Clear Cell Cancer: Predictors of Sensitivity and Resistance

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Background: Ovarian clear cell carcinoma (OCCC) is an aggressive histological subtype of epithelial ovarian cancer (EOC). A potent poly(ADP) ribose polymerase (PARP)-inhibitor, olaparib, has been shown to be active in cancers with dysfunctional homologous recombination (HR) DNA repair, including tumours with loss of BRCA1, BRCA2 or PTEN function. The aims of this study were to determine whether a subset of OCCC cell lines would be sensitive to PARP inhibition and to identify potential mechanisms of loss of competent HR in OCCCs.

Design: The ability of 12 OCCC cell lines to elicit HR DNA repair in the presence of DNA double stand breaks was determined using the γH2AX (a surrogate marker for DNA double strand breaks) and RAD51 (a surrogate of marker HR repair) foci formation assay following irradiation or treatment with cisplatin or olaparib. Cell viability was assessed following treatment with cisplatin and olaparib using Celltitre Glo, BRCA1, BRCA2 and PTEN expression by western blotting. Co-treatment of cells with olaparib and verapamil (a p-glycoprotein inhibitor) was performed to evaluate the role of MDR1 expression on olaparib resistance. A tissue microarray containing 50 OCCC primary tumours was assessed for the expression of PTEN and MDR1.

Results: Of the 12 OCCC cell lines, TOV-21 and KOC-7c cells lacked PTEN expression, and none showed loss of BRCA1 or BRCA2 expression. Five of the 12 OCCC cell lines (KOC-7c, TOV-21, KK, RMG-1, and SMOV-2) were unable to elicit HR in the presence of DNA double strand breaks induced by radiation. These cells, with the exception of KOC-7c, had significantly higher sensitivity to cisplatin and olaparib than HR-competent OCCC cells. Upon treatment with cisplatin or olaparib, however, KOC-7c cells did not form γH2AX foci and RAD51 foci, but expressed high levels of MDR1. Treatment of KOC-7c with the MDR1 inhibitor verapamil sensitised these cells to olaparib. Loss of PTEN expression was noted in 8% (4/49) primary OCCCs, of which 3 expressed moderate-to-high levels of MDR1.

Conclusions: A subgroup of OCCC cells are sensitive to PARP inhibitors *in vitro*, and this sensitivity can be predicted by a γH2AX/RAD51 foci formation assay. PTEN loss of function was associated with a defect in HR repair in OCCCs. Expression of known drug efflux pumps (e.g. MDR1) may cause resistance to olaparib. These results provide a rationale for the testing of PARP inhibitors as a targeted therapy in a subset of OCCCs.

1114 Wolffian Tumors of the Female Genital Tract: A Study of 32 Cases

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Background: Wolffian tumors of the female genital tract (FGT), including mesonephric adenocarcinoma (MA) and female adnexal tumor of probable Wolffian origin (FATWO), arise from remnants of the mesonephric (Wolffian) system. Their diagnosis is often problematic due to their low frequency as well as overlapping histologic features with other FGT tumors. Behavior is also not well known as only small series have been reported.

Design: Files of 2 institutions were searched, including Dr. R. Scully's consultation files. Glass slides and medical records were reviewed and relevant clinicopathologic features were recorded.

Results: A total of 32 tumors were identified, including 26 FATWOs and 6 MAs. Average patient age was 45 years. FATWOs originated from ovary (n=16), fallopian tube (n=9), and pelvis/retroperitoneum (n=1). All MAs arose in the cervix (n=6). FIGO stage at presentation was as follows: I:23, II:4, III:2, and IV:3. Average tumor diameter was 7.6 cm and average mitotic index (MI) was 4.65 /10 high power fields (HPF). Mesonephric rests were identified in all 6 MAs and 6 FATWOs. Eosinophilic secretions and necrosis were present in 20 and 12 tumors respectively. One FATWO had focal sarcomatous transformation. Almost all of both FATWOs and MAs (29/32) had more than one growth pattern, the most common being tubular, cystic, or sieve-like. Other growth patterns included solid, spindled, sertoliform, retiform, and pseudoendometrioid. Two MA and one FATWO had only tubular growth. The stroma was frequently hyalinized. FATWOs in the ovary usually had a multinodular growth pattern compared to other locations. Immunohistochemical studies were available in selected cases with the following positive results: inhibin 4/6, calretinin 8/11, (both usually focal) ER 1/11 (focally), PR 0/11, CD10 10/10, CK7 5/5, PAX8 5/5, EMA 3/4, p16 0/7, CEA 0/3, p53 0/5, HNF1-β 2/6, FOXL2 1/1. Follow-up was available for 23/32 patients (average 73 months); 16 had no evidence of disease, 5 are alive with disease (AWD), 1 died of disease (DOD), and 1 died of another cause. Patients DOD or AWD presented at advanced stage and had an average MI of 14/10 HPF, these included 4 FATWOs from the ovary (including the tumor with sarcomatous transformation) and 2 MAs.

Conclusions: FATWOs and MAs usually present at low stage and have a favorable clinical outcome. They often show a background of mesonephric rests, heterogeneous growth pattern, eosinophilic secretions, and a low MI. Factors associated with malignant behavior included high stage at presentation and high MI.

1115 Endometrial Clear Cell Carcinomas with and without Aberrant p53 Expression: A Study of 16 Cases

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Background: Clear cell carcinoma is a relatively rare tumor type which classically does not demonstrate a mutation in *TP53* in ovarian examples. Endometrial serous carcinomas (EMSC) nearly always show these mutations, almost all of which correlate with overexpression (OE) of the protein or complete loss of expression (null phenotype) by immunohistochemistry (IHC). Little is known, however, about p53 expression in endometrial tumors with clear cell histology.

Design: Endometrial carcinomas which showed typical clear cell histology (EMCC), diffuse positivity with HNF-1β by IHC, and aberrant p53 expression (OE or null phenotype), also by IHC, were included. Mixed epithelial and morphologically ambiguous tumors were excluded. Clinical characteristics and immunohistochemical results for p16 (OE or not) and ER were also recorded. For comparison, EMCCs without aberrant p53 were also analyzed.

Results: Eight cases each of HNF-β-positive EMCC with and without aberrant p53 expression were identified. The clinical and immunohistochemical results are shown in the tables below.

Tumors with aberrant p53 expression (1 tumor had null phenotype)

Case #	p16	ER	Stage	Age	Outcome
1	-	-	IVb	65	AWD
2	equivocal	-	IVb	61	DOD
3	OE	-	IVb	71	AWD
4	-	-	IVb	74	DOD
5	-	-	IVb	65	DOD
6	-	-	IVb	78	DOD
7	equivocal	focal +	IVb	61	AWD
8	-	-	IVb	67	AWD

Tumors without aberrant p53 expression

Case #	p16	ER	Stage	Age	Outcome
1	-	-	IIIc2	61	DOD
2	-	-	Ia	65	NED
3	-	-	Ic	73	NED
4	-	-	Ib	83	DOD
5	-	-	Ia	51	DOD
6	-	-	Ia	55	NED
7	-	-	IIIc1	75	AWD
8	-	-	IIIc2	56	NED

All patients whose tumors showed aberrant p53 presented at advanced stage due to peritoneal metastases and are either dead of disease (DOD) or alive with disease (AWD) on follow-up. Only 3 of the patients whose tumors lacked aberrant p53 presented at advanced stage and in each case this was due to lymph node metastases, not peritoneal dissemination. Half of the patients without aberrant p53 have no evidence of disease (NED). The average age of patients in both groups was not significantly different.

Conclusions: EMCCs with aberrant p53 expression present in a fashion similar to that of EMSC (peritoneal dissemination) and have poor prognoses. EMCC without aberrant p53 present in a similar manner to endometrioid adenocarcinoma (low stage or lymph node metastases) and appear to have better overall prognoses than those with aberrant p53 expression. Whether these tumors represent clear cell carcinomas with tumor progression due to the aquisition of *TP53* mutations or hybrid serous/clear cell carcinomas is unknown at this time.

1116 Molecular Changes in Endometrial Clear Cell Carcinomas and Carcinomas with Clear Cell Features

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Background: Little is known about the molecular changes in endometrial clear cell carcinoma and endometrial carcinoma with clear cell changes. A group of 44 cases of endometrial carcinomas originally diagnosed as clear cell (CC), mixed clear cell (MEC), or carcinoma with clear cell changes were previously studied to evaluate diagnostic interobserver variability and immunoreactivities of the markers HNF-1β, p53, ER, and p16. Based on the consensus diagnoses of a panel of 5 gynecologic pathologists, the tumors were reclassified as CC, endometrioid (EC), serous (SC), undifferentiated (UD), carcinosarcoma (MMMT), or no consensus diagnosis (NCD). We sought to evaluate certain molecular changes in this group of tumors.

Design: Thirty-seven of these cases had adequate material for sequenom analysis for hotspot mutations in the genes *PIK3CA*, *PIK3R1*, *KRAS*, *NRAS*, and *PTEN*. These included 11 CC, 9 EC, 14 SC, 1 UD, 1 MMMT, and 1 NCD.

Results: Of the 37 studied cases, mutations were detected in 15 (41%) tumors. The results are shown in the table below as well as the corresponding immunophenotypes.

Consensus	Mutation (s)	HNF1-ß	ER	p53	p16	Outcome
CC	PIK3R1, PIK3CA, KRAS	+	-	-	-	DOD
CC	PIK3R1	-	-	-	-	DOD
CC	KRAS	-	-	-	-	DOD
EC	PIK3CA, KRAS, NRAS	+	+	-	-	LTF
EC	KRAS	+	+	-	-	DOD
EC	PIK3R1	-	+	-	-	DOD
EC	PTEN	-	+	+	-	LTF
EC	PIK3CA	-	+	-	-	DOD
EC	PIK3R1, KRAS	-	+	-	-	DOD
SC	PIK3CA	-	+	-	-	DOD
SC	PIK3R1	-	+	-	-	NED
SC	PIK3CA	-	+	+	+	AWD
SC	PIK3R1	-	+	-	+	LTF
SC	PIK3CA	-	+	-	+	NED
SC	KRAS	-	+	+	-	DOD

DOD=Dead of disease, AWD=Alive with disease, NED=No evidence of disease LTF=Lost to follow-up

PIK3CA and *PIK3R1* mutations occurred in all tumor types with detected mutations and 1 CC contained both. None of the CC with detected mutations showed overexpression of p53 by immunohistochemistry (IHC). A mutation in *PTEN* was detected in only 1 EC. *KRAS* mutations were also present in all tumor types and coexisted with both *PIK3R1* and *PIK3CA* in 2 separate EC and alone in 1 EC and 1 SC. Overexpression of p53 by IHC occurred in tumors with mutations in *PTEN*, *KRAS*, *PIK3R1*, and *PIK3CA*. HNF-1ß was positive by IHC in tumors with *PIK3CA*, *PIK3R1*, *KRAS*, and *NRAS* mutations. No mutations were detected in 8 CC, 3 EC, 8 SC, 1 UD, 1 MMMT, and 1 NCD. Mutation status did not appear to correlate with clinical outcome.

Conclusions: Endometrial clear cell carcinomas and carcinomas with clear cell features are a heterogeneous group of tumors and show a spectrum of mutations and corresponding tumor types.

1117 PAX8 Differentiates Gastrointestinal Carcinomas from Mucinous Carcinomas of the Ovary, but Not Mucinous Carcinomas Arising in Ovarian Teratomas

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Background: PAX8, a transcription factor involved in organogenesis, is expressed in tumors of the thyroid gland, kidney, and Mullerian system. Recent data suggests PAX8 is useful in distinguishing mucinous tumors of the ovary from the GI tract, but this has not been confirmed; in addition, PAX8 expression in mucinous carcinomas arising from ovarian teratomas has not been studied. We examined PAX8 expression in a large series of primary GI carcinomas, primary ovarian mucinous tumors, and mucinous carcinomas arising from ovarian teratomas to determine the utility of PAX8 in distinguishing these lesions.

Design: Tissue microarrays (TMAs) containing 174 colorectal adenocarcinomas, 103 gastric adenocarcinomas, and 30 pancreatic ductal adenocarcinomas were stained for PAX8. TMAs and whole sections comprising 20 primary ovarian mucinous tumors (16 carcinomas, 4 borderline tumors) and whole sections of 10 mucinous carcinomas arising in ovarian teratomas were also stained for PAX8.

Results:

PAX8 Immunohistochemistry in GI and Ovarian Tumors

Tumor type	PAX8 Reactivity (%)
Colorectal adenocarcinoma	2/174 (1.1%)
Gastric adenocarcinoma	2/103 (1.9%)
Pancreatic adenocarcinoma	1/30 (3.3%)
Primary ovarian mucinous tumors	13/20 (65%)
Mucinous carcinomas arising in ovarian teratomas	0/10 (0%)

Strong PAX8 expression, comparable to that of lymphocytes, was seen in neoplastic gastrointestinal epithelial cells in 5/307 carcinomas. Endocrine cell staining in pancreatic islets and gastric glands was consistently observed. No PAX8 expression was seen in the mucinous carcinomas associated with teratomas. Within teratomas, PAX8 expression was present in foci of thyroid differentiation (n=2) and Mullerian differentiation (n=1).

Conclusions: PAX8 expression can distinguish primary mucinous tumors of the ovary from GI tract adenocarcinomas and is very specific for primary ovarian tumors in this context ($p<0.0001$). However, mucinous carcinomas arising in ovarian teratomas rarely, if ever express PAX8 and thus cannot be reliably distinguished from gastrointestinal adenocarcinomas which may metastasize to the ovary on the basis of PAX8. PAX8 expression may be found in areas of Mullerian and thyroid differentiation in teratomas. Only moderate to strong nuclear PAX8 expression, comparable to staining in lymphocytes or endocrine cells, should be considered positive.

1118 Conventional Screening Criteria May Miss a High Proportion of Lynch Syndrome Patients with Endometrial Carcinoma Due to PMS2 Loss

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Background: Loss of mismatch repair (MMR) protein expression (MLH1, PMS2, MSH2 and MSH6) occurs in Lynch syndrome, while MLH1 loss can also be found in sporadic endometrial tumors due to promoter methylation. Experience with endometrial cancers with PMS2 loss is limited. In this study, we describe the clinical and pathological features of 7 such tumors, the largest case series to date.

Design: Using MMR immunohistochemistry, we investigated the following two different cohorts of endometrial cancer patients: 154 sequential patients, unselected for age or personal and family history of Lynch associated cancers, and 45 patients with a clinical suspicion of Lynch syndrome (age less than 50 and/or a relative with Lynch associated cancer).

Results: 7 patients with PMS2 loss and intact expression of other MMR proteins were identified. Their clinicopathologic characteristics are summarized below.

Case	Age	Personal Cancer History	Family Cancer History	Tumor Histotype and FIGO Grade	Stage
Unselected 1	87	No	No	Endometrioid 2	1b
Unselected 2	45	No	No	Endometrioid 2	1b
Unselected 3	75	No	Colon ca in mother at 92; Other Non-Lynch ca	Endometrioid 2	1a
Unselected 4	66	No	Non-Lynch ca	Endometrioid 3	1b
Unselected 5	51	No	Non-Lynch ca	Endometrioid 2	1a
Lynch Suspicious 1	58	Non-Lynch ca	Colon ca in mother at 58	Endometrioid 2	1b
Lynch Suspicious 2	70	No	Colon ca in father at 66	Endometrioid 2	3c

The average patient age was 64.6 years, with only one patient younger than 50. Only 3 patients had first degree relatives with colon cancer, with an average age of 72 years. In comparison with published data, MLH1, MSH2 and MSH6 mutation carriers had an average age of 45.8, 45.5 and 51.2 respectively at the time of endometrial cancer diagnosis, and first degree relatives with colon cancer at an average age of 48.0, 46.2 and 60.9 respectively. All tumors with PMS2 loss were of endometrioid histology. In contrast, non-endometrioid endometrial cancers with MLH1, MSH2 and MSH6 mutations have been reported.

Conclusions: Endometrial tumors with PMS2 loss have a predilection toward the endometrioid histotype. The patients tend to be older and either lack relatives with Lynch associated cancers or have relatives with colon cancer diagnosed later in life that may be confused with sporadic tumors. Therefore, Lynch syndrome patients with PMS2 abnormalities and endometrial cancer may be missed by screening criteria that use young patient age and family history as determinants for further testing.

1119 Frozen Section Does Not Reliably Predict the Likelihood of Lymph Node Involvement in Low Risk Endometrial Adenocarcinoma

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Background: The role of lymphadenectomy in surgical treatment of low risk endometrial carcinoma is controversial. Intraoperative frozen section (FS) may be performed to exclude features that confer an increased risk of lymph node (LN) metastases, with the aim of identifying patients in whom lymphadenectomy may not be necessary. The reliability of FS in this setting, however, is not well established.

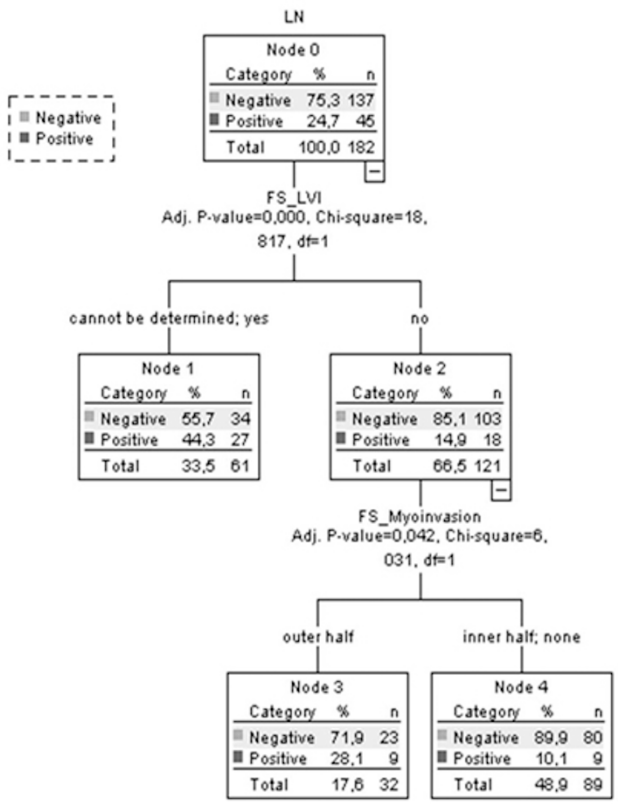
Design: In a multi-institutional study, 45 FIGO grade 1 and 2 endometrial endometrioid adenocarcinomas with LN involvement as the only site of extrauterine spread, and which were assessed by FS, were identified. Tumor grade, depth of myometrial invasion (MI) and presence of lymphatic vascular invasion (LVI) on FS and permanent section (PS) were recorded. The results were compared to a corresponding set of 137 cases without LN metastases.

Results: The results are summarized in the Table.

LN positive, n=45		LN negative, n=137		p value
Grade		Grade		
FS-Gr1	29%	FS-Gr1	29%	
FS-Gr2	71%	FS-Gr2	71%	0.97
PS-Gr1	9%	PS-Gr1	12%	
PS-Gr2	91%	PS-Gr2	88%	0.52
Agreement=76%, Kappa=0.25		Agreement=81%, Kappa=0.42		
MI		MI		
FS-none	0%	FS-none	14%	
FS-inner half	53%	FS-inner half	63%	0.001
FS-outer half	47%	FS-outer half	23%	
PS-none	0%	PS-none	5%	
PS-inner half	42%	PS-inner half	65%	0.0008
PS-outer half	58%	PS-outer half	30%	
Agreement=84%, Kappa=0.69		Agreement=85%, Kappa=0.70		
LVI		LVI		
FS-not reported	24%	FS-not reported	16%	
FS-present	36%	FS-present	9%	<0.0001
FS-absent	40%	FS-absent	75%	
PS-not reported	0%	PS-not reported	0%	
PS-present	91%	PS-present	42%	<0.0001
FS-absent	9%	FS-absent	58%	
Agreement=40%, Kappa=0.06		Agreement=64%, Kappa=0.31		

The rates of grade 1 and 2 carcinoma were comparable between the two groups. The rates of outer MI and LVI were significantly higher in the LN positive group on FS and PS. However, FS underestimated the number of cases with FIGO grade 2, outer MI and particularly LVI. At time of FS, LVI was either not reported or accurately perceived in a large proportion of cases.

Using a decision tree model, MI and LVI status at FS could discriminate between LN positive and LN negative cases with an overall performance of only 75%.



Conclusions: While outer MI invasion and LVI are more frequent in cases with positive LNs, inability to reliably recognize LVI in a large proportion of cases on FS renders FS a relatively inaccurate method for predicting LN involvement in low risk endometrial adenocarcinoma.

1120 Frozen Section Results Do Not Influence the Decision To Perform Lymphadenectomy in Low Grade Endometrial Endometrioid Adenocarcinoma

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Background: The role of lymphadenectomy in the surgical treatment of low grade endometrial endometrioid adenocarcinoma (EEC) is not well defined. In a related study, we have shown that intraoperative frozen section (FS) does not reliably predict the likelihood of lymph node metastases in these tumors. The objective of this study was to assess whether FS results influenced the decision to perform lymphadenectomy.

Design: In a collaborative international study, 9 cancer centers collected 528 cases of FIGO grade 1 and 2 EEC with no evidence of extrauterine disease at the time of surgery. Among these, 17 cases which recurred in follow-up and which were assessed by intraoperative FS were identified. Lymphadenectomy was not performed in 6 cases, while in the other 11 cases, lymphadenectomy was carried out and subsequently yielded negative lymph nodes. Tumor grade, depth of myometrial invasion (MI) and presence of lymphovascular invasion (LVI) reported at FS were recorded for the two groups.

Results: FS was performed in 32%-84% of cases (median 50%) at 6 participating institutions (5 from the United States and 1 from Korea), and in no cases at 3 participating institutions (from Canada, Korea and Mexico).

Among the 17 tumors that recurred, cases that underwent lymphadenectomy versus those that did not, showed no significant difference in the rate of grade 2 tumors, outer MI or presence of LVI on FS, suggesting that FS results did not influence the decision to perform lymphadenectomy. Interestingly, although grade 2 EEC was more common in both groups, both groups also showed a tendency towards inner MI and absence of LVI, features that would normally persuade against lymphadenectomy.

	Grade: 1-2	MI: Inner Half-Outer Half	LVI: Absent-Present-Not Reported
LNs Not Resected, n=6	17%-83%	100%-0%	67%-0%-33%
LNs Resected, n=11	0%-100%	91%-9%	36%-18%-46%
p-value	0.3529	1.0000	0.4667

Conclusions: The rates of FS in FIGO grade 1 and 2 EEC vary widely between institutions. FS results do not appear to influence the decision of whether to perform lymphadenectomy. Furthermore, in such tumors that subsequently recur, FS may have provided misleading information resulting in suboptimal surgical management.

1121 HPV Infectivity of Products of Conception: An Age-Specific Prevalence Study

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Background: Several studies have suggested an association of HPV infection with aborted products of conception (POC). However, previous studies of HPV POC infections have examined limited numbers of specimens and have not addressed the epidemiology of HPV infection in U.S. women. The highest burden of HPV infection is found in females 20-24 years of age; females 25-29 years of age may have almost half the HPV prevalence. The aim of this study was to use a larger scale case-control study design to investigate HPV as a causative factor in aborted POC and to assess HPV infection in relation to patient age group.

Design: The study included 201 specimens: 100 specimens of archival formalin-fixed, paraffin-embedded (FFPE) POC: 20-24 years (n=50) and 25-29 years (n=50), and, 101 controls of term FFPE placenta samples; 20-24 years (n=51) and 25-29 years (n=50). HPV genotyping of purified DNA extracts was performed by GP5+/GP6+ PCR and cycle sequencing. Chromogenic *in situ* hybridization (CISH) was performed for HPV on the PCR positive cases.

Results: HPV was detected in 20/201 (10%) specimens by PCR: 1 low risk type (HPV-11 [n=1]) and 5 high risk types (HPV-16 [n=11], 18 [n=2], 51 [n=1], 59 [n=1], 66 [n=4]). There were no statistically significant differences of HPV prevalence between the two age groups comparing POC specimens, term placenta controls, or POC and controls combined; 20-24 age group: HPV was detected in 16.0% POC samples and in 7.8% term placentas (p=0.23); 25-29 age group: HPV was detected in 10% POC and in 6.0% term placentas (p=0.48). Overall, HPV was detected in 11.9% of the 20-24 group and in 8.0% of the 25-29 group samples (p=0.48). CISH was negative for HPV in all specimens tested.

Conclusions: These data are unsupportive of HPV contributing to the etiology of spontaneous miscarriage. By PCR, HPV was (statistically) as common in control placentas as in POC. The prevalence of HPV in aborted POC was not directly reflective of the prevalence of HPV infections in younger age group females in the U.S. The CISH data are suggestive that the HPV detection by PCR represents a low copy number of incidental or latent infection. Further studies are required to elucidate the factors that contribute to a placental sample testing HPV positive and the significance, if any, for patient or fetal health.

1122 Altered Caspase-14 Expression in Vulvar Squamous Lesions

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Background: Caspase-14 is involved in cell differentiation of stratified squamous epithelia. Its expression is normally restricted to intermediate and upper layers, while

proliferating basal and parabasal layers do not express this protein. We have previously shown that caspase-14 is absent in a subset of cervical squamous cell carcinomas and that a black raspberry extract increases caspase-14 mRNA in various cervical cell lines with and without HPV. Because black raspberry bioactives can be incorporated into topical preparations for the purpose of cancer chemoprevention strategies, we investigated the expression of caspase-14 on vulvar lesions including lichen sclerosus (LS), vulvar intraepithelial neoplasia of differentiated type (dVIN), high grade vulvar intraepithelial neoplasia of classic type (cVIN) and vulvar squamous cell carcinoma (SCC). Expression of AE1, a cytokeratin known to have a pattern of expression indicative of squamous maturation similar to caspase-14, was also determined.

Design: We identified vulvectomy specimens with LS, cVIN, dVIN and SCC from our institution's pathology files. Thirteen areas of histologically unremarkable squamous epithelium (SE) adjacent to these lesions were also examined. Cases were reviewed and selected for either incorporation into a tissue microarray (TMA), recuts for whole sections, or both. Sections were stained with commercially available antibodies to AE1 and caspase-14. The staining pattern was evaluated for each diagnostic entity. In cases that included >1 diagnosis, staining pattern was evaluated separately for each entity.

Results: 12 cases of LS, 27 cases of cVIN, 12 cases of dVIN, and 25 SCC were identified. Aberrant caspase-14 basal layer staining was seen in 5/12 (42%) LS, 8/27 (30%) cVIN, 4/12 (33%) dVIN and 2/13 (15%) SE. 6 cVIN and 4 dVIN showed either full thickness or intermediate and upper layer caspase-14 reactivity while the adjacent SCC was negative. Overall 15/25 (60%) of SCC were negative for caspase-14 including well differentiated keratinizing examples. AE1 generally mirrored caspase 14 but abnormal opposite patterns were also seen.

Conclusions: Abnormal or absent caspase-14 expression was demonstrated in subsets of LS, cVIN, dVIN, SCC and SE. Our findings provide evidence for caspase-14 being a biomarker of differentiation/progression in vulvar lesions and support further research into novel chemopreventive strategies.

1123 Cervical Carcinomas with Neuroendocrine Differentiation: A Report of 29 Cases with Immunohistochemical Analysis and Molecular Genetic Evidence of Common Clonal Origin with Coexisting Squamous and Adenocarcinomas

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Background: Cervical neuroendocrine tumors are a rare, aggressive subtype of cervical cancer, but their immunohistochemical features and relationship to coexisting tumors are incompletely described in the medical literature. Expression of TTF1, c-kit (a potential marker or therapeutic sensitivity), and CD44 (a tumor stem cell marker) by extrapulmonary small cell carcinomas has been recently reported. Cervical neuroendocrine tumors are known to be related to high risk human papillomavirus and p16 immunohistochemical expression is expected.

Design: A search for a 22 year period was performed for all available cervical neuroendocrine tumors. Immunohistochemical staining for synaptophysin, chromogranin A, TTF1, c-kit, CD44, and p16 was performed. Loss of heterozygosity (LOH) analysis for five polymorphic microsatellite markers (D3S1300, D9S171, D11S914, D13S319, and TP53) and X chromosome inactivation analysis were performed.

Results: Twenty-nine cases were identified (17 small cell, 10 large cell, and 2 mixed). The mean patient age was 44 years (range: 18-82 years). There was coexisting adenocarcinoma in 7 cases, squamous cell carcinoma in 2 cases, adenosquamous carcinoma in 1 case, adenocarcinoma in situ in 1 case, and squamous cell carcinoma in situ in 1 case. Of 18 cases with available blocks, 13 (72%) were synaptophysin+, 8 were chromogranin A+ (44%), 9 (50%) were TTF1+, 8 (44%) were c-kit+, and 7 (39%) were CD44+. Strong patchy or strong diffuse p16 staining was seen in all cases. LOH and X chromosome inactivation analysis were performed for 17 cases, 8 of which had a coexisting squamous or adenocarcinoma component. Five of the eight (63%) cases with two components showed allelic loss in both components. All five of these cases demonstrated identical LOH between the neuroendocrine and squamous or adenocarcinoma components. Nonrandom X chromosome inactivation was seen in the neuroendocrine and other components in 4 of the 8 cases. In all 4 cases the pattern of inactivation was identical between the two components.

Conclusions: Cervical neuroendocrine carcinomas have immunophenotypic features similar to other extrapulmonary neuroendocrine carcinomas, including frequent expression of TTF1, c-kit, and CD44. Consistent staining for p16 is also seen. Concordant genetic alterations in X inactivation patterns support common clonal origin for neuroendocrine carcinomas with a coexisting squamous or adenocarcinoma component.

1124 Validation of 3D Glandular Cultures To Investigate Endometrial Carcinogenesis

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Background: Development of three dimensional (3D) epithelial culture systems has emerged as a good approach to study the genes involved in the disruption of gland architecture, loss of epithelial polarity and increase of proliferation. We describe a novel 3D culture system of primary mouse endometrial epithelial cells which could be used to investigate the frequent alterations found in the endometrial carcinogenesis at the light of morphology, such as decreased E-Cadherin or PTEN expression or the effects of estrogenic stimulation.

Design: Epithelial cells from uterus were obtained after a mechanical and enzymatic digestion. After 24 hours of platting in plastic, cells were grown in a medium containing EGF plus Insulin and 3% of a reconstituted extracellular matrix (Matrigel). Matrigel allows cells to display a 3D organization comparable to that observed in vivo endometrium. We analyzed glandular polarity by immunofluorescense staining for different polarity markers. We inhibited E-Cadherin and PTEN expression by lentivirus-delivered shRNAs. We also exposed these cultures to a situation mimicking hyperestrogenism and we studied its effects on cell proliferation.

Results: The endometrial glands developed in our in vitro system conserved cytokeratin expression and were negative staining for vimentin, indicating its epithelial origin. Staining with phalloidin and GM130 indicated correct positioning of actin cytoskeleton and Golgi apparatus respectively. E-Cadherin and β-Catenin staining demonstrated stable cell-to-cell contacts. To validate our 3D culture for the study of disorders associated with tumor formation we infected epithelial cells with PTEN or E-Cadherin shRNAs. PTEN knock-down resulted in consistent increase of proliferation, measured by BrdU incorporation and Ciclin D1 expression. In cultures infected with E-Cadherin shRNA, cells were unable to form glandular structures and caused a complete loss of cell polarity accompanied by the acquisition of features associated with a malignant/migratory phenotype such as β-Catenin translocation, Golgi delocalization and polymerization of stress fibers. An excessive stimulation of estrogen caused an increase of proliferation and an enhanced gland size, but structures retained a correct architecture.

Conclusions: The results confirmed that glandular structures displayed the characteristic apicobasal polarity of glandular tissues, as well as the appropriate formation of cell-to-cell and cell-to-extracellular matrix contact, with patterns similar to those observed in intact endometrium.

1125 The HistoRx AQUAnalysis Platform Is the More Discriminatory Method To Quantify ER/PR Expression: The Example of Low-Grade Serous Carcinoma

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Background: Case reports suggest that hormonal therapy may be an option for low-grade serous carcinomas (LGSC) but data on quantitative hormone receptor expression in LGSC are lacking.

Design: We assessed ER/PR expression on 25 LGSC, 66 high-grade serous carcinomas (HGSC), 31 serous borderline tumours (SBOT), and five normal fallopian tubes (FT) on TMAs using two DAB platforms (Lecia/Bond (SP1, 1E2 antibodies) and DAKO with PharmDx antibodies) and evaluating with the Allred score. Quantitative fluorescent immunohistochemistry, using the DAKO PharmDx antibodies, was performed using the HistoRx AQUAnalysis platform. Mean values were calculated and Anova analysis applied to calculate the 95 CI.

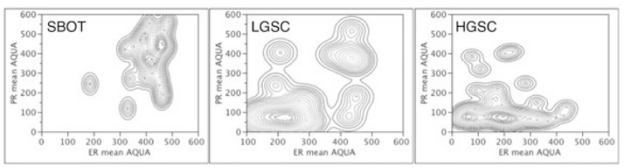
Results:

DAB Allred and AQUA scores

Diagnosis	ER Leica Allred	ER DAKO Allred	ER AQUA score	PR Leica Allred	PR DAKO Allred	PR AQUA score
FT	8.0 (5.9-8.0)	8.0 (6.1-8.0)	402 (318-486)	7.3 (5.0-8.0)	7.8 (5.4-8.0)	474 (361-586)
SBOT	7.7 (7.0-8.0)	7.7 (7.0-8.0)	411 (376-445)	7.3 (6.6-8.0)	7.1 (6.1-8.0)	364 (322-405)
LGSC	7.0 (6.2-7.8)	7.0 (6.1-7.8)	267 (229-304)	5.2 (4.4-6.0)	3.6 (2.5-4.6)	163 (118-209)
HGSC	6.3 (5.8-6.8)	5.8 (5.3-6.3)	195 (171-218)	5.0 (4.5-5.5)	2.5 (1.8-3.1)	111 (81-142)

Means and 95% CI are displayed

ER level of LGSC are significantly lower than FT and SBOT but higher than HGSC, a finding only seen in the HistoRX analysis. LGSC and HGSC do not differ with respect to PR expression; both show a significantly lower PR expression than FT and SBOT.



Nonparametric bivariate density analysis (Figure) revealed two populations with respect to hormone receptor expression within LGSC only when using the ER and PR AQUA data.

Conclusions: Immunohistochemical assays do not distinguish the hormone receptor expression level between LGSC and HGSC, while the HistoRx AQUAnalysis platform showed a significant higher ER expression in LGSC. HistoRx AQUAnalysis reveals two distinct populations with respect to hormone receptor expression within LGSC, which may be useful as a predictive marker for hormonal therapy, assuming that only double high expressors benefit from hormonal treatment.

1126 Stromal Signatures in Endometrioid Endometrial Carcinomas

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Background: The pattern of myometrial invasion in endometrioid carcinomas varies considerably; i.e., from widely scattered glands and cell nests, often associated with stromal desmoplasia, to invasive glands with little or no stromal response. Recently, two distinct stromal signatures, derived from a macrophage-response (CSF1) and a fibroblastic-response (DTF), were identified in breast carcinomas and correlated with clinicopathologic features including outcome. In this study, we explored whether these stromal signatures also apply to endometrioid carcinomas and if the aforementioned expression patterns correlated with morphologic changes.

Design: Stromal signatures were studied by immunohistochemistry on a tissue microarray of 88 endometrioid carcinomas. A case was considered to carry the CSF1-response signature if it showed coordinate expression of CD163, CD16, and CD32. Similarly, co-expression of SPARC and MMP11 was required for inclusion in the DTF-stromal signature group.

Results: Desmoplasia correlated positively with DTF expression signature. Likewise, CSF1 expression signature correlated strongly with the presence of mononuclear infiltrate. The DTF and CSF1 groups accounted for 17 % each of the total (15 of 88). Another 12 cases were positive for both DTF and CSF1 signatures (12/88; 13%). However, over half of the cases (46/88; 53%) failed to express any of the stromal signatures.

Stromal Signatures in 88 Endometrioid Carcinomas

Macrophage response signature (CSF1)	Fibroblast response signature (DTF)	Cases (%)
+	-	15/88 (17%)
-	+	15/88 (17%)
+	+	12/88 (13%)
-	-	46/88 (53%)

The macrophage response (CSF1) was associated with higher tumor grade and *PIK3CA* mutations (P =0.000). In contrast, tumors that evoked little or no inflammatory stromal response were low-grade and had a low rate of vascular invasion and *PIK3CA* mutations (P =0.000).

Conclusions: This study is the first characterization of stromal signatures in endometrioid carcinomas. Our findings shed new light on the relationhip between genetically different endometrioid carcinomas and various types of stromal response.

1127 Predictors of Lymph Node Metastasis or Extrauterine Disease in Low Grade Endometrial Carcinoma, a Multi Institutional Study

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Background: Lymph node metastases (LNM) and extrauterine disease (ED) are infrequent in low grade (FIGO grades 1/2) endometrioid carcinoma (LGEC). This study evaluates predictors of LNM or ED in a large multi institutional study.

Design: For LGEC with and without LNM and ED, each of the 9 participating institutions evaluated pt age,tumor (tu) size, myometrial invasion (MI), FIGO grade, % solid component, % papillary architecture (PA) and presence of microcystic elongated and fragmented glands (MELF), single cell invasion (SCI), lymphovascular invasion (LVI), lower uterine segment (LUS) and cervix (CX) involvement and numbers of pelvic (PLN) and paraaortic (PALN) LNs sampled.

Results: Pt ages ranged from 23-91yrs (median 61). Table 1 summarizes the histopathologic variables. Table 2 provides univariate logistic regression results modeling LNM or ED. There was no evidence of a difference in the number of pelvic or para aortic LNs sampled between groups (p=0.10, 0.64, respectively).

Summary of Histopathologic Variables

	LN+ or ED+ (n=96)	LN-/ED- (n=206)
Variable	Count (%)	Count (%)
Tu≥2cm	93 (97)	149 (72)
MI >50%	54 (56)	56 (27)
MELF	61 (64)	68 (33)
SCI	28 (29)	16 (8)
LVI	77 (80)	52 (25)
>20% solid	31 (32)	44 (21)
PA	58 (60)	100 (49)
LUS involved	64 (67)	75 (36)
CX involved	40 (42)	23 (11)

Univariate Logistic Regression Results Modeling LNM or ED

Variable	Odds Ratio	Confidence Interval	p-value
Tu>2 cm	13.95	3.31-58.89	0.0003
%solid >20%	1.72	0.99-2.93	0.0527
PA	1.59	0.97-2.60	0.0658
%PA *	1.02	1.01-1.03	0.0058
MELF	3.54	2.13-5.87	<0.0001
SCI	4.58	2.36-8.89	<0.0001
LVI	12.00	6.64-21.7	<0.0001
LUS Involved	3.31	1.91-5.75	<0.0001
CX Involved	5.93	3.28-10.73	<0.0001
MI >50%	3.44	2.075-5.717	<0.0001
# PALN sampled	1.09	1.03-1.15	0.0048

*Several cutoffs were statistically significant with the lowest p-value at 0.001 for >30%PA

Conclusions: Tu size ≥2 cm, %PA, MELF, SCI, LVI, LUS and CX involvement, MI>50% and number of PALNs sampled were found to be significant predictors of the odds of LNM or ED.Presence of PA and %solid >20% also suggest increased odds (p=0.0658, 0.0527 respectively). Age, FIGO grade 2 vs 1 and number of PLNs sampled were not significant predictors. This multi institutional study validates MI and LVI as predictors of LNM and ED. Additionally, MELF and SCI invasion patterns as predictors of LNM and ED. Attention to pattern of invasion during frozen section may identify pts who could benefit from LND. Further study is required to determine a multivariate model to predict advanced stage and to study whether these features could also be predictors of recurrence.

1128 Loss of BAF250a (*ARID1A*) Expression in Endometrial Clear Cell Carcinoma: Assessment of Frequency and Clinicopathologic Implications

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Background: SWI/SNF chromatin-modification complexes use the energy of ATP hydrolysis to remodel nucleosomes and to affect transcription in a manner that is critical for proliferation and differentiation. Accordingly, their loss of function has been associated with malignant transformation. *ARID1A* (the expression of whose product, BAF250a, a key complex component, is lost when mutated) has recently been identified as a tumor suppressor gene that is mutated in 50% of ovarian clear cell carcinoma (CCC). The purposes of this study are to assess the frequency of loss of BAF250a expression in *endometrial* CCC and whether this loss has any discernable clinicopathologic implications.

Design: 34 endometrial carcinomas with a CCC component (including 22 pure CCC, 8 mixed carcinomas with a ≥10% CCC component, and 4 carcinosarcomas with a CCC epithelial component), were evaluated by immunohistochemistry using a monoclonal antibody directed against the human BAF250a protein.

Results: 5 (22.7%) of 22 pure CCC were entirely BAF250a[-], whereas the remainder showed diffuse immunoreactivity. None of 4 carcinosarcomas and only 1 (12.5%) of 8 mixed carcinomas were BAF250a[-]. Of the 22 pts with pure CCC, 14, 2, 3, and 3 were FIGO stages 1, II, III & IV respectively. Interestingly, all 5 BAF250a[-] cases were late stage [III,IV], meaning that 83% of all late stage cases were BAF250a[-], as compared with only 1 (6.25%) of the 16 early stage cases (p=.001). 1 of 5 BAF250a[-] cases showed lymphovascular invasion, as compared with 6 of 17 BAF250a[-] cases, an insignificant difference (p>.05). As may be anticipated from the concentration of late stage cases in the BAF250a[-] group, pt outcomes were worsened, at least on univariate analysis, in that group. Pt outcomes for the pure CCC group were as follows: Alive with disease, 6 pts; No evidence of disease, NED, 11 pts; Dead of disease, DOD, 4 pts; f/u unavailable, 1 pt. 60% of the 5 BAF250a[-] pts were DOD (the other 2 were NED), as compared with only 1 (6.25%)of 16 BAF250a[+] pts (p=0.02)

Conclusions: 22.7% of endometrial CCC display complete loss of BAF250a expression. Although formal outcome analyses cannot be performed on this dataset, some noteworthy and intriguing trends *did* emerge, including the disproportionate concentration of BAF250a[-] cases in the late stage group and the attendant possibility of an associated worsened prognosis. These preliminary findings suggest the need for larger analyses to evaluate the prognostic significance, if any, of the loss of BAF250a expression in CCC.

1129 The Pathologic Spectrum of Clinically Cystic Vulvar Lesions: A Single-Institutional, 10-Year Experience with 83 Cases

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Background: Vulvar cysts are known to be uncommon, but a systematic analysis of their full clinicopathologic spectrum as seen in a routine pathology practice is lacking. To facilitate patient counselling in patients presenting with a vulvar cyst, we conducted a study designed to define 1) the spectrum, frequency and proportional distribution of pathologic entities that are seen in patients presenting with a vulvar cyst, and 2) the proportion of those cases that are malignant neoplasms.

Design: An institutional database was queried for all vulvar cases that were designated as cystic in the clinical history or specimen description sections in a 10-year period (2001-2011). All cases (n=83, table 1) were reviewed and classified by gynecologic pathologists.

Results: The average patient age was 46.3 years and the mean aggregate size of submitted tissues was 2.05 cm. The most common cysts were follicular cysts (39.75%) and Bartholin cysts (22.9%). Only 3 cases were malignant (squamous cell carcinomas, SCC), but all 3 cases were recurrences in patients with known vulvar SCC. Specific clinical impressions were stated in 40 cases, and this matched the final pathologic diagnoses in 28. For the groups with ≥3 cases, the highest clinical/pathologic concordance rates were in Bartholin cysts and SCC (100%) whereas the lowest was in pseudocysts, granulation tissue and abscesses (25%). 7% (6/83) of these clinically cystic lesions were entirely solid pathologically.

Distribution of entities presenting as a vulvar cyst (n=83)

Lesion	Number of cases	Patient age (mean)	Average size	Clinical-Pathologic Concordance rate (%)
Follicular (Epidermoid) cyst	33	50.6	1.5	60
Bartholin cyst	19	42.8	2.9	100
Abscess, pseudocyst or granulation tissue	8	39.2	1.8	25
Mucinous cyst	4	37.7	0.95	0
Hidradenoma papilliferum	3	49	2	X
SCC	3	68	2.6	100
Mullerian cyst	2	40	2.1	0
Fibroepithelial polyp	2	39.5	0.6	X
Lipoma	2	45	4.7	X
Angioleiomyoma	1	45	4.5	0
Benign Phyllodes tumor	1	67	2	X
Myofibroblastoma	1	45	2.2	X
Hemangioma	2	35.5	1.4	0
Periurethral (Skene gland) cyst	1	31	1.5	100
Lymphangioma	1	37	4.5	100

X clinical impressions not stated

Conclusions: Clinically cystic vulvar lesions encompass a wide spectrum of pathologic lesions, data on which are outlined, which may be useful in the counselling of patients presenting with a vulvar cyst. Vulvar cysts are only rarely malignant (3.6%), and such lesions are typically recurrences of known malignancies. In this series, there were no patients without a history of malignant vulvar neoplasia presenting with a vulvar cyst that ultimately proved to be malignant.

1130 The Diagnosis of Endometrial Carcinomas with Clear Cells by Gynecologic Pathologists: An Interobserver Variability Study

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Background: The purposes of this study are to assess the level of interobserver variability in the diagnosis of endometrial carcinomas (ca) with clear cells by gynecologic pathologists, and to describe the cases of clear cell ca that display this variability.

Design: 11 gynecologic pathologists (median experience: 10 years) from 11 institutions in the US and Canada rendered diagnoses on glass slides from 35 endometrial neoplasms in a blinded fashion and without predetermined diagnostic criteria. The cases were selected from the files of 3 institutions by 2 authors based on their having *at least* focal clear cells, and had previously been diagnosed as clear cell, endometrioid, serous & undifferentiated ca (CC, EC, SC & UC respectively) at those institutions.
Results: The overall study kappa coefficient (κ) was 0.46, indicative of a "moderate" level of interobserver agreement. κ statistics for CC, SC, EC, mixed CC/SC & mixed EC/CC were 0.6, 0.35, 0.58, -.02, & 0.36 respectively. "Confirmed" CC cases (diagnosed as such by ≥80% of observers; n=12; Group 1) tended to be morphologically "classic": clear cells in ≥33% of tumor (10/12), hyalinized papillae (HP, 6/12), hobnail cells (HC, 6/12), small round papillae (SP, 8/12), tubulocystic patterns (TP, 4/12). Cases diagnosed as CC by at least 1 (9%) but <80% of observers (Group 2; n=13) displayed some of the same features but less frequently: clear cells in ≥33% of tumor (6/13), HP (3/13), HC (2/13), SP (6/13), TP (0/13). The groups did not significantly differ in lesional size, mitotic index, or predominant nuclear grade. EC and mixed ca were the most common non-CC diagnoses in Group 2. 44 semi-quantified morphologic variables were analyzed in a logistic regression model, and some traditionally CC-associated features, including hyaline globules and hobnails, were *not* predictive of confirmed CC from the overall group of 35 cases.
Conclusions: The diagnosis and histotyping of endometrial carcinomas with clear cells, even by experienced gynecologic pathologists, is associated with a moderate level of interobserver variability. For CC, this variabilty is related to the subset of cases displaying morphologic overlap with other histotypes and/or a subtotal composite of diagnostic features.

1131 The Role of Reticulum, Inhibin and Calretinin Staining and *FOXL2* Mutational Analysis in the Diagnosis of Sarcomatoid Granulosa Cell Tumors, Cellular Fibromas and Thecomas

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Background: Typical adult granulosa cell tumors are generally easily and reproducibly diagnosed on morphology alone. It has been recently documented that adult granulosa cell tumors presumably of typical type, contain the single somatic mutation *FOXL2* 402C→G (C134W) in approximately 95% of cases whereas it is detected in only about 15% of thecomas. We recently demonstrated a lower rate of *FOXL2* mutations among spindled or sarcomatoid adult granulosa cell tumors (GCT), diagnosed by pathologist consensus. This study aims to investigate the role the reticulin pattern, the immunoexpression of alpha-inhibin and calretinin, and the presence of *FOXL2* mutation, in the diagnosis of diffuse GCT, cellular fibroma (CF) and thecoma (Th), based on a consensus histological diagnosis.
Design: A total of 60 ovarian SCST of spindle pattern, including 32 GCT of diffuse type, 20 CF and 8 Th were retrieved from archives of Mayo Clinic. Reticulin, inhibin and calretinin stains were performed in all cases. The final diagnosis was established based on a consensus between 5 of 8 experienced pathologists. DNA was extracted from formalin-fixed, paraffin-embedded tissue sections followed by polymerase chain reaction and direct sequencing of *FOXL2* gene.
Results: The median age of the patients was 53 years old (range 20 to 87). Tumor size ranged from 0.6 to 23 cm. The results of reticulin, alpha-inhibin and calretinin are detailed on the table below.

Relation between diagnosis, FOXL2 mutation, Calretinin, Inhibin and Reticulin.

Consensus diagnosis	FOXL2(%)	Calretinin (%)	Alpha-Inhibin	Reticulin
GCT (n=32)	17 positive (53)	30 positive (94)	31 positive (97)	24 nested (75)
Cellular Fibroma	1 positive (5)	10 positive (50)	19 positive (95)	-
Thecoma (n=8)	2 positive (13)	4 positive (50)	7 positive (88)	1 nested (13)
Sensitivity	83	94	100	75
Specificity	36	56	8	93

The presence of a nested pattern on reticulum had a sensitivity of 78% and specificity of 74 % for detection of a *FOXL2* mutation.
Conclusions: These data demonstrate the difficulty in establishing a diagnosis of diffuse or sarcomatoid GCT. Neither the presence of the *FOXL2* mutation, calretinin or inhibin immunostaining or the presence of nests on reticulum stain easily confirmed the morphologic consensus diagnosis. Distinguishing between spindled sex cord stromal tumors remains difficult. Perhaps determining the best methods for their diagnosis may be established only by correlation with biologic behavior in long tern follow-up studies.

1132 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. There is no standardised way to isolate CSCs. Researchers tend to pick one technique and fail to comment on other approaches.
Disease progression of Ovarian Cancer correlates with the predictions of the cancer stem cell hypothesis. For this reason ovarian malignancy was chosen as a system in which to study CSCs, with the intention of understanding the variation of CSCs 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, Hoechst Side-Population and Cell Surface Protein Assays. Cells of interest were isolated via Fluoresence-Assisted Cell Sorting.
Carboplatin and Paclitaxel chemoresistance assays were carried out over 48 – 60 h. Cell viability was measured via MTT assay.
Results: Each screening technique identified putative CSCs (pCSCs) in one or more model systems. For any one model system only one of the three screens identified a pCSC population.
Two of these cell lines have been sorted into their pCSC and non-pCSC subpopulations. In both cases the pCSCs were able to regenerate the non-pCSC phenotype. It was noted that for confident proof of such asymmetric division, single cell plating is required.
There was no difference in chemoresistance between pCSCs and non-pCSCs in either cell line. Both sub-populations were isolated from chemoresistant cell lines.
Conclusions: The various techniques for isolation of CSCs do not mark the same cells within an ovarian cancer context. Each 'type' of ovarian cancer stem cells appears to be mutually exclusive. This may reflect different stages histologies of ovarian disease. pCSCs have been identified within chemosensitive cell lines, and work is in progress to isolate these sub-populations. Investigation of the differences between these chemoresistance and chemosensitive cancer stem cells could elucidate the mechanisms behind chemoresistant relapse.

1133 Incidental Pelvic Lymph Node Lymphangioleiomyomatosis in Women Undergoing Cancer Staging Surgery

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Background: Lymphangioleiomyomatosis (LAM) typically presents as a pulmonary disease in premenopausal women; it may be sporadic or associated with tuberous sclerosis complex (TSC). Rare cases of extrapulmonary LAM have been reported, including lymph node involvement. We report clinico-pathologic features of incidental LAM arising within pelvic lymph nodes removed for cancer staging and its distinction from metastatic cancer.
Design: Patients with extrapulmonary LAM were identified from a search of our pathology database from 1997-2011, with an emphasis on cases with intranodal involvement (LN LAM). Clinical and radiologic data was collected from electronic medical records. Histologic slides were reviewed. Immunohistochemical staining for melanocytic (HMB45) and myoid (desmin and/or smooth muscle actin) markers was performed.
Results: 14 patients had pelvic LN LAM, 13 of whom had no prior diagnosis of TSC or LAM (pulmonary or extrapulmonary). Of these 13 patients, 9 were post-menopausal and 4 pre-menopausal (age range: 39 to 70 years). Surgical indications included: endometrial adenocarcinoma (n=6), cervical squamous cell carcinoma (n=3), ovarian serous or clear cell carcinoma (n=2), ovarian mucinous borderline tumor (n=1), urinary bladder carcinoma (n=1). Among 8 patients with follow-up, none had other TSC lesions by radiographic or clinical report. One patient had LAM within the myometrium. Histologically, the lesions were fascicular proliferations of bland spindle cells with mildly enlarged oval nuclei and flocculent or foamy cytoplasm. Lesion size ranged from 1mm to 17mm. Mitoses were rare (0-1 per 10 high power fields). Multiple LNs, up to 6 total, were involved in 6 patients and 2 had bilateral pelvic LN involved. Metastatic carcinoma to pelvic LN was present in 2 patients and LAM co-existed in the same LN as the metastates but the morphologic and cytologic features were distinct. Intranodal endosalpingiosis co-existed with LAM in 1 patient. Immunohistochemical expression of myoid markers was present in 12/12 cases and HMB45 in 11/12. In the 1 patient with known TSC, that diagnosis preceded the finding of pelvic LN LAM.
Conclusions: Intranodal LAM can be found incidentally during staging surgery for gynecologic or urologic cancer. Unlike pulmonary LAM, most LN LAM occurs post-menopausally. The distinct morphology, lack of atypia or mitotic activity and myoid/melanocytic immunophenotype helps to distinguish intranodal LAM from nodal metastases of the malignancies which prompted nodal dissection.

1134 Prognostic and Pathogenetic Relevance of Embryonal Stem Cell Factors for Serous Ovarian Neoplasms

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Background: Primary resistance to chemotherapy and recurrent disease are the major causes of death for patients with ovarian carcinoma. Tumor stem cells and reexpression of stem cell factors are thought to contribute to both processes Expression of stem cell markers like Sox2 and ALDH-1 was already found in several solid tumors. In this study we performed a comprehensive analysis of Sox2, Nanog and Oct4 as well as ALDH-1, Ki-67, Cyclin D1 in serous ovarian tumors.
Design: 149 cases of ovarian serous carcinoma and 28 serous borderline tumors were analyzed by immunohistochemistry in a tissue microarray. Sox2, Oct4, Nanog, ALDH-1 and Cyclin D1 were evaluated in a semiquantitative score (0=0% positive cells, 1+ = 1-10% positive cells, 2+ = 11-50%, 3+ =51-80%, 4+ = >80% positive cells). For Mib-1/Ki67 the percentage of positive cells was analyzed by counting at least 100 nuclei. Coexpression and correlation of the markers were analyzed. Overall survival was compared by Kaplan-Meier analysis.
Results: For expression of Sox2 we find a significant association with high-grade serous carcinomas if compared to low-grade carcinomas or borderline tumors. We find no correlation between the expression of Sox2 and expression of ALDH-1, Cyclin D1 or Ki67. However, we find a significant correlation between expression of ALDH-1 and Ki67, indicating a role of ALDH-1 in cell proliferation independent from the stem cell phenotype. There was no detectable expression of Oct4 or Nanog. Survival analysis of high-grade serous carcinomas stage II-IV showed a favourable effect of Sox2 expression

on recurrence free survival (median survival 27 months vs. 21 months, p= 0.041) and a positive, but not significant effect on overall survival (39 vs. 25 months, p=0,062). For ALDH-1, however, we demonstrate no effect on recurrence free or overall survival.
Conclusions: Expression of embryonal stem cell factors SOX2 and ALDH-1 can be detected in serous ovarian tumors. We find association of different tumor characteristics with these factors like poor differentiation and improved survival with SOX2 and increased proliferation rate with ALDH. These findings suggest, that expression of several embryonal stem cell factors in serous ovarian tumors does not reflect a unique stem cell phenotype, but rather may show divergent association with biological and clinical parameters.

1135 Maternal Obesity and Gestational Diabetes Are Associated with Reduced Placental Efficiency

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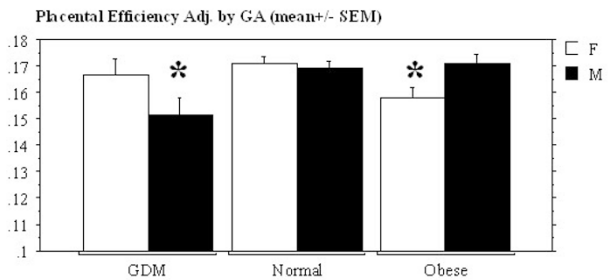
Background: Nearly one-third of reproductive-age women are obese and many of these pregnancies are complicated by gestational diabetes (GDM), which increases the risk of fetal growth abnormalities and stillbirth. The mechanisms underlying these increased risks are poorly understood. We hypothesize that obesity and gestational diabetes may significantly increase placenta growth, thereby decreasing available nutrients to the fetus, so-called reduced placental efficiency.
Design: Retrospective analysis of 518 singleton placentas with available maternal and fetal metrics including pre-pregnancy body mass index (BMI), gestational diabetes diagnosed by glucose tolerance test (GTT), gestational age at delivery, birth weight, placental weight, and neonatal gender (260 males; 258 females). Obesity was defined as BMI of 30kg/m2 or greater. Placental efficiency was defined as birth weight (grams)/ placental weight (grams). Data were analyzed by ANOVA with Bonferroni/Dunn post hoc testing.
Results: Approximately 40% of the mothers in our study were obese before pregnancy (211/518). Forty cases developed GTT proven GDM (8%). Placental efficiency increased with gestational age (R=0.58, p<0.0001) as fetal weight increased faster than placental weight. Therefore, all analyses were performed after adjusting for gestational age. We observed significant gender-effects. Compared to mothers with normal BMI, female babies from obese women without GDM (n=81) had significantly less placental efficiency (p=0.01) due to markedly increased placental weights (p<0.001). In contrast, males from obese women without GDM (n=90) had both increased birthweight and placental weight compared to normals and no significant decrease in placental efficiency. Although we had limited numbers of GDM cases, males (p=0.03), but not females, showed significantly reduced relative placental efficiency. This was a consequence of relatively greater placental weight (p<0.001) in these males compared with their birth weight and controls.



Conclusions: Given the current obesity epidemic, a better understanding between maternal BMI, gestational diabetes, and placental efficiency is required. Our data reveal significant gender effects and the potential importance of reduced placental efficiency in complications such as stillbirth.

1136 Atypical Polypoid Adenomyoma (APA) of the Uterus: A Clinicopathologic Study of 50 Cases

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Background: APA has been regarded as a benign tumor; however, it is often associated with endometrioid adenocarcinoma and its histologic diagnosis and biologic potential have been controversial.
Design: A multicentric study was carried out involving 15 institutions in Japan. Clinicopathologic features, morphology, and the effects of hormonal (medroxyprogesterone acetate) therapy and biologic behavior were studied in 50 APA cases.
Results: The patients' ages ranged from 22 to 66 (mean: 30) years. All but three of the patients were premenopausal. Histologically, the lesions were composed of a biphasic proliferation of architecturally complex and cytologically atypical endometrial glands with a myomatous or myofibromatous stroma. Squamous metaplasia or morules were observed in 37 cases. Eleven had evidence of background endometrial hyperplasia and 15 had endometrioid carcinoma (12 in APA and 3 in the adjacent endometrium). All 6 patients who were initially treated with curettage or polypectomy followed by hormonal therapy had residual or recurrent APA. Cases treated with hormonal therapy exhibited a decreased N/C ratio of epithelial cells, persistent atypical glandular structures, and a markedly edematous stroma. Hysterectomy was performed in 13 cases because a definite diagnosis could not be made preoperatively, the curettages raised the possibility of adenocarcinoma, or because there was a high possibility of residual or recurrent lesions. All showed residual or recurrent APA in hysterectomy specimens. Two showed superficial myometrial invasion, and two showed APA in a focus of adenomyosis. The overall residual or recurrent lesion rate was high (19/50, 38%). All patients were alive and well at 1 to 202 months (mean, 39.6 months).
Conclusions: The rate of recurrent or residual APA was high, and the effects of hormonal therapy were limited. The risk of endometrial carcinoma in women with APA is also high. This study suggests that APA should be carefully evaluated and cannot be automatically regarded as a totally benign entity. The findings indicate a continued risk for the development of endometrial adenocarcinoma in patients in whom complete excision of APA can not be guaranteed. If a confident diagnosis of APA has been made on curettage or polypectomy, hysterectomy is the treatment of choice. However, treatment by complete curettage or polypectomy may be undertaken, thereby, preserving the reproductive function, providing there is subsequent close follow-up.

1137 Ovarian Atypical Endometriosis: A Precancerous Lesion

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Background: Endometriosis is a relatively common condition found in up to approximately 30% of women undergoing laparotomy. But little is known about the incidence of ovarian atypical endometriosis (AEM) and its relation to ovarian carcinomas and the precancerous potential has been discussed.
Design: A series of consecutive cases coded as ovarian endometriosis (EM), ovarian carcinoma, or borderline tumor during the period 1987 to 2009 was retrieved from the surgical pathology files at our hospitals and was clinicopathologically analyzed. The diagnosis of AEM was based on the presence of epithelial features: large hyperchromatic or pale nuclei with moderate to marked pleomorphism; increased nuclear to cytoplasmic ratio; and cellular crowding, stratification, or tufting. Three or more of these criteria must be present.
Results: Atypical glandular changes without ovarian epithelial tumor were observed in 7 (1.1%) of 624 ovarian EM cases. The changes were always focal findings. One patient with ovarian AEM had synchronously endometrioid carcinoma and AEM in the subserosal of the uterus. One patient with ovarian AEM developed endometrioid carcinoma in the abdominal wall 18 months after left oophorectomy. The remaining five ovarian AEM patients without ovarian neoplasm did not develop any malignant epithelial tumors in a follow-up study with average of 6.2 years and a range of 3 to 9 years. Ninety-five (21.5%) of 442 ovarian cancers were associated with ovarian EM; 55 with typical EM and 40 with AEM. Clear cell and endometrioid carcinomas were most frequently associated with EM, with 40.2 % (41 of 102 cases) and 30.9% (21 of 68), respectively. AEM was fund in 20 clear cell carcinomas, in 12 endometrioid carcinomas, four serous carcinomas, three mucinous borderline tumors (Mullerian type) and one serous borderline tumor. In 16 cases, AEM was contiguous with carcinomas. Epithelial metaplasia, including eosinophilic, ciliated and mucinous metaplasia, was more often observed in AEM (47 of 47) than in ME (421 of 622).
Conclusions: Although ovarian AEM without neoplasms is a rare phenomenon, AEM possesses a precancerous potential and is most frequently associated with clear cell and endometrioid carcinomas. Epithelial metaplastic changes are also associated with AEM. Close screening of cellular atypia, hyperplasia, or epithelial metaplasia in ovarian EM is required. It is proposed that a diagnosis of AEM be followed by careful long-term observations of the patient to detect possible concurrent or subsequent development of neoplasia in the ovary or extra-ovarian sites.

1138 Choriocarcinoma at a First Trimester

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Background: The number of cases of choriocarcinoma (CC) with antecedent pregnancy of hydatidiform mole has been decreasing. Choriocarcinoma in situ (CCIS) (intraplacental CC), a neoplastic trophoblastic proliferation localized in the placenta, is a very rare condition and is usually diagnosed after or around a term delivery. CCIS and CC with lung metastases at a first trimester have been rarely studied.
Design: Clinicopathologic analyses were done on four cases of CCIS and a case of CC with lung metastases at a first trimester. The origin of CC and the significance of CC at early gestational stage were discussed.
Results: The patients ranged in age from 28 to 38 (mean: 31) years. They were gravida 2. para 1, G2.P0, G1.P0, G2.P1 and G4.P2. No patients had any past history of gestational trophoblastic diseases. All patients, at the gestational week of 8 to 11, had a dilation and curettage for vaginal bleeding and the absence of intrauterine fetus. No macroscopic abnormalities were noted in any cases. However, histologically, all four CCISs were characterized by localized nodules measuring 3 to 10 mm composed of atypical cytotrophoblasts, syncytiotrophoblasts and intermediate trophoblasts. The nodules appeared to arise directly from normal stem villi and projected into the intervillous space. Fetal elements were not observed in any cases. All of them were diploid by flow cytometry. Urinary beta-hCG levels were within normal range at the diagnosis in all CCISs. Radiographic studies showed no metastatic lesions in any patients. Follow-up study ranging from 1 to 3.5 years showed that all patients were free from disease without therapy. The remaining patient had elevated levels of urinary and serum beta-hCG and developed lung metastases. A dilation and curettage exhibited a proliferation of atypical and mitotically active cytotrophoblasts, syncytiotrophoblasts and intermediate trophoblasts in a large sheet pattern, hemorrhagic necrosis, minimal placental site reaction and the lack of villi. The patient has been treated with chemotherapy.
Conclusions: These results suggest an origin of CCIS from trophoblasts of a stem villus and CC may arise at any stage of pregnancy. It is assumed that almost all CCs at early gestational stage have been expelled as a spontaneous abortion or curettaged. Most of CCs at a first trimester would be unrecognized clinically and pathologically and many patients with CC would have uneventful clinical courses. This study indicates the need to perform thorough microscopic examination of the products of conception submitted, especially in the absence of a fetus or fetal parts.

1139 Characteristic Features of Serous Tubal Intraepithelial Carcinoma and Its Co-Existing Invasive Carcinomas

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Background: In the past several decades, the concept of serous ovarian carcinoma has been progressively revised. However, the exact pathogenesis remains controversial. The most recent concept for pelvic high grade serous carcinoma (HGSC) origin is the fimbriated end of fallopian tube. The objective of our study was to evaluate the characteristic features of serous tubal intraepithelial carcinoma (STIC).

Design: 97 cases with STIC were retrieved from our pathology database by using the keyword "serous tubal intraepithelial carcinoma". The pathology reports and selected slides were reviewed. Detailed clinical and pathological information was collected for analysis. The Standard SEE-FIM protocol was applied firmly in our institution since 2008. Tumors were classified as ovarian, peritoneal, tubal or endometrial primary based on conventional criteria.

Results: Of the 97 STIC cases (2007 to June 2011), 89 cases showed coexisting pelvic HGSCs. The remaining 8 included 3 STIC only cases, one STIC case coexisting with EIC, two cases with endometrioid endometrial adenocarcinoma, one with invasive cervical squamous cell carcinoma, and one with ovarian low grade serous carcinoma. Of the 89 STIC cases associated with HGSC, 51 (57%) were attributed to be of FT origin, 26 (29%) to be of ovarian origin, 9 (10%) of peritoneal origin, and 3 (4%) of endometrial origin based on the presence of the bulk of the tumor. Most of these tumors were widely disseminated at presentation with omental/peritoneal involvement seen in 92% cases, and lymph node and lymphovascular involvement in 49% and 48% cases, respectively. The age, race, and FIGO stage showed no significant difference among the four groups of HGSC.

Conclusions: To our knowledge, this is the largest study evaluating distribution of carcinomas associated with STIC. Although STIC is considered a precursor for most ovarian and primary peritoneal HGSC, the primary tubal cancers are still far more common in presence of a STIC compared with ovarian HGSC. Most ovarian HGSC might originate from the epithelium of the fallopian tube, but substantial percentage of the cases may not be related to STIC. The high percentage of omental involvement (92%) in these tumors suggests direct extension of the tumor cells due to associated STIC.

1140 Proliferation in the Normal Tubal Epithelium Is a Hallmark of the Follicular Phase Not BRCA1 Mutation Status

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Background: BRCA1/2 mutation carriers are at increased risk of ovarian/tubal high grade serous carcinoma. We recently demonstrated that the fallopian tube epithelium (FTE) of BRCA1 mutation carriers have altered signaling pathways compared to controls. We sought to determine whether these differences give a proliferative advantage to the epithelial cells in this high-risk patient population and whether the differences are also due to intraepithelial immune infiltrating cells.

Design: Immunohistochemistry for Ki67 and p53 were performed on histologically normal tubal epithelium (ampulla, TMA n=76, BRCA1/2 versus non-BRCA1/2) and histologically normal fimbria (whole sections, n=18) with known ovarian cycle status at the time of surgery. To determine whether fimbria with cancer precursor lesions have an overall increased proliferative index, we analyzed precursor lesions (n=44) from women with and without BRCA1/2 mutations who underwent risk-reducing salpingo-oophorectomy. We determined the contribution of $CD3^+$, $CD8^+$, $CD20^+$ and $CD68^+$ cells within the tube epithelium in the TMA and fimbria and in 15 cases of Serous Tubal Intraepithelial Carcinoma (STIC). Analysis of the Ki67 stain was performed by 3 independent observers (blinded) using digital slides, where 0 < 1%, 1 = 1%, 2 = 2-4%, 3 = 5-15% and 4 > 4% positive FTE cells.

Results: There was no significant difference (p=0.5711, p=0.7577) in Ki67 positivity between BRCA1/2 (n=42) vs nonBRCA (n=34). A significant increase in $CD68^+$ cells, intercalated in the FTE, was observed in the luteal (n=32) compared to the follicular phase (n=44); (p=0.0027). No significant differences were observed by ovarian cycle or mutational status for $CD3^+$ $CD8^+$ or $CD20^+$ cells. As demonstrated by gene expression profiling (previously published), the FTE-BRCA cases have a higher immune signature particularly in the luteal phase (GO terms: MHC class II p=0.0055, MHC protein complex p=0.021 and chemokine receptor binding p=0.014) than FTE-nonBRCA in either phase of the menstrual cycle.

Conclusions: BRCA1/2 mutation carriers exhibited no significant increase in proliferation of the tube epithelial cells in either the ampulla or fimbriated ends. There was a significant increase in proliferation as measured by Ki67 staining in follicular phase epithelium. A subset of immune cells within the epithelium are increased during the luteal phase. We also demonstrated a marked increase in lymphocytes invading cancer precursor lesions, suggesting lymphocytes may have a role early in tumor formation.

1141 P16/MIB-1 Immunoreactivity and HPV DNA Status in Tubal Metaplasia of Endocervical Epithelium: Is There Any Correlation?

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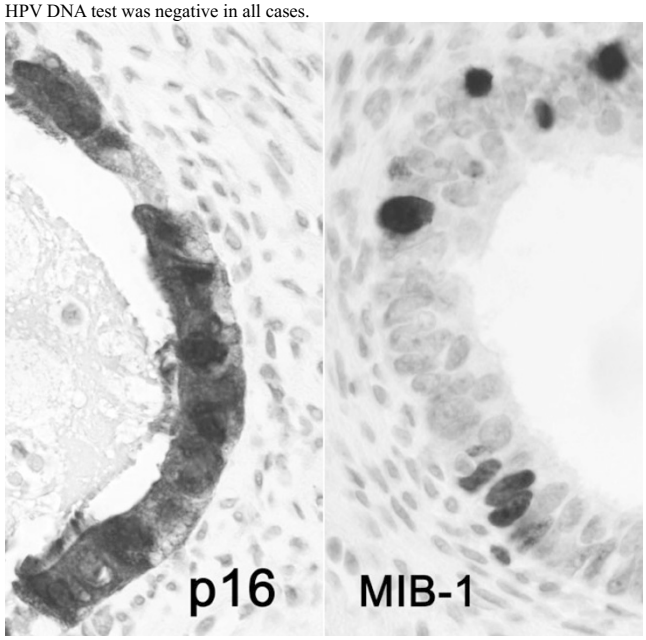
Background: Studies have shown that p16 over-expression occurs in more than half of endocervical tubal metaplasia (TM) and as a result, it is speculated that TM may be a precursor of endocervical glandular neoplasm. In addition, it has been shown that most cases of endocervical glandular neoplasm are associated with HPV infection. In this study, we evaluate p16 over-expression and HPV status in TM to investigate any correlation between these potential neoplastic markers.

Design: From June 2005 to September 2011, all cases diagnosed as AGUS on liquid based cytology with concurrent high-risk HPV DNA testing and subsequent biopsy proven TM were selected. Biopsies containing concurrent squamous dysplasia were excluded due to potential confounding by HPV positivity in dysplastic squamous epithelium. A total of 26 patients with an age range of 21-49 were identified. HPV DNA test results were collected. P16 and MIB-1 immunostains were performed on 17 cases for which the pathology materials were available. MIB-1 stain was scored as <10%, 10-30%, >30% nuclear staining. P16 stain was graded as negative, focal+ (<50% cytoplasmic and nuclear stain) and diffuse+ (>50% cytoplasmic and nuclear stain).

Results: Eight out of 17 cases were negative for p16 stain, 5/17 were focally and 4/17 were diffusely positive. Cases with negative p16 stain demonstrated minimal nuclear changes and architecturally benign endocervical glands lined by cilia. However, cases with focal or diffuse p16 positivity showed significant nuclear atypia including nuclear enlargement, hyperchromasia, and pseudostratification. In all cases, MIB-1 stain was either negative or showed <10% of nuclear staining. Of particular interest, high-risk HPV DNA test was negative in all cases.



Conclusions: This is the first study to evaluate the pattern of p16 over-expression in endocervical TM and its correlation with HPV status. The distribution and intensity of p16 over-expression in TM tended to directly correlate with the degree of nuclear atypia. More importantly, no correlation was found between p16 over-expression and HPV status in TM. The association between p16 over-expression and premalignant potential of TM warrants further investigation.

1142 Does GVHD Involve the Gyn Tract? Immunohistochemical Expression of Elafin as a Marker of Graft-Versus-Host Disease in Gynecological Biopsies

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Background: Graft-versus-Host Disease (GVHD) is a common complication of allogenic bone marrow transplant affecting frequently skin, liver and gastrointestinal tract. Involvement of the gyn tract, particularly vulva and vagina has not been well characterized, and the diagnosis depends on clinical criteria that is sometimes unspecific. Recently, Elafin, an elastase inhibitor secreted in response to cytokines (IL-1 and TNF alpha) has been proposed as a good biomarker to recognize GVHD in skin biopsies. In this study, we describe the morphological findings of 16 patients with GVHD and gynecologic lesions and the use of IHC for Elafin in patients with GVHD.

Design: 30 biopsies (vulva (11), cervix (7), vagina (5), endometrium (3), endocervix(2); 1 areola and 1 perianal region were obtained from 16 patients with lesions suspicious of GVHD, reviewed and stained by IHC with the Elafin antibody. Ten skin biopsies with known GVHD were used as control. A positive Elafin expression was defined as a staining that extend to a depth of >50% of the mucosa or epidermis.

Results: Patients mean age was 35.8 y. Most frequent symptoms included vaginal dryness and discharge, dyspareunia, vulvar discomfort and vaginal scarring. Nine cases had GVHD in the eye, skin, mouth, GI, lung and liver. Morphologically, the vulvar biopsies showed changes similar to GVHD in the skin with occasional ulceration, apoptotic bodies in the basal layer of the epidermis, and chronic inflammatory cells predominantly lymphocytes. The vaginal biopsies showed increased fibrosis, inflammation and variable degrees of VIN and condyloma. Rare apoptotic bodies were also seen. The cervical biopsies had marked acute and chronic cervicitis. One case was involved by leukemia and in another an abnormal lymphoid infiltrate was present. The mucosa had occasional apoptotic bodies. Rare apoptotic bodies were also found in the endometrial glands. HPV was identified in 8 patients. For the Elafin marker, 11 biopsies were interpreted as positive. These biopsies corresponded to those highly suspected of GVHD. Patients received treatment with topical steroids, topical estrogens and dilators.

Conclusions: Our results suggest that the gynecologic tract is affected by GVHD with vulva and vagina being the areas more frequently affected. Morphologic changes are

similar to those occurring in the skin and other organs. Elafin over expression was found in cases with histological changes of GVHD and suggest its potential usefulness to diagnosis GVHD in patients that are clinically suspicious.

1143 Expression of Stem Cell Marker ALDH1 in Cervical Intraepithelial Neoplasia

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Background: Cervical cancer is the second most common type of cancer in women worldwide. The human papilloma virus (HPV) has recently been shown to specifically target cervical cancer stem cell (CSC) s. The ability to identify and characterize CSCs is crucial for understanding cervical cancer. Aldehyde degydrogenase (ALDH) 1, a detoxifying enzyme responsible for the oxidation of intracellular aldehydes, has been shown to be a stem cell marker in several tissue types and tumors. We investigated the expression pattern of ALDH1 in cervical intraepithelial neoplasia (CIN) and its possible role in carcinogenesis.

Design: Fifty-six cervical biopsy specimens with the diagnoses of normal cervical mucosa, koilocytosis, CIN1, CIN2, and CIN3 were collected from the pathology files at Thomas Jefferson University Hospital. All diagnoses were confirmed by at least two experienced pathologists. Immunohistochemical staining for ALDH1 was performed following a previously published protocol. Any cytoplasmic staining of ALDH1 in the epithelial cells was considered positive. Positive and negative controls were used in each study.

Results: Normal cervical mucosa had no ALDH1 expression. In koilocytosis, 2 of 7 cases showed ALDH1+ cells limited to the basal layer. In CIN1, 11 of 20 cases showed ALDH1+ cells limited to the lower 1/3 of epithelium and the other 9 cases were negative for ALDH1. In CIN2, 7 of the 13 cases showed positive cells limited to the basal layer, 4 cases showed positive cells extending to the mid and upper 1/3 of the mucosa. Two cases were negative for ALDH1. All CIN3/carcinoma in situ (CIS) cases were positive for ALDH1. Three of 6 cases show positive cells extending up to mid 1/3 of mucosa and the other three cases show positive cells extending up to the upper 1/3 of mucosa.

Conclusions: Cervical dysplasias show positive staining for ALDH1 compared to normal cervical mucosa. ALDH1 expression and distribution parallels the degree of cervical dysplasia. CSCs, as detected by ALDH1 expression, may play a role in the progression of cervical intraepithelial neoplasia and carcinogenesis.

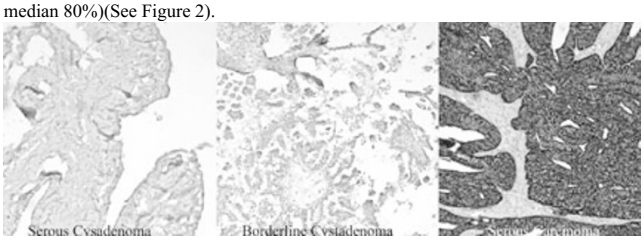
1144 Oncofetal Protein IMP3, a Molecular Marker for the Malignant Progression of Ovarian Serous Neoplasm

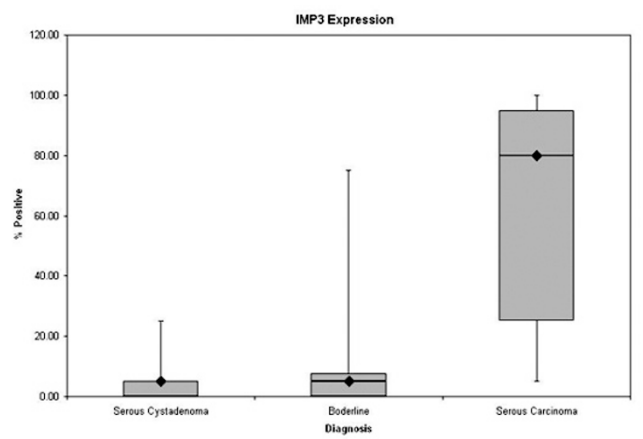
S Goodman, X Yang, D Lu. Umass Memorial Medical Center, Worcester, MA.

Background: The aim of this study was to establish the expression pattern and diagnostic value of IMP3 in benign serous cystadenomas, borderline cystadenomas, and serous carcinomas of the ovary.

Design: A total of 108 cases (oopherectomy specimens) (serous cystadenoma, n=15; borderline cystadenoma; n=17, serous carcinoma; n=74) obtained from the surgical pathology files of the Umass Medical Center were examined by immunohistochemistry for IMP3 expression. The epithelial/tumor cell staining with IMP3 was recorded by percentage for each lesion. Positive staining in greater than 15% of tumor cells is a positive result.

Results: IMP3 demonstrated dark brown cytoplasmic staining(See Figure 1). IMP3 expression averaged 5% in cystadenomas (range 0-25%, median 5%), 12% in borderline tumors (range 0-75%, median 5%), and 64% in serous carcinomas (range 5-100%, median 80%)(See Figure 2).





Conclusions: IMP3 is highly expressed in malignant serous tumors of the ovary but not, or rarely in benign or borderline tumors. The data suggest that IMP3 may play an important role in malignant transformation in serous ovarian cancer. Negative staining for IMP3 can increase the level of confidence in establishing a definitive diagnosis of a benign or borderline tumor, and positive IMP3 staining for a malignancy.

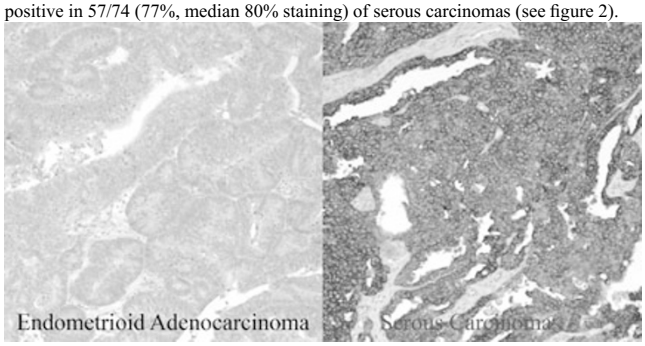
1145 IMP3 Expression Differentiates Ovarian Serous from Endometrioid Carcinoma

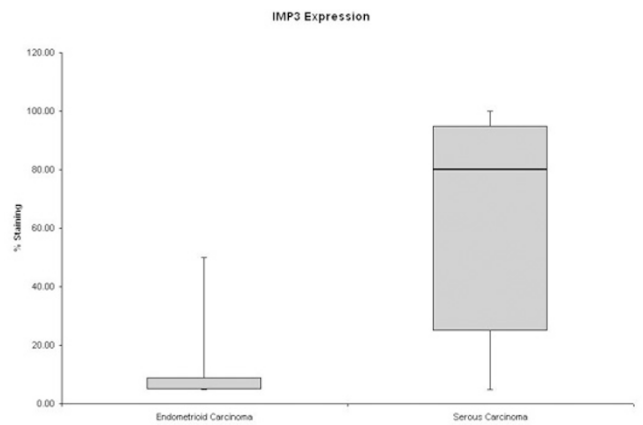
S Goodman, D Lu. Umass Memorial Medical Center, Worcester, MA.

Background: Ovarian carcinoma is characterized by a wide range of tumors. Histologically, differentiation of serous from endometrioid carcinoma can sometimes be difficult, and so far there is no reliable biomarker available. The aim of this study was to compare the expression pattern and diagnostic value of IMP3 in endometrioid and serous carcinomas of the ovary.

Design: A total of 84 cases (endometrioid carcinoma, n=10; serous carcinoma, n=74) obtained from the surgical pathology files of Umass Memorial Medical center were examined by immunohistochemistry for IMP3 expression. The epithelial/tumor cell staining with IMP3 was recorded by percentage for each lesion. Expression in more than 15% of tumor cells is a positive result.

Results: IMP3 showed dark brown cytoplasmic staining (see figure 1). IMP3 expression was positive in 1/10 (10%, median 5% staining) of endometrioid carcinoma cases, and positive in 57/74 (77%, median 80% staining) of serous carcinomas (see figure 2).





Conclusions: IMP3 is expressed in majority of serous carcinoma but only rarely in endometrioid carcinoma. Positive IMP3 staining can increase the level of confidence in differential diagnosis of endometrioid vs. serous carcinoma.

1146 Utility of FISH in the Molecular Diagnosis of Ovarian Germ Cell Tumors

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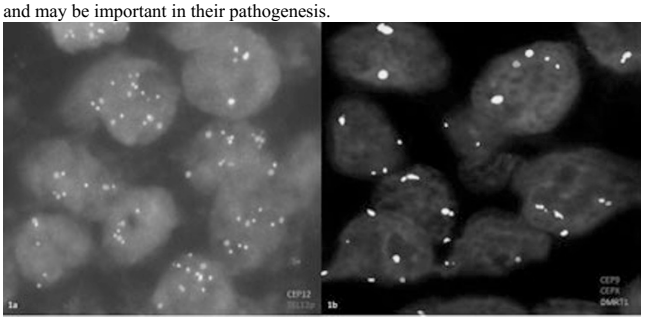
Background: Malignant ovarian germ cell tumors (GCT) are uncommon neoplasms that can be diagnostically challenging, and are important in the differential diagnosis of a variety of tumors. Little is known about their molecular pathogenesis. Fluorescent in-situ hybridization (FISH) has been proven to be useful as a molecular tool for the diagnosis of testicular GCT, and the presence of isochromosome 12 (i(12p)) has been demonstrated in dysgerminoma, the most common malignant ovarian GCT.

Design: Cases of primary ovarian GCT were retrieved: 59 cases, including 4 gonadoblastomas (GNB), 2 embryonal carcinomas (EC), 6 immature teratomas (IT), 2 struma ovarii (SO), 21 yolk sac tumors (YST) and 24 dysgerminomas (DYS), were obtained. Interphase FISH was performed on formalin-fixed paraffin embedded tissue as previously described, using the following probes: spectrum orange centromeric (CEP) 12, spectrum green subtelomeric (Tel12p), spectrum orange CEP 9, and spectrum acqua CEP X (Vysis, Abbott Molecular). We also used a homemade probe for the *DMRT1* gene.

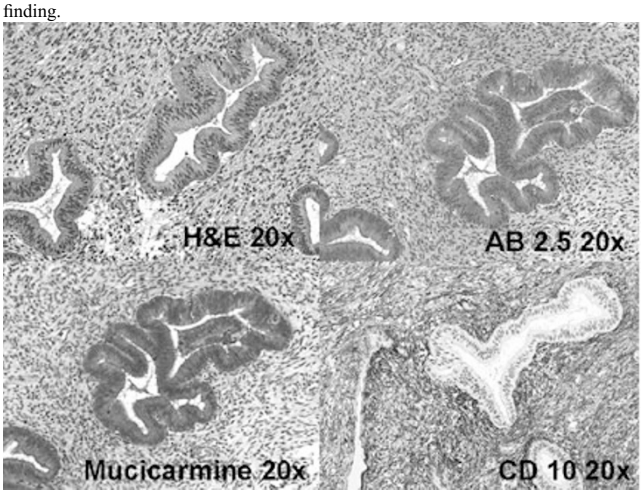
Results: FISH detected several chromosomal abnormalities: all cases of GNB, SO, and the majority of IT lacked i(12p) or 12p overrepresentation. Monosomy X was seen in 50% of GNB and 20% of IT. Polysomy X and 9 was seen in 25% of GNB and 33% of IT. YST had i(12p) or 12p overrepresentation in 28% of cases. 30% had polysomy X and 15% monosomy X. 15% showed either polysomy or monosomy 9. Among DYS, 59% showed 12p overrepresentation (fig.1a) and 29% had i(12p). 27% had either monosomy or polysomy X. 18% showed polysomy 9 and 9% monosomy 9. Two cases (1 YST and 1 DYS) showed *DMRT1* deletion (fig.1b). In the 2 cases of EC,

one show numerical chromosomal gains, and the other was normal. The 2 cases of SO had normal cytogenetic findings. 12p overrepresentation was more common in YST and DYS, compared to GNB, SO and IT (50% vs 9%, p=0.02). 52% of GCT showed numerical changes of chromosomes X and 9, and *DMRT1* amplification was not present.
Conclusions: Contrary to the preexisting evidence, i(12p) or 12p overrepresentation has a relatively low sensitivity (59%) for dysgerminoma diagnosis but a good specificity (83%). Numerical changes in chromosomes X and 9 are very frequent in ovarian GCTs and may be important in their pathogenesis.



1147 Endometrial Glands with Mucinous Metaplasia in the Setting of Chronic Endometritis: A Newly Described Finding

PY Gulavita, B Djordjevic. University of Ottawa, The Ottawa Hospital, Ottawa, ON, Canada.
Background: Mucinous metaplasia is a rare phenomenon in non-neoplastic endometrium. For the first time, we present a case series of mucinous endometrial metaplasia in the setting of chronic endometritis.
Design: We reviewed 77 endometrial biopsies and curettings with chronic endometritis from our institution over a period of 4 years. The histological diagnosis of endometritis was made by establishing the presence of inflammatory cells in the endometrial stroma including plasma cells and any lymphocytes, eosinophils or neutrophils. Presence of endometrial stroma was confirmed with CD10 staining. Mucinous metaplasia in the involved endometrial glands was confirmed with mucicarmine and Alcian blue (pH2.5) staining.
Results: Endometritis was diagnosed in a number of clinical settings, including 44 cases of recent pregnancy with retained products of conception, 28 cases of intrauterine contraceptive device use, 3 cases of uterine leiomyomata, 1 case of uterovaginal prolapse, and 1 case of endometrial hyperplasia. Of these cases, we identified 3 with mucinous metaplasia of endocervical type. Two of these cases were in the setting of dilation and curettage for retained products of conception, and 1 in the setting of intrauterine contraceptive device use. In all 3 cases, mucinous metaplasia was a focal finding.



Conclusions: Mucinous endometrial metaplasia is uncommonly seen in endometritis. In endometrial biopsies, it may be confused with endocervical glands. As diagnosis of endometritis often relies on identification of plasma cells, which may be difficult to detect, awareness of the possibility of mucinous endometrial metaplasia in endometritis may be a useful diagnostic aid.

1148 The Stem Cell Associated Transcription Factor Sox2 as a Diagnostic Marker of Cervical Neoplasia

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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. Similar to other solid tumors, cervical carcinoma contains a heterogeneous population of cancer cells. Recent studies identified and characterized a cancer stem-like population from primary carcinomas of the cervix uteri by RT-PCR, and demonstrated the expression of adult stemness-related genes, including Sox2. In this study, we assed the expression of SOX2 in cervical intraepithelial neoplasia (CIN) grade I-III and invasive squamous cell carcinoma.
Design: Tissue microarrays were constructed with tissue cores from paraffin embedded biopsy material of 54 patients. Included were cases of CIN I (n=12), CIN II (n=14), CIN III (n=9), invasive squamous cell carcinoma (n=14) and normal cervix as control. The tissue microarrays were examined using immunohistochemistry for the expression and localization of Sox2. Expression of SOX2 was semi-quantitatively scored by intensity (0-3) and percentage of cells staining.
Results: In normal cervix, Sox2 was negative or expression confined to the basal cell layer of the epithelium. In low-grade dysplasia (CIN I), Sox2 positivity was seen in the basal 1/3 of the epithelium (100% of cells), mostly of moderate intensity. Cell revealing HPV associated changes usually expressed SOX2. In high-grade dysplasia, moderate to strong SOX2 expression was confined to the basal 2/3 of the epithelium in CIN II (100% of cells), and full thickness expression was seen in CIN III (100% of cells). In invasive squamous cell carcinoma, moderate to strong SOX2 expression was present in 13/14 cases (93%) in 40-100% of tumor cells. One case was negative for SOX2 expression. Squamous metaplasia and endocervical glands were devoid of SOX2 expression in all cases where they were present.
Conclusions: These findings suggest that reactivation of SOX2 is involved in the progression of cervical neoplasia and is an early step in cervical carcinogenesis. SOX2 is a useful marker for detecting cervical dysplasia on biopsy specimens. In addition, SOX2 is helpful for accurate grading of the dysplastic changes. All cases included in this study could be classified, without difficulty, as high-grade or low grade dysplasia based on the staining pattern. SOX2 however is not a suitable marker to distinguish high-grade dysplasia from invasive squamous cell carcinoma, as both reveal strong expression of this marker.

1149 Survey of Mammaglobin Expression by Immunohistochemistry in Gynecologic Carcinomas

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Background: Mammaglobin-A (MGA) has been proposed as a sensitive and specific immunohistochemical marker for breast carcinoma. The extent to which MGA immunoreactivity differs between breast and gynecologic lesions is not known, although the differential diagnosis of breast carcinoma versus a gynecologic primary arises frequently.
Design: We performed a survey of MGA immunoreactivity in 221 gynecologic samples from 191 patients, including 27 benign gynecologic tissues (6 ectocervix, 9 endocervix, 12 endometrium), 13 endocervical adenocarcinomas, 72 endometrial adenocarcinomas, and 88 ovarian adenocarcinomas. Staining intensity was scored as weak (1+), moderate (2+), or strong (3+). The extent of staining was scored as focal (<10% of cells), patchy (10-50%), and diffuse (>50%).
Results: Gynecologic specimens often showed weak (1+) diffuse MGA staining, which we interpreted as being nonspecific. For analysis we thus considered only 2+ or 3+ staining as being significant. In benign tissues, MGA was detected in 0/6 ectocervices, 5/9 endocervices, and 8/12 endometria. Among malignancies, MGA was detected in 1 of 13 endocervical adenocarcinomas (8%; patchy distribution), 12 of 21 endometrial endometrioid carcinomas (57%; 4 focal, 8 patchy), but only 1 of 18 endometrial clear cell carcinomas (5%; focal) and 5 of 31 endometrial serous carcinomas (16%; focal). MGA was present in 6 of 15 ovarian endometrioid carcinomas (40%; 2 focal, 3 patchy, 1 diffuse), 4 of 19 ovarian clear cell carcinomas (21%; 2 focal, 2 diffuse), 1 of 16 ovarian mucinous carcinomas (6%; focal) and 12 of 36 ovarian serous carcinomas (33%; 5 focal, 6 patchy, 1 diffuse). The 3 tumors with the strongest MGA reactivity were all serous carcinomas (ovarian, focal 3+; endometrial, focal 3+; endometrial, patchy 3+). Considering all cases with 2+ or 3+ MGA reactivity, the distribution of this reactivity was focal or patchy (i.e., not diffuse) in 52 of 60 cases. There were no cases with diffuse 3+ MGA expression, as is seen in many breast carcinomas. On the other hand, diffuse 2+ MGA was seen in 4 cases including one endometrioid carcinoma of ovary, one serous carcinoma of ovary, and two clear cell carcinomas of ovary.
Conclusions: A significant proportion of gynecologic carcinomas are immunoreactive for MGA. MGA expression is not specific for breast carcinoma, and gynecologic carcinomas should be considered in the differential diagnosis of MGA-positive malignancies.

1150 Sarcoma Histology, Percentage of Sarcoma, and Tumor Necrosis Are Prognostically Neutral in Uterine Carcinosarcoma

IS Hagemann, D Cao. Washington University School of Medicine, St. Louis, MO.
Background: Uterine carcinosarcoma (malignant mixed Mullerian tumor) is an aggressive variant of endometrial carcinoma which often presents as a necrotic mass, with variable histologies and percentages of sarcoma. The prognostic significance of these factors is uncertain.
Design: We reviewed all uterine carcinosarcomas resected at our institution from 1991 to 2009 for which complete records were available. Reports and slides were reviewed to extract clinicopathologic data including patient demographics, staging, and the histology, relative abundance, and percent necrosis of both carcinoma and sarcoma components. Patient outcomes were retrieved from clinical and public records, with overall survival used as the primary endpoint.
Results: A total of 94 cases were available for inclusion (FIGO stage I, 53 cases; stage III, 27 cases; stage IV, 13 cases), with a mean follow-up interval of 32.7 months. Univariate analysis confirmed the prognostic significance of tumor stage (p=0.0006, log-rank test), of lymphovascular invasion within stage I tumors (p=0.0058), and of depth of myometrial invasion (p=0.0016). Homologous vs. heterologous sarcoma histology was not, overall, prognostically significant, although in patients surviving more than 40 months, homologous differentiation became a favorable indicator. Upon review of intrauterine tumor histology, necrosis was present in the carcinoma component of 49% of cases and, in these cases, averaged 10% in extent. The sarcomatous component

showed necrosis in 65% of cases and, in these, averaged 13% in extent. Cases with and without necrosis of either component did not significantly differ in stage (p>0.05, Student's t), nor did they differ in overall survival when compared stage for stage. The percentage of sarcoma ranged from 1% to 99% (mean 54%), but this factor was also not significantly associated with overall survival.

Conclusions: The presence of tumor necrosis, while dramatic, is not prognostically significant in uterine carcinosarcoma. Likewise, the relative percentage of sarcoma and carcinoma does not predict survival. Homologous differentiation, historically considered favorable, appears in this series to be favorable only in long-term survivors.

1151 Lower Uterine Segment Involvement in Low Grade Endometrioid Endometrial Adenocarcinoma: A Predictor of Disease Progression and Survival

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Background: The clinicopathologic significance of lower uterine segment involvement (LUSi) in management of patients with endometrial adenocarcinoma remains controversial. Although the presence of LUSi is not incorporated into the FIGO staging system as an independent criterion, recent studies have found significant associations with other FIGO prognostic factors. However, an association with adverse clinical outcomes has yet to be elucidated. We investigated the prognostic significance of LUSi in patients with low grade endometrioid endometrial adenocarcinoma, focusing on its association with staging criteria and clinical outcomes, specifically recurrence-free survival (RFS) and overall survival (OS).

Design: Surgical pathology archives of endometrial adenocarcinoma from 2004-2009 were reviewed. Of 417 patients, 294 met inclusion criteria and 130 were excluded due to high grade/aggressive histologic subtypes; synchronous gynecological carcinomas; neoadjuvant therapy. Prognostic data including tumor histology, myometrial invasion, LUSi, lymphovascular invasion (LVI), nodal metastasis, and FIGO stage were recorded. Fisher's exact, Chi-square, and rank-sum tests were used to evaluate the association of LUSi with other prognostic data. Kaplan Meier analysis was used to assess the relationship of LUSi with OS and RFS.

Results: LUSi was present in 95 patients (32.3%) with low grade endometrioid adenocarcinoma. LUSi was associated with presence of LVI (p <0.0001), >50% myometrial invasion (p <0.0001), and grade 2 tumors (p =0.0056). There were 12 local recurrences and no disease-related deaths. Nine patients (10.23%) with LUSi had local recurrence compared with 3 patients (1.79%) without LUSi (p=0.004). RFS at 5 years was 69.5% for patients with LUSi compared with 98.9% for patients without LUSi (p=0.0022).

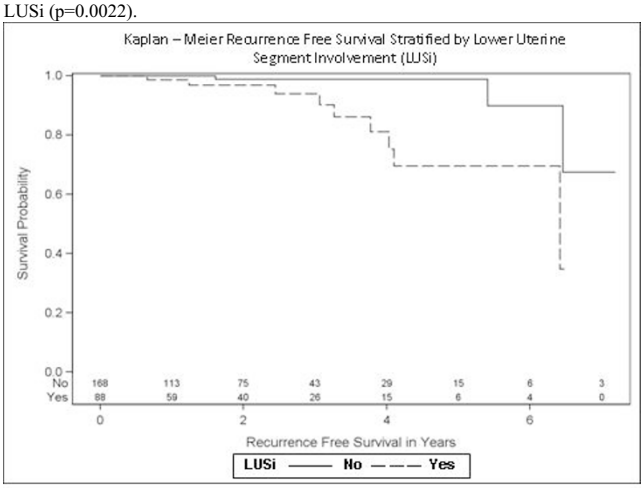

Figure 1. Numbers above x-axis represent patients still at risk for recurrence at one year intervals. There is a significant difference between the curves (p=0.0022) by log-rank test.

Conclusions: The presence of LUSi in patients with low grade endometrioid endometrial adenocarcinoma is associated with significantly decreased RFS and is significantly associated with lymphovascular invasion. Lower uterine segment involvement should be considered a poor prognostic indicator in operative management with regard to surgical staging and clinical treatment.

1152 Claudin-18, MUC1, MUC2, and MUC5AC Are Differentially-Expressed in Ovarian Intestinal- and Endocervical-Type Mucinous Borderline Tumors

SA Halimi, D Maeda, M Fukayama. Graduate School of Medicine, the University of Tokyo, Tokyo, Japan.

Background: Ovarian mucinous borderline tumors (MBTs) are currently classified into two types: intestinal (IMBT) and endocervical (EMBT). Distinguishing between the two types is important, because their histogenesis and clinicopathological features differ significantly. The aim of this study was to analyze the expression of Claudins (CLDNs), which are tight junction proteins known to be differentially expressed in various tumors, in ovarian IMBTs and EMBTs. Mucin (MUC) expression was also examined to reveal the mucin profiles of these two lesions.

Design: A total of 62 cases of ovarian MBTs (40 IMBTs and 22 EMBTs) were retrieved from the archives of the Department of Pathology of the University of Tokyo Hospital. MBTs were classified into IMBTs and EMBTs, based on the review of hematoxylin and eosin stained slides of all the cases. The MBT tissues were arranged in tissue microarrays (TMAs) with duplicate 2 mm tissue cores obtained from the tumor area in each MBT. Immunohistochemistry for Claudins (CLDN1, 3, 4, 7 and 18), MUCs (MUC1, 2, 5AC and 6), CK7, CK20, CA125, ER, PgR and vimentin were performed on the TMAs.

Results: CLDN18 expression was observed in all IMBTs, whereas only one EMBT was CLDN18-positive. There was no significant difference in the expression of CLDNs 1, 3, 4, and 7 between IMBTs and EMBTs. With regards to MUCs, MUC1 was more frequently expressed in EMBTs than in IMBTs. Positive immunoreactivity for MUC2 and MUC5AC were observed more often in the IMBTs. ER, PgR, and vimentin expression were seen almost exclusively in the EMBTs.

	IMBT	EMBT	p-value
CLDN1	32/40 (80%)	20/22 (91%)	0.2638
CLDN3	40/40 (100%)	22/22 (100%)	-
CLDN4	38/40 (95%)	22/22 (100%)	0.2864
CLDN7	40/40 (100%)	22/22 (100%)	-
CLDN18	40/40 (100%)	1/22 (5%)	<0.0001
MUC1	17/40 (43%)	22/22 (100%)	<0.0001
MUC2	19/40 (48%)	3/22 (14%)	0.0077
MUC5AC	37/40 (93%)	14/22 (64%)	0.0044
MUC6	10/40 (25%)	4/22 (18%)	0.5390
CK7	40/40 (100%)	22/22 (100%)	-
CK20	29/40 (73%)	1/22 (5%)	<0.0001
CA125	9/40 (23%)	22/22 (100%)	<0.0001
ER	1/40 (3%)	22/22 (100%)	<0.0001
PgR	1/40 (3%)	16/22 (73%)	<0.0001
Vimentin	0/40 (0%)	22/22 (100%)	<0.0001

Conclusions: Frequent expression of CLDN18, MUC2, and MUC5AC by IMBTs supports a "gastrointestinal" phenotype of the IMBT tumor cells, since CLDN18 and MUC5AC are known gastric epithelial markers and MUC2 is a colonic marker. Furthermore, our study indicates that ER, PgR, vimentin, and CA125 can be used as endocervical-type markers, and CLDN18 and CK20 can be used as intestinal-type markers, for classifying ovarian mucinous tumors into two subsets.

1153 *B-RAF* V600E Mutations in Endometrial Adenocarcinoma

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Background: The *EGFR-KRAS-BRAF* pathway plays a critical role in the development of many types of malignancies including lung, colon and ovarian cancers. Previous studies have only reported the detection of *B-RAF* mutations other than the V600E mutation in endometrial adenocarcinoma. Our current study aims to investigate the prevalence of *B-RAF* V600E mutations in endometrial adenocarcinoma in our patient population.

Design: With IRB approval, nineteen (19) endometrial adenocarcinoma cases were selected for this study. Genomic DNA was extracted from formalin-fixed paraffin-embedded tissue sections that were microdissected to ensure more than 80% tumor cells. *B-RAF* V600E mutations were first screened using mutant allele-specific real-time PCR. Positive cases were then verified by direct sequencing using capillary electrophoresis. *B-RAF* V600E mutation results were also compared to the *K-RAS* results obtained from our previously reported study.

Results: *B-RAF* V600E mutations were detected in 3 of 19 (15.8%) endometrial adenocarcinomas, significantly higher than what has been reported in the literature (P<0.02, Fisher's Exact Test, compared to 0/44, Kawaguichi M et al., 2009). Two of the cases positive for *B-RAF* V600E mutations were endometrioid adenocarcinoma while the third was endometrioid adenocarcinoma with significant mucinous differentiation. These three positive cases did not harbor *KRAS* mutations.

Conclusions: Unlike previous reports in endometrial adenocarcinoma, *B-RAF* V600E mutations were found in our patient group using two different technical approaches. These results, compared to our previous *K-RAS* study (Mod Pathol 2011;24 Supp 1 :270A), indicate that *B-RAF* V600E mutations contribute to the tumorigenesis of endometrial carcinoma at a lower frequency than *K-RAS* mutation. Further investigation of *B-RAF* V600E mutation in sub-types of endometrial adenocarcinoma and its prognostic significance is strongly warranted.

1154 Mutually Exclusive Expression of PAX8 and p63 in Transitional Cell Tumors of the Ovary

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Background: Transitional cell tumors of the ovary(OTCT) include benign, borderline and malignant Brenner tumors(BT) and transitional cell carcinoma of the ovary(OTCC). All are uncommon, collectively comprising less than 2% of ovarian tumors. They histologically resemble urothelial neoplasms of the urinary tract, potentially posing diagnostic pitfalls. PAX8 is a developmental transcription factor and a diagnostic and histogenetic marker for Müllerian epithelial tumors. Its expression has not been fully examined in OTCT. In this study, we investigate the expression of PAX8 in OTCT utilizing immunohistochemistry(IHC) and provide additional information regarding the histogenesis and differential diagnosis of these intriguing tumors.

Design: 25 benign BT, 1 malignant BT, and 23 OTCC were retrieved, and a tissue microarray(TMA) was assembled with 3 cores from each case. Walthard rests(WR) from 5 salpingectomy specimens and TMA containing 41 invasive TCCs of the urinary bladder(UTCC) from radical cystectomy specimens were also examined. IHC with PAX8, p63, and CD44 was performed. Only distinct nuclear staining was considered to be positive for PAX8 and p63. The staining intensity was graded as negative, weak(1+), moderate(2+) and strong(3+).

Results: All of the WR and benign BT were strongly positive for p63, while all of the OTCC were negative. All WR and benign BT were negative for PAX8, while all of the OTCC and the 1 malignant BT were strongly and diffusely positive for PAX8. In

addition, all of the WR and benign BT were 2+ for CD44, while OTCC showed variable staining for CD44(negative in 5, 1+ in 9, 2+ in 8, and 3+ in 1 case(s)). All of the UTCC were consistently negative for PAX8, but had variable staining for p63(negative in 13, 1+ in 25, 2+ in 2, 3+ in 1 case(s)). Staining of CD44 in UTCC was also variable(negative in 9, 1+ in 9, 2+ in 13, and 3+ in 10 cases).

Conclusions: We have demonstrated a mutually exclusive expression of PAX8 and p63 in OTCT. Detection of PAX8 in OTCC confirms that this tumor is of Müllerian origin and thus is a variant of common ovarian carcinomas. Absence of PAX8 in WR and benign BT suggests a non-Müllerian origin of these lesions. The dual positive staining of p63 and CD44 indicates that WR and benign BT immunohistochemically resemble the basal cells of the stratified epithelium. In summary, PAX8, in combination with p63, is a useful marker to distinguish benign BT from malignant BT and OTCC. Furthermore, PAX8 is more reliable than p63 and CD44 in distinguishing OTCC from metastatic UTCC in the ovary.

1155 *K-ras* Mutations in Mucinous Lesions of Uterus

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Background: *K-ras* mutations are often seen in malignancies of lung, pancreas, colon or ovary displaying mucinous differentiation. Our previous study showed that *K-ras* mutation can be found in approximately 70% of mucinous carcinoma and endometrioid carcinoma with significant mucinous differentiation (MC&ECMD), significantly higher than other types of endometrioid carcinoma (EC). Current study expanded the mutation study to the mucinous lesions of uterus in order to gain further understanding of the molecular nature and characteristics of these lesions.

Design: With IRB approval, seven "atypical mucinous (endometrial) hyperplasia (AMH)", six "mucinous (endometrial) metaplasia (MM)" and nine "microglandular hyperplasia of cervix" were obtained from the archival files of the Department of Pathology. Previous MC cases with mutations served as positive control. Six normal cervical biopsy materials were used as negative control. Genomic DNA was extracted from formalin-fixed paraffin-embedded tissue sections that were microdissected to ensure more than 80% of lesional cells. PCR amplification for *K-ras* codons 12 and 13 were performed and followed by sequencing using capillary electrophoresis. The sequencing results were analyzed by "Sequence Scanner v1.0" program.

Results: *K-ras* codons 12 and 13 mutations were detected in 5 of 7 (71.4%) AMH, significantly higher than reported in literature (P=0.005, compared to Feng YZ et al., 2005). One of the six (16.7%) MM presented with *K-ras* mutation. No *K-ras* mutations were detected in normal cervical material or microglandular hyperplasia. These results provided a significant difference of *K-ras* mutation between AMH and MG of cervix (P=0.005, Fisher's Exact Test).

Conclusions: Significantly higher prevalence of *K-ras* mutations was found in the AMH group compared what has been reported in atypical endometrial hyperplasia in literature. Combined with previous result of high prevalence of K-ras mutations in MC&ECMD, it indicated a close association between *K-ras* mutation and mucinous differentiation, and suggesting possible unique pathogenic pathway for MC&ECMD. Detection of K-ras mutations in mucinous metaplasia suggested a possible neoplastic process. Significant difference between AMH and MG of cervix provided valuable molecular aid in differential diagnosis, esp., with limited biopsy material.

1156 Differences in Mismatch Repair Protein Expression of Cervical Adenocarcinoma and Carcinoma of the Lower Uterine Segment

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Background: Carcinomas of the lower uterine segment (LUS) with prominent cervical involvement need to be distinguished from primary cervical adenocarcinoma (PCAC) because of differences in behavior and treatment. Furthermore, an association of carcinoma arising in the LUS with impaired DNA mismatch repair protein (MMRP) expression predictive for Lynch syndrome has been described, while MMRP expression in PCAC is not well characterized.

Design: H&E slides and pathological reports from 42 hysterectomy specimens of cases originally classified as PCAC, were reevaluated for cervical and endometrial precursor lesions and LUS involvement. Cases with LUS involvement (LUS+) in the absence of cervical precursor lesions (CPL-) were subjected to PCR analysis for HPV-DNA. LUS+/CPL-/HPV- cases were reclassified as LUS carcinoma (LUSC). All cases were analyzed immunohistochemically for expression of the MMRP proteins MLH1, MSH2, MSH6, PMS2 as well as for expression of ER, vimentin, CEA, and p16 expression. Immunohistochemical expression patterns of PCAC were compared to LUSC using Fisher's exact test.

Results: A total of five cases with a LUS+/CPL-/HPV- status were reclassified as LUSC (5/42, 11.9%). Of the remaining 37 PCAC, 25 (67%) had an IHC-pattern typical for cervical adenocarcinomas (ER-, Vimentin-, CEA+) and 35 (94%) stained moderately to strongly positive for p16. None of the LUSC cases had an IHC pattern typical for cervical adenocarcinomas and none was diffusely positive for p16. All PCAC had retained MMRP expression, but loss of MMRP expression was found in 4/5 LUSC ($p < 0.005$). One mixed type serous/ endometrioid and one undifferentiated LUSC had loss of MSH2 and MSH6 (suggestive for MSH2 germline mutation). One mucinous LUSC showed isolated loss of PMS2 (suggestive for PMS2 germline mutation), one mixed type serous/ endometrioid LUSC showed loss of PMS2 and MLH1. One LUSC of endometrioid subtype had retained MMRP expression.

Conclusions: Impaired MMRP expression, predictive for Lynch syndrome, is frequent in LUSC and uncommon in genuine PCAC, therefore LUSC should be screened for MMRP expression. As LUSCs with involvement of the uterine cervix are liable to be misclassified as PCAC, uterine carcinoma ambiguous for site of origin need to be subjected to ancillary studies including MMRP expression.

1157 A Candidate Cell of Origin for Cervical Cancer

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Background: Squamous cell carcinoma of the cervix and its precursor (cervical intraepithelial neoplasia) are thought to develop either within, or in close proximity to, the squamo-columnar junction (SCJ) of the ecto and endocervix. Despite this assumption, one issue unresolved in cervical cancer research is the identity of the normal cervical cell type in the SCJ in which tumorigenesis begins.

Design: We discovered an immunophenotypically unique cell population at the SCJ. Using microarray analysis, we compared the gene expression profiles of the ecto and endocervical epithelia with the squamo-columnar junctional cells. Embryo and adult mouse cervical specimens were studied *in situ* to identify, characterize and determine the dynamics of junctional cell development. Candidate biomarkers targeting the junctional population were applied to 90 cases of CIN and 10 cervical squamous and adenocarcinomas. In addition, cervical SCJs following cervical LEEP/conization were analyzed for retention of the SCJ-specific cells.

Results: Human adult cervices contained a immunohistochemically unique population of cuboidal cells at the SCJ. Analysis of embryonic and postnatal mouse tissues revealed specific lining cells in the lower genital tract that over time become exclusive to the region of the SCJ. Expression analysis of laser-captured cells from the ecto and endocervix and the SCJ revealed a panel of markers unique to the junctional cells; this biomarker panel consistently immunostained both low and high grade CINs associated with carcinogenic human papillomaviruses (HPV) via strong expression of $p16^{ink4}$. A second population of low grade CINs were identified that arose caudal to the SCJ. This group was negative for the SCJ-specific biomarkers. New SCJs following LEEP/ conization did not display the immunophenotype of the SCJ cells.

Conclusions: This study establishes, for the first time, the presence of a unique cell population in the cervix that is selectively retained at the SCJ at birth, possesses an immunophenotype identical to carcinogenic HPV-associated neoplasms, and may be lost or reduced following surgical excision of the SCJ. Moreover, it suggests that low grade CINs can be subdivided immunophenotypically into putative ectocervical and SCJ origins. The attributes of the junctional epithelium described above are consistent with a cell of origin for cervical cancer at the SCJ.

1158 Endometrial Biopsy Interpretation Using WHO 2004 and EIN Criteria: An Analysis of 77 Cases with Emphasis on Conservative Management

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Background: Endometrial Intraepithelial Neoplasia (EIN) is a recently described, alternative staging system for endometrial hyperplasia that has been cited as a discerning and reproducible means of predicting the development of endometrial adenocarcinoma. In this study we compare the WHO and EIN classifications using lesions previously classified as hyperplasia, and correlated them with clinical outcome in a patient population treated with hormone therapy.

Design: Seventy-seven WHO classified hyperplasias (all types) from 2004 to 2011 were analyzed. The reviewers were unaware of the initial diagnosis. EIN criteria were applied (gland to stroma ratio at least 55%, cytologic alteration, size > 1mm, exclusion of mimics/cancer). Reclassified cases were compared to the WHO classification, and correlated with clinical treatment and pathologic follow up.

Results: 77 cases (70 with follow up) were available for review; 38 (49%) met criteria for EIN. These 38 were originally classified as complex atypical hyperplasia (CAH) (14, 37%), complex hyperplasia (CH) (23, 60%) and simple atypical hyperplasia (1, 3%). The 39 cases that did not meet EIN criteria consisted of polyps (10, 26%), subdiagnostic atypical or crowded glands (13, 33%), benign hyperplasia (3, 8%) and benign endometrium (13, 33%). Of the 70 patients with available follow-up, 33 (47%) were treated with hormones. Of these, 15 patients (45%) had negative follow up biopsies. 2 patients (6%) went to hysterectomy and were diagnosed with carcinoma (5 & 11 months), and 1 (4%) had residual EIN on follow up. 15 patients (45%) had no biopsy follow up (12 had no evidence of disease clinically, 2 had persistent bleeding but were not re-biopsied, and 1 was lost to follow up). Clinical follow up ranged from 1-83 months with a mean of 16 months. None of the patients who did not meet EIN criteria developed endometrial carcinoma, and all patients who developed endometrial carcinoma were reclassified as EIN during the review.

Conclusions: EIN criteria is sensitive (100%), has an excellent negative predictive value (100%) in that none of the cases that did not meet EIN criteria developed carcinoma, and provides an objective approach to endometrial biopsy interpretation. Medical management of EIN is a viable alternative to surgical therapy in the proper clinical setting, with only 6% progressing to carcinoma over our follow up period. Due to the high negative predictive value of the EIN criteria, patients without EIN may be conservatively managed.

1159 Atypical Uterine Polyps Sub-Diagnostic of Mullerian Adenosarcoma: A Clinicopathologic Analysis of 28 Cases with Long Term Followup

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Background: Mullerian adenosarcoma (MA) is a rare mixed tumor composed of benign glands with papillary (leaf-like) or dilated glands and frequent polypoid intraluminal projections of neoplastic stroma; however, the diagnosis is challenging because many lesions are subtle. We examined the clinicopathologic features and outcome of patients

who had uterine polyps that raised the possibility of, but were not diagnostic for, MA.
Design: A search of institutional archives (1990-2011) for biopsies diagnosed as atypical endometrial or endocervical polyps that had some, but not all, features of MA was performed and 28 cases were retrieved. The frequency of the diagnosis of benign uterine polyp in this time frame was also determined. All 28 cases were evaluated for presence/absence and distribution (focal vs diffuse) of stromal cellularity, altered periglandular stroma, stromal atypia, mitoses, phyllodes-like architecture, intraluminal polypoid projections, and rigid cysts. Partial involvement of the polyp was also recorded. Clinical followup data was obtained when possible.
Results: The diagnosis of atypical endometrial polyp was made in < 0.2% of all uterine polyps diagnosed at our institution. 27/28 (96%) cases had followup (f/u) information; 15 (56%) had f/u biopsy(ies) only, 9 (32%) had TAH +/-BSO, and 3 (11%) had clinical f/u without further pathologic sampling. 1 patient died of pancreatic cancer, but all other patients are alive with NED (range in f/u 5.8-236 mo; mean 142.6 mo). 1 case was reclassified as MA on our review; this patient had benign f/u biopsy and is disease free at 82 mo. 23 (82%) cases showed partial involvement of the polyp. The most common findings were: periglandular stromal alteration (86%; 4 focal, 2 vague); phyllodes-like architecture (79%; 8 focal, 1 multifocal); and rigid cysts (46%). Intraluminal polypoid projections in the absence of phyllodes architecture were uncommon. Stromal atypia was rare (2 cases; 1 focal, 1 multifocal). Mitoses ranged from 0-11/10 HPF; and were not seen in 8 cases. The case with the highest mitotic count had partial involvement of the polyp, focal phyllodes-like architectural changes, and diffuse periglandular stromal alteration.
Conclusions: While periglandular stromal alterations and phyllodes-like architecture often prompt consideration of MA, uterine polyps that have some, but not all features of MA, have a benign clinical followup and need not be treated by hysterectomy if careful followup is feasible. Reconsideration of the threshold for diagnosis of MA also is warranted.

1160 Stathmin, a Microtubule Destabilizing Protein, Is Overexpressed in Most High, but Not Low Grade, Cervical Squamous Intraepithelial Lesions

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Background: A fundamental controversy in using biomarkers to diagnose cervical precursors (ie, cervical intraepithelial neoplasia, CIN) is their specificity for CIN2/3. Stathmin (STMN), a microtubule destabilizingprotein important in mitosis, is overexpressed in a variety of malignancies, and may be associated with poor outcome. STMN expression in cervical neoplasia has never been explored.
Design: Cervical samples (N=191) of non-neoplastic (N=25), non-invasive neoplasia (N=82 CIN, N=19 AIS), invasive squamous cell carcinoma (SCC) (N=31), and invasive adenocarcinoma (ACA) (N=34) were evaluated for STMN, p16, and Ki67 expression by immunohistochemistry (IHC). H&E stained cervical biopsies were independently scored by 3 expert gynecologic pathologists. Squamous lesions were classified as benign, CIN1, CIN2, or CIN3 based on a majority diagnosis (≥2/3 agreeing); cases without agreement were reviewed together and majority opinion was obtained. Each diagnosis was correlated with STMN, p16, and Ki67 expression. IHC interpretation was blindly performed by 2 pathologists.
Results: A majority diagnosis was obtained in 187/191 cases on initial review. Squamous epithelia were classified as benign, CIN1, CIN2, and CIN3 in 25, 56, 11 and 15 cases, respectively. STMN was normally expressed in ectocervical basal cells and was absent in benign endocervix. Positive STMN staining was defined as immunoreactivity in at least 2/3 of the epithelial thickness. STMN was positive in 5/56 (9%) CIN1, 5/11 (45%) CIN2, and 14/15 (93%) CIN3 ($p < 0.001$), and all cases of AIS, SCC, and ACA. In contrast, 25/56 (45%) CIN1, 11/11 (100%) CIN2, and 14/15 (93%) CIN3 lesions were p16 positive. p16 was also expressed in 5 (20%) benign biopsies. All 30 p16 positive benign and CIN1 biopsies were STMN negative. Ki67 was increased in all neoplastic and some non-neoplastic epithelia.
Conclusions: STMN is overexpressed in virtually all cervical carcinomas and CIN3 lesions. In contrast to p16, which can stain CIN1 and reactive epithelia, STMN has greater specificity for CIN3, and will distinguish these lesions from the majority of low grade precursors and negative cervical biopsies. STMN overexpression appears independent of proliferation, maturation, and HPV cytopathic effect, and may be useful in identifying higher grade CIN at greater risk for progression (CIN3). STMN can also be used to confirm AIS or invasive carcinoma. Further studies correlating STMN staining with lesion persistence or morphologic progression, in the context of CIN grade, are warranted.

1161 EGFR Protein Expression and Genetic Amplification in High-Grade Pleomorphic Uterine Sarcomas

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Background: Epidermal growth factor receptor (EGFR) is known to be frequently expressed in undifferentiated endometrial sarcomas (UES) and the sarcomatous component of malignant mixed mullerian tumors (MMMT or carcinosarcoma), but little is known regarding its genetic basis. Only a single case of UES with *EGFR* amplification which showed a significant but temporary response to imatinib has been previously reported (Ann Diagn Pathol. 2007;11(1):49-54). The aim of the current study is to evaluate EGFR copy number and amplification status in a series of non-smooth muscle high-grade pleomorphic uterine sarcomas.
Design: We identified a series of 14 high-grade non-smooth muscle primary uterine sarcomas (hysterectomy specimens) through retrospective review of pathology archive at Vancouver General Hospital since 2000. All cases demonstrated high-grade nuclear features with diffuse and marked nuclear pleomorphism and none showed features of endometrial stromal sarcoma. These included 8 UES, 4 uterine sarcomas with rhabdomyosarcomatous differentiation (US-RMS), 1 uterine sarcoma with osteosarcomatous differentiation (US-OSA) and 1 adenosarcoma that is nearly completely overgrown by high-grade undifferentiated sarcomas (US-AD). EGFR (Dako) immunostaining was performed on representative whole sections and scored using Her-2/neu scoring criteria for breast cancer. Fluorescence *in situ* hybridization (FISH) for *EGFR* and *CEP7* (LSI EGFR/CEP7, Abbott Molecular, 40 tumor nuclei evaluated) was performed in cases that showed 3+ EGFR protein expression.
Results: Seven of the 14 cases showed 3+ EGFR protein expression (4 UES, 2 US-AD, 1 US-RMS and 1 US-OSA). Interpretable FISH was obtained in five cases with 3+ EGFR staining with the remaining two cases to be repeated (data pending). Among these 5 cases, one case (US-OSA) showed *EGFR* amplification (*EGFR*/*CEP7* ratio 2.07) while the 3 other cases (1 UES, 1 US-RMS and 1 US-AD) showed increased *EGFR* copy numbers in >50% of the nuclei evaluated with similar corresponding increase in *CEP7* signals.
Conclusions: A significant subset of non-smooth muscle high-grade uterine sarcomas strongly expresses EGFR and the data here suggest that *EGFR* amplification or increased copy number appear to be the underlying genetic mechanism for the observed protein expression. These findings, together with the prior reported case of *EGFR*-amplified UES provide a compelling rationale for therapeutic targeting of EGFR in these high-grade uterine sarcomas.

1162 Mucinous Carcinoma of the Endometrium: Multi-Institutional Clinicopathologic Study of a Series of 47 Patients

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Background: Mucinous Carcinoma of the Endometrium (MCE) is a rare histologic type representing less than 5% of all endometrial carcinomas. The aim of this study is to evaluate the clinicopathologic characteristics and patients' outcome of this tumor.
Design: A retrospective review of our pathology database of patients with endometrial carcinoma in two large academic centers between 1995 and 2009, was performed. To qualify as MCE, tumors should show at least 50% of mucinous differentiation, with tumor cells showing intracytoplasmic mucin. H and E slides were retrieved and reviewed (2 to 8 slides per case). Clinical and pathological variables including FIGO grade, lymphovascular invasion, lower uterine segment (LUS) involvement, lymph node status, FIGO stage, recurrence, and survival were assessed. Statistical analysis using Chi-Square test and Kaplan Meier method was performed.
Results: Forty seven patients with MCE were identified. Median age was 62 years (range 43-91). The patients' clinicopathologic characteristics are listed in Table 1.

Table 1: The clinicopathologic characteristics of the 47 patients

Variable	Number of patients (%)	
FIGO grade	I	29(60.3%)
	II	18 (39.7%)
	III	0
LUS involvement		11 (23.4%)
Lymphovascular invasion		6 (12.7%)
FIGO Stage	I	36 (76.6%)
	II	3 (6.4%)
	IIIA	2 (4.2%)
	IIIB	0
	IIIC	6 (12.8%)
	IV	0
Recurrence		5 (10.6%)

Median follow up was 62 months (range 15-189). All patients underwent surgical staging with lymph node evaluation. Only three patients received adjuvant chemotherapy after surgery. Recurrence was seen in five patients (10.6%) (4 patients with stage III and 1 with stage II). Median time of recurrence was 13 months (range 8-30). The 5-year relapse free survival was 86.3%. On univariate analysis, predictive factors of recurrence were stage IIIC ($p=<0.0001$), LUS involvement ($p=0.003$), and grade II ($p=0.001$). Multivariate analysis was not attempted due to small sample size and very few events.
Conclusions: To the best of our knowledge, this study is one of the largest series of MCE. Based on our cohort, the predictive factors of recurrence are stage, grade, and LUS involvement. The outcome of patients with FIGO stage I-II MCE is excellent with surgical staging alone.

1163 Papillary Proliferation of the Endometrium: A Clinicopathologic Study of 56 Cases

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Background: Papillary proliferation of the endometrium (PPE) (Am J Surg Pathol 2001; 25:1347-1354) is uncommon and has been the subject of only a few studies. Histologically, it ranges from simple papillae with fibrovascular cores, typically involving endometrial polyps, to complex intracystic proliferations. Some consider the latter appearance nonatypical complex hyperplasia.
Design: To further characterize PPE, with emphasis on the risk of and features associated with underlying neoplasia, the clinicopathologic features of 56 cases of PPE without cytologic atypia were studied. The architectural complexity, extent of the proliferation, and any co-existent hyperplasia of conventional type (WHO classification) were recorded.
Results: The age of patients ranged from 23 to 82 years (median, 53); thirty-six (64%) were postmenopausal. The majority presented with abnormal bleeding. Thirteen patients (23%) were receiving hormonal preparations. The diagnosis of PPE was made in 47 biopsies and in 9 hysterectomies. In 46 cases (82%), there was an endometrial polyp. Fifty (89%) had epithelial metaplasias, most commonly mucinous (39 cases, 78%). Co-

existent non-atypical and atypical hyperplasia was found in 8 and 5 cases, respectively. In 7 cases (12.5%) a low-grade endometrioid carcinoma was found either in the follow-up sample or hysterectomy specimen. In 4 of these, the neoplastic component was separate from the PPE. Features of PPE associated with a concurrent or subsequent atypical hyperplasia or carcinoma included complex or elongate papillae with frequent second and third degree branching, multifocality, and crowded intracystic papillae. Histologic features associated with uneventful outcome included simple papillae with absent or occasional second degree branching, detached or localized papillae confined to the surface of a polyp and a background of atrophic endometrium.

Conclusions: PPE most commonly occurs in postmenopausal women and is commonly associated with an endometrial polyp and epithelial metaplasias. Localized and simple lesions are usually associated with a benign outcome, and may be appropriately labeled as "benign papillary proliferation of the endometrium". Architecturally complex papillae are likely to have an increased risk of carcinoma and should probably be regarded as analogous to complex hyperplasia. Patients with PPE should receive follow-up, including repeat sampling.

1164 mTOR and HIF-1 Pathway Inhibitors: Exploring the Potential in Clear Cell Carcinoma Variant of Ovary and Endometrium, Comparing with That of Kidney

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Background: The inhibitors of mammalian target of rapamycin (mTOR) and hypoxia-inducible factor-1α (HIF-1α) pathway molecules have been approved to treat advanced clear cell renal cell carcinoma (RCC). Ovarian clear cell carcinoma (OCC) and endometrial clear cell carcinoma (ECC) exhibit similar morphology and have been reported to share overlapping gene expression profiles with clear cell RCC. Our objective was to study the expression of HIF and mTOR pathway markers in OCC and ECC, and to compare the patterns to those present in clear cell RCC as a rationale for investigating potentially similar treatment approaches for OCC and ECC.

Design: Immunohistochemical staining using antibodies against mTOR pathway markers mTOR, PTEN, phospho-S (p-S6), and phosphor-4E binding protein 1(p-4E BP1); and HIF-1 pathway marker Glucose transporter-1 (Glut1) were performed on tissue microarrays constructed from 39 clear cell RCC, 33 OCC, and 29 ECC. Nuclear and/or cytoplasmic expression was evaluated for p-S6, p-4E BP1, Glut1 and P27 markers based on the intensity of staining (graded 0-3) and the percentage of positive cells. PTEN immunostain was assessed as expressed or not. Fisher's exact test with two tails was used to compare expression levels of markers between clear cell RCC and OCC, and between clear cell RCC and ECC. The level of significance was assigned at $P < 0.05$.

Results: Comparing clear cell RCC to OCC and ECC, a high expression of p-4E BP1 in all three tumor system was noted. However, OCC and ECC revealed a significantly higher expression of mTOR ($P < 0.0001$ for OCC, 0.001 for ECC), p-S6 ($P = 0.04$ for OCC, 0.0008 for ECC), and Glut1 ($P = 0.006$ for OCC, 0.0006 for ECC) than clear cell RCC, while loss of PTEN expression was significantly higher in clear cell RCC than in OCC and ECC ($P = 0.0006$ for OCC, 0.0008 for ECC).

Immunohistochemical staining results

	clear cell RCC	OCC	ECC
p-4E BP1	71% (n 38)	76% (n 17)	79 % (n 28)
p-S6	54% (n 37)	78% (n 32)	93% (n 27)
PTEN (loss of expression)	82% (n 39)	42% (n 33)	37.5% (n 24)
mTOR	28% (n39)	78% (n32)	70% (n27)
Glut1	49% (n 39)	82% (n 33)	90% (n 29)

Conclusions: Expression of mTOR and Glut-1, and loss of PTEN expression are significantly higher in OCC and ECC, which introduces these tumors as more susceptible for mTOR and HIF-1 inhibitors than clear cell RCC. Over-expression of mTOR and Glut1 in combination with loss of PTEN expression might be used as predictive markers for OCC and ECC response to such inhibitors.

1165 Juvenile Granulosa Cell Tumors: Immunoreactivity for CD99 and Fli-1 and EWSR1 Translocation Status. A Study of Eleven Cases

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Background: The accurate diagnosis of a juvenile granulosa cell tumor (JGCT) can be challenging, as these neoplasms often exhibit morphologic features which overlap other ovarian neoplasms. Additionally, the immunohistochemical profile exhibited by JGCTs is fairly non-specific and may include reactivity with CD99. Recently, we noted that JGCTs can show strong immunohistochemical expression of Fli-1, a transcription factor expressed by Ewing's sarcoma, a neoplasm which is occasionally in the differential diagnosis with JGCT. We evaluated a series of JGCTs, to determine whether Fli-1 is commonly expressed by these tumors, and whether or not they demonstrate chromosomal rearrangements in EWSR1.

Design: Cases diagnosed as JGCT (n=11) were immunohistochemically evaluated for the expression of Fli-1 and CD99. Fluorescence in situ hybridization (FISH) was performed on all cases to look for chromosomal rearrangements involving EWSR1.

Results: All eleven of our cases exhibited positive immunohistochemical staining for Fli-1 and CD99. None of these cases demonstrated a rearrangement in EWSR1 by FISH.

Conclusions: In cases of JGCT which cannot be reliably distinguished from Ewing's sarcoma based on morphology and immunohistochemistry alone, FISH testing for EWSR1 rearrangements can be a useful diagnostic adjunct for their separation.

1166 Use of HPV Insertional Mutation as a Molecular Marker of Circulating Tumour DNA

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Background: The detection of circulating tumour DNA requires the identification of a specific molecular motif on primary tumour and is hampered by the low concentration of circulating tumour DNA (ct DNA) in circulating non tumour DNA.

We looked whether the genomic rearrangement secondary to the clonal integration of HPV DNA sequences into the host genome may be used as a highly specific marker to detect ct DNA in patients with HPV-associated cervical carcinoma.

Design: Nine cases of HPV16-associated invasive cervical carcinoma, corresponding to stage I (2 cases) to stage IV (one case) were analyzed. HPV integration locus was characterized using the Detection of Integrated Papillomavirus Sequenced (DIPS-PCR) method. Cell free circulating DNA was isolated from serum specimens taken before treatment in each case. Sequential serum specimens taken during the course of the disease were also available for 2 of these 9 cases. A PCR-assay was designed to specifically amplify the cell-viral DNA junctions and the minimal amount of tumour DNA detectable with this assay was determined in reconstruction experiments using serial dilutions of tumour DNA into Tris-EDTA buffer.

Results: HPV integration sites were located at different loci in the 9 cases. According to reconstruction experiments, the PCR-assay was able to detect a concentration as low as 0.5 pg/ml of tumour DNA in Tris-EDTA buffer. Using the 9 specific assays, ct DNA was detected in 7 of the 9 serum specimens with concentrations ranging from 0.03 to 42 pg/200 µl. Comparison with clinical data showed that two of the three negative cases corresponded to stage I carcinoma with tumor size ≤ 15 mm. In both cases with sequential specimens, a dynamic of ct DNA according to the tumor mass was observed during treatment and at time of relapse.

Conclusions: HPV insertional mutations can be used to detect ct DNA with a high level of specificity in patients with HPV-associated carcinoma.

1167 Mesonephric Adenocarcinoma of the Uterus and Cervix – A Clinicopathologic Study of 10 Cases

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Background: Mesonephric adenocarcinoma (MA) is a rare type of adenocarcinoma that occurs mainly in the cervix and less often in the uterine corpus. It must be differentiated from diffuse mesonephric hyperplasia and other types of adenocarcinoma. We investigated the clinicopathologic features of 10 mesonephric adenocarcinomas including 7 of the cervix and 3 of the uterine corpus.

Design: Clinical information and follow-up data was obtained from physicians and medical records. The gross pathologic findings were abstracted from pathology reports. Microscopic and immunohistochemical features were evaluated in the tumors and in adjacent mesonephric hyperplasia.

Results: The mean age was 52 years. Nine women had a hysterectomy, with bilateral salpingo-oophorectomy in 3, 5 had lymph-node dissections; 2 pelvic radiation. All patients with cervical MA were stage 1B while 2/3 with corpus MA were stage IIIC or higher. Average follow-up was 5 years. Microscopically, tubular, glandular, retiform, sieve-like, spindle cell and solid growth patterns were observed. Tumor cells were low columnar to cuboidal, had scant cytoplasm and moderately atypical hyperchromatic nuclei. The mean mitotic index was 16/10 HPFs. 30% of tumors were associated with diffuse mesonephric hyperplasia. Immunohistochemical findings are presented in Table 1. The mean Ki-67 index was 25% in tumor, 11% in atypical hyperplasia and 5% in remnants.

Table 1. Immunohistochemical Results

	Stain Intensity				Stain distribution		
Stains	None	Weak	Moderate	Strong	Focal	Intermediate	Diffuse
PAX2	0%	0%	0%	100%	10%	0%	90%
PAX8	0%	0%	0%	100%	0%	22%	78%
CD10	12%	0%	12%	78%	22%	33%	33%
P16	0%	0%	56%	44%	44%	56%	0%
ER	70%	20%	0%	10%	10%	10%	10%
Calretinin	45%	33%	22%	0%	44%	11%	0%
Vimentin	12%	0%	44%	44%	22%	44%	22%
CEA	80%	0%	20%	0%	10%	10%	0%

Conclusions: Mesonephric adenocarcinoma often occurs in a background of mesonephric hyperplasia. Most cases had diffuse, strong staining for PAX2 and PAX8 but no staining pattern was diagnostic of MA. Areas of confluent growth with one or more of the characteristic patterns differentiate MA from hyperplasia and other types of adenocarcinoma. When there is a purely tubular pattern the boundary between hyperplasia and MA is ambiguous, but greater nuclear atypia, a higher mitotic count and Ki-67 index differentiate MA from hyperplasia and remnants. Diffuse growth of mesonephric tubules in a small specimen raises the possibility of MA and requires further evaluation. The clinical behavior of cervical MA appears similar to other types of adenocarcinoma of similar stage but uterine corpus MA may be more aggressive.

1168 The Desmoplastic Stromal Response as Defined by Positive a-Smooth Muscle Actin Staining Is Predictive of Invasion in Adenocarcinoma of the Uterine Cervix

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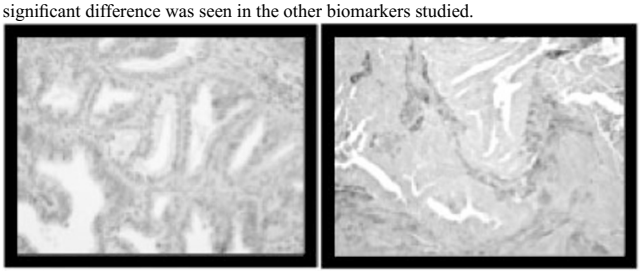
Background: The overall incidence of cervical carcinoma has decreased over the past four decades, however the incidence of glandular lesions of the cervix, both adenocarcinoma *in situ* (AIS) and adenocarcinoma (AC), have increased. The

differentiation between AIS and AC is clinically important because accurate diagnosis leads to appropriate triage and counseling of high risk patients. Unfortunately, histological features described to differentiate between AIS and AC are frequently insufficient. Surrogate histopathologic markers for invasion are needed.

Design: The objectives of this research were to examine the IHC profiles of AIS and early AC to identify biomarkers that enhance accurate diagnosis. The H&E and IHC of 20 women with glandular lesions were independently reviewed by two pathologists fellowship-trained in gynecologic pathology. Biomarkers included: α-SMA, ER, CEA, Ki67, p16, COX-2, and CD1a. Each stain was described according to pattern, intensity of staining, and percentage of positive cells. Statistical analysis was performed using SYSTAT v. 11.0.

Results: A statistically significant increased staining of the periglandular stroma for α-SMA was seen in AC as compared to AIS. Intensity was 1.2 versus 2.2 (p=0.04) and percent of positive staining cells was 18% versus 44% (p=0.05) in AIS and AC, respectively. Figures 1 illustrate the staining patterns of AIS (left) and AC (right). No significant difference was seen in the other biomarkers studied.



Conclusions: The presence of a desmoplastic stromal response as identified by increased periglandular staining for α-SMA is useful in identifying invasive glandular lesions of the endocervix. Further studies are necessary to confirm these findings and to establish biologically relevant cut-off values for α-SMA staining.

1169 Characterization and Comparison of Ovarian Primitive Neuroectodermal Tumors and Immature Teratomas by Immunohistochemistry and Fluorescence In-Situ Hybridization

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Background: Ovarian primitive neuroectodermal tumors (oPNETs) are rare germ cell tumors thought to arise from the immature neuroepithelium (imNE) of teratomas. Limited studies have been performed to characterize the immunohistochemical profile of oPNETs or their presumed imNE precursors. Although oPNETs are thought to mimic central PNETs (cPNETs) rather than peripheral PNETs (pPNETs), it remains unknown whether oPNETs exhibit MYC amplification as seen in cPNETs or EWSRI translocation as seen in pPNETs. Deletion of PTEN was also tested as PTEN deletion is known to promote primordial germ cell proliferation.

Design: Immunostains for markers of germ cell tumors (Sox2, Sall4, Oct-4), cPNETs (CD56, nestin, Bcl2, chromogranin, synaptophysin), and pPNETs (Fli-1, CD99) were performed in 5 oPNETs and 11 immature teratomas. FISH analysis for nMYC, cMYC, EWSR1, and PTEN was performed in all 16 tumors.

Results: Immunostain results are summarized in the table below. No oPNETs (0/5) or imNE (0/11) showed amplification for nMYC or cMYC or an EWSR1 translocation. 20% of oPNETs (1/5) but no imNE (0/8) showed PTEN deletion.

	Diffuse/Strong		Focal		Weak/Negative	
	imNE	oPNET	imNE	oPNET	imNE	oPNET
CD56	11/11	5/5				
Nestin	10/10	4/4				
Chromo			1/11	2/5	10/11	3/5
Synapto	1/11		3/11	2/5	7/11	3/5
Vimentin	10/11	4/5			1/11	1/5
Sox2	10/11	5/5	1/11			
Sall4	7/11	2/5	2/11		2/11	3/5
Oct4			5/11		6/11	5/5
Pax8	11/11	5/5				
Bcl2	10/11	4/5		1/5	1/11	
P53		5/5	5/11		6/11	
CD99					1/1	5/5
Fli1					11/11	5/5

Conclusions: Overall, the immunostaining patterns within the imNE of teratomas were similar to those of oPNETs, with the exception of p53 which consistently showed more expression in oPNETs, and Oct4, which was focally expressed within imNE in 45% of cases, but not within the oPNETs. The increased p53 expression in oPNETs may indicate tumor progression. The focal Oct4 expression within immature teratomas appeared mainly in occult foci of embryonal carcinoma in these tumors. Both tumor types showed strong staining for CD56, nestin, vimentin, Sox2, Pax8, and Bcl2. Unlike pPNETs, imNE and oPNETs were negative for CD99 and only weakly positive for Fli1. Unlike cPNETs, expression of chromogranin and synaptophysin was negative to focal in both oPNETs and imNE. oPNETs expressed Sall4 (40% positive) and Sox2 (100% positive), which represent diagnostic pitfalls as these markers are commonly used to diagnose other germ cell tumors. FISH testing for nMYC and cMYC amplification and EWSR1 translocation was negative in all oPNETs and imNEs suggesting that the molecular mechanisms of oPNETs do not mimic either central or peripheral PNETs.

1170 The Lung-Restricted Marker Napsin A Is Highly Expressed in Clear Cell Carcinomas of the Ovary

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Background: Napsin A is a member of the aspartic proteinase family that is expressed at high levels in type II pneumocytes and pulmonary macrophages and has been recently demonstrated to be a relatively specific and sensitive marker of lung adenocarcinomas. However, napsin A expression has also been identified in a subset of renal papillary and clear cell carcinomas, as well as a small number of thyroid carcinomas. Recently, we identified expression of napsin A in a case of clear cell carcinoma (CCCa) of ovary and, therefore, wished to determine the extent of napsin A in a series of CCCas, comparing results with other gynecologic tract (GYN tract) carcinomas, including endometrioid adenocarcinomas (EnCa) and high grade papillary serous carcinomas (PSCa) of ovary.

Design: A series of 12 CCCa, 10 EnCa and 10 PSCa were tested by immunohistochemistry (IHC) with a monoclonal antibody to napsin A (IP64, Leica), comparing results with antibodies to another lung-restricted marker, TTF-1 (SPT24, Leica), and a GYN-restricted marker, PAX8 (BC12, Biocare). The following scoring criteria were employed: 0 = negative, <1% = rare cell, 1-25% = focal, 26-75% = variable, and >75% = uniform.

Results: All 12 of 12 (100%) of CCCas were napsin A-positive, with all 12 cases showing variable to uniform expression. In contrast, 3/11 (27%) of EnCa, 0/10 (0%) of PSCa were napsin A-positive. The lung-restricted marker, TTF-1, was positive in 0/12 (0%) of CCCa, 1/11 (9%) EnCa, and 1/10 (10%) PSCa, usually either rare cells positive or focally positive. The GYN-restricted marker, PAX-8, was positive in 9/9 (100%) CCCa, 9/10 (90%) EnCa, and 10/10 (100%) PSCa; all cases were uniformly positive.

Conclusions: Napsin A is highly expressed in clear cell carcinomas of ovary, occasionally expressed in endometrioid adenocarcinomas and not expressed in serous papillary carcinomas. This unexpected finding suggests that napsin A may assist in separating CCCa from its mimics in GYN tract and could prove critical in the evaluation of metastatic carcinomas of unknown primary in which ovarian carcinoma is in the differential diagnosis. A potential pitfall, however, could be the rare case of TTF-1-positive/napsin A-positive endometrioid adenocarcinoma. These findings underscore the importance of using a complete antibody panel to include multiple "organ-specific" markers in identifying carcinomas of unknown primaries.

1171 Gastric-Type Endocervical Adenocarcinoma – An Aggressive Histologic Subtype

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Background: Gastric type adenocarcinoma (GA), including minimal deviation adenocarcinoma (MDA), is a rare variant of endocervical adenocarcinoma (ECA) that is not etiologically associated with human papillomavirus (HPV). GA and MDA represent morphologic spectrums of the same tumor and have been reported to have a worse prognosis than usual HPV-associated ECA. We describe the clinical outcomes and distribution patterns of metastases in a series of GA/MDA.

Design: A retrospective review of GA or MDA was performed from the pathology databases of two institutions, spanning 14 years. Stage at diagnosis (DX), recurrence/metastases patterns and overall survival were analyzed.

Results: 31 cases were identified, 29 of which had follow-up data. The patients ranged in age from 30 to 66 years (mean 50). One patient had a family history of Li-Fraumeni syndrome, while one had Peutz-Jeghers syndrome. The stages at presentation were as follows: IA1 (1), IA2 (1), IB1 (9), IIA1 (2), IIA2 (3), IIB (1), IIIB (8), IVB (6). More than half the patients presented at advanced stage IIA-IVB (65%). At the time of diagnosis, 8/17 patients (47%) had positive lymph nodes (LNS), 8/23 (35%) had adnexal (AD) metastases (MET) and 12/26 (46%) had pelvic and/or abdominal disease, of which 7 had concurrent AD or LN involvement. 17/27 (63%) had at least one site of MET at DX. MET sites included LNS, adnexa, omentum, bowel, peritoneum, diaphragm, abdominal wall, bladder, vagina and appendix. All patients were treated with radical surgery, chemoradiation or a combination. Follow-up ranged from 1 to 89 months (MOS) (mean 24); 14 (48%) have no evidence of disease, 4 (14%) are alive with disease, and 11 (38%) died of disease. Survival ranged from 2 to 89 MOS (mean 30). Ten patients (33%) recurred between 6-70 MOS (mean 20) involving retroperitoneal and inguinal LNS, liver, lung, vagina, bladder, colon, pelvis, omentum, brain, bone and appendix.

Conclusions: GA represents a distinct, biologically aggressive type of cervical adenocarcinoma. The majority of patients present at advanced stage (65% >=stage II), intraabdominal and pelvic MET are not uncommon and the disease carries a high mortality rate (38%). The rate and sites of metastases and recurrence are unusual for cervical ACA and may require a different clinical approach from usual ECA.

1172 Immunohistochemical Profile of Gastric Type Endocervical Adenocarcinoma, Including HER2/Neu Status

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Background: Gastric type (GA) and minimal deviation adenocarcinoma (MDA) are subtypes of endocervical adenocarcinoma not related to infection by human papillomavirus (HPV) and are characterized by expression of gastric pyloric gland type mucin as evinced by HIK1083 immunoreactivity. The extent to which these tumors express other Mullerian or gastrointestinal (GI) immunophenotype has not been previously studied. We evaluated a series of GA/MDA for expression of such markers. Since gastric and ovarian mucinous carcinomas have been shown to overexpress HER2/neu with concurrent amplification (and are therefore amenable to targeted therapy with traztuzumab), we also included this stain in our panel.

Design: A retrospective review of GA or MDA from the pathology databases of two institutions was performed. Using standard protocols, immunohistochemical (IHC) studies were performed on formalin-fixed, paraffin embedded tissue for CK7, p16, CK20, CDX2, p53, PAX8 and Her2/neu. Her2/neu expression was scored according to the ASCO/CAP guidelines (0 to 3+). All others were scored semiquantitatively as POS (>50%), NEG (0), FOC (any to 50%).

Results: 16 cases with blocks available for staining were identified. Results are summarized in Tables 1 and 2. Four cases also had tissue from metastatic sites available for staining.

Table 1

	POS (%)	FOC (%)	NEG (%)
CK7	16 (100)	0	0
CK20	3 (19)	2 (13)	11(69)
p16	0	1 (6)	15 (94)
CDX2	0	4 (19)	12 (81)
p53	3 (19)	13 (81)	0
PAX8	7 (47)	3 (20)	5 (33)

POS >50% staining; NEG no staining; FOC <=50% staining

Table 2

	3+ (%)	2+ (%)	0/1+ (%)
Her2/neu	1 (6)	1 (6)	14 (82)

The metastatic tumors had similar staining patterns to the primary, except for 3 cases where CK20/CDX2 was either lost or focally gained. One case also lost PAX8 in the metastasis.

Conclusions: Gastric type and minimal deviation adenocarcinoma are characteristically CK7 and many, but not all, are PAX8 positive as well. p16 is negative, further supporting HPV-independent tumorigenesis. A minority of tumors express focal "GI markers" CK20 and CDX2 but are usually negative. p53 is overexpressed in 19% of these tumors which may indicate presence of TP53 mutation. Her2/neu shows 2+ or greater expression in 12% of cases which may be indicative of Her2 amplification.

1173 Histologic Evaluation of Prophylactic Hysterectomies in Women with Lynch Syndrome

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Background: Women with Lynch syndrome (LS) are at increased risk for endometrial (EC) and ovarian carcinoma (OC).Current surveillance recommendations for detection of EC and OS in LS patients are not effective. Small studies have shown that prophylactic hysterectomy and bilateral salpingo-oophorectomy (P-TAH-BSO) are the most effective and least expensive preventive measures in these patients. Data regarding histologic findings in prophylactic specimens in these patients are lacking.

Design: All LS patients who underwent P-TAH-BSO at MSKCC from 2000 to 2011 were identified. Slides were evaluated for the presence of endometrial hyperplasia (EH), EC, OC or any other recurrent histologic findings.

Results: 25 patients were identified with (age 36-61 years). 16 patients had a history of colon carcinoma and 2 patients had a history of sebaceous carcinoma. Focal non-invasive FIGO grade 1 endometrioid ECs were detected in 2 patients, age 54 yrs (MSH2 mutation) and 56 yrs (MLH1 mutation). Focal complex atypical hyperplasia was seen in 2 patients, age 35 (MLH1 mutation) and 53 years (MSH2 mutation). One patient (44 year old with MSH2 mutation) showed a mixed endometrioid/clear cell OC and simple hyperplasia without atypia in the endometrium. The OC was adherent to the colon, but did not show distant metastasis. Other notable uterine findings included mucinous metaplasia (MM) and increased lymphoid aggregates (LA).

Table 1.

	EH	EC	OC	MM	LA
n (%)	3/25 (12%)	2/25 (8%)	1/25 (4%)	4/25 (16%)	5/25 (20%)

Conclusions: In our study, prophylactic uteri and ovaries in LS revealed incidental EC and/or EH in 20%, and OC in 4% of cases. The endometrial carcinomas were low grade, confined to endometrium and seen in patients older than 50 yrs. Prophylactic hysterectomy allows recognition of early lesions in LS which appear to be small, focal and not diffuse. MM and LA were also frequently noted in benign uteri.

1174 A Clinicopathological and Immunohistochemical Study of 54 Cases of Dysgerminoma and Gonadoblastoma

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Background: In the ovary, dysgerminoma (DG) most often develops as a pure tumor, a mixed germ cell tumor or from a gonadoblastoma (GBL) in a patient with dysgenetic gonads. The purposes of this study are to assess the clinical, gross, and histologic features of DG and GBL and determine the best immunohistochemical markers to delineate the types of germ cell neoplasia present in these tumors.

Design: We searched the Pathology records for cases of DG and GBL from 1980 to 2011. Immunohistochemical stains for OCT4, SALL4, NANOG, PLAP, TSPY, CD117, D2-40, AFP, Glypican-3, HCG, AE1/AE3 and CAM5.2 were performed in all tumors. GBL were also stained for WT1, inhibin A (INHA), calretinin, SF1 and CK18. Stains were scored for extent (percent) positivity (0-4) and intensity (0-3), and a composite score (CS) was calculated as the product of the two scores (0-12).

Results: We identified 54 tumors: 32 pure DG, 9 DG mixed with other germ cell elements, 6 DG arising in GBL, and 5 pure GBL. Mean age was 21 years for patients with DG (range 5-51) and 16 years for patients with GBL (range 11-21). Mean DG size was 16 cm; mean weight, 1.3 kg. For DG patients, 27 patients (57%) were stage I, 2 (4%) stage II, 12 (26%) stage III, 1 (2%) stage IV, and 5 (11%) had unknown stage disease. Mean mitotic activity in DG was 22 per 10 HPF (median 18, range 0-61). The most sensitive markers for DG were OCT4 (CS=11.6), D2-40 (CS=10.9), SALL4 (CS=9.6), NANOG and CD117 (CS=8.6) and PLAP (CS=6.8). Similarly in GBL, germ cell nests were best highlighted by OCT4 (CS=11.6), CD117 (CS=11.5), D2-40 (CS=11.4), SALL4 (CS=9.5), PLAP (CS=8) and TSPY (CS=7.5). AE1/AE3 (CS=6.4) and INHA (CS=4.7) were the most sensitive markers of sex cord-stromal cells in GBL. Interestingly, the Y chromosome-specific protein TSPY was expressed in 4 (10%) DG not associated with GBL. It was exclusively cytoplasmic in contrast to its cytoplasmic and nuclear localization in GBL.

Conclusions: OCT4, D2-40 and CD117 are the most sensitive markers for malignant germ cells in DG and GBL. AE1/AE3 and INHA are the most sensitive markers of sex cord-stromal cells in GBL. The expression of TSPY in some pure dysgerminomas raises the possibility that these patients may harbor regions of the Y chromosome.

1175 Transitional Cell-Like Growth in Ovarian Endometrioid Carcinoma: Clinicopathological, Immunohistochemical and Behavioral Features Distinguishing It from High Grade Serous Carcinoma

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Background: The endometrioid subtype of ovarian cancer (OEC) is distinguished from high grade serous (OSC) by its genetics, clinical presentation, histologic precursors, immunophenotype, and behavior. Ribbon-like growth resembling transitional cell carcinoma (TCC) of the urinary bladder has been described in OSC, ovarian TCC (OTCC) and endometrioid endometrial carcinoma (EEC). The purpose of this study was to determine whether transitional cell-like morphology (TCLM) occurred in OEC and describe the clinical, histological and immunophenotypic characteristics that distinguish it from OSC and OTCC.

Design: All pure OECs from our institute between 1982 and 2009 were examined for TCLM: undulating ribbons or compact nests resembling OSC and OTCC. To exclude OSCs that were misdiagnosed as OEC, high grade tumors that expressed the WT1, a marker of OSC, in the transitional cell component were excluded. We examined the morphologic and immunohistochemical features specifically in the TCLM.

Results: Of 71 OECs examined, we identified 10 tumors (14%) that showed TCLM. The women were 39-79 years old (mean, 52 years). Five tumors were stage I, 2 stage II, and 3 stage III. The tumors ranged from 8.5 to 23 cm (mean, 15 cm). One tumor was FIGO grade 1, 3 grade 2 and 6 grade 3. The TCLM occupied 5% to 90% of the overall tumor (mean, 41%). Varying degrees of squamous differentiation were present in the TCLM in 9 tumors. None contained small papillae or benign Brenner tumor, and only one had rare psammoma bodies. Two were associated with endometriosis. Within the TCLM, WT1, p63, inhibin, calretinin and SF1 were negative in the all cases. ER was positive in 8 tumors. One grade 3 tumor was diffusely positive for p53, but this tumor was p16 negative. One tumor was negative for MLH1 and PMS2. Two patients (20%) with the transitional cell pattern died (average follow-up, 47 months), and 14 (23%) without the transitional cell pattern died (average follow-up, 41 months).

Conclusions: Some OECs show a transitional cell growth pattern that resembles OSC and OTCC, but this does not appear to affect behavior. Admixed squamous differentiation and absence of diffuse WT-1, p53 and p16 expression are the most useful features to prevent over-diagnosis of this pattern as OSC or OTCC.

1176 Prognostic Significance of Primary Tumour Factors in Stage III High Grade Endometrial Cancer

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Background: The implications of surgical and pathologic findings in stage III high grade endometrial cancer (HEC) are controversial. Whether adjuvant treatment such as chemotherapy (CT) or radiation (RT) should be included based on high-risk primary tumour factors remains to be determined. This study sought to determine the contribution of primary tumour factors (PTF) versus treatment (TT) received in recurrence and survival outcomes.

Design: A retrospective cohort study was performed, including all patients with surgical stage III HEC treated at our institution (1999 - 2009). PTF assessed included: stage, histologic subtype, lymphovascular invasion (LVI), extent of myometrial invasion (MI), cervical (CI), serosal (SI), adnexal (AI), parametrial (PI) and vaginal (VI) involvement. Nodal status and margins (MR) were also assessed. Recurrence-free survival was calculated using the methods of Kaplan and Meier, and hazards ratios were determined using Cox proportional hazards modeling.

Results: The study included 82 patients (mean age = 66 ± 11 years) with surgical and pathologic stage III HEC (IIIA - 38%, IIIB - 7%, IIIC - 55%). The median follow-up duration was 22 months and median time to recurrence was 8.5 months. HEC comprised 24% grade 3 endometrioid, 23% carcinosarcoma, 22% mixed, 21% serous, 9% clear cell and 1% undifferentiated carcinoma. The prevalence of PTF assessed was: 74% deep MI, 27% SI, 52% CI (stromal), 48% AI, 6% VI, 5% PI, 85% LVI and 16% positive MR. Thirty-two percent of patients received pelvic with or without vaginal vault RT, 17% of patients received CT, and 35% of patients received both RT and CT. Fifty-nine percent of patients recurred (IIIA - 40%, IIIB - 12%, IIIC - 48%).

In univariate analyses, recurrence was associated with SI (p = 0.04), positive MR (p = 0.0003), CI (p=0.001) and histology (p = 0.01). When controlling for PTF and TT received, clear cell and carcinosarcoma histologic subtypes (HR 8.6 and 6.0, respectively) as well as cervical stromal invasion (HR 3.7) were associated with an increased risk of recurrence. Meanwhile, patients who received either CT or RT experienced significantly decreased risk of recurrence (HR 0.42 and 0.48, respectively).

Conclusions: When controlling for high-risk PTF and TT received, PTF such as histologic subtype and cervical stromal invasion are associated with risk of recurrence, while adjuvant treatment with CT, RT, or both, may decrease risk.

1177 HPV Infection Analysis of Concurrent Glandular and Squmaous Lesions and Adenosquamous Carcinoma of the Uterine Cervix

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Background: High-grade cervical intraepithelial neoplasm (HGCIN) is a precursor lesion of squamous cell carcinoma of the uterine cervix. CIN lesions are often found adjacent to glandular lesions of adenocarcinoma and adenocarcinoma in situ (AIS). We analyzed the human papillomavirus (HPV) infection status of concurrent glandular and HGCINs and adenosquamous carcinoma of the uterine cervix to know their histogenensis.

Design: During 8-year period 2003 to 2010, 13 surgical cases of the uterine cervical adenosquamous carcinoma and 17 cases of glandular lesions with concurrent HGCINs of the cervix (11 cases of concurrent adenocarcinoma and HGCINs, and 6 cases of concurrent AIS and HGCINs) were examined in this study. The squamous and glandular components were separately microdissected from paraffin sections using a microtome blade.

Results: Of 13 adenosquamous carcinoma cases, HPV infection was positive in 12 cases. On 12 cases, the HPV type of each adenocarcinoma and squamous cell carcinoma component demonstrated the same type. The detection types were HPV 18 of 6 cases, HPV 16 of 5 cases, and 1 case of HPV 45. On remaining one cases, HPV was not detected in both components. Among 17 cases of glandular lesions with concurrent HGCINs, same HPV type (HPV-18) was presented in only one case. On 17 cases, different HPV types were demonstrated in 13 cases. On remaining 3 cases, HPV was detected in the component of HGCINs, but not detected in the component of glandular lesions. The glandular lesions in these 3 cases are adenocarcinoma, but not AIS.

Conclusions: We showed that many concurrent glandular lesions and HGCIN are formed separately. However, according to the result that same HPV type were detected in adenosquamous carcinoma, the glandular and squamous parts of the concurrent lesions do not develop into glandular and squamous components of adenosqumous carcinoma.

1178 Specialized Pathology Review in Patients with Ovarian Cancer: Highly Recommended To Assure Adequate Treatment. Results from a Prospective Study

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Background: In view of retrospective findings on second opinion pathology in ovarian cancer, it seems certain that a considerable number of ovarian borderline tumors (BOTs) or metastatic non-ovarian primaries are being erroneously diagnosed as ovarian carcinomas. If BOTs are misdiagnosed as cancer, patients may not only suffer from non-beneficial morbidity at unnecessary high cost but may have to cope with an incorrect diagnosis of cancer for the rest of their lives. In cases of metastatic disease mistaken for an ovarian primary, more adequate therapeutic modalities may be withheld from some patients. Finally, clinical trials may be biased through unintended disregarding of histological inclusion criteria. We hypothesized that 5% of all patients in clinical trials of ovarian carcinomas have lesions other than epithelial ovarian cancer, namely BOTs, non-epithelial or metastatic cancer. This is the first such study with a prospective approach.

Design: Patients who were enrolled into a chemotherapy trial of ovarian carcinoma were asked to consent to a translational subprotocol. Contributing pathologists were asked to submit all original slides as well as paraffin material. Specialized central pathology review of all cases was performed by two experienced gynecopathologists. In cases of clinically relevant diagnostic discrepancies, the contributing pathologist was contacted. If a given discrepancy could not be resolved, a panel of experts was available for clarification.

Results: 454 patients with an outside diagnosis of ovarian epithelial cancer were recruited. In 6.8% (n=31), a major diagnostic discrepancy of potential clinical relevance was found. Most frequently (n=15), serous BOTs had been misdiagnosed as invasive cancer. Ovarian metastases constituted the second most frequent misdiagnosis (n=12). As minor discrepancies, a divergent histological typing of ovarian carcinomas was found in 28.2% (n=128).

Conclusions: This study clearly shows that central pathology review by experienced gynecopathologists is highly recommendable if overtreatment with chemotherapy of patients with BOTs and inadequate treatment of patients with ovarian metastases is to be avoided in the future. Specialized pathology review should become standard procedure in study protocols prior to randomization. In order to further optimize the quality of care, a high throughput infrastructure for specialized pathology review will have to be established. The authors propose a new internet-based ovarian cancer network, capable of providing specialized second opinion pathology within 10 working days.

1179 FOXL2 Molecular Testing in the Diagnosis of Ovarian Neoplasms

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Background: Recently a single, recurrent somatic mutation (402C→G) in FOXL2 was identified in almost all adult-type granulosa cell tumors (aGCT) but not other ovarian neoplasms. The histopathological features of aGCT can be mimicked by a variety of other tumors, making diagnosis of aGCT challenging. It has been suggested that molecular testing for FOXL2 mutation might be a useful tool in the diagnosis of aGCT. The aim of this study is to demonstrate how testing for the FOXL2 mutation can be used in a gynecological pathology consultation service.

Design: Immunohistochemistry (IHC) for FOXL2 was done using an anti-FOXL2 polyclonal antiserum. If IHC was positive, the FOXL2 mutation status was subsequently analyzed using a TaqMan PCR assay. In cases with a scattered growth pattern or minimal tumor only, laser capture of tumor cells was done prior to DNA extraction.

Results: To date, 10 problematic cases have been assessed where the differential diagnosis after the initial investigations included aGCT. The differential diagnoses included: Sertoli Leydig cell tumor (SLCT), juvenile granulosa cell tumor (jGCT), endometrial stromal sarcoma (ESS). and follicular cyst. In all cases FOXL2 IHC was positive, however in only two samples was the FOXL2 mutation detected, thus confirming a diagnosis of aGCT in these 2 cases.

Conclusions: The analysis of the FOXL2 mutational status in clinical samples was a useful diagnostic tool in situations where the differential diagnosis was between aGCT and SLCT, jGCT, ESS and follicular cyst. The informative value of this test is limited to the question of whether a given tumor is an adult type granulosa cell tumor or not, but it is highly specific in this context.

1180 Successful External Validation of the Calculator for Ovarian Subtype Prediction in a Clinical Trial Case Series

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Background: With the growing certainty of future ovarian cancer treatment being type specific it becomes more and more desirable to reliably type ovarian carcinoma. Recently it has been shown, that the application of a set of nine immunohistochemical markers can objectively support the classification of a tumor into one of the five major types of ovarian cancer by entering immunoscores into the Calculator for Ovarian Subtype Prediction (COSP). It was the aim of this study to externally validate the nine-marker panel's prediction equation for the first time.

Design: A cohort of 408 cases which were derived from an international chemotherapy trial including all histological types was immunohistochemically stained for PR, p53, DKK1, WT1, p16, Vimentin, HNF1B, TTF3 and MDM2. Scoring was performed by one of the authors who was not previously involved in generating the original data for development of the COSP. In this validation experiment, two antibodies (HNF1B & MDM2) had to replaced for of lack of availability or quality reasons.

Results: Of the 350 tumours that were assigned to the serous subtype, the COSP predicted 315 of these to be high-grade serous and 5 to be low-grade serous (table1). The overall concordance rate between the COSP preciction and specialized gynecopathology review is 87.7%.

Table 1: Central review diagnoses versus predicted histo type

	Predicted Diagnosis				
Expert Review	CCC	ENDO	HGS	LGS	MUC
CCC (n=21)	16	1	3	0	1
ENDO (n=15)	0	6	7	0	2
MUC (n=6)	2	1	1	0	2
SEROUS (n=350)	14	11	315	5	5
Undifferentiated (n=16)	1	0	15	0	0

CCC: Clear Cell Carcinoma; ENDO: Endometrioid Carcinoma; HGS: High Grade Serous Carcinoma; LGS: Low Grade Serous Carcinoma; MUC: Mucinous Carcinoma

Conclusions: This experiment demonstrates the external validation of the COSP equation. The results are consistent with all internal validation exercises so far and suggest that this nine marker immunopanel can be used as an diagnostic adjunct in determining ovarian carcinoma cell type.

1181 TP53 Mutations in Uterine Atypical Leiomyomas

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Background: Most uterine smooth muscle tumors can be broadly subdivided into two groups: leiomyoma (including variant subtypes) and leiomyosarcoma based on cytologic atypia, mitotic activity and coagulative tumor cell necrosis. A small number defies classification into unequivocally benign and unequivocally malignant categories and are classified as "atypical leiomyoma" and "STUMP". These two latter tumor types generally behave in a benign fashion but some are malignant. Morphologic and immunohistochemical studies designed to identify the malignant subset have thus far been unsuccessful. Although p53 overexpression as determined by immunohistochemical staining has been reported in these tumors, it is uncertain whether atypical leiomyomas harbor *TP53* mutations. We therefore performed a mutational analysis of atypical leiomyomas to determine their *TP53* mutation status.

Design: A total of 13 atypical leiomyomas were analyzed for *TP53* mutations. In 7 cases marked cytologic atypia was diffusely present and in 6 it was focal. In tumors with focal atypia, manual microdissection was employed to separately dissect the atypical and conventional leiomyoma areas. DNA samples were sequenced and *TP53* mutations were analyzed from exons 4 to 9.

Results: *TP53* mutations were detected in 8 (62%) of 13 atypical leiomyomas. Of the 8 tumors with mutations, 5 had morphologically distinct components composed of atypical and conventional leiomyoma areas. In three of these 5 cases, a *TP53* mutation was detected in the atypical component but not in the conventional leiomyoma area. In addition, there were three atypical leiomyomas that were associated with conventional leiomyomas and of those, one atypical leiomyoma harbored a *TP53* mutation and the concurrent conventional leiomyoma did not. The other two contained wild-type *TP53* in both the atypical and conventional leiomyomas.

Conclusions: The findings in this study indicate that, as currently defined, the group of tumors designated "atypical leiomyoma" is heterogeneous at least based on the presence of *TP53* mutations. It is tempting to speculate that those harboring *TP53* mutations represent the subset of atypical leiomyomas that are malignant. As follow-up information was not available in this study we were unable to confirm this hypothesis. Accordingly, future correlated molecular genetic and clinicopathologic studies in which follow-up information is available are necessary to clarify this important finding.

1182 Brenner Tumors. A Mutational and Immunohistochemical Analysis of 39 Cases

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Background: Benign Brenner tumors (BTs) are classified as epithelial tumors of the ovary, based on the presence of nests of epithelial cells resembling urothelium. They are thought to be derived from Walthard cell nests because of their similar appearance. In addition, they have a prominent fibromatous stroma. The aim of this study was to evaluate their immunohistochemical and molecular genetic features with the view that this might shed light on their pathogenesis.

Design: A total of 39 BTs were retrieved from the Johns Hopkins Hospital and the Washington Hospital Center surgical pathology files. Immunohistochemistry (IHC) for WT1, ER, PR, calretinin, SF1 and alpha-inhibin was performed on FFPE tissue sections on 39 tumors. In addition, the nests of transitional-type epithelium from 20 tumors were laser-capture microdissected. The stromal component was manually microdissected in all cases as well. Genomic DNA was extracted for sequence analysis of *BRAF*, *KRAS*, *PIK3CA*, *CTNNB1*, *HER2, PPP2R1A* and *FOXL2* mutations.

Results: A somatic mutation of *PIK3CA* was detected in the stromal component in one tumor. No other mutations were detected in either the epithelial or the stromal component in the remaining tumors. IHC analysis revealed weak WT1 immunoreactivity in the epithelial nests in 3 cases. A similar IHC pattern of WT1 was found in the stromal cell component in 12 cases. In contrast, there was weak expression of ER in 28/39 (72%) and PR in 26/39 (67%), and strong expression of calretinin in 36/39 (92%), SF1 in 37/39 (95%) and alpha-inhibin in 34/39 (87%) in the stromal component. In contrast, expression of these biomarkers in the epithelial component was generally absent. Notably, calretinin, SF1 and alpha-inhibin showed particularly strong staining in the stromal cells that immediately surrounded the epithelial nests in the majority of the tumors.

Conclusions: Our findings suggest that mutation of the genes analyzed is unlikely to be a common molecular genetic aberration underlying the development of BTs. Moreover, the periepithelial IHC pattern of calretinin, SF1 and alpha-inhibin, markers of steroidogenic cells, suggests that the stroma is not a passive component but may play an important role in the pathogenesis of this tumor. It is conceivable that the stroma is responsible for inducing the epithelium or vice versa. Further studies are necessary to identify common molecular genetic aberrations in this tumor.

1183 Cyclin D1 Is a Sensitive and Specific Diagnostic Immunomarker for YWHAE-FAM22A/B Endometrial Stromal Sarcoma

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Background: We recently described a novel genetic fusion *YWHAE-FAM22A/B* resulting from translocation t(10;17)(q22;p13) in a subset of endometrial stromal sarcomas (ESS) which are histologically higher-grade and clinically more aggressive than *JAZF1*-rearranged ESS. We describe here the utility of cyclin D1 as a diagnostic immunomarker for *YWHAE-FAM22A/B* ESS.

Design: Gene expression profiling was performed by 3' end sequencing in 3 *YWHAE-FAM22A/B* ESS, 4 *JAZF1* ESS and 4 uterine leiomyosarcomas. Significance analysis of microarray data was used to identify genes (immunomarkers) substantially upregulated in *YWHAE-FAM22A/B* ESS. Immunomarker specificity and sensitivity were evaluated in a series of *YWHEA-FAM22A/B* ESS and other uterine tumors.

Results: The 3' end sequencing profiles demonstrated cyclin D1 overexpression in *YWHAE-FAM22A/B* ESS compared to *JAZF1* ESS and leiomyosarcomas: this candidate immunomarker was selected for further evaluation because of its general availability in pathology laboratories. Immunohistochemically, > 70% of nuclei in the round cell/epithelioid cell component of all *YWHAE-FAM22A/B* ESS (N = 10) demonstrated diffuse moderate-to-strong nuclear cyclin D1 staining (comparable to that seen in Mantle cell lymphoma) and such diffuse positivity was rarely seen (0.4%) in other uterine tumors (Table 1). Other than *YWHAE-FAM22A/B* ESS, most uterine sarcomas (98%) displayed cyclin D1 straining in < 20% of tumor nuclei.

Cyclin D1 (Thermoscientific) immunostaining results in 267 uterine tumors.

	Number of cases examined	Cyclin D1 positive cases (>70% moderate-strong nuclear positivity)
ESS with JAZF1/SUZ12/PHF1/EPC1 rearrangement	34	0 (0%)
ESS with YWHAE-FAM22A/B rearrangement	10	10 (100%)
Sarcoma NOS	12	0 (0%)
Adenosarcoma*	25	0 (0%)
Leiomyosarcoma	111	1 (1%)
Malignant mixed mullerian tumor	14	0 (0%)
Leiomyoma	49	0 (0%)
Endometrial stromal nodule	3	0 (0%)
Polypoid endometriosis	7	0 (0%)
Uterine tumors resembling ovarian sex cord tumor (UTROSCT)	2	0 (0%)

* 8 cases showed sarcomatous overgrowth

Conclusions: We have identified a cyclin D1 as a sensitive and specific diagnostic immunomarker for *YWHAE-FAM22A/B* ESS. When evaluating uterine sarcomas where the differential of ESS with high grade histologic features is considered, cyclin D1 should be included in the immunohistochemical work-up to evaluate the possibility of *YWHAE-FAM22A/B* ESS.

1184 Ancillary Techniques in Distinction of Androgenetic/Biparental Diploid Mosaic Conceptions from Hydatidiform Moles

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Background: Recent studies have demonstrated the value of ancillary techniques, including p57 immunohistochemistry (IHC) and short tandem repeat (STR) genotyping, for distinction of hydatidiform moles (HM) from non-molar specimens (NM) and for subtyping HM as complete hydatidiform moles (CHM) and partial hydatidiform moles (PHM). With rare exceptions, CHM are p57-negative and androgenetic diploid; PHM are p57-positive and diandric triploid; and NM are p57-positive and biparental diploid. Androgenetic/biparental diploid mosaic conceptions (ABDMC) have some morphologic features that simulate HM but are genetically distinct in that they contain an admixture of p57-negative androgenetic diploid and p57-positive biparental diploid cells. Combined p57 IHC and STR genotyping of ABDMC has not been reported.

Design: This study characterizes 9 ABDMC identified in a series of 390 products of conception specimens subjected to p57 IHC and STR genotyping. Fluorescence in situ hybridization (FISH) was performed on 4 to further assess ploidy.

Results: All cases were characterized by hydropically enlarged, variably sized and shaped villi, with some having distinct stromal hypercellularity. In 4 cases, the villi lacked trophoblastic hyperplasia whereas in 5 there was a focal villous component with trophoblastic hyperplasia and features of CHM. The villi lacking trophoblastic hyperplasia were characterized by discordant p57 expression within individual villi (p57-positive cytotrophoblast and p57-negative villous stromal cells) whereas the villous components having trophoblastic hyperplasia were uniformly p57-negative in both cell types. STR genotyping of multiple villous areas in each case demonstrated variable paternal:maternal allele ratios of >2:1. FISH demonstrated diploidy in 4 cases, including 2 with a CHM component. These results are consistent with an admixture of androgenetic diploid (p57-negative) and biparental diploid (p57-positive) cell lines. In 2 cases with a CHM component, persistent gestational trophoblastic disease (GTD) developed, with one patient requiring hysterectomy despite chemotherapy; no metastatic GTD was found in either.

Conclusions: ABDMC, including those with a CHM component, can be recognized by their distinctive p57 expression patterns and confirmed as such by their unique genotyping results, thus preventing misclassification as typical CHM, PHM, or NM. The presence of androgenetic cell lines, particularly in those with a CHM component, warrants follow-up due to some risk of persistent GTD.

1185 Follow-Up Findings of Abnormal Vaginal Pap Tests from Post-Hysterectomy Women and Their Correlation with hrHPV DNA Test Results

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Background: Vaginal Pap tests are mainly performed in females with hysterectomy due to dysplastic/neoplastic lesions of genital tract. Abnormal vaginal Pap tests are managed in the same manner as abnormal cervical Pap tests; however, vaginal Pap tests differ in two respects: 1) they are obtained from elderly populations, 2) most patients have a history of dysplastic/neoplastic lesion.

Design: Vaginal ThinPrep Pap tests reported as ASC-US, ASC-H, LSIL, or HSIL with Hybrid Capture 2 (HC2) HPV DNA test were retrieved from our pathology database over 5 years period. Follow-up findings (histology and cytology) were analyzed and correlated with HPV test results.

Results: During the study period, 1,864 vaginal Pap tests were reported as ASC-US, ASC-H, LSIL, or HSIL with simultaneous HPV test results. The prevalence of HPV positivity and the age range are shown in Table 1.

HPV Test Results in Abnormal Vaginal Pap Tests

Abnormal Pap	Tested Cases	Positive Case (%)	Age, yrs (range)
ASC-US	1,614	365 (22.6)	56.7 (17 - 94)
ASC-H	49	29 (59.2)	58.6 (22 - 87)
LSIL	185	142 (76.8)	56.2 (27 - 92)
HSIL	16	13 (81.3)	62.6 (42 - 93)
Total	1,864	549 (29.5)	56.8 (17 -94)

A total of 779 women, including 333 HPV positive cases, had follow-up results with an average follow-up period of 16.2 months (0.5-94).

Follow-up results (histopathological and cytopathological)

	hrHPV positive			hrHPV negative		
	Total F/U #	LSIL/ VAIN1 (%)	HSIL/ VAIN2/3 (%)	Total F/U #	LSIL/VAIN1 (%)	HSIL/VAIN2/3 (%)
ASC-US	214	93 (43.5)	8 (3.7)	401	18 (4.5)	2 (0.5)
ASC-H	21	14 (66.7)	2 (9.5)	17	4 (23.5)	1 (5.9)
LSIL	88	59 (67.5)	8 (9.9)	26	14 (53.8)	1 (3.8)
HSIL	10	2 (20)	7 (70)	2	1 (50)	1 (50)

Sensitivity, specificity, positive and negative predictive values (PPV and NPV) of HPV test for subsequently developed high-grade lesions were calculated.

Conclusions: 1. This is the largest study on HPV prevalence and subsequent follow-up results for post hysterectomy women with abnormal vaginal Pap tests.

2. The prevalence of HPV increased with increased degree of vaginal Pap abnormalities.

3. For ASC-US, ASC-H and LSIL, the NPV of HPV for the subsequent detection of CIN2/3/HSIL were very high (99.5%, 94.1%, and 96.2%).

4. HrHPV testing is a reasonable and cost-effective approach for women with vaginal ASC-US, even with ASC-H.

5. Patients with vaginal ASC-H or LSIL and negative hrHPV may be more efficiently managed by following up with Pap and HPV testing rather than colposcopy, especially for older women.

1186 Identification of an Effective Immunohistochemical Panel in Distinction of Breast Carcinoma from Ovarian Serous Carcinoma

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Background: When working on a tumor of unknown origin, breast carcinoma (BCA) versus ovarian serous carcinoma (OSCA) may present a diagnostic challenge because of the overlapping morphological features and immunostaining profile. In this study, we re-evaluate the expression of an extensive panel of biomarkers including recently described markers GATA3, Trefoil factor 1 (TFF1) and Trefoil factor 3 (TFF3) using a single immunostaining system (Dako).

Design: We immunohistochemically evaluated the expression of 1) epithelial markers (AE1/3, CAM5.2, CK7, CK20, CK17, CK19, CK903, EMA); 2) mucin gene products (MUC1, MUC2, MUC4, MUC5AC, MUC6); 3) tumor suppressor genes and transcription factors (ER, PR, p53, beta-catenin, WT-1, CDX2, pVHL); and 4) tumor-associated proteins (TTF-1, napsin A, GATA3 [Santa Cruz; sc-268], TFF1 [Epitomics; AC0045], TFF3 [Epitomics; AC0103], FOXA1, ERG, HepPar1, glypican 3, SALL4, OCT4, PAX2, PAX8, RCC GCDFP-15, mammaglobin, S100P, IMP3, maspin, MOC31, CEA, CA19-9, CA125, CD10, CD15, villin, and P504S) on 146 cases of breast carcinoma (98 ductal carcinomas and 48 lobular carcinomas) and 41 cases of ovarian serous carcinoma on tissue microarray sections. The staining intensity was graded as weak or strong. The distribution was recorded as negative (<5% of tumor cells stained), 1+ (5-25%), 2+ (26-50%), 3+ (51-75%), or 4+ (>75%).

Results: The positive staining results from selected antibodies, which demonstrated diagnostic value, are summarized in Table 1. When combining ductal and lobular carcinomas, the positive staining results for GTAT3, TFF1 and TFF3 were 95%, 77%, and 88%, respectively, with a strong and diffuse staining (3+ or 4+) in 131 cases (90%), 79 cases (56%) and 103 cases (72%), respectively. For ovarian serous carcinomas, 38 cases (93%) %) were strongly and diffusely (3+ or 4+) positive for PAX 8, and 30 cases (73%) were strongly and diffusely (3+ or 4+) positive for WT-1.

Table 1. Summary of immunostaining results on selected antibodies

Antibody	Breast DCA	Breast LCA	OSCA
GATA3	90/98 (92%)	48/48 (100%)	0/41 (0)
TFF1	68/95 (72%)	41/47 (87%)	0/41 (0)
TFF3	81/96 (84%)	45/48 (94%)	3/41 (7%)
PAX8	0/98 (0)	0/48 (0)	41/41 (100%)
WT-1	0/98 (0)	0/48 (0)	36/41 (88%)

DCA-ductal carcinoma; LCA-lobular carcinoma

Conclusions: These data demonstrate that GATA3, TFF1, TFF3, PAX8 and WT-1 are the most effective diagnostic panel for distinguishing breast carcinoma from ovarian serous carcinoma.

1187 Histologic Patterns of Myometrial Invasion in FIGO Grade 2 Endometrioid Endometrial Adenocarcinoma

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Background: Endometrioid adenocarcinoma of the endometrium (EEC) is the most common histologic type of endometrial cancer; however few studies have evaluated the importance of patterns of myometrial invasion. Therefore, we were interested in evaluating this parameter in cases of grade 2 EEC and correlating it with clinical outcome.

Design: 68 cases of grade 2 EEC (January 2002 - September 2009), with at least 2 years of clinical followup were retrieved from our files and reviewed to identify those with myoinvasion. Myoinvasion was subclassified into the following patterns: infiltrating glands (IG), broad front, adenomyosis-like, microcystic elongated and fragmented glands (MELF), and adenoma-malignum. Depth of invasion and presence/absence of lymphovascular invasion were confirmed, and clinical follow up was obtained.

Results: 56/68 (82%) cases of grade 2 EEC were myoinvasive. Of these, 11 (20%) were superficially invasive (<10% myoinvasion), 28 (50%) invaded 10-49%, and 17 (30%) invaded >50% into the myometrium. The invasive patterns consisted of IG (32; 57.1%), both IG and a second minor component of MELF (4, 7.1%), broad front (11; 19.6%), MELF alone (3, 5.4%), adenomyosis-like (6; 10.7%). No cases of adenoma malignum-like pattern of invasion were identified. Lymphovascular invasion was noted in 21 cases (30.9%); 15 (71%) of which had IG. 11 (16.2%) cases contained cervical stromal invasion; 8 (72%) of which had IG. Six (8.8%) patients had hysterectomy and bilateral salpingo-oophorectomy while sixty-two (91.2%) also underwent a lymphadenectomy (LND). Forty-five (66.2%) patients received adjuvant therapy (chemotherapy, radiation therapy, or vaginal brachytherapy). 6/62 patients with LND had metastases; 4/6 (66%) had IG. None of the cases without myoinvasion recurred while 8 (14.3%) cases with myoinvasion recurred (follow-up mean: 45 months). Of the patients with recurrence, all had an IG (including one case with a minor MELF component).

Conclusions: In this series, the majority of patients with grade 2 EEC had myometrial invasion (56/68; 82%) which is in distinct contrast to our previous study (Mod Path 24 (1), 2011; 264A, Abstract 1119) where 30% of Grade I cases were invasive. The presence of infiltrative gland pattern of myoinvasion is associated with recurrence and other pathologic variables of poor outcome including LVI, cervical stromal invasion and lymph node involvement.

1188 Site of Origin and High-Grade Pelvic Serous Carcinoma

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Background: Although classified as "ovarian cancers", the origin of high grade serous carcinomas in the pelvis has been controversial. Recent studies implicate the distal oviduct in a significant percentage of these tumors by the presence of a serous tubal intraepithelial carcinoma (STIC). However, the magnitude of the role played by the fallopian tube remains unclear. We performed a detailed analysis of consecutive cases of serous cancer to determine 1) the maximum number of cases that could be assigned to a non-tubal source by exclusionary criteria and 2) if there was immunohistochemical evidence of an origin in the ovary.

Design: Consecutive cases of high-grade carcinoma accessioned between 2005 and 2007 were analyzed. Reports were scrutinized for whether the distal fallopian tubes were completely analyzed. Cases containing STIC or in which the fimbria could not be fully examined to exclude STIC, were placed in the non-exclusionary category. Cases in which the distal tubes were reported examined and were negative were systematically reviewed to confirm this and exclude STIC. In a separate subset of cases in which both the fimbria were visualized and STIC excluded, the ovaries were immunostained for PAX2 and p53 to identify normal PAX2 positive epitheium in continuity with adjacent p53 positive or PAX2-null cancers or putative precursor epithelium (either PAX2-null or p53 positive).

Results: 203 cases of high-grade carcinoma were identified of which 143 (70%) either reported a STIC or did not identify both fallopian tubes. Of the remaining 60, 34 were availble for review. Of these, 9 contained inadequate or no fimbria in the tubal tissue blocs, 5 contained a STIC, and 16 contained negative fimbrial histology from both the right and left fallopian tubes. Analysis of the ovaries from 20 cases with no clear tubal primary revealed PAX2-null and p53-positive surface serous carcinomas. However, in no case was a carcinoma detected in continuity with either normal or immunohistochemically altered (PAX2-null, p53+) mullerian epithelium, in keeping with surface implantation rather than de-novo neoplastic change.

Conclusions: In approximately 16% of high-grade pelvic mullerian carcinomas, both fimbria can be identified and both will be histologically benign. Analysis of the ovarian cortices do not implicate a source in the ovarian surface epithelium using the existing precursor models described for the fallopian tube. The possibility of other origins and the impact of meticulous sampling the oviduct will be discussed.

1189 Re-Evaluation of Immunohistochemical Markers in Endometrial Adenocarcinomas

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Background: When working on a tumor of unknown origin, a metastatic adenocarcinoma from the endometrium may present a diagnostic challenge because of the inconsistent data from the literature and complicated immunostaining profiles for endometrial adenocarcinoma (EAD). In this study, we re-evaluate the expression of an extensive panel of biomarkers in endometrial adenocarcinomas using a single immunostaining system (Ventana XT).

Design: We immunohistochemically evaluated the expression of 1) epithelial markers (AE1/3, CAM5.2, CK7, CK20, CK17, CK19, CK903, EMA); 2) mucin gene products (MUC1, MUC2, MUC4, MUC5AC, MUC6); 3) tumor suppressor genes and transcription factors (ER, PR, p53, beta-catenin, ERG, GATA3, WT-1, CDX2, pVHL); and 4) tumor-associated proteins (TTF-1, napsin A, p16, HepPar1, glypican 3, SALL4, OCT4, PAX8, RCC, GCDFP15, mammaglobin, S100P, IMP3, maspin, MOC31, CEA, CA19-9, CD10, CD15, villin, and P504S) on 128 cases of endometrial adenocarcinoma endometrioid type (FIGO I 32 cases, FIGO II 58 cases, and FIGO III 38 cases) on tissue microarray sections. The staining intensity distribution was recorded.

Results: The positive staining results from selected antibodies are summarized in Table 1. Twelve of 128 cases (9.4%) showed diffuse (4+) and strong p16 positivity. All cases were negative for CK20, SALL4, OCT4, MUC2, HepPar1, villin, GCDFP15, and S100P.

Table 1. Summary of immunostaining results on selected antibodies

Antibody	EAD FIGO 1 (N=32)	EAD FIGO 2 (N=58)	EAD FIGO 3 (N=38)	Positive Cases (%)
CK7	29/32	52/58	34/38	89.8%
CK17	2/32	0/58	0/38	1.6%
MUC1	32/32	58/58	38/38	100%
Vimentin	31/32	52/58	35/38	92%
ER	32/32	58/58	35/38	97.7%
PR	30/32	57/58	38/38	97.7%
Mammoglobin	19/32	45/58	18/38	64%
TTF-1	0/32	2/58	0/38	1.6%
Napsin A	3/32	0/58	0/38	2.3%
Glypican 3	0/32	0/58	4/38	3%
pVHL	0/32	2/58	0/38	1.6%
Maspin	12/32	15/58	4/38	24%
CEA	4/32	9/58	5/38	14%
p16	32/32	57/58	38/38	99%
P504S	7/32	34/58	24/38	50.8%
CDX2	0/32	1/58	0/38	0.8%
PAX8	32/32	58/58	37/38	99%
RCC	0/32	4/58	2/38	4.7%
ERG	0/32	1/58	0/38	0.8%

Conclusions: These data demonstrate that 1) mammaglobin and other tumor/organ-specific markers can be positive in a significant percentage of cases; 2) nearly all cases are positive for ER, PR and PAX8 regardless of tumor grade; 3) 10% of cases can be negative for CK7 and vimentin; and 4) caution should be taken when using p16 to differentiate endometrial primary from endocervical primary since close to 10% of cases are diffusely and strongly positive for p16.

1190 *MiR-182* Mediated *BRCA1* Dysregulation in Ovarian Serous Carcinoma. I. Molecular Analysis

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Background: *BRCA1/BRCA2* mutations are hallmarks of high grade papillary serous carcinoma (H-PSC). Approximately 40-50% of H-PSC have altered expression of *BRCA1/BRCA2* (germline, somatic and epigenetic alterations). Furthermore, women with germline mutations of *BRCA1/2* have a 30%–70% chance of developing H-PSC. If inactivation of *BRCA1/2* is critical in the early tumorigenesis of H-PSC, there must be other mechanisms whereby *BRCA1/2* is downregulated in the remaining 50% of H-PSC. Recent findings of *miR-182*–mediated repression of *BRCA1* expression and significant overexpression of miR-182 in H-PSC suggest that miR-182 may play an important role in the tumorigenesis of H-PSC through its negative regulation of *BRCA1*.

Design: To explore the role of *miR-182* in the early stages of H-PSC tumorigenesis, we conducted *in vitro* molecular and cellular analyses of *miR-182* in human fallopian tube secretory (FTSE) and ovarian surface epithelia (OSE) cell lines. We 1) established stable cell lines with stable *miR-182* overexpression; 2) examined the oncogenic properties of *miR-182 in vitro*; 3) investigated DNA damage response in cells with *miR-182* expression after ionizing radiation; 4) examined and characterized several predicted target genes of *miR-182*, including *BRCA1, HMGA2* and *MTSS1*; 5) studied tumor growth and metastasis *in vivo* (in nude mice) with and without *miR-182* overexpression.

Results: 1) Introducing *miR-182* overexpression in normal immortalized cell lines (both FTSE and OSE) resulted in tumor transformation and significantly enhanced cell invasion *in vitro*. 2) FTSE and OSE cell lines with *miR-182* overexpression had impaired repair of DNA double-strand breaks when treated with low doses of IR. 3) We further demonstrated that the oncogenic properties of *miR-182* in ovarian cancer were mediated by its negative regulation of *BRCA1* and *MTSS1* expression and its positive regulation of oncogene *HMGA2.* Knockdown of *miR-182* expression in ovarian cancer cell lines (SKOV3, and HEY) could therefore partially restore BRCA1 and MTSS1 expression and reduce aggressive tumor growth *in vitro*. Ovarian cancer cell line SKOV3 with *miR-182* overexpression enhances lung metastasis *in vivo*.

Conclusions: *MiR-182* confers a powerful oncogenic potential in normal and malignant ovarian/fallopian tube cell lines through its negative regulation of several critical target genes in H-PSC, including BRCA1, MTSS1 and HMGA2. Therefore, *MiR-182* likely plays a significant role in the early tumorigenesis of H-PSC. Our findings provide a novel target and a new potential therapeutic modality in treating H-PSC.

1191 Utility of the SNaPshot Assay in Ovarian Carcinoma Genotyping

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Background: Ovarian carcinoma (OC) is the leading cause of death among gynecological malignancies with a 46% overall 5 year survival. Lack of effective methods for its prevention/early detection (75% stage III/IV at diagnosis) and common development of resistance to first line agents has launched studies to better understand its pathogenesis and identify targeted therapies. TP53 and KRAS are among the most frequently mutated genes while PIK3CA, although not as prevalent, is actively being investigated as promising therapeutic target. Recently, a multiplexed allele-specific assay (SNaPshot) has been developed to detect simultaneous somatic mutations in tumor DNA extracted from formalin-fixed paraffin-embedded specimens to obtain rapid/effective tumor genotyping and select patients that may respond to specific therapies. The aim of this study is to determine prevalence of mutant genes in resistant OC using this new assay.

Design: The cohort included 63 tumors from 62 patients (1 with 2 different tumors) with resistant OC and ECOG 0-2 performance score. Samples were tested for common hot-spot mutations using SNaPshot assay that detects mutations in 68 different loci as well as insertions and deletions from 15 cancer genes (AKT1, APC, BRAF, CTNNB1, EGFR, ERBB2, IDH1, KIT, KRAS, MAP2K1, NOTCH1, NRAS, PIK3CA, PTEN and TP53).

Results: Most patients (47/62) had high-grade (HG) serous or mixed serous and transitional cell carcinoma (SE-TCC), 5 HG endometrioid carcinoma (EC), 3 clear cell carcinoma, 2 MMMT, 2 small cell carcinoma of hypercalcemic type, and 1 low-grade (LG) EC, LG serous, TCC, and mucinous carcinoma (Muc-Ca) respectively. 18 tumors (29%) showed one or more mutations with a total of 28 mutations identified: TP53 in 10 (6 HG serous or SE-TCC, 1 TCC, 1 MMMT, 1 HG EC, and 1 Muc-Ca), KRAS in 7 (3 serous or SE-TCC, 2 HG EC, and 1 LG serous and 1 Muc-Ca), PIK3CA in 5 (2 HG EC, 2 serous or SE-TCC, and 1 clear cell carcinoma), CTNNB1 in 3 (2 HG and 1 LG EC), PTEN in HG EC, AKT1 in LG EC and NRAS in HG serous or SE-TCC.

Conclusions: TP53, KRAS and PIK3CA were the most frequently mutated genes in OC, TP53 being most common in HG serous carcinomas and KRAS in LG and HG serous carcinomas, while mutations in CTNNB1 and PTEN were only seen in ECs and PIK3CA mutations in HG tumors. As patients with PIK3CA mutations have been considered for enrolment in clinical trials with PI3 kinases inhibitors, this assay can be used in clinical settings to determine the prevalence of mutant genes in resistant OC and identify patients that may respond to target therapies.

1192 Should High-Risk Adolescents Have Pap Tests?

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Background: The 2009 ACOG guidelines state that cervical cancer screening (CCS) commence at age 21-years owing to a very low incidence of cervical carcinoma in this age group and the potential for harmful follow-up treatment. Our institution serves a large population of high-risk adolescents (HRA), with early onset of sexual activity and pregnancies. We investigated outcomes and demographic data of HRA with HSIL Pap tests in order to identify any subgroups who may benefit from CCS.

Design: Computer-based search for years 2000-10 was done to identify Pap results from women under 21-years-old. Chart review of women with at least one HSIL Pap test was done to obtain biopsy and LEEP results and sexual and pregnancy history when available.

Results: Of 56,785 adolescent Pap tests, 45,276 (79.5%) were negative, 11,232 (20%) were ASCUS or LSIL and 277 (0.5%) were HSIL; Of the HSIL group, 89 (32%) had no biopsy, 35 (13%) had no dysplasia on biopsy, 57 (21%) had CIN 1, 40 (14%) had CIN 2, and 56 (20%) had CIN 3. One case of microinvasive cervical carcinoma (MIC) was found on LEEP.

Adolescent Demographic Data

Grade	No. Lifetime Partners (n)	Age of First Intercourse (n)	Age of First Pregnancy (n)
CIN 1/No Dysplasia on Bx	3 (9)	16 (8)	18 (6)
CIN 2	4 (10)	18 (11)	18 (6)
CIN 3/MIC	7 (14)	15 (15)	17 (9)

No differences in demographic data were statistically significant between any subgroups.

Conclusions: Our rate of HSIL in adolescents was 0.5%, consistent with other studies. However, our study did find 56 (20%) CIN 3 lesions in the HSIL subgroup. CIN 3 behavior in adolescents has not been well described, but current guidelines from ACOG and the ASCCP states that CIN 3 lesions in adolescents should be ablated. This subset of adolescents was detected by cervical cancer screening and, in line with current guidelines, was treated with ablation. This study shows that the ACOG guidelines for adolescents may be reasonable for the majority of the adolescent population, but a subset of HRA with CIN 3 would be not be detected. The demographic data failed to identifiy a subgroup of adolescents at higher risk for CIN 3. We cannot suggest a way to select out the high-risk adolescents that would require CCS. Because there is a subset of HRA with CIN 3 lesions, we feel that more study is needed to create a process that can select out these high-risk adolescents for CCS.

1193 Microcystic Stromal Tumor Is a Distinct Ovarian Neoplasm Characterized by ß-Catenin Alteration

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Background: Microcystic stromal tumor (MCST) is a recently described subtype of ovarian tumor characterized by a prominent microcystic histological pattern and diffuse immunoreactivity for CD10 and vimentin. Descriptions regarding the histological features of MCST are limited, and the pathobiology remains unclear. Here, we report four cases of ovarian MCST in which we have performed extensive histological, immunohistochemical, and genetic investigations to determine its distinctiveness among ovarian neoplasms.

Design: In addition to the morphological characterization of ovarian MCSTs, we performed immunohistochemical comparisons between ovarian MCSTs and a variety of sex cord-stromal tumors. Markers examined in this study include vimentin, CD10, β-catenin, WT-1, α-inhibin, calretinin, ER, PgR, EMA, and cytokeratin AE1/3. Further, two of the MCSTs were analyzed using direct sequencing for a mutation in exon 3 of the β-*catenin* gene.

Results: The patients' ages ranged from 32 to 41 years. One patient had a past history of familial adenomatous polyposis (FAP). Microscopically, all four tumors showed similar histological features: generally bland tumor cells with round to ovoid nuclei growing in microcystic, macrocystic, and solid patterns. Intervening thick fibrous stroma were observed. Immunohistochemically, tumor cells were diffusely and strongly positive for CD10, vimentin, and WT-1, but negative for α-inhibin, calretinin, ER, PgR, and EMA. Cytokeratin expression was variable. Importantly, we detected aberrant nuclear expression of β-catenin protein in all four cases. In contrast, none of the sex cord-stromal tumors examined showed nuclear expression of β-catenin. Mutation analyses performed in two of the non-FAP patient cases revealed the presence of the c.98C>G mutation in exon 3 of β-*catenin*.

Conclusions: Our immunohistochemical analyses showed that MCST is a distinct ovarian neoplasm characterized by aberrant nuclear expression of β-catenin. The results of our immunohistochemical and mutation analyses, along with the presence

of MCST in an FAP patient, strongly suggest that dysregulation of the Wnt/β-catenin pathway plays a fundamental role in the pathogenesis of ovarian MCST. Finally, by comparing the immunophenotype of MCST with other ovarian sex cord-stromal tumors, we were able to identify unique features of MCST and a panel of markers useful for differential diagnosis

1194 Do Deeper Sections Increase the Yield of Detection of Serous Tubal Intraepithelial Carcinoma (STIC) in the "Sectioning and Extensively Examining the Fimbriated End" (SEEFIM) Protocol?

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Background: Several studies have demonstrated an association between STIC and pelvic (i.e. ovarian, endometrial and peritoneal) serous carcinoma. It has been suggested that the adoption of the SEEFIM protocol should enhance the detection of STIC since it allows for the histomorphological evaluation of the entire fallopian tube with added emphasis on the fimbrial mucosa. To our knowledge, however, no study has yet examined the potential effect of deeper level H&E sections of the entire fallopian tube on the yield of STIC from a sample of salpingectomies associated with ovarian, endometrial and peritoneal serous carcinomas.

Design: Two deeper level H&E sections were cut from all blocks of the entirely submitted fallopian tubes (as per the SEEFIM protocol) and were reviewed by a panel of expert gynaecological pathologists. The 55 cases from which these deeper sections were obtained were initially diagnosed as STIC-negative serous carcinomas of ovarian, endometrial and primary peritoneal origin. These cases were part of a larger study of the frequency of STIC in 300 consecutive bilateral salpingectomies of which 67 were associated with serous carcinoma of the ovary, endometrium or peritoneum. Of these 67 cases, 12 had shown STIC and 55 were negative.

Results: Four additional cases of STIC were identified. All four STICs were intratubal without involvement of the fimbriae. No additional cases of STIC were identified in those cases associated with an endometrial serous carcinoma.

Frequency of STIC in serous gynaecologic malignancies before and after deeper sections

Cases of Serous Carcinoma		Associated Salpingectomy Specimens		
Primary Site	Number	Number of cases with STIC by single H&E (%)	Number of additional cases in which STIC was found on deeper sections	Total Number of cases with STIC (%)
Ovary	32	6 (19)	3	9 (28)
Endometrium	28	4 (14)	0	4 (14)
Peritoneum	7	2 (29)	1	3 (43)

Conclusions: The single H&E section SEEFIM approach detected only 75% (95%CI: 51-90%) of the cases of STIC in our sample. We conclude that the yield of the SEEFIM protocol is indeed increased with deeper sections. Furthermore, none of the extra cases of STIC would have been detected had deeper level H&E sections been performed only on the fimbrial blocks.

1195 Predetermined Search Methods Can Increase the Yield in Counting Mitotic Figures in Uterine Leiomyosarcoma (ULMS)

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Background: Diagnosis of ULMS is based on cytologic atypia, presence of coagulative tumor cell necrosis and mitotic index, all features with a variable degree of subjectivity. Frequently, no guidance or search plan is used when performing a mitotic count and a drop and run (linear) or drop and weave (random) search pattern is used. This study assessed accuracy and reproducibility in utilizing specific search patterns for mitotic figures in ULMS.

Design: 11 cases of ULMS (chosen to assure abundant mitotic figures and desired tumor patterns) were culled from divisional files. Each case was evaluated at low power (4X) and examples of each tumor pattern were selected for counting. The tumor patterns were designated random, linear, along fascicles, fascicle cross-section, reactive (including areas near necrosis), hypercellular, and interface (tumor & normal). Slides were screened by 3 observers at 20X for the presence of mitotic figures, and, at the first mitotic figure seen, one 40X mitotic count (10 high power fields) was performed for each available search pattern. The data was then compiled as an average count per pattern along with a maximum inter-observer range (MIR) for each case.

Results: Counting in hypercellular areas and along fascicles yielded the highest counts, while fascicle cross section, linear, and random patterns yielded the lowest. 13 Non-diagnostic counts, i.e. less than 10 mitosis per 10 40X fields, were obtained (5 into fascicles, 3 random, 3 linear, 2 reactive\necrosis). Pattern based counting did not improve reproducibility based on MIR scores, with counting along the interface of ULMS and normal tissue faring the worst (see table 1).

	Random	Linear	Hypercellular	Interface	Along Fascicle	Into Fascicle	Reactive \ Necrosis
MIR	22	29	23	37	24	12	19
Average	26	20	44	28	33	12	23

Conclusions: Mitotic figure assesment in hypercellular areas and along fascicles yields the highest number of mitotic figures. Mitotic activity should not be assessed in random or linear patterns and cross sections of fascicles should be avoided as counts may not reach the diagnostic threshold. As a diagnostic criteria, mitotic figure counting shows poor reproducibility, and, therefore, care should be used in selecting regions in which mitotic figure assessment will be made.

1196 MELF Pattern of Myoinvasion in Endometrioid Endometrial Adenocarcinoma Is Associated with Low-Grade Tumors, Deep Myometrial Invasion and a Low Proliferative Index

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Background: Low-grade and early-stage endometrioid endometrial adenocarcinoma (EEC) usually has a favorable outcome. There is a subset of patients with a microcystic, elongated and fragmented (MELF)-pattern of invasion that has been implicated with increased lymphovascular invasion (LVI) and lymph node metastases. Changes in immunohistochemical markers within MELF areas suggest these areas are distinct from the original tumor. This study characterizes the frequency of MELF-type invasion, its relationship with depth of myometrial invasion, LVI, changes in estrogen receptor (ER) status, E-cadherin and Ki67 expression.

Design: 286 cases of pure EEC were retrieved between 2001- 2011. The histological slides and clinical records were reviewed to record FIGO grade, depth of invasion, pattern of myometrial invasion, LVI and clinical outcome. A representative slide was selected to determine the expression of ER, E-cadherin and Ki67 within the tumor and in the MELF areas. Fisher's exact tests and student t-tests were used for statistical analyses.

Results: MELF was identified in 47 of 286 (16%) cases including 11 with focal areas of MELF. Tumors with MELF pattern were more likely to be grade 1 (76% v 51%), contain LVI and show invasion into outer half of myometrium. In FIGO grade 1 tumors, the MELF group was still more likely to contain LVI (62% v 26%, $p<0.001$) and invade into outer half of myometrium (65% v 33%, $p<0.001$). Disease recurrence and mortality were not significantly higher in MELF group. There was no significant difference in expression of ER and E-cadherin in MELF areas with the rest of the tumor. The proliferative index Ki67 appeared to be lower in at least 50% of cases in the MELF areas.

Table 1

	LVI present	Outer half myometrial invasion
Total EEC (all grades)	29%	38%
MELF cases (all grades)	72%	74%
Grade 1 EECs	26%	33%
Grade 1 with MELF	62%	65%

LVI and myometrial invasion in all tumors (grade 1-3) versus grade 1 tumors

Conclusions: MELF pattern of myoinvasion in EEC is more common in low-grade tumors. Within grade 1 tumors MELF pattern of myoinvasion is associated with deep myometrial invasion and LVI. Expression of ER and E-cadherin within the main tumor and MELF areas was similar in the majority of cases. However MELF areas demonstrated a lower proliferative index. This finding although appears unexpected for areas of active invasion, has been demonstrated in other malignancies.

1197 Coexisting High-Grade Vulvar Intraepithelial Neoplasia (VIN) and Condyloma Acuminatum: Independent Lesions Due to Different HPV Types

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Background: The majority of vulvar intraepithelial neoplasia (VIN) is high-grade and high-risk human papillomavirus (HRHPV)-related (most commonly HPV16), and is considered the precursor of HRHPV-related squamous cell carcinoma of the vulva. Vulvar condyloma acuminatum is low-risk HPV (LRHPV)-related (most commonly types 6 and 11) and has virtually no risk of neoplastic progression. While infections with multiple high- and low-risk HPV types have been reported for cervical squamous intraepithelial lesions, coexisting vulvar condyloma and adjacent high-grade VIN have not been well-characterized.

Design: Eleven cases of concurrent condyloma acuminatum and adjacent flat high-grade VIN and four cases of high-grade VIN with prominent warty/condylomatous architecture were analyzed using immunohistochemical (IHC) analysis of p16 expression and in situ hybridization (ISH) for HPV detection (probes for HPV6/11, HPV16, and HPVWS [types 6,11,16,18,31,33,35,45,51,52]).

Results: All patients had underlying conditions with evidence of immunosuppression (human immunodeficiency virus infection, post-transplant therapy, or autoimmune disorder). Data are summarized in Table 1.

Lesion Type	**p16 (n/n tested)**	**HPV type (n positive/n tested)**
Condyloma with adjacent high-grade VIN (n=11)		
Condyloma	Negative (few focal/weak) (11/11)	HPV6/11 (10/10)
High-grade VIN	Positive (diffuse) (11/11)	HRHPV* (5/5)
High-grade VIN with warty/ condylomatous features (n=4)	Positive (diffuse) (4/4)	HRHPV* (4/4)

* HRHPV detected either by positive HPV16 and/or positive HPVWS in conjunction with negative HPV6/11 (additional HPV ISH and HPV genotyping by PCR is ongoing)

Conclusions: The restriction of LRHPV to condylomatous components and HRHPV to high-grade VIN components of adjacent lesions suggests these are independent and a result of infection with multiple HPV types. A diffuse pattern of p16 expression can highlight small foci of high-grade VIN which may be overlooked in more abundant condylomatous tissue from immunosuppressed patients. The presence of HRHPV in lesions with prominent warty/condylomatous architecture supports their classification as forms of pure high-grade VIN and distinguishes them from condyloma acuminatum.

1198 HPV73-Mediated CINII/III in a Gardasil Vaccinated Patient

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Background: Human papillomaviruses (HPVs) are responsible for about 500,000 cases of cervical cancer, 10 million further cases CIN II/III, and 30 million anogenital warts or low grade CIN each year. Two prophylactic HPV vaccines have been developed to prevent this disease: Gardasil(R), a quadrivalent vaccine (targeting HPV6, 11, 16, 18) and Cervarix(R), a bivalent vaccine (targeting HPV-16 and -18). Both vaccines contain

L1 virus-like particles (VLPs) derived from HPV16 and 18, which are most frequently associated with cervical cancer. These type-specific vaccines can effectively prevent infection of only two out of 18 potential oncogenic HPV types. Here, we report a case of CINII/III, a precursor to malignancy that a patient developed two years after Gardasil vaccination. HPV testing showed that this premalignancy is caused by HPV73, a high risk HPV type that is not detected by most commercial tests. To our knowledge, this is the first evidence of CINII/III following Gardasil vaccination.

Design: A 21 year old female with a history of normal Pap smears for two years had the recommended three injections of Gardasil vaccine within six months. No HPV testing was performed because of the normal Pap history.

Results: Two years following the Gardasil vaccination, the patient developed LSIL. No HPV testing was performed for this abnormal cytology, instead, the patient was directly referred to colposcopic biopsy examination, in which CINII/III was confirmed. COMPLeTe Care HPV, a HPV test that simultaneously detects and types all 15 oncogenic HPV types, was performed on the biopsy tissue and HPV73 was detected. The patient returned to normal Paps following the removal of the affected areas by a laser procedure.

Conclusions: The current L1-VLP vaccines are type-specific and therefore, either a multivalent L1-VLP or a broad-spectrum vaccine against all oncogenic HPV types would be the ultimate strategy to prevent HPV related diseases.

An HPV test that detects all oncogenic HPV types is an important component to prevent false-negative results in managing HPV related diseases. Additional tools such as typing can confirm the persistency of infection and multiple infections, type prevalence, type-specific etiology, and type-specific risk assessment to better manage the disease. Finally, even small DNA viruses like HPV have salvage and backup pathways that have been honed over thousands of years of evolution and many replicative cycles. With increasing vaccination, one can predict an imbalance of the existing oncogenic types and potential emergence of new ones.

1199 Loss of ARID1A Expression Correlates with Stages of Tumor Progression in Uterine Endometrioid Carcinoma

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Background: ARID1A is a recently identified tumor suppressor which participates in chromatin remodeling that is required to regulate a variety of nuclear activities including gene expression. We previously reported inactivating mutations in 40% and loss of expression of *ARID1A* in 26% of low-grade uterine endometrioid (FIGO 1) carcinomas.

Design: A total of 70 cases of endometrial lesions were studied for the immunostaining pattern of ARID1A. They included 36 complex atypical hyperplasia (CAH) and 34 high-grade endometrioid (FIGO 2 and 3) carcinomas. Among the 34 high-grade endometrioid carcinomas, there were 8 cases that contained concurrent components of low-grade (FIGO 1) carcinoma. An antibody that was specific for the ARID1A encoded protein was used for immunohistochemistry. Complete loss was defined as absence of ARID1A immunoreactivity in all tumor cells whereas clonal loss was lack of the staining in a discrete tumor area(s) in the background of ARID1A positive tumor cells.

Results: We found that 5 (14%) of 36 cases of CAH displayed clonal loss of ARID1A, but none showed complete loss of ARID1A expression. In contrast, complete and clonal ARID1A loss was detected in 13 (38%) and 2 (6%) of 34 high-grade endometrioid (FIGO 2 and 3) carcinomas, respectively. Among them, 8 cases had concurrent components of low-grade carcinoma. In the low-grade areas from those 8 mixed cases, complete loss of ARID1A was recorded in 2 (25%) and clonal loss in 5 (62.5%) of cases. In three of those 8 cases, ARID1A retention or clonal loss was observed in the low-grade component while complete loss was detected in the adjacent high-grade area.

Conclusions: In summary, clonal loss of ARID1A was found in 14% of CAH but complete loss in 0%. In contrast, complete loss of ARID1A expression was found in approximately 25% of low-grade carcinomas and 38% of high grade carcinomas. These findings strongly suggest that loss of ARID1A staining (presumably due to a mutation) plays an important role in tumor progression in endometrioid carcinoma which may have significant implications to further understand the pathogenesis of uterine endometrioid carcinoma.

1200 DPEP1, a Useful Immunohistochemical Tool in Distinguishing Primary from Metastatic Endometrioid and Mucinous Ovarian Tumors

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Background: Metastasis of colorectal adenocarcinoma (ADC) to the ovary is not an uncommon occurrence. In particular, mucinous and endometrioid ovarian ADC phenotypes have proven to be diagnostically challenging in the differential diagnosis with metastatic colon ADC. From recent proteonomic studies that demonstrated significant upregulation in colonic ADC relative to matched normal tissue, DiPeptidase-1 (DPEP1) has emerged as a potential diagnostic marker for colonic ADC. This finding has direct relevance to cases wherein the differential diagnosis predicament previously described are encountered, as DPEP1 may function to further help delineate metastatic colonic ADC from primary ovarian mucinous or endometrioid tumors.

Design: Four cases of mucinous colonic ADC and ten cases of conventional colonic ADC from female patients were retrieved from archival files as well as 4 cases of endometrioid ovarian tumors, 6 cases of endometrioid uterine tumors, and 4 cases of mucinous ovarian tumors. All sections were subjected to immunohistochemistry with antigen retrieval using an antibody to DPEP1 (Sigma) at a titer of 1 : 1500.

Results: The conventional colonic ADC demonstrate diffuse, strong expression for DPEP1 in a polarized (apical/luminal centric) fashion. The mucinous colonic ADC showed some cytoplasmic staining with patchy apical/luminal staining. All four mucinous ovarian tumors were negative. Half of the ovarian endometrioid tumors expressed DPEP1, but in a punctuate fashion, with a circumferential pattern on the positive cells. Only 1 of the 4 endometrial endometrioid ADC cases expressed DPEP1, and in a manner similar to the ovarian endometrioid tumors.

Conclusions: DPEP1 has utility in distinguishing colonic ADC from mucinous and endometrioid ADC of the ovary and is based on the presence of expression and the pattern of staining. The characteristic diffuse apical/luminal centric staining noted in the colonic ADC was only focal in the mucinous colonic ADCs, but still present in contrast to its complete absence in mucinous ovarian tumors. For those ovarian and endometrial tumors that did express DPEP1, the staining was focal and circumferential, in contrast to the apical/luminal staining pattern noted in colonic ADCs.

1201 Correlation of CXCL12/CXCR4 Expression and FOXP3 Cell Infiltration in Normal Endometrium, Typical and Atypical Hyperplasia and Endometrioid Adenocarcinoma

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Background: Chemokines and chemokine receptors are also known to play a role in both immunity and the inhibition or growth of cancer cells. Recent studies on human cervical and ovarian cancer have shown there to be an overproduction of the chemokine CXCL12 and its receptor (CXCR4) which is now known to be related to cancer progression.

Design: AIM: to investigate whether a correlation between CXCL12/CXCR4 expression and FoxP3+ Treg cell infiltration in normal endometrium (NORMAL), simple hyperplasia (SH), complex non-atypical/ typical (CTH), complex atypical hyperplasia (CAH) and endometrioid adenocarcinoma (EA) exists.

METHODOLOGY: Our study consisted of building a progressive TMA with:17 NORMAL patient cases,20 SH + CTH patient cases,16 CAH patient cases and 40 EA patient cases.This investigation compared a total of 93 patient cases from the extraction of 259 tissue cores which were subjected to IHC staining. Slides were scored via light miscopy to measure the percentage of positive Foxp3 T- cells and the percentage area x intensity of CXCL12/CXCR4 expression.

Results: One-way analysis of variance (ANOVA) was followed by post hoc analysis using Tukey's Multiple Comparison Test which revealed statistically significant differences between the amount of CXCL12 expression, CXCR4 expression and Foxp3 T cell infiltration between Normal, SH + CTH, CAH and EA patient groups ($P < 0.001$). As for FoxP3 Treg infiltration post hoc analysis by Tukey's Multiple Comparison Test identified: Normal vs SH + CTH ($q = 5.26$, $P< 0.05$), Normal vs CAH ($q = 8.853$, $P< 0.05$), Normal vs EA ($q = 6.735$, $P< 0.05$), SH + CTH vs CAH ($q = 4.043$, $P< 0.05$) and CAH vs EA ($q = 3.929$, $P< 0.05$) to have statistically significant differences, with the exception of: SH + CTH vs EA groups which showed no statistically significant difference ($q= 0.74$, $P >0.05$).

Conclusions: We observed increasing CXCL12 and CXCR4 expression which correlated with increasing endometrial per-neoplastic changes and cancer. Trends for Foxp3 Treg infiltration showed to increase with increasing prE-neoplastic morphology, however unexpectedly higher Foxp3 levels were seen in CAH when compared to EA patients. Despite these differences, a weak positive correlation was observed between increasing Foxp3 Treg infiltration and increasing CXCL12 expression (Spearman correlation, P value <0.004**, 2 tailed test, $r = 0.30$), indicating potential chemoattractant properties of CXCL12 regarding Tregs.

1202 Extrauterine Endometrial Stromal Sarcoma: A Clinicopathologic Study of 63 Cases

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Background: Extrauterine endometrial stromal sarcoma (EESS) is an uncommon tumor that occurs in patients (pts) over a wide age range. The extrauterine location, non-gynecologic symptoms/signs at presentation and confounding histologic features can pose a diagnostic challenge. In this study, we present the clinicopathologic features of 63 such cases.

Design: 63 cases of EESS from a period of 20 yrs were retrieved. Clinical information was obtained from the pts' charts and questionnaires sent to physicians. The following clinical parameters were recorded: pts' age, clinical presentation, treatment (tx), recurrences and current status. In 46 cases slides were re-reviewed. The following pathologic parameters were recorded: size and location, gross and microscopic features, and evidence of endometriosis.

Results: The pts' ages ranged from 27 to 87 yrs (median 50). The most common symptoms/signs were: abdominal/pelvic mass or pain, vaginal bleeding, and gastrointestinal symptoms. Tumor size ranged from 1.2 to 24.5 cm. The most common sites of involvement included ovaries (24), bowel wall (27), abdomen/peritoneum (37), pelvis (20) and vagina (6). Multiple sites of involvement were present in 34 cases. 42/45 cases had a classic microscopic pattern and 1 had dedifferentiation; 16 had vascular invasion. Sex-cord elements were seen in 8, smooth muscle differentiation in 3, myxoid change in 4, and hyaline plaques in 23 cases. Stromal fibrosis was present diffusely in 5 and focally in 21 cases. Endometriosis was noted in 29/63 cases. CD10 was diffusely (+) in 26 and focally in 3 of 29 cases. Desmin was focally (+) in 7/15 cases. ER was (+) in 27/28 cases; PR was (+) in 33/34 cases. In 25% of cases an initial diagnosis other than ESS was made: GIST, leiomyosarcoma, liposarcoma, synovial sarcoma, MPNST and various sex cord stromal tumors. 61/63 pts had cytoreductive surgery, 31/48 had hormonal tx, 13/45 had chemotx, and 7/45 had radiation tx. Follow up ranged from 5 to 336 months; 14 cases were lost to follow up. 29 pts had recurrent disease; the time to recurrence ranged from less than 12 mos to 192 mos (median 42 mos). 7 pts died of disease (DOD) with a median of 70 mos from dx to death, 14 pts are alive with disease, and 27 pts are alive without disease.

Conclusions: EESS is commonly associated with endometriosis and tends to be indolent with a propensity for recurrence. 6/7 pts who DOD had bowel involvement and 1 had dedifferentiation. Unusual histologic features or presentation and the extrauterine location lead to an inaccurate dx in a significant number of cases.

1203 P504S (AMACR-alpha-Methylacyl-coA Racemase): A Novel Marker of Clear Cell Carcinoma of the Female Genital Tract

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Background: Clear cell carcinoma (CCC) of the female genital tract (FGT) poses a diagnostic challenge as its histologic features can overlap with other carcinomas particularly when they demonstrate clear cell change. Currently, CCC is diagnosed primarily on morphologic features with poor interobserver reproducibility. As yet, there are no specific immunohistochemical markers to assist in the diagnosis of CCC. P504S (AMACR-alpha-methylacyl-coA racemase) is widely used in the diagnosis of prostate cancer and papillary renal cell carcinomas. In addition, studies have show the expression of this marker in CCCs of the urinary bladder and urethra. However, P504S expression has not been extensively studied in tumors of the FGT, particularly CCC. The aim of our study is to determine if P504S is expressed by tumors of Müllerian origin with attention to its value, if any, in the diagnosis of CCC.

Design: 30 CCCs of FGT were retrieved from our pathology files covering a period of 26 years (1985 to the present). CCC from the following sites were included: endometrium (9), ovary (10), cervix (8), pelvis (1), vagina (1) and metastasis to a pelvic lymph node (1).13 serous carcinomas (SC), 15 endometrioid carcinomas (EC) of the endometrium and ovary, and 9 cases of endometriosis were used as controls. All cases were stained for P504S (Zeta Corp., Arcadia, CA). Granular cytoplasmic staining was interpreted as positive. Staining in the neoplastic cells was graded as follows: 0: no detectable staining, 1+: 1-5%, 2+: 6 to 25%, 3+: 26 to 50%, 4+: 51 to 75% and 5+: >76%.

Results: P504S was positive in 19 of 30 (70%) CCCs. Of these, 3 cases showed 1+, 4 showed 2+, 5 showed 3+, 3 showed 4+ and 6 showed 5+ staining for P504S. Only 1 of 13 (8%) SC was positive (1+) for P504S. Ten of 15 (67%) ECs were completely negative for P504S. Three of 5 ECs showed 1+ staining, 1 case showed 2+ staining and 1 case showed 5+ staining in the tumor cells. The positive staining of ECs, including the case with 5+ staining, was mostly confined to areas of necrosis and showed only weak to moderate intensity. None of the SC or EC showed strong diffuse staining for P504S.

Conclusions: We found that P504S is expressed in 70% of CCC of the FGT. Strong, diffuse, granular cytoplasmic staining of P504S, when present, is highly suggestive of CCC histotype. P504S is negative in majority of SC and EC and can be a useful marker to differentiate them from CCC, in challenging cases. Staining in areas of necrosis can be seen in SC and EC and must be interepreted with caution.

1204 Predictive Value of Cervical Cone Biopsy Margins and Endocervical Curettage Results on Persistence of Residual Cervical Neoplasia in Subsequent Hysterectomy Specimens

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Background: We studied the value of margins assessment for cervical cone biopsy specimens and of concurrent endocervical curettage (ECC) results to predict the presence of residual neoplasia in the hysterectomy. Confounding variables may include the type of excisional biopsy performed, i.e, cold-knife cone (CKC) or loop electrical excision procedure (LEEP), and type of neoplasia, i.e, glandular or squamous. We also analyzed the results based on the aforementioned confounding variables.

Design: We performed a retrospective review of records from January 1996-October 2010 of 217 women with a diagnosis of squamous or glandular cervical neoplasia in a cone biopsy. These subjects all subsequently underwent total hysterectomy within 11 years of the diagnosis (range: 2 days-11 years, median: 54 days, mean: 201 days).

Results: Of the 217 women meeting the criteria, 13 had both squamous and glandular neoplasia and were counted in both categories. ECC was performed at the time of cone biopsy in 154 cases. Among patients with involved margins, residual neoplasia was found in 53% of those with squamous neoplasia versus 62% of those with glandular neoplasia. Among patients with negative margins, residual neoplasia was identified more frequently in the glandular group (53%) than in the squamous group (31%). Stratification based on the type of excision did not affect the outcome. Additionally, patients with neoplasia on concurrent ECC were 3.0 (squamous) and 1.7 (glandular) times as likely to have residual neoplasia compared to those with negative ECCs ($p<0.01$ and $p=0.03$, respectively). Among patients with negative ECCs, those with a diagnosis of glandular dysplasia on concurrent cone biopsy were 2.0 times as likely to have residual neoplasia compared to patients with squamous neoplasia ($p=0.01$). Lastly, among patients who had margin involvement by squamous neoplasia, those who also had a positive ECC were 3.8 times as likely to have residual neoplasia compared to those with a negative ECC ($p<0.01$).

Conclusions: Our data is in accord with prior literature showing residual glandular neoplasia to be more frequent than residual squamous neoplasia despite negative margins on cone biopsy. Our study suggests that neoplasia found in a concurrent ECC is an important predictor of residual neoplasia and may ultimately prove to be of greater prognostic value than the cone biopsy margins. Finally, the modality of excisional biopsy did not significantly affect the frequency of residual disease.

1205 *MiR-182* and Its Target Gene Dysregulation in Ovarian Carcinoma: Pathology and Clinical Analysis

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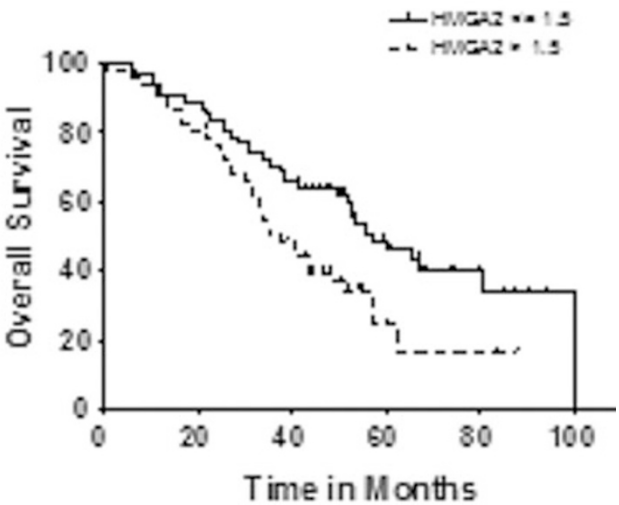
Background: *BRCA1/BRCA2* mutations are hallmarks of high grade papillary serous carcinoma (H-PSC). Nearly 50% of H-PSC shows genomic alterations of *BRCA1/BRCA2*. If inactivation of *BRCA1/2* is critical in the early tumorigenesis of H-PSC, interference with *BRCA1/2* expression by non-genomic mechanisms may exist. We and other investigators have demonstrated that several H-PSC associated genes, including *BRCA1*, *HMGA2*, *FOXO3* and *MTSS1* are directly regulated by *miR-182*. Presently, we intend to investigate the expression of *miR-182* and its target genes in a cohort of advanced ovarian cancer patients.

Design: We aimed to examine whether *miR-182* and its target gene expression are associated with H-PSC histology and outcome. 117 cases with advanced ovarian cancer (88 H-PSC, 29 non-serous carcinoma) and 30 normal fallopian tubes were obtained from patients treated at Northwestern University between 2002 and 2007. Tissue microarrays in duplicate were prepared and stained for *miR-182* and *BRCA1*, *HMGA2*, *FOXO3* and *MTSS1*. Gene expression patterns in association with pathology andclinical outcomes were analyzed by various statistical modalities.

Results: H-PSC demonstrated significantly higher *miR-182* ($p = 0.002$) and *HMGA2* ($p = 0.02$) expression, and significantly lower *BRCA* ($p=0.052$) and *FOXO3* ($p<0.0001$) expression than normal fallopian tube tissue. *miR-182* is significantly correlated with *MTSS1* expression ($r=0.31$; $p < 0.001$), while other target genes did not show a significant correlation with *miR-182*. These findings suggest complicated regulation mechanisms between the genes involved in H-PSC. Among examined *miR-182* target genes, only *HMGA2* was significantly associated with serous type carcinomas ($p<0.01$), ascites ($p<0.009$) and decreased overall survival ($p=0.02$) (Figure 1). *FOXO3* expression was associated with lower stage of disease ($p = 0.04$) and a solid histologic growth pattern ($p=0.03$).

Conclusions: *miR-182* is significantly overexpressed in most H-PSC. The level of *BRCA1* expression has no significant correlation with histological features and clinical outcome. In contrast, *HMGA2* is significantly associated with H-PSC and worse clinical outcome.



1206 Pax 8 Is a Reliable Marker in Making a Tissue Diagnosis of Primary Epithelial Ovarian/Peritoneal Carcinomas for Neoadjuvant Chemotherapy

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Background: Neoadjuvant chemotherapy (NAC) followed by interval debulking surgery is an alternative approach in the management of patients with advanced stage epithelial ovarian carcinoma (EOC)/peritoneal carcinomas (PC) compared to the traditional debulking surgery followed by chemotherapy. Therefore, a tissue diagnosis for malignancy confirmation is crucial. However, due to the wide differential histologic diagnoses, an accurate diagnosis could be challenging. Herein, we are planning to evaluate the utility of the transcription factor protein, PAX8, in the diagnosis of primary EOC/PC for patients eligible for NAC.

Design: This is a retrospective study in which our database was searched for patients diagnosed with EOC/PC who received NAC followed by debulking surgery during a seven-year period (2004-2011). Immunohistochemistry (IHC) was performed on paraffin-embedded tissue from 96 cases (53 EOC/PC and 43 non-ovarian carcinomas). The IHC slides were blindly reviewed and the results of the PAX8 staining intensity were reported as positive (weak, moderate, and strong) or negative (no staining observed).

Results: For EOC/PC cases, 36 cases were biopsy specimens and 17 cell blocks prepared from ascitic fluid (n=10), pleural fluid (n=4) and supraclavicular lymph node aspiration (n=3). The histology was as follows; 45 serous, 3 endometrioid, 3 mucinous, 1 clear cell and 1 carcinosarcoma subtypes. As for non-ovarian carcinomas, the tissue distribution was as follows; cell block from ascitic fluid (n=1), whole section from metastatic adenocarcinoma to the ovary (n=12) and biopsies form various sites (n=30). Overall, PAX8 was positive in 53/96 (55%) cases and negative in 43/96 (45%) cases. 51/53 (96%) of EOC/PC were positive and 41/43 (95%) non-ovarian carcinoma cases were negative. The 2 non-ovarian carcinoma cases positive for PAX8 were renal cell carcinoma. The sensitivity and specificity of PAX8 was 96% and 95%, respectively.

Conclusions: The histologic distinction of EOC/PC from its mimics is clinically important yet not one single stain has been proven to be highly reliable. Our data clearly showed that PAX8 is very highly sensitive and specific in primary EOC/PC, especially

on biopsy and cytology on cell blocks. Therefore, we highly recommend adding it to the immunohistochemical panel for the diagnosis of EOC/PC, especially in institutions where neoadjuvant is gaining popularity.

1207 Assessing Endometrial Hyperplasia and Carcinoma Treated with Progestin Therapy

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Background: Progesterone treatment is an alternative to hysterectomy in some patients with complex atypical hyperplasia (CAH) and well differentiated endometrial carcinoma (WDC). In 2007, Wheeler et al. proposed a classification scheme for assessment of treated CAH and WDC. They concluded that after 6 months of treatment persistent cytologic atypia and architectural abnormalities were associated with treatment failure. This study aims to assess the criteria proposed by Wheeler et al.

Design: With IRB approval, 30 cases of progesterone treated CAH (18) and WDC (12) were assessed. Patients were 18 to 78 years old (mean 49) and 16 were premenopausal. The initial endometrial biopsy, treatment biopsies/currettages, and hysterectomy specimens (when available) were classified by the WHO criteria for CAH and WDC. Applying the features used by Wheeler et. al, progesterone- related changes were assessed at 3-6 month intervals for the following features: gland:stroma, architectural abnormalities, mitotic activity, nucleus: cytoplasm, presence of nucleoli, nuclear chromatin quality, cytoplasmic metaplasia, and cytologic atypia (defined as coarse chromatin, prominent nucleoli, and irregular nuclear contours). Architectural abnormalities included increased gland:stroma ratio and gland confluency (cribriforming/papillary). Outcomes were defined by diagnosis of the last available specimen as resolution (benign/inactive endometrium), regression (hyperplasia without atypia), persistence (CAH or WDC), or progression (CAH to WDC/WDC to poorly-differentiated carcinoma).

Results: Outcomes were: resolution (21 cases), regression (2 cases), persistence (3 cases), and progression (4 cases). Frequently early biopsies showed increased glandular crowding and cytoplasmic metaplasias (eosinophilic, secretory, and squamous) but this did not predict poor outcome. Resolution was most predictably detected by the complete absence of cytologic atypia in the six month post-treatment specimens. All cases with persistence or progression retained cytologic atypia for at least six months.

Conclusions: Our study confirms the results of Wheeler et al. Persistence of cytologic atypia after six months of therapy is a strong indicator of treatment failure.

Since cytologic atypia is subjective, comparison to the patient's previous pre- and post-treatment biopsies is essential for proper assessment.

Increased architectural complexity is common in early progesterone treatment and should not count as progression of disease.

1208 Carbonic Anhydrase Type IX Expression in Lobular Endocervical Glandular Hyperplasia and Related Adenocarcinoma of the Uterine Cervix

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Background: Recent studies have demonstrated that carbonic anhydrase type IX (CA-IX) is a promising bio-marker for early detection of endocervical adenocarcinoma in cases of atypical glandular cells (AGC) on cytology. For the purpose of a triage of AGC cases, CA-IX appears to be a superior to HPV DNA testing and/or p16 cytochemistry since a subset of endocervical adenocarcinomas are high-risk HPV-independent as represented by gastric-type adenocarcinoma of the cervix.

Design: Cases of lobular endocervical glandular hyperplasia (LEGH) (n=10), atypical LEGH (ALEGH) (n=6), and endocervical adenocarcinomas, including usual-type (n=3) and gastric-type (n=7, including 3 minimal deviation adenocarcinomas) were retrieved from the file. Using representative sections of the lesion, expression status of CA-IX was determined immunohistochemically, employing anti-CA-IX antibody (Abcam, MA, USA). Proportion of positive areas and staining intensity, ranging from 0 to 3+, were evaluated.

Results: All 10 adenocarcinomas, including both usual and gastric-types, were positive for CA-IX, with 7 of 10 cases showing 3+ staining, and 6 cases showing positive areas exceeding 50% (3 cases; 100%, 1 case; 80%, 2 cases; 60%). Two adenocarcinomas showed only 30% areas with 2+ positivity, and one showing only 5% areas of 1+ staining. All 10 LEGH without atypia were positive for CA-IX, with all cases showing 50% or more positive areas, although only one case showing 3+ positivity, whereas 5 and 4 cases were 1+ and 2+ intensity, respectively. Five of 6 atypical LEGH were positive for CA-IX, with 4 cases showing 3+ positivity in 100% of areas. Normal endocervical glands, tunnel clusters, and non-specific endocervical glandular hyperplasias were negative for CA-IX, although reserve cell population was positive for CA-IX.

Conclusions: CA-IX appears to be a promising marker for the detection of gastric-type adenocarcinoma and ALEGH, and might play a crucial role in the HPV-unrelated pathway of carcinogenesis, as represented by LEGH-gastric-type adenocarcinoma sequence. From the point of view for practical use of this particular marker, it should be emphasized that; (1) CA-IX staining is rather heterogeneous in cases of adenocarcinoma, and (2) LEGH and ALEGH are also CA-IX-positive, and therefore, results of the staining should be combined with morphology.

1209 Significance of Alterations of the RB1 Pathway in High Grade Serous Carcinoma

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Background: High grade serous carcinoma (HGSC) of the ovary/tube is characterized by a low prevalence of recurrent mutations, numerous DNA copy number changes and promotor methylation events. An understanding of the aberrant genes and pathways should lead to more effective targeted therapies. The mechanisms of one commonly deregulated pathway, the RB pathway, are not well understood and we hypothesize that different mechanisms may identify clinically distinct subgroups of HGSC.

Design: All cases included for study were HGSC, controlled for grade, stage, and treatment, with followup data avalable. Snap frozen tissue samples from 75 cases were obtained for molecular profiling, including RB1 loss of heterozygosity (LOH), gene expression analysis, and SNP and copy number analysis. Immunohistochemistry for RB1, CDKN2A, CCNE1, CCND1, CCND2 was completed using 102 cancer cases and 15 tubal epithelial precursor lesions. Correlations between RB pathway alterations were determined and prognostic significance assessed.

Results: RB1 LOH was frequent, seen in 76% of 42 cases, with RB1 hemizygous deletions present in 33% of 75 cases. CDKN2A hemizygous deletions were seen in 8%, and amplification in 16% of cases. Immunohistochemistry for p16 showed a diffusely strong homogenous pattern of positivity in 51%, low or negative staining in 20%, and a heterogeneous pattern in 29%. RB1 staining was positive in 60%. RB1 LOH did not correlate with RB1 protein expression, but there was a strong correlation with p16 staining. Clinical outcome was predicted by three patterns of RB1/ p16 staining. The RB1+/p16 homogeneous pattern subgroup identified 38% of tumors with the shortest recurrence free survival at 14 months, while the RB1+/p16 heterogeneous subgroup (30%) and the RB1-/p16 homogenous subgroup (32%) had an improved recurrence free survival of 22.5 months. Patterns of RB1/p16 staining seen in HGSC were identical in synchronous Tubal Intraepithelial Carcinomas.

Conclusions: Deregulation of the RB pathway is frequent in HGSC, and occurs through various non-redundant mechanisms. A strong homogeneous pattern of CDKN2A protein expression identifies a significant percentage but not all of HGSC, and correlates with RB1 LOH. RB1 protein expression stratifies CDKN2A staining into 3 clinically distinct groups, reflecting differences in tumor biology which may have treatment implications. The presence of similar staining patterns in the immediate precursor lesion of HGSC suggests RB pathway abrogation is an early genetic alteration.

1210 Significance of Complement Split Product C4d Deposition in Paraffin-Embedded Placenta of Systemic Lupus Erythematosus (SLE) and Pregnancy Induced Hypertension (PIH)

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Background: SLE and PIH are both related to premature labor and intrauterine growth retardation (IUGR) with similar histological findings of the placentas. The mechanism of placental injury of these different disorders, however, is still unclear. The purpose of this study was to investigate the utility of C4d immunostaining in placentas of SLE and PIH.

Design: Immunostaining of C4d was performed on paraffin-embedded tissue sections of placentas from 21 patients with SLE including 2 curettage cases because of uncontrollable clinical conditions, 21 with PIH, and 20 control cases. We used H-score for the evaluation of C4d immunoreactivity: The score was obtained by the formula: 3 x percentage of strongly staining trophoblasts plus 2 x percentage of moderately staining trophoblasts plus percentage of weakly staining trophoblasts, giving a range of 0 to 300. Clinical data were compared to H-score in both SLE and PIH.

Results: Immunoreactivity of C4d was significantly greater in villous trophoblastic membranes in SLE and PIH cases compared to control cases ($P<0.05$). Twelve (57%) of SLE including 2 curettage cases and 7 (33%) of PIH showed intermediate to strong-reactivity of C4d, with an H-score ranging from 14–270 and 15–105, respectively. All H-scores of control cases were less than 4. Placentas with intermediate to strong reactivity of C4d showed significantly low-placental weights (SLE, $P<0.01$; PIH, $P<0.05$), low-birth weights (SLE, $P<0.01$; PIH, $P<0.05$), and preterm birth in SLE ($P<0.05$) compared to low H-score (0-3) cases for each disorder.

Conclusions: The degree of C4d immunoreactivity was associated with low birth and placental weight in both PIH and SLE, and with preterm birth in SLE. These findings suggest that complement cascade is related to placental abnormality of both PIH and SLE. The evaluation of C4d in paraffin-embedded placenta, including abortion and curettage specimens, may be important information for subsequent pregnancies in these patients.

1211 Inadequate Endometrial Biopsies Follow-Up and Proposed Adequacy Criteria

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Background: Endometrial biopsies and curettings are among the most common specimens received in the pathology laboratory. In several aspects, these specimens present a unique challenge for surgical pathologists, and one of them is the adequacy. Since there is no defined criteria for adequacy, pathologists should exercise judgment in reporting adequacy based on the clinical information and age of the patient.

Design: We reviewed all endometrial biopsies from January 2009 to August 2011 that were performed at our institution. We selected the cases that were diagnosed as insufficient or inadequate. The cases with the available follow up were reviewed. The final diagnoses on these cases were evaluated in order to confirm the inadequacy of the samples and to further categorize the causes of inadequate or insufficient diagnosis.

Results: A total of 141 endometrial biopsies were found. 60 cases had available follow up biopsy or curetting. 41 (68%) of these patients were 45 years old or older. In the

subsequent follow up, 17 cases (28%) had significant findings, which consisted of 2 cases of endometrial adenocarcinoma, 8 cases (13%) with complex hyperplasia with atypia, and 7 cases (11%) of endometrial polyp. The remainder of the cases revealed benign findings mostly composed of atrophic endometrium in post menopausal status. From the above 60 cases, 39 cases (65%) showed no endometrial tissue. These biopsies consisted of blood, mucus and endocervical tissue. 15 cases (26%) showed superficial or minimal endometrial tissue and 6 cases (10%) were from the lower uterine segment mixed with endocervical glands.

Conclusions: This study emphasizes the importance of avoiding diagnostic terms such as "no significant pathological findings" and the importance of appropriate description of the microscopic findings. We strongly suggest that the endometrial biopsies be qualitatively and quantitatively evaluated. The absence of enough well preserved endometrial tissue from the body and fundus should be clearly transferred to the clinicians with the appropriate use of terms insufficient, inadequate or unassessable.

In our experience the absence of the above mentioned statements in the final report, can be interpreted as "negative for hyperplasia and malignancy" by some clinicians, and these patients will not be appropriately managed. In our study, the three most common instances which these statements should be used are: 1) No endometrial tissue present, 2) Scant endometrial tissue from the lower uterine segment, or 3) Only endometrial stroma with minute fragments of endometrial glandular strips.

1212 Endometriosis-Associated Carcinomas Exhibit Significant Site-Specific Differences: Analysis of 396 Cases

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Background: Recent studies document a shared increased rate of ARID1A mutations in endometriosis and endometriosis associated carcinomas and a common endometriosis-carcinoma pathway has been proposed. However, the relative proportions, association with other histologic types, and site of origin (ovarian vs extra-ovarian) have not been well examined.

Design: We performed a retrospective, consecutive review of pathology reports to identify all malignancies associated with concurrent endometriosis in the past 10 years using the following parameters in our search criteria: "endometriosis", "endometriotic", "endometrioma" in association with the following: "carcinoma", "adenocarcinoma", "borderline", "low malignant potential", "sarcoma", "adenosarcoma", and "carcinosarcoma". In each case, the primary site and type of tumor was recorded in addition to the site(s) of endometriosis.

Results: 396 cases of simultaneous endometriosis and gynecologic malignancy were identified. The most common site of malignancy was the ovary (67%), followed by uterus, fallopian tube, pelvis/peritoneum, and cervix. The 3 most common types of malignancies were endometrioid (45%), clear cell (23%) and serous carcinomas (13%). Analysis of the entire 10 year data set suggested the majority of endometriosis-associated ovarian carcinomas were endometrioid (53%), followed by clear cell (17.5%), and serous (15.6%). However, subanalysis of the ovarian cancers diagnosed in the last five years (almost all of which were diagnosed by gynecologic pathologists using standard diagnostic criteria, often in conjunction with immunohistochemistry), showed a preponderance of clear cell carcinomas (37%) with fewer serous (16%) and endometrioid (33%) carcinomas. In contrast, extra-ovarian endometriosis-associated cancers were more commonly endometrioid (64% and 68%, 10-year and 5-year analyses). Extra-ovarian endometriosis-associated clear cell carcinoma was extremely rare (4% and 6%, 10-year and 5-year analyses).

Conclusions: Clear cell carcinoma is the most common ovarian cancer associated with endometriosis when tumor classification is based on uniform diagnostic criteria informed by immunohistochemistry and subspecialty-trained gynecologic pathologists. However, this subtype is extremely rare in extra-ovarian endometriosis associated cancer. These data suggest that the endometriosis-carcinoma pathway is non-uniform and differs depending on site of disease.

1213 Patients with Vaginal Recurrence of Low Grade Endometrial Adenocarcinoma Do Not Have the Usual High Risk Factors Found in Patients with Other Recurrences: A Multi-Institutional Study

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Background: The most common site of recurrence of low grade endometrial adenocarcinoma is the vagina; however, there is no clear explanation of how the tumor cells colonize the vagina.

Design: In this multi-institutional study we compiled data from 113 patients from 1991-2011 with FIGO 1 and 2 endometrial adenocarcinomas with recurrence, and 187 control cases that did not recur and had similar follow-up time (44 and 59 months, respectively). We further stratified this group and compared multiple risk factors for patients with vaginal recurrence, patients with recurrence in other sites, and control patients.

Results: There were no significant differences in the age of patients, type of surgical procedure, gross appearance or grade of the neoplasm.

There were significant differences in many high risk factors associated with extravaginal recurrence compared with our control group and patients with vaginal recurrence. These include average tumor size, average depth of myoinvasion, presence of microcystic, elongated and fragmented (MELF) pattern of myoinvasion, presence of lymphovascular invasion (LVSI), documented cervical involvement, and death secondary to disease (DOD).

Risk factors in cases and controls:

Site of Recurrence	Tumor Size (cm)	Depth Myoinvasion (%)	MELF n(%)	LVSI n(%)	Cx Involvement n(%)	DOD
Extravaginal	8.1	50	39(54.2)	51(70.8)	32(44.4)	30(41.7)
Vaginal	3.9	28	12(29.3)	9(22)	10(25)	4(9.8)
Controls	4.1	27	61(32.6)	49(26.2)	15(8)	0(0)

Conclusions: 1. The known risk factors in low grade endometrial adenocarcinoma for patients with vaginal recurrence are similar to control cases, not to those of patients with recurrences in other sites.

2. The only parameter seen in patients with vaginal recurrence that is significantly different than controls is the presence of cervical involvement (25% vs 8%).

3. Only 9.8% of patients with vaginal recurrence died of disease. This suggests that this site of recurrence is not an indication of aggressive behavior.

4. The presence of increased cervical involvement, but absence of other high risk factors and low incidence of mortality in patients with vaginal recurrence raises the hypothesis of contamination of the vagina during the surgical procedure.

1214 miRNAs Regulate Myometrial Invasion in Endometrioid Endometrial Carcinoma

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Background: Recently, distinct miRNA expressions signatures have been identified in different stages of endometrioid endometrial carcinoma (EEC). The PTEN/AKT pathway is activated in EEC and induces deregulation of various FOXO proteins, a family of transcription factors that controls cell fate. FOXO1 which is involved in cell cycle regulation, apoptosis, and cell differentiation, is also controlled by miRNAs. For the first time, we study the expression of miRNA signatures and FOXO1 in a series of non-invasive and invasive EEC.

Design: miRNA expression signatures were studied in a series of 25 EEC and 5 normal endometria. Results, were validated in a series of 41 additional EEC by quantitative PCR for miR-27 and FOXO1, and immunohistochemistry for FOXO1 and active Caspase 3. Also PIK3CA and PTEN gene mutations were assessed by sequencing analysis for exons 9 and 20 of PIK3CA, and exons 1-9 of PTEN genes.

Results: In unsupervised hierarchical clustering analysis, normal endometria and non-invasive EEC were grouped together and separately from invasive and advanced stage tumors. A signature of 20 miRNAs was found to be differentially expressed in invasive and non invasive tumors (adjusted p value <0.001). One of them, miR27, was overexpressed in invasive carcinomas when compared to non invasive EEC (p=0.0013) and its expression increased linearly in each stage (p=0.001). Differences between non-invasive and invasive EEC were also observed in the validation group (p=0.045). Moreover, FOXO1, the main target of miR27, was downregulated in invasive tumors when compared to non-invasive tumors (p=0.033). Accordingly, we found that expression of active caspase 3 was higher in non-invasive than in invasive EEC (p=0.023). By immunohistochemistry, all tumors lacked FOXO1 immunoreaction whereas normal endometria were immunoreactive. In invasive tumors, miR27 overexpression (p=0.032) and FOXO1 downregulation (p=0.014) also occurred in unmutated PIK3CA tumors (n=27).

Conclusions: Different miRNA signatures were found in invasive and non invasive EEC. The miR27-FOXO1 tandem inhibits apoptosis and represents an alternative mechanism of tumor cell survival in unmutated PIK3CA cases.

1215 P16 Expression in Early Müllerian Serous Carcinogenesis

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Background: Mutation of tumor suppressor gene p53 is believed to be responsible for the initiation and progression of serous endometrial (ESC) and ovarian (OSC) carcinomas as well as their early/localized forms: endometrial intraepithelial carcinoma (EIC), tubal intraepithelial carcinoma (TIC) and the putative precursor p53 signature (p53S). In the majority of cases, p53 mutation is associated with p53 immunoreactivity; however, some molecular events do not result in p53 protein accumulation. Recent studies demonstrated P16 expression in ESC and OSC but data on its expression in early serous carcinogenesis is limited. We aimed to examine p16 immunoreactivity in EIC, TIC and p53S.

Design: We studied p53 and p16 expression in EIC (n=27; 9 pure EIC and 18 with adjacent ESC), TIC (n=10) and p53S cases (n=11), using immunohistochemistry. Expression [percent staining (score 0-4) + intensity (score 1-3)] was assessed in the neoplastic epithelium and categorized as positive if the combined score was ≥5.

Results: Among EIC cases, 22/27 (81%) were positive for p53 and 27/27 (100%) for p16. Among ESC cases 14/18 (78%) were positive for p53 and (17/18) 94% for p16. Among TIC cases, 9/10 (90%) were p53 positive and 10/10 (100%) were p16 positive. P53 was negative in 4 cases on both EIC and ESC components, one pure EIC and one TIC case. All p53 negative cases had typical morphologic features of serous/intraepithelial carcinoma. P53 was positive in all 11 p53S by definition, while p16 was universally negative.

Conclusions: We analyzed the gene products of p53 and p16 with pivotal role in cell cycle regulation and tumorigenesis. There is a high level of concordance between p53 and p16 expression in ESC and EIC component. These results suggest that p16 has a role in early müllerian serous carcinogenesis but is absent in non-committed lesions such as p53 signature. P16 immunohistochemistry is more sensitive than p53 in identifying early/localized forms of serous carcinoma and can be used as an adjunct confirmatory tool in p53 negative cases.

1216 Multiplex Short Tandem Repeat (STR) Genotyping of Complete Hydatidiform Moles: Analysis of Zygosity and Presence of Invasive Gestational Trophoblastic Disease at Presentation

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Background: The vast majority of complete hydatidiform moles (CHM) are androgenetic diploid, with most (>80%) arising from fertilization of an egg lacking maternal DNA by a single sperm that duplicates (monospermic/homozygous 46,XX) and a small subset arising via fertilization by 2 sperm (dispermic/heterozygous 46,XY or 46,XX). It remains controversial whether dispermic/heterozygous CHMs have a significantly greater risk of persistent gestational trophoblastic disease (GTD). Analysis of zygosity of CHMs with and without invasive GTD at presentation has not been specifically addressed.

Design: In a series of 390 products of conception specimens analyzed by p57 immunohistochemistry and STR genotyping for diagnosis and subclassification of hydatidiform moles, 125 CHMs were diagnosed. Of these, 69 were genotyped (including all with invasive disease). Zygosity was compared between those with and without invasive GTD at presentation.

Results: Of the 69 genotyped CHMs, 7 (10%) were invasive CHMs at presentation (in hysterectomy specimens); 2 of these were accompanied by choriocarcinoma in the uterus, 1 with pulmonary nodules consistent with metastatic GTD. 10 of 69 (14%) CHMs were dispermic (all XY) and 59 (86%) were monospermic (XX). 5 of 59 (8.5%) monospermic CHMs and 2 of 10 (20%) dispermic CHMs were invasive at presentation (p=0.27). The 2 invasive CHMs accompanied by pathologically documented choriocarcinoma were monospermic.

Conclusions: Dispermic XY CHMs, which represent only ~15% of CHMs, have a greater risk of being invasive at presentation but this difference did not reach statistical significance. However, the 2 examples of invasive CHMs associated with choriocarcinoma were monospermic XX, indicating that this form is not without risk of significant GTD. Follow-up analysis is required to assess the risk of persistent GTD in these 2 genetic types of CHMs.

1217 P-ERM, a Marker of Cell Polarity, Distinguishes Tubal Intraepithelial Carcinoma from Benign Oviductal Mucosa

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Background: Serous tubal intraepithelial carcinoma (STIC) is a non-invasive phase of pelvic serous cancer at risk for metastasizing. Because of its biologic significance, its accurate distinction from non-malignant mimics is important. This can be achieved in part by the application of histologic criteria and immunostaining with biomarkers (p53, Ki-67). However, consistently distinguishing STIC from proliferative or expansile lesions with p53 mutations (p53 signatures) remains difficult. Loss of cell orientation is an important feature of STIC. Certain proteins, such as Ezrin-Radaxin-Moesin (ERM) are involved in cytoskelaton organization and when activated (phosphorylated or P-ERM) concentrate on the apical cytoplasmic membrane of polarized epithelia. We sought to determine if P-ERM would be useful in distinguishing STIC from its benign couterparts in oviductal mucosa.

Design: A range of oviductal epithelia, including STICS (20), serous carcinomas (10), benign secretory outgrowths (SCOUTs) with (p53 signatures, 4) and without altered p53 staining (10), and expansile or proliferative p53 signatures (3) were immunostained with an antibody to P-ERM. Staining patterns were compared with attention to their location and intensity.

Results: P-ERM staining was linear and luminal in normal mucosa, with weaker staining of the cell membranes. In STICS, the luminal staining was either lost or disrupted, producing a discontinuous pattern, with increased staining of individual cell membranes, a feature also seen in invasive carcinomas. Expansile or proliferative p53 signatures maintained the apical staining with variable cell membrane staining. Other SCOUTs and normal epithelium displayed a similar staining pattern. The intensity of P-ERM staining in the former was less but not accompanied by conspicuous staining of individual cell membranes. There was no apparent relationship between P-ERM staining and proliferation (MIB1).

Conclusions: We show, for the first time, that an immunohistochemical correlate of cell polarity (P-ERM) shows an altered expression pattern in STIC that will distinguish it from its benign counterparts, including proliferative p53 signatures. If confirmed this finding warrants further analysis of this and other indices of cell polarity as objective markers for diagnosis and mapping the evolution of early pelvic serous cancer.

1218 Prognostic Impact of an Adenofibromatous Component in Stage I Ovarian Clear Cell Adenocarcinoma

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Background: Ovarian clear cell adenocarcinoma (CCA) is frequently associated with endometriosis, and less often with an adenofibromatous component (AF). While one study suggested that AF might be a factor for less aggressive behavior, the other study showed contradictory results. We conducted a retrospective study to examine prognostic impact of AF in Stage I CCA.

Design: Eighty-nine cases (age: 32-84yrs, average 54.9yrs) of Stage I CCA surgically treated between 2000 and 2007 were included in the study. All H-E slides of the ovarian tumors were reviewed for the presence of AF and endometriosis associated with carcinoma. The number of low-power (x40) fields (LPF) with AF was counted. Peritoneal cytology at the time of laparotomy was also reviewed. A multivariate Cox proportional hazards model was used to assess the association of potential prognostic factors (AF, endometriosis, peritoneal cytology) with disease-free survival.

Results: Of 89 cases 27 (30.3%) were associated with AF, 54 (60.7%) with endometriosis, and 27 (30.3%) with positive peritoneal cytology. The number of LPF with AF varied between 1-80 (median 5, mean 13). Of 89 cases, 15 (17%) recurred in a follow-up period (range 4.1-111.1 months, median 38.7 months). Multivariate analysis identified that AF >15 LPF was associated with improved disease-free survival (HR=0.23, 95% CI 0.47-1.15, p=0.074), and positive peritoneal cytology with recurrence (HR = 9.05, 95% CI 2.44-33.49, p=0.001), while endometriosis had no prognostic significance (HR = 0.76, 95% CI 0.24-2.38, p=0.63).

Conclusions: Our results suggest that association with AF > 15 LPF may predict favorable prognosis in Stage I CCA.

1219 Free Fetal DNA Incites a Local Inflammatory Response in Murine Placentae Via TLR-9 Resulting in Fetal Loss and Preterm Delivery

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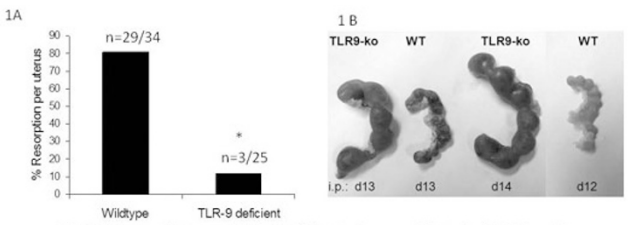
Background: Free fetal DNA is in the maternal circulation during normal pregnancy. Inflammatory disorders such as pre-term delivery and pre-eclampsia have been have higher levels than normal pregnancy. Since fetal DNA has increased hypo-methylated CPG content compared to adult DNA, we questioned whether it may act as a ligand for TLR-9 in an *in vivo* model.

Design: Wildtype and TLR-9 deficient female C57/bl6 mice were mated and became pregnant. On day 10- 14 of gestation, they were injected intra-peritoneally with 300µg/ml of free fetal DNA. After 48 hours, a post-mortem hysterectomy was performed and grossly examined. Immunohistochemistry techniques were applied to cross sections of placental sites and collected sera.

Results: The resorption rate of pregnancy in the wildtype mice was 85% compared with the TLR-9 deficient mice (12%).



(Fig 1 A) Both wildtype and TLR-9 deficient mice were injected with 300ug of fetal DNA, Within 48 hours resorptin and deliverey rates were measured. * Indicates statistically significant (B) Grossly, The gestational sacs were counted and measured WT is wildtype and TLR-9 ko represents those mice deficient for TLR-9.

In 2 of the 4 wildtype injected, the sacs were remarkably smaller in size and weight suggesting preterm delivery and cannibalisation. Staining the placental site for IL-6 and TNF demonstrated a marked increase in inflammatory cells and protein within the wildtype mice compared to the TLR-9 deficient mice.

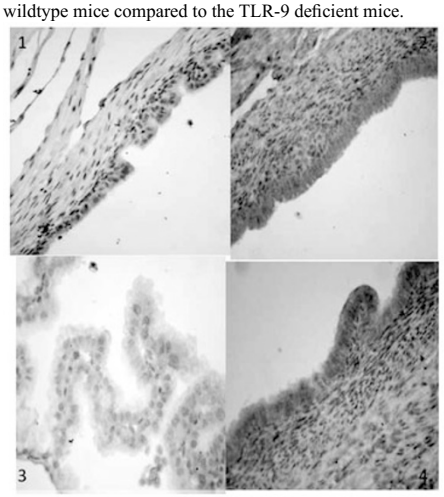


Figure 2:

Micrograph 1 is a cross section of the Placenta/endometrial complex of a TLR-9 deficient mouse 2 days after injection with fetal DNA. It has been stained for IL_6 Compared to a wildtype mouse at the same gestational age which has been stained for IL_6 on the right. (Micrograph 2) Brown staining indicates presence of protein IL_6.

Micrograph 3 is a cross section of placenta/endometrium in a TLR-9 deficient mice stained for TNF-α undered the same conditions as above. Compared with micrograph 4 which is from a wildtype mouse. Brown staining indicates the presence of TNF-α

Conclusions: High Concentrations of Free fetal DNA are immunotoxic to fetuses in pregnant wildtype mice. The presence of which results in death, resorption, and pre-term delivery. The absence of this response in TLR-9 deficient mice suggests it is likely to be TLR-9 dependant. Considering the flux of free fetal DNA in human pregnancies and the high levels noted in those complicated by pre term labour and pre-ecampsia, it is likely that TLR-9 has a role to play in these processes.

1220 A Clinicopathologic Analysis of 419 Consecutive Endometrial Carcinomas with Emphasis on Lower Uterine Segment Tumors

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Background: The clinicopathological features of lower uterine segment (LUS) endometrial carcinomas are incompletely defined.

Design: 419 consecutive cases of primary endometrial cancer were stratified by site of tumor involvement (LUS vs corpus vs LUS and corpus) and evaluated for patient age at time of presentation, tumor type, grade, stage, and mismatch repair protein status. Real-time PCR (Methylight) DNA methylation analysis was performed in cases with MLH1/PMS2 deficiency.

Results: Patients with LUS only tumors were younger than those with corpus only tumors (p=0.0004) or tumors involving the corpus and LUS (p=0.001). The proportion of non-endometrioid tumors tended to be increased in the LUS compared to the corpus, but only the proportion of undifferentiated carcinoma reached statistical significance (7.7% in LUS vs. 0.6% in corpus; p=0.03). The proportion of Lynch syndrome (LS)-associated cancer in the LUS was higher than in the corpus, but this did not meet statistical significance.

	LUS	Corpus	LUS and Corpus
Cases	26 (6.2%)	312 (74.5%)	81 (19.3%)
Age (yr)	55.4 +/- 15.3	64.2 +/- 11.8	65.8 +/- 13.3
Non-Endometrioid	34.6%	19.6%	19.8%
Grade 3	46.2%	31.1%	43.2%
Stage III/IV	23.1%	19.6%	35.8%
dMMR	30.4%	29.6%	28.4%
LS-associated	13.0%	7.7%	5.4%

dMMR = mismatch repair protein deficiency

Conclusions: Carcinomas restricted to the LUS form a unique group of endometrial cancer characterized by younger age at presentation and more frequent undifferentiated histology. LUS tumors also trend towards high grade, non-endometrioid histology and more frequent association with Lynch syndrome, but this does not appear to be statistically significant.

1221 CTNNB1 Mutation, ß-Catenin and E-Cadherin Expression and the Relationship with Clinical and Histopathological Prognostic Factors in Endometrial Adenocarcinomas and Hyperplasias

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Background: Endometrial cancers are the most common cancers of the female genital tract that more than 80% of it consist of endometrioid type adenocarcinomas. In endometrial carcinomas, the underlying factors and their correlation with several histologic and clinical parameters are guiding the patients follow up and new treatment methods. In our study, CTNNB1 mutation, β-catenin and E-cadherin expressions are examined and their relationship with the prognostic parameters and survival are studied.

Design: To the study, ninety three endometrioid adenocarcinoma, and fifteen atypical endometrial hyperplasia cases were included and those cases were diagnosed at Cukurova University, Medical Faculty at the Pathology Department between the years 2002-2007. We have investigated the expression of β-catenin, E-cadherin by immunohistochemistry and CTNNB1 mutation by PCR in all cases.

Results: In 93 endometrioid adenocarcinoma cases, membranous and cytoplasmic β-catenin expression was found in 74 cases, nuclear β-catenin expression was found in 14 cases, negative β-catenin expression was found in 5 cases. All of the 15 atypical endometrial hyperplasia cases displayed cytoplasmic and membranous β-catenin expression. In univariete analysis, β-catenin was found to have prognostic significance with survival. In multivariete analysis, negative β-catenin expression was found as an independent prognostic factor. CTNNN1 mutation was found in 34 (36.6%) of 93 endometrioid adenocarcinoma, and it was found in 3 (20%) cases out of 15 atypical endometrial hyperplasia cases. There was a significant correlation between CTNNB1 mutation and histologic grade. Decreased mutations were found in high grade tumors. There were statistical significance between E-cadherin expression and tumor grade, lymphovascular invasion, and metastasis of lymph node.

Conclusions: In this study, it has been shown that CTNNB1 mutation take a role early steps of carcinogenesis in endometrioid adenocarcinoma and negatif β-catenin expression was found to be an independent prognostic factor for survival.

1222 Downregulation of CYP27A1 Expression and Activity in Human Endometrial Carcinoma Implicates an Abnormal Bioactivation of Vitamin D in Advanced Endometrial Carcinogenesis

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Background: The association between vitamin D insufficiency and a higher risk to develop several types of human carcinomas has been attributed to an impaired production of 1,25-dihydroxyvitamin D, the vitamin D hormone, from its precursor 25-hydroxyvitamin D and reduced vitamin D receptor (VDR) levels in highly proliferating cells. This study examined whether impaired local bioactivation of vitamin D to 25-hidroxyvitamin D by CYP27A1 contributes to endometrial cell carcinogenesis.

Design: The immunohistochemical expression of CYP27A1, the Vitamin D receptor (VDR), and 24-hydroxylase (CYP24, the enzyme that degrades vitamin D metabolites) was examined in tissue microarrays (TMA) from 80 samples of normal endometrium, and 157 endometrial carcinomas (ECs) with different histological types, FIGO grades, and pathological stages. Three EC cell lines (IK, RL95, HEC1A) were incubated with vitamine D3, and subjected to clonogenic assays.

Results: In normal endometrium (NE), average CYP27A1 and VDR expression levels were lower in the proliferative phase compared to the secretory phase (p=0.06 and p< 0.000, respectively). Interestingly, CYP27A1 expression was significantly higher in EC than in normal endometrium (p = 0.0002) suggesting an attempt by EC cells to compensate for the marked reductions in average VDR levels (EC: 90.7 vs. NE: 142.5; p = 0.0002). In fact, the functional relevance of local vitamin D activation by CYP27A1 in the control of cell carcinogenesis was demonstrated by a dose dependent reduction in the number of EC colonies formed upon a 48 h exposure of the human EC cell lines IK, RL95 and HEC1A to vitamin D3. Furthermore, CYP27A1 was significantly decreased in FIGO grade III tumors (p = 0.072) and stage III carcinomas (p = 0.003). The expected reductions in the levels of CYP24 in parallel with those of VDR expression rule out a role for enhanced catabolism of vitamin D metabolites in EC carcinogenesis in vitamin D deficient states.

Conclusions: Defective local bioactivation of vitamin D, due to vitamin D deficiency or reduced CYP27A1 expression, contributes to endometrial cell carcinogenesis through an impaired control of cell proliferation.

1223 Mesonephric-Like Endometrioid Glandular Proliferations: A Morphologically Distinct Form of Metaplasia

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Background: Endometrioid proliferations exhibit a wide variety of morphological variants, including spindled, corded, sertoliform, and microglandular. We have recently encountered a form of endometrial metaplasia that demonstrates morphology reminiscent of mesonephric hyperplasia in the uterine cervix, similar to that previously described as a deceptive pattern of cervical invasion by endometrial carcinoma. Herein we describe the features associated with this unique form of metaplasia.

Design: A total of 14 cases featuring discrete proliferations of small, rounded and uniform tubules with cytologically bland nuclei were identified by retrospective review of recent consultation files. H&E stained slides were reviewed and each case was classified as simple metaplasia, hyperplasia, or carcinoma. In selected cases, immunohistochemical studies were performed to confirm endometrial origin with a panel of 3 markers (ER, vimentin, p16).

Results: Of the 14 cases, 8 were associated with carcinoma, 3 were associated with hyperplasia and 3 were associated with benign processes. In each case, the characteristic glands were small, somewhat crowded in relation to the adjacent uninvolved endometrium, and round or tubular in contour. The gland lumens often contained pale staining, occasionally dense eosinophilic secretions. Nuclei were only mildly atypical, but mitotic figures were almost always present. The gland clusters tended to occur in the basalis of the endometrium or lower uterine segment, intermingling with normal uninvolved glands and sparing the superficial zone even when associated with an endometrioid carcinoma.

Conclusions: Mesonephric-like endometrial glandular proliferations represent a unique form of metaplasia, often arising along the basalis of the endometrium. This predilection for basalis and lower uterine segment (which may reflect a muted response to prevailing hormonal mileu) may account for the previously recognized unusual microglandular pattern of cervical spread by some endometrioid carcinomas.

1224 Endocervical Adenocarcinoma – Proposal for a New Pattern-Based Classification System with Significant Clinical Implications: A Multi-Institutional Study

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Background: The treatment of endocervical adenocarcinoma (EAC) is largely based on tumor depth of invasion (DOI); however, this crucial prognostic parameter is pathologically difficult to measure accurately or consistently. Greater than 95% of lymph nodes (LN) resected in EAC are negative, yet such aggressive LN dissections cause significant morbidity without obvious clinical benefit to patients, many of whom are at a young age. Therefore, we investigated other pathologic parameters that may better identify patients at risk of developing LN metastases.

Design: Cases diagnosed and treated as EAC from 12 institutions were reviewed. Clinical information and pathologic features were assessed, including: DOI, tumor size, LVI and pattern of tumor invasion using a newly devised system, defined as follows:

Pattern A = well-demarcated glands, regardless of DOI

Pattern B = early stromal invasion arising from well-demarcated glands

Pattern C = diffuse, destructive invasion

Results: 360 cases were identified (stage IA1 to IVB). Ages ranged from 20 to 83 years (mean 44.9) and DOI ranged from 0.5 to >40mm (mean 7.7mm). LVI was present in 145 cases.

Table 1 shows outcome data comparing the standard method of tumor evaluation (DOI) vs. the newly proposed pattern-based method:

Table 1

	Patients	Patients with Pos LN	Total LN	# Pos LN	Stage I	Stage II-IV
Standard Method	360	53 (15%)	8187	83 (1%)	327 (91%)	33 (9%)
Pattern A	79 (22%)	0	1651	0	79 (100%)	0
Pattern B	91 (25%)	7 (8%)	2153	11 (0.5%)	89 (98%)	2 (2%)
Pattern C	190 (53%)	46 (24%)	4383	72 (2%)	157 (83%)	33 (17%)

Pos= positive; LN=lymph nodes

Conclusions: 1- Classifying EAC by histologic pattern would identify 22% of patients who do not need lymph node resection (pattern A, all stage I disease).

2 - Patients with pattern B rarely have lymph node metastases and 98% have stage I disease.

3- Aggressive treatment should be offered to patients with pattern C since 24% of these patients have lymph node metastases and all patients with high stage disease have pattern C tumors.

4- This pattern-based classification of adenocarcinoma is simple, reproducible and clinically significant.

1225 Endometrial Intraepithelial Neoplasia and Secretory Change: Diagnostic Features and Underlying Mechanisms

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Background: Endometrial Intraepithelial Neoplasia (EIN) and subdiagnostic areas of gland crowding (GC) are difficult to interpret in the presence of secretory differentiation. We determined how often EIN and GC lesions with secretory differentiation are associated with background changes referable to circulating progestins.

Design: We identified all sequential cases over a 69 month interval in which the diagnostic report contained the descriptor "secretory" and either "EIN" or "gland crowding". 36 EIN and 24 GC cases had slides available. Morphologic features in lesional and background endometrium, and presence within a polyp, were recorded by two observers blinded to prior report diagnoses. All observations were analyzed separately for EIN and GC groups.

Results: EIN patients studied averaged 45.2 years of age, and those with GC 46.6 years, both significantly younger (p<0.01) than historical average (53 years) of all patients diagnosed with EIN at our institution.

Table 1: Comparison of secretory differentiation within lesion (EIN or GC) and background glands.

		BACKGROUND			
		NON-SECRETORY	SECRETORY	TOTAL (%)	P VALUE
EIN	NON-SECRETORY	1.6	22.6	24.2	0.159
	SECRETORY	19.4	56.4	75.8	
	TOTAL (%)	21.0	79.0	100.0	
GC	NON-SECRETORY	11.8	14.7	26.5	0.012
	SECRETORY	2.9	70.6	73.5	
	TOTAL (%)	14.7	85.3	100.0	

Pos= positive; LN=lymph nodes Table 1 shows that in 76% of EIN cases secretory differentiation was intralesional and not significantly (p=0.159) associated with circulating progestins (secretory background endometrium). 74% of GC lesions were secretory, and positively associated with a secretory background (p=0.012). Polyps tended to be more frequent within the GC (29%) than EIN (19%) series (p=0.272), but both groups were less frequent (p=0.039, p<0.001, respectively) than the 43.4% polyp rate seen for all patients with EIN at our hospital. 15% of GC lesions were located within an EMP (p=0.091), compared to only 4% of EIN lesions (p=0.366).

Conclusions: Secretory EIN and EIN in a secretory background are rare lesions which occur in younger patients, and are less likely to occur in polyps than their non-secretory counterparts.

Secretory differentiation within EIN does not require actively circulating progestins, as indicated by the histology of the background endometrium.br]Secretory differentiation in GC lesions parallels that of the background endometrium, supporting a hormonal cause. Accurate examination and dating of the background endometrium may be useful in the diagnosis of EIN with secretory differentiation and in the classification of sub-diagnostic (GC) lesions.

1226 Immature Metaplastic CIN1: A Variant with Intense P16 Staining and Low Proliferative Index

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Background: Epithelial maturation traditionally is used to grade CIN in histologic sections. However, in some an immature epithelium displays atypia that is low in proportion to the level of epithelial maturity. Although MIB1 and p16ink4 are helpul in establishing the diagnosis of CIN in this setting, classifying such lesions can be problematic. We analyzed a subset of these atypias, and this study summarizes a correlation between morphology and p16 and MIB1 immunostaining.

Design: Immature metaplastic atypias were divided into those with putative low (uniform nuclear spacing, minimal nuclear variation, absent or mild nuclear hyperchromasia) and high-grade (irregular nuclear spacing, heterogeneous nuclear morphology, increased nuclear chromasia) features. Immunohistochemical staining for p16 was classified as patchy or diffuse (horizontally) and MIB-1 proliferation index was recorded as percentage of positive cells and location of elevated proliferative index as a function of basal, middle and superficial third of the epithelium. Staining patterns and histologic grade were correlated.

Results: Forty-three cases were classified without knowledge of the immunohistochemistry. Immature metaplastic low grade CINs exhibited strong and diffuse staining for p16 and but unlike High grade CINs, the proliferating (MIB1+) cells were concentrated in the more basal 1-2 thirds of the epithelium and the proliferative index was less than 30%. Variable columnar differentiation was observed in some, with strong staining of both the columnar and squamous cells by p16.

Conclusions: A distinct subset of immature CINs displays a uniform cell population and based on both cytology and proliferative index, warrants classification as low grade CIN (CIN1). p16 immunohistochemistry, although helpful in the recognition of these lesions, will not distinguish them from higher grade CIN (CIN2/3). Attention to regularity in nuclear morphology with absence of noticeable differences in cell size and shape, combined with MIB1 staining, is helpful,. The presence of columnar differentiation, which also stains positive for p16 is consistent with bidirectional differentiation in the transformation zone epithelium. Further studies of this entity are warranted to precisely determine its biologic behavior.

1227 IMP3, EGFR and E-Cadherin in High Grade Ovarian Serous Carcinomas To Predict Disease Progression and Survival

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Background: Most high grade serous carcinomas (HGSCA) of ovary are advanced stage tumors with early recurrences. However, there are some unusual cases that do not recur and have excellent survival. This study aims to compare cases of HGSCA that showed rapid recurrences with those that did not recur, and also to evaluate the potential appropriateness of Epidermal Growth Factor Receptor (EGFR), E-cadherin and IMP3 to predict recurrence and survival.

Design: Thirty one cases of Stage III HGSCA were available for review satisfying our inclusion criteria (6 cycles of Taxol and carboplatin and < 1 cm of residual disease). Of the 31 cases, 17 recurred within 18 months follow-up and 14 cases did not recur (with a minimum follow-up of 49 months). The patterns of invasion, such as pushing and infiltrative, were assessed in all cases. The primary tumors were then subjected to a panel immunohistochemical (IPOX) marker such as IMP3, EGFR and E-cadherin. The staining results were recorded in semiquantitative fashion. Disease free survival (DFS) and overall survival (OS) of these cases were compared with the results of IPOX for individual stains using Log-rank test. Logistic regression analysis was performed to compare IPOX results of individual stains with recurrence status and with the pattern of invasion.

Results: Twenty one of 31 (67.7%) HGSCA expressed IMP3, 9 of 31 (29%) expressed EGFR and all cases expressed E-cadherin. The EGFR-negative tumors showed higher recurrence rate than EGFR-positive tumors. The cases with a higher percentage of destructive invasion showed higher IMP3 positivity and higher chances of recurrence whereas cases with higher percentage of pushing invasion showed lower IMP3 positivity and lower chances of recurrence (p=0.02). IMP3-negative tumors had lower odds of recurrence than IMP3-positive tumors (Odds Ratio: 0.1; 95%CI:0.016-0.615; p=0.01). Kaplan-Meier plots showed that patients with negative IMP3 staining had a significantly higher OS than those with IMP3 positive tumors (p=0.01).

Conclusions: - IMP3 may serve as a useful prognostic marker that can stratify patients of advanced stage, high grade serous carcinomas into two distinct subsets: majority with early recurrence (<24 months) with an infiltrative pattern of invasion and IMP3 positive; and a smaller subset that do not show disease recurrence for at least 49 months follow-up with pushing borders and IMP3 negative.

- IMP3-positive cases showed a significantly lower overall survival as compared to IMP3-negative tumors.

1228 Interobserver Agreement in the Diagnosis of Ovarian Carcinoma Types: Impact of Sub-Specialization

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Background: Ovarian carcinomas are a diverse group of tumors with different morphologic and molecular features. Correct classification has a potential impact on therapy since not all lesions need adjuvant chemotherapy, and not all tumors respond equally to standard chemotherapy. The aim of this study was to determine interobserver agreement in the diagnosis of ovarian carcinomas between specialized gynecological (GYN) pathologists and general (GEN) surgical pathologists.

Design: We selected 58 ovarian carcinomas surgically resected at SBUMC between 1/1/1999 and 6/1/2011, including 23 high grade serous carcinomas, 19 clear cell carcinomas, and 16 endometrioid carcinomas. All cases were reviewed by a senior GYN pathologist, and all diagnoses were confirmed with immunostains for WT1, p53, ER, and hepatocyte nuclear factor 1-beta. Immunostains were done in tissue microarrays containing three random cores per tumor. Only cases in which all three cores had the same immunoprofile were included in the study. One representative slide from each case was sent to 3 GYN pathologists and 3 GEN surgical pathologists, who were asked to classify the lesions according to their own criteria. The reliability of agreement between the pathologists was assessed using Fleiss' kappa value.

Results: The reliability of agreement was "substantial" for the GYN pathologists (Fleiss' kappa value = 0.67), and "moderate" for the GEN pathologists (Fleiss' kappa value = 0.54). The most common problem was to confuse serous carcinoma with either clear cell carcinomas or endometrioid carcinomas. This happened in 13% of cases in the GEN group, but only in 0.7% in the GYN group. The accuracy rate for diagnosing serous carcinoma was 84.5% in the GYN group and 58.33% in the GEN group (p= 0.0003).

Conclusions: Interobserver agreement was better among GYN pathologists than general surgical pathologists. Diagnosis of ovarian carcinoma tumor type is more accurate when done by GYN pathologists than GEN pathologists. However, there is a small percentage of cases in which classification by light microscopy alone is difficult even for GYN pathologists. If tumor type becomes an important factor in treatment decisions cases should be reviewed by a gynecological pathologist, and confirmatory immunostains may be necessary when the H&E morphology is ambiguous.

1229 Genetic Instability in Serous Tubal Intraepithelial Carcinoma and Tubo-Ovarian Dysplasia from Prophylactic Oophorectomies for Genetic Risk

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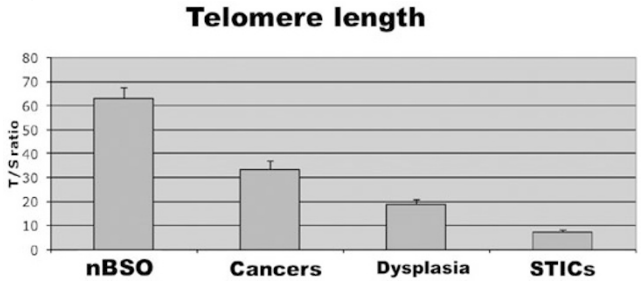
Background: Through *BRCA* and *P53* alterations, genetic instability plays an important contribution in ovarian carcinogenesis. We sought to study telomere shortening and surrogate markers of genetic instability in early and pre-invasive stages of ovarian cancer, serous tubal intraepithelial carcinoma (STIC) and tubo-ovarian dysplasia (TOD).

Design: 51 tubo-ovarian dysplasia from bilateral prophylactic oophorectomies (pBSO) with *BRCA1* (36) or *BRCA2* (15) mutation (defined by morphological score and immunohistochemical expression of p53, ALDH1 and gH2AX), 12 STICs, 53 ovarian high grade serous carcinoma and 36 control bilateral oophorectomies (nBSO) were laser-capture microdissected on formalin-fixed paraffin-embedded sections and analyzed by comparative genomic hybridization (CGH array) and for telomere length (T/S using quantitative real-time polymerase chain reaction based on the Cawthon technique (Nucleic Acid Res 2002 30:1-6)).

Results: We found few subtle genomic alterations in dysplastic epithelium in opposition to the important genomic imbalances in STICs and ovarian cancers. STICs had the shortest telomeres followed by TOD in pBSO (respectively, T/S= 7.23 and T/S= 18.78). Ovarian carcinoma (T/S= 30.68) had shorter telomeres than nBSO (T/S= 52.75) but longer than STICs and dysplasia.



All these differences were statistically significant. When comparing telomere lenght in dysplasia from *BRCA1* and *BRCA2* pBSO, the shortest telomeres were observed in *BRCA1* carriers.

Conclusions: These findings suggest that genetic instability occurs at early stages of ovarian and tubal carcinogenesis. Targeting genetic instability might be an option for prevention in high risk patients, but requires further studies.

1230 Value of SATB2 in the Differential Diagnosis of Intestinal-Type Mucinous Tumors of the Ovary

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Background: Primary mucinous adenocarcinomas of the ovary are a diagnostic challenge since their histological and immunohistochemical features usually overlap with metastatic tumors. SATB2 is a recently identified protein with restricted expression in the glandular cells lining the lower gastrointestinal tract. The aim of this study is to further explore the differential expression of SATB2 in primary and metastatic tumors of the ovary.

Design: From the pathology files of the Instituto Nacional de Cancerología de México, mucinous ovarian tumors of intestinal type were retrieved, a double reading of H&E slides was performed in order to confirm diagnosis, detailed review of the clinical chart was performed to define the primary origin of the tumor (ovarian vs metastatic). Immunohistochemical staining for CK20, CDX2 and SATB2 were performed and evaluated by two gyneco-pathologist.

Results: Twenty-four mucinous tumors were identified; 11 were classified as metastatic according to clinical features (7 well-differentiated colon adenocarcinomas and 4 well-differentiated adenocarcinomas of the appendix), the rest were catalogued as primary neoplasms of the ovary (at least 2 years of follow-up during which there were no carcinomatosis, pseudomixomas, or tumors at other sites). The primary neoplasms of the ovary were positive to CDX2 in 38% of cases, positive to CK20 in 92%, and all were negative to SATB2. The metastatic neoplasms were positive to SATB2 in 90% of cases, positive to CDX2 in 81%, and to CK20 in 100%. Specificity SATB2 for differentiation of metastatic from primary was 96.4%, sensitivity 87.5%, PPV 95% and NPV90%, for CDx2 specificity 61,5%, sensitivity 81,8%, PPV 64% and NPV 80%, CK20 specificity 10%, sensitivity 95,8%, PPV 47,9% and NPV75%, respectively.

Conclusions: SATB2 appears to be a useful antibody in the differential diagnosis between primary and metastatic mucinous intestinal-type neoplasms, and highly sensitive in detecting lower gastrointestinal tract metastasis.

1231 Number of Involved Lymphatic Vessels and Their Distance from Tumor Are Independent Prognostic Factors of Lymph Node Metastases in Low Grade Endometrial Adenocarcinoma. A Multi-Institutional Study

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Background: Metastasis to lymph node in low-grade endometrial adenocarcinoma (LGEC) are uncommon. Clinical stage is the most important prognostic factor in predicting nodal disease regardless of histological grade. The aim of this study was to investigate whether histological features exist that can predict lymph node involvement.

Design: This is a multi institutional case-control study where 153 patients with LGEC and nodal disease were compared to 336 controls with negative lymph nodes. Studied variables included size and appearance of tumor, depth of myometrial infiltration, cervical stromal and or lower uterine segment involvement (LUS), stromal reaction, intratumoral necrosis, LVI (divided as 0, 1 vessel, 2-5 vessels and more than 5 vessels), distance to LVI (adjacent to neoplasm and deeper than invasive front of tumor). Difference between mean estimates were evaluated by the student´s-T, Chi-square or Fisher exact tests. Multivariate analysis was performed using logistic regression model. P<0.05 was considered statistically significant.

Results: Mean age in the case group was of 58.2 years vs 61 in controls (p=0.010). Average tumor size 5.4 cm vs 4.2 cm (p=0.001), myometrial invasion 58% vs 35% (p=0.0001).

Table 1

	Cases with LNM (n=153)	Control cases (n=336)	P
LVI (more than 5 foci)	39%	9%	0.0001
Distant LVI	28%	6%	0.0001
Necrosis	60%	35%	0.0001
Stromal reaction	84%	55%	0.0002
Stromal cervical invasion	33%	9%	0.0001
LUS involvement	60%	37%	0.0002

In multivariate analysis, the distance to LVI (deeper than tumor invasive front) had OR 2.34(CI:1.231– 4.447)(p=0.009), cervical invasion OR 1.588(CI:1.14-2.214) (p=0.006) LVI (more than 5 foci) OR1.57(CI:1.076-2.296)(p=0.019), and myometrial invasion OR 1.124(CI:1.069-1.182)(p=0.0001) were independent predictors of lymph node metastasis.

Conclusions: Patients with larger tumor size, deeper myometrial invasion, LVI, especially deeper than the tumor front, intratumoral necrosis, stromal tumor reaction, and cervical stromal invasion are significantly associated with lymph node metastasis. LVI involving more than five vessesls and LVI deeper than the tumor front are independent factors for nodal metastasis in LGEC.

1232 Body Mass Index Correlates with Mismatch Repair Protein Expression in Endometrial Carcinomas

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Background: Mismatch repair protein (MMR) immunohistochemistry (IHC) of endometrial carcinomas (EC) is routinely used to screen for Lynch syndrome at our institution. Body mass index (BMI) can be used to estimate the estrogen milieu because adipose tissue production of estrone. There is some evidence linking estrogen with MMR regulation in colorectal carcinomas (CRC) and similar mechanisms may be operative in EC.

Design: We retrospectively reviewed MMR IHC including MLH1, PMS2, MSH2 and MSH6 on 517 consecutive hysterectomy specimens with EC. Each IHC was classified as positive or negative. Equivocal IHC cases were resolved by staining sections of another block, two cases remained unresolved. Clinical databases were accessed to extract BMI data at the time of surgery. Relationships between MMR IHC, BMI, age and tumor type were explored.

Results: Women <50 years constituted 13% of the cases, had a significantly higher BMI (n=69, BMI=40.4 +/- 12.8) than women ≥50 years (n=448, BMI=36.4 +/- 9.95), p=0.013 and 60 (87%) of their tumors were type 1 (considered estrogen driven). Regardless of age, a significantly higher BMI was seen in 356 cases with normal MMR IHC (BMI=37.6 +/-10.5) when compared to 159 cases with any MMR absent on IHC (BMI=35.5 +/-10.2) p=0.027. When age was analyzed as a continuous variable: (1) women with absent MLH1 (n=116) and/or PMS2 (n=130) were significantly older than women with both proteins present (both p<0.001) and (2) women with absent MSH2 (n=12) and/or MS6 (n=29) were significantly younger than women with both proteins present (p=0.025 and p=0.002 respectively). A significant individual MMR loss-BMI association was found for MSH6 in women <50 with lower BMI correlating with negative MSH6 IHC (p=0.003).

Conclusions: BMI showed multiple statistically significant associations across our 517 hysterectomy cases. Overall, a higher BMI correlated with normal MMR indicating a possible role for estrogens in the maintenance of DNA repair in EC as has been suggested in CRC. These findings indicate particular BMI significance on MSH2 and/or MSH6 expression in women <50 years with EC.

1233 Endometrial Intraepithelial Neoplasia Involving Polyps: A Followup Study

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Background: Endometrial intraepithelial neoplasia (EIN) is a precancerous neoplasm of the endometrium that carries a 45-fold higher risk of endometrioid carcinoma on followup. For this reason, when EIN is detected, management includes either hysterectomy or hormonal therapy and close followup. EINs have also been reported in endometrial polyps (EMPs), but the implications of this diagnosis are unclear. We examined a consecutive series of EINs associated with EMPs to determine their likelihood of persisting or progressing to adenocarcinoma on followup sampling.

Design: Consecutive cases coded as both EMP and EIN in the pathology files between 2009 and 2010 were identified. Cases designated as arising in polyps were culled from this group, examined histologically and followup determined by a search of the pathology records.

Results: Eighty-four cases coded as both EIN and EMP were identified. Of these, the diagnosis of EIN occurring in EMP were confirmed in 37. Followup endometrial sampling was obtained in 29 (78%). Of these 29, 13 (45%) showed no evidence of EIN, 12 (41%) contained persistent EIN, with or without a polyp and 4(14%) contained a well differentiated endometrioid adenocarcinoma.

Conclusions: Close followup of women with EIN apparently confined to polyps will reveal the absence of EIN in nearly one-half of cases. Nevertheless, a nearly equal proportion will have persistence of their disease and 14% will be shown to have endometrial adenocarcinoma. Thus, while the possibility of complete removal of the EIN by polypectomy exists, the risk of persistence/recurrence requires close followup. The range of EIN morphologies seen in polyps will be illustrated.

1234 Serous Tumor of Low Malignant Potential of the Ovary – Is the 10% Cut-Off Reliable?

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Background: Conventionally, ovarian serous neoplasms of low malignant potential (OV-SLMP) are diagnosed when epithelial proliferation and detached cell clusters are present in ≥10% of the tumor. Some authors have suggested the terms "focal proliferation" or "focally atypical" when the epithelial proliferation comprises <10% of an otherwise typical serous cystadenofibroma (SCA). Using these terms at frozen section (FS) does not typically prompt staging and the peritoneal cavity is left unexamined. However, we have encountered rare cases of SCA with <10% proliferation associated with non-invasive and/or invasive peritoneal implants (Ni-IM; I-IM). The aim of our study is to evaluate the incidence of extraovarian disease in cases of "focal OV-SLMP" and determine its correlation with clinical outcome.

Design: Cases of focal OV-SLMP were selected from a cohort of OV-SLMP from 1985-2006. Focal OV-SLMP is defined as epithelial proliferation and cell detachment, diagnostic of LMP, but present in <10% of the tumor. The presence of proliferation without cell detachment was designated as "focal proliferative areas". The following parameters were evaluated- age, size, laterality, status of the opposite ovary, pathologic staging (if performed), the presence of extraovarian disease (at time of presentation and subsequently) and clinical follow up.

Results: 1,123 OV-SLMP were identified, of these 20 (1.8%) met criteria for focal OV-SLMP. Patients were 32-81yrs (median 62.5); 14 (70%) were post-menopausal (PM). Ovarian size was 1.2-18cm (7.9cm mean); laterality was: left (10), right (9) and bilateral (1). External surface of ovary was smooth (18), granular (1) and not known (NK) in 1 case. The cyst wall was smooth (11), granular (4), had focal papillary excrescences (4) or NK (1). Opposite ovary had endosalpingiosis (3), SCA (9), SCA with focal proliferative areas (2), focal OV-SLMP (2), normal (1), NK (1), Sertoli Leydig cell tumor (1) not removed (1). Peritoneal sampling (n=14) revealed 2 cases with endosalpingiosis and implants (1 Ni-IM and 1 I-IM). 1 case developed SCA with focal proliferative areas and endosalpingiosis in the pelvis 5yrs later. Follow-up on 17 cases ranged from 60-138 mos (median 74mos). 15 pts. were alive with no evidence of disease, 1 pt. with I-IM died of disease, 1 pt. was alive with cervical cancer.

Conclusions: Extraovarian disease in focal OV-SLMP is rare and appears to be associated with endosalpingiosis. Although rare, it can result in an unfavorable outcome depending on the nature of the implants. Focal OV-SLMP, if identified at FS should prompt examination of the peritoneal cavity, particularly in PM women.

1235 SALL4, Glypican-3 and CDX2 Expression in Endometrial Endometrioid Adenocarcinomas: An Immunohistochemical Study of 57 Cases

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Background: Yolk sac tumor (YST) can share histological features with endometrioid carcinoma which can pose a diagnostic challenge. This is especially true in cases of YST at unusual anatomical sites such as the endometrium. SALL4, glypican-3, and CDX2 are known to be expressed in ovarian YSTs. While CDX2 has been found to be expressed in the squamous morules of endometrial endometrioid proliferations, the other two markers have not been evaluated in endometrial endometrioid adenocarcinomas. In this study we evaluate the expression of SALL4, glypican-3, and CDX2 in endometrial endometrioid adenocarcinomas.

Design: Fifty-seven cases of endometrial endometrioid adenocarcinomas (FIGO grades: 1, 2 and 3) were retrieved from the pathology files of our institution covering a period of 15 years (1996 to the present). All the cases were stained for SALL4 (6E3, 1:100, Biocare Medical, Concord, CA), glypican-3 (1G12, predilute, Cell Marque, Rocklin, CA), and CDX2 (CDX2-88, 1:50, BioGenex, Freemont, CA). Granular cytoplasmic staining for glypican-3, and nuclear staining for SALL4 and CDX2 were interpreted as positive. Staining in the glandular component of the neoplastic cells was graded as follows: 0: no detectable staining, 1+: 1-5%, 2+: 6 to 25%, 3+: 26 to 50%, 4+: 51 to 75% and 5+: >76%. For grading of CDX2, staining in the squamous morular component was excluded.

Results: Of the 57 endometrial endometrioid adenocarcinomas, only 2 showed 1+ staining for SALL4, while 55 (96%) were negative. Of the glypican-3 cases 6 showed 1+ staining, 1 showed 3+ staining, 48 were negative and 2 cases had excessive background staining and were not interpretable. Staining for CDX2 in the glandular component of the tumors was 1+ in 9 cases and 48 cases were negative. CDX2 staining exclusively in squamous morules was observed in 8 cases and were included in the negative group.

Conclusions: SALL4 is a reliable immunohistochemical marker to distinguish YST from endometrial endometrioid adenocarcinoma, as it is positive in less than 5% of the latter. Glypican-3 also appears to be a useful marker for this distinction, however, a rare case of endometrial endometrioid adenocarcinoma can show diffuse staining for glypican-3. As expected, we found that CDX2 was strongly positive in squamous morules, and was also focally expressed in the glandular component of endometrial endometrioid adenocarcinoma, in up to 15% of the cases.

1236 Comparison of Hybrid Capture 2 HPV Borderline or Low Positive Results with the Cobas® HPV Test Results in the ATHENA Trial

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Background: The Digene Hybrid Capture 2 assay (HC2, Qiagen, MD) is often used as a standard for new HPV detection tests, but at the lower limits of detection the reproducibility, sensitivity and specificity for detection of cervical intraepithelial neoplasia (CIN) is suspect and samples with RLU values of greater than or equal to 1 but less than 2.5 have be retested. In this study we compare the performance of the new FDA- approved Cobas® HPV test (Roche, CA) against the HC2 assay in the borderline or low positive range.

Design: This study is taken from the baseline data of the ATHENA HPV study which enrolled 47,208 women 21 years and older between 2008-2009 who had cytology, HPV testing with the HC2 test, Roche Cobas® HPV Test and Roche Linear Array High Risk Genotyping assay (LA) performed in 5 accredited clinical labs. This sub-analysis includes 331 patients with RLU/CO values on the HC2 test between 1 and 5.0 RLU. When repeat testing for HC2 did not yield a definitive result or had inadequate volume, results were reported as indeterminate (59 patients total, RLU available for 55). For these 331 patients, cytology results were as follows: 194 Negative, 111 ASC-US and 26 ≥ASC-US.

Results: There were no indeterminates or inadequates with the Cobas® HPV Test compared to 55 indeterminates for the HC2 test. Of the 56 negative samples by the LA assay, 2 were negative by the HC2 Test compared to 48 negative by the cobas HPV Test (Negative Percent Agreement [NPA] of 3.6% for HC2 compared to 85.7% for the Cobas® HPV Test). Sequencing revealed NPA of 4.3% for HC 2 compared to 52.2% for the Cobas® HPV test. Results from biopsies adjudicated by 3 expert pathologists revealed sensitivities in this low range was 96.8% for the HC2 test and 90.3% for the Cobas test, but the specificity of the cobas® HPV Test was 24.3% compared to 1.6% for HC2.

Conclusions: In the low RLU positive range, the Roche Cobas® HPV Test showed comparable sensitivity, greater specificity and better NPA than HC2 without the need for retesting when HPV detection or biopsy-confirmed CIN is considered as an end-point. Additionally, the Cobas® HPV Test provides HPV16/18 genotyping result which eliminates the need for reflex testing in patients who are HPV positive and cytology negative.

1237 Fluorescence In Situ Hybridization (FISH) for Detection of MAML2 Rearrangement in Patients with Adenosquamous Carcinoma of Uterine Cervix

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Background: Cervical adenosquamous carcinomas are defined as tumors containing an admixture of malignant squamous and glandular cells. It is a relatively uncommon tumor with aggressive behavior. Mucoepidermoid carcinoma (MEC) of salivary glands shows translocation t(11;19). In view of morphological similarities between adenosquamous carcinoma of uterine cervix and some MEC's of the salivary glands, and the possibility of development of drug therapy targeted to the gene rearranged in MEC, we decided to evaluate for t(11;19) translocation in patients with adenosquamous carcinoma of uterine cervix.

Design: After obtaining ethics approval, we identified 19 cases of adenosquamous carcinoma in our archives. Patient age ranged from 30 to73 (average of 46). Of 19 cases, 2 were grade 1, 8 grade 2 and 9 grade 3. Fusion of the MECT1/MAML2 genes, resulting in t(11;19)(q21;p13) was evaluated in the 19 formalin fixed paraffin embedded tissue (FFPE) samples of tumors by a dual color fluorescence in situ hybridization (FISH) assay using the following bacterial artificial chromosome (BAC) clones: RP11-27C9 and RP11-1071A4 for MECT1 gene and RP11-277H22, RP11-111I14 and RP11-145B9 for MAML2 gene.

Results: Interpretable FISH results were obtained for all specimens by scoring 60-100 cells/sample. All samples were negative for the MECT1/MAML2 fusion. However, 2/19 cases showed MECT1 amplification, 3/19 cases had an extra copy of the MAML2 gene, and extra copies of both MECT1 and MAML2 genes were found in 5/19 cases.

Conclusions: Our study demonstrates that all cases with moderate to poorly differentiated adenosquamous carcinoma are negative for t(11;19); however it appears that copy number changes/amplification/translocation of MECT1 and MAML2 may be involved in the pathogenesis of this disease. Further evaluation of the changes observed in these individual genes, and possible translocations with other partners such as CRCT2 and CRCT3 are currently being undertaken.

1238 Invasion Patterns of Metastatic Pelvic High-Grade Serous Carcinoma Are Associated with BRCA Alterations

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Background: BRCA-associated high grade serous carcinoma (B-HGSC) is associated with relatively better clinical outcomes than sporadic HGSC, at least in part because of increased sensitivity to platinum chemotherapeutic agents. It has recently been reported that B-HGSC has a distinctive histologic appearance, characterized by solid and transitional cell-like architecture. Interestingly, ovarian transitional cell carcinoma has also been reported to be relatively prognostically favorable and chemosensitive; it has also been suggested that there is a tendency for peritoneal metastasis in the form of circumscribed nodular masses amenable to optimal debulking. We hypothesize that peritoneal metastatic B-HGSC is associated with characteristic morphologic features indirectly associated with favorable outcomes.

Design: The morphologic patterns of peritoneal metastatic sites were studied by reviewing 123 HGSC cases. Genetic subgroups represented were: *BRCA1* germline mutation (n=13); *BRCA1* somatic mutation (n=5); *BRCA1* promoter methylation (n=10); *BRCA2* germline mutation (n=5); *BRCA2* somatic mutation (n=1); negative for *BRCA1* germline mutation (n=20) and other HGSCs with no BRCA abnormality (n=69). Number of metastatic sites ranged from 1 to 9 (average of 4 per case). Tumor architecture (solid, cribriform/pseudoendometriod, papillary and pseudopapillary) and pattern of invasion (infiltrating vs pushing) were recorded for each metastatic site. Histologic evaluation of metastases was evaluated without knowledge of genotype.

Results: A pushing invasive pattern was seen in 24/34 (71%) metastatic cases with *BRCA1* or *BRCA2* abnormality, as compared with metastatic sites of negative BRCA1 germline or other high grade serous cases with no BRCA abnormality, which showed characteristically non-micropapillary, infiltrating invasion (89/89 cases, 100%). Micropapillary infiltration was found in 24% (8/34) of the metastatic sites with *BRCA1* or *BRCA2* abnormality as compared with no sites in the negative *BRCA1* germline or other HGSC with no BRCA abnormality. Similar tumor architecture (solid, cribriform/pseudoendometriod and pseudopapillary) was found across all subgroups.
Conclusions: Patterns of invasion had greater association with BRCA abnormalities than tumor architecture. A pushing pattern of invasion is a highly prevalent and specific morphologic characteristic of metastases of pelvic HGSC with associated *BRCA* abnormality. This finding may have diagnostic, prognostic and therapeutic value if independently confirmed.

1239 HER-2 and EGFR: Possible Targets for Vulvar Carcinoma

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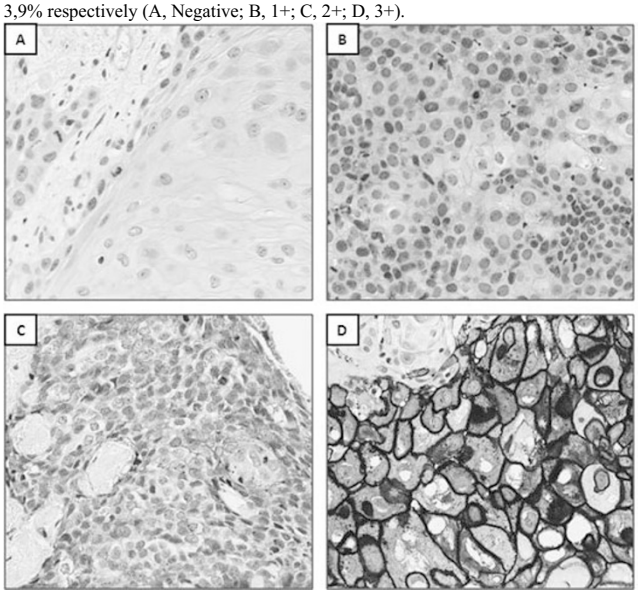
Background: Vulvar carcinoma is a rare disease. Being radical surgery the standard treatment approach, often accompanied by considerable morbidity, knowledge on prognostic and predictive factors is criticaly necessary. The aim of this study was to identify alterations in EGFR and HER-2 oncogenes and its protein expression in order to determine prognostic and predictive values of new therapies for vulvar cancer.
Design: 140 invasive squamous cell carcinoma of the vulva were selected for this study, confirmed by pathologist and collected in two TMAs, in which immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) were performed for HER-2 and EGFR. All results were associated with clinical data of patients and with well-established prognostic factors in anatomic pathology.
Results: A 100% correlation between FISH and IHC for HER-2 and EGFR was observed in our cases. HER-2 and EGFR overexpression (3+) by IHC were present in 1,6% and 3,9% respectively (A, Negative; B, 1+; C, 2+; D, 3+).



Amplification was present in 2,1% for HER-2 and 6,4% for EGFR. Overexpression of HER-2 corresponded to poorly differentiated tumors (p=0,0221), presence of vascular invasion (p=0,0314), recurrence (p=0,05) and death by cancer (p=0,0349). Lower survival rates were observed in tumors scored as 3+ for HER-2 (p=0,0445), but it was not significant for EGFR. EGFR expression showed no association with the analyzed clinical and histological data.
Conclusions: Our study shows a novel data regarding the 100% correlation between FISH and IHC for HER-2 and EGFR in vulvar carcinoma which allows the distinction between two entities of negativity (score 0, 1+ and 2+) and positivity (3+) of these oncogenes in vulvar cancer. Despite of the rare overexpression found for HER-2, it can possibly be associated with a more aggressive phenotype of the tumors. The lack of association between EGFR expression with the analyzed clinical and histological data may be due to the heterogeneity of the vulvar carcinomas. Still, the overexpression of both membrane receptors, although rare, could benefit a small group of patients with therapy against these tyrosine kinase receptors.

1240 Is It Possible To Differentiate Endometrial Serous Carcinoma from Endometrioid Type in Liquid-Based Preparation? A Cytomorphologic Study

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Background: Although the Pap smear is not a sensitive screening test for endometrial cancer, positive Pap smear may be the first presentation in these patients. The objective of this study is to characterize the cytomorphologic features of endometrial serous carcinoma in Pap smears. In addition, we correlate the Pap smears findings with stage of the disease and other histologic prognostic predictors.
Design: The pathology files were searched for the time period 2001 to present, for cases of adenocarcinoma of endometrial origin in Pap smears, for which there was tissue diagnosis available. All cases were reviewed for nuclear, cytoplasmic, background and architecture features. In addition, thirty randomly selected cases of preoperative negative Pap smears in patients with serous carcinoma were included as control group.
Results: There were a total of 90 cases of Liquid-based preparation Pap smears with the diagnosis of adenocarcinoma, endometrial origin, without further classification. Histologically, 40 cases turn to be serous carcinoma and 50 cases of endometrioid carcinoma. In serous group, the average age was 68 years; while in endometrioid group was 59 years. In serous group, positive Pap smears strongly correlated with lymphovascular invasion (35/40, 88%; P< 0.001), lower uterine segment (33/40, 83%; P< 0.001) and cervical involvement (27/40, 68%; P< 0.01); in comparison to negative cytology group (6/30, 20%; 8/30, 27%; and 5/30, 17%; respectively). Regarding the endometrioid group, cervical involvement was seen in 12/50 (24%), lower uterine segment in 15/50 (30%), lymphovascular invasion in 34/50 (68%). High grade endometrioid carcinoma was seen in 18/50 (36%), and low grade in 32/50 (64%). The presence of foamy macrophages in the background, low grade nuclei with spindle cell features and presence of foamy cytoplasm (mucin) are features correlated with endometrioid diagnosis. On the other hand, the presence of micropapillary architecture (rosette/acinar formation), with hob-nail nuclei, prominent nucleoli, dense cytoplasm and multinucleated cells are features consistent with serous carcinoma.
Conclusions: In our study, both serous and endometrioid carcinomas have different cytologic features in liquid based preparation. Patients with endometrial serous carcinoma who have malignant cytology detected by preoperative cervical cytology are at increased risk of having known poor prognostic factors such cervical involvement, presence of lymphovascular invasion and high stage tumor.

1241 RecQL1 DNA Repair Helicase: A Potential Therapeutic Target and Proliferative Marker Against Ovarian Cancer

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Background: RecQL1 helicase belongs to the RecQ DNA helicase family which participates in the maintenance of genomic integrity. RecQL1 is also highly up-regulated in rapidly proliferating cells, including various cancers such as liver, lung, pancreas, head & neck and colon. Silencing RecQL1 in cancer cells by RNA interference with siRNA induces mitotic catastrophe and death in a wide range of cancer cells that have defects in the cell cycle checkpoint system. However in ovarian cancer (OC), the role of RecQL1 has not been studied.
Design: 1) RecQL1 expression and cell growth were examined in 4 OC (e.g., serous and clear cell types) cell lines with or without RecQL1-siRNA transfection. 2) Immunohistochemistry for RecQL1 and Ki-67 were performed on 118 cases of surgically resected OC, i.e., 50 of serous, 26 of endometrioid, 21 of clear cell, 15 of mucinous, and 6 of others. The relationship among RecQL1 and Ki-67 expressions, and clinicopathological parameters was investigated.
Results: 1) Both cell lines showed strong RecQL1 expression. When they treated with RecQL1-siRNA, reduced levels of RecQL1 and various degrees of cell death were observed. 2) The expression of RecQL1 was seen in 90% of the all cases, including 47% of strong (diffuse and intense) RecQL1 expression cases. The strong expression was significantly more frequent in serous type OC (p=0.03), and in OC cases with high Ki-67 expression (p=0.02) and high FIGO stage, although the latter lost value when adjusted by histologic type.
Conclusions: RecQL1 is often expressed in OC and the expression level correlated with proliferative activity. Also it would be an excellent molecular target for OC therapy.

1242 Transitional Cell Tumors of Ovary: Immunohistochemical Profile with Reference to Markers for Transitional Cell Carcinoma

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Background: Transitional cell tumors of ovary are a set of tumors that resemble tumors of the urothelium. They are relatively uncommon and comprise approx. 1-2% of all ovarian tumors. In rare instances one may have to differentiate these tumors from metastatic transitional cell carcinomas (TCC) to ovary. There has been recent interest in numerous markers, especially PAX8 and PAX2. The immunohistochemical profile of these tumors is not well studied.
Design: Paraffin blocks from 15 cases of benign Brenner tumor were selected for staining. In addition, we studied 4 cases that were diagnosed in our institution as being transitional cell carcinomas (TCC) of ovary and one case that was a metastatic TCC from the bladder. Six immunostains were performed on these cases - PAX2, PAX8, CK7, CK20, p-63 and uroplakin. Immunostains were graded visually for extent of staining 0 (0%), 1+ (1-33%), 2+ (34-66%) and 3+ (>67%); and for intensity of staining as 1+ (weak), 2+ (moderate) and 3+ (strong).
Results: Benign Brenner tumors showed positive staining with CK7 in 92% (12/13), p63 in 92% (11/12) and PAX8 in 7% (1/14). These tumors showed no staining with CK20 (0/12), uroplakin (0/8) and PAX2 (0/12). Cases of ovarian TCC showed positivity for CK7 and PAX8 in 4/4 cases; and p-63 in 1/4 cases. None of these cases stained with CK20, uroplakin and PAX2. One case of metastatic TCC from bladder showed positivity for CK7, PAX2, PAX8 and p63, with negative staining for uroplakin and CK20.
Conclusions: Transitional cell tumors of ovary appear immunophenotypically different from transitional cell tumors of the urinary tract. Brenner tumors are CK7+/CK20-/p63-/uroplakin-/PAX2-/PAX8- and do not share immunophenotype of urinary tract TCCs. PAX8 is known to be strongly positive in thyroid tissues and our one case of Brenner tumor that showed PAX8 positivity had concomitant struma ovarii. TCCs of ovary similarly are CK7+/PAX8+/CK20-/p63-/uroplakin-/PAX2-. The positivity noted with PAX8 stain likely reflects the mullerian origin of tumor. These tumors were high grade and the possibility of admixed serous carcinoma is not excluded. The metastatic TCC

showed an immunophenotype of its parent tumor and this is distinct from the staining pattern of all the other tumors. We did not have any cases of borderline and malignant Brenner tumors. Additional immunostains (ER and WT-1) are in process for further immunophenotypic characterization of these tumors.

1243 Endometrial Stromal Sarcomas with Distinct Low and High-Grade Components: A Clinicopathologic Review of 11 Cases

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Background: Endometrial stromal tumors (ESTs) represent a group of neoplasms composed of cells morphologically resembling proliferative-phase endometrial stroma. Cytogenetically, many of these tumors show a reciprocal balanced translocation between chromosomes 7 and 17 involving the *JAZF-1* and *JJAZ-1* genes. While ESTs includes endometrial stromal nodules and (low-grade) endometrial stromal sarcomas (ESS-LG), the existence and classification of a true high-grade endometrial stromal sarcoma has been debated. Cases showing both low and high-grade components are exceptionally rare, and the morphologic criteria for distinction are variable depending on the study. Herein we report 11 cases of high grade endometrial sarcoma with histologically distinct low and high-grade components.

Design: After obtaining IRB approval, we reviewed institutional and consultation cases interpreted as "endometrial stromal sarcoma" or "undifferentiated stromal sarcoma" between 1994 and 2011. Only hysterectomy cases showing characteristic histologic ESS-LG features with a component sharply deviating from classic ESS-LG, or high grade tumors with a pattern of myometrial invasion similar to ESS-LG were included, yielding a total of 11 cases. The histologic and clinical features of each case were reviewed. Cytogenetics for t(7;17)(p15;q21) are pending.

Results: Each case showed an ESS-LG component, manifested as either an architectural pattern of tongue-like infiltration resembling ESS-LG or characteristic ESS-LG tumor cytology. In 11/11 cases, the high-grade component lost the typical infiltrative pattern of ESS-LG and formed necrotic sheets. The high-grade component showed increased eosinophilic cytoplasm in 11/11 cases but was otherwise cytologically heterogenous, featuring a combination of spindled (5/11), epithelioid (8/11), or pleomorphic (2/11) cells. The mean mitotic index in the high grade component was 45 mitoses/10hpf (range 21-84). Heterologous elements in the form of rhabdomyosarcoma were seen in 2/11 cases. In those patients with available clinical data, 8/8 patients presented with advanced disease and 7/8 died of disease.

Conclusions: We report 11 cases of a high-grade uterine sarcoma with a distinct ESS-LG component. Although the high-grade component was cytologically variable, they all featured increased cytoplasm, abundant tumor cell necrosis, and a high mitotic rate. The presence of both low and high-grade components, in conjunction with the aggressive behavior of these neoplasms, supports the existence of a true high-grade endometrial stromal sarcoma.

1244 PAX8 and PAX2 Expression in Endocervical Adenocarcinoma In-Situ and High-Grade Squamous Dysplasia

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Background: The transcription factors, PAX2 and PAX8, are expressed in Mullerian glandular epithelial cells including the secretory cells of the fallopian tube and endometrial glandular epithelium. Currently, there is limited knowledge regarding the expression of PAX8 and PAX2 in neoplastic cervical squamous and glandular lesions. In-situ carcinomas of the cervix are exemplified by endocervical adenocarcinoma in-situ (AIS) and high grade squamous intraepithelial lesions (HSILs). Both AIS and HSIL present histologically as hyperchromatic crowded groups of epithelial cells displaying loss of polarity and hyperchromatic nuclei with coarse chromatin. Occasionally, small strips of neoplastic epithelium can be difficult to precisely classify as glandular or squamous. Hence, we sought to investigate the expression of PAX8 and PAX2 in AIS and HSIL.

Design: We identified 66 cases of AIS from the pathology archive. In 18 of these 66 cases, concurrent HSILs were present. Immunohistochemistry for PAX8 and PAX2 were performed and nuclear expression of these markers were examined in all cases. Semiquantitative scores with respect to staining intensity (0, negative; 1, weak; 2, moderate; 3, strong) and extent of staining (0, no staining; 1, <25% cells positive; 2, 25-50% cell positive; 3, >50% cells positive) were recorded for benign endocervical glandular epithelium as well as foci of AIS and HSIL. For each, the two scores were added for a combined score of 0 to 6. A combined score of 2 or less was considered negative whereas a combined score greater than 2 was considered positive.

Results: PAX8 positivity was observed in 64 (97%) of 66 cases of AIS. PAX2 was positive in 3 (5%) cases of AIS; PAX2 expression was lost in 63 (95%) of the 66 foci of AIS. In 62 of these 66 cases, benign endocervical glandular epithelium was present in the immunostained slides; PAX8 and PAX2 positivity was seen in 62 (100%) and 60 (97%), respectively. Seven (39%) and 0 (0%) of the 18 HSILs were positive for PAX8 and PAX2, respectively. The difference in PAX8 expression of AIS versus HSIL was statistically significant (Fisher exact test, $p<0.05$).

Conclusions: Immunohistochemistry for PAX2 is effective in confirming the diagnosis of AIS, as benign endocervical glandular epithelium is typically PAX2(+) whereas neoplastic endocervical glandular epithelium is usually PAX2(-). The far majority of AIS lesions and a subset of HSILs are PAX8(+). With regards to the distinction between AIS and HSIL, a PAX8(-) immunophenotype is particularly predictive of high grade squamous dysplasia.

1245 Morphological Effects of Chemotherapy on Ovarian Serous Adenocarcinoma

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Background: Ovarian serous carcinomas have traditionally been treated with surgical cytoreduction and post-operative chemotherapy with carboplatin/taxol. Recently, there is a growing tendency to treat advance ovarian cancers with neoadjuvant chemotherapy followed by debulking surgery. In these cases, the initial diagnosis is made on a biopsy, or cytological examination of the peritoneal fluid. The aim is to minimize tumour volume and obtain optimal cytoreduction. Treatment effects in such resections are reported, however, these chemotherapy induced effects on ovarian carcinomas have not been well studied. We sought to 1) assess chemotherapy changes in ovarian serous adenocarcinomas by comparing pre- and post-chemotherapy samples; and 2) examine grading post-chemotherapy.

Design: Archival cases of serous adenocarcinomas were reviewed, including: group a) pre-treatment biopsies (n=9); group b) subsequent post-chemotherapy resections of the same patients as in group a (n=9); and group c) resections without chemotherapy (n=18). We compared non-treated biopsies and resections (groups a and c) to post-chemotherapy resections (group b). Cases were evaluated for treatment effects (epithelial-stromal ratio, old hemorrhage, intratumoural lymphocytes, blood vessel proliferation, tumour necrosis, bizarre nuclei, giant cell reaction, foamy macrophages, ballooning and cholesterol clefts); treatment response (none, minimal or marked) and grading (Silverberg).

Results: The epithelial to stromal ratio was increased in all treated cases and correlated with the degree of treatment response ($p=0.0005$). Blood vessel proliferation (+2) and old hemorrhage and hemosiderin were significantly increased in all treated cases ($p=0.0005$ and $p=0.015$, respectively). Intra-tumoural lymphocytes ($p=0.0001$), bizarre nuclei, cholesterol clefts, giant cell reaction and foamy macrophages were nearly exclusively seen in treated cases. Four cases showed lobular carcinoma-like features. Post- compared to pre-chemotherapy grade remained the same or increased in 6/7 cases. Bizarre nuclei were a pitfall for nuclear atypia post-treatment.

Conclusions: Our study highlights specific morphological changes in serous adenocarcinomas treated with chemotherapy, which may be linked to treatment response. We identified three chemotherapy associated changes not reported previously, (epithelial to stromal ratio, blood vessel proliferation and intratumoural lymphocytes). Grading post-treatment was similar to pre-treatment (remains high grade 2 or 3).

1246 Interobserver Agreement on High-Grade Endometrial Carcinoma and Correlation with ER, p53 and p16 Expression

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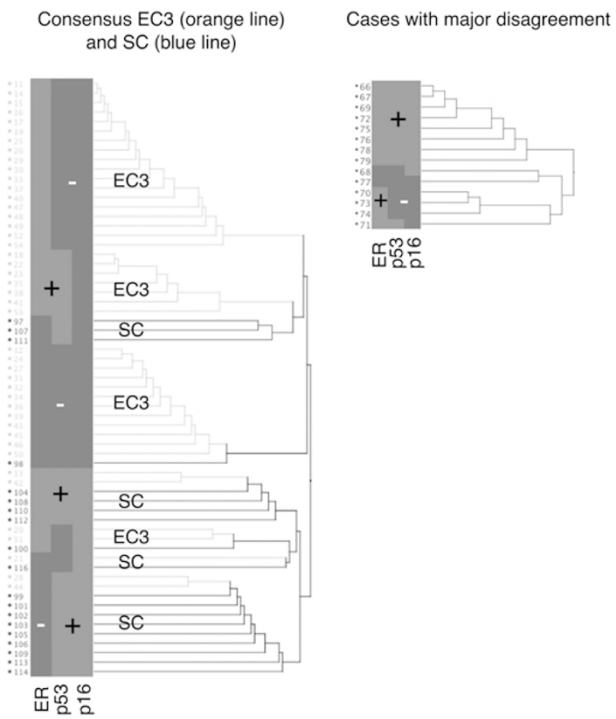
Background: High grade endometrial carcinomas (HGEC) are a heterogeneous group. Inclusion criteria for clinical trials such as PORTEC 3 include high-grade tumor with FIGO grade 3 endometrioid (EC3), serous (SC) or clear cell (CCC) histology. We aim to assess interobserver reproducibility in diagnosing HGEC histologic types and whether routine immunohistochemical marker may aid diagnosis.

Design: 116 cases of HGEC were identified from gynecologic oncology consultation files at Tom Baker Cancer Centre. Representative slides (1-3 /case) were independently reviewed by 5 pathologists according to practice. The diagnoses were summarized into categories: endometrioid low-grade (ECLG), EC3, SC, CCC and other. Interobserver reproducibility was assessed by kappa analysis. p16 (0<80%, 1≥80%), p53 (0<50%, 1≥50%) and ER(0<50%, 1≥50%) expressions were assessed using tissue microarray.

Results: A consensus diagnosis (≥ 80% agreement) was established in 78 cases (67%) with the reviewers' submitted diagnosis, and in 85 cases (73%) with the categorized diagnosis. The kappa values are shown in Table 1. Of the 31 cases with no consensus agreement, 14 (12%) had major disagreement (high-grade vs. low-grade), 17 (15%) had minor disagreement in high-grade tumor type. Among the major disagreement cases, the main problems were ECLG vs. EC3 and ECLG vs. SC. A panel of ER, p16, p53 correlated with histologic diagnosis of EC3 vs. SC in 89% of cases (Figure 1). Six cases with major disagreement showed an immunophenotype suggestive of endometrioid type. The other 8 cases were triple positive, a combination that is unlikely for ECLG.

Kappa Value for Each Pair of Reviewers

Reviewer	Submitted Diagnosis	Categorized Diagnosis
1-2	0.47	0.49
1-3	0.56	0.62
1-4	0.44	0.52
1-5	0.50	0.55
2-3	0.62	0.67
2-4	0.45	0.57
2-5	0.50	0.59
3-4	0.54	0.60
3-5	0.61	0.68
4-5	0.82	0.89



Conclusions: 1. Interobserver disagreement exists in 1/3 to 1/4 of cases in grade and type. 2. Major disagreement on high-grade vs. low-grade is seen in 12% of cases with potentially significant clinical consequences. 3. A panel of ER, p16, and p53 may help to assign endometrioid or serous type.

1247 Histopathologic Features of 164 Failed Endometrial Ablation Cases

RA Simon, MR Quddus, C Zhang, MM Steinhoff, WD Lawrence, CJ Sung. Brown University/Women & Infants Hospital, Providence, RI.

Background: Dysfunctional uterine bleeding (DUB) is common and not always successfully managed medically. Hysterectomy is an option when medical therapy fails; however, endometrial ablation is a minimally invasive alternative. Although the failure rate is low, continued DUB after ablation does occur. We analyzed the histopathologic features of hysterectomy specimens after ablation.

Design: We retrieved cases of hysterectomy after ablation between 2001 and 2010. Control cases were defined as post-ablation hysterectomies for indications other than DUB. In each case, the ablation-hysterectomy interval and detailed histologic features comprising changes of ablation were recorded. The presence of these features was compared between study and control groups, and with the ablation-hysterectomy interval. Statistical analysis was performed via Student's t-test and Fisher Exact Test.

Results: We identified 164 cases (including 35 control cases) in patients 23-62 yrs of age (median=42 yrs). In 53 cases, the entire endometrial surface was examined histologically. Table 1 lists the histopathologic features noted. Significant differences in the ablation-hysterectomy interval were noted between: those with coagulative necrotic debris (median: 5 mos., range: 0.75-72 mos.) and those without (median: 23 mos., range: 1-132 mos.); those with superficial, congested, viable blood vessels (median: 2 mos., range: 0.75-53 mos.) and those without (median: 18 mos., range: 1-132 mos.); and those with dense fibrosis (median: 26 mos., range: 4-84 mos.) and those without (median: 9.5 mos., range: 0.75-132 mos.). Patients with prior tubal ligation were more likely to have continued DUB (p=0.0021).

Finding	Study (n=129)	Control (n=35)
% surface ablated		
<26%	62 (48.1%)	9 (25.7%)
>75%	46 (35.7%)	23 (65.7%)
Fibrosis	23 (17.8%)	13 (37.1%)
Coagulative necrotic debris	45 (34.9%)	14 (40.0%)
Superficial congested vessels	15 (11.6%)	5 (14.3%)
Ablated LUS only	12 (9.3%)	2 (5.7%)
Cornual region spared	2 (1.6%)	1 (2.9%)
Adenomyosis	67 (51.9%)	12 (40.0%)
Leiomyoma	63 (48.8%)	16 (45.7%)
Prior tubal ligation	63 (48.8%)	7 (20.0%)
History of endometriosis	12 (9.3%)	5 (14.3%)

Conclusions: Possible sources of continued DUB include: coagulative necrotic debris, which may persist for years; superficial, congested blood vessels—persistent and viable even after the remainder of the endometrium is ablated; re-epithelialization of the endometrium via adenomyosis; endometriosis associated with proximal dilatation of the fallopian tube after ligation; and residual endometrium present due to spared cornual regions, ablated LUS only, or irregular ablation due to submucosal leiomyoma.

1248 PTEN Status and Frequency of Endometrioid Carcinoma and Its Precursors Arising in Functional Secretory Endometrium: An Immunohistochemical Study of 29 Cases

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Background: Well-differentiated (WD) endometrioid adenocarcinoma (EMCA) and its precursors, complex hyperplasia without atypia and atypical hyperplasia (endometrial intraepithelial neoplasia, EIN), often arise in a background of proliferative, but particularly anovulatory, endometrium secondary to prolonged unopposed hyperestrinism. Although endometrial hyperplasia or EMCA is not typically associated with functional secretory endometrium, the frequency of such lesions has, to our knowledge, not been described in the literature. Mutations of the *PTEN* tumor suppressor gene may occur in up to 80% of EMCA and 2/3 of EIN, resulting in loss of immunohistochemical expression of PTEN. However, the PTEN status of typical EMCA and its precursors arising in physiologic secretory endometrium has not been reported, and forms the basis for our study.

Design: From our institutional archives we identified 29 endometrial biopsy, curettage, or hysterectomy specimens, over 8 ½ years, showing EMCA and/or endometrial hyperplasia arising in physiologic secretory endometrium. We reviewed routine H&E slides of these cases to confirm the diagnoses for PTEN immunohistochemistry (Cascade Bioscience; 1:100 dilution). PTEN-null cases were defined by an absence of immunostain within the lesional glands.

Results: A total of 1601 cases of endometrial carcinoma and 528 cases of atypical endometrial hyperplasia were identified over the study period. Twenty-nine cases of endometrial hyperplasia or carcinoma arising in secretory endometrium were identified in patients 37-54 yrs of age (median: 44 yrs). Diagnoses included 7 complex hyperplasia without cytologic atypia (CH), 9 atypical complex hyperplasia (ACH), 11 FIGO grade 1 EMCA, one FIGO grade 2 EMCA, and 2 tumors of mixed cell type (serous and WD endometrioid). Twenty-one of 29 cases had tissue available for immunostaining. PTEN-null lesions were identified in 1 of 6 (16.7%) cases of CH, 5 of 5 (100%) cases of ACH, and 7 of 8 (87.5%) cases of grade 1 EMCA. The WD endometrioid component of one mixed cell type tumor was PTEN-null, while the grade 2 EMCA was PTEN-positive.

Conclusions: Our findings confirm that typical EMCA and its precursor lesions only rarely arise within a functioning secretory endometrium (frequency of <2% in our study). We found the rate of PTEN-null cases of these lesions to be comparable to that of the usual type reported in the literature. Loss of PTEN expression was noted more frequently in atypical hyperplasia or low grade carcinoma than in non-atypical hyperplasia or grade 2 EMCA.

1249 HNF-1ß in Ovarian Carcinomas with Serous and Clear Cell Changes

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Background: Many ovarian tumors show clear cell change including high grade serous carcinomas (SC). Accurate diagnosis is important, however, as ovarian clear cell carcinoma (CCC) is well-known to be less responsive than SC to traditional types of ovarian carcinoma chemotherapies. In a previous study, 32 ovarian carcinomas originally diagnosed as pure CCC (n=11), mixed serous and clear cell (MSC) (n=10), or pure SC (n=11), were evaluated to determine cell type. Parameters evaluated included clinical, morphologic, and immunohistochemical features. In this study, MSC showed mitotic rates, stages of presentation, and immunoreactivities of the markers WT1, ER, and p53 similar to that of SC. It was thus concluded that MSC represent SC with clear cell change. HNF-1β, an immunohistochemical marker which has been shown to be rather specific for CCC was not evaluated in this study. We thus sought to analyze this marker in this group of tumors.

Design: One block of each of the SC and CCC was stained with the immunohistochemical marker HNF-1β. In the cases of MSC, 2 blocks were evaluated when the serous and clear cell components were not present on the same slide.

Results: The results of the immunohistochemical analysis are shown in the table below.

Tumor type	# HNF-1ß + cases
CC	11/11
SC	0/10
Clear cell component of MSC	0/10
Serous component of MSC	0/10

All (11/11) of CCC were positive for HNF-1β and showed a diffuse pattern. None (0/11) of the SC showed immunoreactivity for HNF-1β. In the cases of MSC, both the serous and clear cell components were negative for HNF-1β.

Conclusions: HNF-1β appears to be a sensitive and specific marker for CCC and is not expressed in SC with clear cell change. The pattern of immunoreactivity of HNF-1β in tumors with both serous and clear cell changes supports the conclusion that MSC are SC with clear cells.

1250 Detection of the JAZF1-JJAZ1 Fusion Transcript in Endometrial Biopsy Material Several Years Prior to the Clinical Presentation of Endometrial Stromal Sarcoma

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Background: The JAZF1-JJAZ1 fusion transcript has been identified in approximately 80% of endometrial stromal nodules, 60% of endometrial stromal sarcomas and 20% of undifferentiated uterine sarcomas. Because the malignancy of these sarcomas is believed to increase over time, with a subset of stromal nodules developing into stromal sarcomas, and a subset of these tumors developing into undifferentiated stromal sarcomas, early

detection and diagnosis is crucial. Our hypothesis is that the fusion transcript may be present and identifiable in endometrial biopsy material years prior to the clinical presentation of a uterine mass.

Design: Formalin fixed paraffin embedded tumor material was retrospectively identified in the archives of the Karolinska University Hospital. Cases were included in this study if the JAZF1-JJAZ1 fusion transcript was identified in the endometrial stromal tumor (EST) material and there was one or more prehysterectomy endometrial biopsy available for analysis. The fusion transcript was detected through real-time PCR.

Results: Two patients were identified where the fusion transcript could be detected in the EST (diagnosed at the time of hysterectomy) and where there was an earlier endometrial biopsy performed for another indication. H&E review of both biopsies revealed no histologic evidence for the presence of an EST. In one patient, an endometrial curettage was performed for dysfunctional uterine bleeding 5 years prior to the diagnosis of the EST. The JAZF1-JJAZ1 fusion transcript was identified in this biopsy material. In the second patient an endometrial curettage was performed 6 years prior to hysterectomy for an EST. No fusion transcript was identified in this material.

Patient	Indication EMBx	H&E DxEMBx	Transcript Detected EMBx	Time between EMBx and EST Diagnosis	Transcript Detected EST
1	DUB	EMP	Yes	5 years	Yes
2	DUB	EMP	No	6 years	Yes

Conclusions: This work demonstrates that detection of JAZF1-JJAZ1 transcript can allow diagnosis of ESTs years prior to their clinical presentation. This is of potential great importance since treatment success is strongly dependent on stage at diagnosis. Furthermore, the detection of the transcript as early as 5 years prior to diagnosis suggests that translocation is an early event in tumorigenesis and supports the notion that stromal sarcoma may develop from a benign precursor such as a stromal nodule.

1251 The Histologic Spectrum of Grossly Visible Pigmented Lesions of the Uterine Cervix: A Prospective Study

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Background: Pigmented lesions of the uterine cervix (UC) are rarely encountered and represent a wide spectrum of congenital, reactive and neoplastic conditions.

Design: We conducted a prospective study of 2,118 consecutive hysterectomy specimens from 02/2010 to 08/2011which uncovered by routine gross inspection a total of 32 pigmented lesions of the UC.

Results: Of the 32 grossly visible pigmented lesions of the UC, routine histology revealed 31 histologic correlates including 25 (81%) blue nevi, 1 (3%) lentigo / melanosis, 1 (3%) focal vasculitis, 2 (6.4%) previous biopsy site reactive lesions with hemosiderin-laden macrophages, 1 (3%) hemorrhagic Nabothian's cyst, 1 (3%) hemangioma, and 1 (3%) case of multinucleated giant cell reaction to India-ink like material. Almost one third of the cases required deeper levels to reveal the nature of the pigmented lesions (9/31). The overall incidence of blue nevus in the UC was 1.2 %. More than half of the patients were Caucasians (13/25, 52%). The mean age of the UC blue nevus patients was 47.4 years (range 31 to 64 years). The number of blue nevi per UC varied between 1 to 3 and the size ranged from 0.1 cm to 2 cm (mean : 0.68 cm). All UC blue nevi were located in the stroma of the endocervix. These blue nevi of the UC appeared to evolve through 3 distinct morphologic phases: 1- stromal melanocytic foci composed of fine spindle cells (9/25, 36%), 2- mixed phase with fine spindle, plump spindle, and epithelioid cells (15/25, 60%), and 3- nevus-like with epithelioid cells (1/25, 4%). Of note, all (100%) of UC blue nevi cases were not reported in the original hysterectomy surgical pathology report or were misinterpreted as biopsy site. Compared to blue nevus, lentigo of the cervix was significantly less common and located in the squamous mucosa of the ectocervix. Lentigo of the UC was characterized by hyperpigmentation of the basal keratinocytes admixed with scattered slightly enlarged melanocytes.

Conclusions: This study demonstrates that most grossly visible pigmented lesions of the UC are benign and have a histologic correlate. Therefore, careful macroscopic and microscopic examination, in many cases with the help of deeper levels, is required to characterize these pigmented lesions and facilitate the exclusion of a more ominous pathologic process such as UC malignant melanoma.

1252 Association of Isolated Single Umbilical Artery with Maternal Health and Placental Pathology; a Retrospective Study of over 6,500 Placentas

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Background: While single umbilical artery (SUA) has an overall incidence of 1%, isolated SUA (iSUA) occurs in only 0.37-0.5% of pregnancies. Isolated SUA is independently associated with fetal intrauterine growth restriction (IUGR), prematurity, multiple gestations, and maternal chronic (CHTN) or gestational hypertension (GH) and diabetes mellitus (DM). It is of questionable clinical significance in the newborn period and has not been associated with long term, adverse growth and neurologic outcomes. However, we queried whether noninfectious placental pathologies seen with CHTN, GH, DM, and placental dysfunction, and/or those fetal conditions linked to adult onset cardiovascular and chronic renal diseases were more common with iSUA.

Design: A retrospective review of all institutional placental and previable fetoplacental (gestational age <20 wk) surgical pathology accessions from 1/01/2006 to 12/31/2010 was undertaken. Patient demographics, obstetrical history and pathologic diagnoses were extracted from the medical records.

Results: There were 6534 cases with evaluable umbilical cords. Overall incidences of SUA and iSUA were 3.2% (n=211) and 1.9% (n=110) respectively, with iSUA significantly associated with multiple births (p=0.011), CHTN (p=0.0270), GH (p=0.032), DM (p=0.023), IUGR (p=<.002), low placental weight (p=<.002), and non-central cord insertion (p=0.001) compared to normal cords. Chronic chorionic villous (CV) ischemia was significantly more common in singleton placentas with iSUA versus 3-vessel cord cases (p=0.024); CV infarction was more frequent, but the difference was not significant (19.4% versus 14.4%, p=0.178). In singletons with non-central cord insertion and CHTN, DM, and/or GH, 82.4% of iSUA cases had CV ischemia versus 65.0% with 3-vessel cords (p=0.249). No association with prematurity, fetal thrombotic vasculopathy (FTV), or villitis of unknown etiology (VUE) was found.

Conclusions: We confirmed the association of iSUA with IUGR, multiple birth, CHTN, DM, and GH. We found significant independent association between iSUA and chronic CV ischemia and an increased rate of CV infarction; however, no significant association was found for preterm birth, FTV or VUE. The higher rate of CV ischemia after controlling for insertion site and CHTN, GH, and DM suggests that iSUA cord placentas may be more susceptible to ischemia than 3-vessel cord placentas. The higher association with CV infarction did not reach statistical significance, possibly due to sampling.

1253 Does Hormonal Therapy Affect the Morphology of Uterine Smooth Muscle Tumors?

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Background: The reported histopathologic findings in leiomyomas (LM) among women treated with hormones vary. Degenerative (necrosis/infarction, myxedema) and/or atypical (cytologic atypia, increased mitotic activity) changes have been observed in uterine smooth muscle tumors in association with oral contraceptive (OC) use. We compared LM with and without degenerative and/or atypical changes to assess the association of hormonal therapy with these morphologic features.

Design: We searched the pathology database at the Yale School of Medicine between 1/1/2005 and 9/1/2011. 1815 female patients with a diagnosis of LM were identified. Cases associated with concurrent cancer, pregnancy or previous uterine artery embolization were excluded. The medical records for the remaining patients' were examined for documentation of any prior hormonal therapy, along with its duration and dosage when available.

Results: 875 cases (733 hysterectomies,142 myomectomies) were eligible for the study (age range [19-95 years]; median [47 years]). 210 (24%) patients were on homonal therapy within 3 months of surgery, 48 with degenerative changes and 162 without degenerative changes. Hormonal therapies included progesterone/estrogen OC (102), progesterone (17), estrogen (29), leuprolide (45) and Tamoxifen (17). Prior hormonal therapy was significantly associated with degenerative (Table 1,2; p=0.002, Fishers exact [FE]), but not atypical changes (p=0.164, FE). Significant associations were attributable only to leuprolide (p=0.0001, FE), Loestrin (p=0.007, FE), and medroxyprogesterone (p=0.034, FE).

Conclusions: Hormonal effect on LM morphology varies with the particular hormone used. Certain OC are more likely to be significantly associated with increased degenerative changes, including necrosis/infarction and myxedema. Hormonal therapy did not appear to be significantly associated with increased cytologic atypia or increased mitotic activity in LM in this group of cases.

Morphologic features in LM exposed to any hormone or a single hormonal agent

Degenerative features?	Any hormone	Progesterone	Norethindrone	Estrogen	Leuprolide	Tamoxifen
Yes # (%)	48(100)	0 (0)	1 (2)	1 (2)	19 (40)	1 (2)
No # (%)	162(100)	4 (2.5)	12 (7)	28 (17)	26 (16)	16 (10)

Morphologic features in LM exposed to combination progesterone/estrogen OC (specific progesterone agent listed)

Degenerative features?	Unidentified OC	Loestrin	Norgestrel	Medroxyprogesterone	Levoenorgestrel	Other*
Yes # (%)	11 (23)	5 (10)	1 (2)	7 (15)	2 (4)	0 (0)
No # (%)	37 (23)	4 (2.5)	3 (2)	14 (9)	3 (2)	15 (9)

* Includes OC with Etonogestrel, Drospirenone, Norgestimate, Desogestrel and Norelgestromin.

1254 Mismatch Repair Protein Expression in Clear Cell Carcinoma of the Endometrium: Frequency and Clinicopathologic Correlation of 41 Cases

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Background: Lynch Syndrome is associated with a >40% risk of developing endometrial carcinoma, with quite variable frequency of the different histologic subtypes reported, including high-grade endometrioid carcinomas (EEC), clear cell and serous carcinomas. Recently, an increased incidence of clear cell carcinomas (CCC) of the ovary has also been described in the setting of Lynch Syndrome. However, no large series of endometrial CCC have been studied. The goals of this study are to evaluate the overall incidence of loss of DNA mismatch repair (MMR) protein expression in a series of endometrial clear cell carcinomas and its potential association with Lynch Syndrome.

Design: After IRB approval, 41 endometrial CCC were retrieved from our files from 1986 till 2010. All available H&E slides (2-12 per case) were reviewed by two gynecologic pathologists and clinicopathologic parameters were recorded. Immunohistochemical expression of MMR proteins (MLH1, MSH2, MSH6, and PMS2) was assessed.

Results: The mean age of the patients was 67.8 years (range: 40-92; 5 patients < 50). Twenty patients were diagnosed at early stage (FIGO I and II), and 21 at advanced stage (FIGO III and IV). Combined loss of MLH1 and PMS2 expression was seen in 2 patients (40 and 59 years), while combined loss of MSH2 and MSH6 expression was seen in 2 other patients (46 and 48 years). A predominant solid growth was seen in 3/4 tumors (also present in 8/37 CCC with preserved MMR proteins) and abundant peri- and intratumoral lymphoplasmacytic infiltrate in 2/4 (both with absent MSH2/MSH6) (also seen in 9/37 CCC with preserved MMR proteins). All 4 patients were

diagnosed with FIGO IA tumors, and had no evidence of disease with a mean follow-up of 148 months (range: 80-220). None of these 4 patients had metachronous ovarian or colorectal carcinoma. Eleven out of the other 16 patients with early stage CCC also had no evidence of disease with a mean follow-up of 96 months (range: 6-205). Two patients with a history of colorectal carcinoma had preserved MMR protein expression and advanced stage CCC.

Conclusions: In this study, the overall frequency of DNA MMR protein abnormalities in endometrial CCC is relatively low (4/41; 9.8%) compared to a mean of 25% reported in EEC. Among patients aged ≤ 50 in our cohort, 3/5 (60%) showed loss of DNA MMR protein expression and they did not have high-stage tumors, however, clinical behavior was similar to patients without abnormal MMR protein profile, and they had not developed other evidence of Lynch Syndrome.

1255 Validation of an Algorithm for the Diagnosis of Serous Tubal Intraepithelial Carcinoma

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Background: It has been reported that the diagnosis of serous tubal intraepithelial carcinoma (STIC) is not optimally reproducible based only on histologic assessment. Recently, we reported that the use of a diagnostic algorithm that combines histologic features and coordinate immunohistochemical expression of p53 and Ki-67 substantially improves reproducibility of the diagnosis. The goal of the current study was to validate this algorithm by testing a group of 6 gynecologic pathologists who had not participated in the development of the algorithm (3 faculty, 3 fellows) but who were trained in its use by referring to a website designed for that purpose.

Design: H&E, p53, and Ki-67 slides, which contained 41 mucosal lesions of the fallopian tube, were reviewed by all observers.

Results: Overall consensus (≥4 of 6 pathologists) for the 4 categories of STIC, serous tubal intraepithelial lesion (our atypical intermediate category), p53 signature, and normal/reactive was achieved in 76% of lesions with no consensus in 24%. Combining diagnoses into 2 categories (STIC vs. non-STIC) resulted in overall consensus in 93% with no consensus in 7%. The kappa value for STIC vs. non-STIC among all 6 observers was also high at 0.67 and did not significantly differ whether for faculty (κ=0.66) or fellows (κ=0.60).

Conclusions: These findings confirm the reproducibility of this algorithm by a group of gynecologic pathologists who were trained on a website for that purpose. Accordingly, we recommend its use in research studies. Before applying it in routine clinical practice, the algorithm should be evaluated by general surgical pathologists in the community setting.

1256 A Population-Based Study of Ovarian Serous Borderline Tumors (SBTs) with Uniform Pathology Review and Long-Term Follow-Up

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Background: The behavior of SBTs and their relation to low-grade (micropapillary) serous carcinoma remain uncertain. We undertook a population-based study involving the entire country of Denmark, with uniform pathology review, **no** patients lost, and long-term follow-up.

Design: 2 nationwide registries, which cover 100% of the female population in Denmark, were searched for all cases with a diagnosis of SBT over a 25-year period (n=1,516). In order to find SBTs misclassified as carcinoma, cases with a diagnosis of "well-differentiated serous carcinoma" from 1997 to 2002 were also retrieved (n=114), resulting in an additional 64 SBTs. Slides of the ovarian tumors and implants from all cases were re-reviewed, and 1,009 SBTs were confirmed. The cohort was followed for up to 31 years (mean, 12.7 yrs). We subdivided SBTs into atypical proliferative serous tumor (APST) and non-invasive micropapillary serous carcinomas (MPSC) based on the amount of micropapillary (MP) growth. Because the threshold amount of MP growth that correlates with aggressive behavior has not been firmly established, we compared survival using a cut-point of at least 5 mm of MP growth vs. a cut-point of 1 mm as the criterion for a diagnosis of MPSC.

Results: Overall survival for patients with APST was significantly lower than the general female population using either the 1 mm or 5 mm cut-point to distinguish APST from MPSC (p=0.001, 0.003, respectively). Similarly, the overall survival for MPSC using the 1 mm cut-point was significantly lower than that of APST (p=0.007), but survival using the 5 mm cut-point, although lower, was not significant (p=0.15). Using both the 1 mm and 5 mm cut-points, MPSC compared to APST had a higher frequency of advanced stage disease (p<0.0001, 0.001, respectively), invasive implants (p<0.0001, 0.0001, respectively), and microinvasion (p<0.0001, 0.003, respectively).

Conclusions: The mortality of patients with APST with <1 mm of MP growth is lower than that of the general population in Denmark. Mortality for tumors with 1-5 mm of MP growth was significantly increased compared to APST. Based on these findings, consideration should be given to reducing the threshold for the diagnosis of MPSC from 5 mm to 1 mm.

1257 HER2 and GRB7 Expression in Serous and Endometrioid Carcinomas of the Endometrium

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Background: Expression and amplification of HER2 is associated with high grade and stage endometrial cancer with the highest rate in serous carcinomas (20-80%) and the lowest in grade 1 endometrioid carcinomas (< 5%). Growth factor receptor-bound protein-7 (GRB7) gene located on chromosome 17 is in the immediate vicinity of the HER2 gene and promotes HER2 mediated signaling and tumor formation. The aim of this study was to evaluate HER2 and GRB7 expression in serous and endometrioid endometrial carcinomas.

Design: 53 cases of endometrial carcinoma from 2004 to 2010 were selected (31 cases of serous carcinoma (SC-58.5%) and 22 cases of endometrioid carcinoma (EC-41.5%). 5 of 22 cases of EC (16.1%) were grade 1 and 17 (83.8%) were grade 3. Immunohistochemical stains for HER2 and GRB7 were performed. HER2 was scored as: score 3 uniform intense membrane staining of > 30% of tumor cells; score 2 equivocal: complete membrane staining that is either non uniform or weak in intensity but with out obvious circumferential distribution in at least 10% of the cells; and score 0 or 1: negative: no staining or weak, incomplete membrane staining in any proportion cells. Scores 0 and 1 were considered negative, score 2 equivocal and score 3 positive. For GRB7 percentage of positively staining cells and intensity of staining were noted. < 10% cells with weak staining were considered negative.

Results: HER2 was positive in 13 of 31 (41.9%) of SC and equivocal in 4 cases (12.9%). GRB7 was expressed in 5 of 53 (9.4%)cases; all were SC of which 4 cases were HER2 positive and 1 case was HER2 equivocal. GRB7 positive cases showed > 30% cells with moderate to strong staining. Neither HER2 nor GRB7 expression was detected in EC. 5 of 22 EC (22.7%) were HER2 equivocal and the rest negative. 9 of the 13 HER2 positive (69.3%) SC were stage T3 or M1, one (7.7 %) case was T2 and 3 (23.0%) cases were T1. Of the 14 HER2 negative SC, 7 (50%) cases were T3, 2 (14.5%) cases were T2 and 5 (35.7%) were T1. 3 of 5 both HER2 and GRB7 positive SC were stage T3, the other two cases were stage T1 and T2.

Conclusions: Only SC showed overexpression of HER2 (41.9%) and GRB7 (6.0%) compared with EC (0.0%). Co-expression of HER2 and GRB7 was present in 30.7% cases of SC. Majority (69%) of HER2 positive SC presented as high stage (T3 or M1) cancers compared with HER2 negative SC (50%), although the differences were not statistically significant. Our findings suggest that there is a common biological pathway between HER2 and GRB7 in SC of the endometrium and that GRB7 in addition to HER2 could serve as target for therapy.

1258 Negative for Dysplasia in Loop Electrosurgical Excision Procedure (LEEP) and Cold Knife Cone Biopsy: Review of 380 Cases

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Background: Loop electrosurgical excision procedure (LEEP) and cone are often used for treatment of high-grade cervical intraepithelial lesions (CIN 2+). Pathologists sometimes see the complete absence of dysplasia in a LEEP or cone specimens during their practice. There are several hypotheses to explain the absence of dysplasia, such as postbiopsy regression and small foci of dysplasia not sampled in the histologic sections. However, very few publications in the literature address the negative for dysplasia rate in LEEP and cone.

Design: LEEP and cone cases were retrieved from the database of Department of Pathology during January 1, 2008 to December 31, 2010. Cases without previous in-house tissue (biopsy or cytology) of high-grade intraepithelial lesions (HSIL) were excluded. Fisher exact and student t tests were used for statistic analysis.

Results: A total of 451 cases of LEEP and cone biopsies (257 cases of LEEP and 194 cone biopsies) were reviewed. 71 cases were excluded due to lacking in-house tissue of HSIL and/or CIN2+. Of the 380 studied cases, 219 LEEP and 161 cone, patients received cone biopsy were statistically older than patients with LEEP (37.2 vs 29.3 year-old, p=0.0001). 24 cases were negative for dysplasia (24/380, 6.3%) and there was no significant difference (p=0.67) between LEEP (15/219, 6.8%) and cone (9/161, 5.6%). 65/380 cases had only HSIL cytology without biopsy confirmed CIN2+. The negative for dysplasia was higher in cytology cases than those with biopsy confirmed CIN2+ (7/65 vs. 17/215), but it was not statistically significant (p=0.09). Reviewing the previous cervical biopsy or cytology of the 24 negative for dysplasia cases confirmed HSIL/CIN2+ in 22 cases and LSIL/CIN1 in the remaining 2 cases.

Conclusions: Negative for dysplasia in LEEP or cone is not an uncommon finding in daily practice, and the negative for dysplasia rate in our study was 6.3% (5.6% for cone and 6.8% for LEEP), which is lower than literature reports (14% -16.5%). Based on our study, misinterpretation of previous biopsy or cytology was an unlikely contributing factor for the negative LEEP and cone. Our results also support the current treatment guideline for HSIL cytology-the "see and treat" approach without biopsy confirmation of CIN2+.

1259 Occult Gynecological Cancer in Prophylactic Risk-Reducing Salpingo-Oophorectomies from BRCA Mutation Carriers

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Background: Women with BRCA mutations are at increased lifetime risk for breast, ovarian, tubal, and peritoneal carcinoma (CA). Bilateral risk-reducing salpingo-oophorectomy (RRSO) is an effective strategy to signifcantly reduce this risk. We describe occult gynecologic cancers (OGC) diagnosed at prophylactic RRSO in BRCA mutation carriers.

Design: 210 consecutive BRCA+ women who underwent RRSO were identified in our oncology registry. Medical records and pathology were reviewed, demographics and pathological findings were charted, and the two diagnostic RRSO groups (OGC and

benign) were compared for mutation, ethnicity, age at RRSO, prevalence of breast CA, and age at diagnosis of breast CA.
Results: 14(6.7%) of the 210 RRSO from BRCA+ women had OGC and 196 RRSO were benign. OGC was present in 13(6.7%) of 194 RRSO that had been serially sectioned and entirely submitted for microscopy and in 1(6.3%) of 16 RRSO that had routine sectioning and sampling. Women with OGC ranged from 43 to 76 years in age at RRSO, 10(71.3%) were of Ashkenazi Jewish descent, 12(85.7%) were BRCA1+ and 2(14.3%) were BRCA2+. The OGC included serous tubal intraepithelial carcinoma/STIC (8), invasive tubal CA (2), STIC and bilateral ovarian CA (1), ovarian CA [bilateral (1); unilateral (1)], and primary peritoneal CA (1). STICs were the most frequent tumors, measured up to 0.3 cm, and involved the distal tube/fimbria. Most STICs were unifocal. All OGC were serous and high grade. The largest tumor was a 4 cm tubal CA. Post RRSO followup (4 to 114 mos) is available in 13 OGC cases;no followup is available in one case. Two women with stages II and III CA at RRSO had recurrent CA at 21 and 28 months, respectively. Of the 8 women who had only STIC at RRSO, 6 have had no recurrence, 1 returned with mullerian CA in an isolated celiac lymph node at 32 months, and 1 was diagnosed with stage 3 peritoneal CA at 42 months. In both cases, the adnexa had been entirely submitted for microscopy and the excised uterus/cervix were benign. When the OGC and the benign groups were compared, there was a significant difference in the mean age at RRSO (55.9 vs 48.4 yrs, respectively p=0.0085). No significant difference (p>0.05) was observed in the prevalence of BRCA1 mutation, Ashkenazi Jewish origin, or breast CA, or in the mean age at breast CA diagnosis between the two groups.
Conclusions: -OGC was present in 6.7% of asymptomatic BRCA+ women who underwent prophylactic RRSO.
-STIC is the most frequent OGC diagnosed in these women.
-Women with OGC, even if confined to STIC when found at prophylactic RRSO, warrant ongoing surveillance for CA.

1260 Evaluation of Histological Types of Endometrial Carcinomas: Experiences from Endometrial Biopsies of 358 Consultation Cases

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Background: Uterine serous carcinoma (USC, type II), accounts for 10% of all endometrial cancers, and is more aggressive than the endometrioid cancer (EMC, type I). Since USC has a higher propensity for lymphovascular invasion, extrauterine spread than EMC, meticulous staging is important. For this reason, accurate diagnosis of the tumor type for endometrial carcinomas in endometrial biopsies/curetting specimen is particularly valuable for surgical management. While consultation of endometrial neoplasm biopsies is a standard protocol in gyn-oncology practice, the value and importance of agreeing upon the type of endometrial carcinoma deserve further investigation.
Design: A total of 358 biopsies of endometrial carcinomas were reviewed from the last three years of consultation records. The cases were from 49 hospitals, medical centers and private groups. Patients' ages ranged from 19 to 86 years old. For those cases with no agreement upon the type of endometrial carcinomas between the original diagnosis and consultation, one or more relevant immunomarkers were examined, including b-Catenin, CEA, cytokeratin, ER, PR, P16, P53, Vimentin, and WT1. Most cases with revised diagnoses for tumor types were further correlated with hysterectomy specimen.
Results: Among the 358 endometrial carcinomas, original diagnoses of type I carcinoma accounted for 91% of cases (327/358) and type II carcinoma for 9% (31 cases). A total of 41 cases (11.5%) were questioned for tumor type based on histology alone. All 41 cases were further examined by immunohistochemistry. Of the 41 cases, 36 cases (10.1%) were reclassified based on histology and immunohistochemistry (10 cases were changed from USC to EMC; 18 cases from EMC to USC; 8 cases resulted in various other outcomes (clear cell vs Secretory or squamous differentiation; Carcinosarcoma vs EMC with dedifferentiated; endometrial EMC vs cervical usual type) and 5 cases (1.4%) remained undetermined.
Conclusions: Overall, 10% of endometrial carcinoma cases from our consultation were reclassified. Interpretation of type I and II endometrial carcinomas remains a common problem in general practice. Common errors include: 1) lack of awareness of the importance of tumor types; 2) bias towards a single histologic feature, 3) sole reliance upon p53; 4) lack of examination of relevant immunomarkers. In addition, there are a small proportion of endometrial carcinomas that have a low grade 'endometrioid' appearance, aggressive growth behavior and inconclusive immunostains. Further clinical and molecular studies may help define the nature of these tumors.

1261 Secretory Endometrial Intraepithelial Neoplasia (SEIN) Arising in Secretory Endometrium: Histologic & Immunohistochemical Features of a Rare EIN Variant

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Background: Diagnosis of EIN in a background of secretory endometrium (SE) is difficult due to gland crowding. In addition, a rare variant of EIN with secretory features has been suggested. In this study, we evaluated the histologic features of this uncommon and difficult to diagnose entity and evaluated the distinction between SEIN and other types of EIN from SE.
Design: 77 endometrial biopsies diagnosed as hyperplasia (all types, WHO criteria) were evaluated using EIN criteria. We searched for cases of possible SEIN, as well as EIN associated with a background of SE. Histologic features (including luminal complexity and cytoplasmic and nuclear features) were evaluated in all cases. PAX2 staining was performed on all available blocks to investigate previous claims that PAX2 may aid in the diagnosis of EIN. PAX2 staining was defined as 3+ (strong nuclear staining, >90% nuclei), 2+ (weak nuclear staining, >50% nuclei), or 1+ (faint nuclear staining, <50% nuclei).
Results: Of the 77 cases, 3 (4%) were classified as SEIN. The defining features included luminal complexity; vacuolated, voluminous cytoplasm; nuclear overlap and vesicular chromatin. SEIN is distinct from SE because the glands are larger, more complex, and haphazardly arranged. Three additional cases were classifed as EIN arising in a background of SE. These lesions were histologically distinct from SEIN because the neoplastic glands lack luminal complexity and resemble proliferative glands, but they arose in a background of SE. Luminal secretions were present in both types of EIN. Lesional EIN tissue was available for PAX2 staining in 5 cases. All cases of EIN showed altered PAX2 staining compared to background non-neoplastic glands, which are strongly PAX2 positive. Three cases showed negative PAX2 staining in EIN. One case showed increased EIN PAX2 staining compared to the background. The remaining case showed decreased EIN PAX2 staining (2+) compared to normal glands. Five of the six EIN cases were treated with hormones, with negative subsequent samples (rebiopsy or hysterectomy). On follow-up, all EIN cases had no evidence of disease (mean: 7 months).
Conclusions: This is the first detailed description of SEIN, which is an uncommon lesion distinct from traditional EIN, and its distinction from SE using both histologic criteria and PAX2 staining. All EIN (including SEIN) displayed altered PAX2 staining when compared to normal glands, primarily negative or decreased staining. Hormone therapy appears to be effective in treating EIN, although follow-up in this study is short.

1262 Architectural vs. Nuclear Atypia Defined FIGO Grade 2 Endometrial Endometrioid Adenocarcinoma (EEC): A Clinicopathologic Comparison of 154 Cases

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Background: FIGO grade 2 EEC carries a 88% five year survival rate. They are defined by >5% but <50% solid epithelial component. A small subset may display <5% solid growth, but display marked nuclear atypia and are therefore designated grade 2. We compared tumor characteristics, staging, and clinical outcome of patients with architectural versus atypia defined grade 2 EEC.
Design: 154 cases of grade 2 EEC were reviewed to confirm grade; percent solid growth, and presence/absence of atypia. Only marked atypia (characterized by significant nuclear pleomorphism identifiable at low power magnification or a pattern of enlarged nuclei, 1.5-2 x normal, with irregular nuclear contours, dispersed chromatin and prominently enlarged nucleoli) increased the FIGO grade one level. Depth of invasion, tumor stage, lymph node status and clinical outcomes were then compared.
Results: 154 cases were evaluated. 23 were eliminated based on review (6 to grade 3, 17 to grade 1). Of the remaining 131 FIGO II cases, 19 (15%) were grade 2 based on the presence of severe atypia and 112 (85%) met the architecturally defined criteria (Tables 1 & 2).

Pathologic features of architecturally and atypia defined tumors

	Invasive	<50% Invasive	>50% Invasive	Cervix +	Nodes +	Recurrance
Atypia Defined	17 (89%)	10 (53%)	7 (37%)	2 (11%)	2 (11%)	1 (5%)
Architecturally Defined	89 (79%)	63 (56%)	26 (23%)	3 (12%)	5 (4%)	8 (7%)

Recurrent cases - Clinicopathologic Features

Atypia	% Solid	Depth (%)	LVI \ LN	Stage	Recur site \ Time (months)	Adjuvant	Status
Severe	0	55	- \ 0	IB	Vaginal Cuff \ 6	No	NED
NS	5	0	- \ 0	IB	Unknown \ 10	No	NED
NS	30	25	+ \+	1A	Lung \ 16	+	LFU
NS	5	40	+ \ 0	1A	Lung, LN\ 27	+	DOD
NS	5	90	+ \ 0	1B	Unknown \ 46	+	LFU
NS	10	20	- \0	2	Port Site \ 24	+	NED
NS	10	50	+ \ 0	3	Lung \ 7	+	LFU
NS	40	100	+ \ 0	2	Unknown \ 12	+	AWD
NS	5	>90	0 \ 0	4	Lung \ 1	+	LFU

LVI-Lymph Vascular Invasion, LN-Lymph Node (0= not available), NS-Not severe, NED-No Evidence of Disease, LFU-Lost to Follow Up, DOD-Died of Disease, AWD-Alive with Disease

Conclusions: Atypia defined vs. architecturally defined grade 2 ECC's show no significant difference in stage and prognosis. An increase in grade based on presence of nuclear atypia stratifies patients at increased risk since 89% of these patients have myoinvasion at the time of hysterectomy which is in distinct contrast to our previous study (Mod Path 24 (1), 2011; 264A, Abstract 1119) where 70% of Grade I cases were non-invasive. No significant correlation between percentage of solid component and risk of recurrence was identified in this study.

1263 Negative Loop Electrosurgical Cone Biopsy Following a Biopsy Diagnosis of High-Grade Squamous Intraepithelial Lesion: Frequency and Clinical Significance

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Background: Loop electrosurgical excision procedure (LEEP) is a therapeutic option following a biopsy diagnosis of high-grade squamous intraepithelial lesion (HSIL). Most LEEPs confirm the HSIL biopsy diagnosis but some will not show HSIL or carcinoma. Such negative findings suggest the possibility of an incorrect biopsy diagnosis, removal of the lesion by biopsy or insufficient LEEP sampling. We studied a series of LEEPs to determine the frequency of negative LEEPs after HSIL biopsies and the clinical follow-up of these negative LEEPs.
Design: The Department of Pathology's records were searched for all patients undergoing LEEP who had prior cervical biopsies and subsequent clinical follow-up.
Results: Three hundred seventy-eight women were found who had index cervical biopsies, subsequent LEEPs and clinical follow-up. Clinical follow-up averaged 25.8 months. Three hundred seven women had HSIL on biopsy with 234 (76%) showing HSIL or carcinoma on LEEP and 73 (24%) LEEPs being negative or disclosing low-grade squamous intraepithelial lesion (LSIL) on LEEP. Thirty-two of 223 (14%) patients with

HSIL biopsies and LEEPs had HSIL on clinical follow-up. Eight of 73 (11%) patients with HSIL biopsies but negative or LSIL LEEPs had HSIL on follow-up.

Conclusions: Twenty-four percent of patients with HSIL biopsies are negative or LSIL on LEEP. There is no statistical difference in development of HSIL after LEEP for those with positive biopsies and LEEPs (14%) versus positive biopsies and negative LEEPs (11%). A negative LEEP following a positive biopsy is frequent (24%) and does not predict a different clinical follow-up than a positive biopsy and positive LEEP.

1264 Endocervical-Type Mucinous Borderline Tumors Are Related to Endometrioid Tumors Based on Mutation and Loss of Expression of ARID1A

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Background: Nongastrointestinal-type mucinous borderline tumors have been described as displaying endocervical and serous differentiation and hence have been termed "endocervical-type" mucinous borderline tumors, "mixed epithelial papillary cystadenoma of borderline malignancy of mullerian type" or "atypical proliferative seromucinous tumors". A striking feature of these tumors is their frequent association with endometriosis. This is an unusual finding as pure endocervical and serous tumors are not usually associated with endometriosis. *ARID1A* is a recently identified tumor suppressor, which frequently loses its expression and is mutated in endometrium-related carcinomas including ovarian clear cell, ovarian endometrioid and uterine endometrioid carcinomas. Although *ARID1A* mutations and expression have been studied in gynecological cancer, its expression pattern has not been investigated in ovarian atypical proliferative (borderline) tumors.

Design: In this study, we analyzed ARID1A expression in serous, gastrointestinal-type and endocervical-type (seromucinous) mucinous, and endometrioid atypical proliferative (borderline) tumors using immunohistochemistry and performed mutational analysis in selected cases.

Results: We observed loss of ARID1A staining in 8 (35%) of 23 seromucinous tumors. In contrast, ARID1A staining was retained in all the other 32 tumors except in one endometrioid tumor ($p<0.01$). Mutational analysis was performed on two representative seromucinous tumors, which showed complete loss of ARID1A. Both tumors harbored somatic inactivating *ARID1A* mutations. Previous studies have reported loss of expression and/or mutation of ARID1A in 30-57% of endometrioid and clear cell carcinomas but only rarely in serous tumors.

Conclusions: We showed a significantly higher frequency of loss of ARID1A expression in endocervical-type (seromucinous) tumors, presumably due to mutation, than the other histologic types, suggesting that they are molecularly related to endometrioid and clear cell tumors. If other investigators confirm our findings, we propose designating them "atypical proliferative (borderline) papillary endometrioid tumors of mixed cell type" based on their morphologic and molecular genetic features.

ARID1A immunoreactivity in ovarian atypical proliferative (borderline) tumors.

	serous	endometrioid	gastrointestinal type mucinous	seromucinous
cases with loss of ARID1A staining	0	1	0	8
total case number	13	8	12	23
% of cases with loss of staining	0	13	0	35

1265 Increased Expression of Hypoxia-Inducible Factor-1 Alpha in the Late Third Trimester Human Placenta from Patients with Chronic Hypertension

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Background: Hypoxia-inducible factor 1(HIF-1) is a critical transcription factor involved in oxygen homeostasis that regulates adaptive responses to hypoxia, including angiogenesis. In a normal pregnancy, there is extensive angiogenesis and vascularization of placenta. The expression of alpha submit of HIF-1 (HIF-1α) is increased under physiological hypoxia in early placentas and it is abnormally elevated in pre-eclamptic placental tissue. Since both diabetes and hypertension are known risk factors for preeclampsia, we studied the protein expression of HIF-1α in these conditions and evaluate its use as a marker of fetal hypoxia.

Design: Immunohistochemistry studies for HIF-1α stains were performed on late third trimester (≥ 35 weeks) placenta parenchyma. Consecutive cases of patients with hypertension and diabetes delivered at our institution from January to August 2010 were included, 10 age/race/parity comparable cases without significant medical history were selected as negative controls. Only nuclear staining of HIF-1α was considered positive, and Fisher's exact test was used to compare the percentage of positive cases between groups.

Results: 69 cases were included in our study, including 14 cases of diabetes, 45 cases of hypertension and 10 control cases. 20 patients presented with preeclampsia at the time of delivery. Nuclear expression of HIF-1α was seen in 34 cases (49%) of all 69 placentas, including 6 cases of diabetes (43%), 24 cases of hypertension (53%) and 4 cases of control patients (40%). 8 out of 20 cases with preeclampsia showed positive HIF-1α staining (40%). Upon further stratifying the hypertension patients into chronic hypertension group (15 cases) and pregnancy-induced hypertension group (30 cases), 11 cases of chronic hypertension placentas (73%) showed HIF-1α staining, while only 13 cases of pregnancy-induced hypertension placentas (43%) was positive; however, neither hypertension group had statistically higher HIF-1α staining than the control group ($p>0.05$). We did not observe any correlation between HIF-1α expression and microscopic vasculopathy.

Conclusions: There was increased HIF-1α protein expression in the placentas from patients with chronic hypertension, while its expression in pregnancy-induced hypertension and diabetes appeared to be comparable to that of placentas without significant medical history. Our data suggest there are different pathophysiological mechanisms between chronic hypertension and pregnancy-induced hypertension that affect late trimester placenta.

1266 Endometriosis: Is It Benign or Pre-Neoplastic? Analysis of Molecular Genetic Alterations in Ovarian Endometriosis

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Background: A number of studies have demonstrated that endometriosis is a risk factor for ovarian clear cell and endometrioid carcinomas, and postulated that benign endometriosis may progress to atypical endometriosis, and eventually to ovarian clear cell and endometrioid carcinomas. Atypical endometriosis has been thought to be a pre-neoplastic lesion for these malignancies based on the fact that multiple molecular genetic alterations that are present in these cancers have also been identified in adjacent atypical endometriosis, such as mutations of ARID1A (encoding BAF250a, a recently identified tumor suppressor for these cancers), upregulation of hepatocyte nuclear factor (HNF)-1b, and loss of hormonal receptors expression, etc. A naturally arising but important question is whether benign endometriosis also harbors these molecular genetic alterations or not.

Design: Formalin-fixed paraffin-embedded blocks of ovarian resection specimens were selected. Endometriosis (N=36) was chosen as well as clear cell carcinoma (CCC, N=26). Atypical endometriosis was present in 13 out of 26 cases of CCC. Normal endometrium (N=5) was also included as a control. Immunohistochemical staining for BAF250a (ARID1A), HNF-1b, ER and PR was performed.

Results: CCC had loss of BAF250a expression (57.7%), HNF-1b upregulation (92.3%) and loss of ER and PR expression (92.3% and 85.6% respectively). Similar immunostaining profiles were present in atypical endometriosis and even in a small portion of benign endometriosis, but not in normal endometrium (table 1).

Table 1 Immunoprofiles of BAF250a, HNF-1b, ER and PR in endometriosis and CCC

	BAF250a N(%)	HNF-1b N(%)	ER N(%)	PR N(%)
Normal endometrium (N=5)	5(100%)	0(0)	5(100%)	5(100%)
Benign endometriosis (N=36)	28 (77.8%)**	12 (33.3%)**	28 (77.8%)**	18 (50%)**
Atypical endometriosis (N=13)	5 (38.5%)*	7 (53.8%)	2 (15.4%)*	3 (23.1%)
CCC (N=26)	11 (42.3%)*	24 (92.3%)*	2 (7.7%)*	4 (15.4%)*

* $p<0.01$ vs benign endometriosis, ** $p<0.01$ vs normal endometrium (Fisher's exact test).

Conclusions: Our study demonstrated that a small portion of benign ovarian endometriosis has already accumulated multiple molecular genetic alterations, similar to that in atypical endometriosis and CCC, suggesting that benign endometriosis might be pre-neoplastic rather than benign. In addition, these biomarkers used in this study can potentially help us identify high-risk endometriosis and facilitate appropriate clinical intervention for these patients.

1267 BAF250a *(ARID1A)* Combined with HNF-1b, ER and P53 Can Distinguish between Ovarian Clear Cell Carcinoma and Papillary Serous Carcinoma

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Background: Ovarian epithelial carcinoma consists of a heterogeneous group of different types of carcinoma. Most studies have shown that clear cell carcinoma (CCC) and endometrioid adenocarcinoma might have similar genetic pathway and more commonly associated with endometriosis, while high grade papillary serous carcinoma (PSC) has a different tumorogenesis pathway with common P53 mutation. However, morphologically, high grade clear cell carcinoma and papillary serous carcinoma are not always readily distinguishable. Recent studies suggest that loss of expression of BAF250a, a tumor suppressor encoded by *ARID1A*, and up-regulation of hepatocyte nuclear factor (HNF)-1b were commonly present in CCC, but not PSC. In our study, we would like to show whether we could differentiate these 2 carcinomas by using a panel of immunohistochemical stains including BAF250a, HNF-1b, P53, estrogen receptor (ER) and progesterone receptor (PR).

Design: Formalin-fixed paraffin-embedded blocks of ovarian resection specimens were selected. Cases of CCC (n=26) and high grade PSC (n=24) were selected. Immunohistochemical staining for BAF250a, HNF-1b, P53, ER and PR was performed by our diagnostic lab.

Results: BAF250a, HNF-1b, P53 and ER all have different expression patterns between CCC and PSC ($P<0.01$), as shown in table below. Most CCCs are negative for BAF250a, ER, P53 and positive for HNF-1b, while PSC are positive for BAF250a, ER, P53 and negative for HNF-1b.

Table 1 Immunoprofiles of ovarian CCC versus PSC

	BAF250a	HNF-1b	ER	P53	PR
CCC (N=26)	42.3% (11/26)	92.3% (24/26)	7.7% (2/26)	7.7%(2/26)	15.4% (4/26)
PSC (N=24)	100% (24/24)*	4.2% (1/24)*	91.8% (22/24)*	62.5% (15/24)*	16.7% (4/24)

* $p<0.001$ by Fisher's exact test

Conclusions: Our data support that ARIDA and HNF-1b involves ovarian CCC carcinogenesis but not in PSC. PSC and CCC are two biologically different carcinoma types and can be readily differentiated by a panel of immunohistochemical stains of BAF250a, HNF-1b, P53 and ER in morphologically challenged cases.

1268 The Clinical Significance of *K-Ras* Mutation in Endometrial "Surface Epithelial Changes" and Their Associated Endometrial Adenocarcinoma

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Background: The entity of so-called 'surface epithelial changes' (SECs) was first described in 1995. Morphologically, SECs usually arise directly from malignant glands at

the most superficial aspect of endometrioid carcinomas (EC) and impart the appearance of a 'maturational' phenomenon at the surface of the cancer. SECs typically show bland mucinous, syncytial, squamoid, and papillary features that mimic a variety of benign entities, particularly endocervical microglandular hyperplasia. Studies have reported SECs to be associated with approximately half of ECs many of which showed focal mucinous differentiation. Therefore, despite their morphologically benign histology, some workers have questioned whether the presence of SECs represents a 'marker' for an underlying malignancy. Since the biologic nature of SECs is unknown, we aimed to study the prevalence of *K-ras* gene mutations in SECs and the underlying EC from which they directly arise.

Design: 14 cases with biopsy proven SECs and ECs in their subsequent hysterectomies were retrieved from our institutional archives. In 8 of 14 cases, only SECs were present in the endometrial biopsy but ECs were found in each uterine corpus after hysterectomy. 6 endometrial samplings showed concomitant SECs and ECs. All tumors associated with SECs were so-called 'type 1' ECs. Genomic DNA, derived from microdissected SECs and ECs to ensure >80% lesional cells, was extracted from formalin-fixed paraffin-embedded tissue sections. PCR amplification for *K-ras* codons 12 and 13 was performed with subsequent sequencing by capillary electrophoresis.

Results: *K-ras* codons 12 and 13 mutations were detected in 11 of 14 (78%) SECs and 11 of 14 (78%) ECs. 9 of 11 point mutations were identified at codon 12 with the most prevalent mutation (4 of 9) being G12D (codon 12**;** GGT > GAT**)**. Only 2 point mutations were seen at codon 13, both being G13D. All SECs had the same k-ras mutation as their underlying EC.

Conclusions: 78% of cases with SEC**s** had K-ras mutations, and in each case the mutation in the SECs was identical to that in the associated underlying ECs. These findings strongly suggest that SECs, despite their bland appearance and varied histology, are neoplastic surface manifestions of the underlying EC. Therefore, their presence in endometrial biopsies/curettages, particularly in postmenopausal women, should raise suspicion for an associated EC.

1269 Array CGH Analysis Reveals Amplification of Met and AKT2 in Clear Cell Carcinoma of the Ovary

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Background: Clear cell carcinoma of the ovary (OCCC) is a chemo-resistant tumor with relatively worse prognosis and is frequently associated with endometriosis. Frequent mutations of the ARID1A gene and activating mutations of the PI3CA gene are reported in OCCC, but positive array CGH results for specific gene amplification have rarely been reported.

Design: In this study, we performed an array CGH analysis using formalin-fixed, paraffin-embedded samples from 13 OCCC patients to comprehensively evaluate gene copy number changes in OCCC samples. We also performed Taqman gene copy number analysis, fluorescence in situ hybridization, immunoblotting, and immunohistochemistry to confirm the array CGH results.

Results: Array CGH analysis revealed that Met gene amplification was present in 4 / 13 OCCC patients and 2 / 8 OCCC cell lines. Amplification of the AKT2 gene, which is a component of one of the downstream signaling pathway of Met together with PI3CA, was also detected in 3 / 13 OCCC patients and 2 / 8 OCCC cell lines. Totally 73 OCCC cases were examined by Taqman PCR for Met amplification, and 37.0% revealed to have Met gene amplification (>4 copies). Furthermore, stage 1 and 2 patients with Met gene amplification had significantly worse overall survival than patients without Met gene amplification (p=0.037).

Conclusions: We demonstrated for the first time Met and AKT2 amplification by array CGH in approximately half of the OCCC cases. Met amplification was significantly related to worse prognosis. Activation of the Met/PI3CA/AKT pathway may be one of the most important molecular events in OCCC carcinogenesis.

1270 Co-Expression Patterns of HPV L1 and p16 in Anal Squamous Intraepithelial Neoplasia

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Background: HPV L1 produces capsid protein to protect the viral genome and confer contagiosity. It is mainly expressed in the episomal stage and its expression is suppressed after HPV integrated in host genome. HPV L1 expression is inversely correlated with the expression of E6 and E7 oncoproteins. Elevation of E7 and subsequent degradation of pRb in turn triggers p16 overexpression. AIN is a precursor of the anal squamous carcinoma linked to HPV infection. Histopathologically, it is often challenging to accurately grade AIN. To investigate the role of HPV L1 and p16 in anal dysplasia, we studied the immunohistochemical co-expression patterns of HPV L1 and p16 in anal condyloma, AIN1 and AIN2-3 lesions.

Design: Anal biopsies with condyloma and AIN were retrieved and histologically reviewed. Expression of nuclear HPV L1 was studied using combined immunohistochemistry and in situ hybridization technique from Advanced Medical. p16 immunohistochemistry was performed using Ventana antibody. Only diffuse, full-thickness nuclear and cytoplasmic p16 staining was considered as positive. Patch p16 cstaining was excluded in this study.

Results: Co-expression of HPV L1 and p16 was analyzed in 53 anal lesions, including 10 condylomas, 21 AIN1 and 22 AIN2-3. There were 18 cases immunoreactive to HPV L1 and 23 cases showing p16 positivity. The immunostaining patterns of both HPV L1 and p16 are tabulated below.

HPV L1	p16	Condyloma	AIN 1	AIN 2-3
positive	positive	0	0	1
positive	negative	7	10	0
negative	positive	0	1	21
negative	negative	3	10	0

Briefly, expression of HPV L1 and p16 diffuse staining pattern was mutually exclusive in all the cases except one, which showed focal high grade AIN2 in a background of extensive AIN1. Expression of HPV L1 but lack of p16 expression was seen in 70% condyloma and 47.6% of AIN1, but none of AIN2-3 lesions. In contrast, diffuse p16 expression with lack of HPV L1 staining was seen in all high grade AIN and only in 4.7% (1/21) of AIN2-3. None of the condyloma cases showed diffuse p16 staining. Lack of both HPV L1 and p16 expression was seen in 30% of condyloma and 47.6% of AIN1.

Conclusions: Our study indicates that the expression of HPV L1 and p16 is mutually exclusive in a majority of anal condyloma and AIN. Expression of HPV L1 represents a biological episomal stage and usually seen in low grade lesions and diffuse p16 expression represents an integrated stage of malignant transformation. Our data suggests that application of both HPV L1 and p16 biomarkers can not only facilitate accurate grading of AIN, but also add risk assessment value of the anal lesion.

1271 Clinical Outcome of Patients with Insufficient Sample on Endometrial Biopsy or Curettage

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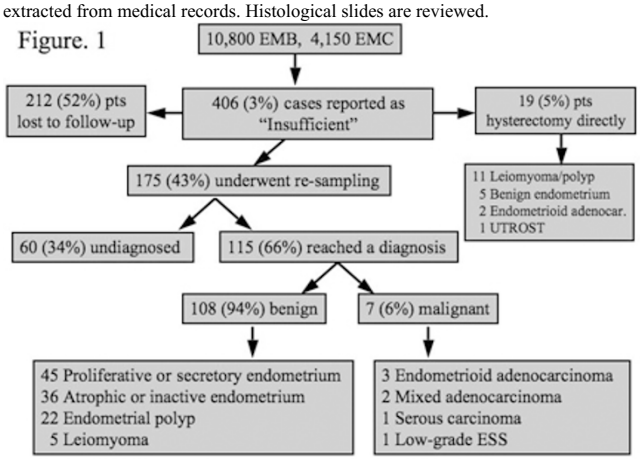
Background: Two commonly used procedures in the assessment of abnormal uterine bleeding are dilation & curettage and biopsy. As the latter gradually gains in popularity, pathologists face the increasing challenge of making a diagnosis on minute material. In certain instances, the sample is so minimal that pathologists report it as "insufficient for diagnosis". Such report triggers either repeat sampling, follow-up, or even, hysterectomy. Alarmingly, a considerable percentage of patients are lost to follow-up and remain undiagnosed. Therefore, accurate triage of these patients is critical and yet remains problematic. Here we reporte the clinical outcome of 406 such patients at our institute over the past six years.

Design: UMASS database (2005-2010) was searched. The patients' follow-up data extracted from medical records. Histological slides are reviewed.

Figure. 1



Results: Result details are showed in Figure 1. A total of 10,800 endometrial biopsies and 4150 curettages were received. "Insufficient" or "nondiagnostic" was reported in 406 cases (3%), including 321 biopsies and 85 curettages.

212 patients were lost to follow-up. The remaining 175 patients repeated sampling. 60 (34%) remained undiagnosed. The other 115 (66 %) acquired a diagnosis: 108 (94%) benign, seven (6%) malignancy. Ninteen patients underwent hysterectomy directly. The outcomes were sixteen benign, three malignancy. In total, ten patients (5%) were found malignancy with additional procedures.

Thirty-six cases reported "rare atypical cells" in additional to "insufficient tissue". Immunostaining (p53, Ki67) are performed but remain inconclusive. These resulted in 10 patients re-sampling; 12 hysterectomy; 12 lost to follow-up. The outcomes were seven (31%) benign, two (9%) atypical complex hyperplasia, one (4.5%) cervical adenocarcinoma, ten (45%) endometrioid adenocarcinomas, two (9%) serous carcinomas.

Conclusions: Our results outline the potential outcomes of patients with non-diagnostic endometrial sample. A small but significant portion of these patients has malignancy (5%). If rare atypical cells present, the chance of malignancy is significantly higher (69%). For the adequate management of these patients, it is critical to be aware of the risk and to clearly communicate the uncertainty in these cases.

1272 Pelvic High Grade Serous Carcinoma and Association with Serous Tubal Intraepithelial Carcinoma

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Background: Serous tubal intraepithelial carcinoma (STIC) is considered a precursor of pelvic high-grade serous carcinomas (PHGSC). However, data regarding true prevalence of STIC and specific association of STIC with PHGSC are limited. The aim of the study was to determine the frequency of STIC in women with HGSC at various sites from a large women's hospital and determine associated clinical-pathologic features.

Design: We searched our institutional pathology database from 2005 to 2010 to retrieve cases diagnosed as HGSC of tubal, ovarian, peritoneal and endometrial origins. The prevalence of STICs and their association with HGSCs in every specific group were analyzed. The data obtained before and after institution of SEE-FIM protocol (sectioning and extensively examining the fimbriated end of the fallopian tube) were also compared. The SEE-FIM protocol was instituted at our hospital in 2008. Tumors were classified

as ovarian, peritoneal, tubal or endometrial primary based on the conventional criteria.
Results: During the study period 472 cases of PHGSC were identified and classified as ovarian in origin in 225 cases (48%), tubal in 130 (27%), peritoneal in 17 (4%), and endometrial in 100 (21%) cases. There were only 11 (4%) cases of STIC reported in the 3-year period of 2005-2007 (i.e. prior to SEE-FIM protocol). In contrast, in the period of 2008-2010, the number of STIC cases was significantly increased to 43% (table 1). The frequency of STIC at each site ranged from 6% (endometrial) to 88% (ovarian) in the cases from 2008-2010. STIC was identified in 29% cases of ovarian HGSC.

Table 1 Frequency of STIC in HGSCs for each primary site

Years	Fallopian tube	Ovary	Peritoneum	Endometrium	Total
2005-2007	7/73 (10%)	1/121 (1%)	2/7 (29%)	1/52 (2%)	11/252 (4.0%)
2008-2010	50/57 (88%)	30/104 (29%)	5/10 (50%)	3/48 (6%)	93/219 (43%)

Conclusions: 1) Standard SEE-FIM protocol is necessary for accurate detection of STICs; 2) STIC is the precursor lesion of tubal HGSC; 3) The association of STIC with ovarian HGSC in the current study is lower (29% in the current study versus 40-60% in prior studies), suggesting that a significant proportion of ovarian HGSC lack a tubal precursor lesion. This could also be due to fused tubes and ovaries where it is impossible to do the SEE-FIM protocol and obviously STIC will not be determined 4) STIC is unrelated to endometrial HGSC.

1273 Papillary Mucinous Metaplasia as a Possible Precursor of Low-Grade Mucinous Adenocarcinoma of the Uterine Corpus

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Background: Mucinous adenocarcinoma is an uncommon type of malignant neoplasm of endometrium, which includes specific variants such as low-grade mucinous adenocarcinoma, and microglandular adenocarcinoma simulating microglandular endocervical hyperplasia. However, their precursor lesions have not yet been identified. During the review of endometrial lesions in postmenopausal women, we have noticed that endometrial mucinous metaplasias have variable degrees of proliferative change with or without intraglandular micropapillary architecture, some of which show histological similarity to the recently described low grade mucinous adenocarcinoma. The histological similarity raised the suspicion that papillary mucinous metaplasia could be a precancerous lesion of low-grade mucinous adenocarcinoma.
Design: We have searched 21 endometrial mucinous metaplasia from women in age of 50 or older during 3.5 year periods. In an effort to explain the pathogenetic significance of mucinous metaplasia without intraglandular papillary tufts (designated as simple muinous metaplasia, n=7) and with papillary tufts (designated as papillary mucinous metaplasia, n=14), we analyzed immunohistochemical expressions of estrogen (ER) and progesterone receptors (PR), Ki67, PTEN, β-catenin, P16, P53, and PAX2 using paraffin embedded tisssue sections. In addition, mutational analyses for KRAS and PTEN were performed in 7 simple mucinous metaplasia and 9 papillary mucinous hyperplasia using paraffin embedded tissue to explain the relationship with endometrial adenocarcinoma.
Results: Intraglandular papillary tufts in papillary mucinous metaplasia showed selectively decreased or lack of PAX 2 and PR expressions, and an increased expression of P16 compared to the surrounding glandular epithelium or to the simple mucinous metaplasia. PTEN expressions in glandular epithelium were decreased in both groups. Papillary mucinous metaplasia showed KRAS mutation of codons 12 or 13 in 89% compared to 14% in simple mucinous metaplasia. Ki-67 labeling index, ER, and β catenin expressions were not significantly changed in both groups.
Conclusions: Although we could not directly compare these results of mucinous metaplasia with those of mucinous adenocarcinomas, immunohistochemical expressions, mutational status, and histological similarity suggest that papillary mucinous metaplasia could be a precancerous lesion of endometrium, especially of mucinous adenocarcinoma.

1274 How Often Does Positive Peritoneal Wash Cytology Independently Upstage and Impact Clinical Management of Ovarian Malignancies?

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Background: Findings of diagnostic tumor cells in peritoneal wash cytology (PWC) specimens can result in upstaging of ovarian carcinomas (OvCa) to FIGO Stage IC or IIC. Positive PWC, however, is not the only parameter for designating an OvCa as Stage IC. Ovarian capsular rupture and/or surface involvement by the tumor can also result in Stage IC, irrespective of PWC status. Also, upstaging alone will not necessarily lead to altered clinical management according to National Comprehensive Cancer Network (NCCN) guidelines. The aim of this study was to document how often PWC alone caused upstaging and altered clinical management of OvCa at a large academic women's hospital.
Design: We searched our institutional database for cases of positive PWC in primary OvCa with comprehensive staging over a 5-year period from July 2006 to July 2011. Cases with incomplete staging and questionable synchronous ovarian and endometrial carcinomas were excluded. Stage, histologic tumor type, ovarian capsule status, surface tumor involvement, and character of extra-ovarian implants were documented and analyzed.
Results: A total of 215 qualified cases were identified, including 25 cases of stage IC (11.62%), 7 of IIC (3.26%), and 183 of III and IV (85.12%) OvCa. Of 25 IC cases, 21 had either a ruptured capsule or surface involvement in one or both ovaries, only 4 (16% of Stage IC cases, 1.86% of all cases) were upstaged solely based on a positive PWC. Histopathologic subtypes of the 4 cases were: clear cell carcinoma, mixed epithelial carcinoma (clear cell, high grade serous, and endometrioid carcinoma), serous borderline tumor, and mucinous adenocarcinoma. Based on current NCCN practice guidelines, grade 3 OvCa will undergo chemotherapy even if Stage IA or IB; borderline tumors regardless of stage will be observed if no invasive implants are found; and all stage 2 OvCa will be treated similarly regardless of substaging. Therefore, positive PWC in 10 of the 11 upstaged cases did not alter the immediate management based on NCCN guidelines. The only case where positive PWC findings resulted in both upstaging and alteration of management was a mucinous OvCa treated with chemotherapy, a tumor that otherwise would have been Stage IA and observed.
Conclusions: Positive PWC very infrequently provides additional information impacting staging of comprehensively staged OvCa tumors, when all relevant features impacting staging were considered, particularly capsular integrity and ovarian surface tumor involvement. Furthermore, positive PWC by itself only rarely altered NCCN guideline-designated treatment.

1275 Survivin Expression in Cervix Carcinoma Correlates with Residual Disease after Neoadjuvant Radio-Chemotherapy

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Background: Radio-chemotherapy followed by surgery represents a therapeutic option for advanced cervical cancer. The identification of potential markers of response is crucial for planning the treatment. Survivin is a family member of the inhibitor of apoptosis (IAP) which is the primary mode of cell death induction by several classes of anticancer agents and ionizing radiation. The relationship between survivin immunohistochemical expression on pre-treatment biopsy and pathological assessed response to radio-chemotherapy on surgical specimens is investigated.
Design: The study included 52 primary uterine cervix cancer patients treated with radio-chemotherapy followed by surgey. Immunohistochemistry was performed on the pre-treatment biopsy using the polyclonal rabbit anti-survivin antibody. Results (Hscore) were evaluated according to Pallares 2005 [1].
Histological response to therapy was classified on the hysterectomy specimen as follows [2]: pR0: Pathological Complete Response, pR1:Pathological Partial Response, pR2: Pathological No Response.
After the examination of all surgical specimens, all patients were restaged according to TNM and FIGO: FIGO 0 were considered as "No Residual Disease" (NRD); FIGO stages I-II were considered as "Local Residual Disease" (LRD); FIGO stages III-IV were considered as "Metastatic Disease" (MD).
Results: The histological examination of the completely sampled cervix disclosed 25 pR0 cases (48,07%), 17 pR1 cases (32,69%) and 10 pR2 cases (19,23%). pR2 patients showed a significant higher Hscore compared to both pR0 (135,2±76,38 Vs 200±58,3; p=0,021) and pR1 (124,11±63,84 Vs 200±58,3; p=0,011). No statistically significant differences were observed between pR0 and pR1. After the examination of all the surgical specimens, 24 patients (46,16%) were staged as NRD, 23 (44,23%) as LRD (44,23%) and 5 (9,61%) as MD. MD patients showed a significant higher Hscore compared to both NRD (134,58±77,96 Vs 228±48,68; p=0,01) and LRD (130,11±60,23 Vs 228±48,68; p=0,004). No statistically significant differences between NRD and LRD were observed.
Conclusions: Patients with higher survivin expression show a worse local and systemic response than patients with lower expression. These findings confirm the role of survivin in cancer cell resistance to therapy.
[1] Pallares J et al. Int J Gynecol Pathol. 2005 Jul;24(3):247-53.
[2] Zannoni GF et al. Int J Gynecol Pathol. 2008 Apr;27(2):274-81.

1276 Sex-Determining Region Y-Box 2 (SOX2) Expression Predicts Poor Prognosis in Human Ovarian Carcinoma

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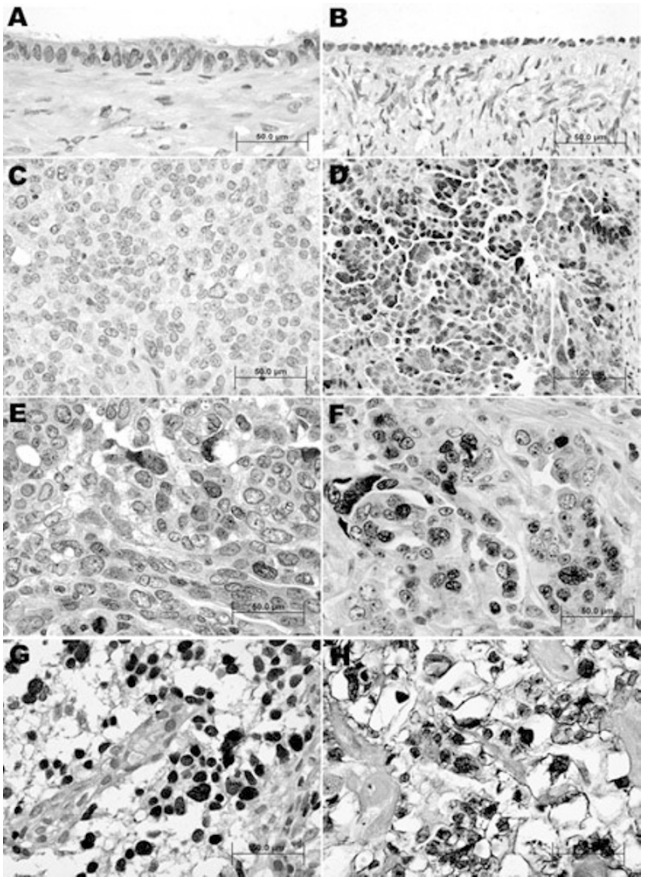
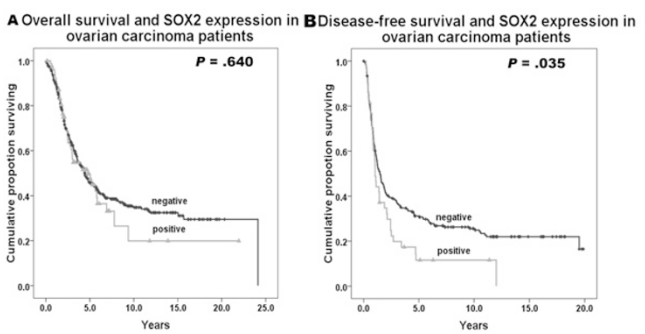
Background: Sex-determining region Y-box 2 (SOX2) is proposed to be a key transcription factor in embryonic stem cells. The known roles of SOX2 in development and cell differentiation suggest that it is relevant to the aberrant growth of tumor cells. Thus, SOX2 may play an important role in tumor progression. However, its clinical significance in human ovarian carcinoma has been uncertain until recently. The aim of the present study was to clarify the clinical role of SOX2 expression in ovarian carcinoma.
Design: Immunohistochemical staining of 540 human ovarian carcinoma samples for SOX2 was performed using tissue microarray. The associations among SOX2 expression and clinical factors (diagnosis, tumor grade, International Federation of Gynecology and Obstetrics stage, and response to chemotherapy), overall survival, and disease-free survival were analyzed.
Results: We observed SOX2 expression in 15% of the ovarian carcinoma samples. Use of the Fisher exact test and one-way analysis of variance suggested that SOX2 expression was associated with high-grade carcinoma ($P = 0.009$), especially high-grade serous carcinoma ($P = 0.048$); International Federation of Gynecology and Obstetrics stage (II-IV, $P = 0.005$); and malignant mixed müllerian tumors ($P = 0.048$). SOX2 expression was also associated with decreased disease-free survival durations ($P = 0.035$; log-rank test).
Conclusions: Our results showed that SOX2 expression is closely related to poor clinical outcome in patients with ovarian cancer. It may be a potential marker of ovarian cancer stem cells related to tumor recurrence, similar to its role in regarding embryonic stem cells.





1277 Liquid-Based Cytology and High Risk HPV Screening Test Histories Preceding 2827 Histopathologic Cervical Intraepithelial Neoplasia 2/3 Diagnoses

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Background: Cervical screening in the US increasingly involves newer FDA-approved cytologic methods and adjunctive HPV testing. Since the primary goal of cervical screening is to prevent cervical cancer by identifying and treating precancerous cervical lesions, we decided to carry out a large scale audit of screening test histories for women with established histopathologic CIN2/3 diagnoses.

Design: A computer-based search of our CoPath database was conducted over a study period of 65 months between July 2005 and November 2010 to identify patients with histopathologic CIN2/3 diagnoses. Preceding screening test histories for women screened with HC2 HPV testing and/or computer-imaged LBC ThinPrep Pap Tests were analyzed.

Results: 2827 patients with histopathologic CIN2/3 diagnoses were identified. The average patient age was 30.7 with a range of 16 to 91 years. Abnormal Pap tests ≤4 months before histopathologic CIN2/3 diagnoses were identified in 2074 of 2827 patients (Table 1). HSIL was the most common abnormal result (41.6%). Additional Pap test results, ≤ 3 years before histopathologic CIN2/3 diagnoses, were identified in 1488 patients (Table 2). Prior HPV results ≤4 months before CIN2/3 diagnoses were identified in 807 patients, with 97.4% hrHPV-positive. Additional HPV results, ≤ 3 years before CIN2/3 diagnoses, were identified in 454 patients. 83% patients had at least one positive HPV results, but 24.2% had at least one negative HPV result.

Abnormal Pap Test Results Proximate to Histopathologic CIN2/3 Diagnoses

Abnormal Pap	Case No	%
HSIL	862	41.6
LSIL	464	22.4
ASC-US	445	21.5
ASC-H	288	13.9
AGC/AIS	15	0.7
Total	2074	100

Prior 3 year-Pap Test History

Pap Results	# Patients	%
At least one abnormal smear	978	65.7
At least one normal smear	911	61.2
Both normal and abnormal smears	401	26.9
Total # patients	1488	100

Conclusions: 2074 of 2827 patients with histopathologic CIN2/3 had recent abnormal Pap results with HSIL in 41.6%. 97.4% of 807 patients with CIN2/3 diagnoses and recent hrHPV results tested hrHPV-positive, most often after reflex testing and ASC-US Paps. Among patients with additional screening tests ≤3 years before CIN2/3 diagnoses, a significant percentage of patients had negative Pap or negative HPV test results, consistent with rapid onset of at least some CIN2/3 lesions, inadequate sampling, or sensitivity issue of the tests. Our results support the value of co-testing in detection of small or inaccessible CIN2/3 lesions.

1278 Overexpression of Enhancer of Zeste Homolog 2(EZH2) and Focal Adhesion Kinase (FAK) – In High Grade Endometrium Carcinoma

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Background: It has been reported that the deregulation of E-cadherin is associated with Src/FAK signaling axis and histone deacetylase (HDAC)/EZH2 activity. However, the association between EZH2 and FAK and the clinical significance of such an association in endometrial carcinoma has not been reported.

Design: 202 archived cases of endometrial carcinoma (1996-2000) were reviewed and divided into subtype (Type I and Type II). TMAs were developed as per established procedures. EZH2, FAK, pFAK immnuohistochemical stain were performed and expression was scored as negative (0), low (1) and high (2).

Results: A total 141 cases (69.8%) Type I tumors and 61 cases (30.2%) type II tumors were identified. The expression of EZH2 and FAK was detected in 68. 8% and 46.1% of tumors, respectively, while pFAK was expressed in 64.7% of tumors. EZH2 overexpression was identified in 7.56% of type I tumors *vs.* 62.96% of type II tumor ($p<0.001$). FAK and pFAK overexpression was only seen in 24.79 % and 1.68% of Type I tumors as compared to 72 % and 58.82% of type II tumors respectively ($p<0.001$). There was a positive correlation between the expression of EZH2, FAK and pFAK ($p<0.0001$ for all paired association). The overexpression of EZH2, FAK, and pFAK were significantly associated with high histologic grade, angiolymphatic invasion, lymph node metastasis, myometrium invasion and cervical involvement (all p values <0.01). Kaplan-Meier curves showed that overexpression of EZH2 (Log-rank test, p=0.0024), FAK and pFAK (p=0.0001) were significantly associated with decreased overall survival. On multivariate analysis including the overexpression of EZH2, FAK and pFAK, tumor stage, tumor type, vascular invasion and tumor grade, only high tumor grade persisted as an independent prognostic factor for poor overall survival.

The Expression of EZH2, FAK and pFAK in Endometrial Carcinoma

		Type I (%)			Type II (%)		p-value
	Negative	Low	High	Negative	Low	High	
EZH2	49(28.3)	61(35.3)	9(5.2)	5(2.9)	15(8.7)	34(19.7)	<0.001
FAK	88(52.7)	29(17.4)	0(0)	2(1.2)	12(7.2)	36(21.6)	<0.001
pFAK	54(31.8)	63(37.1)	2(1.2)	6(3.5)	15(8.8)	30(17.6)	<0.001

Conclusions: Our study thus suggests that overexpression of EZH2, FAK and pFAK correlate with several well established pathologic risk factors and might predict a more aggressive biologic behavior in endometrial carcinoma making them potential therapeutic targets for treatment of endometrial cancer.

Head & Neck

1279 Primary Signet-Ring Cell (Mucin-Producing) Adenocarcinoma of Minor Salivary Glands: A Clinicopathologic, Immunohistochemical and Molecular Survey

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Background: Primary salivary signet-ring cell (mucin-producing) adenocarcinomas (SRCA) are extremely rare and poorly understood. We evaluate the clinicopathologic features of 4 cases and evaluate immunohistochemical and molecular features modeled after common profiles in mucinous/signet ring adenocarcinomas of other sites.

Design: Four cases were retrieved. Histochemical and IHC staining using standard technique was performed and included the following: mucicarime, PASD, AE1/3, CK7, CK20, CK5/6, CAM 5.2, CDX2, ER, AR, PSA, TTF-1, thyroglobulin, mammaglobin, HER2/neu, synaptophysin, chromogranin, actin, AMA, p63, calponin, PsAP, s100, GCDFP, Ki-67 and E-Cadherin. Additionally, fluorescence in situ hybridization for *ALK* gene rearrangements using a break apart probe 2p23 was performed (Abbott Molecular, Des Plaines, IL). Cases with more than 20% of tumor cells showing a rearrangement were considered positive.

Results: The male:female ratio was 3:1. The mean age was 56 (range: 18-81). Sites involved were buccal mucosa (2), soft palate (1), and deep parotid (1). Perineural and angiolymphatic invasions were present in (3) and (2) cases respectively. The patient