Design: From January 2011 to August 2013, 162 patients underwent robotic laparoscopic radical prostatectomy for clinically localized prostatic carcinoma at our institution. Periprostatic fat pads, yielded during defatting of the prostate, were dissected and sent to pathology for histopathologic examination in 133 cases. Clinical and pathological staging was recorded according to the 2009 American Joint Committee on Cancer (AJCC) criterion.

Results: Of 133 patients whose periprostatic fat was examined, 32 (24%) patients had lymph nodes in the periprostatic fat pads. Metastatic prostatic carcinoma to periprostatic lymph nodes was detected in 5 individuals (3.8%). All 32 patients had bilateral pelvic lymphadenectomy. 3 of the 5 patients with positive periprostatic lymph nodes had no metastasis in pelvic lymph nodes, thereby upstaging 3 cases from T3N0 to T3N1. No relationship exists between the presences of periprostatic LNs and prostate weight, patient age, pathological staging or Gleason score. When compared to cases with tumor free periprostatic LNs, the tumor in cases with metastatic periprostatic LNs has following features: 1) higher postoperative Gleason score (P=0.01); 2) higher pathological staging (p=0.001); 3) higher rate of seminal vesicle invasion (p=0.01); 4) higher rate of extracapsular invasion (p=0.00001); higher rate of positive surgical resection margin (P=0.001).

		Pt with tumor free LNs in periprostatic fat	Pt with metastatic LNs in periprostatic fat	P value
Number of Patient		27	5	
Age				0.79
	mean	60	59.2	
	range	49-75	50-62	
prostate weight				0.33
	mean	45.1	55.6	
	range	23-123	40-75	
postoperative Gleason score				0.01
	3+3	7	0	
	3+4	12	0	
	4+3	4	4	
	>4+3	4	1	
Seminal vesicle invasion (+)		6	4	0.01
Extracapsular invasion (+)		2	5	0.00001
Surgical resection margin (+)		7	5	0.001
Pathology stage				0.001
	pT2	22	0	
	pT3a	2	1	
	pT3b	3	4	

Conclusions: Despite the relative low incidence of positive periprostatic lymph node, routine pathological work up of periprostatic fat pads should be performed to guarantee adequate lymph node staging.

1127 Immunoexpression of Napsin A in Renal Neoplasms

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Background: Immunohistochemistry (IHC) for Napsin A has been widely used to support a diagnosis of lung adenocarcinoma with high sensitivity (93.9%). Previous reports have stated that Napsin A is also highly specific for lung adenocarcinoma (up to 94.7%). However, a recent report demonstrated that Napsin A is expressed in papillary renal cell carcinoma (RCC). In this study, we evaluated the immunoexpression of Napsin A in renal neoplasms.

Design: Duplicate tissue microarrays (TMA) of 159 surgically excised renal neoplasms of various types were constructed. IHC for Napsin A was performed on TMAs with appropriate positive and negative controls. Tumors with more than 10% cells staining positive for Napsin A were considered as immunoreactive.

Results: See Table 1.

Table 1. Expression of Napsin A in renal neoplasms.

Neoplasm	No.	Napsin A (%)
Acquired cystic disease associated RCC	2	100.0
Chromophobe RCC	45	11.1
Clear cell RCC	23	43.5
Clear cell papillary RCC	19	47.4
Metanephric adenoma	3	100.0
Mucinous tubular and spindle cell carcinoma	1	0.0
Oncocytoma	23	56.5
Papillary RCC	37	83.8
TFE/MITF RCC	1	0.0
Urothelial carcinoma	6	0.0

Conclusions: Napsin A is expressed in various types of renal neoplasms with sensitivity up to 100.0%. It is, sometimes, difficult to distinguish oncocytoma from chromophobe RCC on fine needle aspiration and/or needle core biopsy of a renal tumor. IHC for Napsin A may be useful in distinguishing oncocytoma from chromophobe RCC with positive labeling favoring oncocytoma. Based on our results, Napsin A is not specific for lung adenocarcinoma. When an metastatic carcinoma of unknown primary is positive for Napsin A, the differential diagnosis should include tumors of both renal and lung origin.

Gynecologic and Obstetric Pathology

1128 Recurrent Grade I, Stage Ia Endometrioid Carcinomas of the Uterus: Analysis of Pathology and Correlation with Clinical Data

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Background: Low-grade and low-stage endometrioid carcinoma of the uterus is thought to have an excellent prognosis in the vast majority of patients. However, despite the lack of myometrial invasion or superficial invasion, some patients develop recurrent disease, often in the vaginal apex. There is limited literature on these tumors, but recent analyses have implicated the pattern of myoinvasion as a prognostic factor. The emphasis of the current study is non-invasive or stage Ia, grade I endometrioid adenocarcinomas.

Design: The pathology data base at Virginia Mason Medical Center (VMMC) was searched for cases of grade I endometrioid carcinoma from 1998 to 2012, along with prior biopsy diagnosis. Cases with concurrent ovarian and carcinoma were excluded as were cases with 50% or > myometrial invasion. Pathological and clinical data was collected. Histological evaluation was performed, when possible, on either the original biopsy, hysterectomy and/or follow-up biopsies.

Results: At VMMC, there were 243 cases of grade I stage Ia endometrioid adenocarcinoma. Of these, 85 had lymph node dissections, 37 had omental and/or peritoneal biopsies. Peritoneal washes were performed on all patients and 12 were positive for tumor. Angiolymphatic invasion was present in 9 patients, lower uterine segment involvement was present in 5 (3 -epithelial only, and 2 - invasive) and endocervical gland involvement in 2. From these, only 9 patients experienced recurrent disease (4%), the majority in the vaginal cuff (67%). Non-myoinvasive tumors accounted for 5 cases and 4 patients had superficial (<50%) invasion. Angiolymphatic invasion was present in 2 patients, lower uterine segment invasion in one and one patient had a positive peritoneal wash. None of the patients had post-hysterectomy treatment. After recurrence, 7 patients had pelvic and vaginal radiation therapy, 2 chemotherapy (1 with radiation); 1 has not received therapy yet. Average follow-up time was 20 months (39 to 8 months), 2 were lost to followup. Six patients had NED, 1 died with disease.

Conclusions: The majority of patients with grade I and stage Ia endometrial adenocarcinoma have an excellent outcome with no treatment. Rare cases have recurrence of disease. Treatment after recurrence in our study resulted in good clinical outcomes for patients where long term followup was available. Further analysis is need in order to determine why some patients recur after surgery while the majority do not.

1129 Uterine Serous and FIGO 3 Endometrioid Carcinomas: A Multi-Institutional Comparative Study of 335 Cases

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Background: Although uterine serous carcinoma (USC) and FIGO grade 3 endometrioid carcinoma(E3C) constitute a minority of all endometrial carcinomas, they account for a disproportionate number of deaths. The study aimed to compare clinicopathologic features and outcome of USC & E3C.

Design: 335 endometrial carcinomas were identified from 4 institutions. Only E3C and USC reviewed and agreed on by 2 GYN pathologists(average of 4 slides/case) using WHO criteria were included in the study. 99 E3C and 167 USC were included in the final cohort. Clinicopathologic variables were analyzed, including patient demographics, tumor type, myometrial invasion, lymphovascular invasion(LVI), cervical involvement(CI), lymph node(LN) status, FIGO stage, recurrence & overall survival.

Results: Median age of patients with E3C and USC was 61 & 69 yrs, respectively. Outer half myometrial invasion was identified more often in E3C compared to USC(p=.001). Absence of myometrial invasion was seen more frequently in USC(p=.002). CI(p=.015), metastases to fallopian tubes(p=.001) & recurrence(p=.001) were seen more frequently in USC. Although there was no significant difference in LVI,a higher number of positive LNs was identified in E3C(p=.001). Omental involvement was seen more in USC than E3C(p=.05). Early FIGO stage(I-II) was seen more frequently in E3C, whereas more cases of advanced stage (stage IV) were seen in USC(p=.009).There was no statistical difference in overall survival between the 2 cohorts matched by stage(p=.264)

Clinicopathologic features

	E3C,n=99(%)	USC,n=167(%)	p value
Median Age	61y	69y	
Race*			.121
AA	18(42.9)	64(55.7)	
Other	24(57.1)	51(44.5)	
Myometrial Invasion			.002
Yes	90(90.9)	126(75.4)	
No	9(9.1)	41(24.6)	
Depth of Invasion*			.001
None	0(0)	41(24.6)	
Outer half	40(44.4)	76(45.5)	
Inner half	50(55.6)	50(29.9)	
Cervical Involvement*			.015
Yes	24(24.5)	66(39.5)	
No	74(75.5)	101(60.5)	
LVI			.151
Yes	67(67.7)	97(58.1)	
No	32(32.3)	70(41.9)	
Total Positive LN*	87(98.9)	106(63.9)	.001
Mets to ovaries*			.096
Yes	8(17.4)	50(29.9)	
No	38(82.6)	117(70.1)	
Mets to fallopian tubes			.001
Yes	0(0)	28(16.8)	
No	99(100)	139(83.2)	
Recurrence*			.01
Yes	21(21.2)	40(44.4)	
No	78(78)	50(55.6)	
Omental Involvement			.05
Yes	3(6.7)	21(19.6)	
No	42(93.3)	86(80.4)	
FIGO Stage			.009
I&II	69(69.7)	88(52.7)	
III	24(24.2)	51(30.5)	
IV	6(6.1)	28(16.8)	

*Complete follow up data was not available AA, African American; LN,Lymphnode; LVI,lymphovascular invasion

Conclusions: USC and E3C present various clinical and morphologic differences. However stage-by-stage both tumors have an adverse outcome.

1130 Immunohistochemical Analysis of Stathmin Expression in Uterine Smooth Muscle Tumors: Potential Utility in Distinguishing Leiomyosarcomas from Leiomyomas

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Background: The oncogenic phosphatidylinositol 3-kinase (PIK3CA) pathway is known to be highly dysregulated in uterine leiomyosarcomas. The oncoprotein stathmin (STMN1) is a microtubule destabilizer, promoting cell proliferation, migration and metastases. Experimental studies have identified STMN1 as a robust biomarker of PIK3CA pathway activation. Accordingly, we hypothesized that STMN1 is frequently expressed in uterine leiomyosarcoma, and that STMN1 immunohistochemistry (IHC) may have diagnostic utility in classifying smooth muscle tumors regarding their malignant potential.

Design: STMN1 IHC was performed on sections from 97 mesenchymal tumors involving the uterus, and results were scored on a 0-12+ scale based on staining intensity (1-3+) multiplied by staining extent (0-4+). The tumors included 32 spindle cell leiomyosarcomas, 30 conventional leiomyomas, 19 "unusual" smooth muscle tumors (see results), and 13 miscellaneous non-smooth muscle sarcomas involving the uterus. Cases with scores >1+ were classified as STMN1-positive.

Results: 100% of the leiomyosarcomas were STMN1-positive, as compared with 37% (11/30) of the leiomyomas ($p<0.0001$). The average STMN1 score was significantly higher in leiomyosarcomas (8.7) than in leiomyomas (1.6, $p<0.0001$), consistent with the morphologic finding that STMN1 expression tended to be strong and diffuse in leiomyosarcomas but when present, was weak in leiomyomas. Among the 19 "unusual" smooth muscle neoplasms, 4 cases classifed as STUMP, 5 atypical (symplastic) leiomyomas, and 7 highly cellular leiomyomas were all STMN1-negative. However, 2 of 2 mitotically active leiomyomas (average MI: 9 MF/10 HPF) were STMN1 positive (average score 12), as were 2 of 3 benign metastasizing leiomyomas (average score 2.5), and a cotyledonoid dissecting leiomyoma (score 12). Amongst smooth muscle neoplasms, STMN1 had a sensitivity for leiomyosarcoma of 100% (95% CI 87-100), a specificity of 69% (55-81), a positive predictive value of 67% (51-79), a negative predictive value of 100% (87-100), and an odds ratio of 144 (8-2493, p=0.0006). All non-smooth muscle sarcomas were also STMN1-positive (average score 7.1).

Conclusions: STMN1 is a highly sensitive marker for leiomyosarcoma and has a high negative predictive value. Lack of STMN1 expression in a putative case of leiomyosarcoma argues strongly against the possibility. Positive expression, however, is not sufficiently specific to be used in isolation to support this diagnosis.

1131 Impact of Histological Subtype in Early Stage Cervical Cancer: A Multi-Instituitional Study

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Background: Cervical adenocarcinomas(ADC) are viewed as more aggressive than cervical squamous cell carcinomas(SCC). This study aimed to determine the impact of SCC vs. ADC in early stage(ES) cervical cancer.

Design: 283 patients(pts) diagnosed with ES (IA1-IB2) cervical ADC(132) & SCC(151) during 2000-2010 from 2 inner city hospitals (Mexico and Detroit) was included.Age,stage,tumor size,depth of invasion,lymphovascular invasion (LVI),lymph node status (LNS),recurrence rate(RR) & overall survival(OS) were analyzed.GYN pathologists at the respective hospitals reviewed the slides.

Results: The Mexican cohort (165) had 101ADC and 64SCC while the Detroit cohort (118) had 31ADC and 87SCC.Median f/u was 4 & 5 yrs respectively.In the Mexican cohort,ADC had higher grade than SCC(p=0.01) while SCC had larger tumors with deeper invasion (p<0.001).RR [ADC(5%, 5/101), SCC (8%,5/64)], LVI and LNS were not statistically different.OS was comparable in ADC and SCC (98.7% and 95.2%).ADC received more radiation therapy(RT) than SCC (71.3%vs.37.5%).In comparison,ADC and SCC in the Detroit cohort showed no significant difference in grade, tumor size, depth of invasion, LVI and LNS.RR [ADC (16%, 5/31), SCC (9 %, 8/87), p=0.7] and OS(87% and 86%)were not statistically different.52% with SCC and 42% with ADC received RT (p=0.16).

Table1: Clinicopathological parameters of cervical ADC and SCC

		Mexican Hospital			Detroit Hospitals		
		ADC,n= 101(%)	SCC,n= 64(%)	p-value	ADC,n= 31(%)	SCC,n= 87(%)	p-value
Median age(range)		51(35-80)	51(22-86)	NS	44(30-65)	46(27-90)	NS
Stage	IA1	3(3)	1(1.6)	NS	0(0)	8(9.1)	NS
	IA2	1(1)	0(0)		3(9.6)	5(5.7)	
	IB1	96(95)	54(84.4)		26(83.9)	58(66.7)	
	IB2	1(1)	9(14)		2(6.5)	16(18.5)	
LNS	Positive	16(15.8)	13(20.3)	NS	4(12.9)	22(25.3)	NS
	Negative	85(84.2)	51(79.7)		23(74.2)	43(49.4)	
	Unknown	0	0		4(12.9)	22(25.3)	
Grade	1	19(18.8)	14(21.9)	p=0.01	6(19.4)	8(9.2)	NS
	2	50(49.5)	45(70.3)		13(41.9)	48(55.3)	
	3	32(31.7)	5(7.8)		8(25.8)	21(24.1)	
	not recorded				4(12.9)	10(11.4)	
LVI	Positive	26(25.7)	17(26.5)	NS	10(32.3)	32(36.8)	NS
	Negative	75(74.3)	25(39.1)		15(48.4)	37(42.5)	
	Unknown	0(0)	22(34.4)		6(19.3)	18(20.7)	
Tumor Largest Diameter(Mean cm)		2.0(1.8-2.4)	2.8(2.5-3.03)	p<0.0001	2.5(0-8)	2.6(0-13)	NS
Invasion Depth (Mean cm)		0.8(0.7-0.86)	1.6(1.3-1.6)	p<0.0001	0.7(0-2.5)	0.5(0-2)	NS

Conclusions: Prior studies suggested poor outcomes for ADC when compared to SCC.However, this study reveals comparable RR and OS in early stages of ADC and SCC in two different geographic and ethnic cohorts.The paradigm of more aggressive therapy for early stage cervical ADC warrants further investigation to potentially reduce morbidity in these pts.

1132 Characterization of Immune Cell Infiltrate in Different Subtypes of Ovarian Cancer

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Background: Recent investigations of tumor microenvironment have have shown that the immune contexture of the primary tumors has prognostic value and helps in predicting response to therapy in different types of cancer. The aim of this study is to provide a detailed evaluation of density and composition of immune infiltrate in specific histological subtypes of ovarian cancer, exploiting advanced digital image analysis systems.

Design: Ninety-seven ovarian cancer specimen including 51 high-grade serous carcinomas (HGSC), 27 endometrioid carcinomas (EC) and 19 clear cell carcinomas (CCC) were collected from the archive of the Department of Pathology, Spedali Civili di Brescia. CD3, CD163 and BDCA2 immunostained slides obtained from FFPE tumor tissue, recognizing respectively T-cells, macrophages and plasmacytoid dendritic cells (PDCs) were analyzed using a digital microscopy approach. The count of tumor-associated immune cells was performed processing 1 cm^2 tumoral area.

Results: Tumor-associated macrophages were the most represented immune cell type in all cancer subtypes (average/cm^2: $64.9x10^3$ in HGSC; $40.5x10^3$ in EC and $34.7x10^3$in CCC), followed by T-cells (average/cm^2 : $30.2x10^3$ in HGSC; $33.5x10^3$ in EC and $14.5x10^3$ in CCC) while PDCs were extremely rare in all subtypes (average/cm^2: 67 in HGSC; 30 in EC and 35 in CCC). The density of the total immune cells infiltrate ($CD3^+$+$CD163^+$+$BDCA2^+$cells) was highest in HGSC (average/cm^2: $95.2x10^3$), intermediate in EC (average/cm^2: $74.1x10^3$) and lowest in CCC (average/cm^2: $46.6x10^3$). The T-cells/macrophages ratio ($CD3^+$/$CD163^+$) was significantly lower in CCC (0.33) ($p<0.05$) compared to HGSC (0.55) and EC (0.54). However the variation of T-cells density was significantly proportional to the variation of macrophages density in all ovarian cancer subtypes ($p<0.05$). Conversely there was no correlation between T-cells and PDCs density ($p>0.05$) and between macrophages and PDCs density ($p>0.05$).

Conclusions: The digital microscopy approach proposed here might improve the evaluation of the cancer "immune contexture" by analyzing large tumor areas. The variability in the density of T-cells and macrophages found in the different cancer subtypes might indicate heterogeneity in chemokine production and suggest different levels of immune editing taking place at the primary tumor site.

1133 Different Immunohistochemical Patterns of p16 in Endometrial Stromal Tumors and Leiomyosarcoma

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Background: Distinguishing endometrial stromal tumors from leiomyosarcoma can be challenging due to similar morphologic features. Immunohistochemical studies are helpful but not specific in differentiating these two commonly seen myometrial neoplasms due to overlapping staining patterns. Our previous study and others show overexpression of p16 in leiomyosarcoma. To assess the utility in applying p16 immunostaining in distinguishing leiomyosarcoma from endometrial stromal tumors, we evaluated the expression pattern of p16 in a cohort of 30 cases of endometrial stromal tumor in comparison with that of 35 cases of leiomyosarcoma.

Design: Following approval from the Institutional Review Board, a database search was performed for endometrial stromal tumor (including stromal nodule and stromal sarcoma) and leiomyosarcoma. Clinical information and histopathologic features were reviewed and tabulated. Immunohistochemical staining for p16 was performed using Ventana MTM p16 kit. P16 immunostaining patterns were recorded as nuclear and/or cytoplasmic and were semiquantitatively evaluated as negative (<5% of tumor cells), focally positive (5-50%), or diffusely positive (51-100%). Intensity scores of 0 to 3 were assigned as follows: negative staining as 0, weak as 1, moderate as 2, and strong as 3. A mean intensity score was calculated as the sum of intensity scores divided by the total number of cases.

Results: Sixty-five total cases were applied to this study, including 30 cases of endometrial stromal tumors and 35 cases of leiomyosarcoma. Majority of endometrial stromal tumor cases (26/30; 86.7%) showed negative immunoreactivity to p16 antibody. Four cases (13.3%) of endometrial stromal tumor (1 stromal nodule and 3 stromal sarcomas) revealed focal (10-35% tumor cells) p16 nuclear staining. In contrast, all 35 cases (100%) of leiomyosarcomas stained diffusely positive for p16 with moderate to strong intensity. A calculated endometrial stromal tumor mean intensity score was 0.4 compared to leiomyosarcoma mean intensity score of 2.9.

Conclusions: The majority (86.7%) of endometrial stromal tumors, including stromal nodules and stromal sarcomas, lacks expression of p16 protein. A small percentage of endometrial stromal tumors shows focal p16 staining pattern, which is distinct from the diffuse and strong nuclear staining patterns seen in most leiomyosarcoma cases. Our study indicates that p16 immunostaining is a useful tool in differentiating endometrial stromal tumors from leiomyosarcoma.

1134 IDO1 Is Strongly Expressed in Placental Chronic Villitis

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Background: Chronic villitis (CV) is a common pattern of placental injury, and causes both intrauterine growth restriction and recurrent fetal death. The etiology of CV is poorly understood; however, it has been suggested that it represents a host-versus-graft reaction. Indoleamine 2,3- dioxygenase 1 (IDO1) metabolizes tryptophan to kynurenine, which inhibits T cell proliferation and, by extension, regulates self-tolerance. Placental IDO1 expression has been documented, including its critical role during implantation, but the role of IDO1 in CV has yet to be studied.

Design: IDO1 immunohistochemistry was performed, using a polyclonal anti-IDO1 rabbit antibody (Sigma HPA023072), diluted 1:2000, on formalin fixed, paraffin embedded tissue from 32 singleton placentas with CV retrieved from our departmental archives. Tissue sections were scored on the following parameters: 1) presence of CV on H and E stained slides, 2) the number of foci of CV present, 3) percentage of block involved by CV detectable by IDO1 staining, and 4 to 6) cellular localization of IDO1 staining in CV, endothelial cells, and villous trophoblasts.

Results: In 10 of 32 cases, CV could not be identified on additional sections. In 22 of 32 cases, foci of CV (from 1 to > 10) were identified, composing 1-30% of the entire tissue section. In 100% of the cases with identified CV, a strong, diffuse, cytoplasmic pattern of staining was identified within the focus/foci of CV. In contrast, this staining pattern was absent in 100% of cases where CV was no longer present. Background staining was characterized by weak to strong nuclear staining of endothelial cells in all cases, as well as weak-moderate staining of villous trophoblasts (in 86% of cases).

Conclusions: IDO1 is strongly, diffusely expressed in regions of CV, predominantly in the cytoplasm of mononuclear cells. This specific staining pattern contrasts with nuclear staining in unaffected regions, where the strongest expression is in fetal villous endothelium. While decreased IDO1 expression might have suggested a mechanism for CV based on failure of tolerance (i.e., rejection), other studies have suggested that IDO1 is inducible in a variety of inflammatory states. Therefore, increased IDO1 in CV is inconsistent with the host-versus-graft theory and raises the possibility of a more complex reactive process to a yet to be identified inciting event. Elevated IDO1 levels observed in CV also may have important implications for transport of the essential amino acid tryptophan through the placenta to the fetus.

1135 PAX-8 Staining in a Spectrum of Mesothelial Lesions, Including Well-Differentiated Papillary Mesothelioma and Malignant Mesothelioma

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Background: PAX-8 is a transcription factor important in organogenesis of the thyroid gland, kidney, and Mullerian system. Many gynecologic lesions label with PAX-8, including serous carcinoma and clear cell carcinoma, and benign proliferations like endometriosis. The differential diagnosis often includes mesothelial lesions, including malignant mesothelioma and a variety of benign mesothelial proliferations. Unfortunately, though PAX-8 reliably marks gynecologic cases positively, it has also been shown to label select mesothelial lesions, possibly resulting in confusing staining pattern.

Design: PAX-8 expression of was assessed in a range of mesothelial lesions including malignant mesotheliomas (MM), well-differentiated papillary mesotheliomas (WDPM), peritoneal inclusion cysts (PIC), adenomatoid tumors (AT), and mesothelial hyperplasia. Staining was performed with both a rabbit polyclonal PAX-8 (Proteintech Group, Chicago, IL) and a mouse monoclonal antibody (clone: BC12, Biocare Medical, Concord, CA) when feasible. Calretinin (rabbit polyclonal, Cell Marque, Rocklin, CA) immunostaining was also performed on all cases. All stains were performed on fully automated immunostainers. Slides were evaluated by two pathologists.

Results: All MM and AT were negative for PAX-8, while most borderline lesions (WDPM) were positive. The large majority of benign benign lesions were also negative with three cases showing positive staining of mesothelial cells (See table 1). All cases were positive for calretinin.

PAX-8 staining in mesothelial proliferations (n=112+)

	Number of cases PAX-8+
WDPM (N=13)	9
Adenomatoid tumor (N=10)	0
Peritoneal inclusion cyst (N=5)	1
Reactive mesothelium (N=51)	2
Malignant mesothelioma (n=33)	0

Conclusions: All MM were negative for PAX-8, as were a majority of benign lesions. In WDPM, polyclonal PAX-8 showed more diffuse staining in a majority of cases compared to the monoclonal antibody. The four cases of WDPM that were PAX-8 negative were histologically similar small (1-3 mm), papilliform proliferations with a densely fibrotic core and bland mesothelial lining, while the positive WDPMs tended to be more complex, larger, and showed at least mild nuclear atypia. This raises the question whether these smaller lesions would be better classified as papillary mesothelial hyperplasia. Overall, PAX-8 is a useful marker for distinguishing MM from gynecologic malignancies, but not is reliable for distinguishing benign and borderline mesothelial lesions from small/superficial epithelial proliferations of the gynecologic tract.

1136 GATA-3 Expression in Trophoblastic Tissues: An Immunohistochemical Study, Including Diagnostic Utility

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Background: Immunohistochemical expression of GATA-3 is seen predominantly in non-neoplastic bladder and breast epithelium and their respective carcinomas; however, expression in normal and lesional trophoblastic tissues is unknown.

Design: Immunohistochemical expression of GATA-3 was assessed in a range of normal/lesional trophoblastic tissues (n=209), including non-molar products of conceptions and 2nd and 3rd trimester placentas, ectopic pregnancies, partial and complete hydatidiform moles, placental site nodules, normal and exaggerated implantation sites, epithelioid trophoblastic tumors, placental site trophoblastic tumors, and choriocarcinomas. Extent (0, <5% [+] cells; 1+, 5-24%; 2+, 25-49%; 3+, 50-74%; 4+, 75-100%) and degree of intensity of expression were recorded.

Results: GATA-3 was expressed in 96% of cases, with the different cell types from normal and lesional tissues generally exhibiting a decreasing extent and/or intensity of expression in the following order: intermediate trophoblast (IT) [implantation and chorionic types] > villous trophoblastic columns > cytotrophoblast (CT) > syncytiotrophoblast (ST). Increasing gestational age was associated with a decrease in extent and intensity of expression in normal CT/ST while normal IT maintained diffuse and strong expression from early to late gestation. Expression was typically absent in decidua and myometrium.

GATA-3 Staining in Trophoblastic Tissues

	0	1+	2+	3+	4+	% positive
Non-molar conceptions/placentas, (n= 77)	0	0	5	2	70	100
Hydatidiform Moles (n= 24)	0	0	2	3	19	100
Implantation site (n= 37)	0	1	3	6	27	100
Placental Site Nodule (n= 16)	0	3	8	4	1	100
Placental Site Trophoblastic Tumor (n= 6)	2	2	0	0	2	67
Epithelioid Trophoblastic Tumor (n= 17)	1	1	1	6	8	88
Choriocarcinoma (n= 32)	5	7	5	6	9	84

Conclusions: GATA-3 is frequently expressed in normal and lesional trophoblastic tissues. This study expands the spectrum of neoplasms known to express GATA-3. Thus, recognition of expression in trophoblastic tumors is important because it can present a diagnostic pitfall in the assessment of suspected metastatic bladder or breast carcinomas involving the gynecologic tract. In the evaluation of diagnostically problematic tumors for which trophoblastic neoplasms are in the differential diagnosis, GATA-3 can be included as part of an immunohistochemical panel particularly when other trophoblastic markers are either not available or yield ambiguous results.

1137 The Expression of Receptor Tyrosine Kinases in Endometrial Clear Cell Carcinomas: Frequency and Clinicopathologic Significance

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Background: There are no effective molecularly-targeted therapies approved for use in endometrial cancer, but the recent identification of activating FGFR2 mutations in a subset of endometrioid tumors has renewed investigative efforts into the larger group of receptor tyrosine kinases (RTKs) as potential targets. RTKs are a subclass of transmembrane growth-factor receptors with an intrinsic, ligand-controlled tyrosine-kinase activity. Dysregulation of RTKs is common in epithelial neoplasms, and has been associated with tumor development and progression. Accordingly, anti-cancer therapies that are directed at RTKs or their associated pathways have shown promising efficacy

in a variety of tumors. The aim of this study is to assess the frequency of expression and the clinicopathologic significance of an expansive group of RTKs in endometrial clear cell carcinomas (CCC).
Design: Immunohistochemical analyses for selected class I (HER2/neu, EGFR), class III (PDGFRα, PDGFRβ, c-kit), class IV (FGFR2) and class V (VEGFR) RTKs were performed on 54 CCC, 17 endometrial serous carcinoma (ESC), 49 endometrioid carcinomas (EEC; 18 grade 1, 19 grade 2, 12 grade 3) and 25 non-neoplastic endometrial (NNE) samples in a TMA. HER2/neu scores were assigned according to the current breast ASCO/CAP scoring criteria, while all others were scored on a 0-12+ scale (intensity of staining [1+ to 3+] multiplied by extent of staining [0 to 4+]).
Results: All CCC were negative for HER2/neu and c-kit. Only 1 CCC was PDGFRβ-positive. Scores of≥1+ were seen for PDGFRα and EGFR in 61% and 93% of CCC respectively. There were no significant differences between CCC and a) EEC of any grade, b) ESC and c) NNE regarding either the frequency or the semiquantitatively determined level of expression of any RTK. VEGFR and FGFR2 expression at scores≥4+ was present in all cases. None of the RTKs showed statistically significant associations with clinicopathologic variables or patient outcomes.
Conclusions: The current study documents the expression of RTKs in CCC. HER2/neu, PDGFRβ, and c-kit are not, or are only rarely expressed in CCC, whereas the expression of VEGFR and FGFR2 is apparently ubiquitous. PDGFRα (61%) and EGFR (93%) are expressed in the majority of CCC. There is no association between the assessed RTKs and patient outcomes or clinicopathologic factors. The frequent expression of some RTKs in CCC suggests that investigations are warranted into the potential efficacy of RTK inhibitors (or modulators of their downstream effectors) in this rare histotype of endometrial carcinoma.

1138 A Morphologic Analysis of 95 Ovarian Clear Cell Carcinomas: Evaluating Features of Possible Prognostic Significance

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Background: With the exception of ovarian clear cell carcinomas (O-CCC), correlation between tumor grade and prognosis is well documented in surface epithelial carcinomas. When compared to the other carcinomas, advanced stage O-CCCs are associated with a poorer prognosis, whereas early stage disease has a comparable prognosis. We aimed to identify which, if any, morphologic features are prognostically significant and whether there is value in grading these tumors.
Design: 95 pure O-CCCs with clinical follow-up were retrieved from institutional archives and personal consultation files. Features evaluated included size, architectural pattern(s) (tubulocystic (TC), macrocystic, papillary, solid, non-specific glands), degree of pleomorphism (graded semiquantitatively 1-3), nucleolar grade (at 10x, 1: non visible, 2: less than 50% visible/not prominent, 3: >50% prominent), mitoses, necrosis, background endometriosis/adenofibroma, and pTNM stage. Survival differences were analyzed using the log rank test and Kaplan-Meier estimator.
Results: Of all variables evaluated, TC architecture was the only morphologic feature statistically associated with a favorable prognosis (p=0.0137). TC architecture was predominant in 56.5% (35/62) of stage I tumors versus 33.3% (11/33) of advanced stage tumors. Stage at presentation was also of prognostic significance (p<0.0001) with a five year survival of >80% in stage I tumors compared to 20% for stage III/IV (average follow-up of 78.7 months). Recurrences developed in 31.6% (30/95) of patients at an average interval of 24.6 months from time of operation. 19.4% (12/62) with stage I tumors developed recurrences, compared to 54.5% (18/33) with advanced disease. No other morphologic features evaluated approached statistical significance including pleomorphism, nucleolar grade, mitoses, or background endometriosis/adenofibroma.
Conclusions: This study shows that a TC pattern is more often seen in patients with stage I tumors and is associated with a more favorable outcome. As with prior studies, a nuclear/nucleolar grading system failed to correlate with outcome. Given that the majority of patients present with disease confined to the ovary and have an overall favorable prognosis, the contention that O-CCCs be considered high grade must be challenged. Instead, pathologic stage should be emphasized, with the option of adding a comment if there is an predominance of a TC pattern, since both of these criteria are independently predictive of outcome.

1139 Role of Y-H2AX Expression in Prediction of Radiation Resistance in Endometrial Carcinoma

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Background: Adjuvant radiotherapy is administered to endometrial carcinoma (EC) patients after surgery, depending of histological type, grade, and stage. Phosphorylation of H2AX (Y-H2AX) has an important role in the recruitment and localization of proteins involved in DNA repair, after exposure to ionizing radiation. After radiation, H2AX phosphorylation is retained longer in radiosensitive tumors. Y-H2AX is considered a good marker of DNA damage and a good predictor of radiation-resistance but has never been applied to prospectively predict radiation resistance in EC.
Design: Fresh tumor tissue from 27 primary ECs was subjected to irradiation and Y-H2AX analysis. The series included 16 grade 1-2 endometrioid carcinomas (EEC), 5 grade 3 EECs, 2 serous carcinomas (SC), 1 mixed EEC-SC, and 3 malignant mixed müllerian tumors (MMMT). Four different samples were obtained for each case. Two samples were irradiated, incubated in culture medium (30 minutes the first sample, and 24 hours the second sample), fixed in formalin, and embedded in paraffin. Two paired samples were not subjected to irradiation but managed identically to the irradiated ones. Y-H2AX was assessed by immunohistochemical (IHC) staining by comparing Y-H2AX expression between the two irradiated samples (30 minutes versus 24 hours in cultured medium) after having normalized IHC scores with the corresponding non-irradiated samples. The pathologic and IHC (PTEN, p53, Ki-67, NFkb, HIF1a) features of the tumors were evaluated in correlation with the levels of Y-H2AX.
Results: Overall, an increase of Y-H2AX expression after 24h of irradiation was detected in 15 of the 27 cases (55%). It was seen in 9 of 16 grade 1-2 EEC, 3 of 5 grade 3 EEC and 2 of 3 MMMT and 1 SC, while there was not increase of Y-H2AX expression in one mixed EEC-SC and one pure SC. Pathological and IHC features were not different between EC with decreased or increased Y-H2AX after radiation, with the exception of PTEN, which was significantly lower in EC with maintenance or increase of Y-H2AX after radiation.
Conclusions: Y-H2AX expression assays may be applied to EC samples to prospectively assess resistance to radiation therapy. Results suggest that EECs with PTEN loss are more sensitive to radiation.

1140 *MED12* and HMGA2: Two Independent Genetic Alterations in Uterine Leiomyoma

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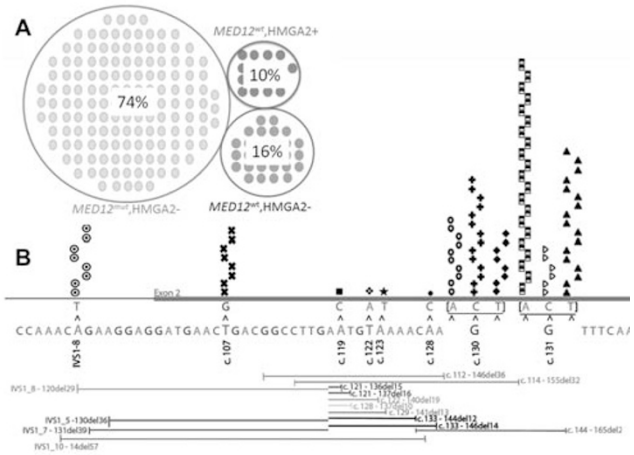
Background: Uterine leiomyoma are genetically heterogeneous and to date many significant mutations have been identified including mutations of HMGA2. Recent identification of *MED12* mutations in uterine leiomyomas provides a promising candidate gene for understanding tumorigenesis of leiomyomas. In this study, we examine the spectrum of *MED12* mutations in leiomyomas, correlate those gene mutations with MED12 protein expression, and compare the expression pattern of HMGA2 with *MED12* mutations.
Design: We collected 178 usual type leiomyomas for *MED12* mutation analysis. All DNA samples were extracted from formalin-fixed, paraffin-embedded tissue samples. Tissue microarrays were made from each tumor and matched myometrial controls. Expression of HMGA2 and MED12 were examined by Western blot, immunofluorescence and immunohistochemistry. Moderate immunoreactivity for HMGA2 in >50% of nuclei was read as HMGA2 positive.
Results: *MED12* mutations were found in 74.7 % (133/178) of leiomyomas. Of those, point mutations accounted for 88% (117/133) cases and complex mutations for 12% (16/133).



Western blot and immunohistochemistry demonstrated that leiomyomas with complex *MED12* mutations had significant lower immunoreactivity for MED12 (p<0.01) than myometrial controls. Conversely, leiomyomas with point mutations of *MED12* showed immunoreactivity for MED12 similar to those tumors without *MED12* mutation. A total of 18 leiomyomas were strongly immunoreactive for HMGA2, accounting for 10.1% of cases. HMGA2 overexpression was exclusively found in those leiomyomas without *MED12* mutations.
Conclusions: Through this study, we demonstrated *MED12* mutations are very common in leiomyomas with a wide range of simple to complex mutations identified in intron 1 and exon 2. Although there is a reduction of MED12 protein in tumors with *MED12* mutations, immunohistochemistry cannot separate leiomyomas with and without *MED12* mutations. Most importantly, HMGA2 overexpression was exclusively found in tumors without *MED12* mutations suggesting that *MED12* mutations and HMGA2 overexpression represent independent molecular pathways for uterine leiomyomas.

1141 Development of a Response Scoring System to Quantify the Effect of Neoadjuvant Chemotherapy in Ovarian Cancer – Ovarian Cancer Response Scoring (OCRS) Study

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Background: Histopathological response scoring systems for solid tumours treated with chemotherapy are an objective measure of the response to therapy. To date, in ovarian cancer (OC) such scoring has not been established. Neoadjuvant chemotherapy (NACT) is given in advanced OC to reduce tumor burden in order to achieve complete cytoreduction at subsequent surgery (interval debulking surgery, IDS). With new therapies being developed and tested for different histotypes of ovarian cancer which may not be associated with raised serum CA125 or measurable lesions on imaging there is a need for standardization of tissue-based response criteria. Such a system has to be a) easily applicable in routine histopathological practice and b) reproducible among different centres. The primary endpoint of the present study was to develop

a histopathological scoring system that standardizes response to neoadjuvant chemotherapy in ovarian cancer with good agreement between different pathologists.
Design: An Ovarian Cancer Response Scoring System (OCRS) was developed based on the system proposed by Dworak et al. H&E slides from IDS of 42 high-grade serous ovarian cancer (HGSC) patients treated at teaching hospital cancer centre were retrieved with informed consent and independently scored from 0-5 by three pathologists. Pathologists were blinded to each other as well as to clinical outcome and CA-125 response. A single section representing the area of lowest response at two sites (omentum and adnexa) was selected for the scoring. Fleiss' kappa statistics (κ) and Kendall's coefficient of concordance (KCC) between pathologists were calculated separately for each site.
Results: The five score OCRS levels of inter-observer agreement in omental metastasis scoring were $\kappa = 0.36$, ($p<0.001$) and KCC = 0.91 ($p<0.001$). Ovarian/pelvic tumor scoring resulted in $\kappa = 0.30$ ($p<0.001$) and KCC = 0.85 ($p<0.001$). A condensed three item OCRS (combining scores 0-1, 2-3 and 4-5) increased agreement on omental scoring to $\kappa = 0.75$, ($p<0.001$), KCC = 0.89 ($p<0.001$) and absolute agreement to 81%. Values for ovarian/pelvic three item OCRS were $\kappa = 0.55$, ($p<0.001$), KCC = 0.79 ($p<0.001$) and absolute agreement was present in 68%.
Conclusions: OCRS can be easily integrated in routine diagnostic practice and shows a good level of inter-observer agreement. Results of analysis of an independent validation set and correlation of OCRS with outcome data will be presented.

1142 HLA Ligandome Analysis Reveals HDAC1 as a Tumor-Associated Antigen in High-Grade Serous Ovarian Carcinomas and Potential Candidate for a Multipeptide Vaccine

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Background: Quantity and composition of intratumoral lymphocyte subsets in high-grade serous ovarian carcinomas (OvCa) are of significant prognostic relevance. However, little is known about whether this is due to specific tumor antigens which are presented to intratumoral T-cells. The goal of our study was to use HLA ligandome analysis to identify tumor–associated antigens, and their correlation with T-cell subsets and prognosis in serous OvCa.
Design: HLA presented peptides were obtained by immuno-affinity-chromatography of HLA molecules using a special protocol developed for solid tissue analysis. Tumor-associated and overrepresented peptides were identified by mass spectrometry and further tested in immunogenicity analysis. Finally, the combined prognostic effect of tumor infiltrating lymphocytes (TILs) and the expression of specific antigens was evaluated with immunohistochemistry in a tissue microarray of 136 cases of serous OvCa.
Results: One of the most commonly presented antigens identified in OvCa tissues was histone deacetylase 1 (HDAC1) which is able to induce T-cell responses in healthy donors. When tested by immunohistochemistry, high HDAC1 expression impacted negatively on prognosis (median 26 vs. 42 months, p=0,011, log rank). However, high numbers of intratumoral T-cells were able to overcome the negative prognostic effect, potentially reflecting the specific, antigen-triggered anti-tumoral immune response.
Conclusions: Our study demonstrates the identification of specific tumor-associated antigens from primary OvCa by HLA-ligandome analysis with mass spectrometry. The combined approach of proteomics and immunohistochemical analysis not only allows to analyze the interplay of immune cell infiltrates and tumor antigens but is a valuable tool for the development of a multipeptide vaccine for ovarian cancer.

1143 Overexpression of Tubulin β-3 in Endometrial Carcinomas

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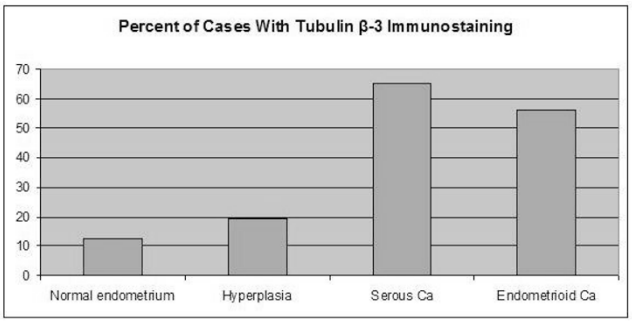
Background: Paclitaxel is a widely used chemotherapeutic agent in gynecologic tumors to stabilize microtubules and inhibit microtubule depolymerization. Overexpression of tubulin β-3 has been linked to paclitaxel resistance and has been previously described in a limited number of endometrial serous carcinomas.
Design: We assessed tubulin β-3 expression by immunohistochemistry in normal and hyperplastic endometrial samples and in endometrial carcinomas using a tissue microarray. Tubulin β-3 expression was scored semiquantitatively as 0, 1, or 2, corresponding to absence of staining, weak/focal staining, and strong/diffuse staining, respectively.
Results: A total of 63 serous and 185 endometrioid type endometrial adenocarcinomas, 40 normal and 26 hyperplastic endometrial samples were included in the study. Among endometrial carcinomas 41 of 63 serous (65%) and 104 of 185 endometrioid (56%) tumors showed tubulin β-3 expression (score 1 or 2), compared to only 19% of endometrial hyperplasia and 13% of normal endometrium, respectively (Figure 1). Tubulin β-3 expression showed statistically significant correlation with lymph node metastases in serous carcinomas. However, no significant correlation was observed between tumor stage and lymphovascular invasion and tubulin β-3 expression.
Conclusions: Tubulin β-3 overexpression is present in a significant proportion of endometrial carcinomas – both serous and endometrioid subtypes, as opposed to absence of staining in most endometrial hyperplasias and normal endometria, indicating that tubulin β-3 may play a role in the pathogenesis and disease progression. Tubulin β-3 expression is associated with increased chance of nodal metastases in serous carcinomas, thus it may be a marker of a more aggressive disease course.



1144 FIGO Grade 3 Endometrioid Carcinoma Versus Serous Carcinoma: Morphologic Mimicry Involves Only Specific Genomically Defined Subsets of Endometrial Carcinoma

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Background: The Cancer Genome Atlas (TCGA) identified four major genomic groups of endometrial carcinomas (ECs), the POLE ultramutated class 1; C1), microsatellite instable hypermutated (class 2, C2), copy-number low (class 3, C3), and copy-number high (serous-like; class 4, C4) groups. The aims of the study were i) to determine the agreement between the reported diagnosis of FIGO grade 3 endometrioid carcinoma (Gr3-EMC) and independent review of these cases by two pathologists and ii) to study the TCGA genomic classes of discrepant cases.
Design: Seventy-two Gr3-EMCs from the TCGA dataset were morphologically assessed by two gynecologic pathologists from two academic institutions. Virtual whole slide images publicly available at cBioPortal (http://www.cbioportal.org) were examined. The histologic type of a given case was recorded by each pathologist independently without knowing its TCGA genomic class, which was subsequently obtained from cBioPortal.
Results: Among the 72 cases Gr3-EMCs reviewed, there were 49 agreements, 5 disagreements and 16 deferrals between the 2 pathologists. One case showed no tumor. The interobserver agreement results and correlation with the TCGA genomic classes are described in Table 1.

TCGA class	Concordant DX (n=50)	Discordant DX (n=5)	Deferrals (n=16)
C1 (n=16)	9 (56%); 8 EMC, 1 SC	2 (13%)	5 (31%)
C2 (n=33)	25 (76%); 24 EMC, 1 SC	1 (3%)	7 (21%)
C3 (n=9)	8 (89%) EMC	0	1(11%)
C4 (n=13)	8 (62%); 4 EMC, 4 SC	2 (15%)	3 (23%)

Concordant results were observed in 9 C1 (56%), 25 C2 (76%), 8 C3 (89%) and 8 C4 (62%) Gr3-EMCs cases. Of the 13 Gr3-EMCs classified as of C4 genomic subtype, 4 cases had a consensus morphological diagnosis of serous carcinoma (SC) upon re-review, and in additional 3 cases, 1 of the pathologists suggested a SC diagnosis.
Conclusions: As many as half of C4 EMCs originally classified as G3-EMCs may instead represent SC, whereas C3 tumors were never interpreted as SC. It remains a challenge to reproducibly distinguish SC from C1, C2, and C4 G3-EMCs. These data confirm that C1 and C2 G3-EMCs are frequently morphologically ambiguous. If clinical studies can confirm that C4 G3-EMCs are inseparable from SC, then accurate diagnosis of SC given a histologically ambiguous tumor should rely not only on p53 immunohistochemical stains, but also on the exclusion of C1 and C2 tumors by morphologic evaluation and ancillary testing.

1145 Developmental Role of BRCA1 on Platinum and Taxane Resistant Models for Ovarian Cancer: MDR Related Mechanisms

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Background: Ovarian cancer (OC) is the leading cause of death from a gynecological malignancy.The standard taxol and carboplatin chemotherapy often fails and patients relapse with chemoresistant disease. ABC transporters including P-glycoprotein (P-gp), MRP2 and BCRP have been shown to be involved in chemoresistance in drug resistant cell lines. Patients with BRCA1 deficiencies have an increased lifetime risk of approx. 39-46% for developing OC. BRCA1 has a role in many cellular functions including DNA damage repair. An inverse resistance relationship exists between platinum and taxane therapies in relation to BRCA1. Cells with non-functional BRCA1 are sensitive to platinums but resistant to taxanes. The opposite is true for cells with functional BRCA1.
Design: OC cell-lines UPN251 (BRCA1 wildtype) and OVCAR8 (BRCA1 methylated) were used to develop novel taxane and platinum resistant cell lines. A selection strategy was set up to investigate the role of BRCA1 in resistance development in relation to, the inverse resistance relationship between platinums and taxanes and alternating doses of platinums and taxanes.
Results: Doses of taxol and carboplatin were selected after examining clinical trial papers and pharmacokinetic studies to elucidate suitable doses with clinical relevance. OVCAR8 sublines did not produce significant resistance and were not focused on post selection. Resultant UPN251 sublines displayed notable resistance for both drugs. R7-U-C was 3.4 fold resistant to carboplatin and R7-U-T was 8 fold resistant to taxol. UPN251 sublines developed using taxol were all significantly resistant to taxol and vinblastine (known P-gp substrates). Resistance was reversed with the adition of elacridar (a known P-gp/BCRP inhibitor). Cross resistance in UPN251 carboplatin models were seen with cisplatin and $CuSO_4$.

Conclusions: Overexpression of P-gp/BCRP are a possible resistance mechanism for our UPN251 models selected with taxol as elecridar reverses taxol and vinblastine reisitance. MRP2 may play a role in carboplatin resistant sublines due to cross resistance to cisplatin. $CuSO_4$ cross resistance indicates possible involvement of copper transporters. Western blotting is planned for P-gp, BCRP and MRP2 to confirm involvement in our resistant models. BRCA1 wildtype cells (UPN251) developed resistant mechanisms easier than BRCA1 methylated cells (OVCAR8). UPN251 saw quicker development of possible MDR related mechanisms. UPN251 resistant sublines will be used to profile miRNA markers for OC resistance.

1146 MicroRNA Profiling Reveals Increased MiR34 and C-Myc Downregulation in Obesity Associated Low Grade Endometrioid Adenocarcinomas

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Background: Epidemiologic evidence has revealed an association between adiposity and endometrial cancer risk. The most common endometrial cancer histologic type, low grade endometrioid adenocarcinoma (EC), is especially frequent amongst obese women. However, morphologically indistinguishable EC are also seen in some non-obese women. This highlights the currently incomplete understanding linking EC and its major risk factor. MicroRNAs (miRNA), a class of single-stranded non-coding RNA molecules, play critical roles in various biological regulation pathways including cell differentiation, proliferation and apoptosis. We investigate miRNA in FIGO 1 EC from obese vs non-obese women.

Design: 11 FIGO 1 EC from obese women (BMI≥ 30 kg/m^2) and 13 FIGO 1 EC from non-obese (BMI<30 kg/m^2) were randomly selected. RNA was analyzed with Nanostring. RT-PCR with Taqman probes was used to confirm microRNA regulation. RT-PCR on target genes was also performed.

Results: Nanostring miRNA profiling of FIGO 1 EC from obese vs non-obese women identified a single differentially-expressed miRNA: miR34c3p. This miRNA was found at 2.91 fold in tumors from obese women. Up-regulation of this miRNA was confirmed by RT-PCR. In silico studies showed that miR34c3p targets c-myc proto-oncogene. Down regulation of c-myc in EC of obese women was also found by RT-PCR.

Conclusions: Gaining a better understanding of the relationship between obesity and EC can provide new insights into pathogenetic mechanisms. Here we found a specific miRNA signature of obese vs non obese FIGO 1 EC and suggest a new possible role for miR34c3p as centerpiece involving c-myc.

1147 Specific MicroRNA Gene Signature Identifies Putative Role of BCL2 in the Tumorigenesis of Uterine Serous Carcinomas

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Background: Molecular mechanisms explaining the development and progression of uterine serous carcinoma (USC) are incompletely understood. MicroRNAs (miRs) function as sequence-specific regulators of gene expression through translational repression and/or transcript cleavage. MiRs play key roles in cellular processes of differentiation, proliferation, apoptosis and metabolic homeostasis.

Design: 5 USC and 24 low grade endometrioid adenocarcinomas (EC) were randomly selected. RNA was analyzed with Nanostring. RT-PCR with Taqman probes was used to confirm miR regulation. RT-PCR on target genes was also performed.

Results: Only 7 miRs were differentially expressed: 6 downregulated in USC (miR521, miR522-3p, miR31-5p, miR18a-5p, miR660-5p, miR146b-5p) and 1 upregulated (miR375). The most significantly regulated miR was miR375. Downregulation of: miR31-5p, miR 146b-5p and the upregulation of miR375 was confirmed with RT-PCR. In silico studies showed that miR146b-5p targets RRM2B (repairing damaged DNA in a p53/TP53 dependent manner) and notably, miR375 targets BCL2. We ran RT-PCR on BCL2 and showed inverse correlation between miR375 and BCL2.

Conclusions: MiRs expression distinguishes USC and EC. In particular we identified miR375-BCL2 as a possible specific mechanistic pair in USC.

1148 HPV Genotyping Does Not Predict Absence of CIN in the Conization Specimen in Women Treated for CIN2-3

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Background: Conization is the mainstay technique for the treatment of cervical intraepithelial neoplasia grade 2 and 3 (CIN2–3). Even with the use of strict criteria approximately 10-20% of patients with histologically confirmed CIN2-3 in the pre-conization biopsy have no residual CIN in the cone specimen. The role of high-risk human papillomavirus (hr-HPV) genotype in this outcome and its importance in the pre-treatment prediction of no residual CIN in the cone specimen remains to be evaluated. This study aims to evaluate whether certain genotypes or the presence of multiple hr-HPV co-infection are associated with absence of CIN in the conization specimen in women treated because of CIN2-3.

Design: From 687 conizations performed between 2008 and 2011, 110 women showed absence of CIN in the surgical specimen. We selected all patients who fulfilled the following criteria: 1) diagnosis of CIN2-3 in a pre-conization biopsy confirmed by a positive p16 staining; 2) absence of lesion in the conization specimen with negative p16 result; and 3) available material for hr-HPV typing in both specimens (n=43). The control group consisted of one randomly selected age-matched control for each case with confirmed diagnosis of CIN2-3 and positive p16 staining both in the pre-conization biopsy and in the conization specimen. HPV detection and genotyping in all histological specimens was performed using the highly sensitive SPF10 PCR technique.

Results: All pre-conization biopsies from the study and the control group were positive for hr-HPV. In the conization specimens, hr-HPVs were identified in 42/43 (97.7%) cases from the control group but only in 14/43 (32.5%) of the study group (p<0.001). Previous to conization, no differences in HPV genotypes or in multiple HPV co-infection were observed between the study and the control group (HPV16-18, 27/43 [62.8%] vs. 25/43 [58.1%]; p=0.410; multiple genotypes 8/43 [18.6%] vs. 9/43 [20.9%]; p=0.500).

Conclusions: Neither specific hr-HPV genotype, nor multiple infection are associated with absence of CIN 2-3 in the conization specimen. hr-HPV genotyping does not add any additional information which could predict absence of CIN2-3 in the conization specimen before treatment.

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1149 SLC7A10, GEM and TAGLN: A Novel Immunohistochemical Panel for the Differential Diagnosis of Endometrial Stromal Sarcoma Versus Leiomyosarcoma

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Background: Endometrial stromal sarcoma (ESS) and uterine leiomyosarcoma (LMS) are the two most common uterine sarcomas. Overlapping morphology and immunohistochemical expression patterns may pose a diagnostic challenge. Recently, Davidson et al. used gene expression analysis to identify groups of genes overexpressed by ESS and LMS. Within the identified genes, *SLC7A10 (solute carrier family 7, member 10)* was overexpressed in ESS, and *GEM (GTP binding protein overexpressed in skeletal muscle)* and *TAGLN (transgelin)* were overexpressed in LMS. We hypothesized that examination of ESS and LMS by immunohistochemistry for expression of these markers may contribute to the differential diagnosis of ESS and LMS.

Design: Cases were retrieved from the Pathology files and reviewed by two gynecologic pathologists. Only cases with a consensus diagnosis were included in the study. Paraffin embedded tissue from 24 ESS and 24 LMS were examined by immunohistochemistry for expression of SLC7A10, GEM and TAGLN. Antibody expression was examined for nuclear versus cytoplasmic staining, strength of expression and semi-quantitatively scored as 0, 1+ to 3+. Appropriate negative and positive controls were used. Cases were considered positive with 2+ or 3+ expression.

Results: Antibody expression patterns:

Endometrial Stromal Sarcoma versus Leiomyosarcoma by IHC

SLC7A10			
Tumor type	Negative	Positive	p-value (Fisher's-exact test)
ESS	9	15	< .001
LMS	0	24	
GEM			
Tumor type	Negative	Positive	p-value (Fisher's-exact test)
ESS	18	6	< .05
LMS	10	14	
TAGLN			
Tumor type	Negative	Positive	p-value (Fisher's-exact test)
ESS	19	5	< .02
LMS	10	14	

Expression in normal tissue

Antibody	Endometrial stroma	Smooth muscle	Myocytes
SLC7A10	Negative	Negative	Negative
GEM	Weak/Focal	Positive	Positive
TAGLN	Negative	Positive	Negative

Conclusions: Statistically significant differences in IHC staining patterns were observed for SLC7A10, GEM and TAGLN. All antibodies were more frequently expressed in LMS than ESS. Based on the gene expression profile by Davidson et al., this was hypothesized for GEM and TAGLN, but unexpected for SLC7A10. Of note, GEM and TAGLN were validated by quantitative real-time PCR in this publication which was not performed for SLC7A10. Our data support that immunohistochemically, all three markers, SLC7A10, GEM and TAGLN, can contribute to distinguish ESS from LMS in difficult cases.

1150 Loss of PAX2 Nuclear Expression: A Useful Diagnostic Feature for In Situ and Invasive Endocervical Adenocarcinoma

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Background: PAX2 is a transcription factor necessary for the development of Wolffian duct system. It is ubiquitously expressed in normal endocervical glandular epithelium. Recent studies have suggested that PAX2 expression is frequently lost in endocervical adenocarcinoma. To substantiate this finding and assess its usefulness as a diagnostic feature in our laboratory, we evaluated PAX2 expression in a group of endocervical adenocarcinomas in comparison with endometrial adenocarcinomas and ovarian serous carcinoma.

Design: Immunohistochemical evaluation of PAX2 expression was performed on 229 cases of gynecological adenocarcinomas (ADCs) on tissue microarray sections including 60 endocervical ADCs (16 were adenocarcinoma in situ), 128 endometrial ADCs and 41 ovarian serous carcinomas (CAs). The presence or absence of nuclear staining in tumor cells was scored by three independent pathologists. The data was statistically analyzed using Fisher exact test.

Results: A complete loss of PAX2 nuclear staining was observed in 16 of 16 (100%) endocervical adenocarcinoma in situ (AIS) and 42 of 44 (95%) invasive endocervical ADC. This is in sharp contrast with benign endocervical glands, all of which show strong PAX2 expression. In AIS cases, absence of PAX2 expression was often observed in the cytologically atypical portion of a gland only with retained expression in benign appearing glandular cells. PAX2 loss was found in 5 invasive adenocarcinomas that were

negative for p16 immunostaining. It was also found in 6 of 8 unusual type endocervical adenocarcinomas (3 of 3 minimal deviation ADCs and 3 of 3 gastric type ADCs). The 2 tumors retained strong PAX2 expression were unusual type ADC with high grade clear cell carcinoma morphology. In comparison, PAX2 loss was a less frequent event in endometrial ADCs (45%, $p<0.05$) and ovarian serous CAs (42%, $p<0.05$). Among endometrial ADCs, PAX2 loss was found in 8 of 24 (33%), 37 of 58 (64%), and 13 of 38 (34%) tumors with FIGO grade 1, 2 and 3 morphology, respectively.

Conclusions: Loss of PAX2 expression is a useful diagnostic feature discriminating malignant endocervical lesions including unusual type endocervical ADC from benign/reactive process. It is also potentially useful in differentiating enodocervical ADC from endometrial ADC.

1151 Risk of Secondary Malignancy (Including Breast) in Patients with Mismatch Repair Deficiency

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Background: Loss of mismatch repair (MMR) proteins by immunohistochemistry (IHC) is a sensitive and specific way to identify defective DNA mismatch repair (dMMR). Germline mutation in MMR genes is characteristic of Lynch syndrome, which is associated with significant increased risk of colorectal and endometrial cancer. Less commonly, malignancy has been reported in a variety of other organs, including breast, in patients with Lynch syndrome. Very little is known about the MMR status of these secondary tumors when they do occur. This study evaluates MMR protein expression in secondary tumors from a series of patients with known dMMR.

Design: 1,476 tumors (index tumors) as well as all secondary malignancies associated with these index tumors were tested for MMR by IHC (MSH2, MSH6, PMS2, MLH1) between 2002 and 2013. Index tumors were predominantly non-selected endometrial (retrospective and prospective) and colon (prospective) carcinomas. All tumors with MLH1/PMS2 deficiency were tested for hypermethylation or BRAF mutation, where appropriate.

Results: Of the 1477 index tumors, 162 were dMMR, and of that subset 32 secondary tumors were identified (19.7%). In contrast, 80 secondary tumors were identified in the intact MMR group (6.0%). Though additional malignancies were more common in the dMMR group ($p=0.0001$), these patients were not more likely to have any specific subtype of tumor. In particular, breast cancer was not overly represented in the dMMR secondary tumors group and when it did occur, trended towards intact MMR. When secondary tumors had dMMR, they were more likely to have deficiency in MSH2/MSH6 than MLH1/PMS2 ($p=0.01$). No patients with tumors with sporadic MLH1 deficiency had secondary tumors with dMMR. There was no significant difference in age in patients with secondary tumors in the dMMR and intact MMR groups (mean, 61 vs 66 years). 32% of the secondary tumors in the intact MMR group occurred in men, compared to 3.7% in the dMMR group ($p=0.0018$).

Conclusions: Patients with tumors exhibiting mismatch repair deficiency are more likely to have additional tumor(s) than those whose tumors have intact MMR ($p=0.0001$). This effect is more pronounced in women. Breast cancer does not appear to be overly represented in patients with MMR deficiency. MSH2/MSH6 deficiency is more commonly associated with a secondary tumor than MLH1/PMS2 deficiency, even when methylation/BRAF status is taken into account.

1152 The LAST Guidelines in Clinical Practice: Community Experience of Implementing Recommendations for p16 Use

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Background: HPV-associated squamous lesions of the lower anogenital tract present a diagnostic and terminological challenge. In 2012, a 2-tiered system, LSIL and HSIL, was proposed by the LAST Project. The biomarker p16 was also recommended for cervical biopsies: 1) to differentiate HSIL from benign mimics, 2) to establish a diagnosis of CIN-2, 3) professional disagreement, and 4) high-risk Paps where the biopsy is interpreted as ≤ LSIL. We compared p16 use before and after implementation of the LAST guidelines and assessed HSIL detection attributable to the new protocol.

Design: We reviewed all cervical biopsies diagnosed by two pathologists for 1 year prior to (Year A) and after adoption of (Year B) the p16 guidelines. All cases with p16 were categorized into 4 groups per the LAST guidelines. We calculated the percentages of and reason for p16 use across all biopsies, and the difference in HSIL detection by p16. Percentages were compared using chi-square analysis.

Results: 1829 and 1728 biopsies were reviewed in Years A and B, respectively. p16 use significantly increased from A to B, with significant changes in reasons for p16 use (Table 1). In Year A, p16 was used primarily for category 1 with fewer cases in category 2. In Year B, the frequency of category 1 decreased with concomitant increases in categories 2 and 4; approximately 1/3 of the cases in category 4 were p16 positive and diagnosed as HSIL. Finally, significantly more cases of HSIL were detected by using p16 per the LAST guidelines (Table 2).

Incidence and Indication for p16

	Year A	Year B	p-value
p16 use rate	2.8%	4.9%	.0009
p16 use category			
1. HSIL vs mimic	94.1%	47.1%	<.0001
2. Suspect CIN II	5.9%	27.1%	.0024
3. Professional disagreement	0%	0%	N/A
4. High-risk Pap	0%	25.9%	.0005

HSIL Biopsies Detected with P16

Year A	Year B	p-value
10.8%	17.9%	0.0287

Conclusions: Implementation of the p16 LAST guidelines in our practice resulted in a significant increase in p16 use, nearly doubling the rate from 2.8% to 4.9%, albeit much lower than the 20% predicted in the LAST publication. This increase was largely due to increased p16 use in categories 2 and 4, and resulted in enhanced HSIL diagnosis. Approximately 1/3 of cases in the latter category would have been underdiagnosed without implementation of the LAST guidelines. In summary, there was improved HSIL detection without excessive use of p16. The findings support the implementation of the p16 LAST guidelines in routine clinical practice.

1153 Young Patients with Uterine Serous Carcinoma: A Study of Selected Epidemiological and Immunohistochemical Features Including Expression of DNA MMR Proteins

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Background: Endometrial serous carcinoma (ESC) constitutes approximately 10% of endometrial carcinoma and is unusual in patients under the age of 60. It is typically not an estrogen-driven tumor or associated with obesity. In addition, there have been conflicting reports regarding the presence of DNA mismatch repair (MMR) abnormalities in ESC. We thus sought to establish whether MMR abnormalities play a significant role in ESC or mixed carcinoma with a serous component in patients aged less than 60 years and to characterize the clinical presentation of these patients.

Design: A cohort of sixty eight patients diagnosed with ESC (54 cases of pure ESC, 13 mixed endometrioid endometrial carcinoma (EEC)/ESC, and 3 ESC with clear cell features) and aged less than 60 years was retrospectively identified from an MSKCC clinical database. Epidemiologic, clinical and survival data was recorded on all cases. Immunohistochemical analysis for PMS2 and MSH6 was performed in a subset of cases (n= 39).

Results: The patient age range was 44-59 years with a mean age of 55.7 years. Seventy-two percent of patients were overweight (32%) or obese (40%). Most (79%) patients were post-menopausal. Six patients (9%) had a history of breast carcinoma and five (7%) had taken Tamoxifen. Tumor stage at diagnosis was as follows: 38% Stage I, 7.3% Stage II, 32% Stage III and 21% Stage IV. All Stage I patients were alive with no evidence of disease at a median follow up of 36 months; in contrast, at a median follow up of 33 months, 20% of Stage III and IV patients were alive with disease, while 49% were dead of disease. The median time to death was 22 months. Only 1 of 39 cases demonstrated MMR abnormalities, in a patient with Lynch Syndrome. This tumor showed loss of MSH2 and MSH6 by immunohistochemistry.

Conclusions: Young patients with ESC show a clinical course similar to that of older patients and do not frequently have a history of tamoxifen use. The presence of DNA MMR abnormalities is rare in this group of tumors. While obesity is classically associated with endometrioid tumors, a subset of ESC occurs in overweight and obese patients and may be estrogen-driven, especially in young patients. Further immunohistochemistry for p53, PTEN and ARID1A will now be performed on this patient cohort to further characterize this rare tumor of young women.

1154 MiR31 Related RET Onco-Addiction in Neuroendocrine Carcinomas of the Uterine Cervix

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Background: Neuroendocrine carcinomas of the uterine cervix (NEC) are highly aggressive cancers. As in squamous cell carcinomas (SCC), in NEC p53 and Rb are down and p16 is up. MicroRNAs (miRs) are noncoding RNAs with roles in posttranscriptional regulation. Here, we identify a specific miR signature of NEC by comparing to SCC and adenocarcinoma of the uterine cervix (AC).We show that such signature reveals novel endocrine dependent tumorigenic pathways.

Design: 4 NEC, 4 SCC and 2 AC were selected from tissue archives. RNA was analyzed by Nanostring. The deregulated miRs were confirmed with RT-PCR. Targets were identified by in silico studies and confirmed by RT-PCR. MCF7 cell line (breast cancer) was transfected with miR31 and RET expression was assessed by Western Blot.

Results: In the NEC vs AC analysis 6 miRs only were deregulated: 4 down (miR-215, miR-31-5p, miR-192-5p, miR-194-5p) and 2 up (miR-9-5p, miR-7-5p). 5 miRs were deregulated in NEC vs SCC, 3 down (miR-31-5p, miR-203, miR-205-5p) and 2 up (hsa-miR-375, hsa-miR-135a-5p). Mir31-5p, down in both analyses, by similar fold difference was validated by RT-PCR, as were miR203 and miR205-5p for NEC vs SCC. On the other hand, RT-PCR did not confirm miR135a-5p deregulation. RET is target of miR31-5p (in silico). Noteworthily, estrogen receptor alfa 1 (ESR1) is target for all deregulated miRs identified except miR205-5p. Transfection of miR31 in the MCF7 cell line was followed by downregulation of RET as seen on Western Blot.

Conclusions: MiR profiling generated a short list of deregulated genes in NEC vs both SCC and AC showing that NEC has a specific miR signature. One miR in particular, miR31-5p, was down by comparable amounts in NEC vs both SCC and AC. Interestingly, RET is a putative target for miR31-5p (in silico). Downregulation in similar amounts of miR31-5p was validated in both analyses, NEC vs AC and NEC vs SCC. Hence, NEC tumorigenesis might partially be RET driven, similar to other neuroendocrine tumors. Transfection of miR31-5p in MCF7 was followed by Western Blot confirmed downregulation of RET, showing that this mechanism might play a role in many other cell types. Of note, in silico shows that all deregulated miRs (except miR205) target ESR1. Therefore, the miR signature of NEC reveals a dual nature: neuroendocrine and estrogen-sensitive. This is a fundamental progress in the understanding of this cancer and its potential treatment.

1155 Placental Fetal Thrombotic Vasculopathy in Association with Gastroschisis

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Background: Gastroschisis is a full thickness abdominal wall defect associated with evisceration of fetal intestines and herniation of the gastrointestinal structures into the amniotic cavity The etiology of the defect is unknown, but some common risk factors are low socieoeconomic level, young maternal age and smoking. The maternal immune response to new paternal (fetal) antigens may also play a role in the development of gastroschisis. The overall survival rate is about 90%. Fetal thrombotic vasculopathy (FTV) is characterized by thrombosis, avascular villi, intimal fibrin cushions, and fibromuscular sclerotic changes in the placenta. Multiple conditions have been associated with the condition including preeclampsia, intrauterine growth restriction, intrauterine fetal demise, and amputation necrosis.

Design: To evaluate and determine what placental changes are associated with gastroschisis, hospital based databases at Texas Children's Hospital and Ben Taub Hospital were searched to identify cases of gastroschisis in which the placenta was sent for pathological review.

Results: Five cases of gastroschisis were identified from our database search. The mean maternal age of the fetuses was 22 years old.The gestational ages of the cases in the study ranged from 33 to 38 weeks gestation. Findings of the postmortem and postnatal examination of the five cases identified were found to have thrombi in the umbilical vessels. Other features of FTV were also identified including areas of avascular villi, intimal fibrin cushions,& fibromuscular sclerotic changes in the placenta, and hemorrhagic endovasculosis. Increased nucleated red blood cells were frequently identified within the villi of the placenta.There was a strong association between gastroschisis and vacuolization and hemosiderin-laden macrophages in the membranes.

Conclusions: Gastroschisis is a ventral body wall defect that occurs early in the development of the fetus. Due to its location, cord compromise may occur. It is known that cord compromise, primarily in the venous circulation, can lead to thrombosis of the umbilical vessels. In our study, fetal thrombotic vasculopathy was identified in each of the five cases of gastroschisis. To our knowledge, this is the first case series showing a possible association between the two entities. The etiology of the association is unknown, but compromise of the cord in association with the abdominal wall defect may be likely. If the thrombotic events are severe enough in the fetal circulation, intrauterine fetal demise may occur as a consequence.

1156 Uterine Leiomyomas with Bizarre Nuclei. A Clinico-Pathological Study of 68 Cases

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Background: Leiomyoma with bizarre nuclei (LM-BN) is an uncommon LM variant with histologic features (mono- or multinucleated bizarre cells that may have a diffuse distribution, prominent nucleoli, and karyorrhectic nuclei that may mimic atypical mitoses) that often cause confusion with leiomyosarcoma.

Design: 68 LM-BN were collected from 2000 to 2010. Features recorded included patient age, therapy, tumor size and border, gross appearance, density (low ≤ 30%, intermediate 30-70% and high>70%) and distribution (diffuse, multifocal or focal) of bizarre nuclei, mitotic count, karyorrhectic nuclei, presence of necrosis, and cells with conspicuous dense eosinophilic cytoplasm (rhabdoid-like). Follow-up was obtained in 54 patients.

Results: Patients ranged from 25 to 75 (average 48) years. Fifty-one underwent hysterectomy and 17 myomectomy. For 52 tumors gross findings were known. A white, whorled and rubbery cut surface was noted in 43/52 (82%), 4 were polypoid (8%) and prominent cystic degeneration was seen in 5 (10%). Hemorrhage/ necrosis was seen in 9 tumors (17%). In 54 LM-BN the margin was well circumscribed and in the others could not be evaluated with certainty. Twenty-five (37%), 25 (37%) and 18 (26%) LM-BN showed low, intermediate and high bizarre nuclei density respectively. Twenty-two (32%) tumors showed diffuse, 27 (40%) multifocal and 19 (25%) focal atypia respectively. Mitotic count ranged from 0 to 7/10 (average 1 to 2)/ 10 HPFs. Forty-three had <2/10 HPFs, 22 showed 2 to 5/10 HPFs and in 3 tumors mitotic count was 6, 7, and 7/10 HPFs respectively (two-focal and one-diffuse atypia). All but 3 LM-BN showed karyorrhectic nuclei that were striking in 12 mimicking atypical mitoses. Ischemic but not tumor cell necrosis was detected in 28 tumors (41%). Rhabdoid-like cells were noted in 23 tumors (striking in one). Follow up was available in 54 patients and they had no evidence of recurrence, 38 treated by hysterectomy and 16 by myomectomy, ranging from 1 to 13 years (overall average 5; in patients with myomectomy average 5.7 with range from 2.8 to 11).

Conclusions: Our results corroborate that LM-BN is associated with a favorable outcome even in those patients only treated by myomectomy and highlights that a conservative approach can be undertaken in these patients as many of them are of reproductive age. Because of the favorable outcome, the term LM-BN is preferable to alternative terminology including "atypical leiomyoma".

1157 SATB2 Immunohistochemical Staining Is Useful in Distinguishing Primary from Metastatic Mucinous Tumors in the Ovary

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Background: Primary ovarian mucinous neoplasms as well as metastatic mucinous carcinomas to the ovary, irrespective of their site of origin, often express enteric markers (CDX2 and CK20), making it difficult to distinguish a primary ovarian mucinous neoplasm from a metastatic mucinous carcinoma. SATB2 (special AT-rich sequence binding protein 2) is a nuclear matrix-associated transcription factor which is expressed in glandular cells of the lower gastrointestinal tract. The aim of this study was to evaluate the utility of SATB2 immunohistochemical (IHC) staining in distinguishing primary ovarian mucinous neoplasms (carcinomas and borderline tumors) from metastatic mucinous carcinomas to the ovary from the colon, appendix or pancreas.

Design: IHC staining for SATB2, CDX2 and CK20 was performed on whole tissue sections from 84 resected mucinous neoplasms: 25 ovarian neoplasms (12 mucinous carcinomas and 13 borderline tumors, intestinal type), 19 metastatic carcinomas to ovary (10 appendiceal, 5 colonic, 4 pancreatic) and 40 primary non-ovarian carcinomas (20 colonic, 10 appendiceal, 10 pancreatic). The extent of staining was graded as 0 (<5% nuclear staining), 1+ (5-25%), 2+ (26-50%) or 3+ (>50%), and intensity was graded as weak, moderate or strong.

Results: IHC stains for CDX2 and CK20 were each positive in 92% of primary ovarian mucinous tumors and were positive in 90% and 85%, respectively, of all metastatic mucinous carcinomas to the ovary. SATB2 was expressed in 80% and 100% of mucinous colonic and appendiceal carcinomas metastatic to the ovary, respectively, with 3+ strong nuclear staining in all but 2 cases. Similar staining with SATB2 was seen in primary mucinous carcinomas of the colon (95%) and appendix (100%). In contrast, mucinous ovarian metastases from the pancreas were all SATB2 negative, as were all primary pancreatic carcinomas, 75% of primary ovarian mucinous carcinomas, and 85% of mucinous borderline tumors of the ovary.

Conclusions: 1) CDX2 and CK20 are not helpful in distinguishing primary ovarian mucinous neoplasms from metastatic mucinous carcinomas from the colon, appendix, or pancreas, since they are commonly expressed by each of these tumors. 2) Although SATB2 cannot separate a primary ovarian mucinous tumor from a metastatic pancreatic carcinoma, it is extremely helpful in distinguishing primary mucinous ovarian neoplasms from metastatic mucinous carcinomas of colonic or appendiceal origin.

1158 Persistent Low Expression of hZip1 in Mucinous Adenocarcinomas of the Ovary, Colon, Stomach and Lung

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Background: Zip1 is an zinc uptake transporter involved in the extraction of zinc from circulation and its accumulation intracellularly. We have previously demonstrated that ZIP1 is decreased in prostate cancer. However, the role of hZIP1 in carcinomas of the other organs has not been comprehensively examined. The goal of the current work is to study the expression of hZIP1 in mucinous carcinomas from a variety of organs as compared with non-neoplastic and conventional adenocarcinomas in these organs.

Design: hZip1 protein expression in mucinous adenocarcinomas of the ovary, colon, stomach and lung in tissue microarrays (TMAs) were determined by IHC using an anti-hZip1 antibody. The TMAs were comprised of 35, 51, 31 and 21 mucinous adenocarcinoma of the ovary, colon, stomach and lung, respectively, 152 ovarian serous, 129 conventional colonic and 4 conventional gastric adenocarcinomas. Normal ovarian (n=17), colonic (n=12) and gastric (n=5) tissue were also present in the TMAs. hZip1 immunoreactivity in the epithelial cells were scored as follows: negative, 0; 1+, < 10%; 2+, 10-50% and 3+, > 50% positive cells.

Results: hZip1 showed consistently low expression in mucinous adenocarcinoma of the ovary, colon, stomach and lung with an average score of 1.3, 1.7, 2.2 and 2.4, respectively. The expression of hZip 1 was significantly lower in mucinous adenocarcinomas as compared to 1) ovarian serous carcinoma (P<0.001) and normal ovarian tissue (P=0.015), 2) colonic adenocarcinoma and normal colonic mucosa (P<0.001), and 3) conventional gastric adenocarcinoma (P<0.05) and normal gastric epithelium (P=0.027). The mean score of hZip1 was significantly lower in low and intermediate grade mucinous carcinomas of the stomach, as compared to high grade counterparts (2.7 versus 1.9, p<0.01). There was no association between stage and hZip1 scores in any of the mucinous tumors. 152 ovarian serous carcinomas revealed hZIP1 scores of (0), 1+, 2+ and 3+ in 1 (0.7%), 13 (9%), 32 (22%) and 106 (70%) cases, respectively. There was a statistically significant lower expression of hZip1 in low compared to high grade serous carcinomas (P<0.05).

Conclusions: hZIP1 expression appears to be consistently decreased in mucinous adenocarcinomas from a variety of organs. However hZIP1 is also decreased as a function of increasing grade in both mucinous and non-mucinous tumors. These findings suggest that hZIP1 plays a fundamental role in the carcinogenesis of some organs that is particularly accentuated in mucinous tumors.

1159 The Prognostic Effect of MLH1 Loss in Endometrial Endometrioid Adenocarcinoma

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Background: Microsatellite instability (MSI) arises due to loss of mismatch repair (MMR) protein function. 15-20% of sporadic endometrial carcinomas have MSI due to methylation of the *MLH1* gene promoter with subsequent transcriptional silencing. The value of MLH1 as a prognostic parameter is not well established. The aim of this study was to assess the effect of MLH1 loss on recurrence free survival (RFS) and overall survival (OS) in a large cohort of endometrial endometrioid adenocarcinomas.

Design: Immunohistochemistry for MMR proteins, MLH1, MSH2, MSH6 and PMS2 was performed in 106 early stage (I and II) and 106 advanced stage (III and IV) endometrial endometrioid carcinoma cases. The cases were closely matched by tumor grade and patient age. The patients had no known history of Lynch Syndrome. Tumors with negative nuclear immunohistochemical staining for any one of the four MMR proteins were classified as having MSI, with the remainder classified as microsatellite stable (MSS).

Results: MSI was identified in 30.7% of all patients (22.2% MLH1, 2.8% MSH2, 2.4% MSH6, 2.8% PMS2 and 0.5% MSH6/PMS2). MLH1 loss was detected in 16.0% of early stage and 28.3% of advanced stage patients (p=0.065). Cases with non-MLH1 MMR loss were excluded from subsequent analyses. Follow-up was censored at 5 years. By multivariate Cox proportional hazards regression model, MLH1 loss was not significantly associated with RFS (p=0.767) or OS (p=0.770) in the early stage cases. However, in advanced stage cases, MLH1 loss conferred reduced RFS (HR 2.02 [95% CI, 1.08-3.78], p=0.028). This effect was independent of tumor grade and patient age. MLH1 loss was not significantly associated with OS in the advanced stage group (p=0.220).

Conclusions: MLH1 loss in advanced stage endometrial endometrioid adenocarcinomas is an independent negative prognostic factor of recurrence. As such, identification of MLH1 loss in these tumors in the preoperative setting may help guide patient management decisions.

1160 Are Endometrial Clear Cell Carcinomas Type I or Type II Cancers?: A Clinicopathologic Analysis of Hormone Receptor Expression in 49 Cases

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Background: Recent large-scale epidemiological studies have found that endometrial clear cell carcinomas (CCC) are associated with some of the same estrogen-dependent risk factors as Type I endometrial carcinomas, albeit to a lesser degree, which suggests that their traditional categorization as Type II cancers may be an oversimplification. To provide some insights into whether CCC have a hormone-independent pathogenesis, we performed the largest and most comprehensive analysis reported to date of hormone receptor profiles in CCC. We also assessed the expression of NcOA3, a nuclear receptor coactivator that interacts with hormone receptors to enhance their transcriptional activator functions.

Design: Immunohistochemical studies for NcOA3, androgen receptor (AR), estrogen receptor-alpha (ERα), estrogen receptor-beta (ERβ), and progesterone receptor (PR) were performed on 49 CCC, 17 endometrial serous carcinomas (ESC), 46 endometrial endometrioid carcinomas (EEC) of all grades, and 25 non-neoplastic endometrial samples, all in a TMA. Cases were scored using a semi-quantitative system based on the percentage of nuclear-immunoreactive epithelial cells: 0 (0%); 1+ (1-25%); 2+ (26-75%); 3+ (>76%).

Results: At the≥1+ threshold for positivity, 30%, 100%, 84%, and 8% of CCC expressed ERα, ERβ, PR, and AR respectively. However, with the exception of ERβ, less than 7% of CCC expressed any hormone receptor in >25% of cells. 62% of CCC were NcOA3-positive (49% in >25% of cells), and typically in a diffuse pattern. In EEC, the rate of NcOA3 expression increased with increasing grade and decreasing ERα and PR expression. ERβ expression was ubiquitous. See table.

Percentage of Cases Showing Significant (≥2+) Immunoreactivity Within Epithelial Cells

Tissue Types	ERα	ERβ	PR	AR	NcOA3
CCC	6	100	2	4	49
ESC	50	100	56	57	50
EEC grade 1	94	100	88	50	0
EEC grade 2	84	100	88	42	10.53
EEC grade 3	67	100	67	50	33
Proliferative Endometrium	100	100	100	0	11
Secretory Endometrium	78	100	90	0	50
Atrophic Endometrium	100	100	100	40	0

Conclusions: A significant subset of CCC express hormone receptors, but generally at low levels and in a small proportion of tumor cells. However, a significantly larger subset of CCC (62%) express NcOA3, and typically in a diffuse pattern. These findings raise the possibility of a novel role for hormone receptor coactivators such as NcOA3 in CCC, whereby the upregulation of NcOA3 may allow the pathogenesis of this cancer to be driven at least partially by hormones, even if demonstrable hormone receptor expression is low.

1161 Intraoperative Diagnosis of Hysterectomies for Endometrial Carcinoma; How Reliable in Identifying Patients at Risk?

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Background: Hysterectomies for endometrial carcinoma are submitted for intraoperative consultation to identify patients who are at risk for extrauterine spread and require further staging. High risk parameters include high tumor grade or serous/clear cell types;deep myometrial invasion and cervical extension.Among these tumor grade and histological subtyping were found to be less reliable for intraoperative diagnosis. The aim of this study is to evaluate the accuracy of intraoperative diagnosis at identifying the high risk parameters that will lead to surgical staging in patients with endometrial adenocarcinoma.

Design: Frozen section and permanent diagnosis of 50 hysterectomies performed for endometrial adenocarcinoma at Marmara University Hospital between 2011-2013 were evaluated. As a routine practice depth of myometrial invasion,presence of cervical extension and tumor histological subtype were reported intraoperatively in all cases. Intraoperative reporting of tumor grade was present in only 27 of the cases.This was either because the clinicians did not demand it or due to the lack of pathologists experience. In all cases frozen section and final pathology reports are compared for high risk parameters.

Results: The agreement between intraoperative diagnosis and final pathology for depth of myometrial invasion and histological type were 86% (n:43) and 88% (n: 44) respectively. In 6 (12%) cases the depth of myometrial invasion changed in final report but in only 2 (4%) the error would affect patient management. In 5 cases diagnosis changed from endometrioid to serous/clear cell or malignant mixed mullerian types in the final report. In 4 of these cases deep myometrial invasion was also reported intraoperatively so the error in tumor type did not affect patient management. The agreement between intraoperative and final pathology for tumor grade was 77% (n:21). All errors related to grade were between grade 1 and 2 which would not affect operative procedure. Overall when all high risk parameters are evaluated together in only 3 (6%) cases the errors in intraoperative diagnosis leads to suboptimal operative management. All the error cases but one were evaluated by pathologists not experienced in gynecological pathology.

Conclusions: As a result intraoperatie evaluation of myometrial/cervical invasion, histological subtype and grade together combined with experience in gynecological pathology gives excellent results and very reliable in identifying patients for further treatment.

1162 Synchronous Ovarian and Appendiceal Mucinous Neoplasms in the Absence of Pseudomyxoma Peritonei

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Background: Synchronous ovarian and appendiceal mucinous neoplasms sometimes occur in patients without clinical evidence of pseudomyxoma peritonei (PP). It is unclear whether these neoplasms are related. We aimed to characterize and compare the immunophenotype of these synchronous tumors.

Design: We identified 10 cases of synchronous ovarian and appendiceal mucinous neoplasms with no clinical PP from our institutional archive over past 12 years. These cases were centrally reviewed and separated into subgroups 1 and 2 based on with or without microscopic peritoneal/ovarian surface mucin deposits (cellular or acellular). A 9 marker-immunopanel (CK7, CK20, CDX2, PAX8, MUC1, MUC2, MUC5AC, P53 and P16) was performed on representative tissues sections of the two tumors.

Results: Between subgroup 1 (4 cases) and subgroup 2 (6 cases), there were no significant differences in age, laterality, size and histologic type (benign to borderline) of ovarian tumors, and size of appendiceal tumors (all LAMN). In subgroup 1 cases, 2 developed PP later and both had ovarian surface and contralateral ovarian involvement, appendiceal perforation with microscopic serosal mucin deposits and acellular or cellular mucin on peritoneum. No subgroup 2 patients developed PP so far. The key immunoprofiling findings are summarized in Table 1.

Table 1: Immunophenotype Comparison of Subgroups 1 and 2

	Subgroup 1 (Case#)	Subgroup 2 (Case#)
Appendiceal / Ovarian Concordance		
9/9 markers (100%)	2 (50%)	1 (16.7)
8/9 markers (89%)	2 (50%)	-
7/9 markers (78%)	-	1 (16.7%)
6/9 markers (67%)	-	-
5/9 markers (56%)	-	
4/9 markers (44%)	-	2 (33.3%)
3/9 markers (33%)	-	2 (33.3%)
2/9 markers (22%)	-	-
1/9 markers (11%)	-	-
Anticipated Patterns --		
Appendiceal / Ovarian / Combined (if independent)		
A(PAX8-) / O(PAX8+) / Combined	4 / 0 / 0	6 / 3 / 3
A(PAX8-CDX2+) / O(PAX8+CDX2-) / Combined	4 / 0 / 0	6 / 2 / 2
A(CK7-CK20+) / O(CK7+CK20-) / Combined	3 / 0 / 0	2 / 3 / 2
A(CK7-CK20+CDX2+) / O(CK7+CK20-CDX2-) / Combined	3 / 0 / 0	2 / 2 / 1
A(MUC2+MUC5AC-) / O(MUC2-/MUC5AC+) / Combined	2 / 0 / 0	3 / 3 / 3

All subgroup 1 cases showed a high degree of concordance in immunoprofile between appendiceal and ovarian tumors, with identical expression of appendiceal pattern in >90% of markers. In subgroup 2 tumors, however, the concordance was poor, with only one case showing concordance in all markers. Two cases showed CDX2+/PAX8- LAMNs and CDX2-/PAX8+ ovarian mucinous cystadenomas, and another case showed contrasting appendiceal/ovarian patterns for CK7 (-/+), CK20 (+/-), CDX2 (+/-), MUC2 (+/-), MUC5AC (-/+), and p16 (-/+).

Conclusions: When there is peritoneal and/or ovarian surface involvement, even only microscopic, the synchronous tumors were all of appendiceal primary. Of those without peritoneal lesion, however, they were heterogeneous with some most in keeping with truly independent primary tumors. Further molecular characterization is needed to confirm these interpretations.

1163 Is Recurrent Endometrial Carcinoma Due to Occult Lymph Node Metastases?

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Background: Low grade endometrioid adenocarcinoma (EC) is typically confined to the uterus and rarely recurs. To determine if occult or micrometastases are missed by standard sectioning or could play a role in recurrent EC, the negative LNs in patients (pts) with recurrent EC were subjected to our institutional ultrastaging protocol.

Design: 15 FIGO stage I/II EC with documented recurrence from 1990-2008 with available LN blocks were submitted for ultrastaging as follows: 5 H&E slides at 250µ intervals with 2 unstained slides at the first level for immunoperoxidase studies if needed. 22 FIGO stage I/II EC with negative LNs and no recurrence served as a control. %myometrial invasion (MI), cervical (CX) stromal involvement, lymphovascular invasion (LVI), #pelvic LNs an #para aortic LNs were recorded.

Results: The histologic variables for recurrent and non recurrent EC are summarized in table 1. Recurrence sites included: vagina, 5 pts; lung, 5 pts; pelvis, 4 pts; para aortic LN, 2 pts; liver, 1 pt. A total of 378 LNs were reviewed: 146 in recurrent EC (111 pelvic; 35 para aortic) and 232 in non recurrent EC (181 pelvic; 51 para aortic). No evidence of occult or micrometastatic EC was detected in any of the LNs.

Histologic Variables in Recurrent and Non Recurrent EC

Variable	Recurrent EC (n=15)	Non Recurrent EC (n=22)
%MI (median)	10	37.5
CX involvement	2 (13%)	2 (9%)
LVSI	4 (27%)	9 (40%)
#Pelvic LNs (median)	6	7.5
#Para aortic LN (median)	2	1

Conclusions: Contrary to what would be anticipated, the non recurrent EC group had greater MI and a higher percentage of tumors with LVI. There was little difference in the median numbers of LNs obtained between recurrent and non recurrent EC. Occult metastases appear to play no role in recurrent EC based on this limited sample. The development of recurrent EC may be related to non lymphatic modes of spread and possibly molecular factors. Additional studies are necessary to elucidate factors that might help predict recurrences in EC.

1164 Comparative Analysis of Napsin A, AMACR, and Hepatocyte Nuclear Factor 1β as Diagnostic Markers of Ovarian Clear Cell Carcinoma: An Immunohistochemical Study of 260 Ovarian Tumors

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Background: The purpose of this study is to comparatively assess the diagnostic utilities of 3 putative markers of ovarian clear cell carcinoma (CCC) - Napsin A, Alpha-methylacyl-CoA racemase [AMACR], and hepatocyte nuclear factor 1-beta [HNF1β] - in a large cohort of ovarian tumors.

Design: Immunohistochemical studies for Napsin A, AMACR, and HNF1β were performed on TMA slides from 260 ovarian neoplasms, including CCC (n=65), serous carcinoma (n=101), endometrioid carcinoma (n=19), mucinous carcinoma (n=33), adult-type granulosa cell tumor (n=18), yolk sac tumor (n=7), dysgerminoma (n=8), and miscellaneous other tumors [transitional cell carcinoma (n=3), sertoli-leydig cell tumor (n=2), and one each of carcinosarcoma, neuroendocrine carcinoma, thecoma, and strumal carcinoid]. Cases that displayed immunoreactivity in≥1% of tumor cells were classified as positive.

Results: Table I

Percentages of Immunoreactive Cases

Tumor type	HNF1β (% positive)	Napsin A (% positive)	AMACR (% positive)
Clear cell carcinoma	92	82	63
Serous carcinoma	7	1	1
Endometrioid carcinoma	58	5.3	0
Mucinous carcinoma	67	0	0
Granulosa cell tumor	0	0	0
Yolk Sac Tumor	100	0	0
Dysgerminoma	0	0	0
Miscellaneous other types	0	0	0

Table 2

Clear Cell Carcinoma Versus All Other Tumors: Diagnostic Performances Of The Various Immunoprofiles

Immunoprofiles	Number of cases	Odds ratio (OR; 95% CI)	P value	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
HNF1β[+]	103	42 (16-112)	<0.0001	0.92	0.78	0.58	0.97
AMACR[+]	54	165 (37-725)	<0.0001	0.63	0.99	0.95	0.89
Napsin[+]	55	857 (109-6740)	<0.0001	0.82	0.99	0.98	0.94
HNF1β[+]/ AMACR[+]/ Napsin[+]	42	707 (42-11872)	<0.0001	0.65	1	1	0.89
HNF1β[+]/ AMACR[-]/ Napsin[+]	13	43.9 (5.6-345)	0.0003	0.18	0.99	0.92	0.79
HNF1β[+]/ AMACR[-]/ Napsin[-]	47	0.47 (0.2-1.1)	0.0825	0.11	0.79	0.15	0.73
HNF1β[+]/ AMACR[-]/ Napsin[-]	1	1.02 (0.4-25)	0.99	0	0.99	0	0.75

Conclusions: Although Napsin A, AMACR, and HNF1β are all viable markers of CCC, Napsin A most optimally combines high sensitivity and high specificity. HNF1β is highly sensitive but is limited by its suboptimal specificity; AMACR is highly specific but is relatively insensitive. Using CCC as a diagnostic end-point relative to all other tumors, the best performing immunoprofiles are Napsin A positivity (OR 857) and the co-expression of all three markers (OR 707). The latter, however, has a suboptimal sensitivity of only 0.65. Therefore, Napsin A (sensitivity 0.82; specificity 0.99) is the single best marker for CCC.

1165 Are Clear Cell Carcinomas of the Ovary and Endometrium Phenotypically Identical? A Proteomic Analysis

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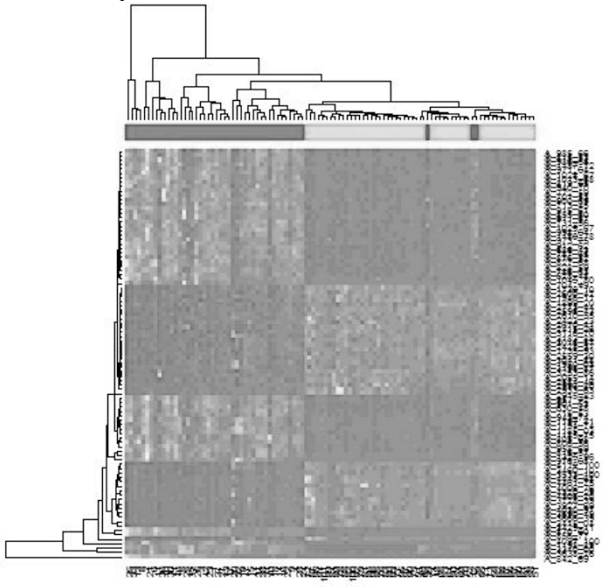
Background: Clear cell carcinomas of the endometrium (E-CCC) are extraordinarily rare, which is a limitation on investigative efforts into their molecular basis and the development of molecularly-directed therapies. Clear cell carcinomas of the ovary (O-CCC), in contrast, are significantly more common, and accordingly have been studied more extensively. Although these tumors are recognized to display broad phenotypic overlap, it is unclear how readily findings in O-CCC can be extrapolated to E-CCC. In the present study, the authors utilized a proteomic technology, matrix-assisted laser desorption ionization imaging mass spectrometry (MALDI IMS), to assess and quantify the differences between O-CCC and E-CCC. MALDI IMS supplements gene expression approaches in that it accounts for postranslational modifications to proteins, and also supplements immunohistochemical approaches by screening for large numbers of proteins without a specific target.

Design: Slides from TMAs containing 54 O-CCC and 47 E-CCC were analyzed by MALDI IMS and unique spectral peaks were identified for every 0.05 mm^2 of tissue. Non-viable tissues and tumor-associated stroma were excluded prior to subsequent analyses. A linear mixed effect model with random intercept was fit for each spectral peak, and effect size (mean peak area difference between the two groups, or ratio of mean peak area of E-CCC to O-CCC) for each peak were determined. p values were obtained from each model, and were subsequently adjusted using the false discovery rate.

Results: From 730 normalized peaks in samples from both groups, 93 (12.7%) showed a≥1.5 fold difference (and at an adjusted p value cutoff of $1e^{-10}$) between E-CCC and O-CCC. Figure 1 shows results from a cluster analysis of these peaks (red, left side of panel is E-CCC; green is O-CCC). The underlying heat map is based on the median peak area of each patient.



Conclusions: Our findings suggest that there are demonstrable proteomic differences between O-CCC and E-CCC, quantified here at 12.7%. Although this figure may be modulated by a variety of analytic factors, it may also be explained by the different microenvironments in which these tumors originate, with a significant subset of O-CCC arising from the highly oxidative environment of endometriotic cysts.

1166 Increased Expression of Monocarboxylate Transporters 1 and 4 Correlates with Histologic Type of Uterine Cervical Cancer

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Background: Cancer cells are dependent on anaerobic glycolysis for their high energy needs. This leads to production of large quantities of lactate, which is transferred in/out the cells by Monocarboxylate Transporters (MCTs). MCTs have been shown to be upregulated in various tumors. In cervical cancer, a lactate-rich microenvironment has been associated with poor prognosis. Furthermore, MCT immunoexpression has been described to increase from preinvasive to invasive carcinomas. Our aim was to study the role of MCTs in cervical cancer by evaluating the immunohistochemical expression of MCT1 and MCT4 in a large series of cervical lesions (preneoplastic and neoplastic) and assessing its clinical-pathological value.

Design: Tissue microarrays with 11 CIN3, 70 invasive squamous cell carcinomas(SCC), 4 adenocarcinomas in situ and 37 invasive adenocarcinomas(ADC) were immunohistochemically stained for MCT1 and MCT4. Immunoreactions were evaluated quantitatively. Clinical data and follow-up were recorded.

Results: Tissue from 122 patients (median age: 46 years old; mean of follow-up: 54 months; FIGO stages I(55%), II(21%), III(6%) and IV(6%)) was included. We excluded ten cases from the analysis because they were either lost to follow-up or tissue was lost. MCT1 was present in 72% and 32% of SCC and ADC cases, respectively. MCT4 expression was found in 37% and 68% of the cases in SCC and ADC. We found a

significant association of the expression of MCT1 with SCC ($p<0.001$) and of MCT4 with ADC ($p<0.001$). There was evidence of increasing expression of MCT4 from CIN3 lesions to SCC (0% vs. 59%, $p<0.05$) but not of MCT1 or from adenocarcinoma in situ to ADC. Increased MCT1 expression was correlated with metastasis in ADC ($p<0.05$). No correlations were found between the association of MCT1 or MCT4 expression and stage, local recurrence or death from disease.

Conclusions: The association between MCT1 and MCT4 expression and the histological type might underline a different metabolic profile in these two histological types. MCT4 expression was found to increase from preinvasive squamous lesions to invasive squamous cell carcinomas. MCT1 expression is associated with lymph node or distant metastasis in adenocarcinoma. These findings suggest that MCT1 and MCT4 may have an important role in cervical cancer progression.

1167 Aldehyde Dehydrogenase Positive Cancer Stem Cells Are at the Center of an Ovarian Cancer Stemness Hierarchy

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Background: Chemoresistant relapse is the main cause of mortality in ovarian cancer. A residual population of cancer stem cells (CSCs) is believed to drive recurrence. Therapeutically targeting CSCs may avert chemoresistant relapse. Multiple CSC populations have been identified within ovarian cancers. A single therapeutic approach may be able to target multiple CSC populations if they are part of one stemness hierarchy.

Design: Two models of ovarian cancer were screened for CSCs. The A2780 cell line is a model of cisplatin sensitive ovarian cancer. The A2780cis cell line is a cisplatin resistant model derived from the A2780 cell line. ALDEFLUOR™ (ALDH) was used via flow cytometry/fluorescence-activated cell sorting to detect/isolate putative CSCs (pCSCs). pCSCs were validated in NOD.SCID mouse tumorigenicity assays (malignant potential) and single cell asymmetric division (AD) assays (differentiation and self-renewal potential). Gene analysis was done using Affymetrix HuGene2.0 microarrays.

Results: ALDH+ pCSCs and ALDH- non-pCSCs, isolated from the A2780 and A2780cis cell lines, were validated both *in vivo* and *in vitro*. Tumorigenicity studies showed that both populations grew tumours from 500 cells with equal efficiency. AD assays showed that ALDH+ cells are capable of self-renewal and differentiation. Additionally, it showed that the ALDH- population consisted of two cell types: i) ALDH_NegA cells, which can differentiate and self-renew to produce ALDH+ and ALDH- cells and ii) ALDH_NegB cells, which can not differentiate and only produce ALDH- cells. Xenograft studies indicate that ALDH_NegB have a reduced malignant potential. Microarray analysis identified overexpression of ALDH1A2, CXCR4 and retinoic acid (RA) signalling genes in ALDH+ cells relative to ALDH- cells.

Conclusions: These results are indicative of an ALDH_NegA□ALDH+□ALDH_NegB ovarian cancer stemness hierarchy. In which both ALDH_NegA and ALDH+ populations are tumorigenic. Overexpression of CXCR4 in ALDH+ cells indicates a possible link to metastasis. Overexpression of RA signalling genes indicates that RA may be able to differentiate ALDH+ cells. This suggests RA could be used to treat ovarian cancer patients with ALDH+ CSCs. It is advantageous to identify such hierarchies, as targeting a stemness hierarchy, as opposed to multiple CSC populations, may be sufficient to remove the malignant potential from a tumor. ALDH_NegA/NegB markers have yet to be identified. Further microarray analysis is under way to identify such markers.

1168 Molecular Characterization of Melanomas Originating from Gynecologic Sites

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Background: Melanoma does not respond to traditional chemotherapy/radiation, so targeted therapies are very important. Advances in molecular diagnostics have identified mutations in *BRAF*, *NRAS* & *ckit* genes. The rates of mutations vary by anatomic site & sun-exposure status. *NRAS* mutations tend to occur with chronic sun exposure, *BRAF* in non-sun exposed skin and *ckit* in mucosal sites & chronically sun-exposed skin. Targeted therapies exist for *BRAF* & *ckit* mutated tumors. To our knowledge, no study to date has systematically characterized the incidence of molecular mutations in these genes in melanomas originating from gynecologic sites (MOGS): vulva, vagina and cervix. Vulvar melanomas often originate in skin, while vaginal, cervical (and some vulvar) melanomas arise in squamous mucosa. This study examines whether MOGS have similar mutations as a group & whether mutational status matches those previously reported for skin & mucosal melanomas.

Design: 60 patients with MOGS were identified & H&E stained sections were reviewed for the accuracy of diagnosis, histopathologic features, & presence of adequate tumor for molecular analysis. 32 cases had adequate FFPE tissue for analysis. Utilizing the Pinpoint Slide DNA Isolation System™ followed by PCR suitable amplified DNA was extracted from 27 cases. Sanger sequencing was performed for the 3 genes in question & analysis of known mutations sites was undertaken. We compared rates of mutation for skin vs mucosal lesions & reviewed histopathologic features of tumors with a mutation looking for similarities.

Results: We found 5 of 27 MOGS (18.5%) harbored a mutation in the *ckit* gene: 3 insertion/deletions (two in exon 14, one in exon 15) & 2 point mutations (exon 14). None of the cases had mutations in the *BRAF* or *NRAS* genes. Two cases had been previously tested for *BRAF* & *ckit* mutations, one had a *ckit* mutation in exon 14. The 6 tumors with *ckit* mutations included 1 arising in the vagina (mucocsa) & 5 arising in the vulva (2 involving skin & mucosa and 3 skin only). Of note, 4 cases had co-existing atypical nevi present.

Conclusions: Though MOGS can arise from either skin or squamous mucosa, they are molecularly more similar to each other & follow the mutation pattern previously described in mucosal melanomas (*ckit* mutations). While 65% of all melanomas from the skin harbor a mutation in *BRAF*, not a single case in our study was mutated. These findings my help guide clinicians when choosing molecular testings for patients with MOGS. The patients with confirmed *ckit* mutations may be benefit from tyrosine kinase inhibitors such as imatinib mesylate (Gleevec).

1169 Malignant Mesonephric Tumors of the Female Genital Tract: A Clinicopathologic Study of 14 Cases

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Background: Malignant mesonephric tumors (MMTs) are rare neoplasms of the female genital tract, arising primarily in the cervix, and they are considered to be derived from remnants of the mesonephric duct. Because of their rarity, the biologic behavior of MMTs is not well known. Extrauterine tumors have been rarely reported.

Design: Fourteen cases of MMT were clinicopathologically studied to further understand the histologic diversity, histogenesis, and biologic behavior.

Results: The patients ranged in age from 46 to 73 years (mean, 58 years). There were 10 adenocarcinomas (ACs) and 4 malignant mixed mesonephric tumors (biphasic tumors with a sarcomatoid component) (MMMTs). The ACs were found in the cervix (6), meso-ovary or ovary (3), and uterine body(1). The MMMTs involved the meso-ovary or ovary (2), uterine cervix (1), anduterine body (1). ACs showed variable proliferative patterns, including glandular, tubular, papillary, retiform, and solid. One of the ACs exhibited cartilaginous differentiation in the lung metastasis. Among the MMMT subgroup,3 were homologous and 1 was heterologous (cartilage). One each of AC and MMMT had mixed epithelial elements, including serous and endometrioid adenocarcinomas. Mesonephric remnants or hyperplasia was observed in 2 cervical ACs and 1 meso-ovarian MMMT. One ovarian AC was associated with endometriosis. Immunohistochemically, all tumors were positivefor CAM5.2, CD10, and calretinin and negative for ER, PgR, and CEA. Of the 11 patients with follow-up information available (mean, 60 months), 3 patients with MMMT (2 in meso-ovary or ovary, 1 in uterine body) died of spread disease at 25, 51, and 100 months, respectively, after surgery. No patients with AC and follow-up information died of disease. One patient was alive with lung metastases and the remaining 7 had no evidence of disease 6 to 76 months postoperatively.

Conclusions: The diversity of histologic patterns was characteristically observed. Seven cases arose outside of the uterine cervix, and mesonephric remnants or hyperplasia was found in only 3 cases. Since two cases exhibited mixed epithelial elements and one was associated with endometriosis, some MMTs without mesonephric remnants may be considered adenocarcinoma with mesonephric differentiation. The possibility of a mesonephric tumor should be considered when encountering an unusually appearing carcinoma, malignant mixed tumor, or even an endometrioid adenocarcinoma. All of the patients with AC and follow-up information survived. In contrast, 3 of 4 patients with MMMT died of disease. MMMTs may be more aggressive than ACs.

1170 Early Stage Placental Mesenchymal Dysplasia: A Clinicopathologic Study of 10 Cases with Gestational Age Less Than 20 Weeks

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Background: Placental mesenchymal dysplasia (PMD) is characterized by placentomegaly and may be mistaken for partial hydatidiform mole (PM) or complete mole (CM) with co-twin both clinically and macroscopically because of the presence of "grape-like vesicles". It may be associated with a normal fetus, a fetus with intrauterine growth restriction, or a fetus with features of Beckwith-Wiedemann syndrome (BWS). However, PMD is both underdiagnosed and underreported. PMD with gestational age less than 20 weeks is rarely reported.

Design: In order to elucidate the clinical presentation, complications, microscopic features, and differential diagnoses, 10 cases of PMD with gestational age less than 20 weeks were clinicopathologically analyzed. Immunohistochemical study of p57 (Kip2) (p57) and TSSC3, both are products of a paternally imprinted, maternally expressed gene, was also done.

Results: Maternal ages ranged from 23 to 40 years (mean age, 32 years). Five patients were initially diagnosed as PM and one was as CM with twin on ultrasound examination. The gestational periods ranged from 12 to 19 weeks. One case was associated with BWS. Histologically, early stage PMD was characterized by moderate swelling of stem villi with cistern formation, myxoid change, dilated veins, mild stromal cell proliferations, and the absence of trophoblastic hyperplasia. Dilated subchorionic vascular vessels with or without luminal thrombosis, or chorangiosis, which were observed in the third trimester PMD, were not found. Cytotrophoblasts were positive for p57 and TSSC3 in all cases. Regarding p57, villous stromal cells were diffusely positive in 3 cases, focally positive in 5, and uniformly negative in 2. TSSC3 was negative in stromal cells in all cases.

Conclusions: PMD presents with a wide spectrum of clinicopathologic findings. It is important to identify PMD cases prenatally to reduce fetal morbidity and mortality. Early stage PMD can be clinically or pathologically misdiagnosed as abortion, PM, or CM with a twin. Histologic features in early stage PMD are less distinctive compared with those of PMD in the third trimester. The diagnostic clues are moderate swelling of stem villi with cistern formation, myxoid change, dilated veins, and mild stromal cell proliferations, and the absence of trophoblastic hyperplasia. The p57 and TSSC3 immunohistochemical study is useful for differential diagnoses in equivocal cases. It is important to identify PMD cases in early stage to reduce fetal morbidity and mortality.

1171 Twin Placenta with Complete Hydatidiform Mole and Its Mimics: Histologic Diagnosis and Immunohistochemistry for the Imprint Gene Products P57 (Kip2) and TSSC3

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Background: Twin pregnancies with a normal placenta and complete mole (CM) are very rare, and they may pose a diagnostic challenge. Microscopically, they resemble partial mole. The differential diagnosis is very important for patient management. p57 (Kip2) (p57) and TSSC3 are products of paternally imprinted, maternally expressed genes.

Design: Twelve cases of morphologically putative twin pregnancies with CM, all of which were in the first trimester, were retrieved from a file of 526 CMs, and they were analyzed by the immunostaining of p57 and TSSC3. DNA ploidy in six of the 12 cases was also analyzed by flow cytometry, and all of them were diploid.

Results: The admixture of large hydropic villi with circumscribed trophoblastic hyperplasia and smaller, normally appearing villi without trophoblastic hyperplasia was observed in five cases. The cytotrophoblasts and stromal cells in larger villi were negative for p57 (androgenic) and TSCC3, whereas these cells were positive in the smaller villi (biparental). The five cases were diagnosed as CM with twin (twin pregnancy with a coexisting normal placenta and CM). In the remaining seven cases, some villi had histologically typical CM features and some showed edema, stromal cell hyperplasia, and the absence of trophoblastic hyperplasia. Some villi exhibited hydropic stroma and focal trophoblastic hyperplasia. The villi with CM features showed typical immunohistochemical profiles of p57 and TSCC3. In villi with edema, stromal cell hyperplasia, and the absence of trophoblastic hyperplasia, cytotrophoblasts were positive for p57 and TSSC3 (biparental) and stromal cells were negative for them (androgenic). In villi with hydropic stroma and focal trophoblastic hyperplasia, TSSC3 was focally expressed. The seven cases had been erroneously diagnosed as CM with twin. The findings suggested that the seven cases were CMs due to androgenic/biparental chimera or a mosaic, although molecular cytogenetic analysis was not done.

Conclusions: The findings support the hypothesis that misexpression of p57 and TSSC3 is involved in the abnormal development of androgenic CMs. The immunohistochemical analysis is a useful tool for the differential diagnosis of twin placenta with CM and morphologically challenging CM cases. CMs probably due to androgenic/biparental chimera or a mosaic histologically mimic twin with CM. Immunohistochemistry for the imprint gene products p57 and TSSC3 can be a useful screening tool for cytogenetic analyses.

1172 Ovarian Neoplasia Associated with Lynch Syndrome Is of Non-Serous Histotypes

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Background: Ovarian carcinoma is the third most common tumor type in patients with Lynch Syndrome (LS), after colorectal and endometrial carcinomas, and patients with LS have a 10-12% lifetime risk of developing ovarian carcinoma. Few cases series describing the histotype of the ovarian tumors in patients with LS have been reported and these typically lacked either central pathological review or application of current diagnostic criteria for determination of ovarian carcinoma histotype. This study was undertaken to determine the spectrum of ovarian carcinoma histotypes seen in patients with LS.

Design: We collected 20 cases of ovarian carcinoma in patients known to have LS. All slides from these cases were reviewed and ovarian carcinoma histotype assigned, without knowledge of the previous diagnosis.

Results: In 19 of 20 patients information regarding the exact LS mutation was available: MSH2 mutations were most common, being present in 11 patients, while 3 patients had MLH1 mutations and 2 had MSH6 mutations. The ovarian carcinomas, based on review, were of the following histotypes: 10 endometrioid carcinomas, 4 clear cell carcinomas and 5 mixed carcinomas (3 mixed endometrioid/clear cell, 1 mixed endometrioid/malignant Brenner, 1 mixed endometrioid/seromucinous borderline tumor). The final tumor was a de-differentiated carcinoma. There were no serous carcinomas or serous borderline tumors. Although mucinous differentiation was common in the endometrioid carcinomas, there were no mucinous tumors of intestinal type. In 2 cases the histotype diagnosis changed based on review (from mucinous to endometrioid and from high-grade serous to endometrioid, respectively).

Conclusions: All ovarian carcinomas associated with LS in this series were of non-serous histotypes, and thus completely non-overlapping with the histotype of ovarian carcinomas associated with the other common autosomal dominant hereditary cancer susceptibility syndrome, Hereditary Breast and Ovarian Cancer syndrome. The carcinomas were of histotypes associated with endometriosis. These findings have implications for screening of ovarian carcinomas for mismatch repair enzyme expression by immunohistochemistry, as well as genetic counseling and mutation testing for LS.

1173 Application of p16 Immunostain in Classification of Adenoid Basal Tumors of the Cervix

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Background: Our understanding of adenoid basal tumors of the cervix has evolved over time. Most of the proliferations referred to as "adenoid basal carcinomas" have a clinically benign course - leading some to suggest the term "adenoid basal epitheliomas". However, it has been acknowledged that rarely, these may be associated with invasive carcinomas. Here we investigate the role of p16 in the classification of these tumors.

Design: A database search was conducted from January 1990 to June 2013 for adenoid basal lesions of the cervix. The hematoxylin and eosin stained slides were reviewed. Immunohistochemical stain for p16 was performed on representative sections with adequate controls. The histologic features, staining patterns and the clinicopathologic data were recorded.

Results: Ten cases of adenoid basal tumors of the cervix were retrieved. The patient age ranged from 53 to 77 years and the average age was 67.7 years. The specimens included 4 hysterectomies, 3 loop electrosurgical excisions, 2 biopsies and 1 polypectomy. Seven cases revealed bland histology consistent with adenoid basal epithelioma. Other three cases showed an invasive cytologically malignant component (2 adenoid basal/squamous carcinomas, 1 papillary squamous cell carcinoma) intermixed with a bland epithelioma component. All non-invasive adenoid basal epithelioma cases showed either negative or patchy cytoplasmic p16 immunostaining pattern. In contrast, all invasive carcinomas showed strong and diffuse nuclear p16 immunostaining pattern. Follow-up was available in seven patients and showed no evidence of disease (duration: 3-60 months, average: 13 months).

Conclusions: Distinct p16 immunostaining patterns exist between non-invasive adenoid basal epitheliomas and invasive adenoid basal carcinomas. Diffuse and strong nuclear p16 staining associates with invasion. Our study indicates that p16 immunostaining can be a useful tool in differentiating between non-invasive and invasive adenoid basal tumors along with careful histopathologic evaluation.

1174 Immunohistochemical Expression of GATA3 in Primary Extramammary Paget Disease of the Vulva

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Background: GATA binding protein 3 (GATA3) plays a critical role in breast development by promoting luminal cell differentiation and has proven to be a useful immunohistochemical marker for breast carcinomas. Breast tissue has a close resemblance to apocrine/eccrine glands due to similar embryologic derivation. Further, it is speculated that extramammary vulvar Paget disease has an apocrine/eccrine origin. Given these considerations, in this study, we investigated the expression of GATA3 protein in primary vulvar Paget disease.

Design: A database search was conducted from January 2000 to September 2013 for biopsies/excisions of vulvar Paget disease. Clinical history of any other internal malignancy was recorded and cases of secondary Paget disease were excluded. The majority of cases that were included for further study had undergone immunohistochemical workup for confirming primary nature of the disease. Immunohistochemical stain for GATA3 was performed on representative sections and the staining patterns and clinicopathologic data were recorded. Staining was recorded as positive (nuclear staining) or negative with the intensity as weak, moderate or strong and the distribution as focal (< 50% tumor cells) or diffuse (≥ 50% tumor cells).

Results: Thirty-three cases of vulvar Paget disease from 20 patients were retrieved: 30 cases with intraepithelial Paget disease and 3 cases with invasive carcinoma. Patient age ranged from 57 to 85 years with a mean of 72 years. Eleven patients had multiple recurrences of Paget disease. No history of urothelial carcinoma or colorectal adenocarcinoma was present in any of the patients. GATA3 immunostain exhibited diffuse nuclear staining in Paget cells in all the 33 cases - with strong intensity in 31 cases and with moderate intensity in 2 cases. The Paget cells were easily distinguishable from the surrounding keratinocytes due to their abnormal nuclear morphology and more intense staining pattern. There was no difference in staining patterns between the intraepithelial and the invasive components.

Conclusions: Our study demonstrates that GATA3 protein is overexpressed in vulvar Paget cells. Given its expression in both the intraepithelial and the invasive components, it may be involved in the early pathogenesis of vulvar Paget disease. GATA3 is a highly sensitive immunohistochemical marker for primary extramammary vulvar Paget disease and can be employed as a useful ancillary tool in its diagnosis.

1175 Clinico-Pathologic Characteristics of Female Anal Pap Smears in an Urban County Hospital

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Background: It is projected that over 4000 women will be diagnosed with anal cancer in 2013. There are no national screening guidelines or consensus criteria for anal cytology (AC); however, cervical cancer screening guidelines are often used. In this study, we aimed to delineate the characteristics of women who have AC screening at an urban hospital.

Design: A retrospective chart review for all female AC smears was performed between July 2004 to July 2013. Demographic information, cervical pap, genital histology, and information related to HPV and HIV status were also obtained.

Results: A total of 139 AC smears from 88 patients were identified, including five transgender patients. The mean age was 42 . The mean time between AC was 14.6 months. The vast majority were African American (85.1%) with smaller numbers of Caucasian (10.4%), Hispanic (3.4%), and American Indian/Alaskan (1.1%). The data on AC diagnoses are located in Table 1. 22% of AC had a corresponding anal histology, of these, 32% had AIN2 or 3. 88.6% of patients were HIV positive and 44.3% had AIDS. Table 2 contains data concerning AC diagnosis and HIV/AIDS status. There was no statistical significance between AC diagnosis and HIV/AIDS status. The HPV status was known for 56 patients, with 79% being positive. Of the patients with cervical cytology, 70% with an abnormal AC also had an abnormal cervical pap. Clinically visible lesions were associated with 20% of AC.

	Unsat	*NILM*	*ASC-US*	*LGSIL*	*ASC-H*	*Adenocarcinoma*
% of total paps	6.5%	27.4%	30.2%	30.2%	4.3%	1.4%

Table 1

	NILM	*ASCU-US*	*LGSIL*	*ASC-H*
HIV+	34	38	41	6
HIV-	4	4	1	0
AIDS	12 (p=0.1)	20 (p=0.7)	23 (p=0.3)	4 (p=0.7)
Non-AIDS	20	17	17	2

Table 2

Conclusions: Our study shows a predominantly African American population with a large burden of HIV. Our population had a high pre-test probability of abnormal AC due to the prevalence of HIV or the presence of clinical lesions. More than 60% of patients had an abnormal AC, but no patients had an AC diagnosis of HSIL. All of the HSIL diagnosed on subsequent histology were missed by AC. This may indicate that less conservative diagnostic criteria than the Bethesda system should be applied to AC . We found that there was no statistical significance between a positive HIV/AIDS status and severity of AC diagnosis. Historically patients who are immunosuppressed have a higher burden of SIL. Further study may be needed to define diagnostic criteria specific for AC.

1176 Gastrointestinal- and Müllerian-Type Epithelium in Ovarian Mucinous Cystadenoma: Immunohistochemical Analysis of 139 Cases

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Background: Ovarian mucinous tumors show stepwise malignant transformation from mucinous cystadenoma (MA) to mucinous borderline tumor (MBT) and to mucinous adenocarcinoma (MCa). Two subsets of MBT have been established: gastrointestinal (GI) and Müllerian (M) types. However, no such distinction is made for MAs. An association between Müllerian derivatives and M-type mucinous tumors has been reported, while the origin of GI-type mucinous epithelium is unclear. This study analyzed a series of MAs immunohistochemically using gastric, intestinal, and Müllerian markers to elucidate the direction of the differentiation of mucinous epithelium that arises in the ovary and to seek a link between GI- and M-type epithelium.

Design: We retrieved 139 cases of MA. The tumors were initially evaluated morphologically for presence or absence of GI- and M-type epithelium. Representative slides of morphologically pure GI- and M-type MAs were chosen. If a transition from M-type epithelium to GI-type epithelium was observed, slides containing such areas were sent for immunohistochemistry. The expression of the gastric marker claudin-18 (CLDN18) and intestinal (CDX2) and Müllerian (ER) markers was evaluated immunohistochemically. The epithelium that was positive for CLDN18 or CDX2 was designated as "GI type", and the epithelium showing the CLDN18–/CDX2–/ER+ phenotype as "M type".

Results: GI-type epithelium characterized by CLDN18 or CDX2 positivity was present in 93% (129/139) of the MAs. Of these, 14 were associated with teratomas and one with a Brenner tumor. A transition from M-type (CLDN18–/CDX2–/ER+) epithelium to GI-type epithelium was seen in 12 cases (9%). Although most GI-type epithelium was ER-negative, scattered ER positivity was observed in 18 cases (14%). A rare subset of MAs (eight cases) was purely Müllerian type, consisting of epithelium with Müllerian morphology and diffuse ER expression.

	CLDN18	CDX2	ER
-	12	99	101
1+	3	16	11
2+	6	10	8
3+	29	11	6
4+	89	3	13
	127/139 (91%)	40/139 (29%)	38/139 (27%)

Conclusions: Ovarian MAs can be subclassified into GI and M types. In GI-type tumors, the gastric phenotype is predominant. Since the transition from M-type epithelium to GI-type epithelium is seen in some cases, we conclude that GI-type epithelium can arise not only from teratomatous lesions or Brenner tumors but also from Müllerian duct derivatives or ovarian surface epithelium via metaplastic/neoplastic processes.

1177 Undifferentiated Uterine Sarcomas Can Be Divided into Two Prognostically Distinct Groups Using Mitotic Index

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Background: Undifferentiated uterine sarcomas (UUS) are rare primary uterine sarcomas. They appear to have a heterologeneous biology and a poor prognosis. The goal of this study was to examine the role of various clinical and pathological variables in the prognosis of these tumors.

Design: Twenty-six cases of UUS were identified in the archives of the Karolinska University Hospital. All original slides were re-reviewed and a variety of clinical and pathologic parameters were recorded, including age at diagnosis, mitotic rate, presence of tumor necrosis, tumor size, presence of lymphovascular invasion, and grade of nuclear atypia. Follow up data in the form of overall survival was available on all patients.

Results: Analysis of mitotic rate demonstrated that these tumors could be clearly divided into two groups. One group (n=10 cases) had an extremely high mitotic rate (mean 36.8 ± 8.6) while the other (n=16 cases) had a lower rate (mean 8.7 ± 5.8). No cases had a mitotic rate between 18 and 30. Grouping the cases according to mitotic rate high vs. low demonstrated a statistically significant difference in overall survival by Kaplan-Meier analysis (p=0.00391). None of the other parameters evaluated showed a correlation with overall survival, including grade of atypia, presence of tumor necrosis, stage, and age at diagnosis.

Conclusions: These results indicate that UUS can be divided into two prognostically relevant groups using mitotic rate. Although this study involved only twenty-six cases, a statistically significant difference could be demonstrated. Although UUS has previously been considered to have a uniformly poor prognosis, these results indicate that clinically relevant subgroups exist within this tumor type.

1178 Unique ("SET") Histologic Features Correlate with Tubal Intraepithelial Carcinoma in Women with and without BRCA Mutations

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Background: Extra-uterine high-grade serous carcinoma (HGSC) has been linked to the distal fallopian tube via an early preinvasive intraepithelial neoplasm (TIC). TIC is found in approximately 5% of prophylactic salpingo-oophorectomies of women with BRCA1 or BRCA2 mutations (BRCA+) and 40% of unselected HGSCs. We have recently discovered that TICs are paradoxically less commonly found with high stage HGSC, raising questions about the origin of many HGSCs in this population. We sought to validate this discovery in more than one context by correlating specific tumor patterns with TIC frequency.

Design: A series of documented BRCA+ and BRCA- HGSCs that were analyzed by the SEE-FIM protocol were scored for 1) associated TIC and 2) pattern of serous carcinoma differentiation, including SET features (Solid, pseudo-Endometrioid and Transitional; Soslow 2012) and "classic "serous differentiation. Ovaries from a separate subset of tumors lacking TIC were analyzed by calretinin staining to confirm or exclude neoplastic transformation of the ovarian surface epithelium (OSE).

Results: 24 and 30 BRCA+ and BRCA- cases were studied; 5/24 (21%) and 16/30 (53%) were TIC+ (p = .02). Of 20 and 28 informative for optimal histologic classification, 9 (45%) and 2(7%) were SET+ (p=.004). One of 4 BRCA+ TIC+ cases were SET+ and 0 of 14 TIC+ BRCA- cases were SET+. Overall 17 of 18 (94%) TIC+ cases showed classic serous differentiation versus 20 of 30 (67%) TIC- cases (p = .035). Analysis of 100 ovarian histologic sections from 25 additional TIC- cases did not reveal evidence of transformed OSE in proximity to the neoplasm.

Conclusions: SET features correlate both with BRCA+ (as previously reported by Soslow et al) and the absence of TIC, the latter finding paradoxically linked to high stage HGSCs in BRCA+ women. The origin (tube vs ovary) of this significant subset of HGSCs remains unclear but resolution of this question is particularly relevant to the BRCA+ population if prophylactic salpingectomy alone is contemplated as a cancer prevention measure. There is no immunohistochemical evidence from this analysis to implicate a direct transformation of the ovarian surface epithelium.

1179 Endometrial Mucinous Carcinomas: A Characterization of Intratumoral KRAS Mutations in Mucinous and Endometrioid Histologic Components

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Background: KRAS mutations are frequently seen in malignancies with mucinous morphology. Endometrial carcinomas (EC) of the mucinous type (MCs) are defined by the WHO (1994) as a variant of endometrial carcinoma with abundant intracytoplasmic mucin. In our previous study, MCs were associated with a significantly higher frequency of KRAS mutations as compared to matched conventional endometrioid carcinomas. Some workers use predominate mucinous component, viz, >50% to define endometrial MC. We designated those 'mixed' type ECs that show from >10% to <50% mucinous differentiation as *ECs with significant mucinous differentiation* (ECMD). This study expands our previous report by contrasting the KRAS mutational status of the mucinous components of MCs and ECMDs vs. their associated 'usual' endometrioid components.

Design: The 17 KRAS+ cases from our previous report were studied, including 6 MCs and 11 EMMDs. The specimens were microdissected to isolate only a morphologically pure endometrioid component. In addition, morphologically benign endometrium associated with its MC and/or EC SMD was microdissected from 5 cases (3 from MCs and 2 from ECMDs). Direct DNA sequencing for KRAS mutations at codons 12 and 13 using capillary electrophoresis were done. PNA test was used to verify equivocal cases.

Results: KRAS mutations were detected in the endometrioid components of 5/6 (83%) MCs, 3/11 (27%) ECMDs, and 0/5 (0%) of benign endometrium. A statistically significant difference in the frequency of KRAS mutations was found in the ECMD group when the components of mucinous and endometrioid tumor were compared (11/11, 100% and 3/11, 27%), respectively. (p<0.001).

Conclusions: Our current study shows a strong association of KRAS mutations in the endometrioid component of our prior KRAS mutation-positive MCs (83%). When the mucinous component predominates, qualifying for an MC, KRAS mutations appear to be widespread, irrespective of the mucinous or non-mucinous differentiation of the tumor cells. In comparison to the MCs the endometrioid component of ECMD were less frequently associated with KRAS mutations (27%), even when the mucinous component of the same tumor contained a mutation (100%); the difference is statistically significant (p<0.001).

1180 The Diagnostic Accuracy of FIGO Grade 3 Uterine Endometrioid Adenocarcinoma Correlates with Specialty Gynecologic Pathology Practice

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Background: Low rates of interobserver diagnostic agreement are seen in high-grade endometrial carcinomas (EC), including FIGO 3 endometrioid carcinoma (EMC). The aim of our study was to assess the degree of accuracy in diagnosing FIGO 3 EMC between two institutions.

Design: 70 and 65 cases diagnosed as FIGO 3 EMC were identified from pathology archives of institution A (general sign out) and institution B (subspecialty sign out) respectively. An average of 4 slides from each case was reviewed together by 2 GYN pathologists from each institution. The cases were then re-classified according to tumor type and grade. Clinicopathologic f/u was obtained for all cases.

Results: Of 70 cases from institution A, 25 (35.7%) were re-classified. These included FIGO 2 with focal marked nuclear atypia EMC (n=8), serous carcinoma (ca) (n=3), mixed with serous ca (n=12) and undifferentiated ca (n=2). From institution B, 11 (16.9%) of the 65 cases were re-classified. These included serous ca (n=1), mixed with serous ca (n=4), undifferentiated (n=5) and mixed with clear cell ca (n=1) (table 1). The diagnostic accuracy of FIGO 3 EMC, defined by consensus, was better at institution B (p=0.002). The recurrence rate for consensus FIGO 3 EMC (21.2%) and re-classified cases (19.4%) were comparable (p=0.81). More patients (25%) in the re-classified group died of disease (14.5%) p=0.04. Median f/u was 172 mths for consensus FIGO 3 and 137 mths for re-classified cases. Overall survival between the two cohorts was not significant p= .110.

Table1: Clinicopathological characteristics of re-classified cases

Type	Stage	Metastasis	Adjuvant Therapy- chemo &/or radiation	Recurrence	DOD
Institution A, re-classified cases n=25					
Undifferentiated ca, 2/5 (8%)	IB=2	0/2	0/2	2/2	2/2
Serous ca, 4/25 (16%)	IA=1 II=2 IVA=1	1/4	2/4	2/4	1/4
Mixed (EMC & serous) ca, 12/25 (48%)	IA=3 IB=5 II=1 IIIA=1 IIIC=1	2/12	9/12	2/12	4/12
FIGO2 with focal marked nuclear atypia, 7/25 (28%)	IA=1 IB=3 II=1 IIIA=1 IVA=1	1/7	6/7	0/7	1/7
Institution B, re-classified cases n=11					
Undifferentiated ca, 5/11 (45%)	IA=3 IIIC1=1 IIIC2=1	1/5	5/5	0/5	0/5
Serous ca, 1/11 (9%)	IA=1	0/1	1/1	0/1	0/1
Mixed (EMC & serous) ca, 4/11 (36%)	IA=3 IIIC1=1	0/4	4/4	1/4	1/4
Mixed (EMC & clear cell) ca, 1/11 (9%)	IA=1	0/1	1/1	0/1	0/1

ca, carcinoma

Conclusions: Our results suggest that subspecialty sign out may be preferable to general practice in evaluating and grading endometrial ca. Diagnostic reproducibility and accuracy should be optimized for the diagnosis of high-grade endometrial ca since treatment paradigms are becoming increasingly subtype-specific.

1181 Napsin A: A Sensitive Immunohistochemical Marker for Uterine Clear Cell Carcinoma

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Background: Napsin A is a recently introduced immunohistochemical marker that is useful in the diagnosis of pulmonary adenocarcinoma and some renal cell carcinoma subtypes. However, its expression in carcinomas of the gynecologic tract has not been extensively studied. The diagnostic reproducibility of uterine clear cell carcinoma (CCC) is suboptimal due to morphologic overlap with other endometrial carcinoma subtypes (clear squamous and secretory change in endometrioid carcinomas and hobnail cells in serous carcinomas) and benign mimics. The immunoprofile of CCC with commonly used markers is non-specific; thus, additional diagnostic markers are needed. This study evaluated napsin A expression in uterine carcinomas and gestational endometrium.

Design: Sixty-two endometrial carcinomas including 23 clear cell carcinomas, 15 serous carcinomas, and 24 endometrioid carcinomas (14 – FIGO grade 1, 6 – FIGO grade 2, and 4 – FIGO grade 3) were assessed for napsin A expression using monoclonal anti-napsin A antibody (Novacastra). The percentage of tumor cells with cytoplasmic staining was quantified to the nearest 10%. Twelve cases of gestational endometrium with or without Arias-Stella reaction were also studied.

Results: Results are summarized in Table 1. Among uterine carcinomas, napsin A demonstrated a sensitivity of 91% and a specificity of 95% for identifying CCC. All cases of gestational endometrium/Arias-Stella reaction also expressed napsin A.

Table 1. Napsin A immunohistochemical expression in endometrial carcinoma and gestational endometrium

Histologic type	Total cases	Negative cases	Positive cases	
	n	n	n (%)	% positive cells, mean/median (range)
Clear cell carcinoma	23	2	21 (91%)	51%/40% (10-100%)
Serous carcinoma	15	13	2 (13%)	15%/15% (10-20%)
Endometrioid carcinoma	24	24	0	-
Gestational endometrium	12	0	12 (100%)	41%/35% (10-80%)

Conclusions: Napsin A serves as a sensitive and specific marker for distinguishing CCC from other endometrial carcinoma subtypes and could improve the diagnostic reproducibility of CCC. However, it does not distinguish CCC from benign mimics such as gestational endometrium/Arias-Stella reaction. Thus, it cannot be used in peri- and postmenopausal women to differentiate CCC from atypical secretory change/Arias-Stella-like reaction in the setting of hormonal therapy.

1182 GATA3 Is a Sensitive and Specific Marker of Mesonephric Differentiation and Neoplasia in the Lower Mullerian Tract

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Background: GATA3 is a transcription factor critical for embryogenesis, development, and cell differentiation, and has recently been shown to be immunoreactive in urothelial and breast carcinomas, and negative in endometrial carcinomas. We have observed GATA3 expression in the epididymis of the testis (Wolffian), and therefore questioned its expression in mesonephric/Wolffian remnants and neoplasias in the Mullerian tract.

Design: GATA3 immunohistochemistry (IHC) was performed on 27 formalin-fixed paraffin embedded cervical samples containing mesonephric epithelia (11 mesonephric remnants, MR; 14 mesonephric hyperplasias, MH; and 2 mesonephric carcinomas, MCA), 30 endocervical adenocarcinomas (ENCA), 155 endometrial adenocarcinomas (EMCA) on a TMA (152 endometrioid and 3 serous type), 2 EMCA in which the possibility of mesonephric differentiation was mentioned in the final report but based on IHC (Inhibin/CD10 negative and Inhibin/Calretinin negative) was classified as EMCA, 6 fallopian tube (FT) MRs, and 3 female adnexal tumors of probable Wolffian origin (FATWO). Cases were evaluated for nuclear intensity (weak, moderate, or strong), and extent (negative=0-5%, focal=5-50%, patchy=50-75%, or diffuse=>75%).

Results: Benign endocervical and endometrial epithelia were negative for GATA3, while squamous epithelium of the cervix was weakly positive in scattered cells. All cervical mesonephric lesions were GATA3 positive, including 11/11 (100%) MRs and 14/14 (100%) MHs (all moderate-strong and diffuse). One MCA, which was associated with MH, showed strong diffuse GATA3 staining, while the other MCA demonstrated moderate and patchy staining. Five of 6 FT MRs were positive for GATA3; however, the staining pattern was variable (4 patchy, 1 focal; 2 weak, 2 moderate, 1 strong). Only 1/3 FATWOs demonstrated moderate and patchy staining for GATA3. 1/30 (3%) ENCAs exhibited weak-moderate, patchy GATA3 immunoreactivity, while the remaining 29/30 (97%) were completely negative. All EMCAs in the TMA were negative for GATA3, while the 2 EMCA where mesonephric differentiation was entertained showed moderate patchy GATA3 staining.

Conclusions: GATA3 is a highly sensitive and specific marker for cervical mesonephric differentiation, and it can be useful when the differential diagnosis includes endocervical and endometrial adenocarcinomas, as the latter are negative for GATA3. GATA3 appears to be less reliable in mesonephric/Wolffian lesions in adnexa; however, a larger cohort should be evaluated to confirm these findings.

1183 Targeted Genomic Analysis of Mullerian Adenosarcoma (MA)

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Background: MA is a rare mixed mesenchymal tumor of the female genital tract, composed of malignant but often low grade stroma and benign-appearing epithelium. Sarcomatous overgrowth (SO), the only established prognostic histological variable, associates with higher stage and shorter survival. To date, molecular or immunohistochemistry (IHC) tools for the diagnosis of MA have not been elucidated. Our goal is to study genomic mutations and copy number variations (CNVs) in MA to understand better their pathobiology and develop specific diagnostic tools.

Design: DNA was extracted from 17 FFPE tissue samples of MA representing 15 subjects, including two cases in which areas of typical histology and SO were tested separately. Samples were analyzed using OncoPanel, an assay interrogating the exonic sequences of 275 cancer genes for mutations and CNVs as well as 91 introns across 30 genes for cancer-associated rearrangements. Massively parallel sequencing was performed using target enrichment by hybrid capture (Agilent SureSelect) and an Illumina HiSeq 2500 sequencer. MuTect and GATK (Broad Institute) were used to detect single nucleotide variants and indels; VISCAPCancer and CCOPY were used to detect CNVs.

Results: Overall, 2 nonsense, 63 missense, 2 frameshift, and 1 small deletion mutations were identified. The most frequent amplification, involving *CDK4* and *MDM2* on 12q14-15, was seen in 5/15 (33%). Activating alterations in PIK3CA/AKT/MTOR pathway members were seen in 6/15 (4 missense mutations and 2 copy number gains). *STAT6* gain was found in 3/15, with 1 carrying a novel missense mutation. *FGFR1* amplification was identified in 1 case. Notably, *TP53* mutations were not identified in any cases. Predicted chromosomal abnormalities showed increased aneuploidy in SO vs typical MA samples, and included loss of 9q and monosomy of 3, 16, and X. *BAP1* loss was seen in 3/15, including 2 with SO. *BCL6* loss also was noted in both cases of SO. *KIT* and *PDGFRA* co-amplification was present in 1 SO. Single gene amplifications were seen predominantly in cases associated with SO (identified in both matched typical MA and SO samples), while *TERT* gain and *BCL2L12* loss were found only in samples of SO. No rearrangements were identified.

Conclusions: We have identified a number of recurrent genomic alterations in MA, including potential markers associated with, and possibly driving, SO. Although further investigation of these findings is needed, confirmation of one or more may lead to new mechanistic insights and novel IHC markers for this often difficult-to-diagnose tumor.

1184 Evaluation of Uterine Leiomyosarcoma by Next Generation Sequencing Reveals Actionable Genomic Abnormalities and New Routes to Targeted Therapies

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Background: Uterine leiomyosarcoma (LMS) is a rare uterine malignancy that arises from the smooth muscle of the uterine wall. Compared to other types of uterine cancers, LMS is an aggressive tumor associated with a high risk of recurrence and death, regardless of stage at presentation. We hypothesized that comprehensive genomic profiling of clinical LMS samples could identify genomic-derived drug targets of therapy for patients with this lethal cancer.

Design: Hybridization capture of 3,769 exons from 236 cancer-related genes and 47 introns of 19 genes commonly rearranged in cancer was applied to≥ 50ng of DNA extracted from 25 LMS FFPE tumor specimens and sequenced to high, uniform coverage. Genomic alterations (base substitutions, small indels, rearrangements, copy number alterations) were determined and then reported for these patient samples. Actionable GA were defined as those identifying anti-cancer drugs on the market or in registered clinical trials.

Results: There were 25 female LMS patients with a median age 57 years (range 42-70 years). There were 1(4%) Grade 1, 3(12%) grade II and 21 (84%) grade III tumors. Three (12%) LMS were Stage I, 5 (20%) were stage II, 3 (12%) were Stage III and 14(56%) were Stage IV. All patients had sequencing performed on their primary tumors. 23 (92%) of LMS had GA on NGS with A total of 85 GA were identified, with 23/25 cases (92%) harboring at least one genomic alteration with an average of 3.4 GA per tumor. The most common non-actionable GA were alterations in *RB1* (64%), *TP53* (60%), *ATRX* (32%)and *MYC* (16%). Nine (36%) LMS had at least 1 actionable GA with an average of 0.9 actionable GA per patient including: mutation, amplification or homozygous deletion of: *PTEN* (16%), *RICTOR* (12%), *TSC1* (8%), *ATK2* (8%) and *CCNE1* (4%) and *CDKN2A* (4%) In a single case, a *STK32B-ALK* fusion that fused the intact kinase domains of ALK to STK32B was identified.

Conclusions: More than one third of the LMS patients in this study harbored at least one actionable GA with the potential to influence and personalize therapy selection. Given the limited treatment options and poor prognosis of patients with leiomyosarcoma in general, comprehensive NGS-based genomic profiling has the potential to identify new treatment paradigms and meet an unmet clinical need for this disease.

1185 Clinicopathologic Analysis of Endometrial Carcinomas with POLE Hotspot Mutation

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Background: The Cancer Genome Atlas (TCGA) identified four major genomic groups of endometrial carcinomas (EC), including a POLE ultramutated subtype comprising ~10% of endometrioid EC. This subtype is characterized by POLE hotspot mutations, ultrahigh somatic mutation rates, and very favorable clinical outcomes. The aim of this study was to examine the morphologic and clinicopathologic features of ECs harboring a POLE hotspot mutation.

Design: The H&E slides and pathology reports for 8/17 POLE mutant EC cases (EC-POLE) described in the TCGA study were retrieved from 3 contributing institutions; for the remaining cases, virtual whole slide images publicly available at cBioPortal (www.cbioportal.org) were examined. Morphological features, including nuclear grade, tumor heterogeneity, metaplastic changes, and tumor infiltrating lymphocytes (TILs) were assessed. Clinical follow-up information and molecular data were obtained from cBioPortal.

Results: Median age of the 17 EC-POLE cases was 54 yrs (range 33-87). Twelve cases presented as stage I, 1 stage II and 4 stage III. All cases were of endometrioid type. Five cases were FIGO grade I, 4 grade II, and 8 (47%) grade III. Eight cases (47%) showed marked nuclear atypia; some in the form of giant bizarre nuclei. Nine cases (53%) exhibited abundant eosinophilic/squamoid cytoplasm. TILs (>40 lymphocytes/10HPFs) were seen in 13 cases (76%). One case showed a focal undifferentiated component. Four cases showed ambiguous features exhibiting an architecture that suggests endometrioid differentiation, associated with marked increase in TILs commonly seen in microsatellite instable (MSI) tumors, but with nuclear features that suggest serous carcinoma. In fact, the majority of EC-POLEs were microsatellite stable (65%) but 6 cases (35%) showed MSI (3 MSI-high, 3 MSI-low). TP53 mutations were present in 6 of 17 EC-POLE cases (35%), of which 4 and 2 cases had non-sense/frameshift and missense mutations, respectively. The majority of EC-POLEs also harbored mutations in PTEN (94%), FBXW7 (82%), ARID1A (76%), and PIK3CA (71%). All patients were alive without disease and none of the patients developed recurrence at time of follow-up (median 33 months; range 2-102 months).

Conclusions: The recognition of EC-POLE cases is important given their favorable outcome. Our histopathological review revealed that EC-POLEs commonly show high grade and ambiguous morphology. As these cases also frequently harbor TP53 mutations, it is important not to misclassify EC-POLEs as serous carcinoma.

1186 Invasion Patterns of Metastatic Extrauterine High-Grade Serous Carcinoma with BRCA Germline Mutation and Correlation with Outcomes

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Background: Characteristic histopathologic features have been recently described in BRCA-associated high-grade serous carcinoma (BRCA-HGSC). It has been shown that BRCA-HGSC is associated with relatively better clinical outcome, partly because of increased sensitivity to chemotherapy, and possibly due to the tendency to spread in the form of circumscribed rounded metastases amenable to optimal debulking. The aim of this study is to evaluate the significance of invasion patterns in metastatic BRCA-HGSC cases.

Design: Thirty-one cases of advanced stage HGSC with known BRCA1 or BRCA2 germline mutation were retrieved from our institutional files. Nineteen patients had germline mutation of BRCA1 vs 12 with BRCA2 mutation. Pattern of invasion at metastatic sites was recorded and classified as pushing pattern (PP), micropapillary infiltrative pattern (MIP), and infiltrative pattern (IP). Histologic evaluation of metastatses was performed without knowledge of genotype or clinical outcome. Clinical data was abstracted from medical records. Appropriate statistical tests were performed.

Results: Median age was 56 yrs (range 31-73 yrs). All cases presented at stage IIIC/IV and underwent complete surgical staging followed by chemotherapy. All 31 BRCA-HGSC cases showed either PP metastases (25; 81%) or MIP (6; 19%). No cases exhibited IP metastases. Among the 6 MIP cases, 5 had BRCA1 germline mutation vs 1 BRCA2. Median time of follow-up was 26 months (range, 13-49 months). All MIP cases recurred or died of disease (4 recurrences and 2 deaths), compared to 12 of 25 (48%) of PP cases (10 recurrences and 2 deaths) (p value=0.03).

Conclusions: The recognition of invasion patterns of metastatic extrauterine BRCA-HGSC is important and has prognostic and probably therapeutic implications. MIP of invasion is associated with worse outcome and is more frequently seen in BRCA1 associated HGSC. Further studies with a larger cohort are warranted to validate this finding.

1187 Microcystic Stromal Tumor of the Ovary: A Distinct Ovarian Stromal Neoplasm Characterized by FOXL2, SF-1, WT-1, Cyclin D1, and β-Catenin Nuclear Expression and *CTNNB1* Mutations

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Background: Since our first description of the microcystic stromal tumor (MST) of the ovary, a distinctive neoplasm with a predominant microcystic pattern and a CD10+/vimentin+ immunophenotype, 2 MST with β-catenin nuclear localization and *CTNNB1* mutation have been reported. We undertook a detailed immunohistochemical study and *CTNNB1* genetic analysis to further characterize this rare neoplasm.

Design: 12 ovarian MST were stained with ß-catenin,FOXL2, SF-1, WT-1, cyclin D1, e-cadherin, estrogen/progesterone receptors, synaptophysin, chromogranin, CD56, and CD99, and scored as diffuse (≥50% tumor cells) positive, focal (<50%) positive, and negative (0%). Genomic DNA was extracted from formalin-fixed paraffin-embedded tumor samples and *CTNNB1* hotspot mutation analysis performed using primers and PCR that amplify exon 3 of *CTNNB1*, followed by Sanger sequencing.

Results: Immunohistochemical results (positive cases/total cases) are shown in Table 1 and as follows: Synaptophysin, 6/12; chromogranin, 0/12; CD56, 1/12, and CD99, 0/12.

Immunohistochemical staining (positive cases/total cases) in MST.

β-catenin nuclear	FOXL2	SF-1	WT-1	Cyclin D1	E-cadherin	ER	PR
12/12 (diffuse)	12/12 (diffuse)	10/12 (6 diffuse, 4 focal)	12/12 (11 diffuse, 1 focal)	12/12 (diffuse)	0/12	1/12 (focal)	0/12

Heterozygous missense point mutations in exon 3 of *CTNNB1* were detected in 8 of 12 cases, resulting in amino acid changes at codons 32, 34, 35 and 37. There was no apparent correlation between *CTNNB1* exon 3 mutation status and tumor immunophenotype.

Conclusions: 1. MST of the ovary exhibits a characteristic nuclear β-catenin+/FOXL2+/SF-1+/WT-1+/Cyclin D1+/E-cadherin- immunoprofile.

2. The finding of diffuse nuclear FOXL2 and WT-1 immunostaining in all cases and SF-1 in most supports the classification of MST within the sex-cord stromal category.

3. All MSTs of the ovary show aberrant nuclear β-catenin expression, which appears to be the result of stabilizing *CTNNB1* mutations in two-thirds. The cause for nuclear β-catenin accumulation in the remainder is unknown.

4. Activation of β-catenin with upregulation of cyclin D1 may play a role in the tumorigenesis of MST.

1188 Napsin A Is Frequently Expressed in Clear Cell Carcinoma of the Ovary and Endometrium

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Background: Napsin A has been considered as a reliable marker for primary pulmonary adenocarcinoma. Napsin A expression has also been shown in extrapulmonary carcinoma including those of renal and thyroid origin. Furthermore, a few recent studies have identified napsin A expression in a subset of ovarian clear cell carcinoma (O-CCC) as well as in a small number of ovarian and endometrial endometrioid carcinomas. Napsin A expression in endometrial clear cell carcinoma (EM-CCC) remains unknown. This study was carried out to investigate the extent of napsin A expression in O-CCC and in EM-CCC, and to compare the former with other carcinomas of ovary, including endometrioid carcinoma (O-EC) and high-grade serous carcinoma (HGSC).

Design: Eighty-nine cases of resected ovarian and uterine carcinomas (22 O-CCCs, 15 EM-CCCs, 13 O-ECs, 39 HGSCs) were examined using immunohistochemistry with a monoclonal antibody to napsin A (TMU-Ad02), comparing results with antibodies to another lung-restricted marker TTF-1 (8G7G3/1), and gynecologic tract-restricted marker PAX8 (polyclonal) and CA125 (Ov185:1). The immunohistochemical expression was scored using: 0 = negative, <10% = rare, 10-60% = focal, and > 60% = diffuse.

Results: Napsin A was positive in 21/22 (95%) of O-CCCs, and 10/15 (67%) of EM-CCCs, with focal or diffuse positive cells in 19 cases in the former, and 6 in the latter.

In contrast, napsin A was expressed in 1/13 (8%) of O-EC with rare positive cells, and 0/39 (0%) HGSC. TTF-1 was positive in 0/22 (0%) of O-CCC, 1/15 (7%) EM-CCC, 3/13 (23%) O-ECs, and 2/39 (5%) HGSCs, usually with rare or focal positive cells. PAX8 was positive in all 89 cases examined, while CA125 was positive in 22/22 (100%) of O-CCCs, 12/15 (80%) EM-CCCs, 13/13 (100%) O-ECs, and 39/39 (100%) HGSCs, usually with diffuse positive cells. There were no Napsin A-positive/TTF-1-positive cases except one EM-CCC, in which positive cells were focal for both markers. In all napsin A-postive and/or TTF-1-positive cases, both PAX-8 and CA 125 were positive.
Conclusions: Napsin A is frequently expressed in O-CCC and in EM-CCC, rarely in O-EC, and not in HGSC. Although TTF-1 is not expressed in O-CCC, it is rarely expressed in EM-CCC, O-EM, and HGSC. These findings confirm the importance of using a panel of antibodies including Napsin A, TTF-1, PAX8 and/or CA125 in the evaluation of metastatic carcinomas of unknown primaries, especially in which gynecologic and pulmonary adenocarcinomas are in the differential diagnosis.

1189 Histologic Predictors of Clinical Outcome in Vulvar Squamous Cell Carcinoma: A Study of 145 Cases

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Background: There is only limited literature regarding the prognostic significance of patterns of invasion and stromal tumor response in vulvar squamous cell carcinoma (vSCC), although it has been suggested that a fibromyxoid tumor response (FMXTR) is associated with more aggressive tumor behavior. The aim of this study was to identify histologic patterns of invasive vSCC and associated stromal tumor response, and correlate these features with clinical outcome.
Design: One hundred and forty-five consecutive cases of invasive vSCC were reviewed by two gynecologic pathologists. Patterns of invasion were classified as infiltrative or nested/pushing. Stromal tumor response was documented if a prominent FMXTR (defined as myxoid stroma surrounding the tumor) or a prominent band-like lymphoid tumor response (LTR) was easily identified at low power magnification (4x). Age, depth of invasion (DOI), lymph node involvement (LNI), clinical stage and recurrence were obtained by chart review. Statistical analysis was performed using unpaired t-test and 2-tailed chi square test.
Results: Sixty-three (43%) cases had an infiltrative pattern of invasion; the remainder had a nested/pushing pattern. FMXTR or LTR was seen in 67 (46%) and 55 (38%) of cases, respectively (neither in 23,16%). When both FMX and LTR were present in the same tumor (n=27/19%), they were mutually exclusive for any given area. The presence of the infiltrative pattern (compared to nested/pushing) correlated with the presence of fibromyxoid stroma (85%, p<0.0001), greater DOI (1.13 vs 0.65 cm, p=0.0048) and recurrence (41% vs 25%, p=0.0458). Increased LNI was associated with an infiltrative pattern but this was not significant (27% vs 14%, p=0.06). Likewise, the presence of fibromyxoid stroma (in comparison to a prominent LTR) correlated with infiltrative growth pattern (p=<0.0001), greater DOI (p=0.0375) and LNI (p=0.0028), but not recurrence (p=0.2446). Age and clinical stage showed no statistical significance.
Conclusions: This is the first study to demonstrate that infiltrative tumor histology is significantly associated with recurrence, deeper invasion and a fibromyxoid stromal response. Likewise, nested/pushing tumors are associated with an inflammatory stromal response and demonstrate overall a more favorable outcome when compared to tumors with a fibromyxoid tumor response. Recognition and reporting of these features can help to plan more efficient therapeutic protocols for women affected with this devastating disease.

1190 Black Raspberry Extract Induces Caspase-14 in a Vulvar Cancer Cell Line: A Potential Natural Agent to Treat Neoplastic and Preneoplastic Vulvar Squamous Lesions

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Background: Natural compounds have been shown to induce caspase-14, a key protein in maturation of squamous epithelia. We examine the in-vitro effect of black raspberry extract (BRE) on a vulvar squamous cell carcinoma (VSCC) cell line and document altered caspase-14 expression in archival malignant and premalignant vulvar squamous lesions.
Design: VSCC cell cultures (SW954; ATCC, Manassas, VA) were grown and exposed in triplicate vials to varying doses of BRE for 32 hours. Cells were fixed and used to create cell blocks. Immunohistochemistry for caspase-14 (PA1-46317, Thermo, IL) was performed on sections of the cell blocks, archival whole tissue sections, and a tissue microarray (TMA). The whole sections and TMA contained normal vulva, lichen sclerosus (LS), differentiated vulvar intraepithelial neoplasia (dVIN), classic VIN (cVIN), and VSCC. Cell block sections were photographed and manually scored.
Results: Normal squamous epithelium confirmed that caspase-14 expression increases with maturation. 5/11 (46%) LS cases demonstrated abnormal full thickness caspase-14 staining, while one LS case had absent caspase-14 expression. 4/7 (57%) dVIN showed markedly reduced expression, and 6/22 (27%) cVIN had either absent or reduced caspase-14. The majority (26/28, 93%) of VSCC had absent or markedly reduced (limited to isolated neoplastic cells) caspase-14. SW954 treated with varying doses of BRE demonstrated a significant increase in caspase-14 (p=0.013) with predominant paranuclear staining. Specifically, 7.3% (±2.0%) of untreated SW954 cells stained positive for caspase-14, while 21.3% (±8.9%), 21.7% (±4.8%), and 22.6% (±5.3%) of SW954 cells were positive for caspase-14 after treatment with 200, 400, and 800 µg/mL BRE, respectively. Pair-wise comparisons between the treatment groups and the control demonstrated significant differences between no treatment with BRE and each of these treatment concentrations (p-values of 0.024, 0.021, and 0.014, respectively).
Conclusions: Caspase-14 expression is abnormally decreased in many premalignant vulvar lesions and in most VSCC. BRE was shown to increase caspase-14 expression in a VSCC cell line. Further investigation is warranted to elucidate the potential use of BRE to treat preneoplastic and neoplastic vulvar squamous lesions.

1191 The Significance of Apoptosis and Apoptosis-Related Proteins in the Pathogenesis of Endometrial Clear Cell Carcinoma: An Immunohistochemical Study

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Background: Disruption of the equilibrium between pro-apoptotic and anti-apoptotic signaling may eventuate in carcinogenesis through a net reduction in apoptosis, and there are now numerous chemotherapeutic strategies that directly or indirectly target apoptotic pathways. However, a large scale assessment of apoptosis-related proteins has not previously been performed in endometrial clear cell carcinoma (CCC). This study aims to determine the significance of 8 apoptosis-related proteins in the pathogenesis of CCC.
Design: The expression of 5 anti-apoptotic proteins (Bcl-2, Bcl-xL, $FLIP_L$, survivin, NF-kappaB_p65 [NFK]) and 3 pro-apoptotic proteins (Bax, caspase 3, caspase 8) were assessed by immunohistochemistry (IHC) on 49 CCC, 17 endometrial serous carcinoma (ESC), 49 endometrioid carcinomas (EEC); 18,19,12 grades 1,2,3) and 25 non-neoplastic endometrial samples (NNE) in a TMA. Objective IHC scores were assigned by an automated image capture system. Scores were then correlated with clinicopathologic parameters.
Results: Only Bcl-2 showed significantly increased expression in CCC as compared to NNE (p=0.011). $FLIP_L$ and Caspase 3, in contrast, showed significantly decreased expression in CCC relative to NNE (p <0.0001 and 0.002). There were no significant differences between NNE and CCC regarding their expression of the other markers. Among the most noteworthy histotype comparisons, $FLIP_L$ and caspase 3 showed significantly lower expression in CCC as compared with EEC and ESC (p <0.0001 and 0.029). Most markers, including Bax, Bcl-xL, $FLIP_L$, and NFK, showed higher levels in EEC than CCC. Bcl-2 and survivin were significantly more expressed in CCC as compared with EEC, but not ESC. Our correlation analysis in the CCC cohort showed that $FLIP_L$ was negatively correlated with caspase 8 and NFK (p=0.0001-0.0005), nearly negatively correlated with caspase 3 (p=0.06), and positively correlated with survivin (p=0.0092). None of the markers showed a significant association with patient outcomes.
Conclusions: Our analysis of the expression and correlation patterns of a large panel of apoptosis-related proteins suggests that the downregulation of caspase 3 and $FLIP_L$ are significant, since these were seen in CCC relative to all other tissues, including EEC, ESC and NNE. $FLIP_L$ is an important regulator of death receptor signaling, which is an integral component of the extrinsic pathway of apoptosis. The relative downregulation of $FLIP_L$ expression in CCC may be an important component of its pathogenesis.

1192 PAX2 Expression in Endometrial Biopsies Simultaneously Diagnosed Using the World Health Organization (WHO) and Endometrial Intraepithelial Neoplasia (EIN) Classification Systems

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Background: Currently, two classification schemes for precancerous endometrial lesions exist: WHO and EIN. Alteration (predominately loss) of PAX2 staining is useful in diagnosing EIN, while its utility in the WHO system is questionable. The purpose of this study was to evaluate PAX2 staining patterns in a series of endometrial biopsies that were classified using both systems.
Design: Diagnosis text search for all precancerous endometrial lesions (simple and complex hyperplasia with and without atypia, EIN, benign hyperplasia and small focus of atypical glands) from 2005-2013 was performed. 108 cases were identified, stained with PAX2, and reviewed by two gynecologic pathologists each of whom trained in one of the classification systems. Thirty cases were deemed benign by both pathologists (anovulatory, polyp, secretory, etc) and 11 cases lost the tissue of interest on deeper sections. PAX2 staining was scored in lesional tissue as normal or altered (lost, markedly increased or markedly decreased, compared to background).
Results: Forty-four of 67 (66%) cases demonstrated altered PAX 2 staining.

Table 1 - PAX2 Alteration By Category

PAX2 Altered	Yes (44)	No (23)
EIN (39)	37	2
Small Atypical Focus (11)	4	7
EIN+Small atypical Focus (50)	41	9
CAH (25)	23	2
CH (25)	16	9
CAH + CH (50)	39	11

Twenty-seven of those 44 patients had subsequent hysterectomies, and 8 cases contained adenocarcinoma (30%). EIN plus PAX2 loss was seen in 37 of 44 cases (84%) compared to 23 of 44 cases (52%) with complex atypical hyperplasia (CAH) and PAX2 loss. Complex hyperplasia (CH), with or without atypia, showed PAX-2 alteration in 39 cases (89%). Of the 30 cases deemed benign, only 1 (3%) showed PAX2 alteration (complete loss); 21 of the benign cases had follow-up samples, and none contained precancerous lesions.
Conclusions: PAX2 alterations correlate well with EIN (84%), but not with CAH (52%). Regardless, in cases of complex hyperplasia with equivocal cytologic atypia, PAX-2 may be helpful in identifying lesions that may have a higher risk of progression. One third of patients with a PAX2 altered lesion, regardless of type or classification scheme, were found to have adenocarcinoma on subsequent sampling. Normal PAX2 staining is helpful in worrisome biopsies, as 100% of cases with normal PAX2 staining showed no signs of malignancy upon further sampling.

1193 Morphologic Features and 2SC Immunohistochemical Staining in Uterine Leiomyomas from Young Women

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Background: Hereditary Leiomyomatosis and Renal Cell Carcinoma syndrome (HLRCC) occurs due to germline fumarate hydratase (FH) mutations and predisposes patients to skin and uterine smooth muscle tumors (USMTs) and renal cell carcinoma. The USMTs in HLRCC often present in young patients and display characteristic morphologic features (prominent eosinophilic nucleoli, perinucleolar halos, and cytoplasmic inclusions) that have been recently described. Expression of 2-succinyl-cysteine (2SC) has been shown to be both sensitive and specific for HLRCC-associated tumors. We examined the morphologic features and 2SC expression of USMTs in patients less than 40 years of age to determine the frequency of 2SC positive cases in this patient population and to assess the sensitivity and specificity of morphologic features for detection of HLRCC.

Design: USMTs from 120 patients under age 40 were examined. All H&E slides were independently reviewed by 2 pathologists who were blinded to the results of the 2SC antibody staining. The combined presence of prominent eosinophilic nucleoli, perinucleolar halos, and cytoplasmic inclusions was considered highly suspicious for HLRCC. Tissue microarrays (TMAs) were constructed and stained with 2SC antibody.

Results: All the USMTs were classified as leiomyomas. 2SC was diffusely positive in 3 cases (2.5%) and negative in 117 cases. The morphologic features are summarized in table 1. On blinded review, both pathologists classified all 3 2SC positive tumors as highly suspicious for HLRCC. Of the 2SC negative USMTs, the 2 pathologists classified 1 and 4 cases respectively as highly suspicious for HLRCC.

Table 1: Morphologic features of USMTs in young women

	Age	Increased Cellularity	> 2 MF/ 10 HPF	Prominent Eosinophilic Nucleoli	Perinucleolar Halos	Cytoplasmic Inclusions
All cases (n=120)	34 +/- 4	12%	2.5%	30%	18%	11%
2SC Negative cases (n=117)	34 +/- 4	12%	2.5%	28%	15%	8%
2SC Positive cases (n=3)	32 +/- 3 (29, 34, 34)	0%	0%	100%	100%	100%

Numbers reflect the percent of cases that were positive for each feature

Conclusions: The frequency of 2SC positive USMTs in women under 40 years of age was 2.5%, which may be an estimate of the prevalence of HLRCC in this patient group (an effort to correlate with FH mutation analysis is ongoing). The combination of prominent nucleoli, perinucleolar halos, and cytoplasmic inclusions appears to be sensitive, but not entirely specific for detection of HLRCC. However, the extensive presence of all of these features should raise the possibility of HLRCC and may warrant further testing.

1194 Universal Mismatch Repair Protein Testing in Endometrial Cancer: A Single Institutional Experience

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Background: The lifetime risk for Hereditary Non-Polyposis Colorectal Carcinoma (HNPCC) syndrome or Lynch Syndrome patients to develop endometrial cancer is 50-60% as compared to 2-3% in the general population. Microsatellite instability (MSI) is identified in nearly all HNPCC patients by polymerase chain reaction (PCR). Immunohistochemical staining for mismatch repair (MMR) protein expression (MLH1, MSH2, PMS2 and MSH6) provides similar information. We analyzed our one year experience (August 2012-July 2013) with universal testing to determine if this is a clinically useful practice.

Design: The MMR protein immunohistochemistry (IHC) was performed on all endometrial carcinomas. Tumors with indeterminate result on IHC were further tested with PCR and the protein(s) were considered lost if high level MSI was identified on PCR. Tumors with loss of MLH1 protein were further tested for *MLH1* gene promoter hypermethylation. A positive result on hypermethylation testing indicated lack of germline mutation of the *MLH1* gene.

Results: Of the 170 endometrial carcinomas, loss of any MMR protein was identified in 34 cases (20%). The most frequent abnormality was the combined loss of MLH1 and PMS2 (23/34 cases) accounting for 68% of all MMR protein loss. Of these 23 cases, 20 were examined for MLH1 promotor hypermethylation and all 20 were positive. Excluding cases with MLH1 loss, the incidence of MMR protein loss in endometrial cancers was 6% (11 of 170 cases).

	Age range	Tumor Morphology
MLH1 and PMS2 loss (n=23)	44-80 years, 15 patients >60 years	Endometrioid in 22 cases and one with mixed undifferentiated and endometrioid carcinoma
Isolated loss of PMS2 (n=7)	49-64 years, 2 patients >60 years	All endometrioid carcinomas
MSH2 and MSH6 loss (n=2)	48-58 years	One endometrioid and one mixed endometrioid and clear cell carcinoma
Isolated loss of MSH6 (n=2)	46-57 years	Both endometrioid carcinomas

Conclusions: Universal testing for MMR proteins in endometrial cancers predominantly identifies MLH1 protein loss. *MLH1* gene promotor hypermethylation studies were positive in all tested cases with MLH1 protein loss. Therefore, the true incidence of Lynch syndrome in patients presenting with endometrial cancer is low (6%), but consistent with literature (3-5%). Reflex testing for MMR protein in endometrial cancers could possibly be limited to patients younger than 65 years, with tumors of non-serous morphology. Our data also questions the clinical utility of promotor hypermethylation studies for *MLH1* gene in endometrial cancer as they are almost always positive.

1195 High-Grade Ovarian Serous Carcinomas (HGOSC): Significant Correlation of Histologic Patterns with EGFR Signaling Pathway Protein IMP3 and Epithelial-Mesenchymal Transition (EMT) Molecule E-Cadherin Predicting Disease Recurrence

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Background: Most ovarian HGOSC are advanced tumors with early recurrences in spite of adequate therapy. However, some cases do not recur & have excellent outcome. We tried to see the relationship among histologic patterns with recurrence & staging. Also, we sought to explore the role of IMP3 & marker of EMT molecule, E-cadherin in predicting recurrence.

Design: 9 cases of stage I & 31 cases of stage III HGOSC were included. Three predominant histologic patterns, infiltrating micropapillary (MP), non-papillary (NP) & intracystic (IC)) were assesed in the ovary & peritoneum (stage III). Primary & metastatic tumors were stained with IMP3 & E-cadherin. The immunohistochemistry (IHC) slides were evaluated semiquantitatively (0-4). Logistic regression analysis was performed to compare patterns & IHC results with recurrence status.

Results: All stage I cases were predominantly IC. Only one recurred in 59 months. In stage III cases, 17 recurred within 18 months & 14 cases did not recur after 49 months. The main histologic features separating recurrence from non-recurrence cases were seen in the metastases. In recurrence group, 11/17 cases had predominantly MP pattern in the peritoneum, while only 4/14 tumors in the non-recurrence category showed MP pattern in the peritoneum (p=0.02). 6/17 tumors in the recurrence group had predominantly NP or IC pattern in the peritoneum, whereas 10/14 tumors in the non-recurrence group had predominantly NP or IC pattern in the peritoneum. IMP3 positivity in the ovary was observed 15/17 cases in the recurrence group, while 6/14 cases in the non-recurrence group expressed IMP3 (p=0.01). E-cadherin staining was significantly low in the peritoneum of the recurrent cases compared to that in the primary tumor, while no difference was observed in the non-recurrence group.

Conclusions: 1. All stage I cases were IC tumors without MP areas.

2. In stage III carcinomas the histologic pattern of the HGOSC is important as 73% of the tumors with MP pattern recurred, whereas only 27% of non-recurrent tumors showed this pattern. In contrast, NP & IC patterns were seen in 71% of the non-recurrent and 35% of recurrent tumors.

3. E-cadherin showed significant decrease in expression in the invasive component, mainly in the metastases of the recurrent cases. The signaling pathway protein IMP3 is expressed in 88% of recurrent cases, especially in the MP areas, hence it can be used as a predictive biomarker in HGOSC.

1196 Expression of DNA Repair Proteins in Endometrial Cancer Predicts Disease Outcome

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Background: DNA repair and apoptosis are two essential systems for maintaining the integrity of the human genome. Recently, alterations of DNA repair proteins have shown to be involved in endometrial carcinogenesis. The aim of this study is to determine the expression and value of DNA repair proteins in endometrial cancer (EC).

Design: This is a retrospective study conducted during a 10 year period (1999-2009). PARP1, γH2AX, ATM, FANCD2, PTEN, BRCA1, and p53 were evaluated using immunohistochemistry on paraffin-embedded tissue from 357 EC patients. The expression of these biomarkers were correlated with clinico-pathologic parameters (age, tumor size, histologic subtypes, depth of myometrial invasion, lymphovascular invasion, FIGO grade, lymph node positivity, recurrence, disease status, recurrence time and survival time).

Results: PARP1, γH2AX, ATM, FANCD2, PTEN, BRCA1, and p53 were positive in 77%, 66.5%, 59%, 36%, 38%, 31% and 45% of cases respectively. The expression of several of the DNA repair proteins were found to be associated with one another. In type I EC, $PARP1^+$, ATM^+, and $FANCD2^+$ were associated with high tumor grade (p 0.031, p 0.0045, p 0.0062 respectively), $\gamma H2AX^+$ and $FANCD2^+$ with advanced tumor stage (p 0.0004, p 0.0085 respectively), $\gamma H2AX^+$, $FANCD2^+$ and $p53^+$ with the presence of lymphovascular invasion (p 0.0004, p 0.0042, p 0.0098 respectively), and $\gamma H2AX^+$ and ATM^+ with tumor recurrence (p 0.0203, p 0.0465 respectively). In type II EC, only $PARP1^+$ was associated with tumor stage (p 0.0310). In univariate analysis, patients with tumor expression of $p53^+$ and $FANCD2^+$ were more likely to have shorter recurrence-free survival (RFS) and shorter overall survival (OS). Finally, tumors with paired expression of ATM^+ and $p53^+$, or ATM^+ and $FANCD2^+$ were more likely to have shorter RFS.

Conclusions: A positive correlation was identified among several DNA repair proteins in EC. Numerous DNA repair proteins were associated with unfavorable clinico-pathologic parameters and the combination of their expressions predicted an increased risk of earlier recurrence and shorter OS. As a result, identifying patients with positive tumor expression of certain DNA repair proteins could significantly impact patient management and treatment.

1197 Ovarian Carcinoma Histotype Determination Is Highly Reproducible, and Is Improved through the Use of Immunohistochemistry

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Background: To assess the variation in ovarian carcinoma type diagnosis among gynaecological pathologists from Nordic countries, to assess the use of immunohistochemistry in diagnosis, and to determine whether a rationally designed panel of immunohistochemical markers could improve diagnostic reproducibility.

Design: Eight pathologists from four different countries (Sweden, Denmark, Norway and Finland) received an educational lecture on diagnosis of ovarian carcinoma type. All tumor containing slides from 54 archival ovarian carcinoma cases, in which type was predetermined by central review, were then independently reviewed by the study participants, who 1) determined type based purely on routine histology, 2) indicated whether they would or would not apply immunohistochemistry to the case in their routine clinical practice, and 3) determined type after reviewing a report of the immunohistochemical staining results. The immunostaining results for a panel of 6 markers (WT1, TP53, P16, HNF-1beta, ARID1A and PR) were determined for all 54 cases, by staining a tissue microarray containing two 0.6 mm tissue cores per case.

Results: The median concordance with central review diagnosis was 86% (median Cohen's kappa = 0.81) based on routine histology. With incorporation of immunohistochemical staining results, concordance with central review diagnosis significantly improved to a median of 90% (median Cohen's kappa 0.86, p=0.0002). With regards to interobserver agreement, the median was 78% (median Cohen's kappa = 0.71) based on routine histology and significantly improved to a median of 85% (median Cohen's kappa 0.80, p=0.0002) with incorporation of immunostaining results.

Conclusions: This study demonstrates substantial accuracy (as determined by concordance with central review diagnosis) and interoberver agreement in typing of ovarian carcinoma, based on routine histology, among the study pathologists. Immunohistochemistry was used frequently, being requested in 54% of cases, and use of the immunostaining results significantly improved both diagnostic accuracy and interoberver agreement. These results indicate that ovarian carcinoma type can be reliably diagnosed by pathologists from different centers and different countries, and further demonstrates that immunohistochemistry has an important role in improving diagnostic accuracy and agreement between pathologists. This indicates the importance of further studies to standardize and improve interpretation of immunohistochemical staining.

1198 Evolution of Ovarian Carcinoma Diagnosis: Insights from a Clinical Trial Case Series

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Background: Surface epithelial ovarian tumors are divided into eight distinct tumor types by the WHO classification of ovarian tumors. Over the last two decades new insights into molecular features, treatment response and patient demographics led the scientific community to conclude, that the histological types of ovarian carcinomas are best considered to be different disease entities. Today five types of ovarian carcinoma are proposed in the literature, leading to a change in diagnoses. To monitor the evolution of ovarian carcinoma diagnosis a clinical trial cohort, which underwent expert gynecopathology case review a decade ago, has now been re-reviewed applying current criteria of ovarian carcinoma histopathology.

Design: In the year 2002 a patient cohort, which was derived from the AGO OVAR 3 trial (recruitment 1995-97), was reviewed by two experienced gynaecopathologists for translational research purposes. All cases were typed according to the then up-to-date criteria and were classified as either serous, mucinous (MUCI), endometrioid (ENDO), clear cell (CCC), transitional cell (TCC) or undifferentiated (UNDIF) carcinoma. Tumor grade was assessed according to the FIGO grading system. The same cohort was now re-assessed by two gynecopathologists of which one of them was responsible for the original case review. Cases were now diagnosed as either high grade serous carcinoma (HGSC), low grade serous carcinoma (LGSC), MUCI, ENDO or CCC.

Results: 334 cases of advanced-stage ovarian carcinomas were available with central review diagnoses from the year 2000. 60% of the original diagnoses were confirmed after applying current ovarian carcinoma histopathology knowledge. In contrast to the original diagnoses where 44,7% cases were assigned to the serous type (MUCI: 7,9%, ENDO: 19,2%; CCC: 12,3%; TCC: 5,3%; UNDIF: 10,6%) now 85,6% were diagnosed as serous carcinoma, of which 92% were classified as HGSC (HGSC: 79,3%; LGSC: 6,5%; MUCI: 3%; ENDO: 5,5%; CCC 4,8%).

Conclusions: Our study demonstrates the diagnostic shift in ovarian carcinoma pathology with significantly more cases being called "serous" nowadays. With respect to the upcoming era of ovarian carcinoma type-specific trials and finally patient treatment, accurate histotyping will become a key point. To accordingly address this important issue in translational research projects it might be desirable to obtain up-to-date diagnoses rather than accepting what has been provided in the past.

1199 Making Better Use of Resources and Optimizing Quality of Care for Ovarian Cancer Patients Using Internet-Based Second Opinion Pathology – Standardized in an Ovarian Cancer Network

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Background: Based on the current literature one may assume that a considerable number of patients in clinical trials of ovarian carcinoma have histopathological diagnoses in conflict with inclusion criteria possibly causing unintended patient morbidity and study bias. It has been suggested that specialized pathology review prior to randomization should become standard procedure in study protocols. We hypothesized that our new, internet-based high throughput infrastructure would be capable of providing specialized second opinion pathology within 10 days.

Design: Patients scheduled for the AGO OVAR17 ovarian carcinoma chemotherapy trial were registered for expert pathologic case review using a new internet-based central pathology review platform prior to randomization. All original slides were requested from local pathologists. Slides were scanned and uploaded to a secured internet server. A network of internationally recognized gynecological pathologists was connected to the server through a custom-designed software platform. If deemed necessary by expert pathologists, immunohistochemistry was available through a collaborating pathology lab.

Results: 880 patients with an original diagnosis of ovarian epithelial carcinoma were registered through our internet platform for expert pathology review from 10/2011 - 07/2013. In 2.5% (n=22) of cases, a major diagnostic discrepancy of potential clinical relevance was found leading to exclusion from the chemotherapy trial. In 10.7% (n=94) of cases the diagnosis of ovarian carcinoma was confirmed but patients were excluded for other reasons. For second opinion pathology five gynecopathologists from Austria, Switzerland and Germany were available online. The average time from patient registration until completion of pathology review was 5.2 days.

Conclusions: Our results show that the use of a new internet-based infrastructure makes specialized case review prior to patient randomization feasible within less than 10 working days. Our new approach might not only help to avoid disregarding of clinicopathologic inclusion criteria but also to further improve quality of patient care through minimization of overtreatment with chemotherapy of patients with ovarian borderline tumors and inadequate treatment of patients with ovarian metastases.

1200 The Utility of MALDI Imaging Mass Spectrometry for the Histotyping of Endometrial Carcinoma: A Proof-of-Concept Study with Clear Cell and Endometrioid Carcinoma

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Background: Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) is a rapidly emerging tool for the analysis of proteins on formalin-fixed tissue sections while preserving the spatial orientation of the tissue. One of the main advantages of MALDI IMS is the ability to screen for large numbers of proteins simultaneously on a single section, thereby expediting the discovery of proteins that may be characteristic of a particular tumor type or disease, and to accurately type them by IMS. In this pilot study, we assessed the applicability of MALDI IMS to the histotyping of endometrial carcinoma, using a test model of 2 histotypes [endometrioid (EEC) and clear cell (CCC) carcinoma].

Design: 57 CCC and 72 EEC (of all grades) were randomly assigned to 2 equally-sized tissue microarrays (TMA), each containing single 1-mm cores from 27 or 28 CCC and 36 EEC. The TMAs were then analyzed by MALDI IMS. After the exclusion of stromal areas and non-viable tumor areas in each TMA, peptide signatures were identified that differentiated CCC from EEC. The signatures were then cross validated.

Results: 47 CCC and 72 EEC had sufficient tissue for analysis. A total of 598 spectral peaks (m/z) were identified in both arrays, of which 261 (44%) had Wilcoxon p-values <0.05, indicative of significant differences between EEC and CCC. 11 peaks had areas under receiver operator characteristic curves of greater than 0.85, identifying peaks displaying the greatest differences between CCC and EEC. A genetic algorithmic classification model was generated from a mixed dataset derived from approximately half of both TMAs, which classified the dataset with a 96.57% accuracy (95.83% for CCC; 97.3% for EEC). The prediction accuracy of the model was estimated using a cross-validation algorithm in which 20% of the data were randomly left out in each of 10 iterations. After this leave-20%-out cross-validation algorithm was applied, the new model classified the dataset with 87.11% accuracy (82.22% for CCC; 92% for EEC). When the cross-validated signature was applied to the other half of both TMAs, it correctly classified 70.1% of the CCC spectra and 76.4% of the EEC spectra.

Conclusions: Since peptide signatures can be further analyzed to identify their associated proteins, this pilot study shows that MALDI IMS is a promising approach for the study and quantification of the differences and similarities between histotypes of endometrial carcinoma, and potentially, for the rapid discovery of discriminatory biomarkers.

1201 Ovarian Clear Cell Carcinoma and Serous Carcinoma: Immunohistochemical Profile and Correlation with Chemoresistance

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Background: Ovarian Clear cell carcinoma (OCCC), an ovarian carcinoma subtype, poorly responds to platinum-based chemotherapy. OCCC and ovarian serous carcinoma (OSC) arise by different molecular pathways and can be difficult to differentiate due to similar papillary architecture and nuclear pleomorphism. Gene expression studies demonstrated over-expression of HNF1β, FXYD2, and the *IL6-STAT3-HIF1A* pathway in OCCC. Prior studies also report an association of STAT3 with chemoresistance in OCCC patients. This study compares expression of HNF1β, FXYD2, and STAT3 in OCCC and OSC with subsequent comparison of STAT3 expression in OCCC and prognosis.

Design: IHC using FXDY2, HNF1β, and STAT3 was done on tissue-microarrays of material from 52 OCCC and 51 OSC patients. This was scored from 0-3 based on nuclear staining intensity of the tumor cells with 0-1 interpreted as negative/weak and 2-3 as positive. Samples from the same patient were averaged and rounded to the nearest integer. Clinical history was available for 32 of the 52 OCCC patients to correlate with STAT3 expression; patients not receiving chemotherapy were excluded.

Results: HNF1β, FXYD2, and STAT3 were positive in 90%, 86%, and 50% of OCCC and 10%, 77%, and 8% of OSC, respectively.

HNF1β			
Tumor type	Negative	Positive	p-value (Chi-squared test)
Clear cell carcinoma	5(10%)	47(90%)	<0.0001
Serous carcinoma	46(90%)	5(10%)	
FXDY2			
Tumor type	Negative	Positive	p-value (Chi-squared test)
Clear cell carcinoma	7(14%)	44(86%)	0.3102
Serous carcinoma	12(24%)	39(77%)	
STAT3			
Tumor type	Negative	Positive	p-value (Chi-squared test)
Clear cell carcinoma	25(50%)	25(50%)	<0.0001
Serous carcinoma	47(92%)	4(8%)	

Patient Status	**STAT3+**	**STAT3 -**	**p-value (Fisher's Exact Test)**
Chemoresponsive	11	6	0.03
Chemoresistant	2	8	

Conclusions: Staining for HNF1β confirms its use as a sensitive (90%) and specific (90%) immunomarker for OCCC in the differential diagnosis with OSC, while FXDY2 is not. STAT3 may serve as a potential immunomarker in diagnostically challenging cases of OCCC (specificity 92%, sensitivity 50%). Though STAT3 expression appears to positively correlate with responsiveness to chemotherapy, prior studies showed *in vitro* inhibition of IL-6 reduced the phosphorylation and activation of STAT3 (p-STAT3), enhancing tumor cell sensitivity towards cisplatin. Further analysis of p-STAT3 rather than STAT3 overexpression may serve as a more accurate predictor of chemoresistance.

1202 Ovarian Germ Cell Tumors Arise from Pre-Meiotic Stem Cells

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Background: In female gonadal development, primordial germ cells reach their peak number in the second trimester and then cease to proliferate after entering the first meiotic division. Oogonia remain arrested in meiosis I until puberty, after which ovulating cells complete meiosis. In mammals, there is no convincing evidence of a stem cell pool to produce additional oogonia in post-natal life. Ovarian germ cell tumors (OGCT) constitute a broad range of tumors, benign and malignant, which are canonically believed to derive from primordial germ cells. Previous reports have suggested that OGCTs can arise from either pre-meiotic primordial germ cells (or stem cells) or post-meiotic germ cells. The present study uses genetic fingerprinting applied to several histologic types of OGCTs to determine whether they reflect the pre-meiotic genome of the patient or derive from a post-meitotic cell.

Design: After receiving IRB approval, we selected 5 dysgerminomas, 5 mature teratomas, and 5 endodermal sinus tumors (EST) available in 1990-2013 from the pathology archives of the University of Chicago Hospitals. Corresponding non-neoplastic tissue from the same patient was also selected. DNA was isolated from malignant and benign cells that were macrodissected from formalin fixed paraffin embedded (FFPE) tissues. Short tandem repeat (STR) analysis was performed using AmpFlSTR profiler plus kit from Applied Biosystems on all samples with comparison of normal and neoplastic cell DNA profiles for each patient.

Results: STR analysis of 8 ovarian germ cell tumors in parallel with normal tissue from the corresponding patient demonstrated identical DNA profiles in 88% (7/8) of the cases, with one non-identical case suggesting origin from a post-meiotic cell. The remaining 7 cases were excluded for failure of DNA amplification due to the poor quality of the DNA from FFPE tissues.

OGCT Type	**DNA Profile Identical to Normal**	**DNA Profile Not Identical to Normal**	**Total cases**
Dysgerminoma	1	0	1
Endodermal Sinus Tumor	2	0	2
Mature Teratoma	4	1	5

Conclusions: In spite of the general consensus in the literature that there is no reserve pool of primordial oogonia in the human ovary, STR analysis shows that the majority of germ cell tumors in this study arise from a pre-meiotic cell: either a primordial oogonia or another pleuripotent stem cell. One case, a mature teratoma, was consistent with origin from a post-meiotic cell.

1203 A Novel Serum MicroRNA Panel to Discriminate Benign from Malignant Ovarian Disease

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Background: Ovarian cancer is the fifth most common cancer in women and the most frequent cause of gynaecological malignancy-related mortality in women. The vast majority present in advanced stages and this is due to lack of a reliable screening test and the absence of symptoms. Currently, no standardized reliable screening test exists. Pelvic examination is practiced widely, but it is not sensitive enough to be used as a reliable screening tool. There is a need for reliable diagnostic and prognostic markers for this disease. MicroRNA profiling has allowed the identification of signatures associated with diagnosis, prognosis and response to treatment of human tumours. The aim of this study was to determine if a microRNA signature could distinguish between malignant and benign ovarian disease.

Design: RNA extraction from serum was extracted using Qiagen miRNeasy® Mini Kit. A training set of 5 serous papillary ovarian adenocarcinomas (stage 3, grade 3) and 5 benign serous cystadenomas were selected for the initial training. A validation set included 20 serous papillary ovarian adenocarcinomas (stage 3, grade 3) and 20 benign serous cystadenomas. The serum/Plasma focus microRNA Exiqon panel was used for the training set. For the validation set a mix and pick Exiqon panel, which focuses on microRNAs of interest was used.

Results: The panel profiling was successfully completed. The raw data all show good data quality. 5 microRNAs were found to be differentially expressed using a cutoff of p-value < 0.05. Based on the results of the pilot study the top 23 deregulated microRNAs were validated. All samples passed the quality control tests. A panel of 4 microRNAs was significantly down regulated in cancer patients. These microRNAs target WNT signaling, AKT/mTOR and TLR-4/MyD88, which have previously been found to play a role in ovarian carcinogenesis and chemo resistance.

Conclusions: MicroRNAs could act as diagnostic biomarkers in ovarian cancer.

1204 Increasing the Sensitivity of Endocervical Curettings by Performing ThinPrep® Pap on Transport Container Fluid: Is Diagnostic Material Going down the Drain?

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Background: Endocervical curettings (ECC) performed at the time of loop electrocautery excision procedure (LEEP) have a high risk of harboring residual dysplasia and micro-invasive carcinomas. The false-negative rate reported for ECC in patients with cervical intraepithelial neoplasia (CIN) involving the endocervical canal is 45%. ECC samples are transported to pathology in either formalin or saline-filled containers and this fluid is discarded after the specimen is submitted. We evaluated the utility of performing liquid-based pap smears from the left-over ECC transport container fluid as a way to retrieve cells, increasing the diagnostic sensitivity of ECC.

Design: Consecutive ECC specimens received at one of the participating institutions (5/2013-9/2013) were prospectively selected. The surgical pathology mesh bag was placed over a ThinPrep® CytoLyt® Solution container (Hologic, Inc. Marlborough, MA), and the specimen was filtered through the bag, collecting the transport fluid in the container. The specimen in the mesh bag was processed routinely. The CytoLyt® was processed to obtain a ThinPrep® liquid-based pap slide. The pap smears were blindly reviewed by two pathologists and the findings were compared to those from the ECC and follow-up specimens.

Results: The case cohort included 19 patients, mean age of 40.5 years (range 23-70 years). Pre-ECC pap smear diagnoses were: 7 ASCUS, 7 LSIL, 2 HSIL, 2 AGC, and 1 high grade vulvar intraepithelial neoplasia (VIN III). Discrepancies between the ThinPrep® (TP) slide and the ECC were seen in 7/19 (37%); the TP impression was confirmed by biopsy or molecular testing in 4 cases. Discrepancies are summarized in table 1.

Table 1. Cases with discrepant ThinPrep® and ECC diagnoses

ThinPrep® Slide (Container Fluid)	**ECC**	**Follow-up**	**Original Pap/ Biopsy**
HSIL and adenocarcinoma in situ (AIS)	CIN III	CIN III and AIS (cone)	HSIL
HSIL	CIN I	VIN III (biopsy)	VIN III (biopsy)
ASC-US	No dysplasia/Human papillomavirus (HPV) changes seen	CIN I (cone)	LSIL
LSIL	No dysplasia/HPV changes seen	Positive HPV DNA test (molecular	LSIL
LSIL	No dysplasia/HPV changes seen	Negative (cone)	ASC-US
ASC-H	Negative	Negative (cone)	LSIL
ASC-US	Negative	Not performed	AGC

Conclusions: Combining the pathologic evaluation of ECC specimens with liquid-based cytology performed on the transport container fluid can increase the diagnostic sensitivity of the ECC procedure for detecting cervical lesions.

1205 p16 and Ki67 Expression in Squamous Intraepithelial Lesions of the Uterine Cervix Which Cannot Be Qualified (SIL Q)

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Background: Nearly 90% of LSIL (CIN I) is negative or focally positive with p16 and has a low Ki67 proliferation index. In contrast more than 90% of HSIL (CIN 2 /3) is diffusely and strongly positive with p16, and has a high Ki67 index. SIL Q, i.e., SIL

which cannot be graded as low or high grade due to incomplete SIL sampling and/or technical or processing limitations may represent undetected AIS/HSIL/Carcinoma. The role of p16 and/or Ki67 expression in detecting SIL Q outcome as positive or negative for AIS/HSIL/Carcinoma has not been evaluated.

Design: 80 SIL Q cases were randomly selected from a group of 332 with SIL Q as the worst initial visit colposcopy diagnosis and were stained by standard immunohistochemistry (IHC) with p16 and Ki67. 149 controls slides of 44 NILM, 15 LSIL, 75 HSIL and 15 AIS were also stained to validate the IHC. Staining was scored together by 2 observers using 2 separate systems for each biomarker. Scoring was based on the location and number of stained SIL Q cells and categorized as positive or negative. System 1 required a basal layer and 2 did not. Outcome was based on the worst histology in 24 months of colposcopy and/or surgical follow up. Biomarker and outcome status were correlated and sensitivity, specificity PPV and NPV were determined.

Results: Performance in the detection of outcome which was positive in 38 (48.1%) cases and 100 (67.1%) controls with system 1, and system 2 was:

Biomarker	p16		Ki67		p16 and/or Ki67	
	System 1	System 2	System 1	System 2	System 1	System 2
	Case:control	Case:control	Case:control	Case:control	Case:control	Case:control
Sensitivity	74.1:90.8	81.6:91.9	85.2:78.8	70.3:79	88.9:91.8	89.2:91.9
Specificity	41.4:90.2	35:80.9	43.3:95.2	52.2:95.7	37.9:90.2	32.5:82.6
PPV	54.1:95.7	54.4:91	57.5:97.5	57.8:97.5	57.1:95.7	55:91.9
NPV	63.2:80.4	66.7:82.6	76.5:65.6	65.6:68.2	78.6:82.2	76.5:82.6

Amongst the cases, the sensitivity and NPV were high, specificity was low, and the PPV was modest regardless of scoring system, biomarker type and whether the markers were measured singly or in combination. Combined markers scored with System 1 achieved the highest NPV. All performance metrics were high amongst the controls.

Conclusions: In terms of SIL Q management, p16 and/or Ki67 IHC staining of SIL Q is of little value in the detection of a positive outcome, but may be of some value in the detection of a negative outcome.

1206 The miR-34 Family (a, b, and c) and p53 Expression in ER-Positive Breast and Ovarian Serous Cancers

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Background: Among the ER-positive ovarian serous tumors, p53 has been implicated in the development of type II high grade serous carcinoma, whereas type I low grade serous carcinoma is thought to arise in a stepwise fashion from benign serous cystadenoma through serous borderline tumor to invasive carcinoma with KRAS and BRAF mutation. In this study, the expression of miR-34 family (a, b, and c) was studied to define its role in ER-positive ovarian serous cancers in relation with p53. ER-positive Luminal A breast cancers were also studied in comparison.

Design: miR34 families were measured in 94 ER-positive serous carcinomas of the ovary and 110 Luminal A breast cancers by RT-PCR technique. The tumors were divided into the low p53-expressing group and the high p53-expressing group, and further divided into p53 mutant type (p53 overexpression without MDM2 elevation), and p53 wild type (p53 overexpression accompanied by MDM2 elevation).

Results: Among the serous carcinoma of ovary, miR-34a and miR-34b expression of the p53 Mutant tumors (35 cases) were significantly lower than the expression of the p53 Wild tumors (59 cases) ($p<0.0001$ and $p=0.019$). miR-34c expression was slightly lower in the p53 Mutant tumors, but there was no statistical significance ($p=0.058$). The expressions of all miR-34 family in the p53 Mutant tumors (63 cases) were lower than the expressions in the p53 Wild tumors (47 cases) of the ER-positive breast cancers ($p=0.001$, $p=0.027$, and $p=0.023$).

Conclusions: The miR34 family may play the role in the ER-dependent ovarian and breast cancers through p53, suggesting the possible target of the therapy.

1207 Utilization of Gene Expression Data to Confirm Chemoresistant and Disease Progression Biomarkers in Ovarian Cancer: Analysis with Multiplex Quantitative Immunofluorescence

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Background: Ovarian cancer is the leading cause of gynecological cancer related death in the United States. Current biomarker screening techniques are suboptimal, and the majority of patients have advanced disease at the time of diagnosis. Quantitative multiplex immunofluorescent (QMIF) platform utilizing formalin-fixed, paraffin-embedded (FFPE) ovarian tissues was used to evaluate a series of potential predictive biomarkers.

Design: 89 patients with ovarian cancer treated with surgery and adjuvant platinum-based chemotherapy were selected from the Mount Sinai surgical pathology archives and Biorepository. Mean age was 59 yrs, with 80% serous, 88% Grade 3, and 85%>/=Stage IIIC. Overall survival was 51% and platinum resistance was 54% with median follow-up of 32 months. 8 tissue microarrays (TMA) were created with tumor and normal tissues. Kaplan Meier with log-rank test and multivariate Cox proportional hazards were used to evaluate association of marker expression with clinical data. Empiric biomarker selection was compared to gene expression assessment utilizing Significance Analysis of Microarray (SAM) on public datasets with emphasis on grade>/=2, stage>/=III serous adenocarcinoma. Ingenuity Pathways Analysis (IPA) was used for functional analysis.

Results: TP53/CK7 was an independent negative prognostic factor for progression-free survival of serous adenocarcinoma after resection and chemotherapy ($P<0.001$). TP53/DAPI/CK7 was an independent negative prognostic factor for overall survival ($P=0.040$). Additionally, pERK is positively associated with response to platinum chemotherapy ($P=0.006$) and lack of tumor recurrence ($P=0.020$). VEGF is positively associated with response to platinum chemotherapy ($P=0.028$) and tumor recurrence ($P=0.004$). SAM analysis was used to evaluate gene expression in primary tumors vs normal tissue identified upregulation of empirically-selected biomarkers: p53, VEGF, Aurora A, Notch 3, and cyclin E. SAM analysis for primary vs metastasis resulted in 75 genes upregulated in metastases. IPA revealed that these genes are involved in cellular movement, angiogenesis, and cell-cell signaling.

Conclusions: Elevated expression of quantitative TP53 in ovarian tumor epithelial cells was associated with reduced overall survival while increased levels of pERK and VEGF were both linked to platinum response. Future studies with QMIF on primary and metastatic samples using Notch3, Aurora Kinase A and Cyclin E, confirmed by SAM analysis, will assess association with disease progression and chemoresponse.

1208 Squamous Intraepithelial Lesions of the Uterine Cervix Which Cannot Be Qualified: SIL Q

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Background: Incompletely sampled cervical Squamous Intraepithelial Lesions (SIL) and/or substandard fixation and processing of the tissue can limit grading as high or low grade and result in a diagnosis of unqualified SIL or SIL Q. SIL Q outcome based on follow up pathology obtained at colposcopy or surgery and correlates of outcome have not been determined.

Design: From a previously reported cohort of 17, 551 women attending colposcopy over a 5 year period (Am J Obstet Gynecol 2010: 203; 481), 332 (1.9%) had SIL Q as their worst initial colposcopic visit diagnosis and also had at least one follow up colposcopy exam. Demographic, clinical, referral Pap test, and colposcopy data at the initial visit and outcome data based on 24months of follow up was extracted from the cohort's electronic data base. Outcome was based on the worst histology amongst cervical biopsy, endocervical curettage, LEEP and/or hysterectomy procedures and categorized as positive if the worst diagnoses were HSIL, AIS or carcinoma and negative if normal, benign or LSIL.

Results: Mean (median) age at presentation was 39.1 (36.5) years and ranged from 20-79. Most had 1-3 pregnancies/births (155, 54.9%/141, 50%), were non smokers (195, 70.1%) and immunocompetent (260, 98.1%), used contraception (219, 78.8%), and had a referral Pap test of AGC/AIS/ASC-H/HSIL (188, 56.5%), adequate colposcopy (202, 74.5%), and a negative/LSIL (220, 77.2%) colposcopy impression. Outcome was available for 329 and was negative in 195 (59.3%) and positive in 134 (40.7%). The mean age of those with a positive versus negative outcome was younger (37.4 vs. 40.3, $p=0.048$), the referral Pap test was more frequently AGC/ASC-H/HSIL (70.1% vs. 47.2%, $p<0.001$), and a colposcopy impression of HSIL/Malignant was more frequent (33.1% vs. 14.8%, $p<0.001$). Significant differences did not occur amongst other variables.

Conclusions: SIL Q as diagnostic category of histopathology reporting is justified based on the 2% prevalence and a positive outcome in nearly 40%. The referral Pap test and colposcopy impression results could be used to streamline patient management and maximize cervical cancer control.

1209 Stem Cell Marker NANOG Alteration in Recurrent Low Grade Endometrioid Adenocarcinomas

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Background: The majority of endometrial carcinomas (EMCA) present as low grade, early stage tumors. Roughly 75% of these patients are cured by surgery with or without radiation therapy. When relapse occurs, it frequently involves the vaginal cuff (local relapse), or as pelvic or abdominal disease (regional relapse) or as distant metastasis. Identification of a biomarker predicting the risk of recurrence of such cases would have immense clinical utility in formulating treatment strategy upfront to prevent recurrence. Previous studies have reported the presence of cancer stem cells in human EMCA. The role of stem cells in recurrent EMCA has not been investigated. It is possible that these cells my be responsible for recurrence. The overexpression of stem cell marker NANOG has been reported in EMCA, however, the role of NANOG in recurrent disease has not been examined. Animal experiments using siNANOG have shown promising therapeutic benefit in various cancers. If primary and recurrent EMCAs show NANOG expression, targeting tumor cells with siNANOG may be used for the treatment of EMCAs with risk of recurrence.

Design: A retrospective review of all endometrioid EMCAs at our institution over a ten year span revealed 1685 cases. Of these, 944 (56%) were grade 1 (G1), 517(31%) G2, and 224(13%) G3. 87 cases recurred: 27(31%) G1, 27(31%) G2, and 33(38%) G3. Archival paraffin embedded tissue of 13 G1 cases and 6 G2 cases and subsequent recurrences were examined by immunohistochemistry for localization and expression of NANOG. Age and stage match 19 controls were evaluated concurrently. The staining was semiquantitatively scored by combination of intensity and extent (in %) of staining.

Results: NANOG expression was seen in cytoplasm and nucleus of EMCA tumor cells. Expression of NANOG was identified in 2/13 (14.3%) G1 primary EMCA versus 5/14 (35.7%) of recurrent G1 EMCA ($P=0.048$). No significant difference of NANOG expression was noted between G1 primary versus recurrent EMCA, and age and stage matched G1 controls ($p=0.55$). More tumor cells in G2 EMCA demonstrated expression of NANOG compared to G1 EMCA in primary, recurrent and control groups. 4/6 cases (66.7%) of G2 primary EMCA show NANOG expression versus 3/6 cases (50%) of recurrent G2 EMCA ($P=0.58$).

Conclusions: NANOG expression is associated with recurrence in low grade endometrioid EMCA (G1). Higher grade EMCA demonstrates more NANOG expression. These findings suggest that cancer stem cells are involved in the recurrence of low grade EMCA and might be a promising target for therapy.

1210 Morphologic Correlates of Human Papilloma Virus (HPV) Infection in Low-Grade Squamous Lesions of the Female External Genital Tract

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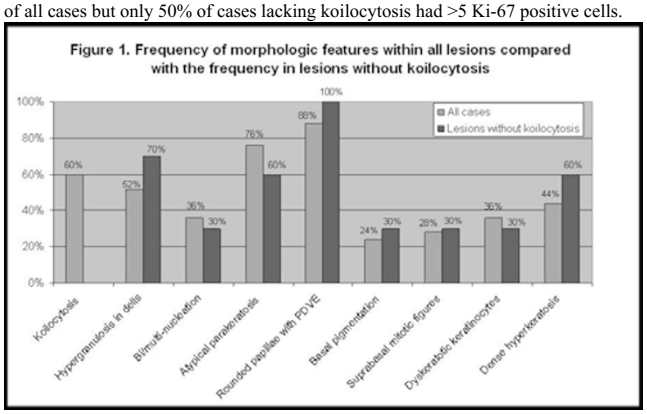
Background: The histologic features of low-grade HPV-associated lesions in the female external genital tract, namely condyloma, are varied and can be subtle. Histologic identification of condyloma in hair-bearing skin can be problematic because the classic cytopathologic effect of HPV, koilocytosis, may be absent. We retrospectively examined a series of HPV positive lesions from this region for nine specific morphologic features, which may be associated with HPV infection.

Design: Twenty-five cases of HPV in situ hybridization (ISH)-positive condylomas involving genital skin were collaboratively reviewed by four gynecologic pathologists. Each case was evaluated for the presence or absence of nine morphologic features (Figure 1). Ki-67 immunostain was performed on each case and suprabasal keratinocytes with nuclear positivity were counted and categorized as 0-5, 5-20, or >20 positive cells.

Results: In all cases, the most frequent features were rounded papillae with papillary dermal vascular ectasia (PDVE) (88%), atypical parakeratosis (76%), koilocytosis (60%), and hypergranulosis accentuated in the dells (52%). In cases lacking koilocytosis, the most frequent findings were rounded papillae with PDVE (100%), hypergranulosis accentuated in the dells (70%), dense hyperkeratosis (60%), and atypical parakeratosis (60%) (Figure 1). Ki-67 positivity in suprabasal keratinocytes was found in the following frequencies: >20 (56%), 6-20 (12%), and <5 (32%). The Ki-67 count was >5 in 78% of all cases but only 50% of cases lacking koilocytosis had >5 Ki-67 positive cells.



Conclusions: The most specific histologic feature of low-grade HPV-associated lesions of the female external genital tract is koilocytosis; however, in our series, 40% of cases lacked koilocytosis. In such cases, identification of rounded papillae with PDVE, hypergranulosis accentuated in the dells, atypical parakeratosis, and dense hyperkeratosis can aid in the recognition of HPV lesions. In addition, Ki-67 expression in suprabasilar keratinocytes can act as an adjunct supportive test when used in combination with the evaluation of specific histologic features.

1211 An Immunohistochemical Comparison of Ovarian and Uterine Endometrioid Carcinoma, Endometrioid Carcinoma with Clear Cell Change and Clear Cell Carcinoma

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Background: Endometrioid and clear cell carcinomas(CCC) of the gynecological tract show distinct clinicopathological profiles. Occasionally, endometrioid carcinomas(EC) exhibit clear cell change, mimicking CCC. This study compares the immunoprofiles of ovarian and uterine EC, EC with clear cell change(EC-CC) and CCC.

Design: Representative sections were selected from 62 CCC(38 ovarian, 24 uterine), 33 FIGO grade 1-2 EC(22 ovarian, 11 uterine) and 33 EC-CC(5 ovarian, 28 uterine) and stained for HNF1β, BAF250a(ARID1a), NapsinA, ER and PR. EC-CC slides showed≥10% CC areas, comprising either of squamoid, secretory or non-specific(NOS) change. Intensity of staining was scored as 0 to 3+ and extent of staining was scored as: 1+=<1%; 2+=≥1%to<10%; 3+=≥10%to<50% and 4+=≥50%. Any nuclear or cytoplasmic staining was considered positive for (HNF1β, ER and PR) and NapsinA respectively. Loss of BAF250a was defined as lack of nuclear staining in >90% of cells.

Results: 55/62(88.7%)CCC, 14/33(42.4%)EC-CC and 17/33(51.5%)EC showed immunoreactivity for HNF1β and staining was≥2+ in≥10% of cells in 49/55, 10/14 and 9/17 cases, respectively. 54/62(87.1%)CCC, 4/33(12.1%)EC-CC and 2/33(6.1%) EC stained for NapsinA. 49/54CCC and 2/4uterine-EC-CC (both with NOS CC) demonstrated≥2+ staining in≥10% of cells. Staining was 3+ in <1% of cells in 2 EC(ovarian). 11/62(17.7%)CCC, 4/33(12.1%)EC-CC and 2/33(6.1%)EC showed loss of BAF250a. Four EC-CC(uterine) showed decreased expression of BAF250a in CC areas (2 secretory and 2 NOS changes). ER was expressed in 10/62(16.1%)CCC, 29/33(87.9%)EC-CC and 31/33(93.9%)EC, while PR was positive in 9/62(14.5%) CCC, 25/33(75.8%)EC-CC and 32/33(97.0%)EC. Decreased ER and PR expression was identified in CC areas of 13/24(54.2%) and 6/21 (28.6%) uterine EC-CC as well as 4/5(80%) and 2/4(50%) ovarian EC-CC. Differences in immunoexpression of HNF1β, NapsinA, ER and PR in CCC versus EC-CC/EC were statistically significant(p<0.0001). EC-CC showed a statistically significant reduced PR positivity when compared to EC(p=0.0268). Although EC-CC also demonstrated a trend towards loss of BAF250a and ER, the difference in expression between EC-CC versus EC was not statistically significant(p=0.67).

Conclusions: EC-CC and EC show similar immunoprofiles. Use of HNF1β, NapsinA, ER and PR can help distinguish EC-CC from CCC.

1212 Somatic *PIK3CA* Mutations in Copy-Number Low Endometrioid Endometrial Adenocarcinomas Are Associated with Better Survival

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Background: The PI3K pathway is implicated in the development of Endometrioid Endometrial Adenocarcinomas (EEC). Recently, The Cancer Genome Atlas (TCGA) Project has stratified EEC into 4 different molecular subgroups with most tumors falling into the low somatic copy-number alterations, microsatellite stable, molecular subgroup (CNL-EEC). The goal of this study was to investigate whether mutations of members of the PI3K pathway are associated with specific survival outcomes in patients with EEC.

Design: Clinical and genomic data of samples from the endometrial carcinoma TCGA study were evaluated with the analytical tools of the TCGA data portal. Association between somatic *PIK3CA* mutations and survival was determined by the Kaplan Meier method. The diagnosis of EEC was confirmed by evaluating high-resolution whole slide H&E images from available frozen and permanent sections. Tumor stage and overall FIGO grade were determined by reviewing H&E images and pathology reports. The effect of specific somatic mutations on *PIK3CA* function was determined by cross-referencing them to previously reported gain-of-function mutations.

Results: The PI3K pathway was altered in 93% (83 of 90) of CNL-EEC as evidenced by mutations in *PTEN*, *PIK3CA*, *PIK3R1* and *AKT1*. Of these, mutations of *PIK3CA* (seen in 48 of 90 CNL-EEC cases) were associated with significant better survival than a wild-type *PIK3CA* genotype in both EEC and CNL-EEC. Better survival in PIK3CA-mutated cases was specific to the CNL-ECC molecular subgroup and not observed in the 3 other TCGA molecular subgroups. There were no significant differences in age, tumor stage and FIGO grade between *PIK3CA* mutated and non-mutated cancers. 79% (38 of 48) of cases contained activating, gain-of-function *PIK3CA* mutations, while 8% (4 of 48) of cases contained new missense mutations in codons previously implicated in *PIK3CA* activation. 13% (6 of 48) of cases had mutations of unknown effect on *PIK3CA* function.

Conclusions: Somatic gain-of-function *PIK3CA* mutations occur in approximately half of the copy-number low, EEC subgroup, and these patients appear to have better survival than those with wild-type *PIK3CA*. Integrated analysis of molecular and histopathological characteristics of Endometrial Adenocarcinomas may better stratify patients who are at increased risk of progression and who may benefit from target-directed adjuvant therapies.

1213 How Reliable Is p16 Staining in Cervical HPV Suspected Lesions?

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Background: Immunohistochemistry (IHC) with p16 evaluation of potential cervical dysplasia has aided significantly with distinguishing normal from dysplastic tissue, and in determining the degrees of dysplasia. Sensitivity for the detection of dysplasia is much higher with p16 staining than histologic interpretation alone; however, there are rare cases where p16 can lead to false positive and negative results. To explore this issue, we conducted an evaluation of cervical biopsies with p16 and Ki-67staining and compared these results to the H&E histologic features.

Design: We reviewed 171 cases of cervical biopsies, all with p16 and Ki-67 stains. These cases were classified as classical dysplasias, classical metaplasias, questionable dysplasias, and probable metaplasias positive for p16. Ten of the 171 cases fell into the latter two categories based on incongruent results between H&E and p16. In these incongruent cases, HPV low and high risk in-situ hybridization (ISH) and specific nucleic acid sequence detection for high risk subtypes using signal amplification methods were performed. In positive cases for high risk HPV, whenever enough DNA was available, reflex tests were performed for subtypes 16/18 and non 16/18. A total of 26 high risk HPV subtypes were investigated.

Results: In 161 cases (94.2%) the H&E findings were congruent with the p16 and Ki-67. In 10 cases (5.8%) the results of the H&E and p16 and Ki-67 were incongruent. Six of the 10 cases were possible dysplasias by H&E but p16 in these cases was positive in only isolated cells. All 6 cases were positive for high risk HPV types. In 4 of these cases, we had enough DNA for reflex which confirmed the presence of non-16/18 high risk subtypes. The remaining four of the 10 cases stained diffusely for p16; however, H&E were not atypical enough for classical dysplasias. There were residual mucinous cells in the upper part of the squamous metaplastic epithelium, and Ki-67 was positive in very few parabasal cells. All 4 cases were negative for HPV by ISH and signal amplification.

Conclusions: 1- Diffuse p16 staining is an excellent marker to confirm cervical high grade dysplasia in 94.2% of the cases.

2- Diffuse p16 can rarely be seen in squamous metaplasia, mainly in cases where residual mucinous cell are present. K-i67 stains positively in very few cells, predominantly at the base of the epithelium.

3- Focal/patchy p16 staining, frequently considered negative, can be associated with high-grade dysplasia due to high risk HPV but not 16-18 types.

1214 Pseudorosette-Like Proliferations in Endometrium

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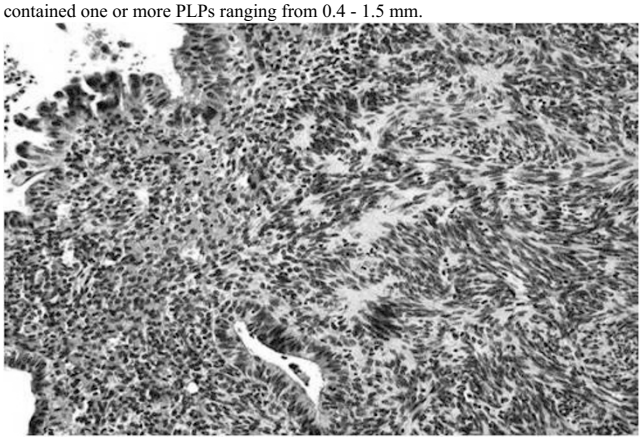
Background: The capricious nature of endometrium results in a wide spectrum of appearances in both neoplastic and non-neoplastic tissue. Unusual stromal proliferations may be encountered leading to misinterpretation. In this study we investigated pseudorosette-like proliferations (PLPs) in the stroma of proliferative endometrium which have not been previously reported.

Design: Fifteen cases of PLPs were identified for this study. Patient age, reason for biopsy, and exogenous hormone use were collected. Characteristics of the endometrium including phase, breakdown, hyperplasia, or endometritis were recorded. The cases were then immunostained for cytokeratin AE1/AE3, CD10, smooth muscle actin (SMA), S-100 and caldesmon to evaluate the nature of these proliferations.

Results: The mean age of patients was 48.3 years (range 37-56). All biopsies were performed for abnormal bleeding. In all 15 cases the endometrium was in the proliferative phase with no evidence of hyperplasia or endometritis. Only one case showed significant breakdown and epithelial metaplasia. Each endometrial sample contained one or more PLPs ranging from 0.4 - 1.5 mm.



In 9 cases, a pseudorosette-like focus was available for immunostaining. Eight of the 9 cases showed strong (3+) staining of the PLP for SMA and one case showed moderate (2+) staining. Six cases showed negative or weak (1+) staining for CD10 in the PLP. The remaining 3 cases showed 2+ staining for CD10. In all cases, staining for SMA was stronger than for CD10. In all cases, PLPs were negative for cytokeratin, S-100, and caldesmon.

Conclusions: Pseudorosette-like proliferations may occur in the stroma of proliferative endometrium. Such proliferations appear unrelated to use of exogenous hormone, breakdown, endometritis or hyperplasia. While PLP may weakly or moderately express CD10, they invariably strongly express SMA, suggesting smooth muscle differentiation. Recent work has shown that human endometrial stem cells will successfully differentiate into smooth muscle cells when supplemented with myogenic growth factors in vitro. Further studies are needed to elucidate the factors contributing to smooth muscle differentiation of endometrial stromal proliferations in vivo.

1215 The RNA-Binding Protein IMP3: A Novel Molecular Marker Which Predicts Recurrence of Endometrial Adenocarcinoma

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Background: Recurrence of localized endometrial adenocarcinoma of the endometrioid type is often unpredictable. We aimed to investigate whether an oncofetal protein, IMP3, can serve as a biomarker to predict recurrence for patients with endometrial adenocarcinoma.

Design: A total of 248 patients with a diagnosis of primary endometrial adenocarcinoma, endometrioid type, who underwent hysterectomy were studied. All cases were collected between September 1997 and August 2005 in a large tertiary academic medical center. The expression of IMP3 in endometrial adenocarcinomas was evaluated by immunohistochemical staining. All patients were further evaluated for outcome analyses. The median follow-up was 59 months.

Results: IMP3 was significantly increased in a subset of endometrial carcinomas that subsequently recurred. Patients with IMP3-positive tumors (N=95; 38%) were over 6 times more likely to recur [hazard ratio (HR) 6.68; 95% CI 2.90 – 15.41; p<0.0001] and were nearly twice as likely to die (HR 1.68; 95% CI 1.06 – 2.67; p=0.027) compared with patients with IMP3-negative tumors. The 5-year recurrence-free survival rates were 93% for patients with IMP3-negative endometrial carcinomas compared to 60% for patients with IMP3-positive tumors. Importantly, none of patients with stage 1 tumors without IMP3 expression recurred whereas 27% of patients with stage 1 tumors comprising of IMP3 protein were found to have recurrences within 5 years follow-up. In a multivariable analysis adjusting for age, FIGO stage, tumor grade and treatment, patients with IMP3-positive tumors were still over 6 times more likely to recur (HR 6.64; 95% CI 2.63 – 16.77; p<0.001) compared with patients with IMP3-negative tumors.

Conclusions: IMP3 is an independent prognostic biomarker that can be used at initial diagnosis of endometrial adenocarcinomas, particularly stage 1 tumors, to identify a subgroup of patients who have a high potential to develop recurrence.

1216 Utility of Claudin-18 Immunohistochemistry for Detecting Benign Endocervical Glandular Lesions Exhibiting Gastric Differentiation (So-Called Lobular Endocervical Glandular Hyperplasia)

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Background: Lobular endocervical glandular hyperplasia (LEGH) was initially proposed to be a benign hyperplastic/metaplastic lesion consisting of bland mucinous glands with lobulated architecture. Subsequently, the gastric phenotype of LEGH was identified. Now, the term LEGH is frequently used to refer to the proliferation of gastric-type mucinous glands that express gastric markers such as MUC6. Furthermore, LEGH is recognized as a possible precursor of gastric-type adenocarcinoma of the endocervix. Therefore, the need for accurate diagnosis of the lesion is increasing. However, the extent of the lobular architecture is variable across cases, and it is often difficult to diagnose LEGH from small specimens that contain only non-lobulated glands. In this study, we examined the utility of a new pan-gastric immunohistochemical marker, claudin-18 (CLDN18), in the diagnosis of LEGH.

Design: Seven cases with benign endocervical glandular lesions exhibiting gastric differentiation were retrieved. The diagnosis of LEGH was made when the proliferation of glands with gastric-type morphology, either foveolar or pyloric type, was present, and when at least focal lobular architecture was seen. Immunohistochemistry for CLDN18, MUC5AC, MUC6, and ER was performed. The immunophenotypes of the glands of LEGH and background endocervical glands were compared.

Results: The diagnosis of LEGH was made in all cases, although a significant number of non-lobulated glands were present in many of them. All LEGHs were immunoreactive for the gastric markers CLDN18, MUC5AC, and MUC6. CLDN18 was usually negative in the background endocervical glands, whereas the focal expression of MUC5AC and MUC6 was observed frequently. ER expression was lost in most LEGHs.

Immunophenotype of LEGH

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
CLDN18 (LEGH)	95%	90%	95%	100%	90%	100%	80%
CLDN18 (BG)	0%	10%	0%	0%	0%	0%	0%
MUC6 (LEGH)	60%	60%	60%	25%	80%	90%	60%
MUC6 (BG)	90%	10%	20%	30%	65%	30%	30%
MUC5AC (LEGH)	90%	90%	70%	70%	85%	90%	90%
MUC5AC (BG)	15%	10%	0%	0%	30%	5%	5%
ER (LEGH)	0%	5%	30%	40%	0%	1%	20%
ER (BG)	100%	100%	100%	100%	100%	100%	100%

LEGH; lobular endocervical glandular hyperplasia, BG; background

Conclusions: CLDN18 is a sensitive, specific gastric marker for LEGH. Positive CLDN18 immunoreactivity and negative ER immunoreactivity suggest the possibility of LEGH, even when the lobular architecture is inconspicuous morphologically.

1217 *MED12* Mutations in Adnexal (Ovarian and Paraovarian) Leiomyomas

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Background: Adnexal (ovarian and paraovarian) leiomyomas are rare and their histogenesis is uncertain. No study has evaluated the genetic alterations of a series of ovarian/paraovarian leiomyomas in detail. Recent reports have shown that the *MED12* (mediator complex subunit 12) gene is frequently mutated in uterine leiomyomas, but not in leiomyomas at other sites, such as the gastrointestinal tract and soft tissues. This study elucidated the frequency of *MED12* mutations in adnexal leiomyomas, and examined a possible link in the pathogenesis of adnexal and uterine leiomyomas.

Design: Five cases of adnexal (three ovarian, two paraovarian) leiomyomas were retrieved from the archives of the Department of Pathology of the University of Tokyo Hospital. All of the tumors presented as unilateral adnexal masses. None of the tumors were attached to the uterus. Immunohistochemistry for desmin, smooth muscle actin (SMA), WT-1, ER, PgR, vimentin, calretinin, α-inhibin, and CD10 was performed to confirm the diagnosis of leiomyoma. We analyzed all of the tumors for *MED12* mutations. Four-μm-thick sections were cut from paraffin-embedded blocks containing leiomyomas and blocks containing background non-neoplastic tissues. Representative areas of the sections were dissected. Genomic DNA was extracted from the sections. The samples were subjected to polymerase chain reaction (PCR) using a pair of primers encompassing the frequently mutated region of *MED12* (5'-ACTCTCCCACCCCTTCCCCC-3' and 5'-GGCAGGATTGAAGCTGACGTTC-3'). Then, the PCR products were sequenced.

Results: Immunohistochemically, all of the tumors were positive for desmin, smooth muscle actin (SMA), WT-1, ER, PgR, and vimentin and negative for calretinin, α-inhibin, and CD10. Somatic *MED12* mutations were detected in all five leiomyomas. Four of the tumors harbored missense mutations. In one of the cases, the tumor harbored an in-frame deletion mutation.

MED12 mutations in adnexal leiomyomas

	Age	Location	MED12 mutation
Case 1	41	Right ovary	c.131 G>A (p.G44D)
Case 2	49	Right ovary	c.130 G>A (p.G44S)
Case 3	43	Left ovary	c.116_151del36 (p.L39_V51del insF)
Case 4	44	Left paraovarian region	c.131 G>A (p.G44D)
Case 5	66	Right paraovarian region	c.130 G>T (p.G44C)

Conclusions: *MED12* exon 2 mutations are frequent in adnexal leiomyomas. Our data suggest that the pathogenesis of adnexal leiomyomas is similar to that of uterine leiomyomas. Based on our study and reports on *MED12* mutations in smooth muscle tumors from various sites, we speculated that pelvic and extra-pelvic smooth muscle tumors are distinct in terms of their background genetic alterations.

1218 FISH Analysis of PTEN in Endometrial Carcinoma. Comparison with SNP Arrays and MLPA

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Background: PTEN inactivation is frequent in Endometrial carcinoma (EC), and may be caused by several mechanisms. PTEN copy number may be assessed by FISH.

Design: We have used a standardized protocol of PTEN FISH in 31 EC, in comparison with SNP array (aSNP), Multiple Ligation Probe Amplification (MLPA), and immunohistochemistry (IHC).

Results: FISH analysis showed 2 PTEN copies in 17 cases, 3 copies in 9, hemizygous PTEN deletion in 2, and more than one cell population with different PTEN copy number in 3 cases. A good correlation was seen between FISH and aSNP, particularly in cases with 3 PTEN copies. FISH identified 2 cases with deletion of whole chromosome 10, but did not identify a focal deletion of PTEN. Five cases with PTEN deletion and duplication of the second allele by aSNP were interpreted as normal by FISH. Concordance between FISH and MLPA was seen in 15 cases with 2 PTEN copies, while two cases were discordant. Concordant pattern of PTEN deletion by FISH and MLPA was seen in 2 cases, while results were discordant in 2. Six cases were interpreted as amplified by MLPA, but showed polyploidy by FISH. One case with complex MLPA pattern (amplification and deletion) was interpreted as normal by FISH. FISH was superior to aSNP and MLPA in assessing the 3 tumors with more than one cell population with different PTEN copies. IHC correlated quite well in cases with 1 and 2 PTEN copies. However, tumors with 3 PTEN copies did not show increased PTEN protein levels.

Conclusions: In general, results show good concordance between FISH, aSNP and MLPA, particularly regarding tumors with 2 or 3 PTEN copies. aSNP was superior in tumors with deletion of one copy and duplication of the second allele. FISH was superior to aSNP and MLPA in interpreting molecular results in correlation with morphological features, and assessing tumor heterogeneity.

1219 Malignant Mesothelioma of the Female Peritoneum: A Clinicopathologic Study of 95 Cases

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Background: Malignant mesothelioma of the female peritoneum (MMFP) is an uncommon disease with a variable clinical course. In this study, we present the clinicopathologic features of 95 cases seen in our institution.

Design: A search of the database of our department identified 98 cases of MMFP from a 23 year period (1990-present). H&E slides were reviewed in 96 cases (range 1-80) and the diagnosis of MMFP was confirmed in 95 cases. Clinical information was obtained from the patients'(pts) charts or from the treating physicians. The following clinical features were recorded: pts' age, clinical presentation, family history of cancer, asbestos exposure, elevated CA 125, anatomical site of involvement, treatment (TX) and follow-up. The following pathology features were examined: architectural pattern, cell type (epithelioid, spindle), mitotic index (MI) per 10 high power fields (HPFs), the presence of invasion, psammoma bodies, and nuclear grade (0,1,2,3).

Results: The pts' age ranged from 3 to 85 years (median, 49). Most cases presented with either abdominal/pelvic pain or mass, ascites or abdominal distension. Rare symptoms included: back/neck pain, leg swelling, weight loss, vomiting and changes of bowel habits. 12 cases were incidentally found. A vague history of asbestos exposure was noted in 3 cases. A positive family history of cancer was obtained in 30/42 cases. An elevated CA125 was noted in 24/38 cases. The involvement of multiple sites was common while the involvement of a single site was noted in 8 cases. Most tumors had a combination of architectural patterns including: papillary, tubular, cystic, solid, single cells, clusters of cells or trabeculae; four tumors showed a single architectural patttern (solid, 3; cystic,1). 79 cases were composed of epithelioid cells and 16 cases had also spindle cells. 44 cases had marked variation in the nuclear grade (0 to 3), 31 cases had a nuclear grade 0-1, and 18 had a nuclear grade 2-3. The MI ranged from 0 to 27 per 10 HPFs (median, 1). Psammoma bodies were seen in 23 cases. Invasion was documented in 83 cases. TX included surgery with or without chemotherapy or intraperitoneal chemotherapy including the hypethermic modality. Outcome: 24, DOD (2-168mos); 18, AWD (4-108mos); 19, ANED (4-99mos) and 3, alive, status unknown (7,18 and 41mos).

Conclusions: MMFP is an uncommon disease seen in pts of a wide age range. MMFP can be incidentally found. The indolent behavior of some cases of MMFP could be related to focal rather than extensive involvement and the use of new treatment modalitites such as hypethermic intraperitoneal chemotherapy.

1220 Female Adnexal Tumor of Probable Wolffian Origin and Mimics: An Immunohistochemical Study with Mesonephric and Mullerian Markers

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Background: Female adnexal tumor of probable wolffian origin (FATWO) is a rare tumor, believed to be of wolffian (mesonephric) origin. It lacks a specific immunohistochemical profile with variable expression of cytokeratins, calretinin, androgen receptors and CD10, and usual lack of EMA. Considering the important roles of PAX2, PAX8 and GATA3 in the development of the mesonephros, recent studies describing expression of PAX2 and PAX8 in mesonephric lesions, as well as GATA3 in cervicovaginal mesonephric lesions, we studied its expression in FATWOs and FATWO-like tumors. In addition, we explored the expression of these markers in rete ovarii, another proposed site of origin of FATWO tumors.

Design: A database search (1990-2013) was conducted for cases of FATWO and tumors with FATWO-like areas. In addition, 11 recent cases of ovary with rete ovarii were included. PAX8 immunostain was performed in all cases, while PAX2 and GATA3 immunostains were performed in cases with available blocks or unstained slides. For all the stains, only nuclear staining was considered positive. Stains were interpreted as negative (<5%), focal (5-20%), patchy (20-50%), diffuse (>50%); weak or strong intensity.

Results: Thirteen cases were identified: 9 FATWOs and 4 ovarian/paraovarian endometrioid adenocarcinomas with FATWO-like areas. PAX8 was negative in all 9 cases of FATWO. PAX2 and GATA3, studied in 6 and 5 FATWO tumors, respectively, were also negative. PAX8 was positive in all 4 endometrioid adenocarcinomas: 2 strong and diffuse, 1 strong and diffuse in the glandular areas but weak and focal in spindled areas, and 1 weak and focal. PAX2 and GATA3 were studied in 3 endometrioid adenocarcinomas. PAX2 was positive in two, one strong and diffuse and one weak and focal. GATA-3 was negative in two and showed rare positive cells in one case. All rete ovarii showed strong and diffuse positivity for PAX8, while negative for PAX2 and GATA3.

Conclusions: 1. Our study reveals that PAX8 and PAX2 are not expressed in FATWOs and can be employed as useful tools in differentiating these tumors from Mullerian tumors such as endometrioid adenocarcinomas with FATWO-like areas.

2. The difference in PAX-8 staining suggests that FATWOs do not arise from rete ovarii.

3. It is curious, however, that FATWOs do not express mesonephric markers, including GATA3 that stains cervicovaginal mesonephric lesion, which questions their probable mesonephric origin - it is possibly related to an origin from a distinctive portion of the Wolffian duct.

1221 Undifferentiated Carcinomas of the Endometrium-Immunohistochemical Markers of Diagnostic Utility and Implications for Genetic Testing

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Background: Undifferentiated carcinoma of the endometrium (UCA) is an aggressive, under-recognised tumor that occurs alone or in conjunction with endometrioid adenocarcinoma, where it represents de-differentiation. UCA is composed of monotonous cells arranged in solid sheets with lack of gland formation, and frequent mitoses and necrosis. UCA morphologically resembles basal-like carcinomas of the breast (BLC). Immunohistochemical stains (IHC) have shown inconsistent expression for keratins and EMA, and focal staining for neuroendocrine markers. There is limited experience with expression of mismatch repair proteins (MMRP) in UCA. The aim of our study was to perform an extensive IHC panel including keratins, BLC markers and MMRP in a cohort of UCA.

Design: 18 cases of UCA with available slides and blocks were retreived from our files (1988-present). H&E slides were reviewed. The following IHC stains were performed: Keratin cocktail (KC), CK5/6, CK8/18, PAX8, ER, PR, HER2/neu, EGFR, c-KIT, MLH1, MSH2, MSH6, PMS2, with appropriate controls. Genetic testing results were obtained from patients' charts when lack of MMRP staining was detected. Staining was graded as negative (<5%), focal (5-25%), patchy (26-75%), diffuse (>75%).

Results: PAX8 was negative in 78% of UCA with moderate diffuse staining in 3 and patchy in 1 case. KC was diffusely positive in 55%, CK8/18 in 61% and CK5/6 was negative in 78% of cases. ER and PR were negative in 83% and 94% of cases respectively. EGFR was negative in 66%, and HER2/neu and c-KIT were negative in all cases. Concurrent loss of MLH1 and PMS2 staining was identified in 11/18 (61%) cases. MSH2 and MSH6 staining was retained in all cases. Genetic testing was performed only in 4/11 patients with MLH1/PMS2 loss, and all 4 showed MSI-high tumors.

Immunohistochemical staining in UCA

IHC stain	Diffuse	Patchy	Focal	Negative
PAX8	3	1	0	14
Keratin cocktail	10	3	1	4
CK8/18	11	3	1	3
CK5/6	0	3	1	14
ER	1	0	2	15
PR	0	1	0	17
HER-2neu	0	0	0	19
EGFR	4	0	2	12
c-KIT	0	0	0	18

Conclusions: PAX8 appears to a reliable marker in distinguishing UCA from other high grade carcinomas, as it is negative in majority of cases. Although UCA is reportedly negative/focally positive for KC, we found it to be diffusely positive in over half the cases, limiting its utility in this differential. Despite the morphologic resemblance to BLC, UCA does not have a similar IHC profile. Given that 2/3rd of patients in our study had MLH1/PMS2 loss, genetic testing in all patients with UCA should be considered.

1222 Mutation Profiling of Uterine Carcinosarcoma – Insights into Tumor Biology and Therapy

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Background: Uterine carcinosarcoma is a biphasic tumor characterized by the presence of carcinoma and sarcoma components. In this study, we performed a comprehensive mutation analysis of 25 genes previously implicated in the oncogenesis of uterine cancer/sarcoma.

Design: We studied 15 uterine carcinosarcomas with matched frozen tumor, formalin-fixed paraffin-embedded tumor and normal tissue. The carcinoma and sarcoma areas were separately evaluated in 9 cases and peritoneal tumor metastasis was analyzed in

7. An Illumina Custom TruSeq amplicon panel was used, which covered all exons of 25 genes (*ABCC9, AKT1, AKT2, AKT3, ARID1A, CCND1, CHD4, CSMD3, CTCF, CTNNB1, EP300, FBXW7, FGFR2, MAP3K4, MED12, PIK3CA, PIK3R1, PIK3R2, POLE, PPP2R1A, PTEN, SPOP, TP53, TSPYL2* and *ZFHX3*). Libraries were constructed with genomic DNA extracted from matched tumor and normal tissue, pooled and run on the MiSeq (Illumina). All somatic non-synonymous mutations were manually checked in bam files using Integrated Genome Viewer.

Results: Mutations were detected in all 15 primary tumors (1 to 14 genes, median of 4 genes mutated per tumor). In the 9 cases where the carcinoma and sarcoma components were analyzed separately, 8 shared identical mutation(s) in at least one gene (1 to 11 genes, median of 2 genes); the remaining tumor showed a single mutation in the sarcoma component only. These ancestral mutations were also identified in their respective metastatic tumors. There were a greater number of genes mutated in the sarcoma component (average 5.1 genes) compared to the carcinoma component (average 4) (paired t-test, $p = 0.04$). Mutations involving PI3K pathway genes were identified in 9 of the 15 primary tumors, most commonly involving *PIK3CA* (7), followed by *PTEN* (4) and *PIK3R1* (4). The carcinoma and sarcoma components, as well as the metastatic tumors all shared the same PI3K-pathway mutation(s). *TP53* mutations were observed in 7 primary tumors. One tumor harbored a *POLE* mutation and displayed a hypermutation phenotype (mutations in 14 of 25 genes).

Conclusions: Our results suggest that uterine carcinosarcomas arise occur through transdifferentiation of carcinoma into sarcoma. We observed frequent PI3K pathway mutations in uterine carcinosarcoma which are acquired prior to tumor transdifferentiation and metastasis. These findings provide further support for the use of therapy that target PI3K signaling and downstream pathway in uterine carcinosarcoma.

1223 HER2 Immunohistochemistry Significantly Overestimates HER2 Amplification in Uterine Papillary Serous Carcinoma

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Background: Although optimal therapy for uterine papillary serous carcinoma (UPSC) remains unknown, studies have shown overexpression of HER2 in a subset of these tumors, leading to a current clinical trial targeting this pathway(*NCT01367002*). To date, criteria for grading HER2 expression in UPSC are lacking, and most studies have utilized currently accepted algorithms for breast carcinoma that call for screening all cases with immunohistochemistry (IHC), followed by FISH in equivocal cases. However, it is well-known that interpretation of HER2 IHC is prone to significant subjectivity, often leading to false positive results. To better correlate HER2 IHC results with underlying amplification in UPSC, chromogenic in situ hybridization (CISH) was performed on a large number of UPSC and compared to IHC.

Design: 69 cases of UPSC were retrieved from the archives and clinical histories reviewed. All were included in a tissue microarray that was constructed with four 0.6 mm cores per tumor. Both HER2 CISH and IHC were performed using standard techniques. Each case was scored according to current ASCO/CAP criteria for breast carcinoma, with an average HER2:CEP17 >2.2 representing gene amplification, and strong membranous staining in >30% of cells representing protein overexpression. To account for intra-tumoral heterogeneity, whole tissue sections were examined with both IHC and CISH on all amplified cases following initial CISH screening as well as an equivalent number of negative cases for control.

Results: HER2 amplification was detected by CISH in 7 cases (10%). Overexpression was detected by IHC in 14 cases (20%), with an additional 16 cases (23%) showing equivocal staining. The overall concordance rate was 50%. Of the CISH amplified cases five were positive by IHC and two equivocal. Intra-tumoral heterogeneity was seen in one case, and was confirmed on both CISH and IHC with whole section analysis. None of the control whole section slides picked up additional foci of heterogeneous expression or amplification.

Conclusions: Although HER2 overexpression was detected in 20% of cases, gene amplification was only detected in half of these. While confirming that HER2 amplification is found in a subset of UPSC, these data suggest that screening with IHC may lead to misclassification of cases lacking true amplification. Since studies have shown amplification to be the best correlate of response to anti-HER2 therapy, we suggest that ISH is a superior method for identifying eligible patients. Further clinical trials with outcome data evaluating both methods are clearly needed.

1224 Fumarate Hydratase Assist to Recognize Smooth Muscle Tumors Associated with Hereditary Leimyomatosis and Renal Cell Cancer (HLRCC) Syndrome

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Background: Fumarate hydratase is an enzyme that catalyzes the reversible hydration/dehydration of fumarate to malate. The enzyme is normally present in all tissues but when mutations occur there is predisposition to somatic deletions resulting in truncated, non-functional or absent proteins. Mutations in this gene have been associated with hereditary uteri and skin leimyomatosis and renal cell cancer (HLRCC). The morphologic spectrum of uterine SMT has recently been described. We investigated the role of IHC for FH in diagnosis to recognize smooth muscle tumors associated with HLRCC.

Design: Thirty-one uterine SMT from 17 patients with confirmed germ line mutations in the FH gene were evaluated for FH protein expression by immunohistochemistry. Fourteen sporadic smooth muscle tumors from seven patients were stained as control. Patients presented with heavy bleeding, abdominal pain, or uterine tumors were identified as part of clinical screening. Ages ranged between 24 to 50 years (m 33.7). Immunohistochemistry was performed using a mouse monoclonal antibody against human FH protein (Clone J-13, Santa Cruz Biotechnology).

Results: IHC was interpreted as positive or negative when cytoplasmic staining was identified. Histologically, tumors were classified as atypical leimyoma, and one SMT of unknown malignant potential. All patients had multiple uterine SMT with maximum diameters ranging from 1.8 to 8.9 cm (m 5.84 cm). Twenty-seven out of 31 uterine SMT cases associated with HLRCC showed negative FH staining. Four cases showed occasional positive cells. Normal endometrium showed positive staining. All the control leiomyomas without FH mutation showed diffuse positive staining of muscle fibers.

Conclusions: Recognition of the spectrum of HLRCC associated smooth muscle tumors is quite important considering that they occur predominantly in women of reproductive age and that may have high predisposition for the development of other tumors. IHC for FH is a helpful tool to identify patients that may be members of a hereditary family. Recognition of these families is important for early screening and therapy.

1225 Does BRCA+ Pelvic Serous Cancer Have a Dual Pathogenesis? The Paradox of Tubal Intraepithelial Carcinoma (TIC), Early and Advanced Disease

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Background: Women with germ-line BRCA1 and BRCA2 mutations (BRCA+) are at increased risk of developing high-grade serous carcinoma (HGSC) and 6% will manifest with an early tubal carcinoma at risk-reduction salpingo-oophorectomy (RRSO). Of these, approximately 85% originate in the distal fallopian tube within a TIC. However, the lower frequency of TIC in symptomatic women with more advanced disease has yet to be fully explained and raises the possibility that there is a subset of HGSCs in BRCA+ women that develops via a pathway that does not involve a TIC.

Design: We first compared the mean and median ages of 22 asymptomatic BRCA+ women with TIC discovered following RRSO with 17 consecutively accessioned BRCA+ women with high-stage HGSC. We then analyzed 27 BRCA+ and 30 BRCA- (confirmed by genotype) tumors with high-stage disease in which the fallopian tubes were analyzed by the SEE-FIM protocol. For each group, frequency of TIC was determined by review of the pathology reports and original pathology slides.

Results: The mean and median ages of BRCA+ women undergoing RRSO were 46 and 48 years respectively; and with early and advanced disease, 54 and 54 and 51 and 51 years respectively. In the second dataset, the frequency of TIC in advanced HGSCs from BRCA+ and BRCA- women was 5 of 27 (19%) and 19 of 30 (63%) respectively ($p = 0.001$). Mean and median ages for TIC+ vs. TIC- advanced cancers in BRCA+ women were 57 and 54 and 57 and 51 years; and for BRCA- women 60 and 63 and 61 and 63 years respectively. Age differences in TIC+ between BRCA+ and BRCA- were not significant. Differences in TIC- between BRCA+ and BRCA- were significant at $p = 0.0125$.

Conclusions: The study suggests, for the first time 1) that TIC is significantly less likely to be identified in advanced HGSCs of women with BRCA mutations and 2) the subset of BRCA+ advanced HGSCs without TIC develops at a younger average age relative to both BRCA+ (3 yrs) and BRCA- women (7 yrs) with TIC. This lends support to the novel hypothesis that despite the high frequency of TIC in early or asymptomatic BRCA+ malignancies, BRCA+ tumors discovered in an advanced stage are younger, less likely to be associated with TIC, and more likely to evolve rapidly. Further study of potential precursors in the fimbrial-ovarian region is warranted to explain this paradox and more accurately estimate the effectiveness of salpingectomy alone in preventing HGSC in BRCA+ women.

1226 Defining Thresholds for Histologic Criteria Associated with Poor Neonatal Outcome in Fetal Vascular Thrombosis

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Background: Placental fetal vascular thrombosis (FVT) is associated with prenatal fetal monitoring abnormalities, neonatal neurologic impairment, thromboembolism, and stillbirth. The distribution and frequency of histologic features associated with FVT have not been well characterized in samples of random placentas, nor placentas associated with poor neonatal outcome; therefore, thresholds for FVT histology predictive of neonatal morbidity/mortality have yet to be determined.

Design: Placentas from 104 term non-anomalous singletons delivered at a major tertiary obstetrical facility were selected randomly from our archives and blindly reviewed for gross and histologic features associated with FVT, including umbilical cord abnormalities; (umbilical cord (UCT), chorionic plate (CPT), and stem villous (SVT) thrombosis; intimal cushions (IC); and distal changes (villous stromal karyorrhexis (VSK), avascular villi (AVV)). Maternal and neonatal clinical parameters were recorded. 19 additional placentas diagnosed with FVT were reviewed for identical gross, histologic, and clinical features. Criteria defining early poor neonatal outcome (first UA pH $\leq$ 7, Apgar @1 min $\leq$ 6, seizures/thromboembolism within 1 day, stillbirth) were applied to these 123 placentas.

Results: 13 of 104 (12%) random and 11 of 19 (58%) selected placentas met clinical case definition criteria, resulting in 99 controls and 24 cases. $\geq$ 1 CPT or SVT per block was seen in 5/104 (4.8%) and $\geq$1 IC/block in 9/104 (8.7%) of randomly sampled placentas. UCT was not seen. Any VSK or AVV was seen in 16/104 (15.4%) of random placentas. >0.3 SVT/block, >0.17 CPT/block, and diffuse (>2 foci of $\geq$15 villi in >2 blocks) VSK or AVV were each associated with poor neonatal outcome (Fischer exact, $p<0.05$). Recent CPTs were significantly correlated with poor outcome (Spearman, $p<0.05$) whereas organizing and organized CPTs were not. Neither the presence nor number of ICs/block, nor focal or multifocal VSK or AVV correlated with poor neonatal outcome. Using stepwise multivariate logistic regression, the combination of any recent SVT or CPT and diffuse VSK was best predictive of poor neonatal outcome (LR X^2_3 21.28, $p<0.05$).

Conclusions: Vascular lesions seen in FVT were relatively uncommon in our random sample. Diffuse VSK or AVV in combination with SVT and/or CPT, particularly if recent, were associated with poor neonatal outcome. As the extent of SVT and CPT increased, so did the likelihood of morbidity/mortality.

1227 Monoclonal Anti-Stathmin and Anti-HSP27 Are Reliable Biomarkers for Identification of Cervical Intraepithelial Neoplasia and Cervical Squamous Carcinoma

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Background: p16 immunohistochemical (IHC) staining has been consistently used as a reliable and sensitive biomarker to identify cervical intraepithelial neoplasia (CIN) and cervical squamous carcinoma (CSC). However, specificity of p16 for CIN and CSC is limited, thus providing a need for more complimentary biomarkers that allow for more accurate detection of CIN and CSC within the laboratory.

Design: Cervical biopsy specimens of 29 cases of CINI, 27 cases of CINII, 33 cases of CINIII, and 4 cases of CSC were evaluated by IHC. One full section from each case was stained with monoclonal anti-stathmin, monoclonal anti-HSP27 and monoclonal anti-p16. Staining intensity was scored as 0 (negative), 1-2 (weak), 3 (moderate), 4 (strong); the labeling extent was tabulated as 0 (less than 5% positive cells), 1 (5-25% positive cells), 2 (26-75% positive cells), and 3 (greater than 75% positive cells).

Results:

IHC of Stathmin, HSP27 and p16 for CIN I, CIN II, CIN III and CSC.

	Stathmin	HSP27	p16
	Pos/Total (%)	Pos/Total (%)	Pos/Total (%)
CIN I	0/29 (0%)	9/29 (31%)	13/29 (45%)
CIN II	11/27 (41%)	25/27 (93%)	20/27 (74%)
CIN III	28/33 (84.8%)	33/33 (100%)	32/33 (93%)
CSC	4/4 (100%)	4/4 (100%)	4/4 (100%)

CIN: cervical intraepithelial neoplasia. CSC: cervical squamous carcinoma

As shown in table 1 of 29 cases of CIN I, none was stained with stathmin (0/29, 0%); HSP27 was expressed in 9 cases (9/29, 31%); 13 cases positive (13/29, 45%) for p16. Of 27 cases of CIN II, 11 (11/27, 41%) were positive for stathmin; 25 expressed HSP27 (25/27, 93%); 20 cases (20/27, 74%) displayed staining with p16. Of 33 cases of CIN III, 28 (28/33, 84.8%) demonstrated stathmin expression; 33 (33/33, 100%) were positive for HSP27; 32 (32/33, 97%) displayed positivity for p16. All 4 cases of CSC (4/4, 100%) showed positive expression of stathmin, HSP27 and p16 in neoplastic cells.

Conclusions: Monoclonal antibodies against stathmin and HSP27 are reliable antibodies which compliment p16 in the identification of cervical intraepithelial neoplasia and cervical squamous carcinoma.

1228 Gestational Trophoblastic Tumors Are Characterized by GATA3 Expression

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Background: GATA3 is a zinc-finger transcription factor which has been shown to induce trophoblast differentiation in trophoblast stem cells. In tumors, GATA3 is known to be sensitive and specific for urothelial and breast carcinomas, and recent studies have also demonstrated GATA3 in paragangliomas and lesions with mesonephric differentiation. Despite the known association of GATA3 with trophoblast-specific gene expression and placental function, its expression in trophoblast-related neoplasia has not been evaluated.

Design: GATA3 immunohistochemistry was performed on 22 normal placentas (11 immature and 22 mature), 1 atypical placental site nodule, 11 partial hydatidiform moles, 14 complete hydatidiform moles, and 11 gestational trophoblastic tumors including 5 choriocarcinomas, 3 placental site trophoblastic tumors, and 3 epithelioid trophoblastic tumors. 160 endometrial adenocarcinomas (153 endometrioid, 3 serous, 4 clear cell) on a TMA were also stained for comparison. Strong nuclear immunoreactivity was considered a positive result.

Results: Immature placentas are characterized by strong and diffuse nuclear GATA3 staining of the implantation site (intermediate trophoblasts) and cytotrophoblast layer, with variable staining in the syncytiotrophoblast layer. Mature placentas demonstrate less expression of GATA3 with only scattered positive cells in villous cytotrophoblasts, and little to no expression in syncytial knots. Complete and partial hydatidiform moles show diffuse expression in the cytotrophoblasts and implantation site, and heterogeneous expression in extravillous trophoblastic hyperplasia. All choriocarcinomas, placental site trophoblastic tumors, and epithelioid trophoblastic tumors, as well as the atypical placental nodule, were positive for GATA3. All endometrial adenocarcinomas were negative for GATA3.

Conclusions: All trophoblast lineages (cytotrophoblasts, intermediate trophoblasts, and syncytiotrophoblasts) were positive for GATA3. Extent of GATA3 expression varied significantly in immature and mature placentas, suggesting a role in trophoblast maturation. GATA3 does not help distinguish molar gestations from normal immature placental tissue, nor does it distinguish the different gestational trophoblastic tumors. Nevertheless, GATA3 can help distinguish trophoblasts (neoplastic or non-neoplastic) from epithelial malignancies, and this could be especially useful in biopsy specimens. Lastly, GATA3 may be helpful in subtyping metastatic tumors when a trophblastic neoplasm is in the differential; however, distinction from breast and urothelial carcinomas with additional markers would be required.

1229 IMP3 Oncofetal Protein Expression Can Detect Precursor Lesions in Fallopian Tubes of Patients with BRCA Gene Mutation

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Background: Insulin-like growth factor II mRNA-binding protein 3 (IMP3) is an oncofetal protein almost exclusively expressed in fetal tissue and malignant tumors. IMP3 expression has been shown to be a marker of aggressive behavior and correlated with reduced overall survival in many tumors. In the gynecologic tract, IMP3 expression was reported in high-grade serous carcinoma of the ovary, while negative in borderline and serous cystadenomas. Patients with BRCA gene mutation are at an increased risk for developing high-grade serous carcinomas (HGSC) of the ovary, many of which may represent a fallopian tube (FT) origin. Studies of prophylactic salpingo-oopherectomies have identified precursor lesions in the FT such as serous tubal intraepithelial carcinoma (STIC). Overexpression and mutation of TP53 were also identified in these cases.

Design: Herein, we studied the expression of IMP3 immunohistochemistry in FT specimens of forty-four patients with BRCA gene mutation, and compared it to that of TP53. These included four patients (4/44) with an associated HGSC. The remaining 40 patients had prophylactic salpingectomies. STIC was identified in 2 patients, 1 of whom had high-grade ovarian carcinoma (bilateral STIC), while the other patient had an incidental finding.

Results: Among the four patients with high-grade ovarian carcinomas, 3 (75%) were positive for IMP3, while all cases were negative for TP53. Both patients (100%) with STIC expressed IMP3, one of which, co-expressed TP53 (50%). Among the prophylactic group, 46% (19/41) showed focal expression of IMP3, compared to 15% (6/41) with focal TP53 over-expression.

Conclusions: Our data suggests that alteration in IMP3 protein may represent an early stage in the pathogenesis of HGSC, and may serve as a potential marker for detection of precancerous lesions in this subset of patients.

1230 Impact of Mutation in MYBBP1A Gene Related to Ovarian Cancer Recurrence

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Background: Around seventy percent ovarian cancer (OVCa) corresponds to high-grade serous (HGS-OVCa) histological subtype, accounting for the majority (90%) of ovarian carcinoma deaths. During the last years, high-throughput sequencing technology has generated an enormous amount of data that continues to accumulate somatic mutations in a variety of cancers (Sohn et al. PLoS One. 8(1):e54089.2013).. According to this, an integrated genomic analysis including 316 primary HGS-OVCa has been performed within TCGA project (*The cancer genome project, Nature. 2011 June 29; 474(7353): 609–615*). Nevertheless, an exhaustive genomic study of this specific type of tumors has opened new avenues in the identification of molecular markers involved in tumor progression. Using this approach, our group has been focused on the new potential markers identification not only related to ovarian carcinogenesis but also to metastasis and recurrence in HGS-OVCa.

Design: We performed a preliminary study based on exome sequencing (SOLID technology) of paired samples from the same patient including: primary tumor, distant metastasis and main relapse tumors as well as normal tissue used as control.

Results: In the mutational analysis we discovered 46 mutations with pathogenic effect using Bioinformatic prediction, affecting genes directly or indirectly related to transcription. A relevant finding is the presence of mutations in MYBBP1A (MYB Binding Protein -P160- 1a) which has been involved in tumor recurrence in head-neck tumors (BMC Cancer 2012, 12:72). In order to understand the molecular role of this gene in ovarian tumor progression, we evaluated MYBBP1A expression in a cohort of HGS-OVCa including 50 primary and 45 recurrence samples.

Conclusions: Our results point out for the first time at the potential role of MYBBP1A in tumor recurrence in HGS-OVCa.

1231 Morphologic Features of Endometrial Clear Cell Carcinomas with p53 Overexpression

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Background: Endometrial clear cell carcinomas (E-CCC) are known to have a poor prognosis. Recent studies have shown that E-CCC with p53 positivity (p53+) may behave more aggressively. There are few studies however, that correlated p53+ E-CCC with its morphologic features. Our aim was to compare clinicopathologic and morphologic features of p53+ and p53 wild type (p53-wt) E-CCC.

Design: Hysterectomy specimens with diagnosis of E-CCC were retrieved from two institutions between 1994-2013, yielding 33 cases. Cases were reviewed (1-8 slides/case) and 20 cases met diagnostic features of E-CCC. Immunohistochemical staining of formalin fixed paraffin embedded tissue for p53 was performed, and each case was considered p53+ (>75% tumor cells) or p53-wt (<75% tumor cells). Clinicopathologic variables analyzed included: age at diagnosis, stage, lymph node (LN) status, predominant architectural pattern (>50% of tumor), nucleoli, lymphovascular invasion (LVI), depth of myometrial invasion (MI), cytoplasm type, nuclear grade (1-3), stromal inflammation, necrosis, and mitoses.

Results: Six of the 20 cases (30%) were p53+. Average age at diagnosis was 73y (range 57-82) in the p53+ group vs. 63y in the p53-wt group (range 43-83). Three of the 6 p53+ cases (50%) presented at a high stage (T3 or T4) as opposed to only one of 14 p53-wt cases (7%). Only one p53+ case had LN metastasis (20%), while 3 of 12 p53-wt cases (25%) had LN metastases. Predominant architectural pattern in the

p53+ cases was glandular (4/6, 67%); remaining 2 were papillary (2/6, 33%). P53–wt group showed predominantly solid (7/14, 50%) and glandular (6/14, 43%) patterns with one case being papillary.

Morphologic differences between p53+ and p53-wt E-CCC

	p53+ n=6	P53-wt n=14
Predominant architectural pattern		
Papillary	2 (33%)	1 (7%)
Solid	0	7 (50%)
Glandular	4 (67%)	6 (43%)
Presence of nucleoli	6 (100%)	11 (79%)
MI		
No invasion	0	6 (43%)
<50%	4 (67%)	2 (14%)
>50%	2 (33%)	6 (43%)
Cytoplasm		
Clear	3 (50%)	7 (50%)
Eosinophilic	3 (50%)	7 (50%)
Nuclear grade		
1	0	0
2	1 (17%)	9 (64%)
3	5 (83%)	5 (36%)
Stromal inflammation	1 (17%)	8 (57%)
Necrosis	5 (83%)	4 (29%)
LVI	4 (67%)	3 (21%)
Mitotic index/10HPF at 40X (average)	5.5	2.6

Conclusions: Our data suggests there may be some clinicopathologic differences between p53+ and p53-wt E-CCC. Patients with p53+ E-CCC presented at a higher stage, older age, and were more likely to have LVI, higher nuclear grade, MI and necrosis. They were less likely to have solid pattern and stromal inflammation. No significant difference was seen in presence of nucleoli, cytoplasm type, mitotic index, or lymph node status.

1232 P16 Immunohistochemistry May Significantly Reduce the Frequency of Negative LEEPs Following CIN2+ Colposcopic Biopsies

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Background: We and others have shown that p16 immunohistochemistry improves the reproducibility and accuracy of cervical biopsy diagnoses. Our group began using p16 in 2006; therefore, we hypothesized that more accurate colposcopic biopsy diagnoses may reduce the frequency of negative LEEPs in the interval from 2006-2012 compared with 2000-2005.

Design: Retrospective clinicopathologic review of all 1251 cervical LEEPs performed at our institution from 2000-2012. Sections of the biopsies and followup LEEPs were reviewed to confirm the presence or absence of high grade dysplasia (CIN2+) and LEEP followup. This yielded 1214 cases for final analysis. Chart review recorded patient age, Pap smear diagnoses leading to colposcopy, HPV status when available, the clinical service performing the biopsies, pretest diagnosis (CIN 2 versus CIN 3), sexual risk factors, and smoking history. We compared the frequency of confirmed negative LEEPs in the period before p16 use (2000-2005) to the period after initiation (2006-2012) by X^2 analysis and multivariate logistic regression.

Results: We identified 35/1241 (3%) LEEPs from 2000-2012 that were negative for high grade dysplasia following a CIN2+ colposcopic biopsy diagnosis. P16 was rarely employed before 2006 in our practice (1% of biopsies), but utility rose steadily to a peak of 37% of biopsies in 2012 (mean 25% over 2006-2012 interval). The frequency of negative LEEPs before 2006 was 12/208 (6%), which then dropped to a frequency of 23/1006 (2%) from 2006-2012. The odds ratio of having a negative LEEP following a CIN2+ biopsy using p16-based diagnoses was 0.38 [0.19-0.78] (p=0.006).

Conclusions: Few studies have investigated the frequency of negative LEEPs following a CIN2+ colposcopic biopsy diagnosis, but published data suggest it occurs in 10-20% of cases. We observed a significant reduction in the frequency of negative LEEPs in the period from 2006-2012, which is also when our practice began using p16 to assist with CIN2+ classification. The potential impact of p16 to reduce the number of unnecessary LEEPs has been anticipated and now supported by our retrospective study. Clearly, fewer unnecessary LEEPs improves patient care and the cost-effectiveness of this surgical treatment.

1233 Ovarian Teratomas Associated with Anti-NMDAR Encephalitis Are Distinguished by Neural Teratomatous Elements Containing Lymphoid Aggregates with Germinal Centers

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Background: Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis is a rare severe paraneoplastic limbic encephalitis in young women that is often associated with ovarian teratoma. Limited data suggests such teratomas can be distinguished from teratomas in women without encephalitis by the presence of lymphoid infiltrates and reactive lymphoid aggregates involving the teratomatous neural elements. The aim of this study was to topographically map and quantitatively compare these morphologic features in ovarian teratomas from women with versus without anti-NMDAR encephalitis.

Design: Lymphoid populations were evaluated in 5 mature ovarian teratomas in women with anti-NMDAR encephalitis (average patient age 23) and in 21 mature ovarian teratomas in women without encephalitis (average age 28). The topographic distribution (neural versus non-neural elements), cellularity, and quantity of lymphoid infiltrates and aggregates (with versus without germinal centers) was compared in teratomas from women with versus without encephalitis.

Results: Average tumor size was smaller (2.36 cm) in women with encephalitis than without (6.9 cm). Neural elements were present in all teratomas. Average # of low power fields of neural elements was 1 in women with encephalitis and 3.8 in those without encephalitis. Table 1 shows that lymphoid aggregates with germinal centers involving neural elements distinguished teratomas with encephalitis (4/5) from those without (1/21) but scattered / diffuse infiltrates and aggregates without germinal centers were common in neural and non-neural elements of teratomas either with or without encephalitis.

Lymphoid Populations in Teratomas

	With Encephalitis	Without Encephalitis
Involving Neural Elements:		
Scattered infiltrates	3 / 5	4 / 21
Diffuse infiltrates	0 / 5	1 / 21
Aggregates without germinal centers	5 / 5	11/21
Aggregates with germinal centers	4 / 5	1 / 21
Involving Non-Neural Elements:		
Scattered infiltrates	1 / 5	13 / 21
Diffuse infiltrates	1 / 5	0 / 21
Aggregates without germinal centers	4 / 5	21 / 21
Aggregates with germinal centers	1 / 5	0 / 21

Conclusions: Lymphoid aggregates with germinal centers involving neural elements in an ovarian teratoma are associated with anti-NMDAR encephalitis. However, lymphoid infiltrates and aggregates without germinal centers are commonly present in conventional mature teratomas of the ovary and are not specifically associated with anti-NDMAR encephalitis.

1234 Uterine Tumors Resembling Ovarian Sex Cord Tumor (UTROSCT) Lack Rearrangement of *PHF1* by FISH

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Background: Uterine tumors resembling ovarian sex cord tumor are rare tumors composed of epithelial-like cells with architectural patterns resembling those described in sex cord stromal tumors of the ovary. Since their initial description in 1976, it has been unclear whether UTROSCT represents complete overgrowth of sex cord-like elements within the family of endometrial stromal tumors (EST), as the latter can exhibit varying amounts of sex cord-like differentiation, or a distinct and unrelated uterine neoplasm as by electron microscopy they do not show features of stromal neoplasia and they lack t(7;17) by FISH. Recently, a study revealed that all endometrial stromal tumors with sex cord -like differentiation (n=7) showed rearrangement involving *PHF1*; therefore, the purpose of this study was to address the possibility that if UTROSCT are in fact EST with extensive sex cord-like differentiation, they would show evidence of *PHF1* rearrangement.

Design: Cases of UTROSCT were identified from our multi-institutional archive and included both in-house and consultation specimens. Fluorescence in situ hybridization (FISH) was performed on interphase nuclei obtained from formalin-fixed paraffin-embedded sections utilizing a laboratory developed break apart probe for *PHF1* (at 6p21.3). Performance and interpretation of interphase FISH was done according to a clinically validated protocol.

Results: Seven UTROSCT were identified from our files. They were from patients who underwent hysterectomy and whose age ranged from 31 to 53 (mean 43) years. All seven tumors were negative for rearrangements involving *PHF1* by FISH.

Conclusions: If UTROSCT represented a subset of EST, then one would predict that these tumors would share a similar histologic appearance as well as a similar genetic pathway. While UTROSCT has previously been shown to lack *JAZF1-JJAZ1* translocation seen in most EST with classic morphology, it was possible that they could have represented a variant of EST that arose from a different genetic pathway, namely one involving rearrangements of *PHF1*, which has been shown in all EST with variant sex cord like differentiation examined to date. Our results, combined with published data, therefore support the theory that UTROSCT represents a distinct tumor category separate from EST.

1235 Exome Sequencing Identifies Recurrent Somatic Mutations in *USP15, DSN1, PIK3CA* and Chromosome 19q Gain in Cervical Adenosquamous Carcinomas

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Background: Cervical adenosquamous carcinomas are a relatively uncommon histological subtype of cervical cancer. However, these tumors, like adenocarcinomas, have worse prognosis than squamous cell carcinomas. The identification of somatic mutations may augment patient diagnosis and treatment in this disease. This project is nested within a larger whole exome sequencing (WES) analysis of 100 cervical tumors in Norwegian women.

Design: Seven of the 100 cervical tumors were identified as adenosquamous carcinomas by combining hematoxylin-eosin based diagnostics with hierarchical clustering of tumor gene expression data. Tumor and blood DNA were subjected to Illumina-based WES. Single nucleotide variants and small insertion/deletions were identified by the MuTect and Indelocator algorithms respectively. Somatic copy number (CN) data were derived from WES data using the CapSeg algorithm, and significantly recurrent CN alterations were identified by GISTIC2.0 analysis ($q<0.25$).

Results: Three of the 7 cervical adenosquamous tumors harbored somatic point mutations (E95K, I525L and R529T) in the *USP15* gene. We also observed recurrent R323T mutations in the *DSN1* gene in 2 tumors, while a third tumor had focal deletion peak that included *DSN1*. The *USP15* and *DSN1* mutations occurred only in the adenosquamous tumors within this cohort. Furthermore, we found *PIK3CA* mutations (E545K, L267V) and/or amplification in 4 of the 7 tumors. Six of the 7 adenosquamous

carcinomas harbored broad copy number gains in chromosomal arm 19q, in contrast to 3 of 23 adenocarcinomas (chi squared p = 0.0012).
Conclusions: We have identified novel mutations in cervical adenosquamous carcinomas by exome sequencing. The USP15 protein is a deubiquitinating enzyme that increases the stability of the oncogenic HPV16 E6 protein. Therefore, our data suggest that *USP15* may be an oncogene, at least in the context of HPV-related cancers. The putative loss-of-function mutations observed in the *DSN1* gene are in keeping with the role of the DSN1 protein as an essential kinetochore protein required for proper chromosome segregation. Our findings also support the notion that cervical adenosquamous carcinomas may be included in clinical trials of PI3-kinase inhibitors, alongside other cervical tumors. Finally, our data suggest that chromosome 19q gain should be further investigated as a potential diagnostic marker in cervical adenosquamous carcinomas.

1236 HPV Negative Carcinoma of the Uterine Cervix: A Distinct Type of Cervical Cancer with Poor Prognosis

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Background: Although human papillomavirus (HPV) is considered to cause cancer of the uterine cervix (CC), some CCs are negative for HPV with routine tests, such as Hybrid Capture 2 (HC2). We aimed to analyze with highly sensitive PCR techniques the tumors testing negative for HPV by HC2 and to determine the clinico-pathological characteristics and yhe prognosis of the patients with tumors with confirmed HPV-negativity.
Design: WWe included 136 women with CC (32 adenocarcinomas, 104 squamous cell carcinomas) and a HC2 testing performed either simultaneously or within 6 months before the histological diagnosis. Formalin-fixed paraffin-embedded tumor samples from all HC2-negative cases were reanalyzed and genotyped for HPV using three different PCR assays (SPF10, GP5+/6+ and E7 specific assay). p16 expression was evaluated by immunohistochemistry in all cases. Clinico-pathological features, as well as disease free and overall survival (DFS and OS) were analyzed for women with HPV-negative and -positive tumors.
Results: Fourteen out of 136 (10.2%) women were negative for HPV by HC2. After reanalysis by PCR-based techniques only 8/136 (5.8%) tumors were confirmed to be HPV-negative, whereas in six cases different HPVs were identified; five of them were HPV types included in the probes of HC2 test (HPV 16, 18, 45 and 68). Adenocarcinomas were more frequently in the HPV-negative confirmed group than squamous cell carcinomas (5/32, 15.6% vs. 3/104, 2.9%, respectively; p=0.017). p16 immunostaining was positive in 4/6 (66.7%) HPV-positive tumors and in 2/8 (25%) of the tumors with confirmed HPV-negativity (p=0.227). Patients with confirmed HPV-negativity CC had significantly worse DFS than women with HPV-positive tumors (51.9 months [95%CI: 12.2-91.7] vs. 109.9 months [95% CI 98.2-121.5]; p=0.010), but OS did not reach statistical significance (67.7 months [95% CI 20.0-106.9] vs. 108.9 months [95% CI 97.7-120.0] respectively; p=0.225).
Conclusions: HC2-negative result is an uncommon finding in women with CC. Almost in a half of these cases HPV types included in HC2 test are identified by more sensitive techniques. CCs with confirmed HPV-negativity are more frequently adenocarcinomas and are associated with poor prognosis.
This work was funded by Instituto de Salud Carlos III (ICSIII)-Fondos de Investigacion Sanitaria and ERDF 'one way to Europe' (PS09/1084, PI12/1165, PI12/1231).

1237 Monoclonal Antibody for PAX-8 in Mesothelial Lesions of the Peritoneum: A Study of 39 Cases

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Background: Polyclonal PAX-8 has been shown in multiple studies to be a good marker for gynecological, renal, thyroid and thymic tumors. In most studies, polyclonal PAX-8 has been negative in peritoneal mesothelial lesions, and thus believed to help discriminate ovarian carcinomas from peritoneal mesotheliomas. However, some recent studies have reported positivity in neoplastic and non-neoplastic peritoneal mesothelial lesions. The aim of this study was to test a new PAX-8 monoclonal antibody in a variety of peritoneal mesothelial lesions and compare its specificity and sensitivity to PAX-8 polyclonal antibodies previously used in most studies.
Design: Immunohistochemistry using monoclonal antibody for PAX-8 (BC12 API 438, AA Biocare medical) was performed in 39 mesothelial lesions including 14 malignant mesotheliomas, 9 well differentiated papillary mesotheliomas, and 16 multicystic mesotheliomas (AKA peritoneal inclusion cyst). Slides were reviewed using appropriate positive and negative controls, and were evaluated for percentage of nuclear staining and the intensity of staining, the latter using a two tier system: 1+ = weak nuclear staining, and 2+ = intense nuclear staining.
Results: See Table 1

Table 1

Diagnosis	Total number of PAX-8 positive lesions	% of positive cells	Cases with 1+ intensity	Cases with 2+ intensity
Malignant Mesothelioma	5/14 (35%)	20-80%	1/5 (20%)	4/5 (80%)
Well Differentiated Papillary Mesothelioma	1/9 (11%)	80%	1/1 (100%)	0/9 (0%)
Peritoneal Inclusion Cyst	11/16 (68%)	10-95%	5/11 (45%)	6/11 (55%)

Conclusions: This monoclonal PAX-8 antibody is expressed in a significant number of peritoneal lesions: 35% of malignant mesotheliomas, 11% of well differentiated peritoneal mesotheliomas, and 68% of peritoneal inclusion cysts. In many of these cases the percentage of positive cells and the intensity was striking. Thus PAX-8 is not reliable in discriminating ovarian carcinomas from peritoneal mesothelioma.

1238 Validation of a Novel Subclassification of Endocervical Adenocarcinoma: Pitfalls of Inter-Observer Variability

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Background: The newly proposed subclassification system by EG Silva and colleagues for endocervical adenocarcinoma (EAC) identifies patients who need regional lymphadenectomy (LA), and potentially spares LA (and morbidity) in patients at low risk for metastases. As EAC is rare (1.76 cases/100,000 woman-years), accumulating cases to assess these assertions is difficult. The objective of this multi-institutional study is to determine whether gynecologic pathologists confirm the utility of this classification.
Design: 66 cases were identified from the past 10 years of two academic hospitals. The cases were independently classified by two gynecologic pathologists per institution into:
Pattern A: well demarcated glands, no lymphovascular invasion (LVI)
Pattern B: early stromal invasion arising from well-demarcated glands
Pattern C: diffuse, destructive invasion
Follow-up for nodal metastases was via pathology reports and chart review.
Results: Of the 66 cases, 16 were excluded upon review (missing, misclassified, etc). Age ranged from 27-78 years (mean 43.8). Weighted kappa (K) statistics comparing the two pathologists was determined for each institution: for one (35 cases, 71-77% pattern C) the K was 0.909, and the other (15 cases, 20-27% pattern C) had a K of 0.524. The patterns were A (12%), A vs. B (4%), B (22%), B vs. C (8%), and C (54%). All 5 cases of unanimous A had negative LA. Of 11 cases of unanimous B, eight had negative LA, one had no nodal disease by imaging, while two presented at Stage II and did not receive LA. One of the negative LA patients had LVI. Of the 26 cases of unanimous C, 18 (69%) had negative LA, while four (15%) had positive nodes, and four did not receive LA.
Conclusions: Our review adds data to confirm that pattern A EAC is low risk for nodal disease. For pattern B patients, while metastatic disease was present at diagnosis for two, those with EAC limited to the cervix did not have nodal disease. Pattern C is clearly a significant risk factor for nodal disease. The inter-observer agreement was robust at one institution with predominantly C, and moderate at the other with predominance of B. This may reflect more agreement regarding 'diffuse destructive' invasion and more subjectivity for 'early' invasion. In both institutions there were calendar years with no EAC, suggesting skills may fall into disuse. Further investigation of inter-observer variability in application of this new classification with a group enriched with A and B is needed to confirm applicability in the real world setting.

1239 Clinicopathological Characteristics of Signet Ring Cell Variant of Endocervical Mucinous Adenocarcinoma

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Background: Mucinous adenocarcinoma is the most common type of adenocarcinoma in the uterine cervix, comprised of three variants; endocervical, intestinal, and signet ring cell variants (SRCV) based on histopathological features. However, diagnostic value and clinical significance to separate SRCV as an independent disease entity have not been clarified.
Design: During the histopathological review of adenocarcinomas of the uterine cervix from 150 patients treated at the Asan Medical Center, Seoul, Korea, during 11 year-period, 14 cases of SRCV were identified. We attempted to define the clinicopathological characteristics of SRCV by immunohistochemistries for MUC2, MUC5AC, MUC6, HIK1083, P16, P53, Ki67 and alcian blue pH2.5-DPAS mucin stain. We correlated various clinical parameters of the patients with SRCV including disease-free survival rate, metastatic and recurrence rates, and depth of invasion with those of the patients with endocervical and intestinal variants.
Results: Histologically, SRCV formed well defined solid nests composed of mucin containing cells with signet ring cell like features. However, the cells maintained cohesiveness between the tumor cells, which are different from signet ring cell carcinoma of gastrointestinal tract. All 14 cases were admixed with endocervical variant within the same tumor in variable extent. Notably, SRCV were frequently associated with significantly increased infiltrations of neutrophils or lymphoplasma cells in the stroma. The tumor cells expressed MUC5 (13/14, 93%), MUC6 (10/14, 72%) MUC2 (6/14, 43%) and HIK1083 (3/14, 21%), but all (14/14, 100%) were stained blue in alcian blue pH2.5-DPAS stain, suggesting acid mucin secretion. All 14 cases showed immunopositivity for P16, while none of them showed P53 expression, which were similar to those of endocervical variant. Although Kaplan-Meier analysis showed lower disease free survival rate in cases of mixed SRCV compared to pure endocervical variant (p=0.003), metastatic and recurrence rates, depth of invasion, and lymphovascular involvement were not significantly different.
Conclusions: Although cytomorphologic features of individual tumor cells have some similarities to those of signet ring cell carcinoma of gastrointestinal tract and the secreted mucin has some properties of gastric and intestinal mucins, clinical significance of segregating SRCV as an independent disease entity is not so high. Coexistence with endocervical variant in most cases, and similar immunoprofiles of P16 and P53 may suggest that SRCV is a histologic variant of endocervical type.

1240 Histological Characteristics of Gastric-Type Adenocarcinoma of the Uterine Cervix

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Background: Gastric type adenocarcinoma (GAS) of uterine cervix has been proposed as a specific subtype of cervical adenocarcinoma (EC), characterized by secretion of gastric type mucin, and immunopositivities for MUC6 and/or HIK1083 and having a poor prognosis compared to other subtypes. However, histological characteristics or

required ancillary techniques to confirm the subtype has not been widely acknowledged.
Design: We attempted to define the histological characteristics of GAS determined by immunohistochemical features of MUC2, MUC5AC, MUC6, HIK1083, P16, P53, Ki67 and alcianblue-DPAS stains among 150 patients with EC treated at the Asan Medical Center, Seoul, Korea, during 11 year-period along with a validation of previously described clinicopathologic features of GAS.
Results: Five cases (5/150, 3%) showing positivities for all three parameters, HIK1083 (+), MUC6(+), and gastric type mucin (+), were classified as 'probable GAS' and the microscopic findings showed common characteristic features ('gastric features'); 1) small tubular shape and rarely branching glands, 2) single layered epithelium with pale pink cytoplasm (with or without nuclear atypia), 3) often dilated lumen with occasional intraglandular micopapillary features. Seventeen cases (17/150, 11%) showing positivities for any two parameters, including HIK 1083 & MUC6 (n=9), HIK1083 & gastric mucin (n=2), MUC6 & gastric mucin (n=4), were classified as 'possible GAS', among which 'gastric features' were variably identified ranging from 44%-100%. However, among 17 cases of 'possible GAS' defined by positivities for two parameters regardless of histologic findings, intestinal subtype (n=3), signet ring cell subtype (n=2), and poorly differentiated adencarcinoma were also included, suggesting that these parameters are not specific for GAS. The patients with 'probable GAS' or 'probable and possible GAS' had significantly shorter disease-free survival times in Kaplan-Meier survival analyses (P=0.021), significantly larger tumor size, and deeper invasion of cervical wall than the patients with other subtypes.
Conclusions: Subtyping of GAS appears to be clinically significant due to a significant prognostic difference, and the 'gastric features' may be helpful in the recognition of GAS, however, accurate subtyping should always be combined with ancillary techniques including HIK1083, MUC6, and alcian blue-DPAS. It is still difficult to subtype the cases showing positivities for only one or two parameters since MUC6 and HIK1083 were occasionally positive for intestinal and signet ring cell subtype.

1241 Diagnosis of Endometrial Versus Endocervical Adenocarcinoma: Quantitative Image Analysis Is a Useful Diagnostic Tool

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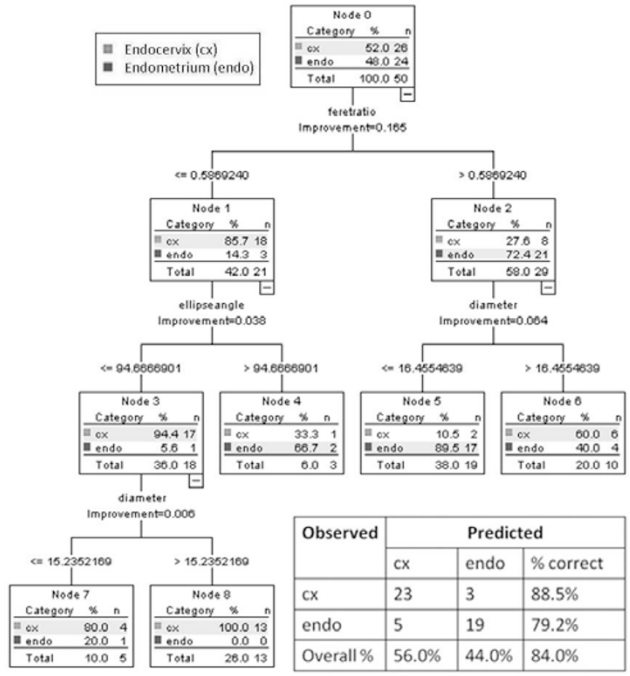
Background: Distinction between endometrial and endocervical adenocarcinoma on biopsy is a common diagnostic issue. While immunohistochemistry is often helpful, up to 50% of endocervical and 70% of endometrial tumors do not fully adhere to expected patterns. Digital image analysis is a novel ancillary tool in surgical pathology, and to date its role in the above differential has not been explored. The aim of this project is to use digital image analysis to uncover distinctive morphologic features between endometrial and endocervical adenocarcinoma.
Design: 24 cases of FIGO grade 1 and 2 endometrial adenocarcinoma and 26 cases of endocervical adenocarcinoma were retrieved from our files. The H&E slides were digitally scanned (Aperio). Two representative tumor areas were selected, and a TIFF image file of each was obtained at 20X magnification. The images were analyzed using digital software (Zen 2011, Carl Zeiss Microscopy, Germany). The following nuclear features were measured: diameter, area, perimeter, area filled, circularity, convexity, ellipse angle, feret ratio, fibre length, form circle, roundness, grey, blue, red and green levels. Collected data was analyzed using statistical software (SPSS v 17).
Results: Of 15 nuclear features analyzed, diameter and feret ratio (a measure of nuclear membrane irregularity) were significantly different between endometrial and endocervical adenocarcinomas by independent samples comparison of means test, while ellipse angle (a measure of relative nuclear alignment) approached significance (Table 1). A classification tree algorithm combining these variables classified 79.2% of endometrial and 88.5% of endocervical adenocarcinomas correctly (Figure 1).
Conclusions: For the first time, we demonstrate that digital image analysis can detect significant differences in nuclear morphology of endometrial and endocervical adenocarcinomas, which are imperceptible to the human eye. As such, it has a potential value as a future ancillary diagnostic tool, and may perform more reliably than immunohistochemistry.

Nuclear feature	Endometrial carcinoma		Endocervical carcinoma		p value
	Mean	StDev	Mean	StDev	
Diameter (um)	15.05	2.57	17.91	4.33	0.007
Feret ratio	0.602	0.02	0.581	0.017	<0.0001
Ellipse angle	91.85	3.65	89.71	4.15	0.06



Observed	Predicted		
	cx	endo	% correct
cx	23	3	88.5%
endo	5	19	79.2%
Overall %	56.0%	44.0%	84.0%

1242 Hormone Receptor Status Predicts Clinical Outcome of High Grade Serous Carcinoma: A Gene Expression Study

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Background: High Grade Serous Carcinoma (HGSC) originates in the hormonally sensitive fallopian tube epithelium. Most HGSC cases express estrogen receptor (ER). Anti-estrogen therapy is often used to treat recurrent disease, but associations between ER expression and tumor response are not well documented. We hypothesize that i) reproductive hormone signaling is altered in HGSC, that ii) reproductive hormone receptor status (ERα/PR) may be an important indicator of clinical outcome and that iii) ER target gene expression will provide further insight into the use of anti-hormone therapy in ovarian cancer.
Design: FFPE tissues (n=334) and snap-frozen tissues (N=43) from HGSC naïve to chemotherapy were obtained from the UHN Pathology Department. Clinical outcome data inclusive of both recurrence-free (RFS) and overall survival (OS) was available on 165 cases. Using tissue microarrays and standard immunohistochemistry, ERα and PR expression was analyzed using automated image analysis. A candidate list of ER target genes was generated by comparing tumor gene expression profiles of the sub-groups based on ER/PR expression. Candidate genes were validated by qPCR and immunohistochemistry on tissue microarrays. Statistical analysis was performed using ANOVA ($p<0.05$) and Fisher's Exact Test ($p<0.05$).
Results: High PR expression cases (31%) had a significant increase in both RFS ($p<0.05$) and OS ($p<0.005$) compared to the PR-low samples. ER expression, positive in 88% of cases, did not predict for survival. Combining expression of PR with ER created four subgroups, with ER-high/ PR-low being the most frequent expression pattern (57%), followed by ER-high/ PR-high (25%), ER-high/ PR-low (12%) and ER-low/PR-high (5%). Overall survival was prolonged in the ER-low/PR-high cases with the shortest OS seen in the ER-high/PR-low patients ($p=0.002$). Gene expression analysis revealed 202 ER target genes with more than a 2 fold-change in expression ($p<0.05$). Five genes (DKK3, SOX11, IGF2, TKTL1, NY-ESO-1) were selected for validation by qPCR and IHC, based on gene ontology and cancer pathway associations.
Conclusions: HGSC is predominantly an ER-high/PR-low cancer, but other patterns of hormone receptor expression exist which predict for an increased overall survival. ER-high differ transcriptionally from ER-low HGSC although some known ER target genes are still transcriptionally activated in the absence of PR. This information should provide further insight into the use of hormone receptors as indicators of anti-hormone therapy response in ovarian cancer.

1243 Carbonic Anhydrase IX Expression Is Common in Epithelial Ovarian Cancer and Is Associated with Abberant Cytoplasmic Expression of the Cell Adhesion Molecule Alpha-T-Catenin (CTNNA3)

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Background: Carbonic Anhydrase IX (CAIX) is associated with tumour hypoxia, which causes dysfunction of a number of cell adhesion molecules, in particular the E-cadherin-catenin complex. The aim of this study was to investigate the relationship between CAIX and the cell adhesion molecule CTNNA3 in epithelial ovarian cancer (EOC) and to compare the frequency of CAIX expression with that seen in breast cancer.
Design: Immunohistochemical analysis (IHC) for CAIX was performed on an EOC

tissue microarray (TMA) (n=61) and on a breast carcinoma TMA (n=89). A cohort of EOC for which full-face sections were available (n=35) were also stained for CAIX and CTNNA3. From the breast cancer cohort, 24 tumours with CAIX expression were identified. Full face sections were obtained from 14 of these and stained with CAIX and CTNNA3.

Results: CAIX was expressed in 44/61(64%) of the EOC TMA cohort and in 34/35(98%) of the ovarian full-face sections. Breast cancers were less likely to show tumour hypoxia with 24/89(28%) expressing CAIX. Loss of membranous CTNNA3 was identified in 5/14 breast tumours staining positive for CAIX. In the remaining 9 tumours, which, on the whole, retained membranous CTNNA3, focal loss was seen specifically in CAIX positive regions in 6/9. Adjacent normal breast tissue showed crisp membranous CTNNA3 in all cases.Loss of membranous CTNNA3 was seen in 20/35(57%)EOCs with hypoxia. Strong expression was seen in 3/35(8%), with weak expression in 12/35(34%). All of the 3 tumours displaying strong membranous CTNNA3 staining, showed focal loss specifically in areas of strong CAIX expression.

Conclusions: CAIX can be more commonly demonstrated in EOC than in breast cancer. Loss of membranous expression of the cell adhesion molecule CTNNA3 is frequent in breast and ovarian cancer. In those tumours retaining membranous localisation of CTNNA3, there was focal loss specifically in CAIX positive regions suggesting a relationship between loss of membranous CTNNA3 and the presence of tissue hypoxia. This is further supported by the *in vitro* finding of loss of membranous CTNNA3 in a chronic hypoxic cell line.

1244 CK17 as an Adjunctive Marker for the Diagnosis of Differentiated Vulvar Intraepithelial Neoplasia

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Background: Vulvar intraepithelial neoplasia (VIN) is a premalignant lesion of vulvar squamous cell carcinoma (SCC). Two subtypes exist in the current ISVVD classification. Usual type VIN (uVIN) is more common, occurring in younger patients, and is associated with human papillomavirus. Differentiated type VIN (dVIN) typically occurs in postmenopausal women, is associated with chronic inflammatory dermatoses, commonly lichen sclerosis, and has a higher risk of SCC. Pathologic diagnosis of dVIN is challenging due to the subtle histologic findings, including parabasal cell atypia and eosinophilia that distinguish dVIN from benign lesions. Cytokeratin 17 (CK17) immunohistochemistry has been used as a marker of dysplasia in the cervix. The aim of this study was to determine the utility of CK17 as an adjunctive marker in the diagnosis of dVIN.

Design: The pathology archives at our institution were searched for cases of dVIN from 2010 to 2013. Histologic slides were reviewed to confirm the diagnosis and select the paraffin embedded blocks for CK17 immunohistochemistry. CK17 antibody (1:50, Dako, Denmark) was used according to the manufacturer's instructions. The staining intensity (weak, intermediate, strong) and location (basal, parabasal, middle or superficial layers) were evaluated.

Results: Fifteen cases of dVIN were identified, 4 biopsies and 11 surgical specimens. Eleven cases included normal mucosa and 2 cases included lichen sclerosis. All cases showed staining with CK17. Thirteen of 15 (87%) dVIN cases showed intermediate to strong staining predominantly in the suprabasal, middle and superficial mucosal layers. Of the remaining 2 cases, one was equivocal with patchy strong staining in the middle and superficial layers, and the 2nd was considered negative with focal weak staining. A sharp contrast between CK17 staining of dVIN and normal mucosa was observed. Normal mucosa demonstrated at most weak staining of the basal layer, although strong staining was noted in normal skin appendages. Two cases with both dVIN and lichen sclerosis demonstrated positive staining of dVIN and negative staining of lichen sclerosis.

Conclusions: Of the premalignant lesions in the vulva, dVIN is more commonly associated with SCC and can be missed on pathologic diagnosis due to subtle histologic findings of atypia. Recognizing dVIN is important for appropriate management and cancer prevention. While current ancillary studies that support a diagnosis of dVIN include p53 and Ki-67, we find CK17 to be a useful marker to add to the immunohistochemical panel.

1245 Primary Endometrial Yolk Sac Tumor (YST): Clinicopathologic and Immunohistochemical Characterization of a Diagnostically Problematic Entity

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Background: Primary endometrial YST is rare, with only 4 cases reported to date. Given its overlapping morphologic features with usual endometrial carcinoma, it can be easily misdiagnosed. We report the morphologic features of a series of cases and assess a panel of immunohistochemical stains for differentiating endometrial YST from Müllerian-type adenocarcinomas.

Design: Database searches for endometrial YST were conducted at two large institutions. H&E slides were reviewed and clinical and morphologic features recorded. Immunohistochemical analysis with antibodies against SALL4, Pax-8, ER, PR, AFP, p53 and Hepatocyte antigen was performed. Immunostaining results were analyzed.

Results: Four cases were identified (3 hysterectomy specimens and 1 endometrial curettage) from 4 patients (age range=35 to 67 y, mean=57). All tumors were pure YST; none had components of Müllerian-type adenocarcinoma or carcinosarcoma. All 4 cases showed a glandular pattern, including 2 with endometrioid-like glandular pattern. Other admixed patterns included hepatoid in 2 cases, solid in 1 case and trabecular in 1 case. Moderate to focally high grade nuclear atypia was present in all cases. Hyaline globules were seen in 3 cases. Of the staged cases, 2 were stage IA and 1 was stage IB. Of the cases with follow-up, 2 patients are alive with no evidence of disease at 5 and 8 months. One patient had a perirenal and mesenteric recurrence and is alive with disease at 14 months. Immunohistochemical results are given in the table below.

Case	SALL4	AFP	Pax-8	p53	ER	PR	Hepatocyte
1	Positive (diffuse)	Positive (patchy)	Negative	Positive (>90%)	Negative	Negative	Positive (focal)
2	Positive (diffuse)	Positive (patchy)	Negative	Positive (>90%)	Negative	Negative	Positive (patchy)
3	Positive (patchy to diffuse)	Positive (patchy)	Negative	Positive (>70%)	Negative	Negative	Positive (diffuse)
4	Positive (diffuse)	Positive (focal)	Positive (diffuse)	Positive (>50%)	Negative	Negative	Negative

Conclusions: Although rare, endometrial YST can mimic endometrioid or serous carcinoma. Recognition of its morphologic features and directed immunohistochemistry will facilitate accurate diagnosis. Endometrial YST has a unique immunostaining profile with diffuse positivity for SALL4 and p53, focal positivity for AFP and Hepatocyte antigen and negativity for Pax-8, ER and PR in the majority of cases. This immunoprofile is distinct from that of endometrioid or serous adenocarcinomas.

1246 Localized Amyloidosis of the Vulva – With and without Vulvar Dysplasia: A Liquid Chromatography Tandem Mass Spectrometric and Immunohistochemical Study

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Background: Primary cutaneous amyloidosis, though common in South East Asia, is uncommon in Europe and North America. Localized vulvar amyloidosis is infrequently reported in the English literature. Since the constituents of such deposits have not been examined previously, this series characterizes amyloid deposits in localized vulvar amyloidosis and their association with vulvar intraepithelial neoplasia (VIN).

Design: All biopsies and excisions of vulva over 18 months were reviewed, and cases with suspected amyloidosis were retrieved after IRB approval. Eleven cases mimicking amyloidosis by H & E stains were selected as controls. All study and control cases were stained with Congo red and immunostained for cytokeratin 5 (CK5) and cytokeratin 14 (CK14). Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) was performed on two localized vulvar amyloidosis and vulvar deposit of one systemic amyloidosis.

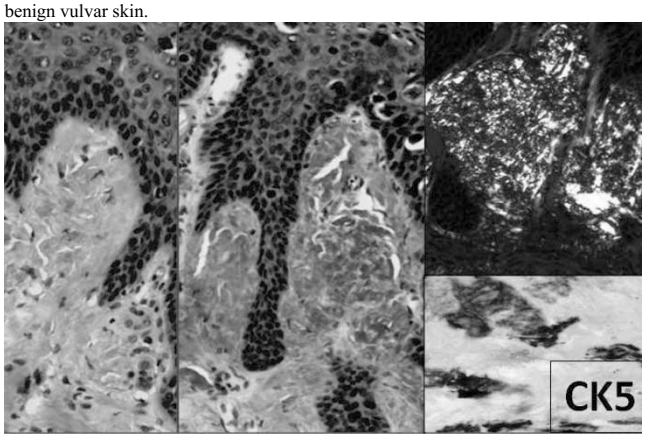
Results: A total of 149 cases were reviewed and 11 cases of incidental localized vulvar amyloidosis were identified. LC-MS/MS study reveals unique peptide profile consistent with keratin-associated amyloid deposition (CK5 and CK14) in addition to serum amyloid P components, apolipoprotein E and apolipoprotein A4 in the two localized amyloidosis. The vulvar deposit of systemic amyloidosis showed AL (lambda)-type amyloid deposit. Immunostain results are tabulated in Table 1. All control cases were negative for Congo red and keratin immunostaining.

Table 1

Vulvar Amyloidosis (N=11) and Congo red Negative Control (N=11)					
		Types of deposit			
		Keratin 5	Keratin 14	Lambda	Others*
Localized Congo red Positive amyloidosis (N=10)	With VIN (N=9)	9	8	0	9
	Without VIN (N=1)	1	1	0	1
Systemic amyloidosis (N=1)	With VIN (N=1)	0	0	1	1

*Serum amyloid P component; Apolipoprotein E; Apolipoprotein A4. All control cases were negative

Conclusions: Keratin-associated amyloid materials (CK5 and CK14) were found to be unique in localized vulvar amyloidosis. This is probably due to leakage of keratins from the basal layer of the epithelium into the superficial dermis and it appears to be associated with high grade and low grade VINs and rarely in lichen sclerosus and in benign vulvar skin.



1247 Intramucosal Growth in Fallopian Tube Fimbriae by Tumors of Non-Gynecologic Origin May Mimic Serous Tubal Intraepithelial Carcinoma and Tubal Mucinous Metaplasia

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Background: Intramucosal growth of high grade serous carcinoma in the fallopian tube (defined as serous tubal intraepithelial carcinoma [STIC]) is viewed as strong evidence for a primary tubal origin of the cancer. Tubal mucosal growth by a cancer originating outside of the fallopian tube traditionally has been regarded as unlikely, but this has not been well studied. The aim of this study is to determine the frequency and anatomic distribution of fallopian tube involvement by tumors of non-gynecologic origin, emphasizing deceptive patterns that may mimic STIC or mimic benign alterations such as tubal mucinous metaplasia.

Design: 95 patients with fallopian tube metastases from tumors of non-gynecologic origin were examined. Primary site of origin included colorectal,35; gastric,10; non-specified upper GI tract,8; pancreatic, 4; cholangiocarcinoma, 2; low grade appendiceal mucinous neoplasm, 7; high grade appendiceal adenocarcinoma, 2; breast, 16; lymphoma, 5; neuroendocrine, 3; mesothelioma, 2; and GIST, 1. Anatomic distribution of tumor growth in the fallopian tubes was evaluated, emphasizing mucosal growth patterns.

Results: Tubal involvement by cancer was grossly occult in 62% of patients. Fimbriae were present in 82/95 patients and contained tumor in 45 (55%) patients while non-fimbriated tube was involved in 83 (87%) patients. Mucosal tumor growth in fimbriae was seen in 27/45 (60%) patients while the fimbrial serosa and/or submucosa were involved in the remainder. Fimbrial mucosal growth was seen in 50% of tumors of pancreatic, biliary, or high grade appendiceal origin; in 37% of colorectal origin or non-specified upper GI origin; in 20% of gastric or breast (ductal) origin or lymphoma. STIC-like growth of high grade tumor cells was seen in 7/13 colorectal tumors growing in the mucosa and in a few cancers of pancreatic, gastric, non-specified upper GI, and breast origin. Mucinous metaplasia-like growth of low grade tumor cells was seen in 10 (37%) specimens with fimbrial mucosal involvement (4 colorectal, 3 non-specified upper GI, 2 pancreatic origin). Ovarian metastases were present in 90/95 patients.

Conclusions: Metastatic high grade cancers of non-gynecologic origin can involve the fallopian tube fimbrial mucosa and mimic serous tubal intraepithelial carcinoma. Likewise, low grade mucinous tumors of non-gynecologic origin can involve the mucosa and mimic tubal mucinous metaplasia. Thus, intramucosal tumor growth in the fallopian tubes is not restricted to tumors of primary tubal origin.

1248 Transgelin, a Novel Marker That Effectively Distinguishes Endometrial Stromal Tumors from Uterine Smooth Muscle Tumors

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Background: Transgelin is a 22 kDa actin-binding protein of the calponin family. It has recently been shown to be a good marker for smooth muscle differentiation. Distinguishing endometrial stromal neoplasms and smooth muscle tumors of the uterus can be challenging. Immunohistochemistry is often not helpful. Many tumors have been shown to express muscle actins and desmin. Others have failed to express markers such as h-Caldesmon and/or CD10. The goal of this study was to determine whether transgelin, a smooth muscle-specific marker, could accurately distinguish endometrial stromal tumors from uterine smooth muscle tumors.

Design: The immunohistochemical expression of transgelin, CD10, Smooth muscle actin (SMA), Desmin, h-Caldesmon and Ki-67 was studied in 11 endometrial stromal tumors (4 low grade endometrial sarcomas, 4 undifferentiated endometrial sarcomas and 3 metastatic endometrial sarcomas) and 10 myometrial tumors (2 leiomyomas and 8 leiomyosaromas). The diagnostic performance of Transgelin was assessed in comparison to other smooth muscle markers in terms of sensitivity, specificity and accuracy.

Results: Upon assessment of the diagnostic performance, Transgelin showed diffuse and strong positivity in all myometria, leiomyomata, and leiomyosarcomas. Of interest, none of the smooth muscle tumors expressed 100% positivity with the other three smooth muscle markers at any given time, as was the case with Transgelin. In contrast, Transgelin expression was totally absent in all endometria and endometrial stromal neoplasms except within blood vessels. However, focal smooth muscle actin positivity was noticed in 2 of the 11 endometrial sarcomas, and 3 of them were negative for CD10.

Conclusions: Transgelin has shown to be a specific marker of smooth muscle differentiation in the uterus, with 100% sensitivity and specificity. The novel marker is useful for distinguishing myometrial from endometrial stromal neoplasms. It could be used as an additional marker for decision making, especially in those tumors with questionable histology and immunophenotypes.

1249 Metastatic Clear Cell Renal Cell Carcinoma to the Gynecologic Tract: Clinicopathologic Analysis and Immunophenotypic Comparison with Mullerian Clear Cell Carcinomas

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Background: Although clear cell renal cell carcinomas (CCRCC) are known to have an expansive group of potential metastatic sites, they only rarely involve the gynecologic (Gyn) tract. In this study, a multi-institutional dataset was analyzed to provide insights into the clinical, morphologic, and immunophenotypic features of this phenomenon.

Design: 17 metastatic CCRCC (met-CCRCC) involving the Gyn tract (9 ovary/fallopian tube, 2 vulva, 3 endometrium, 2 cervix, 1 uterine serosa) were assessed for a variety of clinicopathologic features. Their immunophenotypes were compared with 102 mullerian clear cell carcinomas (Gyn-CCC: 49 endometrial, 53 ovarian) using a panel of 9 potentially discriminatory markers.

Results: The average patient age was 62.3 years (range 45-79 months). In only 1 of 17 cases - a cervical metastasis - was the gyn tract lesion the initial presentation of the renal tumor. Rather, most cases (15/17) presented as a recurrence an average of 19 months (range 6 - 60) after nephrectomy. In 8 (53%) of these 15 recurrences, the Gyn lesion was the first known recurrence. In 10 cases, metastases to other locations (most frequently bone and lungs) were identified concurrent with, or shortly subsequent to the discovery of the Gyn tract lesion. Of the 9 adnexal metastases, only 2 were bilateral, and the average size was 3.7cm. The 2 vulvar metastases presented as ulcerative masses, whereas 5 of the 6 uterine lesions were identified in samples obtained to work up abnormal bleeding. Although met-CCRCC and Gyn-CCC displayed extensive morphologic overlap, 4 statistically significant morphologic differences were identified: i) a diffuse nested/alveolar pattern (14/17 vs 6/102, p<0.0001), ii) mitotic index (6.3 vs 2.9, p=0.04), iii) diffuse hobnail cells (0/17 vs 67/102, p<0.0001) and iv) tubulocystic pattern (0/17 vs 55/102, p<0.0001). Seven of the 9 markers listed below significantly distinguished between the 2 groups, PAX8 and HNF1β being the only exceptions.

Comparative Immunophenotypes of the two Groups

Marker	Met-CCRCC (positive/tested)	Gyn-CCC (positive/tested)	p value
Carbonic Anhydrase IX	7/17	0/102	<0.0001
CD10	15/17	42/102	0.0004
Kidney-specific cadherin	8/17	0/102	<0.0001
RCC-antigen	12/17	0/102	<0.0001
HNF1β	14/17	83/102	1
Napsin A	4/17	90/102	<0.0001
AMACR	1/17	72/102	<0.0001
PAX8	14/17	93/102	0.3755
CK 7	2/17	96/102	<0.0001

Conclusions: Met-CCRCC displays a distinctive set of clinicopathologic and immunophenotypic features that in most cases, should readily facilitate their diagnosis and their distinction from mullerian clear cell carcinomas.

1250 Genetic Variants Linked to Preeclampsia Are Associated with Preterm Labor in Japanese Women

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Background: Our group and others have shown that most cases of preterm labor (PTL) are likely not caused by infection. Instead, placental studies suggest an important underlying mechanism may be placental insufficiency, similar to preeclampsia. Most PTL placentas show accelerated villous maturation and in some cases there is also abnormal spiral artery remodeling. Since angiotensinogen (AGT) Met235Thr and endothelial nitric oxide synthase (eNOS) Glu298Asp variants have been shown to be associated with preeclampsia in Japanese women, we hypothesized that these genetic markers may also be associated with preterm labor.

Design: We identified 97 term negative controls and 42 idiopathic PTL cases leading to preterm birth (mean gestational age 34 +/- 0.54 weeks') from the Pacific Research Center for Human Early Development's (PRCED) obstetric database. Because there is significant variation in allele frequencies between races, these cohorts were restricted to women claiming at least 75% Japanese heritage. Subject metrics included maternal age, newborn sex and measurements, as well as placental measurements. Maternal DNA was genotyped for the Met235Thr single nucleotide polymorphism (rs699) and the Glu298Asp variant (rs1799983) using real-time PCR-based *Taqman* allelic discrimination. Placental sections were reviewed to confirm the absence of acute chorioamnionitis. Data were analyzed by X^2 and odds ratios were reported with 95% confidence intervals.

Results: The AGT Thr235 allele was significantly more common in PTL (q=0.83) compared with negative controls (q=0.70) (OR: 2.7 [1.2-5.7], p-value=0.01). The eNOS Asp298 variant associated with preeclampsia tended be more common in the PTL group (OR=1.5 [0.6-3.7], p=0.2). The uncommon combined TT/AA haplotype (12% of cases) provided an odds ratio of 2.9 [1.0-8.3] (p=0.05) and it was more predictive of reduced placental weight and birthweight than the AGT genotype alone.

Conclusions: The AGT variant associated with preeclampsia is also associated with idiopathic preterm labor, consistent with our working hypothesis that these two common and serious pregnancy complications may share an underlying pathophysiology. The eNOS variant was not as predictive as AGT Thr235, but the combined AGT/eNOS haplotype appears to be more closely associated with fetoplacental growth.

1251 Infarcted Endometrial Polyps with Glandular Atypia: A Potential Diagnostic Pitfall

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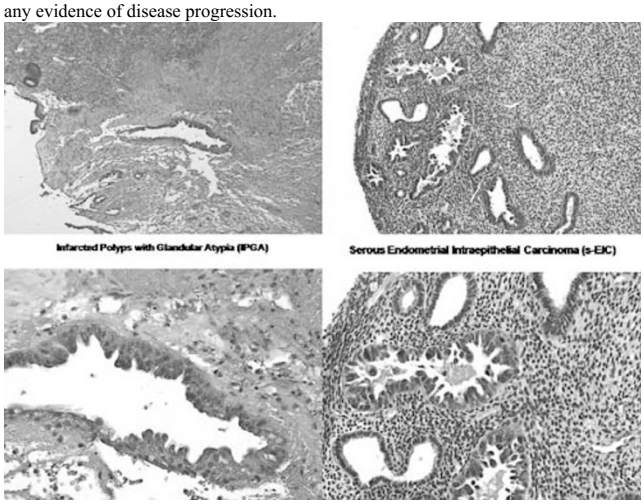
Background: We describe the clinicopathologic features of a group of endometrial polyps that exhibited large areas of infarction, as well as scattered glands within the necrotic zones whose level of atypia prompted some consideration of the possibility of serous endometrial intraepithelial carcinoma (S-EIC).

Design: 13 cases of infarcted polyps with glandular atypia (IPGA) were retrieved from our routine (4), referral (7) and consultation (2) files. A variety of descriptive clinicopathologic features were documented. To comparatively assess their immunophenotypic features, we performed immunohistochemical studies for p53 and MIB1 on all cases, as well as on a 27-case control group comprised of 13 consecutive endometrial polyps without infarction, 10 cases of papillary syncytial metaplasia associated with breakdown (PSM), and 4 endometrial polyps with foci of S-EIC.

Results: The 13 patients with IPGA ranged in age from 41-77 years (mean 62.4). All polyps were diagnosed in biopsies or curettages. The average size of the biopsied sample was 1.9 cm (0.7-4.3). The polyps showed a distinctive pattern of isolated glands within

or on the surface of large zones of necrosis or hyalinization. The glands displayed intraglandular tufting or budding, overlapping nuclei with vesicular chromatin or hyperchromasia, nucleolomegaly, vague syncytia, and no mitotic figures. The proportion of each polyp that was infarcted or hyalinized ranged from 30 to 90% (mean 50). Weak immunoreactivity for p53 was seen in 0-40% of the lesional nuclei in IPGA, and 10-20% were MIB1-positive. In contrast, 80-100% S-EIC cells showed strong staining for p53, and 60-100% were MIB1-positive ($p<0.001$). Wild type p53 staining was also seen in all PSM (0-15%, weak), and non-infarcted polyps (0-20%, weak). Both PSM and non-infarcted polyps showed a low proliferative index. There were no significant differences between IPGA and PSM regarding p53 and MIB1 staining frequency or extent. Follow-up was available in 11 of the 13 IPGA patients, and none has shown any evidence of disease progression.



Conclusions: IPGA is a distinctive reactive lesion whose differential diagnosis includes S-EIC. The p53 and MIB1 staining indices of IPGA suggests that it represents an exuberant form of PSM.

1252 Fibroblastic Variant of Endometrial Stromal Sarcoma – Genetic Reclassification

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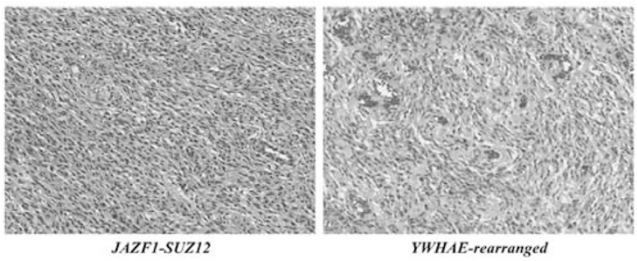
Background: Fibroblastic endometrial stromal sarcoma (ESS) is a known variant of ESS composed of oval-to-spindle-shaped cells set in a conspicuous fibroblastic to fibromyxoid stroma. Given that several genetic rearrangements have been described in ESS and that certain genetic types such as *YWHAE-NUTM2* are associated with more aggressive clinical behavior than others including *JAZF1-SUZ12*, we aim to further define fibroblastic ESS genetically.

Design: Eleven fibroblastic ESSs were retrieved from institutional archives and reviewed; 9 tumors were from hysterectomy specimens, 1 from a resection of recurrent tumor and 1 from an endometrial biopsy. Fluorescence *in situ* hybridization analyses evaluating for genetic rearrangements involving *YWHAE, JAZF1, SUZ12, PHF1, EPC1* and *MEAF6* were performed.

Results: Patients ranged from 21 to 60 years in age at presentation. All 11 tumors contained oval to spindle shaped tumor cells with low-grade monomorphic nuclear features and conspicuous fibroblastic to fibromyxoid stroma. Mitotic rate ranged from 1 to 19 per 10 HPF. Two tumors each showed *YWHAE* and *JAZF1-SUZ12* rearrangements, 3 showed *PHF1* rearrangements, and 4 showed no rearrangements. One of the *YWHAE* ESS cases was a recurrent tumor where only a low-grade fibroblastic component was identified, while the other *YWHAE* ESS was from an endometrial biopsy. Clinically, 2 of 2 *YWHAE* ESS, 2 of 3 *PHF1* ESS, 0 of 2 *JAZF1-SUZ12* ESS, and 0 of 4 non-rearranged ESS recurred. Only 1 patient died from the disease and she had an *YWHAE* ESS.



Conclusions: Fibroblastic ESS is genetically heterogeneous and is a histologic pattern that can be seen in different ESS genotypes with varying biologic potential. Accurate histologic diagnosis can be difficult in biopsy sample without ancillary molecular test. In a resection specimen, thorough tumor sampling coupled with an appropriate immunopanel and/or molecular test can ensure a correct diagnosis.

1253 Different Immunohistochemical Staining Patterns of Primary Mucinous Ovarian Tumors Suggests Multiple Pathways of Differentiation

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Background: The origin of primary mucinous ovarian tumor is enigmatic. Historically, mucinous tumors were thought to arise from metaplasia of ovarian surface epithelium. More recently, authors have proposed origin of mucinous tumors from Brenner tumors as well as teratomas, as both these entities are associated with a subset of mucinous tumors. In this study, we sought to explore the association of these tumors using immunohistochemical stains for Mullerian markers (PAX8 and PAX2), germ cell tumor marker (SALL4) and GATA3 (previously reported in Brenner tumors).

Design: A database search was conducted for cases of mucinous tumors associated with Brenner tumor as well as pure primary mucinous ovarian tumors, intestinal type and endocervical type, in the last three years. Immunostain for PAX8, PAX2, SALL4 and GATA3 was performed with adequate positive and negative controls. For all immunostains, only nuclear staining was considered positive. Staining was classified as focal (<25% of tumor cells) or diffuse (>25%).

Results: Forty mucinous tumors were included in the study; 12 mucinous tumors associated with Brenner tumor, 8 seromucinous borderline tumors and 20 intestinal type mucinous borderline tumors or carcinomas. All mucinous tumors associated with Brenner tumors were negative for PAX8, PAX2, SALL4 and GATA3, while the Brenner counterpart was diffusely positive for GATA3, but negative for other markers. All seromucinous borderline tumors were diffusely positive for PAX8 and PAX2 but negative for SALL4 and GATA3. Of the 20 mucinous tumors of intestinal type, 10 borderline tumors were positive for PAX8, 8 of them diffuse and 2 focal; while the remaining 8 borderline tumors and 2 carcinomas were negative for PAX8.

Conclusions: This study supports different pathways of mucinous differentiation:

1- Mucinous tumors, endocervical type are PAX8 and PAX2 positive, similar to other Mullerian tumors, and are distinct from intestinal type mucinous tumors

2- The mucinous tumors associated with of Brenner tumor lack expression of Mullerian and germ cell tumor markers, similar to the expression of Brenner tumors, suggesting origin from Brenner tumors. As GATA-3 appears to be a squamous and transitional cell marker rather than a marker for Brenner tumors, it may account for the differential staining in the Brenner tumor and its associated mucinous component

3- The immunophenotype of mucinous tumor, intestinal type is variable, where half of them are consistent with a Mullerian phenotype.

1254 Ovarian Brenner Tumors and Their Association with Walthard Nests: A Histologic and Immunohistochemical Study

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Background: The origin of ovarian Brenner tumors is currently uncertain. As in other ovarian surface epithelial tumors, classic theories proposed an origin from surface ovarian epithelium or epithelial inclusion cysts; however, this has recently been disputed, specially for serous carcinoma. As Brenner tumors have squamous or transitional cell morphology, origin from Walthard nests is a possibility. In addition, association of a subset of Brenner tumors with teratomas suggests that they may have germ cell origin. In this study, we performed immunohistochemical staining with markers of varying lineages in an attempt to explore the histogenesis of Brenner tumors and their possible association with Walthard nests.

Design: A database search was conducted for cases of ovarian Brenner tumors in the last three years. H&E slides were reviewed confirming the diagnosis and evaluating for the presence of concurrent Walthard nests. Immunostains with PAX8 and PAX2 (Mullerian and mesonephric markers), SALL4 (pan-germ cell marker), and GATA3 (expressed in urothelial carcinoma and squamous/transitional lesions, such as normal cervix and transitional metaplasia of the prostate) was performed on the Brenner tumors and Walthard nests with appropriate controls. For all the stains, only nuclear staining was considered to be positive. Staining was classified as focal (<50% of tumor cells) and diffuse (>50% of tumor cells) and intensity as weak or strong.

Results: Twenty-eight consecutive cases of Brenner tumors were identified. Eleven of them (40%) had Walthard nests in the parafallopian/ periovarian soft tissue. All Brenner tumors were diffusely positive for GATA3 (strongly positive in 26/28 and weakly positive in 2/28) and negative for PAX8, PAX2, and SALL4. All 11 cases with Walthard nests showed identical staining to the Brenner tumors (GATA3 diffusely and strongly positive; PAX2, PAX8, and SALL4 negative).

Conclusions: 1. Forty percent of Brenner tumors in our study had associated Walthard nests. The similar morphology and immunoprofile of Brenner tumors and Walthard nests suggest a probable link between Brenner tumors and Walthard nests.

2. The lack of staining for Mullerian markers (PAX8 and PAX2) as well as germ cell marker (SALL4) suggests that Brenner tumors and Walthard nests are not of Mullerian or germ cell origin.

3. Brenner tumors express GATA3, and this marker should be investigated in other tumors that have squamous or transitional morphology.

1255 Differential Expression Pattern of GATA3 among Mesonephric Lesions and Endocervical and Endometrial Adenocarcinomas

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Background: Mesonephric lesions of the cervix, especially florid hyperplasia and carcinoma, can morphologically mimic endocervical or endometrial adenocarcinomas. GATA binding protein 3 (GATA3) is a recently described immunohistochemical marker that is expressed in urothelial and breast carcinomas but lost in many other cancers. Considering its role in the development of pro/mesonephros, we hypothesized that it could be expressed in mesonephric proliferations. Here we study

its immunohistochemical expression in uterine mesonephric lesions as compared to endocervical and endometrial adenocarcinomas.
Design: A cohort of 57 cases was selected from the archives including 31 uterine mesonephric lesions, 16 endocervical adenocarcinomas and 10 endometrial adenocarcinomas. Hematoxylin-eosin slides were reviewed and immunohistochemical staining for GATA3 was performed on the most representative section. The immunostaining pattern was recorded as: positive (nuclear staining) or negative with distribution of staining as diffuse or focal (< 20% cells) and intensity as weak, moderate or strong.
Results: Thirty-one mesonephric lesions included 16 remnants, 12 hyperplasias, 1 carcinoma and 2 carcinosarcomas. All but one of them (96.7%) were diffusely positive (>95% of cells, strong, nuclear) for GATA3. One carcinosarcoma case showed focal staining (30% carcinoma cells, 5% sarcoma cells, moderate to strong, nuclear). All the endocervical adenocarcinomas were of the usual type. Of these, 75% (12 cases) were negative and remaining 25% showed focal (<20% tumor cells, weak, nuclear and focal cytoplasmic) staining. The endometrial adenocarcinoma cases included 8 endometrioid and 2 high-grade mixed carcinomas with serous component. Of these, 80% (8 cases) including 6 endometrioid and 2 high-grade mixed carcinomas were negative and remaining 20% showed focal (<10% tumor cells, weak to moderate, nuclear) staining.
Conclusions: Our study demonstrates that GATA3 protein is overexpressed in mesonephric lesions, regardless of their nature (benign or malignant). Loss of GATA3 expression was seen in the majority of endometrial adenocarcinomas (both endometrioid and serous types) as well as in the majority of endocervical adenocarcinomas. Only 20% of endometrial adenocarcinomas and 25% of endocervical adenocarcinomas revealed focal staining. This pattern is very distinct from the diffuse nuclear staining pattern seen in mesonephric lesions. These results support that GATA3 immunostain can be a useful tool in differentiating mesonephric lesions from endocervical and endometrial adenocarcinomas.

1256 Folate Receptor Alpha Expression in Gynecologic Tumors

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Background: A few prior studies have shown Folate receptor-α (FRA) overexpression in ovarian carcinomas. Recent clinical trial data suggest clinical activity of folate receptor antibody in ovarian cancers. However, patient selection criteria for targeted therapy are not well defined. There is also limited information regarding FRA expression in other gynecologic malgnancies.
Design: We studied FRA expression by immunohistochemistry (IHC) in a variety of gynecological cancers and in 41 benign fallopian tubes (FT). The tissue reactivity was scored using the semi-quantitative H-score method [range from 0 (no reactivity) to 300 (diffuse strong reactivity)]. All tissues were represented on tissue microarrays with at least 2-fold redundancy. An H-score of >10 was considered positive.
Results: The most frequently positive tumor was ovarian serous carcinoma followed by ovarian clear cell carcinoma. The endocervical and endometrial carcinomas were less frequently positive compared to ovarian non-mucinous carcinomas (p <0.0001). However; of the 72 positive ovarian carcinomas, only 6 had H-score >200 and 16 had H-scores >150. The predominant pattern of staining in serous tumors was apical, with membranous present to a lesser degree. A typical apical reactivity was seen in the majority of FT.

Folate Receptor-α Expression vs Tumor Types

Tissue Type	FRA+/total(%)	H-score, mean, median, range of positive cases
Endocervical adeno CA	4/34 (12)	47, 44, 40-60
Endometrial endometrioid CA	9/38 (24)	60, 50, 13-130
Endometrial non-endometrioid CA	3/16 (19)	65, 40, 15-140
O-high grade serous CA	52/68 (76)	99, 96, 20-285
O-endometrioid CA	1/13 (8)	190, 190, N/A
O-clear cell carcinoma	16/40 (40)	108, 103, 18-235
O-mucinous CA	0/1 (0)	N/A
O-low grade serous CA	1/1 (100)	20, 20, N/A
O-serous CA (unclassifiable)	2/4 (50)	38, 38, 24-51
O-serous borderline tumor	5/22 (23)	103, 110, 35-150
O-mucinous borderline tumor	0/17 (0)	N/A
Benign fallopian tubes	39/41 (95)	96, 95, 15-175

CA: Carcinoma; O: Ovarian; FRA; Folate Receptor Alpha

Conclusions: FRA expression is more frequent in ovarian carcinomas compared to other gynecological tumors. However, diffuse strong reactivity is uncommon, and was seen only in 8% of positive ovarian carcinoma cases. As folate receptor antibody is tested in clinical trials, we suggest that biomarker assessment with H-scoring be performed to accurately assess response and aid in optimal patient selection for targeted therapy in the future. The preferential expression of FRA for the serous subtype and fallopian tube in combination with the apical staining pattern also provides further evidence supporting FT origin of high grade ovarian serous carcinomas.

1257 Uterine Leiomyosarcomas with Heterologous Elements: The MD Anderson Experience

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Background: Uterine smooth muscle tumors (SMT) with heterologous elements (HE) are rare; experience is limited to small case series. This study presents the largest reported series of such tumors.
Design: A pathology database search (1995-2013) yielded 13 cases. From review of patients (pts) charts, pathology reports and available H&E slides (1-13) the following was recorded: pt age; clinical presentation; tumor stage; tumor size; atypia/mitotic index/coagulative tumor cell necrosis (CTCN) in background SMT; type and % of HE; and follow up.
Results: Pt ages ranged from 30-74yo (median 53). Clinical presentation was known in 12 pts: abnormal vaginal bleeding (8 pts); urinary symptoms (3 pts); uterine mass (3 pts); abdominal distention (2 pts). Tumor size, known in 10 pts, ranged from 7 to 22.5 cm (median 13) with stage known in 13 pts: 3 Stage I; 0 Stage II; 4 Stage III; 6 Stage IV. 11 pts had documented metastases (mts): 4 lung, 4 abdomen, 2 pelvis, 1 liver. Biopsy of a lung mt in 1 pt showed mt of the HE. Follow up from 2 to 55 months was available for 12 pts: 10 dead of disease; 2 alive with disease. Tumor characteristics for each pt are summarized in Table 1.

Histologic Features of LMS with HE

Pt	Tumor Size (cm)	Atypia SMT	CTCN SMT	Mitoses/10HPF SMT	HE Component	%HE
1	18	Moderate	Yes	41	Liposarcoma	25
2	7	Moderate	Yes	50	Osteosarcoma	<10%
3	unkn	Severe	Yes	28	Liposarcoma	10
4	unkn	Moderate	Yes	40	Osteosarcoma	70
5	unkn	Unkn*	Unkn*	Unkn*	Osterosarcoma	*Unkn
6	13	Moderate	Yes	14	Liposarcoma	30
7	22.5	Unkn*	Yes	Unkn*	Benign bone elements	*Unkn
8	7	Severe	Yes	35	Rhabdomyosarcoma + focal liposarcoma	30
9	13.5	Moderate	Yes	12	Chondrosarcoma	20
10	17	Moderate	Yes	20	Rhabdomyosarcoma	5
11	9.3	Moderate	Yes	25	Rhabdomyosarcoma	50
12	7	Severe	Yes	17	Rhabdomyosarcoma	50
13	17	Moderate	Yes	41	Osteosarcoma	15

*Unkn, slides unavailable

Conclusions: All HE in SMT occurred in the background of conventional leiomyosarcoma. Clinical presentation and course are similar to conventional LMS. Most HE are malignant, but rarely may be benign. Bone HE were most frequent in this series followed by rhabdomyosarcoma. Recognition of HE in SMT is important to avoid mischaracterization of tumor type and origin given the HE may be the sole or dominant element in subsequent metastases.

1258 Immunohistochemical Profiling in Assessing Histological Type in Endometrial Carcinoma

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Background: Histological typing is easy in most endometrial carcinoma (EC) cases, but may be difficult in high grade EC, or in EC with mixed endometrioid (EEC) and serous (SC) features. Interobserver agreement improves when immunohistochemistry (IHC) is used.
Design: Tissue microarrays composed of EEC grade 1-2 (46), and SC (25) were subjected to IHC for several proteins, found to be differentially expressed between EEC and NEEC by cDNA and protein analysis: p53, p16, ER, cytoplasmic and nuclear PTEN, IMP2, IMP3, HER2, Cyclin B2 and E1, HMGA2, FolR1, MSLN, Claudin 3 and 4, NRF2. IHC values ranged from 0 to 300, according to intensity and percentage of positive cells. First, differential expression was assessed with a Mann-Whitney test. Second, for each significant protein, a conditional threshold in the IHC value scale was determined to discriminate between EEC and SC, using the Gini index, assessing its significance with a logistic regression model. Third, significant proteins in the models were selected to define a signature based in the number of satisfied conditions. A convenient minimum number of conditions was established to predict the sample type. The usefulness of this classifier was checked in two TMAs composed of EEC grade 3 (33 cases), and mixed EEC-SC (both components) (9 cases).
Results: Nine biomarkers showed significant differences between EEC 1-2 and SC, defining for each one a conditional threshold in the IHC value for predicting SC against EEC 1-2 (p53>35, IMP2>110, IMP3>0, Cyclin E1>215, HMGA2>15, FolR1>50, p16>150, nuclear PTEN>0 and ER<50) (p-values<0.005). All 9 conditions were then combined, specifically considering those cases satisfying 0 to 5 conditions (predicted as EEC) respect to those satisfying 6 to 9 conditions (predicted as SC). This discriminating rule correctly predicted all EEC 1-2 cases and 22 SC cases, but 3 SC cases were wrongly predicted as EEC. Sensitivity was 100% (CI: 89%-100%) and specificity was 88% (CI: 69%-97%). This prediction rule also identified all 33 EEC 3 cases, as EEC, but only identified correctly the EEC and SC components in 4 out of 9 mixed EEC-SCs.
Conclusions: Several proteins are differentially expressed between EEC 1-2 and SC, as single markers. Combination of these markers correctly identified all 46 EEC 1-2, most SC (22 of 25), and all 33 EEC 3 cases. However, expression in mixed EEC-SC was complex, suggesting that they have ambiguous IHC features, regardless of the morphological appearance of the different components.

1259 P16INK4a Immunoexpression as a Possible Predictor of Disease Progression in Squamous Cervical Dysplasia

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Background: A prospective study to determine if degree of $p16^{INK4}$ immunoexpression in patients with cervical dysplasia is related to long-term disease status (7 years).
Design: Prospectively, 155 H&E stained (2005-2006) with pre-neoplastic cervical lesions were reviewed. Primary diagnoses included low-grade (LG) lesions (atypia and CIN I) and high-grade (HG) lesions (CIN II, CIN III). $p16^{INK4}$ IHC was performed. Distribution of expression was interpreted as negative, focal/sporadic, diffuse lower half of epithelium, or diffuse upper half to full thickness of epithelium. Follow-up cervical pathology data (1-7 years) was collected through the electronic pathology database. Current disease status was documented as: follow up diagnosis higher grade than

primary ("progression"); follow up diagnosis equal grade as primary ("persistence"); and follow up lower grade than primary ("regression"). Treatment with LEEP excision within 1 year of primary diagnoses was also documented. Cases without follow up were excluded. Statistical analysis included Chai-squared tests.

Results:

p16INK4 expression in primary pre-neoplastic cervical biopsies and subsequent long term follow up.

All Lesions (n=134)	Progression (n=7; 5%)	Persistence (n=8; 6%)	Regression (n=119; 89%)
p16INK4 Expression			
Negative	14%	-	13%
Focal	14%	50%	16%
Lower Half	29%	25%	29%
Upper Half to Full Thickness	43%	25%	41%
HG Lesions (n=78)	Progression (n=2; 3%)	Persistence (n=5; 6%)	Regression (n=71; 91%)
p16INK4 Expression			
Negative	-	-	3%
Focal	-	40%	10%
Lower Half	50%	20%	27%
Upper Half to Full Thickness	50%	40%	61%
LEEP	50%	80%	51%
LG Lesions (n=56)	Progression (n=5; 9%)	Persistence (n=3; 5%)	Regression (n=48; 86%)
p16INK4 Expression			
Negative	20%	-	29%
Focal	20%	67%	25%
Lower Half	20%	33%	33%
Upper Half to Full Thickness	40%	-	13%
LEEP	20%	33%	52%

Of 134 cases, 58% were HG and 42% were LG. Chai-squared tests comparing distribution of $p16^{INK4}$ expression among LG and HG groups failed to show a statistically significant difference in prediction of outcome. Of the HG cases, 91% regressed, despite 51% treated with LEEP excision within one year of primary biopsy diagnosis.

Conclusions: Many studies correlate full thickness $p16^{INK4}$ expression with more aggressive disease; however, this study demonstrates that $p16^{INK4}$ IHC is not predictive of long-term disease progression. As several previous studies have found, the majority of HG lesions ultimately regressed in long-term follow up.

1260 Prospective Analysis of P16INK4a Immunoexpression and HPV Type in Benign Cervical Biopsies

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Background: The value of $p16^{INK4a}$ as a surrogate marker of high-risk human papillomavirus (HR-HPV) and cervical intraepithelial neoplasia has been well established in recent years, with studies showing increased immunoexpression of $p16^{INK4a}$ in neoplastic cervical epithelial cells and a positive correlation with HR-HPV infection and degree of cervical neoplasia. The objective of this prospective study is to evaluate $p16^{ink4}$ immunohistochemistry (IHC) in benign cervical biopsies and to correlate with HPV status.

Design: 125 archival (2005-2006) H&E stained cervical biopsies with benign diagnoses were reviewed. $p16^{INK4}$ IHC was performed on each case. Distribution of expression was interpreted as negative, focal/sporadic, diffuse lower half of epithelium, or diffuse staining of upper half to full thickness of epithelium. PCR for HPV genotype was performed for each case. HPV subtypes were interpreted as negative, low-risk (LR-HPV), or high-risk (HR-HPV).

Results:

Table 1. p16INK4 expression and HPV genotype of cervical biopsies with benign diagnoses.

	p16INK4 expression			
	Negative (n=78; 62%)	Focal (n=35;2 8%)	Lower Half of Epithelium (n=11; 9%)	Upper Half of Epithelium (n=1; 2%)
HPV Negative	48%	51%	18%	100%
LR-HPV	1%	9%	-	-
HR-HPV	52%	40%	82%	

Of 125 benign cervical biopsies, 62% were negative for $p16^{INK4}$ expression, 8% exhibited focal/sporadic positivity, 9% were diffusely positive in the lower half of the squamous epithelium and 2% showed diffuse positivity extending into the upper half of the epithelium. The one case with $p16^{INK4}$ positivity in the upper half of the epithelium was negative by PCR for HPV. Of the 78 cervical biopsies that were negative for $p16^{INK4}$ expression, 52% were positive for HR-HPV by PCR.

Conclusions: As expected, many of the benign cervical biopsies in this study were negative for $p16^{INK4}$ immunoexpression and HR-HPV by PCR. However, approximately half of the $p16^{INK4}$ negative biopsies were positive for HR-HPV by PCR indicating that many patients harbor HR-HPV infections in the absence of cervical lesions. While the majority of the benign cervical biopsies were negative for $p16^{INK4}$ immunoexpression, many showed positivity in the lower half of the epithelium. The only cervical biopsy that showed full-thickness expression of $p16^{INK4}$ was negative for HPV by PCR, and likely represents false positive staining.

1261 HPV Genotyping in Vulvar Squamous Cell Carcinoma: Comparison of a Luminex-Based Assay vs Linear Array

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Background: High risk human papillomavirus (hr-HPV) causes approximately 50% of invasive squamous cell carcinomas of the vulva (SCCV). High-grade vulvar intraepithelial neoplasia of usual type (uVIN) is a precursor lesion. While many HPV detection and genotyping methods are available, most have been primarily used on cytology specimens. Archived formalin-fixed paraffin embedded tissue (FFPE) from vulvar specimens is available for PCR testing, but can be limited by degradation and cross-linking of DNA and proteins during fixation. Linear Array (LA) (Roche) is a commercially-available genotyping kit. An in-house HPVgenotyping assay, based on the Luminex x-MAP® platform, has been used successfully to genotype HPV in cytology samples. Our objective was to compare LA versus Luminex in FFPE specimens extracted with either QIAamp (QIAGEN) or heat-based method.

Design: 103 FFPE SCCV from 1990-2012 were selected. Five 25-micron curls from representative sections were sent for heat and QIAamp extraction, respectively. All underwent LA and Luminex genotyping. In LA, 450bp region of L1 gene was amplified by PGM09/PGMY11 primer set and chromogen hybridized probes were detected visually. In Luminex, nested PCR with PGMY primers was followed by GP5+/GP6+; colored infrared hybridized probes were detected by automated fluorescence determination. H&E sections were cut before and after DNA testing to confirm that SCCV was present in the tested samples.

Results: 58/103 (56%) SCCV extracted with QIAamp were positive for hr-HPV by Luminex vs 40/103 (39%) by LA ($p=0.0004$). LA identified 1 case missed by Luminex. In heat extracted specimens, 44/103 (43%) Luminex and 2/53 (4%) LA samples were positive. Comparing the 2 highest yielding methods, QIAamp-Luminex detected 13.6% (95%CI 6.7-20.2%) more hr-HPV cases than the heat extraction-Luminex method ($p<0.001$). In QIAamp extracted cases, 53/60 hr-HPV cases had identifiable uVIN, and 10/43 hr-HPV negative cases contained uVIN. Multiple hr-HPV genotypes were detected in 6 cases by Luminex vs 4 by LA. Among blocks from cases with uVIN, hr-HPV was detected in 18/18 (100%), 15/17 (88%), 11/12 (92%), and 7/16 (44%) of blocks aged <5, 5 to <10, 10 to <15, and 15 years or more, respectively ($p<0.001$).

Conclusions: QIAamp is more successful than heat extraction. Luminex assay appears more sensitive than LA for the detection of hr-HPV in FFPE-SCCV. The detection rate with QIAamp-Luminex drops substantially only when blocks are over 15 years old.

1262 *MDM2* Amplification in Endometrial Stromal Tumors: Analysis of 43 Cases by Fluorescence *In Situ* Hybridization

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Background: *MDM2* amplification is known to occur in a variety of neoplasms and its detection by FISH is helpful in distinguishing well differentiated/dedifferentated liposarcoma (LPS) from lipoma. We recently encountered a mesenteric mass diagnosed as dedifferentiated liposarcoma, largely due to the neoplasm's myxoid morphology and MDM2 expression by immunohistochemistry (IHC), from a 46 year old woman with a history of uterine endometrial stromal sarcoma (ESS) with a *JAZF1* rearrangement. Further evaluation of the mesenteric mass also revealed a *JAZF1* rearrangement and a revised diagnosis of metastatic ESS with myxoid and pseudodecidual changes was rendered. Since *MDM2* amplification is not specific for the diagnosis of LPS, we investigated its occurrence in endometrial stromal tumors (EST) in order to appreciate its frequency and potential for misdiagnosis.

Design: Forty three cases of EST were identified in our institutional archive: 14 uterine ESS, 11 metastatic or recurrent uterine ESS, 8 undifferentiated endometrial sarcomas (UES), 5 endometrial stromal nodules and 4 ESS with *YHWAE* rearrangement. FISH was performed on formalin-fixed paraffin-embedded sections with a laboratory developed strategy for chromosome 12 utilizing a centromeric probe and a probe for *MDM2* at 12q13-15. Additionally, 40 of the 43 cases had previously undergone FISH analysis of *JAZF1*, *PHF1* and *YHWAE* using break apart probes. Performance and interpretation of each interphase FISH assay was done according to clinically validated protocols.

Results: Two cases (5%) were found to have *MDM2* amplification. Morphologically, 1 was ESS with fibrous change (*JAZF1* rearrangement) and 1 was an UES (polysomy/copy gain of intact *JAZF1*, *PHF1* and *YHWAE*). Both cases were metastatic lesions to the lung. Patient age was 48 and 73 years in the two cases with *MDM2* amplification. Each patient received adjuvant chemotherapy. At last follow up, both patients had died of disease (19 and 60 months). Interestingly, the proband case with MDM2 expression by IHC was negative for *HMGA2* amplification.

Conclusions: Our study is the first to demonstrate *MDM2* amplification in EST. Awareness of *MDM2* amplification in EST is critical; particularly in locations more common to liposarcoma, to avoid diagnostic errors. Our data also suggest that detection of *MDM2* amplification in EST may help identify potentially new therapeutic targets via the TP53-MDM2 pathway.

1263 SMARCB1-Deficient, Keratin-Positive Tumors of the Vulva in Adults: Epithelioid Sarcoma (ES) or Malignant Extrarenal Rhabdoid Tumor (MERT)? A Clinicopathologic, Immunohistochemical and Molecular Genetic Study of 5 Cases

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Background: ES and MERT share rhabdoid morphology, keratin expression and loss of SMARCB1 tumor suppressor gene product. It has been suggested by some that

many putative MERT may represent instead ES of proximal type (PT-ES). However, as compared to ES, MERT is less often keratin or CD34-positive, and shows *SMARCB1* mutations as compared to homozygous deletions in ES. We studied 5 vulvar tumors classified as ES or MERT for *SMARCB1* abnormalities with the goal of better understanding the proper classification of these lesions.

Design: Five cases previously coded as "ES" (2 PT-ES, 2 classical ES (CT-ES)) or "MERT" (1) involving the vulva were retrieved from our archives. Cases classified as ES showed pleomorphic epithelioid morphology and strong keratin and CD34 expression. The case classified as MERT showed small cell/rhabdoid morphology and weaker expression of keratin and CD34. Cases were tested for *SMARCB1* abnormalities by multiplex ligation-dependent probe amplification (MLPA). Tumors with heterozygous deletions were analyzed by Sanger DNA sequencing to identify *SMARCB1* coding region mutations.

Results: Follow up was available for 4 of 5 patients (range 4-40 months, mean 14). 3 patients were alive with disease and 1 was without evidence of disease at last follow up. Immunohistochemical findings are summarized in Table 1. MLPA and sequencing results are summarized in Table 2.

Table 1

Case	AE1AE3	34BE12	EMA	CD34	Desmin	ERG	SMARCB1
1	S,2+	W,1+	W,1+	S,4+	0	S,1+	Lost
2	S,4+	S,1+	S,1+	S,1+	0	S,1+	Lost
3	W,1+	0	W,1+	S,4+	0	S,1+	Lost
4	S,1+	S,1+	S,4+	S,2+	0	S,1+	Lost
5	W,1+	0	S,4+	S,3+	0	S,1+	Lost

S (strong), W (weak), 1+ (1-25%), 2+ (26-50%), 3+ (51-75%), 4+ (>75%)

Table 2

Case	Original Diagnosis	SMARCB1 MLPA	SMARCB1 Sequencing
1	CT-ES	Heterozygous deletion	Normal (exons 1-7,9)
2	PT-ES	Homozygous deletion	NP
3	CT-ES	Homozygous exon 3-9 deletion	NP
4	PT-ES	Heterozygous deletion	Exon 5 mutation: c.528delC (p.His177Metfs*32)
5	MERT	Homozygous deletion	NP

NP(not performed)

Conclusions: The majority of adult SMARCB1-deficient tumors of the vulva appear to represent ES (including PT-ES), with *SMARCB1* deletions, but not mutations. *SMARCB1* mutations are, however, seen in a minority of cases, suggesting that true MERT may occur in this location. Patient age, tumor morphology and immunoprofile do not reliably discriminate ES from MERT. It remains to be established whether this distinction is of clinical significance.

1264 Problematic Uterine Smooth Muscle Tumors Removed by Myomectomy: A Clinicopathological Analysis of 26 Cases

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Background: Smooth muscle tumors (SMTs) represent the most common group of uterine mesenchymal neoplasms, and when symptomatic, often result in hysterectomy. In women who wish to preserve fertility, or who are not candidates for hysterectomy, myomectomy is an alternative and increasingly common method of treatment. Classification of most uterine SMTs is straightforward though a minority present a significant diagnostic dilemma. Surgical myomectomy, or recent hormone therapy, may aggravate these difficulties. The behavior of atypical leiomyomata treated by myomectomy has been investigated, but reports are few. Outcomes of other problematic uterine SMTs after myomectomy are also unclear.

Design: We searched our institutional archives for uterine SMTs removed via myomectomy between the years 1980 and 2013. We included cases diagnosed as atypical leiomyoma (AL; either of low risk of recurrence, symplastic, or of limited experience), smooth muscle tumor of uncertain malignant potential (STUMP), leiomyosarcoma (LMS), and uterine SMTs shared intra-departamentally as documented in the diagnostic report. All H&E-stained sections were re-examined. Relevant clinicopathological data were recorded. Special attention was given to necrosis which was assigned into 4 categories: none, ischemic/hyaline necrosis, coagulative tumor cell necrosis (CTCN), or uncertain.

Results: Our search yielded 1161 cases, 26 of which constituted our final study group with a mean follow-up of 150 weeks (range 4-642) and mean age of 37 years (range 24-62). There were 15 AL (58%), 8 STUMP (31%), 2 LMS (8%), and 1 apoplectic leiomyoma (~4%). Recent hormone therapy existed in 13 (50%). Cases of AL showed few mitoses/10hpf (mean 2, range 1-6), focal/multifocal severe atypia (13, 87%), but no CTCN, while LMS patients had numerous mitoses (mean 18), diffuse atypia (100%), and CTCN (100%). Uncertainty in type/extent of necrosis often resulted in a STUMP designation (5 patients, 63%), which may have been complicated by prior hormone use. Of the 26 cases, 1 patient died of disease (LMS), 1 is followed for atypical but likely benign liver lesions (AL), and 24 are alive with no evidence of progression.

Conclusions: Uncertainty in the type/extent of necrosis was over-represented in cases diagnosed as STUMP, and was likely complicated by recent hormone therapy rather than sampling. Nevertheless, existing classification schemes accurately predicted tumor behavior. Appreciation for changes associated with prior hormone therapy, particularly those related to necrosis, may be of help in challenging cases.

1265 Epithelioid Smooth Muscle Tumors of the Uterus: Can Histology Predict Behavior?

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Background: Uterine epithelioid smooth muscle tumors (ESMT) are rare. Retrospective studies demonstrate overlapping histologic features between benign and malignant ESMTs. We present the largest series of ESMTs to date.

Design: A database search (1990-2013) yielded 35 ESMT and available H&E slides. The following parameters were recorded: patient (pt) age, tumor (tu) stage, tu size, % epithelioid component (EC), nuclear grade (1-3), mitoses/10 high power fields (MI), coagulative tu cell necrosis (CTCN), tu/myometrial interface, lymphovascular invasion (LVSI), % clear-cell component, hyalinization and follow-up (f/u).

Results: Pts' ages ranged from 27 to 88 yrs (median 44) with 3.5 to 171 months f/u (median 64) for 28 patients. Six had a diagnosis of epithelioid leiomyoma (eLM), 13 of ESMTs of uncertain malignant potential (eSTUMP), and 16 of epithelioid leiomyosarcoma (eLMS). Tu stage was known for 34 pts: I, 25; II, 0; III, 5; IV, 4. Tu size ranged from 1.3-21 cm (median 8 cm) with a median EC of 90%. Thirteen (37%) ESMTs had grade 3 nuclear atypia. Median MI for eLMS was 12/10hpf. 10 tu (29%) had CTCN and 9 (26%) had an infiltrative tu borders/myometrial interface. LVSI was present in 7 (20%) ESMTs. 20 (57%) ESMTs had hyalinization which was prominent in 6 tu. 19 (54%) ESMTs had clear cells (<10% to 75% of the tu). Of the eLMS patients: 7 died of disease (DOD); 8 were alive, disease status unknown (ADU); 3 were alive with disease (AWD), 10 had no evidence of disease (NED). No eLM or eSTUMP had recurrence or DOD. Pathologic features of ESMT by outcome are presented in table 1 and by diagnosis in table 2.

Features of ESMT by Outcome

	Tu Size (median, cm)	% EC (median)	Grade 3 Nuclear Atypia	CTCN	MI (median)	Infiltrative Border	LVSI
NED (n=10)	6.7	90	3 (30%)	3 (30%)	2	4 (40%)	1 (10%)
ADU (n=8)	8.2	70	3 (37.5%)	3 (37.5%)	3.5	1 (12.5%)	2 (25%)
AWD (n=3)	11	90	2 (67%)	1 (33%)	12	1 (33%)	2 (67%)
DOD (n=7)	9.5	80	5 (71%)	3 (43%)	28	3 (43%)	2 (29%)

Pathologic Features of ESMT by Diagnosis

	Tu Size (median, cm)	%EC (median)	Grade 3 nuclei	CTCN	MI	Infiltrative Border	LVSI
eLM (n=6)	6.5	95	0	0	<1	0	0
eSTUMP (n=13)	7.5	90	1	1	2	2	0
eLMS (n=16)	10.5	85	12	9	12	7	7

Conclusions: eLM and eSTUMP tend to have less prominent nuclear atypia and lower MI relative to their conventional counterparts. Unlike previously reported, eLMS in this series had more frequent nuclear atypia, CTCN and a higher MI (12/10hpf). A third of eLMS pts were NED and ~20% were AWD suggesting a more indolent course than conventional LMS.

1266 Diagnostic Accuracy of Endometrial Polyps in Pipelle Endometrial Samples: A Clinicopathologic Study of 195 Cases

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Background: Endometrial polyp is a common cause of abnormal uterine bleeding. Initial investigation in affected patients usually includes a pipelle endometrial (PE) sampling at an outpatient setting. When polyp is suspected histologically, subsequent confirmation would normally be done by diagnostic hysteroscopy (DH) and biopsy, or even operative hysteroscopy under general anaesthesia in some cases. Although the histological features of polyps are well-described, their presence is more variable in small and fragmented PE samples. Diagnostic accuracy in these samples has seldom been studied.

Design: 195 women who had undergone DH and/or polypectomy were identified in a University teaching hospital. All patients had a prior polyp diagnosis in the PE sample. The histology of these samples were compared and analyzed with subsequent DH findings and final hysteroscopic biopsies. Slides were reviewed by 2 gynecological pathologists.

Results: 162 were premenopausal (age 26-58, median, 46) and 33 were postmenopausal (age 44-85, median, 57). The commonest indications for a PE sampling were menorrhagia, metrorrhagia and postmenopausal bleeding. The median time intervals between initial PE sampling and DH for those with and without a final polyp diagnosis were 6 and 7.5 months, respectively. Presence of polyp was confirmed by DH in 56.3% (111/195) cases. Of these, 81.1% (90/111) were confirmed histologically. The commonest histologic feature in PE samples was thick-walled/ectatic vessels, found in 91.8% of cases (179/195). However, a polyp was only detected during the ensuring DH in 59.2% (n=106) of these 179 cases. By univariate analysis, the most reliable histologic features that can predict the presence of an underlying polyp were fibrous stroma (p= 0.01) and focal glandular clustering (p=0.03). The accuracy of detection of polyps in PE samples for premenopausal and postmenopausal women were 53.7% and 72.7%, respectively (p=0.05).

Conclusions: The positive predictive value of PE samples in detecting endometrial polyp was only 56.3%. Although the presence of thick-walled/ectatic vessels was the commonest histologic finding, the only reliable features in our series that can predict the presence of an underlying polyp were fibrous stroma and focal glandular clustering. Pathologists should be cautious in making a definitive diagnosis of polyp in pipelle endometrial samples as this may lead to unnecessary operative procedures.

1267 Lymphadenectomies for Endometrial Cancer: Which Has a Greater Impact on the Final Lymph Node Status, the Number of Lymph Nodes or the Number of Stations Sampled?

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Background: The increasing concern of how many lymph nodes (LNs) should be dissected to assure proper staging of patients with endometrial cancer (EC) has put a greater pressure on pathologists to identify even very small lymph nodes, sometimes submitting not only grossly identified lymph nodes but the entire surrounding fat. Some studies suggested that higher number of LNs is more likely to reveal positive LNs, while others have shown that it is the number of stations sampled rather than the number of LNs that impacts the outcome. We studied the impact of these two factors (the number of LNs and the number of LN stations) on hysterectomies performed for EC at our institution.

Design: The institutional pathology database was searched for hysterectomies with or without lymphadenectomies, performed between January 2003 and December 2006. Clinicopathological features (number of LNs per station and their status, positive (LN+) or negative (LN-) for maligancy, patient demographics, and primary tumor characteristics) were entered into a spreadsheet; the data were dichotomized into < 8 or≥ 8 for total LNs number, and into <4 or≥ 4 for number of stations sampled. Using the Fisher's Exact Test, results were further analyzed, comparing the positive LN rates.

Results: 194 patients with endometrial cancer were identified (age 25 to 91, mean 60 years). 118/194 patients had lymphadenectomy during surgery, 15 of them had positive lymph node status (12.7%). Total LNs number was in the range of 1 to 34 (mean 6.9, median 5, mode 2), while the number of stations dissected ranged from 1 to 5 (mean=median=mode=2).

	Number LN < 8	Number LN ≥ 8	LN stations < 4	LN stations ≥ 4
LN+	10 (12.5%)	5 (12.8%)	12 (10.4%)	2 (66.7%)
LN-	70 (87.5%)	34 (88.2%)	103 (89.6%)	1 (33.3%)
Total	80 (100%)	39 (100%)	115 (100%)	3 (100%)

The differences between total numbers of LNs did not show any significance in predicting positive LNs. However, higher rate of positivity was seen when 4 or more LN stations were sampled, compared to sampling <4 stations (P=0.036).

Conclusions: Our results question the extensive sampling of lymph nodes in hysterectomies for endometrial cancers. A proposal to increase the number of stations sampled rather than extensive lymphadenectomy from limited stations may be suggested as practice for endometrial cancer hysterectomies when staging is required.

1268 Test Characteristics of Specific p16 Clones in the Detection of High-Grade Squamous Intraepithelial Lesions (HSIL)

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Background: p16 has been recommended as an adjunct to morphologic assessment by the CAP-ASCCP Lower Anogenital Squamous Terminology (LAST) Standardization Project for HPV-Associated Lesions. This study evaluates the performance of different p16 clones as compared with CINtec (E6H4) in detecting HSIL.

Design: Of the 53 high-quality articles identified by LAST Work Group 4 addressing the use of p16, 34 evaluated the performance of p16 for detecting HSIL and were included in this study. Sensitivity (SN), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) were calculated for each clone; two-proportion z-tests (pooled) were used to evaluate significance.

Results: Reported clones: E6H4 (17 studies, 3507 cases), K5334 (2, 317), G175-405 (3, 341), 16P04 (4, 715), JC8 (3, 288), 16P07 (2, 374), 7962 (1, 148), and unspecified (2, 219). Compared to E6H4, significantly higher SN, SP and PPV were seen with 16P04. JC8 also showed increased SP and PPV. K5334 showed significantly lower SP and PPV; G175-405, SN and PPV; 7962, SN.

Table 1. Performance characteristics of individual probes

	Negative (p16+/ total)	LSIL (p16+/ total)	HSIL (p16+/ total)	SN% (95%CI)	SP% (95%CI)	PPV% (95%CI)	NPV% (95%CI)
Overall	94/1888	564/1657	2009/2364	85(83-86)	81(80-83)	75(74-77)	89(88-90)
E6H4	83/1340	371/989	1022/1178	87(85-89)	81(79-82)	69(67-72)	92(91-93)
16P04	3/157	14/146	387/412	94(91-96)*	94(91-97)*	96(93-97)	92(88-95)
JC8	1/76	14/83	111/129	86(79-81)	91(85-94)*	88(81-93)	89(83-93)
16P07	0/134	36/115	109/125	87(80-92)	86(80-90)	75(67-82)	93(89-96)
K5334	7/78	77/110	119/129	92(86-89)	55(48-63)*	59(51-65)*	91(84-95)
G175-405	0/94	32/71	122/176	69(62-80)*	81(74-86)	79(72-85)*	71(64-77)*
7962	0/9	2/22	57/117	49(39-58)*	94(77-99)#	97(87-99)#	33(23-43)#
Unspecified	-	18/121	82/98	84(75-91)	85(77-91)	82(73-89)*	87(79-92)*

* p<0.05 for probe vs E6H4. #Sample too small to calculate significance

Conclusions: LAST recommends p16 to improve the detection of HSIL and thus high SN and PPV are required. 16P04 and JC8 clones perform as well or better than E6H4. However, the prevalence of HSIL was lower in the CINtec group (34%) than the 16P04 (58%) and JC8 (45%) groups (p<0.0002) which could affect PPV and NPV. Limitations of this analysis include variation in sampling, processing, definition of p16+ and threshold for HSIL diagnosis. Further studies should be undertaken to directly compare the widely used E6H4 clone with 16P04 and JC8.

1269 Stroma-Associated Fibroblast Activated Protein (FAP) Overexpression Is Independent Predictive Biomarker for Cisplatium Resistance and Shorter 3 Years Overall Survival in Patients with Epithelial Ovarian Cancer

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Background: Fibroblastic activated protein (FAP) is selectively expressed in the stroma surrounding most epithelial cancers with minimal expression in malignant epithelial cells. Tumor-associated stromal AFP overexpression has been associated with poor prognosis in several malignancies. The aims of this study are to evaluate the expression and the predictive value of FAP expression in tumor samples of patients with epithelial ovarian cancer (EOC).

Design: A retrospective study was conducted and 338 EOC patients with adequate follow-up (FU) was retrieved form the archives. Immunohistochemistry (IHC) with FAP monoclonal antibody was one on the paraffin-embedded tissues. The FAP IHC intensity was assed as (-), weak (w), moderate (m) and strong (s). For analysis, (-) and (w) were considered as negative and (m) and (s) as positive staining. Fisher's exact test was performed to test the association between FAP and disease outcomes. Kaplan-Meier method with log-rank test was used to calculate the cumulative survival difference between patients with FAP expression. All reported p values are two sided.

Results: The age of patients ranged from 24-89 years (median 61). 59 were early stages and 277 were late stage disease. 243 were serous subtypes. At last follow-up 135 were alive and 201 were dead. 207 were tumor-associated stroma $(ST)^+$ and 111 were ST^-. Follow-up ranged from 0.36- 175 months, (median 30). FAP-ST^+ was independent prognostic factor to predict cisplatinum resistance; cases with FAP-ST^+ were 1.936 times more likely to be resistant than FAP-ST^- [OR = 1.936; 95% CI (1.115- 3.362) p 0.019]. Patients with FAP-ST^- had a higher OS rate after 3 years FU than those with FAP-ST^+ (p=0.027). High tumor grade was significant independent predictive factor for disease-related death (p 0.02071). As expected, tumor stage and optimal tumor debulking were strongly associated with recurrence, resistance, and death.

Conclusions: 1-Positive expression FAP in the ST of patients with EOC may serve as a negative prognostic factor for clinical outcome and could be a potential biomarker. 2- Due to the frequent presence of FAP in ST, we believe that FAP inhibitors should be further investigated, especially in light of the limited therapeutic options available in the treatment of EOC.

1270 Differentiated VIN (dVIN) Is Under-Recognized on Routine Histopathological Assessment, Resulting in Failure to Correctly Diagnose Positive Surgical Resection Margins

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Background: The precursor lesion of HPV-negative vulvar squamous cell carcinoma, differentiated VIN (dVIN), frequently shows aberrant p53 immunostaining. In □30% of cases it manifests as a complete loss or p53-null pattern. This allows clear demarcation between neoplastic p53-null and non-neoplastic squamous epithelium, with the latter exhibiting wild type (patchy basal) p53 expression.

Design: 14 cases, from 10 patients, were identified from pathology archives of two teaching hospitals based on: 1. presence of dVIN, with or without invasive carcinoma, and 2. p53-null immunostaining pattern. 10 cases had associated invasive carcinoma. All sections from each case were stained for p53 and reviewed together with all H&E sections. The status of the resection margins based on the original pathological assessment was compared to that assessed with p53 IHC.

Results: One case showed p53 loss in the invasive carcinoma but patchy basal positivity in the region diagnosed as dVIN, supporting interpretation as a benign hyperplastic focus, rather than dVIN. In the remaining 13 cases, the areas originally diagnosed as dVIN, as well as associated invasive carcinoma (if present) were p53-null. In 7 of these cases, dVIN was more extensive than originally recognized, based on p53-null immunostaining pattern and subtle morphological abnormalities. In the remaining 6 cases, the regions diagnosed as dVIN correlated well with the extent of p53 loss. The spectrum of morphological changes in p53-null regions that were in continuity with areas originally recognized as dVIN were subtle and typically consisted of an abrupt change in maturation of the squamous epithelium (loss of keratohyaline granules and parakeratosis), tinctorial alterations in the keratinocytes, with cells containing more abundant eosinophilic cytoplasm, and minimal basal nuclear atypia. Margin status changed from negative to positive in 4 of 13 cases, and from focally to more extensively positive in an additional 3 cases.

Conclusions: The clonal in situ component of non-HPV vulvar squamous cell carcinoma can show very subtle morphological abnormalities that are under-recognized based on current diagnostic criteria. As a result, resection margin involvement by dVIN is significantly underestimated. Better tools for diagnosis of dVIN are needed; until such tools are developed the limitations in the current diagnosis of dVIN should be recognized.

1271 Diagnosis of Ovarian Carcinoma Histotype Based on Limited Sampling: A Prospective Study Comparing Cytology, Frozen Section and Core Biopsies to Full Pathological Examination

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Background: There is increasing evidence that different ovarian carcinoma histotypes show different responses to treatment, and this has lead to histotype-specific treatment algorithms. As a result, there is a premium on accurate ovarian carcinoma histotype

diagnosis. Although the gold-standard for ovarian carcinoma diagnosis is examination of the resection specimen, in some clinical settings patients may start on treatment or be enrolled in a clinical trial based on a core biopsy or cytology specimen, however the accuracy of diagnosis based on these more limited samplings is not known.
Design: We prospectively collected 30 cases of adnexal mass identified by the surgeon as highly being suspicious for carcinoma. We performed aspiration and prepared Thinprep smears, took core biopsies, and prepared frozen sections. The 3 study pathologists were asked to place each ovarian tumor into one of six categories (high-grade serous carcinoma, low-grade serous carcinoma, endometrioid carcinoma, mucinous carcinoma, clear cell carcinoma, or other) based on each of these samples. For the core biopsy specimens, the reviewing pathologists permited to order immunostains. These diagnoses were compared to the diagnosis made on the resection specimen.
Results: Diagnoses were most accurately made based on the core biopsy specimens, with the three pathologists making correct diagnoses in 25/29, 26/29 and 26/29 cases. Immunostains were requested in 7, 9 and 9 cases, respectively. Based on frozen sections, the pathologists were able to correctly classify 24/30, 25/30 and 25/30 cases. None of the study pathologists were able to consistently subclassify tumors based on the liquid-based cytology slides. Five cases accounted for a large majority of the diagnostic errors: 2 high-grade serous carcinomas, one with exclusively glandular architecture, and another with prominent fibromatous stromal reaction (misdiagnosed as carcinosarcoma), an endometrioid carcinoma with higher grade nuclear features, a metastasis from a primary endocervical adenocarcinoma, and a poorly differentiated adenocarcinoma lacking features of specific histotype differentiation.
Conclusions: Accurate diagnosis of ovarian carcinoma histotype is possible in most cases, based on either core biopsy or frozen section. There is potential for improved diagnostic accuracy based on awareness of problematic areas in differential diagnosis identified through this case series.

1272 Loss of ATRX and DAXX Expression Identifies Poor Prognosis for Uterine Smooth Muscle Tumors

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Background: Uterine smooth muscle tumors of uncertain malignant potential (STUMP) have variable pathological features and recurrence rates, which results in considerable treatment uncertainty. New prognostic markers are required. Alternative lengthening of telomeres (ALT) maintenance is associated with poor survival in some soft tissue leiomyosarcomas (LMS). ALT maintenance is related to mutations in ATRX (α-thalassemia/mental retardation syndrome X-linked) and DAXX (death-domain-associated protein) genes and their respective proteins are involved in remodelling of chromatin structure. ALT maintenance also produces extremely long protein structures ALT-associated PML bodies (APB).
Design: 40 LMS, 17 STUMPs, and 10 leiomyomas (LM) were studied and follow-up data obtained. They were screened for APBs by immunofluorescent study (anti-PML, H-238, Santa Cruz). Telomere DNA was detected using a Cy3-labelled PNA probe (Life Technologies, Carlsbad). APB positive occur when when both PML protein and telomeric DNA are seen in the nucleus in≥ 0·5% of cells. Immunohistochemistry was done with ATRX and DAXX antibodies (respectively HPA001906 & HPA008736 Sigma-Aldrich, St Louis). Staining in <10% of nuclei were considered loss of expression. p53 (DO-7, Cell Marque) was also done.
Results: Mean patient ages and tumor sizes for LMS, STUMP and LM were respectively 49.5, 44.1 and 50.4 years, and 10, 8.5, and 3.5 cm. Recurrence/death was seen in 35% (6/17) STUMPs and 60% (24/40) LMS. No LMs recurred. For STUMPs, 24% (4/17) were APB+ve and 30% (5/17) overexpressed p53. Loss of staining for ATRX and DAXX was seen in 30% (5/17) and 12% (2/17), respectively. For LMS, 40% (16/40) were APB+ve and 42% (17/40) overexpressed p53. Loss of staining for ATRX and DAXX was seen in 58% (23/40) and 15% (6/40), respectively. All 6 STUMPs and 92% (22/24) LMS followed by recurrence/death showed loss of ATRX or DAXX expression. 60% (17/28) of these cases with adverse outcome were also APB positive. Overexpression of p53 ($p=0.001$) and loss of ATRX and/or DAXX expression ($p<0.05$) were associated with a poor outcome.
Conclusions: Loss of ATRX or DAXX expression in uterine smooth muscle tumors identified a clinically aggressive molecular subtype.

1273 Dermatofibrosarcoma Protuberans of the Vulva: Clinicopathologic and Molecular Study of 8 Cases

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Background: Vulvar dermatofibrosarcoma protuberans (DFSP) is a rare malignancy of fibroblastic lineage. Its occurrence in the vulva is poorly defined with fewer than 30 cases described in the literature. DFSP is known to undergo sarcomatous transformation and to harbor a characteristic COL1A1/PDGFB fusion. However, both events are limited to single case reports in vulvar tumors. Our study aimed to expand the clinical, morphologic and molecular genetic description of vulvar DFSP by compiling the largest series to date of genetically analyzed tumors.
Design: Eight cases were retrieved from the institutional archive. Each was reviewed for its clinical history and histologic features. All cases were immunohistochemically stained with antibodies to CD34, p53, desmin and SMA. FISH was performed on formalin-fixed paraffin-embedded sections utilizing a laboratory developed break apart probe for PDGFB (at 22q12.3-13.1). Performance and interpretation of interphase FISH was done according to a validated protocol.
Results: Patient age ranged 29-75 years (median 49). Six tumors showed classic DFSP, 1 tumor exhibited complete fibrosarcomatous transformation and 1 case comprised classic DFSP transitioning to undifferentiated pleomorphic sarcoma (UPS). All cases of classic DFSP exhibited diffuse positivity for CD34 and patchy, weak positivity for p53 and were entirely negative for desmin. SMA showed patchy positivity in 2 cases of classic DFSP with focal myoid differentiation. In contrast, 2 cases containing sarcomatous transformation showed weak or negative CD34 and strong p53. Six cases including the fibrosarcomatous case were positive for PDGFB rearrangement and two were negative. The case of classic DFSP transitioning to UPS contained additional intact PDGFB fusion signals, possibly indicating additional copies of chromosome 22 or duplication of the gene region. Follow up was available for 6 patients and ranged 1-108 months (median 20). All patients were free of disease at last follow up.
Conclusions: DFSP of the vulva is a rare sarcoma of intermediate malignancy similar to its cutaneous counterpart. Fibrosarcomatous transformation may occur in a subset of cases and demonstrates a distinct morphologic and immunohistochemical profile. COL1A1/PDGFB fusion is detectable in a majority of tumors including those with sarcomatous overgrowth. Copy number gain of PDGFB has been proposed as a marker of progression to fibrosarcomatous transformation in DFSP of other sites. To our knowledge, this is the first description of this finding in the vulva.

1274 Silencing HPV16 E6 Oncogene in a Cervical Cancer Cell Model

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Background: Human papillomavirus (HPV) infection is the major risk factor for development of cervical cancer. HPV16 and 18 are two leading high-risk types that account for about 70% of cervical cancers. Genomic integration with host is a characteristic event in high risk types as it defines the basis for cervical cancer pathogenesis via overexpression of oncogenic E6 and E7. High risk HPV E6 is transcribed as spliced polycistronic transcripts E6, E6*I, E6*II. E6 and E7 subvert normal cellular functions via interactions with tumour suppressor genes p53 and Rb respectively. In this work, short interfering RNAs (siRNAs) were utilized to ablate E6 activity in a HPV 16 infected cell line to acquire a better understanding of the role and mechanism of action of E6.
Design: SiHa cells containing 1-2 copies of HPV16 per cell was the in-vitro cervical cancer cell model system of choice. Five siRNAs targeting varying regions and isoforms of HPV16 E6 were utilized. Cells were forward transfected using Lipofectamine RNAiMax in 6 well plates for 72hours and knockdown levels were assessed.
Results: Over 70% reductions in E6 and E7mRNA levels were achieved in 4 out of the 5 siRNAs. A 2-10 fold increase in p21-downstream biomarker levels in all of the siRNAs compared to the controls. Knockdowns were validated at protein level by evaluating p21, p53 and pRb protein levels via western blot analysis. Other analyses used in examining the effect of E6 silencing in all knockdowns included senescence activation, morphology, apoptosis induction and cell cycle using flow cytometry.
Conclusions: This work illustrates the usefulness of siRNAs as molecular tools to silence effects of oncogenic HPVs in cervical cancer. This approach revealed that the targeting of varying regions in full length E6 and its isoforms yields different responses. The uncoupling of such responses will assist in elucidating the molecular mechanism involved in oncogenic subversion in cervical cancer and facilitate the development of novel therapies for cervical cancer.

1275 Evaluation of Putative Precursor Lesions-SCOUTs, p53 Signature and STIC in Patients with Non-Familial Pelvic Serous Carcinoma by Employing the SEE-FIM Protocol for Evaluation of Distal Fallopian Tubal Epithelium

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Background: The SEE-FIM protocol for detailed evaluation of the fallopian tubes is routinely employed in cases of risk-reduction salpingo-oophorectomy specimen for identification of precursor lesions of ovarian high grade serous carcinomas. The aim of this study was to evaluate this protocol in sporadic pelvic serous carcinomas in comparison to borderline and benign surface epithelial neoplasms and normal controls.
Design: The SEE-FIM protocol was employed prospectively to evaluate 37 cases of high grade pelvic serous carcinoma, seen between January 2012 to March 2013; 56.25% cases had received neoadjuvant chemotherapy. Other groups included normal age-matched controls (10), benign (10) and borderline (6) surface epithelial tumors. Putative precursor lesions including Secretory Cell OUT growths (SCOUTs), p53 signatures and Serous tubal Intraepithelial Carcinoma (STIC) were evaluated by immunohistochemistry for Bcl-2, p53 and Ki-67. The SCOUT index was derived from the SCOUT counts.
Results: A total of 668 sections from the proximal and distal fimbrial fallopian tubes were evaluated morphologically. ***Precursor lesions:*** The **SCOUT count and index** was higher in serous carcinomas as compared to the other groups. It was higher in the fimbrial end as compared to the proximal tube irrespective of the group. In serous carcinomas, SCOUT index in proximal tube was 0.8 and ratio of SCOUTs in the fimbrial/ cross section was 2.12:1. **p53 signatures** were always seen in the tubal fimbrial end. p53 signatures were observed in 21/32 (65.6%) cases of serous carcinoma as compared to 3/10 (30%) controls; it was not observed in benign and borderline surface epithelial tumors. **STIC**s were seen only in the fimbrial end of serous carcinomas and not in any other groups and were more common in the ipsilateral tubes of the serous carcinomas with ovarian origin as compared to tumors with peritoneal origin. STIC lesion was present with an invasive carcinoma component in 25% cases in the fimbrial end and without an invasive component in 6.25% cases of pelvic serous carcinomas.
Conclusions: The distal fallopian tubal fimbrial epithelium shows invasive serous carcinoma as well as its precursor lesions highlighting the importance of its detailed evaluation by the SEE-FIM protocol in cases of non-familial pelvic serous carcinoma.

1276 Villoglandular Endometrioid Carcinoma with Squamous and Mucinous Differentiation and MELF-Type Invasion: Association with Lymph Node Metastasis, and with Microsatellite Instability

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Background: Low-grade, stage IA endometrioid carcinoma is considered to have a low risk of lymph node metastasis. In our institution, we noted an association between a peculiar morphologic type of low-grade endometrioid carcinoma (with villoglandular architecture, focal mucinous and squamous differentiation, intra-tumoral inflammation and microcystic elongated fragmented (MELF) type invasion) and lymph node metastases, sometimes with isolated tumour cells. We hypothesized that the variegated features of these tumors may be related to genetic instability due to microsatellite instability (MSI).

Design: Cases of low-grade endometrioid carcinoma with the features described above were identified. Clinical information was obtained from medical records, and pathologic data were obtained from the gross descriptions and review of HPS-stained slides. Immunohistochemical staining was performed for MLH1, PMS2, MSH2 and MSH6 expression; if expression of any of these was lost, molecular analysis was performed to confirm microsatellite instability. In cases with loss of MLH1 and PMS2 staining, molecular testing for MLH1 promoter hypermethylation was performed.

Results: Thirteen cases were identified in one year. In all but one case with lymph node sampling (11/12), lymph node metastases were observed, in one of two patterns: isolated histiocytoid tumor cells; or scattered metastases, with innumerable isolated tumor cells, as well as discrete glands. In over of half of the cases (7/13), loss of staining for one or more mismatch repair proteins was found, and microsatellite instability confirmed. Six were due to MLH1 promoter hypermethylation, and one was due to inactivating mutation of MSH6.

Patient age and pathologic characteristics

Age	Myometrial invasion (%)	Isthmic involvement	Microsatellite instability
56	45	-	-
62	95	+	-
58	80	-	-
54	60	-	+ (MLH1, PMS2)
61	70	+	-
68	60	-	+ (MLH1, PMS2)
50	60	+	+ (MLH1, PMS2)
66	60	+	-
47	10	+	+ (MLH1, PMS2)
52	40	-	+ (MLH1, PMS2)
52	30	-	+ (MSH6)
60	60	-	-
54	40	Unknown	+ (MLH1, PMS2)

Conclusions: Cases of endometrioid carcinoma with villoglandular architecture, focal mucinous and squamous differentiation, intra-tumoral inflammation and MELF-type invasion were associated with lymph node metastases, even when only superficially infiltrative. Moreover, these cases show a high proportion of MSI.

1277 Pathological Phenotyping of Uterine Leiomyomas from Patients with Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) Syndrome

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Background: HLRCC syndrome is an autosomal dominant disorder resulting from mutations in the fumarate hydratase gene which predisposes patients to cutaneous and uterine smooth muscle tumors as well as renal cell carcinoma. The latter is often aggressive and can present in young patients. Uterine leiomyomas (ULs) are more common, and their recognition may offer an opportunity to identify patients with HLRCC. Recently, the histological features of HLRCC ULs have been described, however, the specificity and sensitivity of these features is not known. The aim of this project was to compare the frequency of previously described HLRCC features in ULs of patients with known HLRCC syndrome to those in patients with sporadic ULs.

Design: 4 patients with known HLRCC and ULs, and 100 patients under the age of 41 with no personal or family history of HLRCC syndrome and ULs (multiple with at least one≥ 3 cm or solitary≥10 cm) were identified. The following tumor pathological features were assessed: tumor number and size, cellularity, mitotic activity, nuclear enlargement, and presence of prominent nucleoli and perinucleolar clearing.

Results: The clinical and pathological features of 4 HLRCC ULs and 100 presumed sporadic ULs are summarized in Table 1. Increased cellularity (8%) and focal cells with prominent nucleoli and perinucleolar clearing (67%) were commonly found in sporadic ULs The most distinguishing characteristic of HRLCC ULs compared to sporadic ULs was the presence of diffusely distributed cells (5-10%) with a *combination of both* enlarged (3x) nuclei with irregular contours *and* eosinophilic nucleoli with perinucleolar clearing. These features were identified in 100% of HRLCC and 3% of presumed sporadic ULs.

Conclusions: Recognition of specific morphologic features in ULs may identify patients who can benefit from genetic testing for HLRCC syndrome. If subsequently diagnosed, these patients and their family members can be followed by renal cancer surveillance programs.

Table 1

Clinicopathologic Feature	Known HLRCC Patients (n=4)	Presumed Sporadic Patients (n=100)
Patient age (years)	Mean 36.7 Range 29-41	Mean 36.4 Range 24-41
Surgical Procedure:		
Hystrectomy	2	2
Myomectomy	50	50
Number of ULs:		
Single	0	17
Multiple	4	83
Size (cm)	Mean 11.0 Range 6.3-16.7	Mean 8.7 Range 3.0-19.2
Cellularity:		
Normal	2	92
Mixed	1	3
Increased	1	5
Mitoses:		
≤ 5 /10 HPF	4	100
< 5/10 HPF	0	0
Nuclear enlargement and irregular contour:		
Present	4	16
Absent	0	84
Prominent nucleoli and perinucleolar clearing:		
None	0	28
Focal	2	67
Diffuse	2	5

1278 MET Protein Overexpression and *MET* Gene Amplification Occur Frequently in Uterine Carcinosarcomas but Are Not Concordant between Their Carcinomatous and Sarcomatous Components

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Background: The MET receptor is a specific tyrosine kinase receptor for hepatocyte growth factor ligand and has been identified as a target for molecular therapy. However, there are only a few recent papers that report MET expression in endometrial carcinomas, and it has not yet been well examined in uterine carcinosarcoma(CS). The aim of this study was to investigate MET protein overexpression and alterations in its gene copy number in the carcinomatous component(CAC) and sarcomatous component(SAC) of CS, acquire insights on the genesis and clonal divergence of both these components and contribute information that could lead to potential molecular therapies for treating CS.

Design: Representative paraffin-embedded tissue blocks were collected from 52 surgically -resected consecutive cases of uterine CS, (30 homologous and 22 heterologous). Tissue microarrays including CAC and SAC and adjacent endometrium were constructed for each case. MET protein overexpression was examined using immunohistochemistry and defined as moderate to strong circumferential membrane staining in≥ 10% of the constituent cells. *MET* gene copy number alterations were examined using brightfield double *in situ* hybridization (DISH) and classified into high-level polysomy and *MET* gene amplification.

Results: MET protein overexpression was detected in 34 cases (65%): CAC only in 25, SAC only in 1, both in 8. High-level *MET* polysomy was present in 11 cases (21%): CAC only in 3, SAC only in 5, both in 1. True *MET* gene amplification was detected in 5 (9.6%) and was always detected in SAC in heterologous type. MET protein overexpression and *MET* gene amplification were double positive in 6 (12%) and 3 (5.8%) of CAC and SAC, respectively. In the adjacent endometrial tissue, *MET* gene alterations were not detected.

Conclusions: MET protein overexpression and *MET* gene amplification were frequently observed in uterine CSs, but their status was frequently discordant between the CAC and SAC. A large part of MET protein overexpression appeared to occur through a mechanism other than *MET* gene copy alteration. In heterologous CS, it was speculated that the SAC frequently occur through clonal divergence in accompanied with *MET* gene amplification. Molecular target therapy for the MET receptor may become a viable treatment option for patients with CS with MET protein overexpression and/or *MET* gene amplification.

1279 Cell-Signaling Pathways in Uterine Sarcomas: Association with High Grade Tumors and Survival

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Background: Uterine sarcomas are aggressive neoplasms classified in three groups according to their origin: endometrial stromal tumors, smooth muscle tumors and miscellaneous mesenchymal tumors. The only recognized prognostic factor so far is the pathological staging. The purpose of this study is to determine whether there are any alteration of the cell signaling pathways and some of the factors associated with invasion such as YB that may be related with outcome.

Design: 75 cases between 1998 and 2013 were selected from our archive: 11 smooth muscle tumors of uncertain malignant potential (STUMPS), 22 endometrial stromal sarcomas (ESS), 15 undifferentiated sarcomas (UES) and 17 leiomyosarcomas (LMS). 10 leiomyomas (LS) were used as control. Clinical follow-up data was obtained by review of hospital records, obtaining a mean follow-up of 152.47 months. Immunohistochemical stains were performed using tissue microarrays. Biomarkers studied comprise: eIF4E, peIF4E, 4E-BP, p4E-BP, 4E-T, pMapK, YB1, pYB1, HER3, mTOR, pS6, GLUT1. In addition, Ki67, CD10, p16 and hormonal receptors immunostains were also performed as routine.

Results: High grade uterine sarcomas overexpress eIF4E, peIF4E, 4E-BP, p4E-BP, 4E-T, pMapK, YB1, pYB1, pS6 and GLUT1 when compared to low grade neoplasms ($p<0.05$). p4EBP expression was the only marker studied which differed amongst LMS

and UES ($p<0.05$). Smooth muscle tumors (LS, STUMPS, LMS) expression of cell signaling markers is different between groups ($p<0.05$), with GLUT1, peIF4E, 4E-BP and YB1 being overexpressed comparing LS to STUMPS ($p<0.05$), whereas eIF4E, pMapK, pYB1 and pS6 are also overexpressed comparing LS to LMS. UES had a higher expression of eIF4E, 4E-T, 4E-BP, p4E-BP, YB1, pYB1, GLUT1 and pS6 compared to ESS ($p<0.05$). Among all cell signaling markers, YB1 overexpression in high grade sarcomas is related to lower survival times (Log rank $p<0.05$).

Conclusions: High grade uterine sarcomas overexpress most signaling markers, regardless of their nature. Interestingly, among them YB1 overexpression has been associated with lower survival times. In the smooth muscle uterine tumors we have observed a gradual increasing expression of those markers along with malignant potential. Moreover, STUMPS are closer to LMS according to their expression. Given the aggressive nature of these tumors, and the overexpression found in those cell signaling pathways, which can be druggable, further studies are required to assess its possible therapeutic value.

1280 127 Uterine Smooth Muscle Tumors: Correlation of Histology and Clinical Outcomes

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Background: Based on the current WHO classification and Stanford scheme, uterine smooth muscle tumors (USMT) can be classified as usual leiomyoma (ULM), mitotically-active leiomyoma (MALM), cellular leiomyoma (CLM), atypical leiomyoma (ALM), uncertain malignant potential (STUMP) and leiomyosarcoma (LMS). Aside from ULM and LMS, the nature of the other variants is largely unknown and remains a challenge for diagnosis and management. In this study, we evaluated the histologic correlation and clinical follow-up in those problematic USMT.

Design: We reviewed the pathology database over the last 20 years at two institutions to retrieve 127 patients with a diagnosis of LMS or one of the USMT variants. Each case was reviewed by two pathologists to confirm the diagnosis based on Stanford scheme and histologic criteria were documented. Each patient's chart was also reviewed up to June 2013 to document the original treatment, additional follow-up, current disease status and survival. The histologic and clinical outcomes were then compared.

Results: Among the original diagnoses of 127 problematic USMT, 18% (23/127) of cases were reclassified, indicating a common problem in rendering a definite diagnosis. Five ALM and 7 STUMP were misclassified due to subjective mitotic counts. Eight CLM were initially diagnosed as ALM due to mild cytologic atypia and 6 MALM were called ALM due to frequent mitoses. (Table 1) Among the 127 cases, 84 cases were reviewed clinically via chart review and included 38 LMS, 30 ALM, 12 CLM, 1 MALM, and 3 STUMP. Clinical data for the remaining cases from the other institute is still under review. Patients' follow-up ranged from 12 months to 230 months. Of 38 LMS, 18 died with metastatic disease, 1 died without LMS, 4 were alive with LMS, and 13 were alive without LMS. Two patients were lost to follow-up. Of the other USMT variants, no deaths were attributed to USMT, but 1 STUMP and 2 ALM had recurrence. (Table 1)

Diagnostic Review of Problematic USMT

	# Cases	Mean age	# Tumor slides reviewed per case (mean)	Reclassified cases	Death with disease	Alive with disease
LMS	38	56	2-39(10)	2/38	18/38	13/38
STUMP	18	42	2-24(12)	7/18	0/3	1/3
ALM	42	44	3-19(8)	6/42	0/30	2/30
CLM	22	46	1-38(12)	8/22	0/12	0/12
MALM	7	46	4-12(14)	6/7	0/1	0/1

Conclusions: Overall, 5% (2/38) of LMS were under diagnosed and 21% (21/89) of other USMT variants were reclassified based on the Stanford scheme. Although there were 9% recurrence of STUMP and ALM, the only deaths attributed to USMT were in patients with LMS.

1281 The Value of Selected IHC Markers in the Diagnosis of Problematic Uterine Smooth Muscle Tumors

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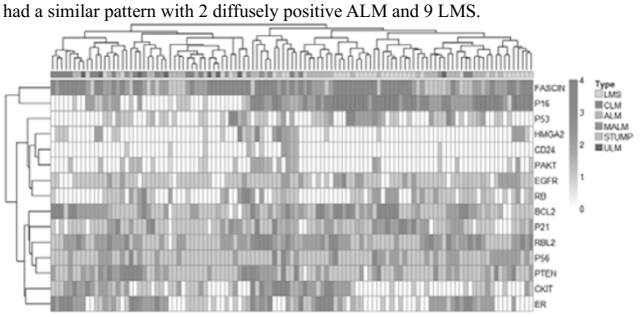
Background: Uterine smooth muscle tumors (USMT) consist of a group of histologically heterogeneous tumors. While benign leiomyoma (ULM) and malignant leiomyosarcoma (LMS) are well-defined, the relationship to other histologic USMT variants, including mitotically-active leiomyoma (MALM), cellular leiomyoma (CLM), atypical leiomyoma (ALM), and borderline tumors (STUMP), is largely unknown. Thus diagnosis often remains a challenge. In this study, we selected relevant IHC markers and investigated their value in the diagnosis of USMT variants.

Design: We collected 111 cases with a diagnosis of either LMS or another USMT variant. The cases included 38 LMS, 13 STUMP, 27 ALM, 18 CLM, 7 MALM, and 8 ULM. Tissue microarrays with 2 mm cores were prepared and immunohistochemical (IHC) stains for 17 markers were performed, including Bcl-2, CD24, C-Kit, EGFR, ER, Fascin, HMGA2, Ki-67, PR, p16, p21, p53, pAKT, PTEN Rb, PS6, and RBL2. The IHC stains were analyzed blindly using a two-tiered scoring system and the results for each IHC and USMT subtype were statistically analyzed.

Results: The immunoprofile of all 17 markers revealed that LMS clustered together. The markers that were significantly different between LMS and other variants included ER, PR, Bcl-2, p16, p21, p53, and Ki-67. When we removed PR, 70% of ALM aggregated with LMS. Interestingly, ALM shared several oncogenic markers with LMS, including p16, p53, Bcl-2, and Fascin (Figure 1). However, ER and PR clearly separated LMS from ALM. In contrast, STUMP had wide range of immunoreactivity for these markers. PR had 0% staining for 1/27 ALM and 32/38 LMS. ER had a similar pattern to PR, but not as dramatic. P16 had a high percent and intensity staining for 15/27 ALM and 30/38 LMS. The other USMT subtypes were mostly negative, including STUMP. P53 had a similar pattern with 2 diffusely positive ALM and 9 LMS.



Conclusions: LMS showed a characteristic immunoprofile different than other USMT variants. ALM shared several oncogenic IHC markers with LMS, including Bcl-2, Fascin, p16 and p53. However, LMS could be clearly separated from ALM by ER, PR and Ki-67. All other USMT variants could not be easily distinguished from each other using the selected IHC markers.

1282 Frozen Section Guidelines for the Evaluation of Mucinous Borderline Neoplasms Based on a Single Institution Study

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Background: Intraoperative evaluation of ovarian tumors is commonly used to guide surgeons to perform the appropriate procedure. A high negative predictive value is of particular interest to surgeons as false negatives can result in additional surgeries and delay in treatment. Ovarian mucinous tumors (MT), in particular mucinous borderline tumors (MBT), may present a challenge and potential for misdiagnosis at the time of frozen section (FS). The goal of our study is to identify guidelines in the appropriate management of MT at the time of FS.

Design: All data about the ovarian tumors submitted to our institution for FS evaluation between January 2009 to July 2013 are retrieved and reviewed. Of 394 cases submitted for FS, 73 were MTs. All information about FS and final pathology diagnoses in addition to tumor characteristics and the number of frozen sections were collected and correlated.

Results: Out of 73 cases, 48 were diagnosed as benign, 18 as borderline and 7 as primary malignant or metastatic at the time of FS evaluation. Of 48 cases interpreted as benign at FS, 9 were borderline and one was malignant. Of 18 cases diagnosed as borderline in FS, 5 showed invasion on additional sections and were signed out as malignant. All of ovarian primary or metastatic mucinous neoplasms were accurately diagnosed. Overall, the agreement between FS interpretation and final diagnosis was observed in 58 out of 73 cases (79%). Average sizes for the accurately diagnosed benign, borderline and malignant tumors were 12.0 cm, 19.0 cm, and 17.0 cm retrospectively. In the accurately diagnosed MBTs, 3 frozen sections were submitted for evaluation in 11 out of 13 cases (85%). Underdiagnosed MBTs (9 cases) were 24.0 cm in average size with complex multiloculations and solid components. In 7/9 (78%) cases, only 2 frozen sections were submitted for evaluation.

Conclusions: Multiple prior studies have shown that inaccurate FS diagnosis occurs more commonly with MT, specifically MBT. This discrepancy is likely due to the larger diameter and heterogeneity of these tumors. Moreover, multiple sections of the most complex appearing foci of tumor can possibility decrease the discrepancy. Also, consultation with a gyn pathologist at the time of frozen section is helpful. We suggest that 3 sections of the MTs that are 20 cm or larger should be examined at the time of FS. These sections should be taken from the solid and most complex foci of the tumor. Based on our findings, this approach can decrease the possibility of sampling errors and rates of false negative FS diagnosis.

1283 Absence or Presence of High Grade Squamous Intraepithelial Lesion in LEEP Specimen: A Clinicopathological Study of 697 Cases

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Background: High grade squamous intraepithelial lesion (HGSIL) on cervical cytology or biopsy samples usually results in a loop electrosurgical excision procedure (LEEP). Occasionally, LEEP specimens are negative for dysplasia or show low grade squamous intraepithelial lesion. Our goal is to compare 2-year follow-up results of patients with negative or positive LEEP and to determine which factors predict the likelihood of a negative LEEP.

Design: Total 697 LEEP specimens at our institution between 2009 and 2012 along with detailed patient demographics, high risk HPV status, prior cytologies and biopsies, colposcopic findings, and follow-up cytology results within two years were studied.

Results: HGSIL was identified in 525 specimens (75%, Positive LEEP) but not in the remaining 172 cases (25%, Negative LEEP). HRHPV for patients with negative LEEP was positive in 38%, equivocal in 6%, negative in 9%, and unknown in 47% of cases. For patients with positive LEEP, HRHPV was positive in 49%, equivocal in 3%, negative in 7% and unknown in 41% of cases. Prior biopsy and cytology results are shown in table 1. Colposcopic findings at the time of LEEP are shown in table 2.

Table 1. Prior Biopsy and Cytology Diagnoses Correlate with LEEP Findings

Prior Dx		LEEP Findings	
Biopsy	**Cytology**	**High Grade**	**Low Grade/Normal**
HG	HG	262 (82%)	56 (18%)
HG	LG or n/a	203 (76%)	65 (24%)
LG or n/a	HG	60 (54%)	51 (46%)

HG=high grade; LG=low grade; n/a=unknown

Table 2. Colposcopic Impression for Positive and Negative LEEPs

	Positive LEEP (n=410)		Negative LEEP (n=63)	
Colposcopic Impression	**n**	**%**	**n**	**%**
Inadequate	3	1%	3	5%
No lesion	9	2%	5	8%
CIN 1	24	6%	25	40%
CIN 1-2	25	6%	8	12%
CIN 2-3	349	85%	22	35%

Most of these patients were followed by cytology for two years. Among 154 patients with negative LEEP, 6 (4%) had recurrent HGSIL, 15 (10%) had ASCUS/LGSIL, and 133 (86%) had negative cytology. Among 420 patients with positive LEEP, 58 (14%) had recurrent HGSIL, 73 (17%) had ASCUS/LGSIL, and 289 (69%) had negative cytology.
Conclusions: Target HGSIL is present in 75% but absent in 25% of LEEP specimens. We have shown that negative LEEP is commonly associated with equivocal HRHPV, prior negative or low grade biopsy, and no lesion or LGSIL on colposcopic examination. Compared to patients with positive LEEP, negative LEEP patients do not show higher risk of persistence/recurrence (4% vs 14%). Therefore patients with positive or negative LEEP could be managed similarly and followed with regular cytology.

1284 The Significance of L1CAM (CD171) Expression, Epithelial-To-Mesenchymal Transition, and Stem Cell Phenotypes in Endometrial Clear Cell Carcinoma

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Background: The L1 cell adhesion molecule (L1CAM) is a 200-220kDa membrane glycoprotein of the immunoglobulin superfamily whose expression has been shown to be related to epithelial-mesenchymal transition (EMT). Recent studies have found that activation of EMT pathways eventuate in increased cellular plasticity, 'stem cellness' and tumorigenicity. The purpose of this study is to evaluate the role of L1CAM expression and related EMT and stem cell phenotypes in the pathogenesis of endometrial clear cell carcinoma (CCC).
Design: 54 CCC, 17 endometrial serous carcinoma (ESC), and 49 endometrioid carcinomas (EEC; 18 G1, 19 G2, 12 G3) in a TMA were immunohistochemically assessed for their expression of the EMT-related markers L1CAM, Snail, Slug, E-Cadherin, BMI1, as well as the putative cancer stem cell markers NANOG, NAC1 and Musashi. In addition to conventional microscopic assessment, objective staining scores were also assigned by an automated image capture system that incorporated staining intensity and extent on tumor cells.
Results: L1CAM expression was present in 81%, 69%, 58%, 26%, and 0% of CCC, ESC, G3 EEC, G2 EEC, and G1 EEC respectively. Analysis of the automatically-generated staining scores showed that statistically significant higher levels of L1CAM were present in CCC as compared with EEC of all grades, G3 EEC, and G1/G2 EEC ($p < 0.0001$ to 0.0226). No differences were found between CCC and ESC, ESC and G1/G2 EEC, and between ESC and G3 EEC (p 0.33-0.88). In all the high grade cancers (CCC, ESC, G3 EEC), there were significant positive correlations between L1CAM and at least 2 of the other EMT (BMI, Slug, Snail) markers. However, CCC, unlike G3 EEC and ESC, did not show any positive L1CAM correlations with stem cell (NANOG, NAC1, Musashi) markers. Additionally, in ESC and G3 EEC, there was an inverse correlation between with L1CAM and e-cadherin, consistent with activation of an EMT-like program. However in CCC, as well as in G1/G2 EEC, there was no significant correlation between the 2 markers. In univariate (but not multivariate) Cox regression analyses, increased L1CAM expression showed a near-significant association with reduced overall (p=0.0698) and relapse-free (p=0.0688) survival in CCC.
Conclusions: L1CAM expression patterns highlight a distinct novelty in the pathogenesis of CCC, whereby a "mesenchymal" phenotype is activated in a manner similar to the other high grade carcinomas (G3 EEC and ESC). However, unlike the latter tumors, and similar to G1 and 2 EEC, there is no concurrent downregulation of e-cadherin.

1285 The Repertoire of TP53 Mutations in Endometrioid Endometrial Cancer

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Background: Mutations in the tumor suppressor gene TP53 are highly recurrent in serous endometrial carcinomas (ECs) but can also be found in a subset of endometrioid ECs. We sought to determine the type and patterns of somatic TP53 mutations in endometrioid and serous ECs, as these might have pathogenetic and diagnostic relevance.
Design: The Cancer Genome Atlas (TCGA) performed an integrated genomic characterization of 232 ECs. These publicly available data were interrogated for mutations using the cBioPortal (http://www.cbioportal.org). Clinical information was obtained from the TCGA Data Portal (file datafile.S1.1.KeyClinicalData.xls; https://tcga-data.nci.nih.gov/docs/publications/ucec_2013/), with a particular focus on the four integrated genomic classes (POLE, MSI, Copy-number (CN)-low, CN-high).
Results: Of the 232 ECs subjected to an integrated genomic characterization by the TCGA, 15% (27/186) of endometrioid, 88% (37/42) of serous and 75% (3/4) of mixed cases harbored TP53 somatic mutations. TP53 mutant serous ECs were significantly less frequently diagnosed at FIGO stage I/II than TP53 mutant endometrioid ECs (41% vs. 74%, respectively; Fisher's exact test P=0.011). In endometrioid cancers, TP53 mutations were most frequently but not exclusively found in grade 3 lesions (i.e. 37% of grade 3, 11% of grade 2, and 3% of grade 1). No difference in the type of TP53 mutations (i.e. nonsense/frameshift vs missense) between endometrioid and serous ECs was found (P=0.769), but endometrioid cancers harbored significantly more frequently deleterious TP53 mutations targeting exon 6 (7/27, 25.9%) than serous ECs (2/37, 5.4%; P=0.029). All TP53 mutant serous ECs but only 56% of TP53 mutant endometrioid ECs were of CN-high (serous-like) genomic subtype (P<0.0001). The remaining TP53 mutant endometroid ECs were of POLE (ultramutated; 6/27, 22%), MSI (hypermutated; 5/27, 19%) or CN-low (endometrioid; 1/27, 4%) subtypes. CN-high TP53 mutant endometrioid ECs more frequently harbored PTEN mutations/homozygous deletions (40%) than TP53 mutant CN-high serous ECs (5%, P=0.005). Within the group of TP53 mutant endometrioid ECs, however, those of CN-high genomic subtype had a lower prevalence of PTEN (P=0.001) and ARID1A (P=0.028) mutations than those classified as of other genomic classes.
Conclusions: TP53 mutations can be found in a subset of endometrioid ECs, and are not restricted to grade 3 lesions or CN-high cancers. Mutations affecting TP53 exon 6 are more frequently found in endometroid than in serous ECs.

1286 Dysregulation of the Mammalian Target of Rapamycin (mTOR) Pathway in Endometrial Clear Cell Carcinoma

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Background: The mammalian target of rapamycin (mTOR) pathway plays an important role in tumor pathogenesis, and is a promising target for directed therapies. The mTOR pathway often begins through inactivation of the *PTEN* tumor suppressor gene and eventuates in increased protein translation through the phosphorylated S6 ribosomal protein (among others), and cell cycle progression through $p27^{kip1}$ (p27) depletion. This study evaluated dysregulation of the mTOR pathway, as inferred from their protein expression patterns, in endometrial clear cell carcinoma (CCC) relative to endometrial endometrioid carcinoma (EEC) and non-neoplastic endometrium (NNE).
Design: 100 endometrial carcinomas (51 clear cell, 49 endometrioid [18 grade 1, 19 grade 2, 12 grade 3], and 24 NNE (9 proliferative, 10 secretory and 5 atrophic) were evaluated by immunohistochemistry for expression of 4 proteins associated with the mTOR pathway (PTEN, Phospho-S6 ribosomal protein [PSRP], p27 and phosphorylated-mTOR [p-mTOR]). Results were scored on a 0-12+ scale for staining intensity and extent, which were then correlated with clinicopathologic parameters.
Results: Decreased p-mTOR (scores ≤4+) were more characteristic of CCC than other tissues, with 61% of CCCs showing decreased p-mTOR, as compared to 0% of EEC ($p<0.01$), and 0% of NNE ($p<0.01$). PTEN was depleted (scores ≤2+) in 33% of CCC, as compared with 61% of EEC (75% of grade 3 EEC [EEC3], $p<0.02$; 57% for EEC grades 1 and 2, $p<0.05$]) and 9% of NNE ($p<0.01$). 44% of CCC showed high (scores≥5+) p27 expression, as compared with 100% of EEC3 ($p<0.01$) and 14% of grdes 1 and 2 EEC ($p<0.01$). 73% of CCC showed high PSRP expression, as compared with 49% of grades 1 and 2 EEC ($p<0.03$), and 50% of EEC3 (p=0.2). PTEN, PSRP and p27 were not associated with patient outcomes. Low p-mTOR was associated with poor outcome in the CCC cohort (5-year disease-free survival 0%, $p<0.03$) on univariate, but not multivariate analysis.
Conclusions: This study provides new evidence of dysregulation of the mTOR pathway in CCC, and highlights the potential utility of mTOR inhibitors in treating this rare histotype. Both CCC and EEC showed evidence of pathway dysregulation, albeit at different levels. Grade 3 EEC had increased mTOR signally with high p-mTOR, low PSRP, high p27, and frequently depleted PTEN. CCC displayed less pathway activation, as evidenced by lower p-mTOR, high PSRP, low p27, and less frequent PTEN depletion. Understanding histotype-related differences in mTOR pathway dysregulation is critical in assessing variations in responsiveness to associated directed therapies.

1287 Proteomic Analysis of Clinicopathologic Factors in Endometrial Clear Cell Carcinoma

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Background: For more than a decade, proteomic techniques have been deployed in trying to understand various aspects of endometrial carcinoma, but very limited work has been done on clear cell carcinoma (CCC). Matrix-assisted laser desorption ionization imaging mass spectrometry (MALDI IMS) utilizes a matrix assisted laser desorption/ionization technique that allows the relative abundance and spatial distribution of proteins and peptides within a tissue sample to be visualised with an extremely high resolution, and to be defined with the molecular specificity of mass spectometry. The purpose of this study is to determine whether mass spectral profiles can be correlated with clinicopathologic features in endometrial clear cell carcinoma (CCC).
Design: MALDI IMS was performed on slides from tissue microarrays comprised of 47 CCC, with spectral information obtained from every 0.05 mm^2 area of tissue. We then assessed whether clinicopathologic variables were significantly correlated with any spectral peaks at the $p<0.05$ threshold for significance, using a regression analysis. The assessed variables included: patient age, FIGO stage, pT classification (AJCC), lymphovascular invasion (LVSI), mitotic index, tumor architectural pattern, the presence of isolated infiltrating tumor cells (in≥10% of tumor), tumor necrosis, and≥10% solid component, depth of myometrial invasion, and presence/absence of lymph node metastases (lymph node status).

Results: From a total of 730 normalized spectral peaks, 152 (20.8%), 92 (12.6%), 71 (9.7%), 66 (9%), 61 (8.3%), 54 (7.4%), 40 (5.5%), 35 (4.8%), 32 (4.4%), 16 (2.2%), 15 (2%) showed statistically significant associations with patient age,≥10% solid component, lymph node status, mitotic index, depth of myometrial invasion, infiltrating isolated tumor cells (in≥10% of tumor), architectural pattern, FIGO stage, necrosis, pT classification, and lymphovascular invasion, respectively.
Conclusions: Our findings indicate that there are spectra that can be associated with clinicopathologic parameters such as patient age and FIGO stage in CCC. This may indicate, for example, that there are significant proteomic differences between low stage and high stage disease in otherwise similar tumors. The analysis also identifies which parameters show the greatest phenotypic differences between its subgroups. Future investigations that utilize these approaches may help to define biomarkers that distinguish one disease state from another, identify the most important drivers of pathogenesis, and ultimately, predict tumor progression.

1288 Biomarker Resolution of Uterine Smooth Muscle Tumor Necrosis as "Benign" vs. "Malignant"

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Background: Uterine leiomyosarcomas are rare malignant tumors with a poor prognosis, while leiomyomas are common and generally exhibit no significant risk of malignancy. Diagnostic criteria for leiomyosarcoma include atypia, mitotic activity, and tumor cell necrosis. Unfortunately, interpretation of necrotic foci as tumor-cell type (leiomyosarcoma) vs. infarct-type (leiomyoma) can be difficult and poorly reproducible. Given the implications of a sarcoma diagnosis and the inherent subjectivity in the interpretation of necrotic foci, we investigated a set of biomarkers that may aid in characterizing the mechanism and vintage of necrosis.
Design: 22 cases of leiomyosarcomas and 18 cases of leiomyomas of the uterus that contained a contiguous interface of viable to non-viable tissue were selected. Preservation of extracellular structure was queried by reticulin stain, and patterns within the nonviable areas scored as "honeycomb" or "degenerated". Immunohistochemical staining for mitotic activity (MIB1) was scored for gradients within viable tissues, as highest staining adjacent to necrotic tissue ("necrotic"), or away from necrotic tissue ("viable").
Results: Reticulin highlighted individual tumor cells, creating a "honeycomb" appearance in the viable tissue of both leiomyosarcomas and leiomyomas. Reticulin pattern in nonviable areas differed significantly ($p<0.001$), with maintenance of a honeycomb pattern predominating (91%, 20/22) in leiomyosarcomas and degenerative loss (61%, 11/18) in leiomyomas. Mitotic gradients, when present, were highest towards the viable tissues of sarcomas (41% viable, 5% necrotic), but highest at the necrotic interface of leiomyomas (0% viable, 22% necrotic).
Conclusions: Preservation of the native "honeycomb" reticulin pattern in nonviable areas is a distinctive feature of sarcomas that suggests either a more recent, or qualitatitively different mechanism of degeneration than in leiomyomas. This pattern was informative in distinguishing benign from malignant tumors and may have diagnostic value. When present, the gradient of mitotic activity is reversed between sarcomas and leiomyomas. The mantle of mitotic activity at the interface of leiomyomas suggests a reparative process at a static interface. Conversely, a zone of mitotic suppression flanking the necrotic interface of sarcomas implies a dynamically migrating process. These findings support the hypothesis that the mechanism of necrosis in benign and malignant smooth muscle tumors is sufficiently different and specific markers may assist in their discrimination.

1289 Comparative Micro Anatomy of the Cervical and anorectal Squamocolumnar Junctions and Potential Correlates of Cancer Risk

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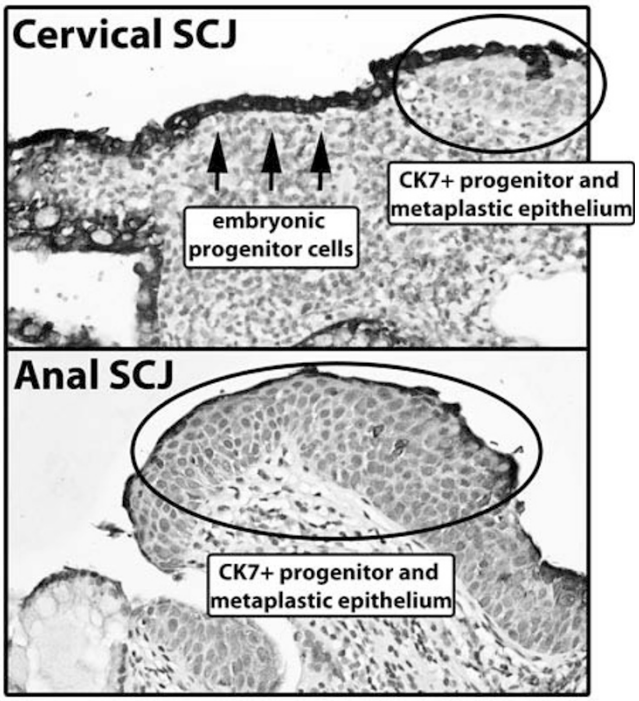
Background: Human papilloma virus (HPV) infection causes cervical cancer and its precursor lesions (HSIL), specifically at the squamocolumnar junction (SCJ). Recent studies have identified residual embryonic cells at the cervical SCJ that share an identical immunophenotype (including CK7) with over 90% of HSILs/carcinomas. In contrast, the majority of vaginal/vulvar carcinomas are SCJ marker-negative implying carcinogenic HPV infection of non-SCJ cells. This disparity in target cell of origin may explain the lower incidence of carcinoma in the vulva/vagina (2.4, 0.65) relative to the cervix (7.9). Interestingly, the incidence of anal cancer is also low (1.5), despite the presence of an anorectal SCJ. We posed the question of whether the anorectal SCJ and its neoplasms have an embryonic cell immunophenotype, as parallelled in the cervical SCJ.
Design: 8 cases with anorectal SCJ and 12 cases of anal intraepithelial neoplasia (AIN)/squamous cell carcinoma (SCC) were analyzed histologically and for the SCJ immunophenotype (CK7+). Benign anorectal SCJ and cervical SCJ were compared. Frequency and distribution of CK7 in anal tumors were compared to a prior database of 58 cervical HSILs/SCCs (Herfs, PNAS, 2012).
Results:



Cervical SCJs demonstrated a distinct, CK7+, cuboidal SCJ cell population residing at the squamocolumnar interface. All HSIL/SCC (58/58) demonstrated strong apical CK7 staining, consistent with neoplastic transformation of the SCJ population and subsequent "top down" differentiation. These SCJ cells were not present in the corresponding regions of the anal SCJ. However, multilayered "metaplastic" epithelium with strong CK7+ was identified in the region of the SCJ (Figure 1). 10/12 (83%) of AIN/carcinomas were CK7(-).
Conclusions: Development of the anorectal SCJ appears to involve the genesis of metaplastic epithelium from CK7+ progenitors; however, there is no discrete residual embryonic cell population remaining. One explanation is that the embryonic cells undergo metaplastic differentiation in this region pre/postnatally, similar to the process of squamous metaplasia seen in the cervix, but without preservation of the embryonic-derived SCJ cells. This would explain why the anorectal junction, despite a high frequency of HPV infection, has a much lower incidence of malignancy.

1290 Endocervical Mucinous Borderline Ovarian Tumors: Immunohistochemical Profile and Clinical Correlations

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Background: Endocervical mucinous borderline ovarian tumors (EM-BOT) are very rare entity, with only few cases series reported until now. Here, we describe a large consecutive series of EM-BOTs, investigating the immunohistochemical profile and clinical correlations of this rare entity.
Design: The electronic database of the Pathology Department of the Catholic University of the Sacred Heart, of Rome, and of the Hospital of the Sacred Heart, Negrar, Italy, were retrospectively reviewed in order to identify women with proven diagnosis of EM-BOT. Specimens were retrieved, and a careful rejoined evaluation of each case was conducted. Immunohistochemistry was performed in order to evaluate the expression levels of the following markers: ER, PR, PAX8, CA125, WT1.
Results: 45 cases of EM-BOT diagnosed between January 2000 and March 2013 were identified. Median age at the time of diagnosis was 37 (21-57), and the median tumor size was 6 cm (2-16). The vast majority of patients showed unilocular masses (89%), with only 4 cases presenting multilocular lesions. All EM-BOT showed a papillary structure, with a diffuse inflammatory infiltration in the stroma, and among the neoplastic cells. A variable percentage of squamous and endometrioid cells was observed, and endometriosic foci were documented in 11 cases (28%). All cases showed a strong immunostaining for ER, PR, PAX8, and CA125. On the other hand, a weak WT1 immunostaining was recognized in only 5 cases (11%), with the remaining 40 patients (89%) resulting negative for WT1 staining. Only 1 patients (2%) showed extraovarian diffusion with a non invasive EM-BOT peritoneal implant.
Conclusions: EM-BOT are characterized by a very favourable prognosis, and a strong association with ovarian or pelvic endometriosis. The immunohistochemical profile of EM-BOT showing strong immunostaining for ER PR, CA125, and PAX8, seems completely different from the profile of intestinal-type mucinous BOTs, which usually show a negative immunostaining for ER, PR, CA125. To our knowledge this is the first series in which the expression of PAX8 was documented.

1291 Genetic Analysis of Atypical Uterine Leiomyoma Suggests a Possible Precursor Lesion of Uterine Leiomyosarcoma

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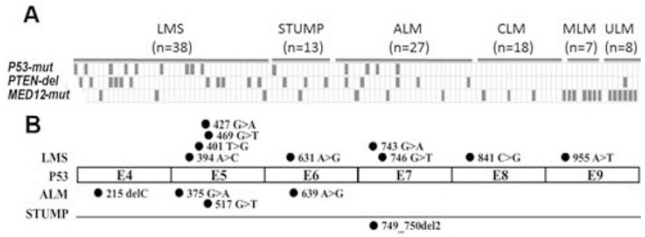
Background: Atypical leiomyoma (ALM) is a variant of uterine smooth muscle tumors (USMT). ALM has been given many different names, largely due to lack of knowledge about its histogenesis and its relationship to leiomyosarcoma (LMS). Thus the diagnosis and clinical management of ALM remains a challenge. Although studies suggest that ALM behaves benign clinically, we sought to characterize the genetic nature of ALM and its relationship to LMS.

Design: We retrospectively collected 127 cases of USMT from two institutions, including 42 ALM and 38 LMS. To further compare the relationship to other USMT variants, we included 40 usual (ULM), 7 mitotically-active (MALM), 22 cellular (CLM) leiomyomas, and 18 leiomyomas of uncertain malignant potential (STUMP). The molecular analyses included *P53* (exons 4-9) and *MED12* (exon 2) mutations, *PTEN* deletion, methylation analysis of 5 selected genes, and 48 selected microRNA. The Mann-Whitney test and Wilcoxon p-value analysis were then performed.

Results: Among 127 USMT, *P53* mutations were detected in 27% of LMS, 8% of STUMP, and 15% of ALM but not in other USMT variants (Figure1). There was no statistically significant difference between *P53* mutations in ALM and LMS (p=0.31). *MED12* mutations varied significantly among MALM (86%), ULM (74%), CLM (14%), LMS (8%), ALM (7%), STUMP (10%) and LMS (8%). *MED12* mutations were most notably separate ULM and MALM from other USMT (Figure1). Loss of at least one copy of PTEN was common in LMS (31%), STUMP (15%), and ALM (20%), but was much less common in ULM (6%), MALM (0%), and CLM (9%). No statistically significant difference of PTEN mutations were found between ALM and LMS (p=0.45). Methylation analysis of RUNX3, CCNA1, WIF1, KLF11, and DLEC1 were still being tested. Unsupervised cluster analysis of 48 selected miRNAs revealed that ALM is more related to LMS than to any other USMT variant.



Conclusions: ALM and LMS share similar genetic alterations of *P53, MED12,* and *PTEN*. These genetic alterations were much less common in other variants of USMT. Our findings suggest that genetically ALM appears to be a precursor lesion of LMS.

1292 PCR-Based *PTEN* Deletion Analysis: A Potential Application for Uterine Smooth Muscle Tumors

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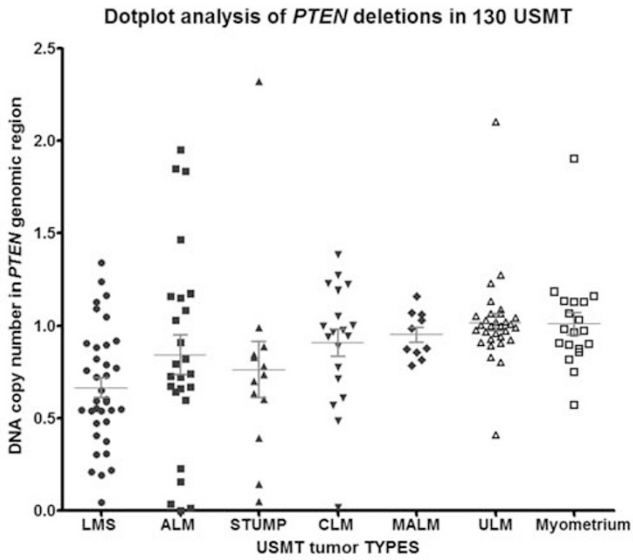
Background: Uterine smooth muscle tumors (USMT) consist of a group of heterogeneous tumors that are currently divided into 6 different subtypes according to the Stanford scheme. These include benign leiomyoma (ULM), mitotically-active leiomyoma (MALM), cellular leiomyoma (CLM), atypical leiomyoma (ALM), uncertain malignant potential (STUMP), and malignant leiomyosarcoma (LMS). Aside from ULM and LMS, the nature of the other USMT variants remains largely unknown and this raises the question as to whether other USMT subtypes differ molecularly. Since *PTEN* genomic deletions (*PTEN-D*) are present in up to 60% of LMS, we sought to examine PTEN-*D* in all other USMT variants.

Design: Absolute quantification PCR is used to detect the accurate copy number of a single gene. Since γ-IFN has stable diploid expression in the human genome, this was used as the control. We designed primers for PTEN and γ-IFN in intron regions and calculated the ratio of PTEN/γ-IFN for copy number determination. The ratio was calculated by the following equation:

DNA (copies/ul) = Concentration (ug/ul) / Molecular weight × (6×10^{14})

If PTEN is diploid, the resulting ratio will be 1 and if PTEN is haploid, the ratio will be 0.5 or less. We extracted DNA from formalin-fixed and paraffin-embedded (FFPE) tissue in 130 USMT cases for use with PCR. After determining the ratios, one-way ANOVA testing was then performed.

Results: *PTEN-D* analysis was first examined on 20 myometrial and 30 ULM specimens. Diploid PTEN was found in 18/20 myometrial (90%) and 28/30 ULM (93%) samples, indicating a reliable test using FFPE tissue. In the USMT subtypes, *PTEN-D* copy number was found to be variable in 31.5% LMS (11/36), 15% STUMP (2/13), 20% ALM (5/25), 12% CLM (2/16), and 0% MALM (0/10). The actual levels of PTEN in each of the 130 cases are listed in Figure 1. Three cases of ALM had PTEN amplification and these cases showed diffuse cytologic atypia with bizarre nuclei histologically. PTEN amplification for these cases will be further investigated using other methods.



Conclusions: The PCR-based DNA copy test is a simple and valuable assay for PTEN-*D* analysis. Significant loss of at least one copy of genomic DNA from the *PTEN* region was frequently seen in LMS, STUMP, and ALM.

1293 Study of 6 Different Types Uterine Smooth Muscle Tumors: Molecular, Histologic and Clinical Analyses

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Background: Based on the current WHO and Stanford scheme, USMT can be classified as leiomyoma (ULM), mitotically-active leiomyoma (MALM), cellular leiomyoma (CLM), atypical leiomyoma (ALM), uncertain malignant potential (STUMP) and leiomyosarcoma (LMS). Aside from ULM and LMS, the nature of the other variants is largely unknown and they remain a challenge for diagnosis and management. Recent studies in molecular biology encourage us to investigate these problematic USMT.

Design: Retrospectively collected 127 cases of USMT, including 7 MALM, 22 CLM, 42 ALM, 18 STUMP and 38 LMS. All cases were reviewed again by 2 pathologists to confirm the diagnosis based on WHO and Stanford schemes. In addition, 40 ULM were also included in this study. Molecular analyses include: *MED12* (exon 2) and *P53* (exons 4-9) mutations; *PTEN* deletion; Methylation analysis of RUNX3, CCNA1, WIF1, KLF11, DLEC1; 48 selected MicroRNA expressions; and immunohistochemistry of 17 biomarkers. Correlation analysis of clinical management and follow-up data.

Molecular and clinical features of 6 different types of USMT

Tumor types	ULM	MALM	CLM	ALM	STUMP	LMS
No. Cases	40	7	22	42	18	38
reviewed tumor slides /case (mean)	-	4-12 (14)	1-38 (12)	3-19 (8)	2-24 (12)	2-39 (10)
P53 mutation %	-	0	0	15	0	27
MED12 mutation %	74	86	14	7	0	8
PTEN deletion %	6	0	9	20	15	31
Diffuse IHC for P16 %	8	14	18	56	18	62
Diffuse IHC for P53 %	0	0	0	7	0	24
Death of disease %	0	0	0	0	0	47
Alive with disease %	0	0	0	7	33	34

Results: Among the original diagnoses of 127 USMT, 18% (23/127) cases were reclassified, mostly due to differing mitotic counts and interpretation of nuclear atypia. *P53* mutations and IHC stain were common in LMS and ALM, while MED12 mutations were extremely common in ULM. Pten genomic deletions were found in 31% of LMS, 15% STUMP and 20% of ALM . MicroRNA signature and cluster analysis showed proximity of LMS and ALM. Immunohistochemical analyses of 17 markers showed LMS different from other USMT, while ALM is the tumor type mostly closely related to LMS. Clinical evaluation showed that LMS had a death rate of 47% (18/38), while ALM had a recurrence rate of 6.7% (2/30) and no deaths attributed to USMT.

Conclusions: This is the first comprehensive study and comparison of 6 different types of USMT [Table1]. ALM is a unique histologic variant of USMT and shares many oncogenic alterations with LMS except for PR (protection), suggestive of a precursor lesion of LMS. STUMP has wide range of histologic and molecular features. CLM is molecularly different from ULM. ALM and STUMP have very low risk of recurrence.

1294 Expression of DNA Mismatch Repair Genes as Independent Prognostic and Predictive Markers for Endometrial Caricinoma

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Background: FIGO staging and grading in endometrial carcinoma(EC) provides the basis for treatment selection and predicting outcome. Lately, biomarkers have paved the way to molecular classifications and have gained prognostic and predictive value. Excision repair cross complementing group 1(ERCC1) and regulatory subunit of ribonucleotide reductase(RRM1) are involved in DNA synthesis and repair, and have also been reported as predictive markers in patients with lung and pancreatic cancers. The study aims to investigate the clinical significance of these markers in EC.

Design: 421 archived cases of EC(1995-2007) were reviewed and TMAs prepared as per established procedures. ERCC1 and RRM1 Immunofluorescence stains were combined with Automated Quantitative Analysis to assess their expression. The average of triplicate core expression was used to determine high and low score cutoff points using log-rank test on overall survival(OS). Association between expression profiles and clinicopathological parameters was tested using Fisher's exact test. The independent prognostic value of ERCC1 and RRM1 was tested using Cox model adjusted for traditional prognostic factors.
Results: 304(72%) type-I EC cases and 117(38%) type-II EC cases were identified. Caucasian women had higher proportion of type-I tumors($p<0.001$) while elderly women were more likely to have type-II tumors ($p<0.001$). ERCC1 and RRM1 expression was observed in 80% of tumors (336 cases & 335 cases,respectively). Kaplan Meier curves showed statistically significant difference in OS between low and high expression of ERCC1 and RRM1. OS remains significantly different using Cox model adjusted for other covariates (age, race, histologic subtypes, lymph vascular invasion and stage) for the two markers. High ERCC1 scores were associated with increased OS when compared to low ERCC1 scores($p=0.007$). In contrast, low RRM1 scores were associated with better OS compared to higher RRM1 scores ($p=0.007$). Log-rank test demonstrated that type-I tumors and advanced stage (FIGO III and IV) tumors could each be subdivided further into better and worse survival groups based on low and high RRM1 expression respectively($p<0.006$). Similarly, lower stage (FIGO I and II) tumors with higher ERCC1expression are associated with better overall survival that those with lower ERCC1 expression($p=0.026$).
Conclusions: We found ERCC1 and RRM1 to be independent prognostic factors for overall survival of EC. They could be utilized for possible future molecular classification and help to tailor optimal individual therapy.

1295 Single PIK3CA Hotspot Mutation Detected in Cervical Squamous Cell Carcinoma: Implications for Targeted Therapy

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Background: The treatment of uterine cervical squamous cell carcinoma (SCC) after surgery is limited to radiation therapy with or without cisplatin-based chemotherapy. Although targeted therapy has been widely explored in many other tumors, it has not been applied to cervical SCC due to lack of data on cancer-specific mutations.
Design: Cancer Gene Mutation Panel with targeted next generation sequencing was performed on 33 cases of cervical SCC to target 2,855 clinically actionable mutations in 50 key cancer genes. The patients ranged from 30 to 80 years of age (mean = 52 years). They all had invasive cervical SCC greater than 0.5cm to ensure adequate DNA extraction. Sequencing libraries were obtained in 28 of the 33 cases with 5 cases failed library preparation due to poor DNA quality.
Results: Seven of the 28 cases (25%) harbored a single point mutation in phosphatidylinositol 3-kinase, catalytic subunit (*PIK3CA*). Six of the seven cases had a single *PIK3CA* E545K mutation and one had a single *PIK3CA* Q546R mutation. Of note, none of the 28 cases had any other hotspot mutation among the remaining 49 genes in the panel, including HPV infection-associated genes such as *TP53, RB1* and *NOTCH1*.
Conclusions: Cervical SCCs harbor a high rate of oncogenic *PIK3CA* mutations. The data strongly suggest that the single point mutations of PI3KCA may drive the carcinogenesis of cervical SCC. More importantly in an era of increasing precision medicine, PI3KCA is potentially targetable for personalized cancer therapy with PI3K inhibitors.

1296 GATA3 Is Expressed in Vulvar Paget's Disease

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Background: GATA-binding protein 3 (GATA3) is a zinc-finger transcription factor involved in cell development and differentiation. Recent studies have shown that GATA3 is a useful marker for breast and urothelial carcinomas. Vulvar Paget's disease (PD) is uncommon but it may show invasion and metastasize. The invasive adenocarcinomatous component associated with vulvar PD shows similar morphology to breast carcinoma and poses diagnostic confusion with the latter in metastatic sites. Here we investigated the status of GATA3 in vulvar PDs with immunohistochemical staining (IHC).
Design: Twenty-four vulvar PDs were included: 6 with an invasive adenocarcinoma component and 18 without. One representative tissue block from each case was used to generate 4um unstained slides for IHC with GATA3. We also stained estrogen receptor (ER), progesterone receptor (PR), and gross cystic disease fluid protein 15 (GCDFP 15) in these tumors for comparison. The staining was semi-quantitatively scored as negative (no tumor cells stained), 1+ (1-25%), 2+ (26-50%), 3+ (51-75%), and 4+ (76-100%).
Results: All 18 PDs without invasivion showed positive GATA3 staining including 1+ in 1, 2+ in 1, 3+ in 1 and 4+ in 15. Positive GCFDP15 staining was seen in 15/18 (83%) PDs including 1+ in 8, 2+ in 3, 3+ in 1, and 4+ in 3. GATA3 stained more tumor cells than GCDFP15 in 11, similarly in 3 and less than GCDFP15 in 1 case, respectively. Only 1/18 PDs was focally positive for ER (1+) and all PDs were negative for PR. All 6 PDs with invasion showed positive GATA3 staining, including 5 positive in both in situ (all 4+) and invasive components (2+ in 1, 4+ in 4) and 1 positive only in the in situ component (1+). GCDFP15 staining was seen in 5/6 cases, including 4 in both components (in situ: 1+ in 1, 3+ in 2, 4+ in 1; invasive: 1+ in 1, 4+ in 3) and 1 only in the in situ component (1+). In the 6 invasive components, 3 showed GATA3+/GCDFP15+ (GATA3 stained more cells than GCDFP15 in 1, similar to GCDFP15 in 1, less than GCDFP15 in 1), 2 showed GATA3+/GCDFP15-, and 1 showed GATA3-/GCDFP15+. One case was positive for both ER and PR in both in situ and invasive components (in situ: 1+ for ER and PR; invasive: ER 4+ and PR 2+) and the remaining 5 cases were negative for both ER and PR.
Conclusions: Positive GATA3 staining is seen in all vulvar PDs. GATA3 staining is generally retained in the invasive component associated with vulvar PDs. GATA3 is more sensitive than GCDFP15 for vulvar PDs. Vulvar PDs only rarely express ER and PR. Vulvar PD should be added to the GATA3+/GCDFP15+ tumor list.

Head and Neck Pathology

1297 Subclassification of Perineural Invasion in Oral Squamous Cell Carcinoma: Prognostic Implications

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Background: Perineural invasion (PNI) is an established independent predictor of adverse outcome in many malignancies including oral squamous cell carcinoma (OSCC) and often results in escalation of treatment. However, detailed histologic analysis and subcategorisation of PNI to select the cohort most at risk has not been attempted.
Design: Clinicopathologic data of OSCC patients were extracted from a prospectively collected database (1995-2012) at a single institution. The Pathology was reviewed for tumor differentiation, tumor depth, patterns of invasion (POI), PNI, lymphovascular invasion (LVI), bone invasion and margin status. The parameters of PNI assessed included: a)uni or multifocal, b)measurement of the size of the involved nerve, c)the location of the involved nerve as intratumoral, at the advancing tumor front, or beyond with measurement of the distance from the tumour for those beyond. Statistical analyses included Chi square test, Kaplan-Meier method, Cox regression analyses.
Results: The study includes 363 patients with OSCC (M:F 223:140, median age 64y, median follow up 6y). PNI was seen in 99 (27%) patients. Presence of PNI correlated significantly with local failure ($p=0.046$) but not with disease specific survival (DSS), regional or distant metastases. On multivariate analysis multifocal PNI (HR:8.7, 95%CI:1.1-70, $p=0.042$) and size of involved nerve (>1mm) (HR:4.9, 95%CI:1.3-18, $p=0.016$) were significantly associated with local failure. Of the 99 patients with PNI, 49 (49%) also showed LVI, bone invasion, or involved margins. 64 (64%) patients received radiotherapy (RT). The use of adjuvant RT amongst patient with PNI did not result in significant differences in the rate of local control, DSS, regional or distant metastases.
Conclusions: The data from this well characterised cohort with a long follow up indicate that presence of multifocal PNI or involvement of nerves >1mm is a significant predictor of local failure. While pathology reports may comment on multifocality of PNI, the size of the nerve is rarely measured. Objective inclusion of these parameters in reports would facilitate larger studies and correlation with clinical outcome.

1298 Significantly Increased Pepsin in Vocal Fold Squamous Cell Carcinoma: An Implication of Carcinogenic Effects of Chronic Laryngopharyngeal Reflux

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Background: Pepsin is primarily synthesized by the chief cells in the stomach as a pro-form zymogen, pepsinogen. Upon being released into the acidic environment of the stomach, pepsinogen is converted into the active form, pepsin, a digestive protease. The larynx is exposed to pepsin, present in the gastric content, following episodes of laryngopharyngeal reflux (LPR). The pepsin will then be internalized by laryngeal epithelial cells by the process of receptor-mediated endocytosis. It has been well established that the presence of pepsin in the upper aerodigestive tract is a sensitive and specific marker for laryngopharyngeal reflux. It has been hypothesized that the presence of pepsin in the larynx could lead to mucosal damages, inflammation and promotion and head and neck carcinogenesis.
Design: A total of 20 cases of vocal fold SCC and 8 cases of benign vocal fold lesions (polyp and keratosis) were retrieved from the Department of Pathology, Central Arkansas Veterans Healthcare System, Little Rock, Arkansas and analyzed by immunohistochemical (IHC) staining using a mouse monoclonal antibody against pepsin (Acris Antibodies, San Diego, CA). The intensity of the cytoplasmic pepsin immunostain was semiquantitatively scored as follow: negative to week (0 to 1); week to moderate (1 to 2); moderate to strong (2 to 3) and strong (3). The individual scores will then be averaged and comparison between vocal fold SCC and benign lesions is made with student's T Test.
Results: Significantly increased pepsin was detected in vocal fold SCC as compared to benign vocal fold lesions. The amount of pepsin as reflected by the IHC staining intensity for vocal fold SCC ranged from 1.5 to 3 with an average score of 2.4 while that for benign vocal fold lesions ranged from 1 to 2 with an average score of 1.25. The difference in the pepsin IHC staining intensity between vocal fold SCC and benign lesions is statistically very significant ($p < 0.001$).
Conclusions: the detection of increased pepsin in most cases of vocal fold SCC supports the co-existence of LPR in these patients and strongly implies that pepsin or chronic LPR may play important role in the carcinogenesis of vocal fold SCC, either alone or more commonly in collaboration with drinking and cigarette smoking.

1299 Promoter Hypermethylation of SOCS1 Gene, a Negative Regulator of EGFR-Signaling Pathway, Significantly Reduces the Survival in Patients with HNSCC

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Background: Epidermal Growth Factor Receptor (EGFR) signaling pathway appears critically important in head and neck squamous cell carcinoma (HNSCC) progression.