including all mature teratoma components, IMP3 may serve as a potential tissue biomarker to distinguish metastatic teratoma from its benign mimickers, especially in the setting of status post chemotherapy for germ cell tumors.

984 Clear Cell Papillary Renal Cell Carcinoma and XP11.2 Translocation-Associated Renal Cell Carcinoma Are Derived from Distal Nephron Tubules and Proximal-Tubules Respectively.

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Background: Clear cell papillary renal cell carcinoma (CCP-RCC) and XP11.2 translocation-associated renal cell carcinoma (XP-TC) are two recently described variants of RCC, but their origin as to which portion of renal tubules they arise from remains unclear. Kidney injury molecule-1 (KIM-1) is a type I transmembranous protein and specific injury marker of proximal tubules. Previous studies have demonstrated that KIM-1 is upregulated in proximal tubule derived RCC (clear cell RCC and papillary RCC) but negative in distal nephron tubule derived tumors (chromophobe RCC and oncocytoma). We attempted to determine the origin of CCP-RCC and XP-TC using KIM-1 expression. Since KIM-1 has a phagocytotic function in injured proximal tubules, we also investigated for correlation with CD68 expression, which is a phagocytic transmembrane glycoprotein, mainly present in macrophages.

Design: The study included three group of RCC. Group 1 had 16 cases which included both clear cell RCC and papillary RCC (KIM-1 positive control group), Group 2 consisted of 11 cases of CCP-RCC (CK7 positive/P504S negative) and Group 3 had 11 cases of XP-TC (TFE-3 positive). Tumors were immunohistochemically stained for KIM-1 (AKG7 monoclonal antibody, JB Bonventre's lab, Brigham and Women's Hospital, Boston) and CD68 (KP1 clone, Dako Cytomation) and staining intensity was graded from 0 to 3+.

Results: KIM-1 showed membranous and/or cytoplasmic staining in all group 1 cases (16/16; 5 with 1+, 7 with 2+ and 4 with 3+ staining). None of group 2, CCP-RCC cases (0/11) expressed KIM-1. In contrast, all group 3, XP-TC cases (11/11) revealed positive KIM-1 expression (1 with 1+, 7 with 2+ and 3 with 3+ staining), suggesting origin from proximal tubules. The cytoplasmic expression of CD68 was present all group 1 cases (16/16, 5 with 1+, 7 with 2+ and 4 with 3+ staining), absent in all group 2 cases (0/11); and present in 9/10 group 3 cases (2 with 1+, 7 with 2+ and 1 with 3+ staining, 1 case was not available).

Conclusions: Using KIM-1 as a differentiation marker, the negative staining in CCP-RCC suggests derivation from distal nephron tubules and positive staining in XP-TC suggests derivation from proximal tubules. CD68 expression closely mirrored KIM-1 expression both in extent and intensity. The expression of CD68 in RCC subtypes is a novel finding and in our opinion this CD68 expression most likely represents a functional relationship with KIM-1, and may not necessarily have a real diagnostic utility; although this finding needs further evaluation.

985 Venous Invasion in Renal Cell Carcinoma: Preoperative Imaging-Gross Correlation.

CX Zhao, KO Ojemakinde, A Bhalodia, M Heldmann, D Elmajian, SM Bonsib. Louisiana State University Health Sciences Center-Shreveport, Shreveport.

Background: Renal sinus vein (SV) invasion is the principal invasive pathway for most renal cell carcinomas(RCC). Emphasis on nephron sparing surgery requires accurate preoperative imaging to optimize surgical strategies. Computed tomography (CT) and magnetic resonance imaging (MRI) are the most sensitive studies for preoperative imaging of RCC. Their accuracy in imaging sinus involvement in RCC has not been investigated.

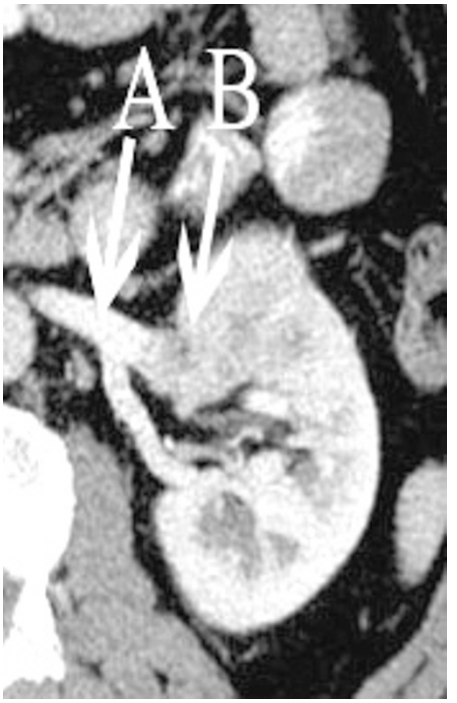
Design: Fifteen cases of RCC were retrospectively selected and staged by 2010 TNM (3-pT1/2, 10-pT3a and 2-pT3b). The original radiology reports were obtained (11 LSUHSC/4 outside hospitals). A radiologist with expertise in urologic imaging received a brief tutorial on sinus invasive properties of RCC and re-examined the CT/MRI images for sinus invovlement. These interpretations were correlated with RCC gross photos.

Results: The mean tumor sizes were 8.7, 8.9, and 8.2cm by original radiologist, expert radiologist and patholgosist gross examination, respectively. SV invasion was not mentioned in any original CT/MRI reports (0/15) but was present in 12/15 cases by pathology review. Upon re-review of CT/MRI by radiology expert following tutorial, SV involvement was identified in 9/15.

RCC Tumor Stage Frequencies

Interpretation	pT1/2	pT3	
		SV	MRV
Original Radiologist	14	0	1
Expert Radiologist	6	3	6
Pathologist	3	3	9

Main renal vein (MRV) involvement was noted in 6/15 cases by expert radiologist but in 9/15 by patholgists. The 3 discrepant cases involved the left kidney.



When compared with radiology images the 3 involved veins were large primary tributaries of the MRV.

Conclusions: 1) Sinus involvement is not routinely mentioned by radiologists 2) Sinus involvement is usually detectable by CT/MRI (9/12) when present 3) Unrecognized sinus invasion by radiologists may lead to overestimate of tumor size and understaging that can be corrected by education on pathologic features of RCC 4) Discrepant MRV involvement by radiologists versus pathologists affecting the left kidney is due to differing definitions of what constitutes the MRV.

Gynecologic & Obstetrics

986 Secretory Cell Expansion of the Fallopian Tube Epithelium May Represent an Initial Cellular Change in Pelvic Serous Carcinogenesis.

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Background: The distal fallopian tube is a common site of origin for tubal intraepithelial carcinoma and pelvic serous carcinoma in high-risk women (i.e BRCA mutation carriers and/or those with a breast cancer history). It has recently been further defined at the cellular level, that the secretory cell as opposed to cililated cell of the fallopian tube is the cell-of-origin of these cancers. However, under normal conditions, secretory and ciliated cells are intimately admixed within the tubal epithelial lining. We hypothesized that a change in ratio between tubal secretory and ciliated cells may represent one of the early steps in the process of female pelvic serous carcinogenesis

Design: Cellular compositions (secretory versus ciliated) in fallopian tubal segments (fimbriated end versus ampulla), *secretory cell expansion* (> 10 but < 30 cells in a row) and *secretory cell outgrowth* (≥ 30 cells in a row) were studied in 3 groups of patients: patients with benign gynecologic diseases ("no-risk"), "high-risk" patients as previously defined, and patients with "ovarian" high-grade serous carcinomas. The numbers of secretory and ciliated cells were counted by 2 methods: light microscopy and immunostainings with PAX8 for secretory cells and tubulin for ciliated cells. The ratio of cellular compositions was statistically compared among the 3 groups

Results: As compared with the "no risk" group, the numbers of tubal secretory cells in the high-risk and cancer groups were increased by a factor of at least 2 and 4 respectively. The frequency of secretory cell expansion was 3 to 4-fold higher in the high-risk and cancer groups, while the frequency of secretory cell outgrowth was 4-fold higher in the high-risk and 6-fold higher in the cancer group, respectively. Overall, immunohistochemistry with PAX8 and tubulin was more sensitive than morphologic evaluation for distinguishing the cell types. There was no significant difference between the tubal fimbriated end and ampulla regarding the number of secretory cells

Conclusions: An increase in the number of secretory cells in fallopian tube epithelium may represent an early, morphologically identifiable event in the process of pelvic serous carcinogenesis, although the underlying molecular mechanism of secretory cell expansion or outgrowth and how they eventuate in cancers, remain unclear.

987 Correlation of the Volume of Carcinomatous Component with the Clinical Stage and Nodal Metastasis in Uterine Carcinosarcoma.

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Background: Carcinosarcoma (CS), also known as malignant mixed Müllerian tumor is an uncommon biphasic neoplasm composed of malignant epithelial and stromal components that can arise in any genital organ but most frequently in the uterus. Prognostic factors that influence the clinical outcome of the patient remain a matter of controversy. However, the most important prognostic factor is the clinical stage at the time of initial treatment. The objective of this study is to evaluate the impact of the volume of the carcinomatous (CA) component represented as percentage of tumor mass on patient's survival represented by the clinical stage as well as its correlation with nodal metastasis.

Design: 49 cases of uterine CS were obtained and evaluated in a collaborative effort of multiple institutions from the year 2001 to 2009. Microscopic slides from all tumor sections of each case were reviewed by at least two pathologists. The volume of CA component was estimated as a percentage on each tumor slide and then calculating the mean of all tumor slides of each case, with good correlation among the different examiners. All cases were staged according to the International Federation of Gynecologists & Obstetricians (FIGO) staging system.

Results: We assessed 49 cases of uterine CS. Over 30% of the cases were Stage I, 10.9% Stage II, 43.5% Stage III, and 15.2% Stage 4. Thirty one cases had lymph node information, 58.1% (18) were positive. The mean percent CA component was 55.4±28.8 (s.d.). The association between stage or lymph node status and percentage CA component was assessed using point biserial correlations, analysis of variance and Student's t-test. There was no association between stage and percent CA component ($r=-0.25$, $p=0.09$). Mean percent carcinoma by stage was 64.8±23.0, 48.8±38.7, 50.8±28.7, and 43.3±31.8 for Stages 1-4, respectively ($F=1.1$, $p=0.4$). There was also no association between lymph node status and percent CA component ($r=-0.32$, $p=0.08$). Mean percent CA by lymph node status was 63.5±19.3 and 45.7±31.0 for cases with negative and positive lymph nodes, respectively ($t=1.82$, $p=0.08$).

Conclusions: This study demonstrates no impact of either CA or sarcomatous components volumes on patient's survival or tumor stage, and also demonstrates no increase in nodal metastasis in cases with higher carcinoma volume.

988 Anti-FOXL2 Antibody Is a Sensitive and Specific Diagnostic Marker for Ovarian Sex Cord-Stromal Tumors.

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Background: We recently identified FOXL2 (402C→G) mutation as being characteristic in adult granulosa cell tumor (aGCT). Testing for this aGCT-specific mutation is not widely available. Therefore, we assessed the potential diagnostic utility of FOXL2 immunostaining by examining the immunohistochemical expression of FOXL2 protein in a broad range of ovarian tumors.

Design: Using a commercially available polycloncal antiserum against FOXL2 protein (Imgenex 1:25 dilution), immunoexpression of FOXL2 was analysed in 501 ovarian tumors, including 130 SCST, using whole tissue sections and tissue microarrays. Immunostaining was also correlated with FOXL2 mutation status. In addition, we compared FOXL2 immunoexpression with that of alpha-inhibin and calretinin in a subset of 89 SCST.

Results: There was positive nuclear staining for FOXL2 in 99/501 (19.8%) cases. FOXL2 immunostaining was present in 96 of 130 (74%) SCST, including more than 95% of aGCT, juvenile granulosa cell tumors, fibromas, and sclerosing stromal tumors. Only 50% (20/40) of Sertoli-Leydig cell tumors (SLCT) stained for FOXL2. Three of three (100%) female adnexal tumors of probable Wolffian origin showed FOXL2 immunoreactivity, while all other non-SCST tested (n=368) were completely negative for FOXL2. Forty five of 130 (34.6%) SCST were positive for FOXL2 (402C→G) mutation, including 39/42 (93%) aGCT, 3 of 40 (7.5%) SLCT, 2 of 5 (40%) thecomas, and 1 of 4 (25%) sex cord-stromal tumors of unclassified type. SCST with a FOXL2 mutation consistently immunoexpressed FOXL2 (44 of 45, 98%), but FOXL2 immunostaining was also seen in many SCST that lacked a mutation (50 of 85, 62.5%). FOXL2 immunostaining showed higher sensitivity for SCST (74%), compared to calretinin (66%), and alpha-inhibin (57%). In the FOXL2-immunonegative SCST, alpha-inhibin and/or calretinin immunostaining yielded positive results in every case.

Conclusions: FOXL2 is a novel immunohistochemical marker that is both sensitive and specific for SCST. FOXL2 immunostaining is present in almost all SCST with a FOXL2 mutation, as well as a majority of SCST without a mutation. The use of FOXL2 together with alpha-inhibin and/or calrentinin should form a panel that yields positive staining with one or more marker in essentially all cases of SCST. Because FOXL2 immunostaining does not generally distinguish among tumors within SCST category, genetic testing for aGCT-specific FOXL2 (402C→G) mutation remains the only diagnostic tool to separate aGCT from other SCST.

989 EZH2 Expression Correlates with Increased Angiogenesis in Ovarian Carcinoma.

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Background: We recently discovered EZH2, a polycomb repressor, to have substantially increased expression in tumor endothelial cells. In this study, we examined the clinical and functional significance of EZH2 and its correlation with VEGF and angiogenesis in ovarian carcinoma.

Design: Cell lines: Mouse ovarian endothelial cells (MOEC) were transfected with the Renilla luciferase plasmid either with or without the EZH2 promoter construct. Cells were then treated with VEGF [conditioned media from SKOV3 ovarian cancer cells (SKOV3-CM)]. EZH2 mRNA was quantified using real time RT-PCR. EZH2 protein levels were evaluated by western blot.

(IHC): 180 paraffin-embedded epithelial ovarian cancer specimens (collected between 1985 – 2004) with available clinical outcome data were identified. IHC for EZH2, CD34, and VEGF was performed. For EZH2 and VEGF, the stained slides were scored based on intensity and percentage of cells stained and categorized as high and low expressors. To quantify micro vessel density (MVD), the number of blood vessels staining positive for CD34 was recorded in 10 high power fields and mean calculated.

Also, clinical parameters and survival data was obtained on these patients from the clinical information system and SEER registry.

Results: In the MOEC cell lines, there was a significant increase in EZH2 promoter activity and EZH2 mRNA expression levels in endothelial cells in response to VEGF (SKOV3-CM). This increases in EZH2 promoter activity and mRNA was blocked with the VEGFR2 specific antibody DC101. Similarly, increased EZH2 protein levels in response to VEGF was blocked by the anti-VEGFR2 antibody.By IHC, EZH2 expression was evaluated in 180 cases in both tumor and endothelial compartments (EZH2-Tand EZH2-E). 66% and 67% of the samples showed high EZH-2 expression in the tumor and endothelial compartments respectively. High expression of EZH2-T and EZH2-E was significantly associated with high-stage ($p < 0.001$) and high-grade ($p < 0.05$) disease. High EZH2-T and EZH2-E was also significantly associated with decreased overall survival (median 2.5 years vs. 7.33 years, $p < 0.001$) and (2.33 vs. 8.33 years, $p < 0.001$) respectively.Tumors with high VEGF expression had significantly correlation with high EZH2-E expression ($p < 0.001$). Moreover, tumors with high EZH2-E expression also had significantly greater MVD ($p < 0.001$).

Conclusions: These findings suggest that EZH2 may be a regulator of tumor angiogenesis and supports the possibility of this being a therapeutic target in ovarian carcinoma.

990 Transitional Cell Carcinoma of the Ovary May Be Related to High-Grade Serous Carcinoma and Is Distinct from Malignant Brenner Tumor.

RH Ali, JD Seidman, S Kalloger, B Gilks. Vancouver General Hospital, BC, Canada; Washington Hospital Center, Washington, DC.

Background: According to the WHO, transitional cell tumors of the ovary include benign, borderline and malignant Brenner tumors (BT), as well as transitional cell carcinoma (TCC) in which a benign Brenner component is not seen. Some TCCs could conceivably be examples of malignant Brenner tumor with overgrowth of a benign Brenner component.

Design: Our objective was to compare the immunophenotype of BT to that of TCC and look for tumors with the immunophenotype of BT, but lacking a benign BT component, among a large cohort of ovarian carcinoma cases. Six BTs (3 benign, 2 borderline, 1 malignant) and 7 TCCs were stained for WT1, ER, p53, and p16. A database of 500 cases of ovarian carcinoma was searched for tumors with an immunoprofile similar to that of BT.

Results: BTs were negative for WT1, p53, ER (except for weak positivity in one case) and negative or weakly positive for p16. In contrast, the seven cases of TCC stained as follows: 4/6 +ve for WT1, 2/6 +ve for p53, 5/7 +ve for ER, 2/7 strongly +ve for p16. Of the 500 cases of ovarian carcinoma, 116 showed the following immunoprofile: WT1 –ve, ER –ve, p16 –ve or weak +ve, p53 –ve. Of these cases, (including 77 clear cell carcinoma, 14 endometrioid carcinoma, 12 mucinous carcinoma, 8 high grade serous carcinoma) none showed morphological features of TCC.

Conclusions: • Immunophenotypes of Brenner tumor and TCC are different, with the latter resembling that of high grade serous carcinoma.

• Tumors with the immunophenotype of Brenner tumor but lacking a benign Brenner component are uncommon and these tumors do not morphologically resemble transitional cell carcinoma.

• We found no evidence of carcinomas with morphological features of transitional cell carcinoma, but with the immunophenotype of BT (i.e. possible examples of malignant Brenner tumor where there has been overgrowth of benign BT components).

• These results suggest that BT and TCC are unrelated, and should not be combined for classification purposes.

991 Stage II-IV Low-Grade Serous Carcinoma of the Ovary: A Clinicopathological Study of 33 Patients.

RH Ali, SE Kalloger, JL Santos, KD Swenerton, B Gilks. Vancouver General Hospital, BC, Canada; British Columbia Cancer Agency, Vancouver, Canada.

Background: Low-grade serous carcinoma of the ovary has only recently been recognized as a disease entity distinct from the more common high-grade serous carcinoma. When confined to the ovary, low grade serous carcinoma is associated with a very favorable prognosis and chemotherapy is typically not recommended. There is little information on the prognosis of patients with low-grade serous carcinoma who have extra-ovarian spread at presentation.

Design: Thirty-three cases of stage II-IV ovarian low-grade serous carcinoma were identified in the Cheryl Brown Ovarian Cancer Outcomes Unit. In 19 cases blocks were available and immunostaining for Ki-67, WT1, E-cadherin, p16, and p53 was performed. Comparison of expression of these markers in low-grade serous carcinoma we made with data on the same immunomarkers in a series of >400 cases of high-grade serous carcinoma.
Results: Mean age of the patients was 56 years. The tumors presented at stage II in 10/32 cases, stage III in 21/32 cases, and stage IV in 1/32 case. On follow up, most patients died of disease, with less than 30% survival at 10 years. Compared to high-grade serous carcinomas, the low-grade serous carcinomas were significantly more likely to express p16 at high levels, and to show abnormal p53 expression ($p=0.004$ and $p<0.0001$, respectively). Ki-67 staining indices were lower in the low-grade serous carcinomas than the high-grade serous carcinomas ($p<0.0001$). There were no significant differences between low-grade serous carcinomas and high-grade serous carcinomas with respect to expression of WT1 and E-cadherin ($p=0.81$ and $p=0.19$, respectively).
Conclusions: This group of patients with low-grade serous carcinoma who had extraovarian spread at the time of presentation had an unfavorable prognosis, similar to that of patients with high-grade serous carcinoma. The immunoprofile of these cases was similar to what has previously been reported for low-grade serous carcinoma, with evidence of lower tumor proliferation and fewer p53 abnormalities than are seen in high-grade serous carcinoma.

992 Rare Cervical Cancers: Immunohistochemistry and Correlation with HPV Infection Status by In-Situ Hybridization and Linear Array.

G Allo, M Tsao, M Rouzbahman, C Wei, S Kamel-Reid, G Rasty. University Health Network, Toronto, ON, Canada.
Background: Human papilloma virus (HPV) is a major etiologic factor in the development of cervical epithelial malignancies. Limited data is available on the less common cervical tumors. Our aim is to examine HPV status, and its correlation with immunohistochemical expression in rare cervical carcinomas and mixed tumors.
Design: Pathology archives at the department of Anatomic Pathology, were searched for primary cervical carcinomas excluding squamous cell carcinoma, adenocarcinoma and adenosquamous carcinoma from 2000 till now. 34 cases were retrieved which include adenoid basal (AB) carcinoma (n=2), clear cell (CC) carcinoma (n=2), glassy cell (GL) carcinoma (n=2), lymphoepithelioma – like (LE) carcinoma (n=3), serous (SR) carcinoma (n=9), villoglandular (VG) carcinoma (n=4), and neuroendocrine (NE) carcinoma (small cell, large cell, mixed carcinomas). The histopathology was reviewed by 2 pathologists. Chromogenic in-situ hybridization (CISH) –INFORM HPV (13 genotypes) and Roche Linear array HPV genotyping (37 genotypes) were performed on all cases. The following immunoperoxidase stains were performed on tissue microarray: (LMWK),CK 5/6,p16, p63, p53, Synaptophysin, Chromogranin, CD56, ER, PR, TTF-1, WT-1 and Ki67.
Results: High risk HPV-DNA is identified in 30% of serous carcinoma and 75% of NE carcinoma. Expression of NE markers in mixed NE carcinoma suggest pluripotential nature of the tumor. p16 is expressed in almost all tumors. p53 is mainly expressed in CC and GL with no expression in serous carcinoma of cervix. WT-1 is negative in all cervical carcinomas.

Expression of IHC markers and HPV

Cases	p16	p63	p53	NE marker	HPV status	HPV+/case #
AB	1+, 30-60%	3+, 100%	<10%	0	-/+	1/2
CC	3+, 100%	0	2+, 30-60%	0	-	0/2
GL	2+, 50%	2+, 100%	2+, 30-60%	0	+	1/2
LE	2+, 100%	3+, 100%	0	0	+	2/3
SR	2+, 100%	0	0	10%	+	3/9
VG	3+, 100%	0	<5%	25%	+	4/4
NE	2+, 100%	3+, 10%	<5%	75%	+	9/12

1-3: intensity, % of tumor cells involved

Conclusions: Our study demonstrates that the majority of rare carcinomas of cervix, including NE variants are positive for high-risk HPV which may suggest a unique carcinogenic pathway induced by the virus. This finding may have a role in tailoring HPV sub-type selection for vaccine and also theraputic strategies.
Our data also suggest that tumors with serous type morphology and p53 expression are possibly derived from uterine corpus and should be treated accordingly.

993 HPV DNA In-Situ Hybridization, p16 and p53 in a Cohort of 128 Vulvar Squamous Cell Carcinomas.

G Allo, AM Mulligan, D Ghazarian, M Tsao, H Mackay, BA Clarke. University of Toronto, ON, Canada; University Health Network, Toronto, ON, Canada; St. Micheal's Hospital, Toronto, ON, Canada.
Background: Vulvar squamous cell carcinomas may develop via two different pathways: the most common pathway is HPV negative well differentiated keratinizing squamous cell carcinoma arising in elderly women, often in a background of lichen sclerosis and differentiated vulvar intraepithelial neoplasia; HPV positive vulvar squamous cell carcinoma occurs in younger women in association with usual vulvar intraepithelial neoplasia. P53 is typically over-expressed in the former and p16 in the latter. We analyzed p16, p53 and HPV integration in a cohort of 128 cases of squamous cell carcinoma of the vulva and determined the prognostic significance of these biomarkers.
Design: An annotated, duplicate core tissue microarray of 128 cases of vulvar squamous cell carcinoma was constructed. HPV DNA in situ hybridization and p16 and p53 immunohistochemical staining were performed. Statistical analysis was performed using JMP v8.0.2 (SAS institute, Carey, NC USA).
Results: HPV and p16 positivity were highly correlated. Thirteen percent of cases were HPV positive. Of these, 87% were positive for p16 and 7% for p53. Twenty five per cent of cases were p16 positive, of which 45% were HPV positive and 18% were p53 positive. Forty-eight per cent of cases were p53 positive and of these only 1.8% were HPV positive and 10.7% p16 positive. HPV was significantly more likely to be positive in p16 positive cancers than p53 positive cancers ($P<0.001$). P53 was more likely to be positive in HPV negative cases compared to HPV positive cases ($P=0.006$). There was no significant difference in median age between the HPV positive and negative groups. There was no significant difference in 5 Year disease specific survival and relapse free survival based on HPV status, p16 and p53 staining.
Conclusions: This data supports a dual pathway of vulvar squamous cell carcinoma progression. Unlike previous studies on vulva cancer and head and neck squamous cancer we did not demonstrate biomarker dependent outcome differences. Correlation with HPV PCR, full section staining and morphology is being performed.

994 Endocervical Adenocarcinoma (EADC) Coexistent with Lobular Endocervical Glandular Hyperplasia (LEGH). A Clinicopathologic Study of 12 Cases.

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Background: Although LEGH was originally described as a distinct hyperplastic glandular lesion of the uterine cervix, recent studies have raised the question that LEGH may be a cancerous precursor of Minimal Deviation Adenocarcinoma (MDA) and other Mucinous Adenocarcinomas (MACs). This study was designated to evaluate the clinical and pathological data of 12 patients with EADCs, the relationship between EADCs and LEGH and the presence of Human Papilloma Virus (HPV) in both lesions.
Design: Using our Institutional database, we identified 250 stage IB EADCs between 1999 and 2009. Pathological diagnoses were reevaluated based on criteria for the diagnosis of LEGH by Nucci et al (Am J Surg Pathol 1999). HPV infection was investigate using the Polymerase Chain Reaction technique.
Results: LEGH components were present in 12 cases (5%). Of the 12 EADCs with LEGH component, 8(67%) were MDAs and 4(33%) were well-differentiated mucinous adenocarcinomas. Patient age ranged from 56 to 72 years (mean:65 years). Chief complaints were, watery and mucinous vaginal discharge in 8 cases and genital bleeding in 4 cases. To note, one patient had concurrent cervical, left ovarian mass (metastatic from the cervix) and a low-grade mucinous tumor of the appendix. Macroscopically, a diffuse (barred-shaped) or nodular enlargement of the cervix was seen in 11(92%) of the cases, multiple small cysts were seen within the nodular component. One case, was entirely cystic. Lesion size ranged from 2 to 3.8cm wide and from 1 to 2.8cm deep. The adenocarcinoma component was identified as a minor lesion within the LEGH component in all 12 cases. HPV (types 16 and 18) was positive in all cases of MACs, but negative in all LEGH and MDAs.
Conclusions: LEGH may be a cancerous precursor of MDA and other mucinous adenocarcinomas of the uterine cervix. MDA and LEGH are probably not related to HPV infection.

995 Comparative Mutational Profiling of Multifocal Low Grade Endometrioid Adenocarcinomas Using Oncogene Point Mutation and Loss of Heterozygosity Analysis.

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Background: Simultaneous endometrial and ovarian adenocarcinomas may represent either independent synchronous tumors or single primary tumors with metastasis. The relatedness of multifocal deposits of adenocarcinoma can be challenging, and discrimination between the two is often difficult based on morphologic features alone. In this study we used comparative mutational profiling guided by microdissection to determine the relatedness of early stage simultaneous endometrioid tumors in clinical settings where the distinction has therapeutic implications (i.e., not widely metastatic).
Design: Departmental records identified 15 patients who presented with simultaneous FIGO grade 1 endometrial and ovarian endometrioid adenocarcinomas diagnosed as independent synchronous stage 1 tumors by established morphologic criteria (i.e., superficial myometrial invasion of endometrial tumors, unilaterality of ovarian tumors, and absence of lymphovascular invasion). DNA was extracted from the tumors, followed by profiling for point mutations in *KRAS,* as well as quantitative genotyping for a panel of LOH cancer-associated markers targeting 1p, 3p, 5q, 9p, 10q, 17p, 17q, 21q, 22q. Tumor pairs with one or more concordant mutations were classified as metastatic. Clinical records were reviewed to establish disease free and overall survival.
Results: Mutational profiling of 7 control cases (mean age: 61; range 45-86 years) confirmed the ability of the technique to detect relatedness in known metastatic disease. Profiling of the 15 study cases (mean age: 51.5; range 23-70 years) showed 9 to be independent synchronous tumors and 6 to be metastatic (overall survival: 10.6 vs. 13.6 years, $p=0.3$; disease free survival: 9.6 vs. 9.5 years, $p=0.9$, respectively). Overall, three mutational signatures emerged which could be classified as 1) highly mutated, 2) minimally mutated with LOH, and 3) MSI. These signatures were equally distributed in the tumors considered to be independent and metastatic by molecular analysis.
Conclusions: By gene mutation and LOH analysis, a significant proportion of patients diagnosed with independent synchronous stage 1 FIGO grade 1 endometrial and ovarian endometrioid adenocarcinomas actually represent cases of metastatic disease. The finding of distinctive mutational signatures in histologically equivalent low grade adenocarcinomas can be used to better understand relatedness in multifocal cancer to potentially predict clinical behavior.

996 Cytology and hrHPV Test Results Associated with 717 Histopathologic Diagnoses of CIN2/3 at a Large Academic Womens' Hospital.

M Amin, X Chen, RM Austin, C Zhao. Magee-Womens Hospital (MWH) of University of Pittsburgh Medical Center (UPMC), PA; Conemaugh Memorial Hospital, Johnstown, PA.

Background: Widely cited studies reflecting use of conventional Pap smears have emphasized the significance of ASC-US Pap tests preceding histopathologic diagnoses of CIN2/3. Data reflecting current widespread U.S. use of liquid-based cytology (LBC), computer-assisted screening, and adjunctive HPV testing remain limited.

Design: A computer-based search was performed for a study period between July 2008 and July 2010 to identify surgical pathology cases reported as cervical CIN2/3 in the CoPath database of a large womens' hospital practice. Previous computer-imaged LBC ThinPrep Pap (TPPT) and hrHPV (Hybrid Capture 2) test results were recorded.

Results: 717 cases of histopathologic CIN2/3 diagnoses were identified, including 600 patients with LEEP/cone biopsies and 42 with subsequent hysterectomies. The average patient age was 33 years with a range of 17 to 91 years. 208 cases (29%) showed no reasons for cervical biopsy in our files. The abnormal Pap test results which led to histopathologic diagnoses of CIN2/3 were available for 509 (71%) women. Of these particular Pap test results, 99 were ASC-US, 66 were ASC-H, 82 were LSIL, 256 were HSIL and 6 were AGC. 314 of 717 CIN2/3 diagnoses had available prior Pap test results (Table 1). Available prior HPV test results are listed in Table 2.

Table 1. Test History Prior to Abnormal Pap Results and Histopathologic CIN2/3 Diagnoses*

Results (prior 3 years)	#Cases	%
At least one abnormal smear	210	66.9
At least one normal smear	184	58.6
Both normal and abnormal smears	80	25.5
Total number of patients	314	100

* The abnormal Pap tests which triggered the surgical procedures are not included.

Table 2. HPV test results of patients with Histopathologic CIN2/3 Diagnoses

	#Cases	#Positive Cases	%
HPV testing immediately before CIN2/3 diagnoses	207	204	98.6
HPV test results in prior 3 years (not including above)	91	75	82.4

Conclusions: LBC Pap test results of HSIL were associated with 50% of 508 histopathologic CIN2/3 diagnoses, followed by ASC-US in 19%, LSIL in 16%, and ASC-H in 13%. Over a three year period preceding CIN2/3 diagnoses, a significant number of isolated negative Pap (58.6%) or negative HPV test (17.6%) results were noted, supporting the value of combined cytologic and HPV co-testing.

997 Factors Predicting Response to Progestin Treatment for Complex Atypical Hyperplasia/Well Differentiated Endometrial Carcinoma in Premenopausal Patients.

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Background: Response rates of complex atypical hyperplasia (CAH) and/or Grade 1 endometrial carcinoma (G1EAC) to progestin treatment are approximately 70 % with median time to resolution being 5 months. Little is known about factors associated with patient's response to this hormone therapy. **Purpose**: To establish clinical and histologic indicators for predicting response to progestin treatment in premenopausal patients with CAH and/or G1EAC.

Design: In 40 premenopausal patients with CAH and/or G1EAC, clinical data (age, ethnicity, parity, BMI) and histologic features from pre- and first-on-(minimum 8 weeks of progestin)-treatment endometrial sampling (ES) were analyzed for distinguishing Responders from Non-Responders. ESs were reviewed by 2 pathologists independently, and the diagnosis, architectural abnormalities (papillary; polypoid; cribriform; back-to-back; budding), nuclear abnormalities (nuclear stratification; nucleolus; N/C ratio >0.67), cytoplasm eosinophilia, cytoplasm squamoid changes, and decidual changes were recorded. Responders were defined by absence of atypia or carcinoma in 2 consecutive ESs and/or hysterectomy.

Results:

Variables associated with Response in univariate analysis

Factor		%(N)	standardized resolution ratio (SRR)	p value
Ethnicity	Hispanic	57.5%(23)	0.98	0.045
	Caucasian	20%(8)	2.28	0.034
	Asian	22.5%(9)	0.25	
Pre-Rx ES	arc abn>/=3	27.5%(11)	0.37	0.028
	arc abn<3	72.5%(29)	1.23	
First on Rx ES	>SH	60%(24)	0.49	0.007
	</=SH	40%(16)	2.25	
First on Rx ES	arc abn(+)	60%(24)	0.5	0.015
	arc abn(-)	40%(16)	1.74	
First on Rx ES	nucleolus(+)	60%(24)	0.64	0.08
	nucleolus(-)	40%(16)	1.44	
First on Rx ES	mitosis >/=2/10 glands	17.5%(7)	0.35	0.02
	mitosis <2/10 glands	82.5%(33)	1.25	
First on Rx ES	lesion>/=1mm	72.5%(29)	0.68	0.04
	lesion<1mm	27.5%(11)	2.38	

SRR>1 indicates response rate higher than baseline

Conclusions: Non-response to hormone treatment in CAH and G1EAC was associated with (1) Pre-treatment ES with significantly abnormal architecture; (2) First-treatment ES with diagnosis worse than simple hyperplasia (SH); (3) First-treatment ES with persistent abnormal architecture; (4) First-treatment ES with persistent nuclear atypia (nucleolus, mitosis ≥ 2/10 glands) and (5) First-treatment ES with ≥1mm lesion.

998 Invasive Endocervical Adenocarcinoma: Combining Depth and Pattern of Invasion for Better Identification of Patients with Lymph Node Metastases.

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Background: The preferred treatment of invasive endocervical adenocarcinoma (Endocx Adenca) is surgical. The type of resection is selected based on the depth of invasion. However, pathologists do not agree on what cases are invasive and how to measure depth of invasion. Over 95% of the resected lymph nodes (LNs) are negative creating an important morbidity problem especially in young patients (Pts).

Design: We reviewed 43 cases treated as invasive Endocx Adenca. Only Pts with hysterectomy and LN resection, or a follow-up >5 years were included. We evaluated tumor size, differentiation, mitosis, necrosis, characteristics of the invasive component, depth of invasion, and vascular invasion (VI). In the area with the main invasive component smooth muscle actin (SMA) and estrogen receptor (ER) were obtained in 25 cases.

Results: The cases were separated into 3 groups: 1) Cases with well or moderately differentiated glands, regardless of their depth or proximity to large vessels. Invasion in this group was based only on the depth of the glands: 13 cases, no VI, ER+, SMA- in 71%. All (263) LNs were negative and no recurrences. 2) Cases with any differentiation but with pushing margins in the main tumor mass and minimal foci (<1 mm) of destructive invasion from the well demarcated margin of Endocx Adenca: 11 cases, 2 had suspicious VI. ER and SMA were noncontributory. All (207) LNs were negative. One Pt had a recurrence in the vagina. 3) Diffuse type of invasion deeper than 1 mm: 19 cases, VI in 7, 1 vessel in 5, and 3 or more in 2. ER- and SMA+ in 70%. 5 had LN mets and/or recurrences. 366 LNs resected in this group. The 2 Pts with 3 or more vessels + had LN mets.

Conclusions: Identifying invasion and determining its depth in Endocx Adenca is extremely difficult, therefore we propose: 1) If the possible invasive area is composed of groups of glands without destructive invasion, regardless of their depth, LN resection is not needed. 2) If there is focal, <1 mm destructive invasion but most of the Endocx Adenca is well demarcated only sentinel nodes need to be obtained. 3) If there is destructive invasion, >1 mm, a complete LN resection is indicated. 4) VI is important only when there are 3 or more vessels involved. Adding the pattern of invasion, which is easily evaluable, to the current method of depth of invasion improves prediction of LN involvement. It would have been possible to identify 24/43 (56%) Pts who did not need extensive LN resections by evaluating the pattern of invasion.

999 Dedifferentiated Carcinoma of the Endometrium and the Ovary: A Molecular Study of 8 Cases.

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Background: Dedifferentiated carcinoma (DC) is an uncommon neoplasm accounting for 1-9% of all endometrial carcinomas (EC) being even more rare in the ovary. It is characterized by the presence of undifferentiated carcinoma (UC) juxtaposed to foci of low-grade endometrioid carcinoma (EEC). Some studies have demonstrated abnormal expression of proteins involved in DNA mismatch repair gene but the molecular features of this type of tumors have not been evaluated. Moreover, a role for p53 has been suggested in the progression from EEC to non-endometrioid (NEEC) carcinoma component, in mixed EEC-NEEC tumors.

Design: Eight DC were included (7 endometrial and 1 ovarian). Immunohistochemical study included MLH-1, MSH-2, MSH-6, PMS-2, and p53. DNA was obtained from paraffin-embedded blocks, and was subjected to microsatellite instability (MI) analysis, methylation specific PCR, and mutational analysis of p53.

Results: p53 immunostaining was positive in seven of the eight cases in the two components (strong staining in two cases, moderate in three, and low in two). Loss of MLH1/PMS-2 expression was seen in four cases (both components), while loss of MSH-2 /MSH-6 expression was seen in one (both components). In three cases, there was normal staining pattern for MLH-1, MSH-2, MSH-6, and PMS-2. MI was seen in all five cases with abnormal staining of mismatch repair genes. MLH-1 promoter hypermethylation was detected in all four cases exhibiting MI and decreased MLH-1 expression. One p53 mutation (T155P, exon 5) was found in one tumor in both components.

Conclusions: DC frequently exhibits microsatellite instability and loss of proteins involved in mismatch repair. Moreover, there is frequent p53 positivity, but with only occasional p53 mutations. The results suggest that alterations in mismatch repair genes and p53 may be associated with development of DC.

1000 Stage IIIC Ovarian Cancer-A Heterogeneous Group of Patients with Different Prognosis.

R Bakkar, D Gershenson, E Silva. MD Anderson Cancer Center, Houston, TX.

Background: Patients suffering from primary ovarian serous carcinoma who present with regional lymph node metastasis without peritoneal involvement outside the pelvis are considered clinically as stage IIIC. Survival of those patients is controversial. We have studied their survival compared to patients with peritoneal involvement beyond the pelvis who are also grouped as stage IIIC. We also included patients with primary peritoneal carcinoma with lymph node metastasis in this study.

Design: Charts of patients with stage III primary ovarian or peritoneal serous carcinoma were reviewed. Patients with primary ovarian serous carcinoma were grouped into patients with lymph node metastasis without peritoneal involvement (group 1, n=13), and patients with ovarian carcinoma with peritoneal involvement beyond the pelvis in addition to lymph node metastasis (group 2, n=43). To compare, group 3 patients (n=38)

were selected with similar criteria as group 2 but with negative sampled lymph nodes. Group 4 patients were those with primary peritoneal serous carcinoma with lymph node metastasis (n=13). Median follow up was 72 months (range 1 to 144 months).

Results:

Table1. Important clinical criteria and survival difference between patient groups

Group	1	2	3	4
Number of patients	13	43	38	13
Number with optimal Tumor Reduction	7 (54%)	26 (60%)	25 (66%)	4 (31%)
Number with preoperative Chemotherapy	0	2	3	0
Median RFS in months	42	9	12	16
Overall survival	62%	26%	36%	46%

Patients with primary ovarian serous carcinoma with regional lymph node metastasis without peritoneal involvement had better recurrence free survival (RFS) and overall survival compared to the rest of the groups (p=0.026). Survival was also better in patients with primary peritoneal serous carcinoma with lymph node metastasis than in those with primary ovarian serous carcinoma with peritoneal involvement and lymph node metastasis. There was no significant age variation and all tumors were histologically high grade. Pelvic lymph nodes were more frequently involved in peritoneal carcinoma versus aortic lymph nodes in ovarian carcinoma.

Conclusions: 1- Patients with primary ovarian serous carcinoma with lymph node metastasis without peritoneal involvement have better survival than those with peritoneal involvement.

2- Patients with primary peritoneal serous carcinoma with lymph node metastasis have better survival than those with primary ovarian serous carcinoma with peritoneal involvement and lymph node metastasis.

Based on this study, stage IIIC ovarian cancer patients need to be stratified to reflect these significant prognostic differences.

1001 Positive PTEN Expression Is Associated with Decreased Survival in Ovarian/Primary Peritoneal High Grade Serous Carcinoma.

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Background: Ovarian/peritoneal high grade serous carcinoma typically presents at advanced stage and has a low 5 year survival rate. Optimal surgical debulking and tumor sensitivity to platinum-based chemotherapy are two well-established prognostics. Molecular markers that identify more clinically aggressive tumors would allow for the development of individualized treatment options. PTEN is a key negative regulator of the PI3K signaling pathway. Loss of PTEN expression in endometrial carcinoma is associated with endometrioid histology; women with endometrioid tumors have a better prognosis than those with non-endometrioid tumors. In breast cancer, loss of PTEN is associated with resistance to Herceptin. Thus, measurement of PTEN expression has the potential to provide important prognostic information. Currently, there is no established clinical scoring system for assessing PTEN expression by immunohistochemistry (IHC). Therefore, we first established a useful PTEN IHC scoring system and then determined if PTEN expression was associated with relevant clinical variables in ovarian high grade serous carcinoma.

Design: PTEN IHC (Dako, clone 6H2.1) was assessed in 126 women with stage III, high-grade serous carcinoma of the ovary/peritoneum who were treated with surgery and then a platinum plus taxane regimen. A 3-tiered scoring system was developed (negative, reduced, positive) based on tumor expression of PTEN in relation to stroma, which was always strongly positive. Immunohistochemistry for anti-phosphorylated (serine 235/236) S6 ribosomal protein (pS6) was performed to validate the PTEN scoring system. PTEN expression was assessed in relation to clinical characteristics using Cochran-Armitage trend test, Fischer's exact test and log-rank trend test.

Results: 11% of the cases had complete loss of PTEN by IHC. The presence of significantly increased pS6 expression in these cases helped to validate the PTEN IHC scoring system. PTEN protein expression was not associated with platinum resistance or surgical debulking status. Positive PTEN expression, which was present in 58% of the cases, was significantly associated with decreased recurrence free survival compared to tumors with negative or reduced PTEN expression (18 months vs. 29 months; p=0.0009).

Conclusions: We have developed a clinically useful scoring system for PTEN IHC in ovarian cancer which can be used for assessing patient eligibility for treatment with PI3K inhibitors. Importantly, positive PTEN expression is associated with decreased survival. This may help to identify patients who can receive more aggressive therapy.

1002 Preinvasive Stage in the Endometrium: Relation and Role of Clonal Expansion and PTEN.

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Background: Physiologic endometrium may undergo proliferation under unopposed estrogenic stimulation that progress to hyperplasia. Some of the hyperplastic glands gain capacity to generate monoclonal proliferation and stromal invasion. The role of PTEN gene in this process still remains uncertain, although there are many theories. In this study, we investigated a neoplastic transition spectrum in 80 cases model to understand the place of clonal expansion and its relation to PTEN functional loss (mutation or promoter methylation) in the way of progression to invasive carcinoma process.

Design: Endometrial samples of 80 hysterectomy specimens diagnosed as benign (n=20), hyperplasia (30 with atypia, 10 without atypia) and endometrioid carcinoma were studied. Due to the performance of applied techniques like laser micro dissection, DNA extraction, PCR, clonality analysis, DNA sequencing and methylation analysis, 25 cases were excluded from the study and replaced by new cases. All hyperplasia cases were selected on the basis of the absence of concomitant carcinoma. Epithelial components were collected by laser micro-dissection. After DNA extraction, clonality was determined by human androgen receptor gene assays based on methylation- specific PCR (HUMAR-MPS). PTEN gene exons were amplified by PCR and sequenced. According to the clonality and PTEN mutation results, cases of only monoclonal and PTEN wild type hyperplasia were submitted to methylation detection procedure.

Results: All benign endometria were polyclonal (PC) and all carcinoma were monoclonal (MC). Glandular proliferation was MC in 31 and PC in 9 cases of 40 hyperplasia. Five of 10 cases of hyperplasia without atypia were PC and remaining 5 were MC. In 30 atypical hyperplasia cases, 26 were MC whereas only 4 were PC. None of the benign endometrial samples – which represented atrophy, secretion, proliferation and polyp – revealed PNEN mutation. In atypical group, only one of 10 hyperplasia cases without atypia revealed PNET mutation whereas PTEN mutations were seen in 8 cases. Four of each low and high grade carcinomas were PTEN-mutated. None of the PC samples were consistent with PTEN mutations. In all 51 MC lesions, 17 were PTEN-mutated and most of the PTEN mutations were in exon 5 and 7. In all 22 monoclonal hyperplasia cases with wild type PTEN, 13 revealed PTEN promoter methylation.

Conclusions: Our findings suggest that there is strong evidence that functional loss of PTEN, either because of mutations or promoter methylation, occurs following monoclonal expansion in the endometrial glandular neoplastic transformation.

1003 Expression Profiling of Paraffin Embedded Lymphoepithelioma-Like Carcinoma of the Uterine Cervix Using Nanostring Arrays Reveals No Evidence of Epstein-Barr Virus Infection.

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Background: Epstein-Barr virus (EBV) is found in nearly all lymphoepitheliomas of the nasopharynx and in a subset of lymphocyte rich undifferentiated carcinomas at other anatomic sites. Its presence in cervical cancer is unclear, and the sensitivity of EBV- Encoded RNA in situ hybridization (EBER-ISH) assays has been questioned. The Nanostring assay allows for detection of gene expression in paraffin embedded tissue without the use of reverse transcription or amplification, making it ideal for studies in which this is the only type of tissue available for analysis.

Design: We examined tumors from 16 patients with lymphoepithelioma-like carcinomas of the cervix using EBER ISH. To further explore the possiblity that EBV infection is present but EBER is not expressed, we designed an array-based assay to detect additional viral gene products. The Nanostring array targets 96 genes, 22 of which are EBV-encoded RNAs including latent and lytic gene products.

Results: In all 16 cases, we found no evidence of EBER expression. Control hybridization showed adequate RNA preservation, confirming that the EBER ISH results are truly negative. RNA was successfully extracted from all 12 of the cervical cancers and from 12 matched normal uterine tissues of the same patients. Nanostring array hybridization was successful in all 24 specimens as judged by recovery of 6 spiked RNAs and 4 housekeeping human transcripts. There was low background signal as confirmed by probing 8 non-human non-pathogen targets. Results of the array studies showed low to absent signal for all 22 virally encoded factors, confirming little if any latent or lytic EBV infection in the 12 benign and matched 12 malignant cervical tissues. Although unrelated to the aim of the study, the Proliferating Cell Nuclear Antigen (PCNA) Gene was uniformly overexpressed in all 12 cancers. This is a gene which is known to be involved in DNA replication.

Conclusions: This study shows that EBV is rarely associated with lymphoepithelioma-like carcinoma in the United States, and it also demonstrates performance characteristics of the Nanostring system for gene expression profiling.

1004 Molecular Alterations in Endometrial Carcinosarcomas (ECS).

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Background: Carcinosarcomas (CS) are uncommon but aggressive neoplasias with biphasic histology of carcinomatous and sarcomatous elements. Although they develop in different organs, they are most common in the endometrium, breast, lung, kidney and upper aerodigestive tract. Due to the relative rarity of Endometrial Carcinosarcomas (ECS) the pathogenesis and the specific genetic alterations underlying these tumors are still not well known.

Design: Samples from 76 ECS were obtained from the Pathology Department Massachusetts General Hospital (Boston, MA, USA). Representative areas were carefully selected from the H&E-stained sections of each tumour and two tissue cores were obtained from each specimen. Immunohistochemistry was carried out on TMA sections by the Envision method (Dako, CA, USA) using the following antibodies: TP53, p16, EGFR and β-Catenin. Fluorescence in Situ Hibridization was performed on TMA sections with Abbott Molecular Probes for EGFR. DNA was isolated from punches of the selected areas. Sequence analysis of the Exon 3 of the CTNNB1 gene was performed on those samples that shown positive nuclear staining for β-Catenin antibody. Aliquots of 41 DNA samples were sent to SEQUENOM® GMBH. SEQUENOM®'s OncoCartaTM Panel is a set of pre-designed and pre-validated SNP assays. It offers analysis of 238 simple and complex mutations across 19 common oncogenes (AKT-1, BRAF, EGFR, KIT, MET, PDGFRα, PIK3CA, H-RAS, K-RAS, N-RAS among others). Data analysis was performed with the MassARRAY® TYPER 4.0 software.

Results: We observed positive staining for TP53 in the 52% of the cases, for p16 in the 81%, for EGFR in the 32% and nuclear β-Catenin was positive in the 20%. FISH demonstrated cytogenetic alterations, like gene amplification or chromosome polysomy in the 32% of samples for EGFR. No deletions or traslocations were observed. No one of the 15 samples sequenced for the Exon 3 of CTNNB1 gene showed any mutation. In total 27 mutations of 9 different oncogenes have been detected in 17 different samples: 2% of samples shown mutations in AKT and BRAF (1 case); 5% in EGFR, MET and NRAS (2 cases); 7% in KIT (3 cases); 10% in KRAS and PDGFRα (4 cases); 20% in PIK3CA (8 cases).

Conclusions: The molecular alterations found in our ECSs suggest that, together with p53, two main pathways could be implicated in the pathogenesis of this neoplasia, like the PIK3CA-AKT and the EGFR-NRAS-KRAS pathways. Supporting by grants from: ISCIII: PI07/90324, PI 080971; RD06/0020/0013; PI-0384/2007; P07-CVI-03100.

1005 Tumor Associated Macrophages and Correlation with Prognostic Factors in Endometrial Adenocarcinomas.

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Background: The pathomechanism and significance of tumor associated macrophages (TAMS) is not fully understood. While some thought TAMS are a result of a host immune response, recent studies suggest that TAMS play an important role in tumorgenesis and angiogenesis. The aim of our study is to evaluate if the number and location of TAMS have any correlation with various prognostic findings in endometrial adenocarcinomas (EAC).

Design: Forty cases of various EAC were selected. Tumor type, grade, stage, presence of lymphovascular invasion (LVI), and clinical outcome were recorded. Immunohistochemical staining for CD68 (DakoCytomation, Carpinteria, CA) was performed on each tumor. The number of TAMS was counted, averaged over 10 high powered fields, and stratified into those associated with tumor epithelium or stroma. The data was then analyzed for correlation with various prognostic factors.

Results: There were 22 endometroid, and 18 serous or clear cell carcinomas included. The number of TAMS in the epithelium and stroma was correlated with tumor characteristics (Table 1).

Table 1: Correlation of Tumor Associated Macrophages and Tumor Characteristics in Endometrial Adenocarcinomas

	Subjects (N=40)	Epithelial TAMs: Mean	p Value	Stromal TAMs: Mean	p Value
Histology			0.127		0.609
Endometroid	22	8.1		17.1	
Serous	15	7.9		15.1	
Clear Cell	3	2.7		14.9	
FIGO Stage			0.870		0.403
I	15	6.6		13.2	
II	5	8.7		15.3	
III	14	8.4		21.0	
IV	6	7.5		13.2	
FIGO Grade			0.867		0.133
1	10	7.4		12.6	
2	9	7.4		23.4	
3	21	7.9		14.8	
Invasion			0.925		0.577
Absent	6	6.5		12.7	
Present	34	7.8		16.8	
LN Mets			0.829		0.964
Unknown	11	5.8		15.2	
Absent	15	8.0		17.1	
Present	14	8.6		16.0	
Lymph-Vascular Invasion			0.416		0.357
Absent	19	6.5		18.5	
Present	21	8.7		14.1	
Alive/Dead			0.988		0.479
Alive	28	7.5		15.5	
Dead	12	8.0		17.7	

Conclusions: Although our study did not show statistically significant correlation between TAMS and any of the prognostic factors in EAC, we concluded the following: There is an overall greater number of TAMS seen in tumor related stroma than in the epithelium. More TAMS (epithelial and stromal) are seen in tumors showing myoinvasion and/or lymph node metastasis. Finally, there are a slightly increased number of epithelial associated TAMS seen in tumors with LVI than those without.

1006 Microsatellite Instability in Advanced Stage Endometrial Endometrioid Adenocarcinoma Is Associated with a Poor Prognosis.

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Background: Microsatellite instability (MSI) arises due to loss of function of mismatch repair (MMR) proteins, commonly MLH1, MSH2 and MSH6, either through genetic loss or epigenetic silencing. There are only a few and conflicting reports on the prognostic value of MSI in endometrial cancer. The aim of this study was to investigate this parameter in endometrioid endometrial tumors with the emphasis on early vs. advanced stage disease.

Design: Immunohistochemistry for MMR proteins, MLH1, MSH2 and MSH6, was performed in 100 endometrioid carcinoma cases. The patients, aged 28-92, had no known history of HNPCC. Immunohistochemistry was scored as positive or negative. Tumors with loss of any one of the three MMR proteins were classified as having MSI, with the remainder classified as microsatellite stable (MSS). Patients were grouped as early (I and II) and advanced (III and IV) stage. Outcomes including depth of myometrial invasion (MI), lymphovascular invasion (LVI), lymph node (LN) status, relapse free survival and overall survival were examined.

Results: The results are summarized in tables 1 and 2.

TABLE 1: OUTCOMES FOR STAGE I,II: MSS VS MSI

	MSS	MSI	p-value
% Alive at last follow-up	96	100	1.00
% Relapse free	73	79	0.7597
% With LVI	29	47	0.2488
% With LN+	0	0	N/A
Median depth of MI (%)	26	33	0.7912

TABLE 2: OUTCOMES FOR STAGE III,IV: MSS VS MSI

	MSS	MSI	p-value
% Alive at last follow-up	95	58	0.0159
% Relapse free	67	25	0.0324
% With LVI	71	100	0.1247
% With LN+	61	78	0.6673
Median depth of MI (%)	42	82	0.0283

MSI was identified in 31 patients (28 MLH1, 1 MSH2, 2 MSH6). Patients were of similar age in the early (59) and advanced stage (61) groups. Unlike the early stage tumors, advanced stage tumors with MSI had significantly shorter overall survival and relapse free survival rates, as well as a greater depth of MI. They also showed a trend toward a higher incidence of LVI and LN metastases. In addition, MSI was significantly more common in grade 3 tumors (58%) than in grade 1 and 2 tumors (30%) (p=0.0441).

Conclusions: Advanced stage endometrioid endometrial carcinomas with MSI are associated with a worse prognosis than their MSS counterparts. MSI may predispose tumors toward an accelerated pace of acquisition of new mutations, leading to more aggressive tumor behavior in advanced stages. As such, identification of MSI in endometrioid endometrial tumors may be instrumental in guiding patient management.

1007 Differential Expression of Survivin and -H2AX in Vulvar Squamous Epithelial Lesions.

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Background: Survivin inhibits apoptosis, is involved in the regulation of cell cycle progression and the mitotic spindle formation. It is overexpressed in many cancers. The histone γ-H2AX is a marker of activated DNA damage and is overexpressed in different cancers and their precursor lesions. It also forms early during apoptosis.

Design: Formalin-fixed, paraffin-embedded archival tissues of 55 patients were immunostained with antibodies to surviving and γ-H2AX to determine their expression in normal squamous vulvar epithelia (NE, n=25), lichen sclerosus (LS, n=10), high-grade classic vulvar intraepithelial neoplasia (VIN, n=16), differentiated VIN (D-VIN, n=16) and vulvar invasive keratinizing squamous cell carcinoma (ISCC, n=20). Immunostaining for both factors was scored for moderate and strong intensities in two cohorts of quantity. Statistical analysis was done by chi-square and Fisher's exact test.

Results: Nuclear survivin expression increased from NE and LS to VIN and D-VIN to ISCC significantly (P=0.0001) and followed the distribution of immature squamous epithelial cells. γ-H2AX reactivity was found in nuclei of cells in all diagnostic cohorts except for NE in any epithelial level and was seen in horn pearls in ISCC, without relevant statistical distributions. Immunoscores did not differ between FIGO stages I and II, grade 1 and grades 2/3, and did not indicate prognosis.

Conclusions: Expression patterns were different for both factors, suggesting their involvement in different biological mechanisms. Expression of survivin in vulvar squamous neoplastic tissues culminating in ISCC and comparably low and insignificant γ-H2AX reactivity reflects the resistance to apoptosis in the oncogenic development of these lesions.

1008 Sertoli-Leydig Cell Tumors: Just Variants of Granulosa Cell Tumors? – FOXL2, SOX9 and SRY Distinguish the Lineage of the Sex Cord Derivatives.

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Background: Sertoli-Leydig cell tumors (SLCTs) of the ovary are composed of sex-cord derivatives resembling those of the testis and a stromal component containing variable amounts of Leydig cells. Based on morphology the sex-cord derivatives have traditionally been regarded as Sertoli cells. However, the pathogenesis has yet to be elucidated and it is unclear if these cells are true Sertoli cells. FOXL2 is a member of the forkhead-winged-helix family of transcription factors and one of the first genes expressed in the development of the female gonad. It is required for proper differentiation of granulosa cells during folliculogenesis. The transcription factor SOX9 is an intermediate downstream target of SRY and is required for testis development by formation and maintenance of (pre-) Sertoli cells. The aim of our study was to further characterize the differentiation of the sex-cord derivatives by evaluating the expression of these two counteracting transcription factors and SRY.

Design: Paraffin embedded material of 10 SLCTs, one Sertoli cell (SC) hamartoma arising in a background of testicular feminization, and a TMA containing 38 granulosa cell tumors (GCTs) were examined by IHC for the expression and localization of FOXL2, SOX9 and SRY.

Results: The sex cord-stromal derivatives revealed strong nuclear staining for FOXL2 in 10/10 cases and were negative for SOX9 expression. Leydig cells were devoid of FOXL2 and SOX9 expression. The SC hamartoma exhibited strong positivity for SOX9 and did not express FOXL2. All GCTs revealed nuclear staining for FOXL2 and SOX9 negativity. SRY was not expressed in any component of the SLCTs, the SC hamartoma or the GCTs.

Conclusions: FOXL2 actively suppresses SOX9 throughout adult life via an upstream regulatory element, leading to mutually exclusive expression of these two transcription

factors. Based on consistent FOXL2 expression in all SLCTs and GCTs, our findings support that the sex-cord derivatives of SLCTs are of granulosa cell lineage. SOX9 expression in the SC hamartoma is consistent with a true Sertoli cell component in this tumor. The encountered SRY negativity in the SCST is in concordance with previous studies. Of interest, SRY was not expressed in the SC harmatoma, however SRY activates SOX9 and might not be co-expressed. Our study suggests that the sex-cord derivatives of SLCTs are of granulosa cell and not of Sertoli cell lineage and may represent a morphologic variant of GCTs.

1009 The Sertoli Cell Associated Molecule Doublesex and Mab-3 Related Transcription Factor 1 (DMRT-1) Is Not Expressed in Sertoli-Leydig Cell Tumors of the Ovary.

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Background: Sertoli-Leydig cell tumors (SLCTs) of the ovary are composed of sex-cord derivatives resembling those of the testis and a stromal component containing variable amounts of Leydig cells. Based on morphology the sex-cord cells have traditionally been regarded as Sertoli cells. However, true Sertoli cells are not present in the adult female gonad and the origin of these cells is unclear. Doublesex and mab-3 related transcription factor 1 (DMRT1) is a key regulator of male development and is expressed in Sertoli cells of the developing and adult male gonad. Mutations in the DMRT1 gene result in defective testicular development and XY feminization.

Design: Archival paraffin embedded material of 10 SLCTs, 3 Leydig cell tumors, one Sertoli cell (SC) hamartoma arising in a background of testicular feminization, a TMA containing 38 granulosa cell tumors (GCTs), and normal ovarian and testicular tissue as controls, were examined by IHC for the expression and localization of DMRT-1.

Results: The sex cord-stromal derivatives of the SLCTs revealed no expression (0/10) of DMRT1. Two cases showed weak cytoplasmic staining in the Leydig cell component. Two cases of Leydig cell tumors were negative for DMRT1 expression, with weak cytoplasmic staining in one case. All 38 GCTs were devoid of DMRT1 expression. The Sertoli cell hamartoma showed moderate (2+) nuclear expression. Sertoli cells in adult testis showed moderate to strong nuclear expression while no expression was seen in the granulosa cells of normal adult ovaries.

Conclusions: DMRT1 is a transcription factor with a Zinc finger-like DNA-binding motif (DM domain) that is a highly conserved component of the male vertebrate sex-determining pathway. Its expression has been demonstrated in Sertoli cells of the testis by ISH in the past. We show that it is also expressed by IHC in Sertoli cells of adult testes and in Sertoli cell hamartomas, but is not seen in so called Sertoli-Leydig cell tumors of the ovary nor is it seen in GCTs or normal granulosa cells of the ovary. This suggests that the sex-cord derivatives of SLCTs are not of Sertoli cell lineage but may be of granulosa cell lineage.

1010 Sex Cord Tumor with Annular Tubules (SCTAT): A Rare Variant of Granulosa Cell Tumors? FOXL2 and SOX9 Distinguish the Lineage of the Sex Cord Derivatives.

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Background: Sex cord tumor with annular tubules (SCTAT) is a rare tumor occurring both sporadically and in association with Peutz-Jeghers syndrome. Morphologically it is composed of simple and complex annular tubules with peripherally oriented nuclei surrounding central hyaline bodies of basement membrane material. The sex cord cells have been described variably as Sertoli or granulosa cells or as "with morphologic features intermediate between those of the granulosa and Sertoli cell tumor". Ultrastructural studies have revealed variable results. FOXL2 is a member of the forkhead-winged-helix family of transcription factors and is expressed early in the female gonad. It is required for differentiation of granulosa cells during folliculogenesis. The transcription factor SOX9 is an autosomal downstream target of SRY and is required for formation of Sertoli cells. The aim of our study was to characterize the differentiation of the sex cord cells in SCTAT by evaluating the expression of these two counteracting transcription factors.

Design: Archival paraffin embedded material of 2 SCTATs were examined by IHC for expression and localization of FOXL2 and SOX9. Both cases were unilateral tumors and occurred in adult females (43 and 49 years of age). There were no stigmata of Peutz-Jeghers syndrome in either of the patients and both cases were thus considered sporadic.

Results: The sex cord cells revealed strong nuclear staining for FOXL2 in 2/2 cases and were negative for SOX9 expression.

Conclusions: SCTAT is a rare sex-cord derived tumor made up of cells variably described as Sertoli or granulosa like. Expression of FOXL2, which is found in granulosa cells and actively suppresses the Sertoli associated SOX9 throughout adult life via an upstream regulatory element, supports a granulosa cell origin for these cells. These findings suggest that sporadic SCTAT may be best classified as a variant of granulosa cell tumor.

1011 Comparison of Fluorescent In Situ Hybridization (FISH), p57 Immunohistochemical (IHC) Staining and Flow Cytometry (FC) in the Work-Up of Suspected Molar Gestation.

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Background: Distinguishing early complete hydatidiform mole (CHM), partial hydatidiform mole (PHM) and hydropic abortus (HA) can occasionally be difficult on a morphologic basis. A variety of ancillary methods (p57 IHC and DNA ploidy analysis by Flow Cytometry and more recently molecular techniques including FISH and molecular genotyping) are utilized to aid in difficult cases. There is limited literature comparing these various methodologies on a single study set of products of conception (POC) cases. The aim of this study was to evaluate the utility of FISH compared to relatively well-established p57 IHC and FC, including accuracy and turnaround time in a community hospital setting.

Design: Thirty-two cases of products of conception (POC) were retrieved from the pathology files at our institution for the time period January 2010-August 2010. All of these cases had morphologic findings that prompted further work-up with either p57 IHC (1:1000; Thermo Fisher, Fremont California), DNA ploidy by flow cytometry and/ or FISH. We retrospectively performed IHC and FISH on all cases to complete the cohort. P57 IHC was considered positive when villous trophoblasts and stromal cells showed strong positivity. FISH procedure utilized 4 centromeric probes (chromosomes 1, 17, X and Y; Abbot Molecular). Trisomy 1 and 17 is incompatible with fetal implantation, unless it is part of a triploidy and this was the rationale in picking these FISH probes over others. X and Y chromosome probes in addition to confirming the findings, allow detection of the sex chromosome complement. DNA ploidy by FC was performed using flow cytometer (BD FACSCanto II) following DNA extraction of paraffin embedded material.

Results:

Final Diagnosis (# of cases)	Flow Cytometry	P57 positivity in villous trophoblast	FISH
HA (20)	Diploid (17); Tetraploid (3)	Positive	Diploid
PM (10)	Triploid	Positive	Triploid
CHM (2)	Diploid	Negative	Diploid
	FC	P57 IHC	FISH
Turnaround time	8 days	2 days	2-3 days

Conclusions: 1. FISH offers the following advantages over FC:

a. Less hands on technical time,

b. Is less susceptible to artifacts of cellular aggregates that can cause misclassification of a diploid case as tetraploid (3/20 cases) by FC,

c. Is more sensitive because ploidy is evaluated in visualized cells, and hence maternal tissue contaminating artifact can be avoided,

d. Shorter turn around time.

2. The combination of FISH and IHC is superior to the previously used combination of FC and IHC.

1012 Non-Fimbrial Localization of Early Fallopian Tube Carcinoma: Implications for Prophylactic Surgery Technique in Women with Hereditary Risk for Pelvic Serous Carcinoma.

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Background: The fallopian tube fimbriae are the major site of localization of the earliest forms of hereditary pelvic serous cancer. Occult cancers in the fimbriae are also common in women with sporadic ovarian or peritoneal serous carcinoma. Bilateral salpingo-oophorectomy is effective in markedly reducing the risk of hereditary pelvic cancer. However, not all such tubal tumors are localized to the fimbriae. Anatomic distribution of early tubal carcinoma carries implications for technique of risk reducing surgery. This study evaluated the topography of occult tubal carcinoma, emphasizing incidence of cases exclusively involving the non-fimbrial tube.

Design: Bilateral tubes were evaluated with an enhanced dissection protocol in 212 women presenting for a variety of benign and malignant gynecologic indications. Tubes were first formalin-fixed. The fimbriae were amputed from the ampulla and sectioned parallel to the fimbrial length. The ampulla was dissected in cross-section. Slice thickness was 2 to 3 millimeters. All slices were examined microscopically.

Results: Tubal carcinoma was present in 15/212 patients. 3 patients had carcinoma exclusively in the mucosa of the tubal ampulla/isthmus whereas 12 patients had carcinoma involving fimbriae. Among the 3 non-fimbrial tumors, 2 were invasive high grade serous carcinoma and 1 was a non-invasive but exophytic high grade serous carcinoma. None were grossly visible. Size was 0.1 to 1.0 cm. None had lymphovascular invasion. Among the 3 patients with exclusive ampullary involvement of the tube, 2 had primary peritoneal serous carcinoma and 1 had primary ovarian carcinosarcoma.

Conclusions: While grossly occult tubal carcinoma most commonly involves the fimbriae, the more proximal tube may occasionally be the exclusive site of involvement. This suggests that the benefit of risk-reducing surgery is maximized if all of the tube, not just the distal portion, is completely removed. Additionally, attention to thin-sectioning and complete histologic examination should be directed to the entire tube, not just the fimbriae.

1013 Expression of RHO Family Proteins in Ovarian Endometriosis.

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Background: Recently, we found that RHO family proteins may contribute to explain the singular biologic behaviour of ovarian clear cell carcinomas, particularly their detection at early stage. Currently, it is believed that most clear cell and endometrioid carcinomas arise from ovarian endometriosis. However, little is known about the expression of RHO family proteins in ovarian endometriosis.

Design: We analyzed by Real Time PCR *RHOA*, *RHOC*, *CDC42* and three *ARHGDIs* (Rho GDI dissociation inhibitors) genes in 20 solitary endometriotic cysts, 12 endometriotic cysts with clear cell carcinoma, and 4 endometriotic cysts with endometrioid carcinoma. Immunoreaction for CDC42 was also investigated in 22 endometriotic cysts (10 solitary cysts, 8 cysts associated with clear cell carcinoma, and 4 cysts with endometrioid carcinoma). Only the non-tumor components were evaluated for RNA and protein expression.

Results: Solitary endometriotic cysts (not-associated with carcinoma) showed higher expression of *CDC42* than endometriotic cysts with cancer (P=0.047). Furthermore,

endometriotic cysts with endometrioid carcinoma had higher levels of *ARHGDIB* mRNA than endometriotic cysts with clear cell carcinoma (P=0.009). CDC42 was exclusively expressed by the macrophages. The proportion of solitary endometriotic cysts with high levels of CDC42-positive macrophages was higher than that of endometriotic cysts with cancer (6/10; 60% vs. 3/10; 30%).

Conclusions: Our results indicated that CDC42-positive macrophages and *ARHGDIB* may play an important role in early endometrioid and clear cell carcinogenesis.

1014 Expression of the Ribosomal Proteins Rplp0, Rplp1, and Rplp2 in Gynecologic Tumors.

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Background: Ribosomes are molecular complexes composed of ribosomal RNA and specific proteins known as ribosomal proteins (RPs). Approximately 80 RP have been identified in human cells and have been implicated in human disorders, particularly in genetic diseases and cancer. The importance of ribosome function and translational control is based on the regulation of ribosome production and the initiation of protein translation by several oncogenes and tumor suppressor genes including p53. Previous work from our laboratory has demonstrated that the expression of the RP Rplp1 immortalizes primary cells and is involved in transformation. This is an acidic RP that belongs to the termed P proteins and is crucial for the efficient assembly of the functional GTPase-associated center of the ribosome.

Design: To investigate the role of the P proteins in tumorigenesis, we examined the messenger RNA expression levels of Rplp0, Rplp1, and Rplp2 in a series of 32 patients with gynecologic tumors. In addition, a total of 140 biopsies of gynecologic cancers (46 endometrioid and 94 ovarian) were studied to evaluate the expression of the P proteins and p53 by immunohistochemistry.

Results: The messenger RNA expression level of all 3 P proteins was significantly higher in the tumor tissue, compared with normal tissue. An up-regulation of P protein expression was observed by immunohistochemistry in an average of 27% of the tumors, as compared with normal tissues. Moreover, the level of P protein up-regulation correlated significantly with p53 expression in serous ovarian cancers. This is an important fact because the level of overexpression of the P proteins correlated with the presence of lymph node metastases in serous ovarian cancers. We also observed that endometrial carcinomas that had invaded the myometrium overexpressed P proteins in the invasive frontat a higher level. In addition, we found that the P proteins are up-regulated in a considerable number of patients with the most common types of cancer.

Conclusions: Our study shows that P proteins are involved in human cancer, and indicates that the expression level of these proteins could be useful as a prognostic marker in specific subtypes of gynecologic tumors. Importantly, the fact that the P proteins are preferentially located in the infiltrating margins of endometrial tumors suggests that they could represent a novel molecular marker of tumor invasiveness.

1015 Micro-RNA Signature of the Epithelial-Mesenchymal Transition in Endometrial Carcinosarcoma.

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Background: Endometrial carcinosarcomas (ECS) are human neoplasias that undergo a true epithelial mesenchymal transition (EMT). The molecular determinants of the EMT in human tumours in vivo are still to be fully established, although a role for some miRNAs in EMT was recently suggested from *in vitro* cellular models, mainly involving the miR-200 family. The aim of this study was to analyze the microRNA (miRNA) signatures associated to EMT in human carcinosarcomas, and to determine their relationships with EMT markers and repressors of *E-cadherin* transcription.

Design: The expression of *E-, P-, and N-cadherin, cadherin-11, p120, vimentin, SPARC, fascin* and *caveolin-1* was studied in a group of 76 ECS by immunohistochemistry. Real-time PCR was used to measure differences in the expression of 384 miRNAs, *E-cadherin, Cadherin-11, SPARC*, *SNAIL,ZEB1, ZEB2, TWIST-1, TCF4, TGFβ1* and *TGFβ2* between the epithelial and mesenchymal component of 23 ECS.

Results: A loss of epithelial characteristics, including cadherin switching and the acquisition of a mesenchymal phenotype, was accompanied by changes in the profile of miRNA expression and the upregulation of all the *E-cadherin* repressors analyzed. There was a more than 5-fold difference in the expression of 14 miRNAs between both neoplastic components. Members of the miR-200 family are involved in epithelial differentiation and they were downregulated in the mesenchymal part of the ECS. In addition, *miR-23b*, *miR-29c*, which are involved in the inhibition of mesenchymal markers, and *miR-203* that is involved in the inhibition of cell stemness, were also downregulated. Upregulated miRNAs included *miR-155*, *miR-369-5p*, *miR-370*, *miR-450a* and *miR-542-5p*.

Conclusions: These data suggest that in human ECS, the interplay between transcriptional repressors of *E-cadherin* and miRNAs provides a link between EMT-activation and the maintenance of stemness. Supporting by grants from: ISCIII: PI07/90324, PI 080971; RD06/0020/0013; PI-0384/2007 and Proyecto de Excelencia P07-CVI-03100.

1016 Histologic Patterns of Recurrent Endometrial Carcinoma.

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Background: As targeted therapeutic agents are tested in recurrent endometrial carcinoma (REC), one cannot assume that patients with recurrence would benefit from targeted therapy based on evaluation of primary tumors. As part of a study evaluating the histologic, immunohistochemical (IHC) and genotypic (GEN) features of primary and REC, we first examine the histologic patterns of recurrence in this disease.

Design: Cases were identified (years 1999-2010) for which tissue blocks were available for both primary carcinomas and their recurrence(s). H&E-stained slides from the primary tumor and recurrence(s) were independently and randomly reviewed for histologic type and FIGO grade. The findings were considered discordant if any single pairing showed differences in either histologic type or FIGO grade.

Results: 31 primary tumors were identified with 39 recurrences. 24 (77%) cases included one recurrence and seven (23%) cases had multiple recurrences. Table 1 summarizes the histologic features of both primary and recurrent endometrial carcinomas. Concordant histology was seen in 20 (65%) cases; 11 (35%) cases were discordant. Two (66%) FIGO I endometrioid carcinomas were FIGO II at recurrence, while one appeared to recur as a tumor with ovarian-like low-grade serous morphology. Four (57%) FIGO II endometrioid carcinomas showed FIGO III (n=3) or ambiguous (n=1) histology at recurrence. One (25%) case, thought to represent FIGO III endometrioid carcinoma, recurred as serous carcinoma. Three (21%) serous carcinomas recurred as tumors with endometrioid patterns (n=2) or undifferentiated (n=1) carcinoma. All three primary carcinomas with ambiguous histology remained so at recurrence.

Histologic features of primary and recurrent endometrial carcinoma

	PRIMARY TUMORS	RECURRENCES
ENDOMETRIOID	14	18
FIGO I	3/14	1/18
FIGO II	7/14	8/18
FIGO III	4/14	9/18
SEROUS	14	14
UNDIFFERENTIATED	0	1
AMBIGUOUS	3	5
OTHER	0	1

Conclusions: In about one third of cases, REC appears histologically different than the primary. Tumor progression or sampling error may account for recurrence morphology of FIGO I endometrioid carcinoma. In high grade carcinomas, morphology is protean and recurrences often do not closely resemble primary tumors. If morphology is linked to immunophenotypic and genotypic features, then primary histology may not be predictive of histology or response to targeted therapy at recurrence. If these preliminary findings were to be confirmed by GEN and IHC studies, then sampling/removal of REC may be required for better determination of eligibility for targeted therapy.

1017 mTOR Expression Is Not Associated with FIGO Grade in Uterine Endometrioid Carcinoma.

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Background: Endometrial carcinoma is the most common cancer of the female genital tract in the US. Currently, there are few molecular prognostic markers. Our previous research has shown that while phosphatidylinositol 3-kinase (PI3K) mutations are common in uterine endometrioid carcinoma (UEC), they do not correlate with FIGO grade. Mammalian target of rapamycin (mTOR) is a growth regulator associated with activation of the PI3K pathway. Previous studies are discordant in proof of mTOR's use as a prognostic marker. In this study, we evaluated UEC for expression of mTOR, to further evaluate mTOR as a possible prognostic marker.

Design: A tissue microarray was constructed using tumor tissue from 68 cases of UEC grade I, 66 cases of UEC grade II, and 55 cases of UEC grade III. Samples of proliferative and secretory endometrium were also included in the analysis. mTOR expression was detected using standard immunohistochemical staining with rabbit monoclonal mTOR antibody (clone 49F9, Cell Signaling technology). A previously described staining index was calculated as the product of staining intensity (0-3) and extent of staining (1=1-10%, 2=11-50%, 3=51-80%, and 4=81-100%) to grade each sample. Institutional IRB approval was obtained for this study.

Results:

mTOR Expression In Uterine Endometrioid Carcinoma

FIGO Grade	Average mTOR score	Range of Score
I (n=68)	4.6±2.8	0-12
II (n=66)	6.2±3.5	1-12
III (n=55)	5.8±3.2	0-12

The average mTOR staining score for grade I tumors was 4.6 (σ=2.8), grade II was 6.2 (σ=3.5), and grade III was 5.8 (σ=3.2). There was a diversity of staining patterns regardless of FIGO grade as indicated by the range of scores. In benign proliferative endometrium there was moderate diffuse mTOR expression, while secretory endometrium epithelium showed strong diffuse mTOR expression.

Conclusions: In this study mTOR expression did not have a significant association with grade in UEC. Of note, previous studies from our laboratory did not find significant association of either *PTEN* or *PIK3CA* mutations with grade. It should be noted that mTOR was expressed in non-neoplastic proliferative and secretory endometrium. Further studies to determine the association of mTOR expression with other prognostic factors and correlation with molecular genetic alterations (e.g., *PIK3CA* and *PTEN*) are needed to determine the utility of this putative biomarker.

1018 Prognostic Indicators of Uterine Adenosarcoma Based on 2010 AJCC Staging Criteria – A Clinicopathologic Study of 39 Cases over 20 Years.

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Background: Uterine adenosarcomas are rare tumors composed of benign epithelium in combination with a malignant mesenchymal component. Due to the rarity of this tumor, American Joint Committee on Cancer (AJCC) staging criteria did not provide specific provisions for staging this lesion until the new 7th edition in 2010. The aim of this study is to determine the prognostic indicators in uterine adenosarcoma based on the new 2010 AJCC staging criteria.

Design: Patients with uterine adenosarcomas from our archive between 1991 and 2010 were retrieved. Parameters including age, follow up period, presence of sarcomatous overgrowth, homologous or heterologous elements, and staging information (as per 2010 AJCC criteria: depth of invasion, extrauterine spread, and lymph nodes status) were extracted. The time interval of recurrence was calculated from initial diagnosis to documented first recurrence of disease.

Results: Thirty-nine cases of uterine adenosarcomas with sufficient staging and follow-up information were identified within the specified study period. The mean patient age was 59.2 years (range: 36-97, median: 58). Twenty-one cases were staged as pT1a, 13 as stage pT1b, 1 as stage pT1c, 1 as stage pT2a, 2 as stage pT3a, and 1 as stage pT3b. Sarcomatous overgrowth was present in 19 cases (49%), and heterologous elements identified in 5 cases (14%). The average follow-up interval was 44 months (range: 1-185, median 25). Seven cases (5 in stage pT1a, 1 in stage pT1b and 1 in stage pT1c) recurred in 4-116 months. All 7 cases exhibited sarcomatous overgrowth, and one of them also had concurrent heterologous elements present.

Conclusions: In this limited study, there was no definitive correlation between recurrent disease and stage as defined by the 2010 AJCC staging criteria. We identified a strong association between disease recurrence and the presence of sarcomatous overgrowth in uterine adenosarcomas. A multi-institutional study with a larger cohort may be required to validate the 2010 AJCC staging criteria for uterine adenosarcomas, particularly with respect to long-term overall and disease-free survival outcomes.

1019 *KIT* Gene Mutation and Amplification in Dysgerminoma of the Ovary.

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Background: Dysgerminoma, the ovarian counterpart of seminoma, is the most common type of malignant ovarian germ cell tumor. The role of *KIT* mutation and amplification in the development of dysgerminoma is not currently established. We analyzed alterations of the *KIT* gene in a large series of dysgerminomas and correlated the findings with clinicopathologic parameters.

Design: Dysgerminoma cells from 22 patients were analyzed for *KIT* mutations at exon 17 codon 816. *KIT* amplification and chromosome 12p anomalies were investigated by dual color fluorescence *in situ* hybridization. KIT protein expression was also examined by immunohistochemistry.

Results: *KIT* exon 17 codon 816 mutations were detected in 6 cases (27%) of dysgerminomas, *KIT* amplification was detected in 6 (27%) cases and KIT expression was detected in 87% of the dysgerminomas. The *KIT* mutation was associated with advanced pathological stage ($P<0.05$), and *KIT* amplification was associated with elevated KIT protein expression ($P<0.05$). Chromosome 12p anomalies were found in 82% of the dysgeminomas and did not correlate with *KIT* abnormalities.

Conclusions: *KIT* mutations occur in approximately one third of cases of dysgerminomas and are associated with advanced stage at presentation. *KIT* is a potential therapeutic target for those dysgerminomas that have the mutation.

1020 Frequency of Known Translocations Detected by Fluorescence In Situ Hybridization in Endometrial and Endometrioid Stromal Tumors (ESTs): A Study of 57 Patients.

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Background: Translocations resulting in gene fusion are often seen in ESTs, particularly endometrial stromal nodules (ESNs) and low-grade endometrial stromal sarcomas (ESSs). While JAZF1-JJAZ1 gene fusion is most common, EPC1-PHF1 and JAZF1-PHF1 gene fusions have been rarely reported. Translocations have not been reported in ovarian endometrioid stromal sarcomas (Ov-ESS). We investigated the frequency of rearrangements involving JAZF1, JJAZ1, EPC1, and PHF1 in ESTs and Ov-ESS and correlated molecular and histologic findings.

Design: 57 patients were included. A tissue microarray was made from 62 tumors, including 19 ESNs, 27 primary ESSs, 14 metastatic ESSs, and 2 Ov-ESSs. Of these tumors, 5 and 2 metastatic ESSs samples belonged to one patient each respectively (primary tumor not available). Variant histologic patterns, including smooth muscle (7), fibrous and/or myxoid (7), sex-cord (6), glandular (3), epithelioid (1), skeletal muscle (1), and fatty (1) differentiation were seen in 7 ESNs, 10 primary ESSs, and 6 metastatic ESSs. FISH was performed on all tissue microarray samples using break-apart and fusion probes for JAZF1, JJAZ1, EPC1, and PHF1 genes.

Results: FISH was successful in 51 samples. Rearrangements of JAZF1, JJAZ1, EPC1, or PHF1 were detected in 31 tumors (61%), including 10/14 ESNs (71%), 12/23 primary ESSs (52%), 7/12 metastatic ESSs (58%), and 2/2 Ov-ESS (100%) of classic (78%) and variant morphology (35%). JAZF1-JJAZ1 gene fusion was found in 21 (68%), including 9 ESNs, 9 primary ESSs, 2 metastatic ESSs, and 1 Ov-ESS. JAZF1-PHF1 gene fusion was found in 1 primary ESS (<1%); EPC1-PHF1 gene fusion in 1 primary ESS and 1 metastatic ESS (1%). 7 cases showed rearrangements of JAZF1 and PHF1 with no apparent fusion partner. Gene rearrangement status in patients with multiple metastatic ESSs remained unchanged. Translocations were not detected in 20 cases. No correlation was found between different translocations and morphology of tumors.

Conclusions: Our results confirm that translocations are present in many ESTs and can be seen in Ov-ESS. JAZF1-JJAZ1 gene fusion is the most common rearrangement, but EPC1-PHF1 and JAZF1-PHF1 gene fusions can rarely be found. Of note, JAZF1 and PHF1 appear to have fusion partners which have yet to be identified. Although no apparent correlation between morphology and specific gene rearrangements was found, gene fusions are more common in tumors of classic morphology than EST variants.

1021 Immunohistochemical and Mutational Analysis of FOXL2 in Uterine Tumors Resembling Ovarian Sex-Cord Tumors.

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Background: Somatic mutations in the FOXL2 gene have recently been described in adult granulosa cell tumors (AGCTs). The FOXL2 C>G mutation, which converts cysteine to tryptophan, is present in the vast majority of AGCTs. Uterine tumors resembling ovarian sex-cord tumors (UTROSCTs) are rare uterine neoplasms with predominant epithelial-like patterns that morphologically, immunohistochemically, and ultrastructurally resemble ovarian sex-cord tumors to variable degrees, particularly AGCTs. It is, however, unknown whether UTROSCTs express FOXL2 protein or harbor the same FOXL2 mutation as seen in AGCTs.

Design: Immunohistochemical stains for FOXL2 protein were performed on 16 UTROSCTs. Percentage of tumor cells with nuclear staining and intensity of staining were evaluated. Mutational analysis using a TaqMan real-time PCR-based allelic discrimination assay was performed on formalin-fixed, paraffin-embedded tissue from 22 UTROSCTs.

Results: Nuclear expression of FOXL2 was present in 4 of 16 UTROSCTs. Percentage of tumor cells with FOXL2 expression ranged from <10% to 40%. Intensity of nuclear staining ranged from 1+ (2 cases) to 3+ (1 case). Mutational analysis was successful in 14 of 22 tumors, but FOXL2 mutation was not identified in any of them.

Conclusions: Although a subset of UTROSCTs show focal FOXL2 nuclear expression by immunohistochemistry, FOXL2 is not mutated in these tumors. Despite overlapping features of UTROSCTs and AGCTs, a strong histogenetic relationship between these two tumor categories cannot be inferred from these results. Thus, UTROSCTs remain in the uncertain histogenesis category of tumors as currently placed by the latest WHO classification.

1022 Persistence of Serous Tubal Intraepithelial Carcinoma after Neo-Adjuvant Chemotherapy: Evidence Based Recommendations for Gross Evaluation of Interval Surgery Cases.

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Background: The origin of pelvic serous carcinomas is a source of continuing controversy. Several recent studies of patients undergoing primary surgery for ovarian, primary peritoneal, and even uterine serous cancer have indicated the value of complete fimbrial sampling in order to detect occult serous tubal intraepithelial carcinoma (STIC). The presence or absence of STIC in specimens from interval surgery after neoadjuvant treatment has not yet, to our knowledge, been addressed.

Design: Fourteen consecutive cases of interval surgery after neoadjuvant chemotherapy were reviewed, using both H&E and, as needed diagnostically, immunohistochemistry for p53 and MIB-1, for the presence or absence of serous tubal intraepithelial carcinoma. Tubal sampling varied among the fifteen cases. Nine archived cases had non-standard sampling of the tubal fimbriae while 5 cases had complete evaluation of the tubal fimbria using a protocol for sectioning and extensively examining the fimbriated end (SEE-FIM).

Results: Residual tumor involved the endosalpinx in five out of nine (55%) cases with non-standard tubal sampling and STIC was identified in one of these. STIC was identified in four out of five (80%) cases with complete fimbrial sampling. Interestingly, p53 positive in situ tubal lesions included both proliferative regions with histologically bizarre cells consistent with chemotherapy effect, and non proliferative regions with minimal to no histologic evidence of chemotherapy effect.

Conclusions: The origin of pelvic serous carcinoma is an area of current controversy. Several lines of evidence indicate that a significant proportion of pelvic serous carcinomas may arise from in situ lesions on the distal fallopian tube. These results are expected to improve the quality of pathologic evaluation by providing data driven recommendations for sampling in interval surgery cases. These results indicate that serous tubal intraepithelial carcinomas can persist through neoadjuvant chemotherapy and can be readily identified during microscopic examination. These results also show the value of a systematic approach to grossing of the fallopian tube.

1023 Platelet Cloaking of Cancer Cells: Platelet Adhesiveness, Effect of Vascular Shear and Access of Chemotherapeutic Agents.

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Background: Recent studies have demonstrated the important role of platelets in metastasis. Tumour cell induced platelet aggregation (TCIPA), where circulating tumour cells (CTCs) may become 'cloaked' with platelets, has implications for chemotherapeutic efficacy, CTC-endothelium adhesion and CTC extravasation. CTC-platelet cloaking

kinetics are poorly understood. We examined (A) platelet-cancer cell adhesion, (B) the role of vascular shear in platelet cloak formation and (C) the impact of platelet cloaking on chemotherapeutic responses.
Design: A: Cell lines representing normal ovarian epithelium and a spectrum of ovarian cancer were studied. Gel filtered platelets were prepared from whole blood from healthy volunteers, loaded with calcein AM dye and applied to cell lines for 45 minutes. Total fluorescence and residual fluorescence following three washes, indicative of platelet adhesion, was recorded. Adhesion was calculated against total fluorescence and a fibrinogen positive control. P-selectin expression as a measure of activated platelets was also assessed by flow cytometry. B: Cone and plate viscometry was used to examine shear and non-shear effects on platelet cloaking of cancer cells. Suspensions of platelets and tumour cells were sheared for 15 minutes at 37°C at a constant shear rate of $200s^{-1}$. C: Cell survival and apoptosis was measured in platelet-cloaked and uncloaked ovarian cell lines treated with paclitaxel or 5-fluorouracil.
Results: Cancer cells demonstrated varying adhesiveness and a dose-dependent ability to activate p-selectin after platelet exposure. Cloaking of cancer cells occurred under venous shear, with infrequent cloaking in static conditions. Cloaked cancer cells demonstrated significantly greater cell survival ($p<0.05$) in the presence of chemotherapeutic agents than uncloaked cells.
Conclusions: Cancer cells form platelet cloaks, which is dependent on vascular shear. Platelet cloaking reduces chemotherapeutic efficacy in cancer cells. The role of platelets in the metastatic highway appears to be important with potentially serious outcomes for patients with cloaked CTCs. *This work is supported the Irish Health Research Board (HRB) PhD Programme in Molecular Medicine: From Genes to Function.*
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1024 Loss of PTEN Expression but Intact Expression of Mismatch Repair Proteins (MLH-1 and MSH-2) in Atypical Polypoid Adenomyomas of the Uterus.

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Background: hosphatase and tensin homolog (PTEN) is a tumor suppressor gene located at 10q23 that encodes a phosphatase that works within the AKT pro-apoptotic pathway and the MAPK pathway to regulate cellular growth as well as other functions. Mutations of PTEN have been implicated in inherited cancer syndromes such as Cowden disease. Patients with this disease typically develop multiple hamartomatous tumors and are at increased risk for breast, thyroid and endometrial cancers. Mutations of PTEN are present in up to 83% of spontaneous endometrial adenocarcinomas, making it the most frequent molecular genetic alteration seen with these tumors. Additionally, deficiencies in mismatch repair proteins are frequently seen with endometrial carcinoma. Recently, we encountered a patient with Cowden syndrome who developed an atypical polypoid adenomyoma (APA) of the uterus which prompted an investigation of the prevalence of loss of PTEN and mismatch repair protein(MHL1 and MSH2) expression in APAs.
Design: A series of APAs were reviewed and diagnoses were confirmed. These included a mixture of curettage and hysterectomy/polypoidectomy specimens. PTEN, MLH1, and MSH2 expression was examined using immunohistochemistry in formalin-fixed, paraffin-embedded tissue. Cases were considered to be PTEN reactive if they demonstrated cytoplasmic staining of the glandular component of the APA and non-reactive if there was no cytoplasmic staining of the glandular component but the stroma or other internal control tissues was reactive. Similarly, MLH1 and MSH2 were considered positive with nuclear staining of the neoplastic cells, and negative if the neoplastic cells were negative but an internal control was positive.
Results: Immunohistochemical staining for PTEN demonstrated a total of 12 of 20 (60%) cases with staining of the glandular component of the lesion. A total of 7 of 20 cases demonstrated stromal and/or squamous metaplasia positive for PTEN but no staining of the glandular component. All of the cases retained expression of MLH1 and MSH2.
Conclusions: PTEN expression is lost in a subset of APAs, albeit less so than is seen with endometrial carcinomas. There was no loss in the expression of mismatch proteins. Uncommonly, APAs develop in patients with Cowden disease.

1025 Alterations in MyD88 and microRNA Expression Are Associated with Chemoresistance in Epithelial Ovarian Cancer.

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Background: The prognosis of epithelial ovarian cancer (EOC) is poor, in part due to the high frequency of chemoresistance. The Toll-like receptor-4 pathway is thought to mediate some of this resistance, particularly via the adaptor protein MyD88. We previously described different clinicopathologic features associated with TLR-4/MyD88 expression in EOC, including predominance of MyD88 expression in serous carcinomas and significantly reduced survival if MyD88+. We examined the expression of MyD88 in chemosensitive and chemoresistant EOC cells, together with two microRNAs that regulate the TLR-4/MyD88 pathway (miR-21 & miR-146a), to assess the direct association between biological behaviour, MyD88 and its molecular regulators.
Design: Archival paraffin-embedded EOC samples (n=22) were divided into type 1 (MyD88+) or type 2 (MyD88-) based on previous immunohistochemical (IHC) expression. Pure tumour samples were obtained using laser capture microdissection, and RNA extracted for gene (n=22) and miRNA analysis (n=18). RNA was extracted from 8 human cancer cells lines, EOC lines 59M, SK-OV-3, A2780 & IGROV-1, and cervical cancer cells KB-3-1, as well as chemoresistant cancer cells A2780cis (Cisplatin-resistant), IGROV-1CDDP (Cisplatin & Paclitaxel-resistant) and KB-8-5-11 (Paclitaxel-resistant). Expression of TLR-4 & MyD88 genes and miRNAs miR-21 & miR-146a was assessed using TaqMan® Real-Time PCR.
Results: TLR-4 & MyD88 gene expression in archival patient samples showed marked heterogeneity but MyD88 expression correlated with previous IHC categorisation into type 1 and 2 tumours. In cell lines MyD88 was expressed (relative to A2780 which is MyD88-) in 59M, SK-OV-3, IGROV-1 & KB-3-1. However, MyD88 expression was significantly increased in all 3 chemoresistant cell lines (A2780cis, IGROV-1CDDP & KB-8-5-11). MicroRNA analysis in archival samples showed significantly increased expression of miR-21 and miR-146a in MyD88- cancers. Similarly, increased miR-146a was detected in those EOC and cervical cells with increased MyD88 expression, particularly A2780cis & KB-8-5-11, although miR-21 was decreased in A2780cis cells.
Conclusions: Our results demonstrate significant alterations in MyD88 expression between chemosensitive and chemoresistant ovarian cancer cells, and significant changes in microRNA expression between MyD88+ & MyD88- cancers. This provides further evidence that MyD88 expression, in part regulated by miR-21 and miR-146a, is associated with adverse biological characteristics including resistance to standard platinum and taxane-based chemotherapies, with consequent reduced survival.

1026 Temporal Relationships between Tamoxifen Use and Endometrial Malignancies.

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Background: The development of uterine malignancies during and after the use of tamoxifen is a well-documented phenomenon. The purpose of the study was to determine if there was a correlation between the duration of tamoxifen therapy, the development of these malignancies in relation to cessation of the drug, and tumor type and/or grade. In addition, we sought to determine any recurrent morphologic features present in these tumors.
Design: All women with a history of breast cancer treated with tamoxifen who subsequently developed a uterine malignancy between 1980 and 2005 were included in the study.
Results: A total of 119 such patients were identified. The distribution of tumor type was as follows: 64 FIGO 1-2 endometrioid carcinomas (EC), 7 FIGO 3 endometrioid carcinomas, 28 serous carcinomas or mixed endometrioid and serous carcinomas, 3 clear cell carcinomas, 2 undifferentiated carcinomas, 13 carcinosarcomas (MMMT) and 2 leiomyosarcomas. There was a higher incidence of high grade tumors in patients who received tamoxifen for 5 years or more (59%), compared to those who used tamoxifen for less than 5 years (26%). Low grade tumors also appear to develop more frequently early on after the use of tamoxifen; the frequency of high grade tumors was more in cases where the interval between cessation of tamoxifen therapy and diagnosis of endometrial carcinoma was more than 1 year (58%) compared to less than 1 year (38%). In the slides available for review, a number of recurrent morphologic features were noted including prominent squamous/morular metaplasia, background polyps and/or polypoid tumors, background endometrial atrophy (also seen in endometrioid tumors) and presence of prominent ovarian stromal hyperplasia.

	FIGO 1-2 EC	FIGO 3 EC	Serous	MMMT	Others
Tamoxifen therapy duration ≥5 years	30	6	24	10	3
Tamoxifen therapy duration <5 years	33	1	5	3	4
Interval between tamoxifen cessation and EC Less than 1 year	44	2	13	8	5
Interval between tamoxifen cessation and EC 1 year and more	19	4	1	5	2

Conclusions: Patients treated with tamoxifen therapy for breast carcinomas can develop a spectrum of endometrial malignancies of all histologic subtypes. Patients who received long duration of tamoxifen therapy had a tendency to develop higher grade tumors, and these often developed later on after tamoxifen cessation. In contrast, patients who used tamoxifen for shorter time periods were more prone to developing lower grade tumors that often presented early after discontinuation of tamoxifen use, with the exception of carcinosarcomas that often presented early.

1027 Overexpression of the Hedgehog Pathway Component Gli2 in Uterine Leiomyosarcoma (LMS) and STUMP: A Morphoproteomic Approach.

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Background: Glioma-associated oncogene 2 (Gli2) is a zinc finger transcription factor whose nuclear expression has been associated with aggressive behavior and invasiveness in melanomas and urothelial carcinomas. Downregulation of Gli2 is associated with the inhibition of proliferation. Gli2 is generally regarded as an effector of the hedgehog signaling pathway but can also be induced by the transforming growth factor-beta/Smad3 signal transduction. Its role in myometrial tumors has not been previously studied.
Design: We examined the expression of Gli2 in a spectrum of uterine smooth muscle tumors. A tissue microarray block was assembled from 11 uterine LMS, 16 smooth muscle tumor of uncertain malignant potential (STUMPs), 14 leiomyomas (LM) and 11 normal myometria. Morphoproteomics uses bright-field microscopy and immunohistochemistry to study the signal intensity, subcellular compartmentalization and extent of distribution in a tumor of protein analytes in signal transduction pathways. Immunohistochemical stains were performed using antibody against Gli2. The intensity (1-3+) and percentage of chromogenic signal was determined, and a composite score (CS), the product of the intensity and percentage was calculated. The significance of difference in means was determined by one way ANOVA.
Results: Gli 2 expression was localized to the nucleus. Gli 2 was positive in 81% (9/11) uterine leiomyosarcomas and majority of Gli2 positive LMS {8/9 (89%)} show moderate to strong (2-3+) signal intensity. The average CS of LMS is 140 (range 0-300). In contrast, 71% (10/14) leiomyomas show mild (1+) positivity with an average CS of 71 (range 0 -100). Three of eleven (27%) of normal myometria showed mild (1+)

positivity with an average CS of 21 (range 0 -100). Fifteen of sixteen (93%) STUMPs showed Gli2 positivity with 33% (5/16) showing moderate to strong (2-3+) positivity and an average CS of 115 (range 0-200). Statistical analysis demonstrated significant difference in Gli 2 expression in LMS versus leiomyoma and normal myometria and in STUMP versus normal myometria.

Conclusions: Gli2 expression with nuclear translocation shows a progressive increase from normal myometria to leiomyoma to STUMP to leiomyosarcoma supporting its role in tumorigenesis and malignant transformation. In this context, targeting Gli2 pathways represents a therapeutic option and should forestall the process.

1028 PAX8 and WT1 Are Superior to PAX2 and BRST2 in Distinguishing Mullerian Tract Tumors from Breast Carcinomas.

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Background: Determining primary site of metastatic carcinoma remains challenging, especially in women with breast and gynecologic cancers. To determine the best panel of immunohistochemical (IHC) markers to identify primary site, we evaluated WT1, BRST2, PAX2 and PAX8 in a large series of primary and metastatic mullerian and breast carcinomas.

Design: IHC for PAX2, PAX8, WT1, and BRST2 were performed on tissue microarrays (TMA) (see table for case breakdown). The 249 uterine carcinomas in the TMA consisted of 207 endometrial and 42 endocervical carcinomas. 10 whole sections of mullerian metastases were also evaluated. TMAs were scored as negative, weak or strong. Decision trees were constructed using Rpart in "R".

Results: The best performing classifier included Pax8 and WT1, achieving an accuracy on the study set of 78%. Accuracy was not significantly improved by adding PAX2 and/or BRST2. Due to the relatively small number of ovarian non-serous cases, this classifier was not useful for discriminating these cases from the other groups but showed 94% accuracy when the analysis was limited to uterine, ovarian serous carcinomas, and breast.

IHC Expression in Mullerian and Breast Carcinomas

	PAX2	PAX8	WT1	BRST2
All uterine carcinomas (n=249)	69 (27.7%)	232 (93.2%)	6 (2.4%)	19 (7.6%)
Ovarian, serous carcinoma (n=146)	44 (30.1%)	138 (94.5%)	134 (91.8%)	4 (2.7%)
Ovarian, non-serous carcinoma (n=66)	8 (12.1%)	48 (72.7%)	14 (21.2%)	4 (6.1%)
Mullerian metastases (n=10)	7 (70.0%)	10 (100.0%)	6 (60.0%)	0 (0%)
Endometriosis (n=5)	3 (60%)	5 (100%)	4 (80%)	0 (0%)
Breast carcinoma (n=210)	0 (0%)	0 (0%)	4 (1.9%)	165 (78.6%)
Breast metastases (n=87)	3 (3.4%)	9 (10.3%)	2 (2.3%)	67 (77.0%)

Conclusions: A combination of PAX8 and WT1 is 94% accurate in differentiating breast, uterine, and ovarian serous carcinomas. However, PAX8 and WT1 negative ovarian non-serous carcinomas are misclassified as breast carcinomas by this algorithm; these cases consist of 28% mucinous, 50% endometrioid, and 22% clear cell carcinomas. Since addition of BRST-2 or PAX2 provides little additional information, identification of primary site by IHC may be more problematic for these histologic subtypes. Only strong nuclear PAX8 staining should be considered positive in the clinical diagnostic setting because weak PAX8 can be seen in 10% of breast metastases, 56% of which are also BRST2(-); weak PAX8(+)/BRST2(-)/WT1(+) phenotype may also rarely occur.

1029 Ovarian Serous Tumors of Low Malignant Potential with Nodal Low Grade Serous Carcinoma.

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Background: Serous tumors of low malignant potential (SLMPs) and low-grade serous carcinoma (LGSC) are part of one biological continuum, whereby SLMP can transform into LGSC. In up to 22% of ovarian SLMPs with lymph node (LN) involvement, LNs are the only site of extra-ovarian disease. We and others have suggested that some nodal SLMPs arise from nodal endosalpingiosis and evolve independently in LNs, rather than being related to the ovarian primary. Furthermore, some cases of ovarian SLMP have been associated with nodal LGSC, which could result from a similar pathogenetic mechanism. This is the first clinicopathologic study of 5 such cases.

Design: We identified 5 cases in the MD Anderson Cancer Center pathology database with ovarian SLMP and nodal LGSC. Clinical follow-up was obtained from chart review. Pathology reports and slides were reviewed to confirm the diagnosis of pure ovarian SLMP and record the location of lymph nodes with endosalpingiosis, SLMP and LGSC.

Results: The patients ranged from 28-68 years in age (median 32). LGSC was identified in supraclavicular (2), cervical (1), periaortic (1) and intramammary (1) LNs, either preceding (by 7 months), concurrently, or following (up to 24 months) the diagnosis of ovarian SLMP. In 3 cases, pelvic and periaortic LNs were collected during the ovarian tumor resection, with nodal SLMP and endosalpingiosis identified in 3 and 2 cases respectively. In the remaining 2 cases, lymph node dissection was not performed. All except one patient received adjuvant platinum-based therapy. Follow up ranged from 1 to 20 years (average 7 years). None of the patients recurred in the pelvis. 3 patients are free of disease. However, one patient with cervical nodal LGSC developed brain metastases and died. Another patient with supraclavicular nodal LGCS is currently living with bilateral malignant pleural effusions. Both patients had pelvic/periaortic nodal SLMP and extensive nodal endosalpingiosis.

Conclusions: For the first time, we present a case series of patients with ovarian SLMP and nodal LGSC. Despite no evidence of LGSC in the pelvis, or any pelvic recurrences, the patients developed extrapelvic nodal LGSC. These patients also had nodal endosalpingiosis and SLMP, suggesting that SLMP/LGSC tumors in LNs may arise independently of the ovarian primary, progress along their own timeline and undergo metastatic spread. Therefore, in patients with ovarian SLMP and extensive pelvic/periaortic nodal SLMP and/or endosalpingiosis, examination and follow-up of extrapelvic LNs is warranted.

1030 Increased Matrix Metalloproteinases-1,-9 in the Uterosacral Ligaments and Vaginal Tissue from Women with Pelvic Organ Prolapse.

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Background: Pelvic Organ Prolapse (POP) is a widespread disease, associated with significant morbidity, but its pathophysiology is still poorly understood. Reduced collagen content, altered ratios of different collagen types, and increased tissue expression of matrix metalloproteinases (MMPs) have been found in samples of vaginal tissue taken from women with prolapse compared with controls. MMP-1 cleaves type I collagen, the most abundant connective tissue protein. Thus, it may play a critical role in the loss of tissue strength in POP. MMP-9 is a rapid gelatinase degrading MMP-1 cleavage products. Our purpose was to investigate the possible association of increased MMPs-1,-9 expression with POP. Additionally, we aimed to evaluate whether inflammatory processes contribute to POP development, possibly by release of various cytokines.

Design: 40 women who underwent hysterectomy, 20 with POP grade 2 and above, and 20 without POP, participated in the study. Biopsies from the uterosacral ligaments and vaginal mucosa were obtained from each woman. Each biopsy was sectioned and stained with MMP-1 and MMP-9 by immunohistochemical methods and with hematoxyline and eosin (H&E). MMP-1,-9 expression was evaluated on the immunostained slides. Possible inflammatory changes (lymphoplasmacytic infiltrate and vascular proliferation) were examined on the H&E stained slides.

Results: A higher stromal (extra-cellular) expression of MMP-1,-9 was found in POP cases compared with controls in vaginal biopsies (MMP-1: p=0.004, MMP-9: p=0.042) as well as in uterosacral ligaments biopsies (MMP-1: p=0.011, MMP-9: p=0.015). Increased intracellular expression of both MMPs was also demonstrated in fibroblasts in vaginal and uterosacral ligaments biopsies of women with POP ($p<0.001$ for MMP-1 and MMP-9). The degree of inflammatory changes, reflected by the number of lymphocytes, plasma cells and capillary-sized blood vessels per 10 high power fields, was similar in specimens obtained from women with and without POP.

Conclusions: The expression of MMP-1,9 appears to be increased in tissues from women with POP. It is possible that MMPs-1,-9 contribute to loss of connective tissue strength and thus participate in the pathogenesis of POP. However, it is also plausible that the biomechanical changes associated with POP resulted in the alteration of MMPs expression. Inflammation does not seem to play an important role in the pathogenesis of POP.

1031 Diagnostic Utility of $p16^{INK4a}$ in Cervical Biopsy Specimens from the Michoacán Cervical Cancer Screening Study II (MECCS II): Correlation with HPV Test Results and Cervical Cytology.

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Background: Surgical biopsy is the gold standard for diagnosis of CIN /carcinoma. CIN 2 is the least reproducible of CIN diagnoses. The $p16^{INK4a}$ (p16) immunostain is a useful objective adjunct in diagnosis of CIN 2. We studied the use of p16 in cervical biopsies from women enrollled in MECCS II.

Design: MECCS II was conducted in 2 sites in Michoacán, Mexico. Pts. ages 30-50, non-pregnant, with varied histories of screening, no history of hysterectomy or pelvic irradiation participated. All pts. had direct samples obtained for HPV testing (Qiagen HC2, Gen-Probe Aptima) and a ThinPrep Pap (Hologic). Pts. + on any test were recalled. At the 2nd visit VIA was done to rule out large (≥3 quadrants) pre-invasive disease or cancer, colposcopy and biopsy followed using the POI directed and random biopsy protocol (≥5biopsies/patient). All HC2 positive subjects eligible by VIA triage were treated with cryotherapy. P16 immunostaining (Cintec) was performed on all CIN biopsies. P16 was considered positive in CIN 2+ when diffuse, strong staining was seen.

Results: 503 pts. with a +HPV test or abnormal Pap were re-called for secondary screening and treatment (34 lost to follow up). 2,350 biopsies were reviewed and many showed marked acute and chronic cervicitis. Table 1 summarizes Pap test results, HPV status and p16 results in patients with CIN 2+. Of 9 patients with CIN 2 and negative p16, 3 were HPV -, 4 had NILM Paps and 5 had CIN 2 in 1 biopsy only. Table 2 shows a comparison of sensitivy, specificity and positive predictive value with and without p16 results.

Table 1: Biopsy Diagnosis of CIN 2+

Diagnosis	HPV+	HPV-	Pap ≥ ASCUS	NILM/ Unsat Pap	p16+	p16-
CIN 3 (n=12)	12	0	9	2/ 1	12	0
CIN 2 (n=22)	18	4	15	5/ 2	13	9
AIM (n=3)	1	2	1	2	1	2

Table 2: Sensitivity, specificity and PPV for CIN 2+

Screening test	Sensitivity CIN 2+	Specificity CIN 2+	PPV CIN 2+
Pre-p16			
HR-HPV	81.4%	92.6%	18.9%
Pap ≥ ASCUS	73.2%	94.9%	22.6%
Post- p16			
HR-HPV	84.4%	92.7%	20.5%
Pap ≥ ASCUS	76.2%	95.0%	24.1%

Conclusions: P16 was helpful in identifying CIN 2 in our study and enhanced the sensitivity of HPV testing and cervical cytology. Pts. with CIN 2 in a single quadrant biopsy were more likely to be HPV – and p16 – than those with multiple quadrant CIN 2. P16 was helpful in the setting of markedly inflamed biopsy samples. All patients with CIN 3 were HPV positive and p16 positive.

1032 Gene Expression Analysis Identifies Two Groups of Ovarian High-Grade Serous Carcinomas with Different Prognosis.

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Background: Gene expression profiling is an important tool to evaluate genetic heterogeneity in carcinomas and is useful for developing expression-based classifications of many cancers as well as markers of disease outcome.

Design: We have examined by Taqman Low-Density Array (TLDA) the expression profiling of 22 genes involved in the PI3K-AKT pathway in 26 high-grade ovarian carcinomas (19 serous and 7 clear cell carcinomas). Gene expression pattern was analyzed by hierarchical clustering analysis and results were correlated with clinicopathological features. To validate the gene expression data, we selected two markers (caspase-3 and XIAP) and investigated their protein expression in 18 high-grade serous carcinomas.

Results: Unsupervised hierarchical clustering divided high-grade ovarian carcinomas into three groups. All clear cell carcinomas clustered into one group. In contrast, high-grade serous carcinomas were segregated into two clusters with different prognosis (P=0.05). High expression of *CASP3*, *XIAP, NFKB1*, *FAS,* and *GSK3B* mRNAs identified high-grade serous carcinomas with better prognosis. These cluster groups were of prognostic significance independent of age, tumor size, and tumor stage in multivariate analysis (P=0.008). Immunoreaction for caspase-3 was concordant with the results obtained by gene expression analysis (Spearman r=0.762, P=0.000). Furthermore, co-expression of caspase-3 and XIAP identified cases with different prognosis (P=0.03).

Conclusions: Our results suggest that there are different subtypes of high-grade serous carcinomas with different biological behavior.

1033 Lymph Node Counts in Endometrial Cancer: Expectations Versus Reality.

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Background: It has been proposed that a thorough lymph node dissection (LND) should yield a median of 35 pelvic LNs (PLNs) and a median of 17 para aortic LNs (PALNs).This resulted in a discrepancy between the expected and reported LN counts at our institution.To address this issue, we began submitting the residual adipose tissue (AT) following routine processing of LNDs to determine if this discrepancy was due to undetected LNs.This study seeks to evaluate whether this approach significantly increases the reported number of LNs in a cohort of patients (pts) treated for EC at our institution.Additionally, we evaluated the association of procedure type, body mass index (BMI) and EC subtype to the number of LNs obtained.

Design: The number of LNs from all hysterectomies for EC with LNDs performed at MDACC from 2006 to the present was recorded and divided into regions as specified in the pathology report.H&E slides from cases in which residual AT was submitted were reviewed to determine the number and size of any additional LNs.Procedure type, histology of the EC, and BMI were also noted.

Results: 1-38 PLNs (median, 11) and 1-25 PALNs (median, 6) were obtained in 258 pts. 47 of 79 cases (59.5%) in which residual AT was submitted were found to have additional LNs (median size 4.0 mm): PLN, median=2; PALN, median=3.There was no significant association between the number of LNs obtained and whether or not residual AT was submitted (PLN, p=0.2; PALN, p=0.78; total LNs, p=0.19). Furthermore, there were no cases in which metastatic EC was present exclusively in the additional LNs.Compared to open hysterectomy, laparoscopically and robotically obtained LNDs had an average of 3 and 0.8 more PALNs respectively (p=0.002).No similar association was found for PLNs or total LNs.No evidence of an association between BMI and total PALNs (p=0.4) or total PLNs (p=0.2) was identified.The same was true for histologic subtype.

Conclusions: Open surgical procedures do not increase the number of LNs obtained compared to minimally invasive techniques.There is no significant difference in numbers of LNs obtained between obese and non obese pts.The median number of PLNs and PALNs in a cohort of EC cases from a tertiary care center fell short of the proposed recommendation for median numbers of PLNs and PALNs.Submitting residual AT did not significantly increase the numbers of reported LNs.These results validate that standard processing of LNDs adequately reflects the actual number of LNs present.The current definition of a thorough LND may be unrealistic and should be revisited.

1034 Reverse Phase Protein Lysate Array (RPPA) Identifies Differential Protein Expression in Uterine Carcinosarcoma.

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Background: Uterine carcinosarcoma (CS) is a highly aggressive tumor characterized by admixtures of carcinoma (CA) and sarcoma (SA). Although the biphasic pattern of CS is diagnostic, individual tumors have marked heterogeneity with respect to differentiation. We adapted RPPA to the use of formalin-fixed, paraffin embedded sections of uterine CS to determine if differential patterns of protein expression correlate with histologic features of CS.

Design: We evaluated 52 uterine CS for histologic subtype of CA, presence/histologic subtype of heterologous elements (HE), ratio of CA to SA, depth of myoinvasion (MI), presence of CA or SA in MI, lymphovascular invasion, presence/histology of lymph node metastases, and presence/histology of recurrences. 50 µg of tumor protein were extracted from each block, and these proteins were subjected to RPPA analysis using a panel of 120 different antibodies previously validated for this assay.

Results: CS with HE showed the most prominent differences in protein expression. Twenty-eight proteins were differentially expressed in CS with chondrosarcoma (ChS) or rhabdomyosarcoma (RS) compared to CS without RS or ChS. These findings are summarized in tables 1 and 2.

Proteins Differentially Expressed in CS with Rhabdomyosarcoma[1]

Protein	p Value
A_RAF	0.01
Caveolin	0.01
CHK1	0.02
ATM	0.01
Cyclin D1	<0.01
EGFR.V	0.01
ER	<0.01
JUNB	0.03
p53	0.03
PAX2	<0.01
SRC	0.02
STAT3	0.01
HER2/neu	0.03
VEGFR	<0.01

[1]proteins uniformly upregulated

Proteins Differentially Expressed in CS with Chondrosarcoma[1]

Protein	p Value
X4EBP1	0.02
AKT.V	0.03
BIM.V	0.01
Casein kinase	0.03
CHK2	0.03
KU80	0.02
PLK1	0.02
YBI_pS102	0.02
Cyclin E1	0.01
FAK_pY925	0.02
GSK3a	0.03
IRS1.V	0.02
NFKB_p65	0.03
SRC	0.02

[1]proteins uniformly downregulated

Conclusions: The presence of HE in CS confers a worse prognosis. Using a novel technology, we have identified a number of different proteins that may be responsible for the more aggressive clinical behavior of CS with HE. Pertinent to therapy, some of the identified upregulated proteins (EGFR, ER, Her2, BEGFR2, AKT and SRC) are cell signalling pathway components that are potential therapeutic targets. These findings underscore the need for pathologists to recognize the presence of HE in CS.

1035 Primitive Neuroectodermal Tumors Primary in the Adnexal Region. The MD Anderson Experience.

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Background: Primitive neuroectodermal tumors (PNET) of the adnexal region are rare.In this study, we present the clinicopathologic features of 18 such cases seen at our institution over a period of 20 years.

Design: 18 cases of PNETs were retrieved from our department files spanning 1990-present.All available pathology material was reviewed.Immunohistochemical studies (IHC) were performed in 15 cases, and Fluorescent In Situ Hybridization (FISH) for EWSR1 translocation in two cases.Clinical information was obtained from patients' (pts) charts.

Results: Ages ranged from 18-81 years (median 31).Clinical presentations included abdominal/pelvic pain, 6; pelvic mass, 5; abdominal distention, 1; incidental mass, 2;unknown, 4.Following surgical resection, 15 tumors were identified in the ovary, and 3 were para ovarian.FIGO stage was known in 15 cases: I (5); II (1); III (8); IV (1). 7 tumors were pure PNET; 11 were combined with mature teratoma (4), immature teratoma or yolk sac tumor (3), carcinoma (3), and carcinosarcoma (1).Histologically, all PNETs were characterized by small, round to spindle shaped cells predominantly in a patternless sheet.Other histologic features included: pseudopapillary structures (4), myxoid background (4), fibrillary background (7) and rosettes (true, 6; Homer-Wright, 7).In PNET combined with immature teratoma, recognition of the PNET was facilitated by an absence of primitive neurotubules and the presence of disorganized sheets of cells.The most commonly used IHC to facilitate diagnosis included: keratin, 2/13 pos;synaptophysin, 2/12 pos;neurofilament 9/9 pos;CD99 2/7 pos;GFAP 4/9 pos;CD56 6/7 pos;and S100 4/7 pos.FISH for EWSR1 demonstrated a translocation in 1 of 2 pts tested.Follow up ranging from 3-107 mos (median 18.5) was available for 16 pts:dead of disease, 9; alive with disease, 2; no evidence of disease, 5. 4 of 5 pts alive without disease were stage I at presentation with 1 currently completing adjuvant chemotherapy.All 5 pts received chemotherapy with 2 treated as neuroblastoma and 2 treated as Ewings sarcoma.

Conclusions: PNET of the adnexal region is aggressive and tends to affect young adult women.It is more commonly encountered in combination with other tumor types, particularly teratoma.Keratin, synaptophysin, and neurofilament are most useful in distinguishing PNET from other entities in the differential diagnosis.The low expression of CD99 suggests that adnexal PNETs more closely resemble central type PNET.

1036 Expression of Tissue Factor and Heparanase in Endometrial Clear Cell Carcinomas: Clinicopathologic Significance and a Possible Role for Tissue Factor in Thromboembolic Events.

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Background: Preliminary analyses suggest that patients with endometrial clear cell carcinoma (CCC) may have an increased risk for thromboembolic events [TVE]. Tissue factor (TF) and heparanase (Hep) are 2 proteins that are involved in activation of the coagulation cascade and several aspects of tumor progression. Hep may also induce TF expression. The purpose of this study is to assess the clinicopathologic significance of TF and Hep expression, especially as they relate to the risk of TVE, in CCC and selected other endometrial cancers

Design: 84 endometrial carcinomas, including 17 CCC, 20 serous carcinomas (SC), 15 grade 1 endometrioid carcinomas (g1-EC), 15 grade 2 EC (g2-EC), 10 grade 3 EC (g3-EC), and 7 mixed carcinomas with at least a 10% clear cell component (mixed CCC), were evaluated for the expression of Hep and TF by immunohistochemistry, and their associated frequency of TVE

Results: 7 of 84 patients experienced 8 TVE during the follow-up period. On univariate analysis, CCC (4/17 cases) was more likely to be associated with a TVE than all other carcinomas (3/67 cases, p=0.03). On multivariate analysis, the CCC histotype (OR 5.2, 95% CI 2.4523-13.6754, p=0.026) was significantly associated with an elevated risk for TVE. TF expression was present in 12 (14.28%) of 84 endometrial carcinomas, including in 41% of CCC, 10% of SC, 14% of mixed CCC, 13% of g1-EC, and in 0% of grade 2 and 3 EC. TF expression was significantly more likely to be seen in CCC than all other cancers (p=0.0018) and all other high grade cancers (p=0.007). On multivariate analysis, TF expression was significantly associated with the risk of TVE (OR 4.8, 95% CI 1.9196-11.93, p= 0.013). TF expression was not associated with patient outcome or any other clinicopathologic parameter. Hep expression was present in 57 (67.8%) of 84 endometrial carcinomas, including 59% of CCC, 43% of mixed CCC, 25% of SC, 100% of g1-EC, 100% of g2-EC, and in 90% of g3-EC, and was significantly associated with the endometrioid histotype, myometrial and lymphovascular invasion, but not outcome or the risk of TVE. For the overall group, there was no significant correlation between Hep and TF expression (Spearman rank correlation, r = 0.311, p=0.4)

Conclusions: Patients with CCC have an increased risk of developing TVE as compared with patients with the other histotypes. This increased risk may be related, at least in part, to an increased rate of TF expression in CCC.

1037 Dysregulation of the Phosphatidylinositol 3' Kinase-Akt-Mammalian Target of Rapamycin (P13K-Akt-mTOR) Pathway in Smooth Muscle Tumors of the Uterus: Clinicopathologic Implications.

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Background: Preclinical analyses strongly implicate the phosphatidylinositol 3' kinase-Akt-mammalian target of rapamycin (P13K-Akt-mTOR) signaling pathway in smooth muscle tumorigenesis and differentiation, indicating that this pathway may be a suitable molecular target for the development of anti-cancer chemotherapeutic agents. The purpose of this study is to define the frequency and patterns of expression of 3 proteins in this pathway [mTOR, Akt and phosphatidylinositol 3' kinase (PI3-K)], in smooth muscle tumors of the uterine corpus, and to establish whether the expression of any of these proteins has independent prognostic significance.

Design: The expression of mTOR, pan-Akt, and PI3-K was evaluated by immunohistochemistry in 31 uterine leiomyosarcomas and 10 leiomyomata, and were correlated with clinicopathologic parameters. Associated peritumoral normal myometrium was present in 27 cases. Cases were scored by multiplication of staining intensity (on a 0-3+ scale) and the extent/distribution of immunoreactivity (on a 0-4+ scale) for potential scores that ranged from 0 to a maximum of 12. Cases with scores of 4+ to 12+ (moderate, 4+ to 8+; high, 9+ to 12+ immunoreactivity) were considered positive.

Results: All 31 leiomyosarcomas were pan-Akt positive with 80.6% of positive cases displaying high scores, whereas all 10 leiomyomata and 27 normal myometria were entirely pan-Akt negative. High pan-Akt scores were associated with high tumor grade but not with advanced stage or outcome. Every tumoral and non-tumoral sample evaluated showed immunoreactivity for mTOR. PI3-K was positive in 20 (64.5%) of the 31 leiomyosarcomas but in none of the leiomyomata. High scores of PI3-K were associated with late pathologic stage (p=0.0022). High PI3-K scores, high pan-Akt scores and PI3-K positivity were not independently associated with reduced disease-specific survival on multivariate analysis.

Conclusions: These findings suggest that relative to normal myometrium, there is indeed dysregulation of the P13K-Akt-mTOR pathway in uterine smooth muscle tumors, and especially in uterine leiomyosarcomas. The expression patterns of proteins in this pathway, especially pan-Akt, may be of diagnostic utility in separating benign from malignant smooth muscle tumors. Pan-Akt, mTOR and PI3-K expression lacked independent prognostic significance in this pilot study, but additional and larger corroborative analyses are required.

1038 Does Subdividing High Grade Ovarian Serous Carcinoma into Silverberg Grade 2 and 3 Have Biologic Validity?

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Background: There are several grading systems for ovarian serous carcinoma (OSC), mostly ternary systems. The Silverberg grading system is based on a composite score of architectural pattern, cytologic atypia and mitotic index. A nominal binary system (high-grade/low-grade) has been proposed reflecting that these are distinct entities rather than a disease spectrum. All high grade OSC correspond to Silverberg grade 2 and 3. Grade categories should reflect therapeutic, prognostic or biologic difference. We have previously shown no prognostic difference between Silverberg grade 2 and 3 OSC and there are no therapeutic differences. It has been suggested that subclassifying high-grade OSC is not relevant, however, molecular studies comparing Silverberg grade 2 and 3 OSC are limited. We analyzed a publicly available microarray dataset of 285gynecological tumors. We compared Silverberg Grade 2 and Grade 3 OSC to determine if they are molecularly distinct entities.

Design: We included 140 arrays in our analysis: 49 Grade 2 and 91 Grade 3 OSC. Data analysis was performed in R (version 2.9.1) using BioConductor. Pre-processing employed the RMA algorithm, and quality-control with boxplots, principal components analysis and clustering indicated that all samples were of good quality. Non-expressed genes were filtered for and excluded based on Y chromosome signals. Two-sided, unpaired, unequal variance t-tests were used to compare grades, followed by an empirical Bayes moderation of the standard error. False-discovery rate adjustments were used to control for multiple testing.

Results: Differential gene expression was not identified between Grade 2 and Grade 3 tumors at either stringent (1% FDR) or relaxed (25% FDR) thresholds. As a control we compared Grade 1 and Grade 3 tumors, and hundreds of genes showed differential expression. We applied a naive p-value threshold of <0.001 to assess functional enrichment. Even at this level only 38 genes were found to be differentially expressed and there was no functional enrichment based on Gene Ontology enrichment analysis.

Conclusions: Based on the current data set, Silverberg Grade 2 and Grade 3 OSC do not differ molecularly (gene expression or pathway analysis), even in a very relaxed statistical analysis. These findings support the use of a two-tier classification system which is in keeping with clinical differences, molecular and morphologic evidence and therapeutic decisions and has been shown to be highly reproducible in practice.

1039 Vascular Pseudoinvasion in Laparoscopic Hysterectomies: Intraoperative Effect or Pathology Grossing Artifact?

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Background: We have previously shown that histologic artifacts including vascular pseudo-invasion (VPI) are significantly more common in laparoscopic than non-laparoscopic hysterectomies and specifically in cases in which a uterine manipulator (UM) is used. Whether or not VPI occurs intraoperatively or is a post-operative grossing artifact is unknown. The aim of this study was to assess the influence of grossing techniques in introducing artifacts including VPI in a subset of prospectively accrued laparoscopic hysterectomies (LH) utilizing a UM. The specimens were processed using an alternative approach aimed at reducing mechanical transport at the time of grossing.

Design: The previously recommended alternative grossing technique was used in 16 LH as follows: the uterus was bivalved on receipt to permit overnight fixation. In contrast to the standard approach of sectioning from mucosa to serosal surface, the uterine wall was sectioned from the serosal aspect towards the mucosal surface with care to clean the scalpel blade between sections. The presence of artefactual changes including nuclear crush artifact, endomyometrial clefting, debris (inflammatory or tumor fragments) in fallopian tube (FT) lumen and VPI, were evaluated and compared with those from 57 LH grossed in the standard way using the Fisher exact test. The number of vessels involved by VPI was compared between the groups using the Student t-test.

Results: No significant difference between the groups was identified for any of the artifacts [Table 1].

Table 1

Artifact	Standard Grossing Technique, N(%)	Alternative Grossing Technique, N(%)	P-value
VPI	16(28)	4(25)	1
Cleft artifact	23(41)	2(13)	0.07
Intratubal material	19(33)	3(23)*	0.36
Serosal carry over	11(20)	5(31)	0.49
Nuclear crush	14(25)	8(50)	0.07

*3 LH did not include FT

In cases with VPI the number of vessels involved ranged from 1 to 13 (average 5.25) in specimens grossed using the standard technique and from 1 to 2 (average 1.25) in specimens grossed using the alternative technique (P=0.04).

Conclusions: Use of the alternative grossing technique described herein was not effective in eliminating the presence of artifacts; however, the extent of VPI was significantly less in these specimens. While the data is limited by small numbers, the findings suggest that adopting this technique may help to reduce VPI and should help to minimize potential misinterpretation of prognostic parameters in these specimens. We intend to accrue more cases to ensure the study is adequately powered.

1040 Biomarkers of Invasion in Cervical Squamous Cell Carcinoma.

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Background: The capacity of neoplastic cells to invade and metastasize depends on different factors, namely on the ability to establish new complex interactions of cellular adhesion molecules and the extracellular matrix (ECM) components. Laminin γ2 chain (lamγ2) plays an important role during tumor invasion and integrins are primary receptors mediating ECM remodeling. Our aim was to identify biomarkers of invasion in cervical squamous cell carcinomas.

Design: Laminin5 (Lamγ2) α2; α3; α5 and αV integrins immunoexpression was evaluated in a tissue microarray (TMA) constructed with representative regions (formalin fixed and paraffin embedded tumor samples) of 81 cervical squamous cell carcinomas (11 being *in situ* and the remaining 70 were invasive) and appropriate controls. Staining was evaluated both in cells and in the ECM.

Results: Lamγ2 was present in the ECM (basement membrane) in all *in situ* carcinomas (CINIII) and also in the cytoplasm of very few cells, in two cases. In contrast, intracytoplasmatic lamγ2 was present in 59 cases of invasive SCC (see table 1). This difference was significant ($p<0.0001$). Regarding integrins, we found that α5 integrin was overexpressed in invasive tumor. This finding was also significantly correlated with invasion ($p=0.0043$). In invasive tumors α5 and αv (cell membrane) integrins were significantly correlated with the presence of intra-cytoplasmatic lamγ2 expression ($p= 0.0009$ and $p=0.0053$, respectively).

Immunohistochemical results

Diagnosis	Nº cases	Location	Lamγ2	Integrins			
				α2	α3	α5	αv
		ECM	100%	36%	not found	not found	9%
CIN III	11	Cell cytoplasm	18%	9%	73%	0%	55%
		Cell membrane	not found	91%	73%	0%	55%
		ECM	86%	21%	not found	not found	29%
SCC	70	Cytoplasm	80%	21%	63%	40%	71%
		Cell membrane	not found	86%	63%	40%	71%

ECM - extracellular matrix; SCC - Squamous cell carcinoma

Conclusions: 1) Intracytoplasmatic laminin γ2 is a good marker of stroma invasion.
2) Invasive cell phenotype in cervical squamous carcinoma is significantly associated with the presence of intracytoplasmatic lamγ2 and α5 integrin.
3) These results suggest that invasion in cervical cancer is predominantly by α5 and αv integrins.

1041 Expression of S100P in Benign Endometrium and Endometrial Adenocarcinoma.

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Background: S100P is a 95-amino acid isoform of the S100 protein family with restricted cellular distribution, first purified from placenta. It activates ERK and NF-kB pathways via the receptor for advanced glycation end-product to promote cellular proliferation and survival. Its expression has been reported in breast, prostate, lung and gastrointestinal malignancies. Recent complementary DNA studies of human endometrium showed S100P is expressed in the postovulatory endometrium. The expression of S100P in endometrial adenocarcinoma (EAC) is largely unknown. We evaluated the expression of S100P in EAC, proliferative endometrium (PE), and secretory endometrium (SE) in this study.

Design: Biopsy and hysterectomy specimens were retrieved from the Department of Pathology archives at Lyndon B. Johnson General Hospital. and a formalin-fixed, paraffin-embedded tissue microarray containing 31 EAC, 29 PE, and 21 SE was constructed. Immunohistochemistry was performed using a mouse monoclonal antibody against human S100P (1:20, R&D Systems, Inc. Minneapolis, MN, USA). S100P expression was semi-quantified by light microscopy regarding staining pattern (cytoplasmic vs nuclear), percent of staining cells (0-100%) and intensity of staining (0-3+). Positive staining is defined as at least 1+ concurrent nuclear and cytoplasmic staining. Cytoplasmic staining alone is regarded as negative.

Results: S100P is expressed predominantly within the nucleus but is also present within the cytoplasm. Minority of EAC (3/31, 9.7%) showed strong (2-3+) and focal to patchy (3 to 50% of cells) S100P staining. Majority of SE (17/21, 81%) showed positive (1-3+) and patchy to diffuse (30 to 90% of cells) S100P staining. All mid to late secretory endometrium (≥ day 20) showed strong and diffuse S100P staining and most (4/6) early secretory endometrium (< day 20) showed negative S100P staining. Minority of PE (5/29, 17%) showed positive (1-2+) and variably distributed (5 to 80% of cells) S100P staining. EAC showed statically significant lower frequency of positive S100P expression compared to SE (P <.001) but not compared to PE (P = .47); and SE showed statically significant higher frequency of positive S100P expression compared to PE (P <.001) by Fisher's exact test.

Conclusions: Majority of EAC do not show S100P overexpression but S100P expression is consistently present within benign SE. These findings indicated S100P expression may be utilized to differentiate difficult cases of EAC and SE with highly complex glandular architecture. Furthermore, S100P immunohistochemical staining may also be an adjunct study in endometrium dating for infertility workup.

1042 Detection of *ALK* Gene Rearrangements Aids in the Diagnosis of Inflammatory Myofibroblastic Tumor of the Uterus.

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Background: Inflammatory myofibroblastic tumor (IMT) is a predominantly benign spindle cell mesenchymal neoplasm that occurs very rarely in the uterus and may be confused with other spindle cell lesions in the gynecologic tract, particularly leiomyosarcoma. A variable staining pattern of many IMTs with keratins and smooth muscle markers contributes diagnostic difficulties.

Design: To better define the histologic features of uterine inflammatory myofibroblastic tumors and to investigate the utility of detection of ALK-1 protein expression and *ALK* gene rearrangements in the diagnosis of uterine IMT we reviewed four cases of atypical inflammatory myofibroblastic tumors seen in our consultation practice. Immunostains for desmin, SMactin, CD-10, keratins and ALK-1 were performed on all cases. FISH detection for *ALK* gene rearrangements was performed in the 3 cases in which tissue was available.

Results: The specimen sites for the IMTs included uterus (2 cases), cervix and cervical polyp and age range was from 26 years to 48 years. Histologic atypia ranged from bland spindle cells to moderate cytologic atypia with pleomorphic vesicular nuclei and prominent nucleoli. All had low mitotic activity, scattered mixed inflammatory infiltrate, and lacked true tumor necrosis. All contained distinct areas of spindle cells embedded in a prominent myxoid background. Immunohistochemical staining varied in regards to CD10, keratin, and smooth muscle markers, however ALK-1 (anaplastic lymphoma kinase 1) staining was consistently positive with a cytoplasmic staining pattern. FISH studies detected *ALK* gene rearrangements in the three cases analyzed.

Conclusions: IMT is a very rare tumor in the uterus, but should always be included in the differential of malignant myxoid lesions of the uterus such as the myxoid variants of leiomyosarcoma and endometrial stromal sarcoma. If a myxoid background is appreciated in a spindle cell lesion of the uterus, especially if inflammatory cells are present, ALK-1 immunohistochemical staining and FISH studies for *ALK* gene rearrangements may aid in distinguishing largely benign IMTs from their malignant mimics.

1043 Germline BRCA1 Mutation Positive Ovarian Cancer Exhibits a Distinctive Highly Specific Phenotype.

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Background: Patients with BRCA1 germline mutation are at increased risk of developing both breast and ovarian cancer. BRCA1 breast cancer is associated with specific tumor morphology, but, other than high grade serous histology, specific histologic features of the ovarian tumors in these patients have not been described. We investigated whether BRCA1 ovarian cancer demonstrate characteristic histologic findings that are predictive of BRCA1 status.

Design: We reviewed the pathologic features of 326 ovarian cancers with known germline BRCA1 mutational status from a population-based ovarian cancer registry. Tumors were evaluated independent of BRCA mutational analysis and classified into 2 categories: "Not compatible with BRCA1" and "Compatible with BRCA1" on the basis of the following criteria: serous/undifferentiated vs. other histology; marked vs. minimal to moderate atypia; abundant mitotic figures vs. few or scattered mitoses; giant, bizarre nuclei vs. monomorphic nuclei; and prominent intraepithelial lymphocytes vs. few or no lymphocytes. Tumors that showed only 2 of these features were separately classified as "Possibly compatible with BRCA1." Tumors that demonstrated "Compatible with BRCA1" histology were additionally investigated for presence/absence of a dominant mass, fallopian tube mucosal involvement, and uterine cornu involvement. Results were correlated with germline BRCA1 status.

Results: 326 ovarian tumors (28/326 [8.6%] BRCA1 positive and 298/326 [91.4%] BRCA1 negative) were reviewed and classified as follows: 240/326 (73.6%) "Not compatible", 44/326 (13.5%) "Possibly compatible"; and 42/326(12.9%) "Compatible." Germline BRCA1 mutations were present in 3.8% "Not compatible" and 26.2% "Compatible." The sensitivity and specificity of "Compatible" morphology were 55.0% and 88.2%, respectively; while the sensitivity and specificity of combined "Compatible" and "Possibly compatible" morphology were 67.9% and 77.5%, respectively. The presence/absence of a dominant mass, fallopian tube mucosal involvement, and uterine cornu involvement did not predict BRCA1 status.

Conclusions: A combination of serous/undifferentiated histology, marked nuclear atypia, high mitotic index, giant, bizarre nuclei and prominent intraepithelial lymphocytes appears to be highly specific for germline BRCA1 mutation positive ovarian cancer (negative predictive value >95%) in this catchment area. Ovarian tumor specific histology may help identify women for BRCA1 mutational analysis in the appropriate clinical setting.

1044 Investigation of Human Papillomavirus Infections in Vulvar Intraepithelial Neoplasia by Polymerase Chain Reaction, Chomogenic In Situ Hybridization and Immunohistochemistry.

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Background: Several studies suggest there are two distinct types of vulvar intraepithelial neoplasia (VIN) that can progress to vulvar squamous cell carcinoma (VSCC): usual VIN (uVIN) is associated with high-risk (hr) human papillomavirus (HPV) infections and lichen sclerosus/differentiated VIN (LS/dVIN) is rarely associated with hrHPV. This study extends the investigation of HPV in these conditions by combining polymerase chain reaction (PCR), chromogenic *in situ* hybridization (CISH), and $p16^{INK4a}$ and p53 immunohistochemistry (IHC) assays.

Design: Random cases of uVIN (VIN grade II/III, n=50) and LS/dVIN (n=50) were collected from Fletcher Allen Health Care archives. HPV genotyping was performed by PCR using GP5+/GP6+ primers followed by cycle sequencing. CISH was performed using a biotinyl-tyramide-based assay. IHC was performed using a polymer detection system.

Results: HPV was detected by PCR in 48 (96%) uVINs (1 low-risk HPV, 6 hrHPV types) and in 11(22%) LS/dVINs (3 hrHPV types) [P<0.0001]. HPV was detected by CISH in 37 (74%) uVINs and in 0 (0%) LS/dVINs [p<0.0001]. Among the uVIN, 2 samples demonstrated diffuse (episomal HPV) CISH signals only, 19 specimens showed punctate (integrated HPV) and diffuse signals, and 16 cases displayed only punctate signals; the patients with lesions showing only integrated HPV (median age=52) were significantly older than patients with integrated and episomal HPV (median age=35.0) [P=0.008]. $p16^{INK4a}$ IHC was positive in 48 (96%) uVIN: in 19 cases staining was confined to the lower third of the epithelium and in 29 cases staining extended up to the full epithelial thickness; staining was unrelated to CISH staining pattern [P>0.05]. Sporadic basal layer $p16^{INK4a}$ staining was noted in 33 (66%) LS/dVINs. Suprabasal staining for p53 was noted in 26.5% uVIN and p53 basal layer staining was noted in 50% of LS/dVINs. The uVIN patients (median age=48.5) were significantly younger than the LS/dVIN patients (median age=60) [P<0.0001].

Conclusions: These findings support the existence of two distinctive pathways. uVIN is HPV driven and occurs in younger women, whereas LS/dVIN is most likely hrHPV

independent and occurs in older women: the CISH and p16 IHC data suggest that hrHPV detectable by PCR in 22% of the LS/dVINs may be incidental to the lesions. The uVIN CISH data indicate that chronic infections may evolve into lesions containing only integrated HPV. $p16^{INK4a}$ and p53 staining may be used qualitatively to distinguish uVIN from LS/dVIN.

1045 The Accuracy of an Intraoperative Diagnosis of Ovarian Borderline Tumor Varies by Histologic Subtype.

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Background: Borderline (BT) ovarian tumors are treated differently depending on the surgeon, patient age, the type of borderline tumor and co- morbid conditions. When the tumor appears to be confined to the ovary, these patients typically undergo conservative surgery. To determine whether additional surgery is needed in this situation, it becomes important to understand the extent to which frozen section diagnoses are overturned on review of permanent sections. The purpose of this study was to assess the sensitivity and positive predictive value of an intraoperative ovarian borderline tumor diagnosis at our institution.

Design: All patients with an intraoperative or final pathologic diagnosis of ovarian borderline tumor of all histologic subtypes between years 2000 to 2010 were included in the study.

Results: A total of 166 patients were included in the study. The median age was 49 years (20.3-91.2 yrs). The sensitivity and positive predictive value of an intraoperative diagnosis of ovarian borderline tumor was 81.6% and 89.9% respectively. 75% of tumors diagnosed as borderline on frozen retained this diagnosis at final pathology (74.6%) while 14 (8.4%) were upgraded from borderline to carcinoma. Twenty cases diagnosed as benign on frozen were upgraded to borderline (12%), while 8 cases (5%) were downgraded from carcinoma to borderline. The rates of undercalling carcinoma as borderline tumor on frozen section varied considerable by histologic type: 5% of tumors diagnosed as serous BTs, 0% of seromucinous BTs, 14% of intestinal mucinous BTs and 80% of small numbers of endometrioid BTs were reclassified as carcinomas on permanent sections. Most under calls were due to sampling error, not interpretive error. Further study of the serous tumors revealed differences related to the presence of micropapillary architecture, with 21% of tumors diagnosed as micropapillary serous BTs reclassified as carcinomas on permanent section compared to 1% of non-micropapillary serous BTs.

Conclusions: One can anticipate an accurate frozen section diagnosis of BT when the tumor is serous or seromucinous and non-micropapillary. This does not provide unmitigated support for performing staging surgeries for these types of borderline tumors when disease appears clinically confined to the ovary, without surface involvement. Significant rates of underdiagnosis can be anticipated with micropapillary serous, intestinal mucinous and endometrioid tumors when diagnosed as BT on frozen section. This provides sufficient support for performing cancer-type staging surgeries at the discretion of the surgeon in these circumstances.

1046 Endometrial Carcinomas with DNA Mismatch Repair Abnormalities: Genotypic Phenotypic Correlations.

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Background: Endometrial carcinomas (ECs) are commonly associated with defective DNA mismatch repair, either due to epigenetic (MLH1 promoter methylation) or genetic alterations (germline mutations in DNA mismatch repair genes resulting in HNPCC/ Lynch syndrome (LS)). Correlations between genotype and morphologic phenotype are poorly understood. In this study, we describe the pathologic characteristics of ECs with DNA MMR abnormalities.

Design: ECs with abnormal DNA MMR IHC that were tested for DNA MMR mutation and/or MLH1 promoter methylation were included in the study (n=11).

Results: Of 11 samples, 7 had absent MLH1 or PMS2 expression. Of these, 4 tumors showed MLH1 promoter methylation; germline testing in the other 3 cases demonstrated a clearly deleterious germline MLH1 mutation in 1 and missence mutations of unclear significance in 2. In the four samples with loss of MSH2 or MSH6 expression, germline testing identified 3 MSH2 mutations and 1 MSH6 mutation. Two of the 11 patients had no personal or family history suggestive of LS while 4 patients met the Amsterdam II criteria for LS (including the 2 patients with MLH1 variants). Six of the 11 tumors were endometrioid (EEC), 1 was clear cell (CC), 1 mixed endometrioid and serous and 3 undifferentiated/dedifferentiated. One endometrioid ca arose in endometriosis. Peritumoral and tumor infiltrating lymphocytes were noted in 2 cases, tumor heterogeneity in 4 cases, lower uterine segment origin in 2 cases, undifferentiated/ dedifferentiated histology in 3 cases and synchronous ovarian clear cell carcinoma (arising in endometriosis) in 1 case. Correlation between genotyping and tumor morphology is shown in **Table 1**.

Correlation between genotype and morphologic phenotype

Genotype (number of patients)	Tumor morphology (patient age in yrs)
MLH1 methylation (4)	1 dedifferentiated ca (35), 1 FIGO 1 EEC (49), 1 FIGO 3 EEC (58), 1 CCC (59)
MSH2 mutation (3)	2 FIGO 2 EEC (41, 47), 1 mixed endometrioid and serous (50)
MSH6 mutation	FIGO 1 EEC (42)
MLH1 deleterious mutation (1)	Dedifferentiated ca (52)
MLH1 missense mutations (2)	1 undifferentiated ca (39), 1 endometrioid ca in endometriosis (55)

Conclusions: Morphologic and topographic characteristics previously described in DNA MMR defective endometrial carcinomas were frequently seen in this patient group. Abnormalities in MLH1, both genetic and epigenetic, appear to confer a particular predilection for tumors with undifferentiated or dedifferentiated histology. It appears that MLH1 promoter methylation can be seen in relatively young patients. Patients with Lynch syndrome also may be at risk for tumors arising in endometriosis.

1047 Molecular Alterations in Morphologically Ambiguous Endometrial Carcinomas.

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Background: Endometrial carcinomas (ECs) are divided into type 1 and type 2 cancers. Type 1 tumors are low grade endometrioid carcinomas with good clinical outcomes, associated with microsatellite instability and mutations in *PTEN*, *K-RAS* and *CTNNB1*. Type 2 tumors are aggressive, high grade ECs that often show *p53* mutations. Both type 1 and type 2 ECs can have *PIK3CA* mutations, that can coexist with mutations in *PTEN* and *p53*. We have previously studied a group of morphologically ambiguous ECs with overlapping features of type 1 and 2 tumors, for interobserver diagnostic concordance and IHC for p53 and p16, and found that p53 overexpression (OE) correlates with adverse clinical outcomes. In this study, we wanted to assess the molecular abnormalities of these ECs and whether they had a prognostic impact.

Design: Twenty three ECs (n=23) with ambiguous morphology that had been stained for p53 and p16 formed the study group. These tumors were subjected to sequenom analysis for hotspot mutations in PIK3CA, KRAS, NRAS, and AKT. Sequencing results for PIK3R1 and PTEN are forthcoming.

Results: Of the 23 cases, mutations were detected in 12 (52%). Correlation between mutation status, morphologic impression and IHC for p53 and p16 are shown in table 1.

Correlation between molecular abnormality, consensus diagnosis, IHC for p53 and p16 and clinical outcomes

Mutation analysis (number of cases)	Consensus diagnosis	p53 OE	p16 OE
RAS mutations	endometrioid	3 of 6 (2 DOD)	0 of 6
PIK3CA exon 9 (2)	Serous	2 of 2 (1 DOD)	1 of 2
PIK3CA exon 20 (2)	1 serous, 1 endometrioid	0 of 2 (LFU)	1 of 2
RAS/AKT/PIK3CA (1)	No consensus diagnosis	0	0
AKT (1)	Endometrioid	0	0
No mutations detected (11)	6 serous, 5 endometrioid, 1 no consensus	6 of 11 (3 DOD)	3 of 11

Of the 13 cases with a consensus diagnosis of endometrioid ca, RAS mutations were noted in 6. 3 of these tumors also showed p53 OE and 2 of these patients died of disease (DOD). Of 8 cases with a consensus diagnosis of serous carcinoma, PIK3CA mutations were noted in 3; 2 with exon 9 mutations also showed p53 OE. The prognosis is difficult to assess due to small numbers and patients lost to follow up (LFU).

Conclusions: A consensus diagnosis of endometrioid carcinoma correlates with presence of RAS mutations, and p53 OE in these cases correlated with worse clinical outcomes. PIK3CA mutations were present in tumors with serous and endometrioid morphology, concurrent p53 OE was seen in cases with PIK3CA exon 9 mutations, but not exon 20 mutations. The impact of PIK3CA mutations on clinical outcome cannot be determined due to small numbers. PIK3CA and RAS mutations can co-exist in ECs.

1048 Evidence for BRCA1 Haploinsufficiency in Expression Profiles from Morphologically Normal Fallopian Tube Epithelium.

S George, A Milea, J Greenaway, C Virtanen, S Shaw, M Sharma, P Shaw. University Health Network, Toronto, Canada.

Background: Histological precursors of High Grade Serous Carcinoma have been identified in fallopian tube epithelium of women at genetic high risk of ovarian/tubal/ peritoneal carcinoma. One of the putative precursors, the p53 signature, is also found frequently in women at low risk. The most immediate precursor to invasive disease, serous tubal intraepithelial carcinoma (STIC) is found more frequently in mutation carriers. We have previously reported that tubal epithelium from mutation carriers (FTE-BRCA) has a differential gene expression signature during the luteal phase of the ovarian cycle. The purpose of this study is to determine whether morphologically normal FTE-BRCA has an expression profile different from non-carriers (FTE-non BRCA).

Design: Morphologically normal tubal epithelium was microdissected from snap-frozen tissues from 12 BRCA1 mutation carriers undergoing risk-reducing surgery and from 12 control samples. All samples were from pre-menopausal women, and an equal number of samples from the follicular and luteal phases were included. Following RNA extraction, reverse transcription and linear amplification, samples were hybridized to Affymetrix GeneChips. Normalized data was subjected to cluster analysis and differentially expressed genes identified using SAM and GeneSpring. Selected candidate genes were validated by real-time RT-PCR and immunohistochemistry on tissue microarrays (TMA) of normal tubes and ovarian cancers. Stained slides were scanned and digital slides (Aperio Scanner XT) analysed using automated image analysis (TMALab, Genie, Aperio, Inc).

Results: 135 genes were differentially expressed, and largely upregulated, with a greater than 2-fold difference and a p-value less than 0.05, in FTE-BRCA compared to FTE-non BRCA. The differences were present in both follicular and luteal phases. The selected target genes are involved in cell proliferation, in DNA damage and in inflammatory signaling. The differential expression of 5 targets was validated by RT-PCR and by immunohistochemistry. Levels of BRCA1 mRNA were not decreased in FTE-BRCA compared to non-BRCA.

Conclusions: We have demonstrated for the first time that FTE from BRCA1 heterozygotes differs from FTE-non BRCA in the absence of histological lesions. These differences suggest that BRCA1 haploinsufficiency exists in normal FTE-BRCA, and that normal FTE-BRCA may respond differently to the microenvironment. These changes may reflect the earliest molecular changes of serous carcinogenesis, and may be markers of increased cancer risk.

1049 Poor Interobserver Reproducibility in the Diagnosis of High-Grade Endometrial Carcinoma.

CB Gilks, E Oliva, R Soslow. Vancouver General Hospital and BC Children's and Women's Hospital, BC, Canada; Massachusetts General Hospital, Boston; Memorial Sloan Kettering Cancer Center, New York, NY.

Background: Patients with endometrial carcinoma who have high-grade subtypes (grade 3 endometrioid, serous, clear cell, carcinosarcoma) have a relatively poor prognosis. There is little information on the reproducibility of subtype diagnosis in cases of high-grade endometrial carcinoma.

Design: Fifty six cases diagnosed as a high-grade subtype of endometrial carcinoma were identified in the archives of VGH. All slides for each case were reviewed independently by three pathologists, who diagnosed the subtype, and assigned a percentage for each component in mixed tumors. Agreement between observers was classified as follows – Major disagreement: A. No consensus for low-grade endometrioid versus high-grade (any subtype) or B. Disagreement with respect to the predominant high-grade subtype present; Minor disagreement: consensus is reached about the cell type of the predominant high-grade component, but there is no agreement regarding a secondary high-grade subtype type.

Results: In 35 of 56 (62.5%) cases there was agreement between all three reviewing pathologists regarding of the predominant subtype of high-grade endometrial carcinoma. In four of these cases (7.1%) there was a minor disagreement i.e. not all observers agreed there was a secondary high-grade subtype present. In 17 of 56 cases (30.4%) there was no consensus between the three observers with respect to the high-grade subtype; in an additional 3 cases (5.4%) there was no agreement between observers with respect to low-grade versus high-grade (any subtype). The final case was diagnosed as low-grade endometrioid carcinoma by all reviewers (but had been previously diagnosed as high-grade). The most frequent areas of disagreement were serous versus clear cell (7/17 cases) and serous versus endometrioid (5/17 cases). Pair-wise comparison between reviewers for cases with major disagreements was as follows: rev. 1 vs rev. 2 – agreement in 5/20 cases, rev. 1 vs rev. 3 – agreement in 7/20 cases, rev. 2 vs rev. 3 – agreement in 9/20 cases.

Conclusions: There were significant major diagnostic disagreements between reviewers in subtyping of high-grade endometrial carcinoma in more than one third of cases. This did not reflect systematic bias on the part of any single reviewer. Although this study was not designed to look at the reproducibility of diagnosis of low versus high-grade endometrial carcinoma, even this distinction proved to be problematic.

1050 Is There a Need for Pre-Operative Evaluation in an Asymptomatic Patient Population Receiving a Supracervical Hysterectomy?

A Gopinath, S Mandavilli, M Assaad, AC Steinberg. Hartford Hospital, CT.

Background: In the United States approximately 550,000 hysterectomies are performed annually with the vast majority done for benign conditions. With the advent of minimally invasive techniques (laparoscopy and robotically assisted laparoscopy), supracervical hysterectomy (SCH)has gained popularity. Urogynecology performs SCH as part of their pelvic reconstruction to decrease the risk of potential complications. Debate has occurred whether in the asymptomatic patient,evaluation (ultrasound,endometrial biopsy) for potential of endometrial pathology is warranted. Urogynecology at our institution does not perform an evaluation in the asymptomatic patients. The aim of this study is:1) find the incidence of incidental uterine carcinoma in SCH specimens 2) evaluate the need for preoperative gynecological workup in asymptomatic patients.

Design: Following IRB approval, the pathologic findings in all SCH performed (October 2006-September 2009) in our institution were analyzed. Two groups were established for comparison,procedures performed by 3 urogynecologists(groupI) vs 29 general gynecologists (groupII). Procedures performed by Gynecology-oncology were excluded from the study.

Results: 320 (groupI-86 and groupII-234) subjects were included. Mean age of groupI-53.7 (27-77yrs) when compared to group2-45.1 (26-65yrs) demonstrated a difference ($p \leq .001$).82 subjects,all in group2, had pre operative endometrial biopsies interpreted at our hospital. In groupII, 1 high grade adenocarcinoma and 1 complex atypical hyperplasia were identified compared to none in groupI (statistically not significant). Other findings in both groups included complex hyperplasia without atypia (1, 0.3%), chronic endometritis (1, 0.3%), benign endometrial polyp (21, 6.5%), leiomyomas (204, 64 %),adenomyosis (113, 35%) and adenomatoid tumor (1, 0.3%).

Conclusions: In patients receiving supracervical hysterectomy the incidence of carcinoma is very low. In this study no significant difference was identifiable between patients who had some form of evaluation and those who did not. This could be attributed to the fact that, in the patients with abnormal findings on preoperative evaluation referral to the gynecology-oncology surgeons occurred. We conclude that with the probability of occult uterine malignancy being very low the need for expensive preoperative evaluation in an asymptomatic population needs to be questioned.

1051 Diagnostic Reproducibility of Hydatidiform Moles: Molecular Genotyping Significantly Improves Morphologic Assessment.

M Gupta, L Wu, A Yemelyanova, R Vang, RJ Kurman, BM Ronnett. Johns Hopkins Medical Institutions, Baltimore, MD.

Background: Distinction of hydatidiform moles (HM) from non-molar specimens (NM) and sub-classification of HMs as complete hydatidiform mole (CHM), early CHM (eCHM), and partial hydatidiform mole (PHM) are important for clinical practice yet diagnosis based solely on morphology is affected by interobserver variability. Molecular genotyping can distinguish these entities by discerning androgenetic diploidy, diandric triploidy, and biparental diploidy to diagnose CHMs, PHMs, and NMs, respectively. This study analyzes the accuracy and inter-observer variability of diagnosis of HMs among experienced gynecologic pathologists relative to genotyping results.

Design: 80 genotyped cases (1 representative slide per case), including 10 CHMs, 17 eCHMs, 27 PHMs, and 26 NMs, were selected from a consecutive series of 200 potentially molar specimens previously diagnosed using p57 immunostaining and genotyping. Slides were classified by 3 pathologists, masked to p57 immunostaining and genotyping results, into 1 of 4 categories: CHM, eCHM, PHM, or NM. Genotyping results were used as the gold standard (true) diagnosis; diagnostic performance was analyzed using kappa (k) statistics.

Results: Using 4 categories, agreement between the individual pathologist's diagnoses and molecular genotyping was fair-moderate (k=0.29-0.49; 50-64%). Using 3 categories (CHM and eCHM combined), agreement was fair-good (k=0.32-0.60; 55-74%). Using a consensus diagnosis (that rendered by 2/3 reviewers), agreement was moderate (k=0.56; 64%) using 4 categories and good (k=0.63; 70%) using 3 categories. Based on all individual pathologists' classifications, agreement varied from moderate to poor among the diagnostic categories, with kappa values of 0.42, 0.40, 0.13 and 0.15 for CHM, eCHM, PHM, and NM, respectively, and 0.59 for CHM and eCHM combined. For 6 cases (1 CHM, 3 eCHMs, and 2 PHMs), there was no consensus diagnosis. Using 3 categories for the 74 cases with consensus, the consensus diagnosis correctly classified 83% of CHMs and eCHMs combined, 84% of PHMs, and 62% of NMs.

Conclusions: Diagnostic reproducibility of HMs by morphology is only fair-good even for experienced gynecologic pathologists, with good agreement only modestly achieved by consensus. Distinction of PHMs and NMs is the most problematic, dominated by over-diagnosis of NMs as PHMs, but failure to recognize all CHMs/eCHMs persists. Genotyping provides a definitive diagnosis for the nearly one-third of cases that are misclassified by morphology.

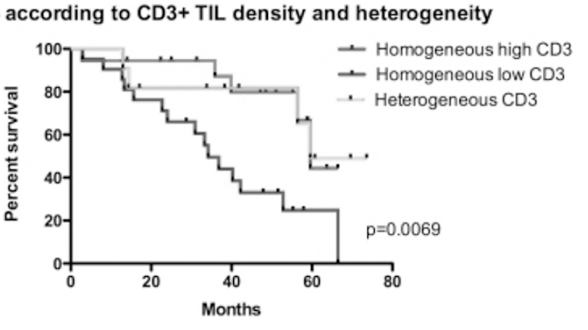
1052 Immunologic Heterogeneity and Survival in Metastatic Serous Ovarian Carcinoma.

IS Hagemann, P Patel, AR Hagemann, L-P Wang, G Coukos, MD Feldman. Univ of Pennsylvania, Philadelphia.

Background: The presence of tumor-infiltrating lymphocytes (TILs) in epithelial ovarian cancer indicates a host antitumor immune response and predicts improved survival. It is not well established whether the presence, distribution, or activity of TILs is different in metastatic tumor deposits as compared to the primary lesion. Between-site heterogeneity could explain the resistance of some tumors to immunotherapy and provide guidance for treating residual disease.

Design: We constructed a tissue microarray containing 1–2 primary tumors and 1–3 metastases from each of 50 patients (stages IIIC and IV) undergoing primary debulking of serous ovarian cancer between 2005 and 2008. Immunostains were performed for the immune markers CD3 (pan T lymphocyte), CD8 (cytotoxic T lymphocyte), and FoxP3 (regulatory T lymphocyte). For each stain, intraepithelial TIL density in triplicate cores was visually semi-quantitated on a scale from 0 to 3. Overall survival (OS) and disease-free survival (DFS) were derived from clinical and public records. Kaplan-Meier curves were compared by the log-rank test. To correct for multiple comparisons on the same data, $p<0.0125$ was considered significant.

Results: High intratumoral CD3+ TIL density averaged across all tumor sites (N=22 out of 50 patients) was significantly associated with favorable OS (p=0.0037), while high CD8+ and high FoxP3+ TIL density (N=29/50 and 22/50 patients, respectively) showed trends toward longer OS (p=0.0458 and p=0.0182, respectively). This effect was slightly less pronounced if only the TIL density of primary sites was considered. In cases having low FoxP3+ TIL density, a low FoxP3:CD8 ratio (seen in N=8/28 patients) predicted longer OS (p=0.0501). The OS of patients with homogeneously high (N=18) and with heterogeneous CD3+ TIL density (N=11) was significantly longer than that of patients with homogeneously low CD3+ density (N=21) (p=0.0069; Figure 1). DFS was not significantly associated with any measure of TIL density in this study.



Conclusions: Lymphocytic infiltration is correlated with overall survival in serous ovarian cancer. This effect is more pronounced when multiple tumor sites are evaluated. While the significance of between-site heterogeneity remains unclear, homogeneously low CD3+ TIL density is an adverse prognostic factor.

1053 BRCA Dysfunction in Ovarian Tumor Cannot Be Reliably Predicted by Morphology.

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Background: Anecdotal data suggests that ovarian tumors with BRCA abnormalities often show tumor infiltrating lymphocytes (TILs), and solid or transitional cell-like

growth pattern. This study examines the possibility of predicting BRCA dysfunction ("BRCAness") based on the presence of these morphologic features.

Design: A consecutive series of 44 ovarian carcinomas were reviewed for tumor subtype, presence of TILs, solid or transitional cell-like growth pattern, and geographic necrosis, without knowledge of BRCA1 and BRCA2 status. TILs were only evaluated in sections from the primary tumor. A prediction of possible BRCA dysfunction was made when a combination of two or more of the above features were present in a high grade serous carcinoma (HSC). The predicted status was then correlated with the actual BRCA1/2 status.

Results: Among the HSCs, morphologic prediction of possible BRCA dysfunction was made on 7/10 cases with (70%) BRCA1 or 2 germline or somatic mutation, 9/10 (90%) cases with BRCA1 promoter methylation, and 8/15 (53%) cases without BRCA mutation or methylation (no BRCA loss group). All 9 cases of non-HSCs were predicted as no BRCA dysfunction and did not show BRCA mutation or methylation. There were significantly more BRCA mutation or promoter methylation cases in the predicted BRCA dysfunction group than in the predicted no BRCA dysfunction group ($p = 0.002$) when considering all cases. However, this difference was not significant when only HSCs were considered ($p = 0.095$). Within the HSC subtype, TILs were more frequently present in the BRCA1 promoter methylation group (9/10, 90%) compared to other groups (4/10 in BRCA1 or 2 mutation group, 7/15 in no BRCA loss group) ($p = 0.015$). Transitional-like morphology was more frequently present in the BRCA1 or 2 mutation group (8/10, 80%) compared to the BRCA1 promoter methylation group (4/10) and no BRCA loss group (6/15) ($p = 0.037$). There was no significant difference in the presence of solid growth pattern or geographic necrosis among the subgroups.

Conclusions: TILs and/or transitional-like growth pattern are seen in the majority of cases with BRCA1 or 2 dysfunction, especially in the BRCA1 promoter methylation group. However, there is tremendous morphologic overlap between tumors with and without BRCA dysfunction. While HSC subtype remains a useful indicator for potential BRCA screening, we cannot, at this point, reliably predict subsets of HSC with BRCA abnormalities based solely on morphologic features.

1054 Factors Influencing Accuracy of Frozen Section Diagnosis of Ovarian Mucinous Tumors; a Review of 100 Cases.

B Harmon, S Hwang, T Parker, M Pearl, C Tornos. Stony Brook University Medical Center, NY.

Background: Ovarian mucinous tumors tend to be large and histologically heterogenous. Many studies point to them as the major source of diagnostic discrepancies between intraoperative and final diagnosis of ovarian masses. The aim of this study was to assess the accuracy of frozen section (FS) diagnosis of ovarian mucinous tumors and to identify possible factors affecting the accuracy rate.

Design: We identified 100 consecutive ovarian mucinous tumors that underwent FS evaluation at the request of our Gynecological oncologists from January 2000 to August 2010. All H&E slides from the FS's and permanent sections were reviewed. Parameters evaluated included: tumor size and gross appearance, bilaterality, previous history of malignancy, number of FS blocks submitted, and pathologist experience in gynecological pathology.

Results: The final diagnosis included 28 mucinous cystadenomas, 27 metastatic mucinous carcinomas, 24 borderline tumors intestinal type, 16 primary ovarian carcinomas, and 5 borderline tumors endocervical type. All benign tumors were classified correctly at the time of frozen section, as were 25 of the 27 metastases, and the other 2 cases were deferred. The table shows results of FS and final diagnosis of all primary ovarian tumors.

Results of FS and final disgnosis of all primary ovarian tumors

Frozen diagnosis	Final Diagnosis		
	Benign	Borderline	Malignant
Benign	28	7	1
borderline	0	13	6
Malignant	0	0	6
Deferred	0	9	3

There were no false positive cases that underwent unnecessary staging. Underdiagnosis occurred in 14/100 (14%). Agreement between frozen section diagnosis and final diagnosis was observed in 72/100 cases (72%). Agreement in non-benign tumors was seen in 44/ 72 cases (61%). The sensitivity was 100%, 44.9%, and 37.5% for benign, borderline, and primary malignant tumors. The corresponding positive predictive value was 77.8%, 68.4%, and 100%. In univariate and multivariate analysis tumor diameter was the only predictor of underdiagnosis.

Conclusions: Intraoperative frozen section diagnosis of mucinous tumors is reliable for benign tumors and metastases, but it has a low sensitivity for borderline tumors and ovarian carcinomas. Surgical management based on intraoperative frozen section diagnosis should be used with caution.

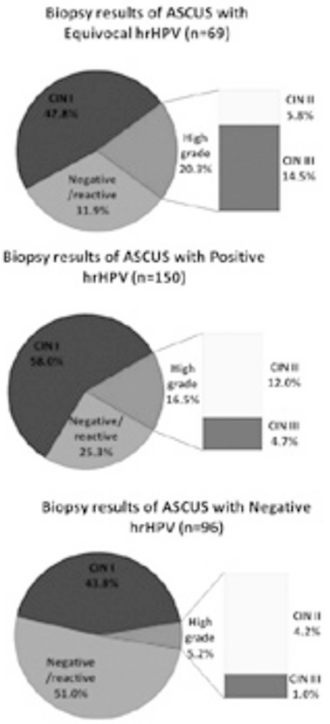
1055 The Significance of ASCUS-Equivocal High Risk HPV DNA Tests in ThinPrep Specimens: A Cytologic/Histologic Review of 315 Cases.

K HooKim, B Smola, D Newton, R Lieberman, S Knoepp. University of Michigan, Ann Arbor.

Background: High risk HPV testing (hrHPVT) improves early detection of precancerous cervical lesions, particularly in patients 30 years or older with normal Pap tests and in patients showing Atypical Squamous Cells of Undetermined Significance (ASCUS) on Pap. Up to 11.4% of the latter are associated with "equivocal" hrHPVTs, for which the current recommendation is repeat testing. To date, no study has adequately analyzed the clinicopathologic significance of "equivocal" hrHPVTs associated with ASCUS cytology in ThinPrep specimens.

Design: All ThinPrep Pap tests diagnosed as ASCUS over a 5-year period were reviewed, and specimens with a corresponding hrHPVT (Hybrid Capture II) and cervical biopsy performed within 2 years were selected. Resulting cases were grouped into three cohorts based on hrHPVT: positive, negative, or equivocal. Equivocal hrHPVT was defined as any positive test below 5 relative light units (RLU/CO). Follow-up histology was correlated with corresponding hrHPVT results. Cases of high-grade dysplasia were reviewed by 2 pathologists.

Results: 9,012 of 274,941 ThinPrep Pap tests were diagnosed as ASCUS, and of these, 7,050 had associated hrHPVTs. HrHPVTs were positive in 2449 (34.7%) cases, negative in 4170 (59.2%) cases, and equivocal in 431 (6.1%) cases. Follow-up histology was available on 945 specimens, of which we selected all 69 equivocal cases, and sequentially 150 positive, and 96 negative cases. High-grade dysplasia was seen in 20.3% of equivocal (CIN II 5.8%, CIN III 14.5%) and 16.5% (CIN II 12%, CIN III 4.7%) of positive hrHPVTs. Comparatively, only 5.2% of negative hrHPVTs showed high-grade dysplasia (CIN II 4.2%, CIN III 1%) on follow-up.



Conclusions: ASCUS with equivocal hrHPVT on ThinPrep specimens correlated with more frequent high-grade dysplasia on follow-up cervical histology than did unequivocal positive hrHPVTs, and moreover correlated with more severe dysplasia (i.e., more CIN 3). Therefore, ASCUS-equivocal hrHPVTs should not be repeated as currently recommended but managed similarly to ASCUS-positive hrHPVT. These tests may indicate a higher risk of dysplasia than those associated with unequivocal positive hrHPVTs.

1056 Utility of P16, KI67 and HPV Test in Diagnosis of Cervical Intraepithelial Neoplasia and Atrophy in Women over 50 Years of Age with 3-7 Year Follow-Up.

JA Jackson, U Kapur, P Rajan, A Salhadar, C Ersahin. Loyola University Medical Center, Maywood, IL.

Background: Differentiating Cervical Intraepithelial Neoplasia (CIN) from atrophy in postmenopausal women is challenging based on morphology alone. p16 and Ki67 help in distinguishing CIN2/3 from atrophy, and a positive HPV test increases the likelihood of follow-up histologic diagnosis of CIN2/3 in women over age 50. Our goal is to further characterize the utility of p16, Ki-67 and HPV tests in women over age 50, particularly in CIN1.

Design: We retrospectively identified consecutive cervical specimens (biopsy, cone, and LEEP) from three one-year time periods (2003, 2004, and 2007). Included were cases from women ≥ age 50 with benign diagnoses, atrophy, and CIN. Slides were stained with p16 and Ki67. p16 was graded as positive or negative, and Ki67 graded as positive or negative. Medical records were reviewed for cytology, HPV test, and histopathologic diagnoses from time of biopsy to 2010.

Results: 97 cervical samples from 86 women (mean age=57) were analyzed and stained. The reason for sampling was either for follow up of abnormal pap smear in 68 (70%) cases or for other reasons (i.e. abnormal biopsy, postmenopausal bleeding). 34 (74%) of CIN1 cases were negative for both p16 and Ki67 (see table). Of CIN1 cases with positive HPV tests, only 1/11(9%) had positive p16 staining, versus 2/2 (100%) of CIN2/3 cases. 39 women with CIN1 had follow-up data available. Of those, 4 (10%) had subsequent histologic progression to CIN2/3 and none developed invasive disease. Of these four cases, p16 was positive in 1 case, and HPV test was positive in a separate case.

P16/Ki67 Staining Pattern and HPV status

	Total Cases	p16-/Ki67-	p16-/Ki67+	p16+/Ki67-	p16+/Ki67+	HPV*
Benign	33	30(90%)	2(6%)	0	1(3%)	6/9
Atrophy	6	5(83%)	1(17%)	0	0	0/2
CIN1	46	34(74%)	7(15%)	1(2%)	4(9%)	11/27
CIN2/3	12	2(17%)	2(18%)	0	8(67%)	2/2

* # positive/total tests performed

Conclusions: In our study, the majority of cases (74%) diagnosed as CIN1 in women ≥age 50 are negative for p16 and Ki67 and do not progress to high grade dysplasia during 3-5 year follow-up. Longer term study, however, is warranted to determine whether cases of p16 and Ki67 negative CIN 1 in this population behave like other benign processes with a low risk of progression, similar to atrophy. A combination of morphology, p16 and Ki67 on cervical specimens in women over 50 years of age, and furthermore, use of these stains on pap smear specimens, in combination with HPV testing may help distinguish CIN from atrophy, and reduce unnecessary invasive follow-up testing.

1057 Increased CD4 Positive T-Cell Recruitment in Primary Chronic Vestibulitis Suggests Potential Disease Triggers.

E Jacobson-Dunlop, C Leclair, M Goetsch, TK Morgan. OHSU, Portland; Oregon Health & Science University, Portland.

Background: Chronic vestibulitis is a common cause of localized introital pain and sexual difficulty. In a tightly controlled prospective study, we recently demonstrated that vestibulitis has more chronic inflammation, neural hypertrophy, and mast cells, than normal control vestibular biopsies. The objective of the current study was to test for significant differences in lymphocyte sub-types in vestibulitis compared with controls.

Design: We used archived tissue sections from our prior prospective study, which recruited adult premenopausal women from the Program in Vulvar Health at Oregon Health and Science University. Patients had severe entry dyspareunia for at least one year and sampling had been performed on tender and nontender vestibule mucosa. Women designated as having primary vestibulitis (n=10) had noted significant pain from the first attempted vaginal entry. Secondary sufferers (n=10) had *de novo* pain onset after no prior history of localized entry pain. An unaffected control group (n=4) had no history of entry dyspareunia. Histologic sections were immunostained in duplicate for CD20, CD3, CD4 and CD8. The study set was scored while blinded to phenotype for the average number of positive cells per high-powered 20x objective field. Differences between groups was evaluated by ANOVA with Bonferroni/Dunn post-hoc testing.

Results: Tender biopsies from women with primary vestibulitis showed a significant shift in the CD8:CD4 ratio ($p<0.01$). They had twice as many CD8 positive cells and twenty times as many CD4 positive T-cells than controls. The number of T-cells was not significantly different in secondary vestibulitis compared with controls, but they did have more CD20 positive B-cells.

Conclusions: This observation suggests CD4 positive T-cell recruitment may provide a clue to understanding potential triggers in primary disease. The predominance of CD20 positive B-cells in secondary vestibulitis suggests a more subacute disease process. Notably, many triggers for vestibulitis have been evaluated in the past without convincing results, perhaps because these studies did not distinguish between primary and secondary disease.

1058 Are Immunoprofile and JAZF1 Genetic Rearrangement of Low-Grade Endometrial Stromal Sarcomas Potential Markers of Aggressive Behaviour?

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Background: Endometrial stromal tumors are uncommon mesenchymal tumors of the uterus. Apart from stage, prognostic factors for endometrial stromal sarcomas (ESS) are not well established. The relationship of tumor genotype (JAZF1-rearrangment) and immunoprofile to clincopathologic parameters is unclear. We compared morphological, immunhohistochemical and genetic profiles of stage 1 versus higher stage low-grade endometrial stromal sarcomas (LG-ESS).

Design: 23 consecutive LG-ESS cases from 23 patients with material available for study were retrieved from pathology files (1990- 2010). A tissue microarray (TMA) paraffin block of duplicate 0.6mm cores was prepared. Immunohistochemistry (IHC) for p53, estrogen receptor (ER), progesterone receptor (PR) and CD10 were done according to standard techniques and assigned an Allred score wherein a score >= 3 was considered positive. For Ki67 a percentage of >10% was considered as positive. All H&E stained slides and IHC stained sections of TMA were reviewed and scored by two pathologists. FISH analysis for the JAZF1 rearrangement was performed. For IHC variables and JAZF1 rearrangement, Fisher's exact test and for mitosis, Wilcoxon's rank-sum test was used to compare stage 1 versus higher stage ESS.

Results: The average age was 48.4 years and all patients were alive at the time of study except one. Follow-up was available in all but one case. Cases included 13 stage I, 3 stage II, 2 stage III, 1 stage IV, 2 primary ovary, 1 extra uterine pelvic mass. More frequent p53 positivity and more frequent JAZF1 rearrangement were seen in higher stage LG-ESS compared to stage 1 diseases. Other parameters showed very similar results in stage 1 versus higher stage disease (Table). None of parameters showed difference when group of patients without recurrence was compared to the patients who had disease recurrence, regardless of stage.

Profile of Stage 1 versus higher than Stage 1 LG-ESS

	p53	ER	PR	CD10	KI67	JazF1	Mean mitoses/10 hpf (STD)
Stage 1	31%	85%	85%	92%	15%	23%	5.06 (6.25)
Higher Stage	83%	100%	100%	100%	0%	66%	5.13 (7.56)

Conclusions: Our data suggest that higher stage ESS is associated with more frequent p53 positivity and more frequent JAZF1 rearrangement. These findings may represent potential markers of aggressive behavior and different underlying mechanisms of tumor progression to higher stage disease.

1059 HPV 16/18 Genotyping in Assisting the Identification of Cervical Precancerous Lesions in Women with Negative Cervical Cytology and Positive High-Risk HPV Screening.

H Ji, C Schneider. Eastside Pathology, Inc., Bellevue, WA.

Background: Co-testing of cervical cytology and high-risk HPV DNA has been widely used for cervical cancer screening in women 30 years and older. A small percentage of those women will have discordant results, i.e., negative cytology and positive HPV. Given the significantly higher cancer risk involved with infections by HPV 16 and 18 compared to infections by other HPV types, the ASCCP has recently advised triaging those women using HPV 16/18 genotyping. If a woman has HPV 16 and 18 detected by genotyping, she will be referred to immediate colposcopy. If HPV 16/18 is negative, she will be followed-up with repeat cytology and high-risk HPV testing in 12 months. In this report, we present our experience with HPV 16/18 genotyping.

Design: During a 12-month period between May 2009 and April 2010, a total of 22,023 cytologically negative liquid-based cervical specimens were tested for the presence of high-risk HPV DNA by the Cervista HPV HR method. A portion of high-risk HPV positive cases were subject to HPV 16/18 genotyping per clinicians' request. A retrospective review was performed to identify all cases with subsequent biopsy diagnosis.

Results: HPV 16/18 genotyping was performed in 326 specimens among 1,214 cases that were positive for HPV DNA. HPV 16/18 DNA was found in 94 cases, with other high risk HPV types in the remaining cases. Subsequent biopsies were performed on 52 patients who were HPV 16/18 positive, 14 patients (26.9%) were found to have cervical precancerous lesions, including CIN 1 in 7 cases, CIN 2-3 in 6 cases and AIS in one case. On contrary, all HPV16/18 negative cases showed normal histology when subjected to histologic evaluation (0/10).

Conclusions: Our findings suggest that HPV 16/18 genotyping is capable of identifying cervical precancerous lesions that would otherwise be missed, including several cases of high grade squamous intraepithelial lesion and one adenocarcinoma in situ. HPV 16/18 genotyping could serve as a clinically useful tool in triaging women 30 years and older who have negative cervical cytology and positive HPV DNA screening results.

1060 Are CIN2 Lesions Overdiagnosed? Selected Immunopanel Can Prevent Unnecessary Cervical Excisions.

R Karabakhtsian, M Cibull, C DeSimone. UKMC, Lexington, KY.

Background: Atypical cellular changes reflecting inflammation, immature squamous metaplasia and atrophy can be diagnostically challenging in small cervical biopsies (bx) when evaluating for high-grade (HG) cervical intraepithelial neoplasia (CIN). Such changes may mimic HG dysplasia and can lead to unnecessary cervical excision. The aim of this study was to retrospectively re-evaluate CIN2 lesions diagnosed on cervical bx in patients with no residual HG lesion in subsequent cervical excision, utilizing a panel of immunohistochemical (IHC) stains.

Design: 211 cervical bx from 151 patients with histologic diagnosis (Dx) of CIN2 who underwent cervical LEEP/Cone excision from 2000 to 2007 were retrieved from pathology files. Residual CIN2 was present in cervical excision in 89/151 (59%) patients (*group 1*, 130 bx*)* and absent in 62/151 (41%) (*group 2,* 81bx). 9/81 bx did not have sufficient tissue for IHC work-up. Tissue blocks of 72/81 cervical bx *(group 2)* were stained for 3 IHC markers: $p16^{INK4a}$ (CINtec, MTM) and proliferation markers MIB-1 (Dako) and proEx^{TM}C (BD). H&E and immunostained slides were independently reviewed by two pathologists. Diffuse nuclear and cytoplasmic staining of cells for p16 in at least half of the epithelial thickness was interpreted as positive for CIN2, and negative otherwise. Co-expression of nuclear staining for MIB-1 and proExC in cells within at least half of the epithelium was interpreted as supportive of CIN2, and negative otherwise.

Results: Histologic Dx of CIN2 *(group 2)* was supported by 3 stains in 61% (44/72) cervical bx and by 2 stains (p16-) in 13% (9/72). In 26% (19/72) of bx stains failed to support the presence of CIN2. Overall, CIN2 was overdiagnosed in 9% (19/211) cervical bx. Immunoreactivity for p16 was present in 83% (44/53) and absent in 17% (9/53) of true CIN2 lesions. In-situ hybridization (ISH) for HPV was performed in p16 (-) group (9 bx). In 4/9 tissue was insufficient for additional work-up. HPV 16/18 by ISH was (+) in remaining 3/5 and (-) in 2/5.

Conclusions: 1) In 25% (53/211) the absence of CIN2 in subsequent excision may be due to small lesion size with complete removal by colposcopic bx, or regression.
2) CIN2 was overdiagnosed in 9% cervical bx in this study.
3) Immunopanel of p16, MIB-1 and ProExC can be useful in separating CIN2 lesions from its benign mimics.
4) While not extremely sensitive for CIN2 (83%), p16 appears highly specific "key" biomarker and critical when dealing with diagnostic challenges.
5) ISH for HPV can be helpful in rare p16 (-) cases.

1061 Revised Histologic Criteria for Ovarian Carcinoma Cell Type Show Improved Correlation with Genotype and Protein Expression Profile.

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Background: In 2004 we reported data emphasizing the prognostic value of *TP53* mutation in FIGO stage I and II ovarian carcinoma (low stage OC). However, tumor histologic subtype, grade, and substage did not correlate with prognosis in this cohort. Histologic recalibration of these tumors using criteria proposed by *Gilks et al*. will allow reassessment of correlations between carcinoma type, genotype and immunophenotype.

Design: The cohort comprised 91 previously identified patients with low stage OC who underwent primary surgical management at our institution from 1980 to 2000. H&E slides were reviewed and histologic type reassigned using *Gilks et al*. criteria.

p53 and WT1 expression was evaluated using standard immunohistochemistry (IHC) techniques on a tissue microarray. p53 expression assessment has evolved between 2004 and 2010. In 2004, p53 expression profile was reported as either positive or negative. In 2010, abnormal p53 expression was scored when there was absolute loss of staining, or overexpression involving at least 50% of cells; p53 expression was considered to be within normal limits if it was focal and involved less than 50% of cells. Direct *TP53* gene sequencing of the entire coding region was performed on all cases with available tissue.

Results: Tumor histologic subtype distributions, as well as correlation between tumor cell type and the other variables are outlined in Table 1.

Table 1.

	2004	2010
Serous	28%	34%
Clear cell	27%	25%
Endometrioid	23%	29%
Mucinous	6%	7%
Malignant Brenner	2%	1%
Mixed epithelial	11%	4%
Carcinoma, unclassifiable	3%	0%
Serous with TP53 mutation	57%	75%
Non-serous with TP53 mutation	24%	13%
Serous with aberrant p53	58%	87%
Non-serous with aberrant p53	31%	9%
TP53 mutation with aberrant p53	71%	83%
Wild type TP53 with aberrant p53	27%	9%
Serous with WT1 expression	65%	77%
Non-serous with WT1 expression	8%	2%

Conclusions: Revised histologic diagnoses correlated more closely with *TP53* mutation status and p53/WT1 expression patterns. *TP53* mutations almost always result in aberrant p53 expression (complete loss or overexpression). Significantly more serous OCs had *TP53* mutation, abnormal p53 expression, and WT1 positivity.These results validate the criteria of *Gilks et al*. and indicate that meticulous histologic assessment allows accurate biologic characterization.

1062 Clinicopathologic Analysis of Low Stage Sporadic Ovarian Carcinoma.

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Background: In 2004 we reported data emphasizing the prognostic value of *TP53* mutation in FIGO stage I and II ovarian carcinoma (low stage OC). However, tumor histologic subtype, grade, and substage did not correlate with 5-year progression-free or overall survival in this cohort; and *TP53* mutation status was not strongly associated with cell type. We hypothesized that histologic recalibration of these tumors using criteria proposed by *Gilks et al*. would disclose closer associations between pathologic findings and prognosis.

Design: The cohort comprised 91 previously identified patients with low stage OC who underwent primary surgical management at our institution from 1980 to 2000. H&E slides were reviewed and histologic type reassigned using *Gilks et al*. criteria. p53 and WT1 expression was evaluated using standard immunohistochemistry (IHC) techniques on a tissue microarray. Direct *TP53* gene sequencing of the entire coding region was performed on all cases with available tissue. Relationships between survival and the following parameters were studied: histologic type, grade, presence of endometriosis, substage, p53 and WT1 expression, and *TP53* mutation. Tumors from known *BRCA*1 and 2 carriers were excluded.

Results: Endometrioid histology and presence of associated pelvic (tubal, peritoneal, or ovarian) endometriosis conferred favorable disease specific (DSS) and progression free survival (PFS) advantage. The presence of *TP53* mutation was prognostically unfavorable for PFS. The findings are summarized in Table1. The other parameters were not statistically associated with survival in this cohort.

Table 1.

	Histologic type		TP53		Endometriosis	
	Endometrioid	Non-endometrioid	Mutant	Wild type	Present	Absent
10-year overall survival (OS)	83.1	60.4	73.4	58.7	77.2	55.2
p	0.1		0.27		0.009	
10-year disease specific survival (DSS)	91.8	62	75.7	58.7	79.5	59.4
p	0.008		0.18		0.009	
10 -year progression free survival (PFS)	92	60.5	76.2	45.7	77.7	59.7
p	0.006		0.04		0.02	

Conclusions: Endometrioid histology and presence of pelvic endometriosis are favorable indicators in stage I – II OC. On the other hand, presence of *TP53* mutation is associated with decreased progression-free survival in low stage OC.

1063 Transformation of Fallopian Tube Epithelial Cells Leads to High-Grade Tumor Formation in a Xenograft Mouse Model.

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Background: High-grade serous ovarian carcinoma (HGSOC) is the most lethal gynecological malignancy in the United States. In order to develop effective screening modalities and novel therapeutic approaches, we must better understand its pathogenesis. Recent studies have implicated the fallopian tube secretory epithelial cell (FTSEC) as a cell-of-origin for HGSOC. However, despite compelling descriptive studies, FTSEC transformation has not been demonstrated experimentally. There is an urgent need to develop a model by which FTSEC transformation can be studied in the lab. Here, we present a proof-of-principle experiment demonstrating that FTSECs can be transformed *in vitro,* producing cells that are highly tumorigenic *in vivo*.

Design: Primary human FTSECs were isolated from fallopian tube fimbriae and immortalized via transduction with retroviral vectors encoding human *Telomerase Reverse Transcriptase (hTERT)* and *Simian Virus 40 T Antigens (SV40 TAg)*. Immortalized FTSECs were then transformed by introducing either *H-Ras G12V* or *c-Myc* oncogenes. Transformation was assessed *in vitro* by measuring cell proliferation, colony formation, and anchorage-independent growth. Tumorigenicity was assessed by xenograft into immunocompromised mice. Tumors were analyzed histologically and by immunohistochemistry.

Results: Both Ras- and Myc-transformed FTSECs exhibited increased proliferation, colony formation, and anchorage-independent growth ability *in vitro* compared to immortalized parental FTSECs. Moreover, both Ras-transformed and Myc-transformed FTSECs formed high-grade tumors when xenografted into immunocompromised mice, with latency periods of approximately 2 months and 4 months, respectively. The resulting tumors were histologically and immunophenotypically consistent with high-grade serous ovarian carcinoma.

Conclusions: Our results show that FTSECs can indeed be transformed into high-grade serous carcinomas. This is significant because: 1) it provides a working model that can be used to query the impact of specific genetic alterations, either alone or in combination, on normal primary FTSECs, and 2) it provides a framework for testing the transformative effects of candidate oncogenes on FTSEC growth, enabling us to identify the alterations that are most critical for driving serous tumorignesis in the fallopian tube epithelium.

1064 Stathmin 1, a Marker of PI3K Pathway Activation and Regulator of Microtubule Dynamics, Is Expressed in Early Serous Ovarian Carcinomas.

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Background: The fallopian tube secretory epithelial cell (FTSEC) is the likely cell of origin for most high-grade pelvic serous carcinomas, including serous ovarian carcinoma. Studies of serous tumor precursor lesions in fallopian tube epithelium demonstrate that *TP53* mutations and accumulation of DNA damage play critical roles in FTSEC transformation. However, few other markers characterize the progression from benign to malignant fallopian tube mucosa. Here, we identify *Stathmin 1* (*STMN1*) as a novel marker of FTSEC transformation. STMN1 is a microtubule destabilizing protein that regulates cytoskeletal dynamics, cell cycle progression, mitosis, and cell migration. Its activity is strongly linked to the activation of growth and differentiation signalling pathways.

Design: We used immunohistochemistry to assess STMN1 and $p27^{Kip1}$ expression in a panel of benign (n = 13) and malignant (n = 12) fallopian tubes containing normal epithelium, p53 signatures, tubal intraepithelial carcinoma, and invasive serous carcinoma. We further examined STMN1 expression in 131 high-grade late-stage serous ovarian carcinomas by tissue microarray analysis and a panel of ovarian cancer cell lines by Western blot.

Results: STMN1 was expressed in neither quiescent cells nor benign lesions of the fallopian tube epithelium, but was robustly induced with progression to tubal intraepithelial carcinoma. STMN1 induction during early tumorigenesis was accompanied by decreased $p27^{Kip1}$ expression, a negative regulator of G_0- to S-phase transition and direct inhibitor of STMN1. STMN1 was expressed in > 80% of high-grade serous ovarian carcinomas and ovarian cancer cell lines, indicating that expression is retained in late disease stages. Additionally, STMN1 may be a useful proliferative cell marker, independent of MIB1.

Conclusions: The dynamics of STMN1 expression observed in early tubal lesions suggest that STMN1 plays a critical role in FTSEC cell cycle progression. Its induction in benign-appearing cells may signal cell cycle entry and identify pre-mitotic cells with proliferative potential. STMN1 expression might contribute to HGPSC pathogenesis by relaying growth signals from oncogenic signal transduction pathways to the cytoskeleton or by potentiating early-stage cell migration and loss of polarity.

1065 HPV Implication Pattern in Coexistent HSIL/AIS Lesion.

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Background: High-grade squamous intraepithelial lesion (HSIL) and adenocarcinoma *in situ* (AIS) are premalignant uterine cervical lesions, and most of them are human papilloma virus (HPV) associated. For the last few decades a panel of theories mentioned the origin of coexistent HSIL and AIS lesion; however there are still few molecular evidences related to those theories. In this study, we examined HPV subtypes as well as the methylation status of the *HPV-16 L1* gene to reveal the difference or affinity of carcinogenetic pathway between HSIL and AIS.

Design: A total of 8 HSIL and AIS lesions were removed by conization or hysterectomy. In each case, HSIL and AIS lesions were isolated individually by micro-dissection from deparaffinized tissue sections. Further, high-risk HPV (HPV-16, -18, -31, -33, -52, -58, and -35) PCR genotyping were performed. Bisulfite-treated DNA was processed to analyze HPV-16-positive cases for the methylation status at the L1 and LCR genes; after a while the methylation status within HSIL and AIS were compared by *P*-value of Fisher's exact test.

Results: Out of 8 cases, 6 (75%) showed same subtype of HPV in both HSIL and AIS: HPV-16, 5 cases; and HPV-18, 1 case. And then 2 (25%) cases contained different subtypes of HPV between HSIL and AIS: HPV-31 (HSIL) and HPV-18 (AIS); and HPV-52 (HSIL) and HPV-16 (AIS). Among the five cases which contained HPV-16 in both HSIL and AIS, no significant difference with one exception in the frequency of

CpG island methylation status in the HPV-16 L1 region were observed; likewise only one case within 6796 genomic position was significantly methylated in HSIL compared with coexistent AIS ($P = 0.0278$).

Conclusions: Our finding suggests that same HPV subtype is involved in the individual carcinogenetic pathway in the majority of the cases coexistent HSIL and AIS lesions. Further more, there is no significant difference between HSIL and AIS in the methylation pattern of HPV-16 L1 gene. These facts suggest that, the two lesions largely arise from same progenitor cells in which recombination between HPV and host cell DNA is occurred. While in the minority of the cases, HSIL and AIS may arise individually with different HPV infectious background and collided with each other.

1066 Microsatellite Marker Polymorphisms Provide Evidence for Germ Cell Origin of Mucinous Ovarian Carcinomas Associated with Concurrent Teratomas.

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Background: Two decades ago, careful studies of chromosomal heteromorphisms and DNA polymorphisms proved that ovarian teratomas are derived from the female host during gametogenesis. When the teratoma arises during meiosis I, chromosomal recombination events result in partial homozygosity in multiple chromosomes. When the teratoma arises during meiosis II, a haploid gamete can either endoreduplicate (resulting in complete homozygosity), or fuse with another haploid gamete (resulting in partial homozygosity). We sought to use these concepts to prove that mucinous carcinomas associated with ovarian teratomas are also germ cell derived.

Design: Six cases of mucinous carcinoma associated with ovarian teratomas were identified. DNA was extracted separately from the teratoma, mucinous carcinoma, and normal tissue after scalpel dissection of tissues from unstained formalin fixed, paraffin embedded sections. Twelve polymorphic microsatellite markers were PCR amplified for each sample; the resulting fluorescently labeled fragments were measured by capillary electrophoresis. Alleles from the teratomas and carcinomas were scored for each marker as non-informative (normal tissue homozygous or PCR failure), heterozygous (two alleles present), or homozygous (one allele present in tumor when normal tissue heterozygous).

Results: Three cases of mucinous carcinoma showed near complete/complete homozygosity for informative markers (8/9, 9/9, and 10/10, respectively). Two of these had identically homozygous teratomas (the third teratoma was unavailable for analysis). Two carcinoma/teratoma pairs were completely heterozygous for all 8 informative markers. The remaining case had a teratoma homozygous for 2 of 10 informative markers, while the matching carcinoma was definitively homozygous for only one of these markers.

Conclusions: Microsatellite polymorphism analysis demonstrates that mucinous ovarian carcinomas genetically match associated teratomas when present. Although this technique does not unequivocally distinguish between homozygosity and loss of heterozygosity (hemizygosity) in the carcinomas, presumed homozygosity across multiple markers in 3 of 6 cases is strong evidence that some mucinous carcinoma/teratoma pairs arise from an endoreduplicated haploid gamete during meiosis II. Similar testing of a larger set of mucinous ovarian carcinomas may show that a subset of mucinous carcinomas have obliterated a pre-existing teratoma and are germ cell (rather than epithelial) derived.

1067 Synchronous Versus Metastatic Gynecological Cancers: A Comparative Mutational Profiling Study.

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Background: Separation of synchronous ovarian primary cancer from a metastatic uterine cancer to the ovary can be difficult and currently relies primarily on the conventional histological examinations, yet such a separation may have important clinical and therapeutic implications. Comparative mutational profiling (CMP) involves the analysis of loss of heterozygosity (LOH) among lesions to facilitate such differential diagnoses. We evaluated the efficacy of CMP in separating a synchronous ovarian primary vs. a metastatic endometrial carcinoma to the ovary

Design: Ten cases of endometrial adenocarcinoma with lesions simultaneously involving the ovary were retrospectively retrieved from our file. All cases were reviewed according to the conventional histological criteria in separation of a synchronous ovary primary with a metastatic endometrial carcinoma involving the ovary. Non-neoplastic tissue and multiple foci of both endometrial and ovarian tumors were microdissected and subject to comparative mutational profiling analysis involving 16 polymorphic loci. Allelic imbalance or LOH at each locus was documented and compared between the endometrial and the ovarian lesions.

Results: Among ten cases selected and histologically reviewed, 2 cases of synchronous endometrial and ovarian tumors, 7 cases of metastatic endometrial carcinomas, and 1 case of metastatic ovarian carcinoma were confirmed based on the conventional histological criteria (morphology, bilateral ovarian involvement, depth of myometrial invasion, lymphovascular involvement, and ovarian surface involvement). CMP analysis revealed an excellent correlation with the histological classification of the tumors in 9 out of 10 cases.

Conclusions: Comparative mutational profiling appears reliable in assessing the relatedness of gynecological cancerous lesions. Such an objective molecular approach has a diagnostic power at least similar to that of the histological evaluation. Additional studies are needed to further confirm the efficacy of CMP in the workup of gynecological tumors simultaneously involving multiple mullerian organs.

1068 Redefining the Gold Standard for Cervical Dysplasia Diagnosis in Risk Factor Analysis.

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Background: Diagnosing cervical dysplasia by conventional hematoxylin & eosin (H&E)-based methods is limited by poor interobserver reproducibility and significant inaccuracy, especially in cases of moderate dysplasia (CIN2). An unrecognized consequence of this problem may be effects on cervical dysplasia risk factor analysis. New molecular tools such as immunostaining for p16 appear to improve diagnostic agreement and predictive value; therefore, we hypothesize that combining p16 and H&E-based diagnoses will significantly change understanding of the relationship between risk factors and cervical dysplasia.

Design: Routine H&E, p16, and Ki67 stained slides were prepared from 252 random colposcopic biopsies obtained from Kaiser Northwest, which had at least five years of clinical follow-up (including negative serial Pap tests or surgical excision). Two expert gynecologic pathologists independently reviewed each case blinded to all clinical data. Diagnoses were reported based on H&E only, H&E and p16, and H&E, p16 and Ki67 after sufficient "wash out periods." Kappa statistic and test accuracy were calculated for each method relative to the gold standard based on clinical follow-up. Odds of at least CIN2 were determined in multivariate logistic regression models using a constant panel of risk factors (eg, age, family income, education level) while varying the diagnostic method.

Results: H&E plus p16-based diagnoses of CIN2 were more reproducible and accurate than H&E only, consistent with recent reports (see table). Ki67 staining was not justified. Low family income was only significantly associated with at least CIN2 if using p16 assisted diagnoses (p=0.02 vs. p=0.61). There was a trend toward significant change in the odds of at least CIN2 if the patient had low family income in regression analysis only if using p16 assisted diagnoses (p=0.057).

CIN2 (*2+)	Kappa	Accuracy*	Sensitivity*	Specificity*
H&E only	0.40	82%	81%	87%
H&E and p16	0.48	95%	94%	100%

Conclusions: Despite the relatively small sample size in this pilot study, the association between low family income and at least CIN2 became significant when diagnoses were made with H&E and p16, and there was a trend toward significance for the odds of at least CIN2 for women with low family income if using H&E and p16. This shifting relationship implies that more accurate diagnoses provided by p16 staining may lead to more valid risk estimation in future epidemiologic studies.

1069 MLH1-Deficient Ovarian and Endometrial Carcinomas Most Often Result from Epigenetic Silencing of MLH1 by Promoter Hypermethylation and Do Not Harbor BRAF V600E Mutations: Implications for Identifying Patients with Lynch Syndrome (LS).

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Background: Mismatch repair (MMR) protein abnormalities occur in both sporadic gynecologic carcinomas and carcinomas from persons with Lynch syndrome (LS). Gynecologic carcinomas may acquire epigenetic alterations, including hypermethylation of the *MLH1* gene promoter, resulting in microsatellite instability. In colorectal carcinoma, 80% of tumors with deficient MLH1 immunohistochemical expression result from *MLH1* promoter hypermethylation which is strongly associated with *BRAF* V600E mutation. We investigated the incidence of *MLH1* promoter hypermethylation and *BRAF* V600E mutations in MLH1-deficient gynecologic carcinomas.

Design: We retrospectively examined 381 ovarian and endometrial carcinomas from 1997 to 2010 for MMR protein abnormalities by immunohistochemistry for MLH1 (clone G168-728, BD PharMingen), MSH2 (clone FE11, Oncogene), MSH6 (clone 44, BD Transduction), and PMS2 (clone MRQ-28, CellMarque). Carcinomas with concurrent loss of expression of MLH1 and PMS2 were analyzed for the V600E mutation of the *BRAF* gene by real-time PCR and post-PCR allelic discrimination melting curve analysis and *MLH1* promoter methylation using real-time PCR (Methylight) for quantitative DNA methylation analysis, as described (Weisenberger DJ et al. Nature Genetics 2006; 38: 787).

Results: Fifty-six of 381 (15%) carcinomas demonstrated loss of MLH1 and PMS2 expression with a median age of 63 years (range 42 to 86 years). None of the 56 cases demonstrated a *BRAF* V600E mutation. *MLH1* promoter hypermethylation was identified in 54/56 (96%) carcinomas. Two carcinomas, one ovarian endometrioid carcinoma diagnosed at 55 years and one endometrial mucinous carcinoma diagnosed at 56 years, demonstrated an absence of *MLH1* promoter hypermethylation.

Conclusions: MLH1 protein deficiency occurs frequently in endometrial carcinomas and is almost always caused by epigenetic silencing of *MLH1* due to hypermethylation of its promoter region. In contrast to colorectal carcinoma, routine screening for MLH1 protein deficiency in gynecologic carcinomas may not be effective in selecting patients for germline genetic testing for LS. In addition, *BRAF* V600E mutation analysis is not useful in distinguishing sporadic gynecologic carcinomas from carcinomas in persons with LS. When identified, MLH1-deficient gynecologic carcinomas should be tested for *MLH1* promoter hypermethylation, although such analysis will not distinguish rare carcinomas with constitutional (germline) *MLH1* epimutation.

1070 An Immunohistochemical Panel Comprised of HNF-1β, WT-1, and PAX 2 To Distinguish Ovarian Clear Cell Carcinomas from Ovarian Serous Carcinomas and Metastatic Renal Clear Cell Carcinomas.

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Background: Ovarian clear cell carcinomas (OCCC) are uncommon ovarian epithelial tumors with unique clinical behavior, including poor response to platinum-based chemotherapy. Although OCCC often display characteristic morphologic features, there

can be considerable overlap with other ovarian epithelial tumors (most often, ovarian serous carcinoma (OSC)) and with metastatic tumors such as renal clear cell carcinoma (RCCC). We studied the pattern of expression of three markers by OCCC, OSC and RCC. In addition to Wilm's tumor 1 protein (WT-1, a mesothelial marker expressed in OSC but uncommonly expressed in OCCC), the immunohistochemical panel includes Hepatocyte Nuclear Factor-1β (HNF-1β) and PAX 2. HNF-1β is expressed in renal tubules and collecting ducts but is not significantly expressed in adult ovaries or testes. It is aberrantly expressed in OCCC, but is rarely expressed in other ovarian epithelial tumors. PAX 2 is expressed in normal endometrium and tubal epithelium. Prior studies have shown its expression in mullerian tumors such as OSC, and have described it as a marker of mullerian origin in metastatic tumors. The aim of our study is to describe the expression of these three markers in OCCC, OSC and RCCC, to facilitate the use of this panel to aid in the diagnosis of morphologically challenging cases.

Design: Cases of OCCC (33), OSC (10) and RCCC (6) were retrieved by searching our pathology database. Immunohistochemical stains were performed on formalin fixed paraffin embedded tissue using three antibodies: rabbit polyclonal HNF-1β (Sigma Prestige), rabbit polyclonal PAX 2 (Invitrogen-Zymed Laboratories) and WT-1 (clone 6F-H2, Ventana Medical). We assigned an intensity score (IS) of 0 (negative), 1 (weak), 2 (moderate) or 3 (strong), and a proportion score of 0 (0 to <1%), 1 (≥1 to 5%), 2 (6 to 20%), 3 (21% to 80%) or 4 (>80%). The cumulative score (CS) is the sum of the IS and PS.

Results:

IMMUNOHISTOCHEMISTRY MEAN CUMULATIVE SCORES

	OCCC	OSC	RCCC
HNF-1β	5.0	1.4	6.8
WT-1	1.5	2.9	0
PAX 2	2.6	7.0	5.8

Conclusions: HNF-1β is strongly expressed in the majority of OCCC, but not in OSC, confirming it as a sensitive marker for OCCC. OCCC tends to express HNF-1β and PAX 2, in contrast to OSC, which tends to express WT-1 and PAX 2. RCCC expresses HNF-1β and PAX 2, but not WT-1. Our study is the first to investigate the expression of PAX 2 in OCCC. This panel of immunohistochemical stains will help to more definitively classify these tumors in routine practice.

1071 Single Isolated Cells Showing Loss of E-Cadherin Expression in the Subtypes of Pseudomyxoma Peritonei; Its Possible Role in the Disease Progression.

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Background: Pseudomyxoma peritonei (PMP) can be classified into two subtypes, disseminated adenomucinosis (DPAM), and peritoneal mucinous carcinomatosis (PMCA) according to the cytomorphologic features of peritoneal lesions and clinical behavior. However, differences in the mechanism of disease progression between the two subtypes have not been explained. We suspected that the difference in the expression of adhesion related protein or epithelial mesenchymal transition, which is crucial for invasion, might be related to the difference of progression rates and prognosis between the two subtypes.

Design: To compare the expression of adhesion related proteins, we performed immunohistochemical stainings for E-cadherin, vimentin, cytokeratin 7 and 20 between the two subtypes in the peritoneal lesions of PMPs using paraffin sections. The cases were 52 pseudomyxoma peritonei treated at Asan Medical Center during 1995-2010. We counted the number of cellular strips composed of cohesive cell clusters and single isolated cells (SICs) floating in mucin pools separately using 10x10 grid micrometer from Olympus BX51 at 400x magnification field on H&E stained and immunostained sections, and then compared the number of SICs with the expression patterns for E-cadherin, and vimentin in cellular strips and SICs, respectively between the two subtypes. CK7 and 20 were used for identification of tumor cells.

Results: Loss of E-cadherin expression was significantly higher in SICs compared to cohesive cellular strips in both subtypes. Median follow up periods was 998 days (range: 14-4631 days). According to the histologic subtypes and the number of SICs, significant difference in the overall survival rates was identified between the two subtypes. The patients having more than 30 SICs/x400 fields had signficantly worse prognosis compared to those having less than 30 SICs, and the number of cases showing more than 30 SICs was significantly higher in PMCA compared to DPAM ($p<0.001$). Vimentin expression was increased in the SICs, but vimentin expressing cells were undistinguishable from the macrophage in the mucin pools.

Conclusions: Slow rate of tumor progression in DPAM subtype of pseudomyxoma peritonei might be attributable to the smaller number of SICs showing loss of cell adhesion molecules, E-cadherin, whereas rapid progression of PMCAs subtype to the larger number of SICs showing loss of cell adhesion molecules.

1072 Low-Grade "Ovarian" Serous Carcinomas Are Derived Mainly from the Fallopian Tube.

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Background: Ovarian serous carcinoma can be broadly categorized into two clinicopathologically distinct groups: high- and low-grade. A significant subset, and possibly the majority, of high grade serous carcinomas are presently thought to originate in the fallopian tube. However, the cell of origin of their low grade counterparts is presently unclear. We compared the phenotypes of ovarian surface epithelia (OSE), ovarian epithelial inclusion cysts (OEIs) and low-grade serous carcinomas (LG-Ca) in order to test the hypothesis that LG-Ca have a tubal, rather than ovarian surface epithelial phenotype

Design: 98 benign ovaries, 50 fallopian tubes, 48 serous cystadenomas, 42 serous borderline tumors, and 28 low-grade serous carcinomas, including the OSE and OEIs of the benign ovaries, were studied by immunohistochemistry using antibodies to PAX8, Calretinin, Ki-67, and tubulin. The secretory to ciliated cell ratio (S/C) was also calculated for each case.

Results: See Table 1.

Secretory and ciliated cells

Features	OSE	OEI	FT	Cystadenoma	Bordeline tumor	LG-CA
S/C ratio^	<0.0001	3.8 +/- 1.1	1.2 +/- 0.5	7 +/- 3.2	35 +/- 12	98 +/- 1.2
PAX8	2/48 (4%)	256/261 (98%)	50/50 (100%)	48/48 (0%)	42/42 (100%)	28/28 (100%)
Calretinin	48/48 (100%)	4/261 (2%)	0/50 (0%)	0/48 (0%)	0/42 (0%)	0/28 (0%)
Tubulin*cells	2 +/- 1.8%	48 +/- 15%	75 +/- 16%	41 +/- 20%	22 +/- 10%	5 +/- 2.3%
ki-67**index	<1%	4 +/- 1.2%	8 +/- 3.5%	5 +/- 2.1%	15 +/- 4.8%	32 +/- 12%

^S/C ratio based on H&E findings. *Tubulin stains ciliated cells, which was presented as an average of sum of all cases studied. **The numbers represent the average Ki-67 index for all cases studied. Cystadenoma, borderline tumor and LG-Ca diagnosed as ovarian based on conventional criteria

Conclusions: The immunophenotype of LG-Ca, OEIs, and normal tubal epithelium, are similar, and are notably different from the immunophenotype of OSE. This suggests that LG-Ca are ultimately tubal in origin. Differential patterns of tubulin expression between tubal epithelium and LG-Ca cells suggest that the cancer develops from a clonal expansion of tubal secretory cells, although detailed mechanisms underlying this process remain unclear. Alternatively, LG-Ca may arise through the direct implantation of tubal epithelium onto the ovary to form OEIs, from which the cancer develops.

1073 The Diagnostic and Biological Implications of Laminin Expression in Serous Tubal Intraepithelial Carcinoma.

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Background: Mounting evidence indicates that serous tubal intraepithelial carcinoma (STIC) is the likely precursor of most ovarian high-grade serous carcinomas (HGSCs). It has been proposed that cells from STICs are shed from the fallopian tube and implant on the ovary developing into a tumor, which simulates a primary HGSC. However, the molecular mechanisms underlying the dissemination of the cells from a STIC are not known. In order to identify the molecules that may be responsible for this critical process, we analyzed the ovarian cancer gene expression and identified several upregulated genes associated with HGSC. Among these, we selected laminin for further study because it has been shown to be involved in cell adhesion, motility and invasion.

Design: RT-PCR was used to assess the expression of different laminin isoforms (LAMA2, LAMA3, LAMC1, and LAMC2) in fresh tissue samples from 8 ovarian HGSCs, 9 ovarian cancer cell lines and 12 primary cultures of normal fallopian tube epithelium (FTE). Immunohistochemistry for LAMA3, LAMC1, p53 and Ki-67 was performed on formalin-fixed paraffin embedded tissue sections from 18 STICs, 16 of which were associated with concurrent ovarian HGSCs. LAMA3 and LAMC1 were scored based on intensity of cytoplasm (0 to 3+), and p53 and ki67 based on percentage of positive cells.

Results: RT-PCR, showed a statistically significant increase of LAMA2 ($p=0.044$) and LAMC1 ($p=0.0006$), and reduction of LAMA3 ($p=0.0032$) and LAMC2 ($p=0.0006$) in the HGSCs samples and the ovarian cancer cell lines as compared to controls. LAMA3 was expressed in normal FTE, STICs and HGSCs and was decreased in intensity in 9 (56%) of 16 HGSCs compared to STICs and FTE. Intense LAMC1 immunoreactivity (2+ and 3+) was detected in 17 (94.4%) of 18 STICs and 14 (87.5%) of 16 of the concurrent HGSCs whereas the LAMC1 staining was undetectable or weak (1+) in all FTE from the same patients. Interestingly, LAMC1 immunoreactivity was intense in 7 STICs in which p53 staining was absent and in 5 STICs with low Ki-67 index (<20%).

Conclusions: LAMC1 appears to play an important role in the development of HGSC. Since it is involved in cell adhesion, motility and invasion, upregulation of LAMC1 may facilitate shedding and dissemination of STIC cells to the ovaries and other peritoneal and abdominal structures. The presence of LAMC1 immunoreactivity in STICs, especially those with negative p53 staining and low Ki-67 labeling index, suggests that LAMC1 could be a reliable tissue biomarker to identify STICs.

1074 *TP53* Mutations in Serous Tubal Intraepithelial Carcinoma and Concurrent Ovarian High-Grade Serous Carcinoma.

E Kuhn, RJ Kurman, RA Soslow, G Han, T-L Wang, I-M Shih. Johns Hopkins Medical Institutions, Baltimore, MD; Memorial Sloan Kettering Cancer Center, New York, NY.

Background: Somatic mutation of *TP53* is the most common molecular genetic alteration in high grade serous carcinoma (HGSC) of the ovary, occurring in more than 95% of cases. As serous tubal intraepithelial carcinomas (STICs) have been proposed to be the most likely precursor of HGSC, we undertook a study to determine whether STICs harbor *TP53* mutations as well. Mutations of *TP53* have been reported in STICs and associated HGSC but that study [1] was limited to 5 cases. We therefore performed *TP53* mutational analysis in a larger series of STICs and concurrent HGSCs and correlated the mutational status with p53 immunoreactivity.

Design: Formalin-fixed paraffin-embedded tissue specimens were obtained from 18 HGSCs with concurrent STICs; 8 of them contained two discrete STICs. Approximately 1,000 cells from STIC and normal-appearing fallopian tubal epithelium were laser-capture microdissected from STICs, while HGSCs were manually microdissected. Genomic DNA was extracted, PCR amplified and sequenced. *TP53* mutations were analyzed from exons 4 to 8. Immunohistochemistry (IHC) for p53 was performed in all the cases.

Results: *TP53* mutations were detected in 15 (83.3%) of 18 HGSCs. Importantly, *TP53* mutations were not detected in the corresponding FTE samples from the same patients, confirming they are somatic mutations in HGSCs. STIC and associated HGSC shared identical *TP53* mutations in 15 (93.8%) of 16 patients including 7 who had two STICs. The discordant case showed *TP53* mutation in the HGSC but not in two separate concurrent STICs. By IHC, all the cases demonstrated the same pattern of p53 immunoreactivity (either all positive or completely negative) except the case with wild-type *TP53* in the STIC and mutant *TP53* in the HGSC. By IHC the STIC was p53 negative and the HGSC p53 positive. The three *TP53* wild-type HGSCs and their associated STICs exhibited undetectable nuclear p53 by IHC. In contrast, four p53-negative HGSCs contained mutant *TP53* with either a deletion or an insertion mutation.

Conclusions: Our findings provide cogent evidence that *TP53* mutations occur in most STICs, and that both STIC and concurrent HGSC share the identical *TP53* mutations in the majority of cases. Future molecular genetic studies are necessary to delineate the clonal relationship and tumor progression from STIC to HGSC.

Reference

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1075 Proliferative Activity in Serous Tubal Intraepithelial Carcinoma Compare to Adjacent Normal Tubal Epithelium and Concurrent High-Grade Serous Carcinoma.

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Background: Serous tubal intraepithelial carcinoma (STIC) has been recently recognized as a potential precursor lesion of ovarian high-grade serous carcinoma (HGSC) but reproducibly diagnosing it, even among expert gynecologic pathologists, can be very difficult. Although proliferative activity as indicated by the Ki-67 labeling index has been reported to be increased in STICs, a direct comparison of the Ki-67 index in the STIC to normal-appearing fallopian tubal epithelium (FTE) and the associated ovarian HGSC in the same patient has not been described. In this study we compare the Ki-67 index of FTE, STIC and ovarian HGSC in the same patient to evaluate whether Ki-67 staining can assist in the diagnosis of a STIC.

Design: A total of 33 STICs were analyzed, and among them 29 were associated with concurrent HGSC. Nine normal fallopian tubes from postmenopausal patients without neoplastic diseases were included as controls. Histological diagnosis of STICs was made according to previously reported morphological criteria (1). The Ki-67 index (using the Mib-1 antibody) was determined by calculating the percentage of cells showing nuclear immunoreactivity, in three random 20X-power fields. A minimum of 250 cells was counted.

Results: Immunoreactivity for Ki-67 in FTE was restricted to a few scattered cells and no statistically significant difference was found between patients with and without HGSC ($p>0.05$). On the other hand, both STICs and HGSCs had significantly higher Ki-67 indices than normal FTE ($p< 0.0001$). STICs were uniformly positive for Ki-67, with an index raging between 11.7%-71.1%. Ki-67 immunoreactivity was predominantly located in the basal layer in STICs. Based on the findings in 42 FTE specimens, we propose to use the mean Ki-67 index (2.4%) + 3 standard deviations (2.8% x 3) which approximated 10% as the cutoff to distinguish STICs (including p53 negative ones) from normal FTE (100% sensitivity and 93% specificity). In 29 cases with concurrent ovarian HGSC, the Ki-67 labeling index was higher in STIC vs. HGSC in 12/29 (41.4%) while it was lower in 17/29 (58.6%) ($p=0.55$).

Conclusions: Our data indicate that STICs have a high Ki-67 index similar to HGSC and that a Ki-67 >10% is a useful adjunct in making the diagnosis.

Reference

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1076 Presence of Sarcomatous Component outside the Ovary Is an Adverse Prognostic Factor for Primary Ovarian Malignant Mixed Mesodermal Tumors: A Study of 47 Cases.

J Kunkel, Y Peng, Y Tao, H Krigman, D Cao. Washington University School of Medicine, St. Louis; University of Texas-Southwestern Medical Center, Dallas.

Background: Primary ovarian malignant mixed mesodermal tumors (POMMMTs) are rare. This study was to investigate their clinicopathologic prognostic factors.

Design: We analyzed 13 parameters in POMMMTs from 47 patients: patient age, FIGO stage, laterality, tumor size, types of carcinomatous component (CC) and sarcomatous component (SC) (heterologous or homologous), percentage of CC and SC, mitotic figures per 10 hpfs in SC, tumor necrosis (percent of tumor), lymph node status (LN), vascular invasion (VI), tumor components outside the ovary (OTO), and surgical debulking status. The patients were followed to the most recent visit or their death. We correlated these parameters with disease-specific survival (DSS) using Kaplan-Meier method and Log-rank test.

Results: The mean age was 69.0 years (> 60 in 33/47). The tumor, average 13.6 cm, was predominantly located in the left and right ovary in 18 and 24 cases, respectively (similar size in both ovaries in 5). FIGO stage was I in 1, II in 5, III in 40, and IV in 1 patient, respectively. LN metastasis and VI was seen in 6/17 and 29/47 patients, respectively. The CC was high-grade serous in 27, endometrioid in 2, mixed high-grade serous and endometrioid in 17, and mixed high-grade serous and clear cell carcinoma in 1. The mean percentage of CC was 70% (10% to 99%). The SC was heterologous in 34 (72%) and homologous in 13 (28%). The mitoses per 10 HPFs in SC averaged 33. Tumor necrosis was present in 45/47 cases (mean 10%, range 1-40%). Tumor OTO contained only CC in 17, only SC in 1, and both in 29 cases. Optimal and suboptimal surgical debulking was performed in 28 and 6 patients, respectively (degree of debulking unclear in 13). The patients were followed from 1 to 183 months: 6 patients lost to followup, 3 died postoperatively, 29 died from disease, 2 died from other causes, 7 still alive (14 to 183 months). The DSS at 1-year, 2-year, and 5-year was 75%, 56%, and 21% respectively. Presence of SC OTO was a significant prognostic factor ($p = 0.03$) whereas stage (only 6 patients with stage I-II) and other parameters were not. After adjusting FIGO stage, presence of SC OTO was still a significant prognosticator for patients with stage III-IV disease ($p = 0.003$) whereas others were not.

Conclusions: Most POMMMTs occurred in older patients with an advanced stage at diagnosis. Presence of SC OTO was a significant adverse prognostic factor. Specific tumor components OTO should be listed in pathology report.

1077 Frequent Loss of Progesterone Response Competence in Isolated Non-Cycling Glands of Normal Secretory Endometrium.

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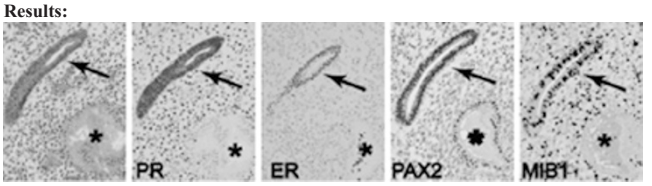
Background: Small numbers of isolated non-cycling (atrophic, proliferative, or very poorly developed secretory) endometrial glands are commonly (45%) seen within the surface layer of progesterone-exposed normal secretory endometrium. We hypothesize that these glands have either lost the progesterone receptor, or developed downstream defects elsewhere in the progesterone response pathway. We here compare expression of a panel of progesterone response genes in these outlier glands to those in background secretory glands.

Design: 20 cases of normal, mid-secretory (23-24 days) endometrial curettings with two or more outlier non-secretory glands on H&E stain were immunostained for mitotic activity (MIB1), and three makers normally downregulated in response to progestins (estrogen receptor,"ER", progesterone receptor,"PR", and PAX2). For each marker, outlier glands were scored as high expressors (indicating defective progesterone associated downregulation) or normal expressors at the low level of the secretory background.

Results:



All or most outlier glands maintained high expression of ER, PR or PAX2 in 83% (15/18), 95% (18/19), and 84% (16/19) of informative cases, respectively, when compared to the diminished levels in background secretory endometrium. Increases in outlier gland mitotic activity (MIB1) were seen in 55% (11/20) of cases. Figure 1 shows marker results for outlier (arrows) compared to adjacent normal (asterisk) background glands.

Conclusions: Acquisition of hormonal incompetence is a common event in small numbers of endometrial glands, which present on routine stains as a dyssynchronous minor element in an otherwise normal secretory endometrium. Outlier glands maintain high levels of ER, PR, and PAX2 expression, in contrast to the low levels in normal background. Affected glands have an increase in mitotic activity. This cannot be explained simply by loss of the progesterone receptor itself, but rather reflects a defective progesterone response within affected glands. The etiology is unknown, but might be explained by a low frequency of inactivating events (mutation, deletion) occurring within the progesterone response cascade during cyclic endometrial gland regeneration.

1078 Intestinal-Type Endocervical Adenocarcinoma In Situ (iAIS): An Immunophenotypically Distinct Subset of AIS Affecting Older Women.

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Background: Conventional endocervical adenocarcinoma in situ (cAIS) is typically strongly and diffusely positive for p16 and has a high Ki67 index in keeping with its frequent association with high risk HPV infection. The intestinal variant of AIS (iAIS) is less common, and its relationship to HPV infection and biomarker expression has not been thoroughly studied. The purpose of this study was to evaluate the frequency and pattern of p16 and Ki67 expression and the HPV status in iAIS in comparison to cAIS.

Design: Fifty-four cases of AIS (14 iAIS, 40 cAIS) were retrieved from our archives. The age of the patient at diagnosis as well as coexisting squamous lesions were recorded. HPV DNA analysis and immunohistochemistry for p16 and Ki67 were performed on cases with available tissue.

Results: The average age at diagnosis was significantly older in iAIS (age 45) versus cAIS (age 34) (p-value = 0.005). In contrast, coexisting squamous intraepithelial lesions were more common in the cAIS than the iAIS (35%, 14/40 versus 23%, 3/13). 15/19 (79%) cAIS showed strong and diffuse staining for p16. Conversely, only 5/12 (42%) iAIS had diffuse strong positivity. 10/10 (100%) cAIS showed >75% nuclei staining for Ki67. In contrast, this degree of staining was only present in 4/11 (36%) cases of iAIS. HPV DNA analysis revealed that 6/7 (86%) of iAIS cases were positive for HPV; five were type 18 and 1 was type 33.

Conclusions: iAIS is strongly associated with HPV infection, sharing a similar pathogenesis with cAIS. However, the distinctly older age is unique, suggesting that age-related factors might influence cell differentiation in these lesions. The less intense

staining for p16 and Ki-67 distinguish this variant from cAIS, and this must be taken into account when using these biomarkers to resolve problematic endocervical lesions with intestinal differentiation.

1079 Fallopian Tube Correlates of Serous Borderline Tumors.

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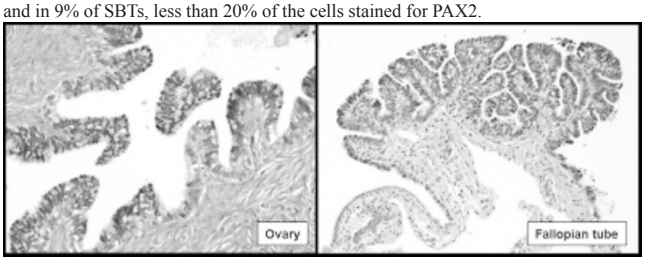
Background: Ovarian serous borderline tumors (SBT) are clonal neoplasms exhibiting secretory and ciliated cell differentiation (Sieben 2006), presumed to arise within mullerian epithelium in the ovarian cortex or peritoneal surface.Epithelial hyperplasia has been reported in fallopian tubes (FT) of patients with SBT (Robey 1989) but its significance is unknown.This study explored 4 parameters possibly linking FTs and SBTs: 1) differentiation characteristics of SBTs, 2) frequency of candidate precursors (secretory cell outgrowths in the FT (SCOUT)) relative to controls, 3) existence of SCOUTs exhibiting ciliated differentiation, and 4) a shared immunophenotype between SCOUTs and SBTs.

Design: 48 SBTs were culled from department files and stained for evidence of ciliated (p73) and secretory (HMFG2 or PAX8) differentiation and PAX2 expression.FTs from 34 SBTs and 65 benign controls were examined for PAX2 (-) SCOUTs.

Results: All SBTs stained for p73 and HMFG2 or PAX8, consistent with origin from a cell capable of secretory *and* ciliated differentiation.SCOUTs were seen in 110/398 (27%) FT cross-sections from SBTs vs 18/161 (11%) in controls (p=<0.001).SCOUTs were heterogenous, ranging from largely secretory (84/110) to mixed ciliated and secretory (26/110). Some SCOUTs had papillary architecture and in two cases, multiple foci of papillary SCOUTs with mixed ciliated and secretory differentiation. All SBTs had heterogeneous PAX2 staining; 35% were >80% positive, 56% were 20-80% positive, and in 9% of SBTs, less than 20% of the cells stained for PAX2.



Conclusions: This study shows for the first time that the FT hosts discrete epithelial alterations that are more frequently associated with SBT, have the capacity for both ciliated and secretory cell differentiation, frequently lack PAX2 expression, and share disturbances in PAX2 expression with SBTs.These findings suggest a precursor condition originating in the FT in a field of multifocal gene dysregulation, a subset of which might gain a growth advantage over pelvic/ovarian surface mesothelium, leading to SBT. If confirmed, this model could explain the origins of both low and high grade pelvic serous neoplasms.

1080 Diagnostic Utility of JAZF1/JJAZ1 Fusion Gene in Endometrial Stromal Sarcoma of the Uterus.

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Background: Diagnosis of endometrial stromal sarcoma (ESS) is usually not problematic in most cases because of their characteristic histopathologic findings. However, when the tumors have extensive secondary differentiation, including smooth muscle, myxoid, fibrous, ovarian sex cord-like/epithelioid, or endometrioid glandular differentiation, correct diagnosis can be very difficult. Recently, the presence of the JAZF1/JJAZ1 gene fusion in endometrial stromal sarcoma has been described, but the incidence has been variably described, and the applicability of the fusion gene in the difficult cases has not been cofirmed. In this study, we analyzed the prevalence of JAZF1/JJAZ1 gene fusion in classical low-grade endometrial stromal sarcomas (n=15) and those with various secondary differentiation (n=13) to examine the applicability of JAZF1/JJAZ1 gene fusion in the diagnosis of difficult cases of ESS showing secondary differentiation.

Design: JAZF1/JJAZ1 fusion transcript were analyzed using nested RT-PCR in 28 cases including classical low-grade ESSs (n=15), those with smooth muscle differentiation (n=3), endometrial glandular differentiation (n=2), and with osseous metaplasia (n=1), undifferentiated endometrial sarcoma (UES) (n=5), UES with sex cord-like differentiation (n=1), combined low-grade ESS and UES (n=1).

Results: The fusion transcript was detected in 18 (85.7%) of 21 low-grade ESSs and in 2 (28.6%) of 7 UESs, and the positive cases of low-grade ESSs included 14 classical ESS, one with smooth muscle differentiation (33%), two with endometrial glandular differentiation (100%), and one with osseous metaplasia (100%), and two UESs combined with classical low grade ESS. Direct sequencing analysis of DNA in 2 cases confirmed a fusion of JAZF1 and JJAZ1 genes forming a new amino acid (glutamic acid), which was previously described in the literature.

Conclusions: JAZF1/JJAZ1 fusion gene was identified in a significant proportion of low-grade ESS regardless of the presence or absence of secondary differentiation, thus, this method can be useful in the diagnosis of difficult cases of ESS.

1081 The Histologic Features of Endometrial Stromal Sarcomas Characterized by *YWHAE* Rearrangement – Distinction from Usual Low-Grade Endometrial Stromal Sarcoma with *JAZF1* Rearrangement.

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Background: Endometrial stromal sarcoma (ESS) is a genetically heterogeneous group of uterine sarcomas; about half harbor *JAZF1* rearrangement while the genetics of the remaining half is undefined. We recently identified a novel translocation-induced genetic rearrangement involving *YWHAE* as the pathognemonic abnormality in a subset of ESS and we describe here the histologic features of this genetic subtype of ESS.

Design: A total of 11 ESS with *YWHAE* rearrangement was identified through multi-institutional review of cytogenetics data and FISH screening. The histology and the immunophenotype (ER, PR and CD10) was examined in 8 primary tumors and 3 metastatic tumors, in comparison to 20 *JAZF1*-rearranged ESS.

Results: All *YWHAE*-rearranged primary ESS had epithelioid areas in which the tumor cells were arranged in a nested pattern with fine stromal capillary network. The cellularity ranged from highly cellular with a small round blue cell appearance to moderately cellular with tumor cells possessing a moderate amount of eosinophillic cytoplasm. In contrast to ESS with *JAZF1* rearrangement which usually display small nuclei with < 5MF/10HPF, the *YWHAE*-rearranged epithelioid cells had larger nuclei with more irregular contour, and all cases showed more than 10 MF/10HPF in addition to tumor necrosis. 5 of the 8 *YWHAE*-rearranged primary ESS contained an admixed cytologically-bland mitotically-inactive spindle cell component in a fibrous/fibromyxoid background. In 3 *YWHAE*-rearranged ESS, only the metastatic tumors were examined: these included two lung metastases with mitotically active epithelioid areas (one with admixed spindle cell component), and one vaginal metastasis where the tumor showed only a low-grade appearance. Immunohistochemically, the spindle cell component (in both primary and metastatic tumors) consistently exhibited ER and PR immunoreactivity with variable CD10 positivity while the epithelioid cell component lacked ER, PR and CD10 immunoreactivity. Staging information was available for 6 primary tumors, of which five were associated with advanced-stage disease (FIGO stage 3) at presentation.

Conclusions: We describe a new molecularly-defined subset of ESS, containing *YWHAE* oncogenic rearrangement, and exhibiting higher-grade histologic features than those in classic ESS with *JAZF1*-rearrangement.

1082 Pap Cytology and Hybrid Capture 2 HPV DNA Testing in 234 Patients with Cervical Squamous Cell Carcinoma.

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Background: Cervical cancer screening with Pap cytology and HPV DNA testing is highly sensitive in predicting cervical precancerous lesions (CIN2/3). However, patients with CIN2/3 or even carcinoma can be missed during the screening. To evaluate the factors that resulted in the screening failure, we compared history of Pap cytology and HPV DNA testing results in patients with cervical squamous cell carcinoma (SQC).

Design: We conducted a computer-based search for a 5-year period and collected the results of surgical pathology, ThinPrep Pap, and Hybrid Capture 2 (HC2) HPV DNA testing from patients with cervical SQC. HPV DNA testing using PCR was performed on the surgical specimens from the cases with negative HC2 test.

Results: 234 patients with SQC were identified, including 35 cases of microinvasive SQC. The average age was 48 years (22 to 84 years). The clinical or Pap findings leading to the diagnostic biopsies are detailed in table 1.

Findings Leading to Histopathologic Diagnoses of SQC

		No. of Patients (% of subcategory)	%
Unknown		**50**	**21.4%**
Clinical Findings		**57**	**24.4%**
	Bleeding	13 (22.8%)	
	cervical lesions	44 (77.2%)	
Pap Findings		**127**	**54.3%**
	ASCUS	8 (6.3%)	
	ASC-H	6 (4.7%)	
	LSIL	3 (2.4%)	
	HSIL	80 (63.0%)	
	SCC/suspicious	27 (21.3%)	
	AGC	3 (2.4%)	
Total		**234**	**100%**

34 patients had prior Pap and the results were listed in table 2.

Pap Histories Over 3 Years Preceding Histopathologic Diagnoses of SQC

Pap Test	Case No.	%
At least one prior abnormal Pap	22	64.7%
At least one prior normal Pap	15	44.1%
Both prior normal and abnormal Pap	3	8.8%

Abnormal Pap tests triggering the biopsy for diagnosis of SQCs were not included.

28 patients had HC2 HPV DNA tests within 5 months before the histologic diagnosis. HC2 HPV DNA was tested positive in 24 patients (86%), while negative in 4 patients (14%). However, HPV DNA was detected by PCR in the surgical specimens from the 4 patients with a negative HC2 test.

Conclusions: HSIL was the most frequent abnormal cytology to trigger the histologic diagnosis of SQCs. 44% of cases had at least one negative Pap test within 3 years prior to the histologic diagnosis. We found a fractional (14%) false negative HC2 HPV rate in patients with cervical SQC, which is similar to the false negative HC2 HPV rate (11%) recently reported in the Kaiser Permanente system after cytology and HPV co-testing. False negative HC2 HPV results argue against the proposed cervical cancer screening with primary HPV screening and secondary Pap cytology.

1083 Uterine/Cervical Embryonal Rhabdomyosarcoma in Adult Women.

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Background: In its classical form, embryonal rhabdomyosarcoma (ERMS) is a vaginal tumor occurring in young girls and is often not considered in the differential diagnosis of uterine and cervical spindle cell tumors in adult women. Experience with consultation cases indicates a need for improved recognition of this tumor.

Design: Clinicopathologic and immunohistochemical features of 12 cases of ERMS identified in women >20 years of age were evaluated, with the goal of improving recognition of this tumor outside of its classical setting.

Results: Patient age ranged from 23 to 86 years (mean, 49.4 ; median,48.5), with 2 patients aged 20-29 years, no patients aged 30-39 years, 5 patients aged 40-49 years, and 5 patients >50 years of age. Tumors originated in the cervix in 7 cases and in the endometrium in 5. These were diagnosed by biopsy in 4, curettage in 1, polypectomy in 6, and hysterectomy in 1. All endometrial tumors and 5 of 7 cervical tumors occurred in women >40 years of age. Tumors were characterized by a spindle cell proliferation in which tightly packed hypercellular foci composed of cells with minimal cytoplasm were scattered throughout an edematous hypocellular spindle cell proliferation, often with condensation beneath epithelial surfaces. Apoptotic bodies and mitotic figures were evident, usually in the hypercellular foci. In 4 tumors, rare to few entrapped endocervical or endometrial type glands were present but these tumors lacked the classical features of adenosarcoma. All tumors coexpressed desmin and myogenin to some degree (desmin was diffuse in 9 and focal in 3; myogenin was diffuse in 6 and focal in 6). Proliferative activity, as assessed by Ki-67 expression, was notably elevated in all tumors, typically concentrated in the hyperpcellular foci.

Conclusions: ERMS has a broader clinical profile than classically expected and can occur as a uterine (endometrial) or cervical tumor in adult women. Thus, ERMS should be considered in the differential diagnosis of uterine/cervical spindle cell tumors, regardless of patient age. The hypocellular background can suggest a low-grade tumor but recognition of characteristic hyperceullular foci in which desmin and myogenin are coexpressed and proliferative activity is elevated is useful for establishing the diagnosis.

1084 Molecular and Genetic Characterization of Diagnostically Challenging Uterine Smooth Muscle Neoplasms.

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Background: Uterine smooth muscle neoplasms are a heterogeneous, partly diagnostically challenging group of tumors with benign, malignant and intermediate tumor subgroups. It is generally believed that uterine leiomyosarcomas (uLMS) arise de novo, rather than from a precursor lesion. However, recently it has been hypothesized that uLMS may arise from preexisting leiomyoma like areas, especially with cellular or atypical morphology. The aim of this study was to focus on the genetic aspects of diagnostically challenging smooth muscle tumors with special interest of atypical uterine leiomyomas (uLM) and uLMS.

Design: 16 uLM (of usual typ, hormonally treated, infracted), 11 atypical uLM and 15 uLMS were evaluated morphologically, immunohistochemically and by array CGH.

Results: As expected, copy number changes were rarely seen in uLM. The only recurrent aberration was loss of chromosome 19 material, which was observed in 7 out of 16 (44%) cases. In contrast, in atypical uLM the number of copy number changes increased significantly involving almost all chromosomes. The most frequent changes (>35%) included gains on chromosomes 1q, 11q, 16p and 17q and a loss of material on chromosome 13q. In uLMS there was a further increase of copy number changes. Here frequent alterations included losses on 1p, 3p, 6p, 10q, 13q, 14q and 16q, and gains of 1q, 11q and 17p.

Conclusions: By comparing three morphologic different uterine smooth muscle tumors we observed a continuous increase of copy number changes from uLM to atypical uLM and uLMS. Between the group of atypical uLM and uLMS several overlapping copy number changes were identified. This suggests that similar or identical pathways may be involved in the evolution of these tumors.

1085 Old Versus New FIGO Staging Systems in Predicting Overall Survival in Patients with Uterine Leiomyosarcoma: A Study of 86 Cases.

D Lim, W-L Wang, C-H Lee, T Dodge, B Gilks, E Oliva. National University Health System, Singapore, Singapore; M.D Anderson Cancer Center, Houston, TX; Vancouver General Hospital, BC, Canada; Massachusetts General Hospital, Boston.

Background: Uterine leiomyosarcoma (LMS) has traditionally been staged using the FIGO system for endometrial cancer. Recently, a new FIGO staging system (modified from the AJCC soft tissue sarcoma staging system) has been proposed. We aimed to compare whether the old or new FIGO staging system is more accurate in predicting overall survival (OS) in patients with LMS.

Design: 86 patients diagnosed with uterine LMS in two tertiary institutions between 1984 and 2010 were retrospectively staged using the old and new FIGO systems. OS was calculated from the date of diagnosis to date of death or last follow up. 5 year survival rates for both groups were estimated using the Kaplan-Meier method. Median OS was also analyzed.

Results: 27 patients were downstaged and none were upstaged using the new FIGO system. Five and 4 patients with old FIGO stages 2 and 3 respectively were downstaged to stage 1 while 18 with old stage 3 were downstaged to stage 2. The median follow-up time was 23.5 months (range, 1 to 216). 30 patients (35%) died of disease.

	New FIGO Stage (No. of patients)				
Old FIGO stage	1	2	3	4	Total No. Patients
1	33	0	0	0	33
2	5	0	0	0	5
3	4	18	5	0	27
4	0	0	0	21	21
Total	42	18	5	21	86

5-year OS rate (%) of patients stratified by stage according to old and new FIGO systems				
	Old FIGO		New FIGO	
Stage	OS Rate	95% CI	OS Rate	95%CI
1	94	86-98	76	61-88
2	50	12-88	64	39-84
3	71	50-86	100	46-100
4	54	32-75	57	34-77
Mean OS rates for old versus new FIGO Staging				
	OS in months (95%CI)			
Stage	Old FIGO		New FIGO	
1	44.7 (28.4-60.5)		45.2 (31.9-58.5)	
2	31.4 (14.8-48.1)		28.1 (18.7-37.5)	
3	32.3 (21.3-43.3)		37.6 (12.4-62.8)	
4	33.9 (15.3-52.5)		34.3 (15.8-52.8)	

Conclusions: Neither staging system is ideal in classifying patients with uterine LMS into 4 clinically significant stages predictive of OS. Differences in OS are not significant between the two systems. Other prognostic factors should be explored and incorporated into the staging of uterine LMS to improve stratification of patients for different therapeutic regimes.

1086 Interobserver Variability in the Interpretation of Tumor Cell Necrosis (TCN) in Uterine Leiomyosarcoma (LMS).

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Background: Distinction of LMS from leiomyoma is based on: 1) nuclear atypia, 2) mitotic rate and 3) TCN. Unlike ischemic-type necrosis which may be seen in benign and malignant smooth muscle tumors (SMTs), TCN is thought to be unique to LMS. The distinction between these two types of necrosis can be challenging, especially during early stages, when necrotic foci are limited or when they exhibit overlapping features. The aim of this study was to assess the interobserver variability in the interpretation of TCN in uterine LMS.

Design: 34 LMS were retrieved and a representative H&E slide showing one or more areas of necrosis was selected from each case. Pathologists from 6 different institutions subspecializing in gynecologic pathology performed a blinded, independent review of the slides. Using the current WHO criteria for assessment of TCN, they had to classify the necrotic foci into: 1) TCN, 2) no TCN or 3) indeterminate for TCN. Agreement among the panelists was categorized as: full agreement—all pathologists in agreement; partial agreement—4 or 5 pathologists in agreement; no agreement—≤ 3 pathologists placing the case into the same category.

Results: Full agreement regarding the presence or absence of TCN was reached in 12 cases (35%) (7 thought to show TCN); partial agreement in 16 (47%); and no general consensus in 6 cases (18%). Overall, the level of agreement was fair (k=0.3948). In 8 of the 34 cases (23.5%), ≥ 1 pathologist made a diagnosis of "TCN" and ≥ 1 pathologist made the diagnosis of "no TCN" in the same case. The number of cases diagnosed as "indeterminate for TCN" by each pathologist ranged from 0 to 10 with a mean of 5.8. In 20 cases, at least 1 pathologist diagnosed "indeterminate for TCN" (59%); at least 2 were undecided in 10 (29%), and at least 3 in 4 cases (12%). Four pathologists diagnosed "indeterminate for TCN" in 1 case.

Conclusions: The level of interobserver agreement amongst experienced gynecological pathologists in the assessment of TCN in uterine LMS is only fair. These results further reiterate the importance of assessing for both nuclear atypia and mitotic activity when differentiating between benign and malignant SMTs and not relying solely on the presence of TCN.

1087 *FOXL2* Mutational Analysis in the Differential Diagnosis of Ovarian Spindled Sex-Cord Stromal Tumors.

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Background: Sex cord-stromal tumors (SCSTs) of the ovary comprise less than 10% of all ovarian tumors. The majority are ordinary fibromas, others types being uncommon or rare. The differential diagnosis of the predominantly spindled and cellular SCSTs, particularly sarcomatoid granulosa cell tumors (GCTs), cellular fibromas (CFs) and thecomas (THs), is often difficult due to their close histologic resemblance and absence of reliable ancillary tests. Recently, the single somatic mutation *FOXL2* 402C→G (C134W) was reported in approximately 95% of adult-type granulosa cell tumors and in 15% of thecomas, but was absent in other ovarian tumors. However, the morphologic subtypes evaluated in these studies were not specified. Most GCTs are easily diagnosed based on morphologic and immunohistochemical features, and the challenge resides in the differentiation of the sarcomatoid variant from other SCSTs. This study evaluates the potential diagnostic application of *FOXL2* mutational analysis, specifically in the setting of spindled and cellular SCSTs.

Design: A total of 86 ovarian tumors, including 12 sarcomatoid GCTs, 27 GCTs of other subtypes, 30 CFs, 9 THs and 8 SCSTs of other categories (4 Sertoli-Leydig cell tumors, 2 steroid cell tumors, and 2 unclassified SCSTs) were retrieved from the archives of the Mayo Clinic. Reticulin, inhibin and calretinin were performed in all cases. The diagnoses were based on blind review of the HE slides and the aforesaid stains by two pathologists with expertise in gynecologic pathology. DNA was extracted from formalin-fixed, paraffin-embedded tissue sections followed by polymerase chain reaction and direct sequencing of the *FOXL2* gene.

Results: The median age of the patients was 55 years old (range 14 to 69). Tumor size ranged from 0.5 to 39 cm. *FOXL2* 402C→G (C134W) was detected in 23 of 27 non-sarcomatoid GCTs (85.2%), 6 of 12 of sarcomatoid GCTs (50%) and 2 of 9 THs (22.2%). The mutation was not detected in any of the CFs and SCSTs of other categories. Mutational analysis was repeated in all GCTs lacking the mutation and the results were confirmed.
Conclusions: The frequency of *FOXL2* 402C→G (C134W) in similar to that reported in the literature for non-sarcomatoid GCTs and THs. However, it is significantly lower for sarcomatoid GCTs, which had detectable *FOXL2* mutations in only half of the cases. Therefore, mutational analysis may prompt re-evaluation of the tumor classification criteria for sarcomatoid SCSTs. Additional studies are warranted to establish the application of molecular testing in the diagnosis of GCTs.

1088 Immuhistochemical Profile of Ovarian Malignant Germ Cell Tumors: Comprehensive Analysis of 14 Markers in 56 Cases.

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Background: Malignant germ cell tumors (mGCTs) still present several diagnostic challenges resulting from diversities in histologic types. Several immunohistochemical markers have been used to aid in the diagnosis of mGCTs. Most of the data available was generated in testicular mGCTs. There is a shortage of literature pertaining specifically to the ovaries. Intuitively, mGCTs of the ovaries are expected to have the same immunoprofile as the ones occurring elsewhere. However, this question has not been specifically addressed. The objective of this study is to present a panorama of the pattern of expression of the most commonly used markers in mGCTs occurring in the ovaries.
Design: An immunohistochemical panel of 14 markers was evaluated in 56 mGCTs of the ovaries selected from the in-house and consultation archives of the Mayo Clinic from 2003 to 2010. The study comprises 23 dysgerminomas, 13 yolk sac tumors, 12 immature teratomas, 3 choriocarcinomas and 5 mixed germ cell tumors. Immunohistochemistry was performed in paraffin-embedded tissue sections with antibodies directed against keratin AE1/AE3, AFP, CAM5.2, OCT-4, D2-40, KIT, PLAP, inhibin, calretinin, MIB-1, synaptophysin, chromogranin, GFAP and hCG.
Results: The median age of the patients was 24 years old (range 9 to 58). Tumor size ranged from 2.3 to 29 cm. All dysgerminomas were diffusely positive for OCT4, KIT, PLAP and D2-40; less than 10% stained for CD30 and less than 5% for AE1/AE3, CAM5.2 and hCG. Yolk sac tumor was the only lesion positive for AFP; a diffuse pattern was present with the exception of 2 cases that showed focal expression. All yolk sac tumors were also positive for AE1/AE3 and CAM5.2; KIT was expressed in 84,6%, PLAP in 77%, D240 in 38,4%, OCT4 in 18% and inhibin in 7,6% of cases. All choriocarcinomas stained positively for hCG and keratins; two were also positive for inhibin and one for CD30. All immature teratomas with the exception of one case were positive for both or either synaptophysin and GFAP; chromogranin was expressed in 1/3 and calretinin in 1/4 of cases. Only one of the mixed mGCT contained embryonal carcinoma, which was diffusely positive for CD30, OCT4, PLAP, D2-40 and keratins, and negative for KIT and inhibin. The other mixed mGCTs showed dysgerminoma, yolk sac and immature teratoma components, which had an immunoprofile similar to the pure tumors in these categories.
Conclusions: The immunohistochemical profile of ovarian mGCTs is similar to the one described for mGCTs of the testis; and, therefore, it can be used in this setting with the same level of reliability.

1089 PLZF Immunostaining Distinguishes Low-Grade and High-Grade Ovarian Serous Tumors.

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Background: PLZF (Promyelocytic Leukemia Zinc Finger), a zinc-finger transcriptional repressor plays an important role in cell cycle regulation. We investigated the PLZF expression in ovarian benign epithelium, borderline tumors with or without micropapillary foci, and in low-grade, and high-grade serous carcinoma.
Design: 55 routinely processed specimens, comprising benign ovary (n=10), serous cystadenoma or adenofibroma (n=10), serous borderline tumor (n=10), serous borderline tumor with micropapillary foci (n=10), high grade serous carcinoma (n=10), and low grade serous carcinoma (n=5) were subjected to citrate-based Ag retrieval followed by exposure to streptavidin-biotin reagents and diaminobenzidine.
Results: In normal ovarian surface, PLZF staining was nuclear and was positive in 80-100% of epithelial lining cells. Nuclear staining was also seen in serous cystadenofibroma, in 90-100% of epthelial cells. In contrast, serous borderline tumors displayed cytoplasmic and perinuclear rather than nuclear staining, with about 10-20% of tumor cells staining. In micropapillary foci, cytoplasmic PLZF staining further decreased to 3-5 % epithelial cells on the long slender micropapillae **(Fig. 1 A – F).**

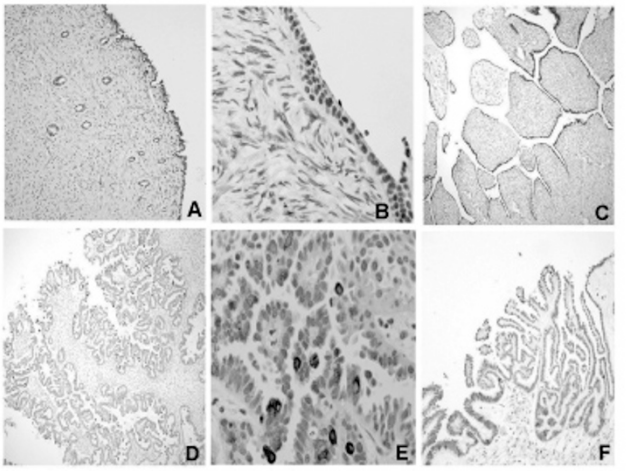

Fig 1 A.B Normal ovary. **C.** Serous cystadenofibroma. **D. E.** Serous borderline tumor. **F.** Micropspillary foci.

Staining of low-grade serous carcinoma was easily distinguished from high grade serous carcinoma: the former displayed cytoplasmic PLZF expression in 5-10% of tumor cells, whereas the latter were completely non-staining **(Fig.2 A-D).**

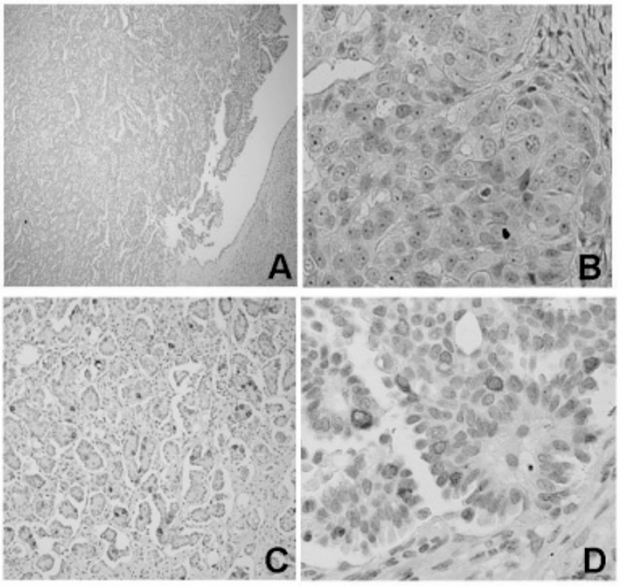

Fig. 2 A, B High grade serous carcinoma. **C, D** Low grade serous carcinoma.

Conclusions: PLZF immunostaining distinguished ovarian normal, low grade neoplastic and high grade malignant epithelia. Our findings are consistent with basic studies that have defined a mechanism of nuclear export of PLZF corresponding to de-repression of cell proliferation. These findings define a new means of distinguishing ovarian serous tumors. Downregulation of PLZF may contribute to uncontrolled serous epithelial cells proliferation; therefore it may play an important role in ovarian serous tumor pathogenesis.

1090 Immunohistochemical Expression of Two Upper Gastrointestinal (GI) Markers (CK17 and DPC4) in Primary and Metastatic Mucinous Ovarian Neoplasms.

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Background: In mucinous neoplasms of ovary, diagnosing metastatic disease can be challenging, especially in the setting of an occult primary. Morphologic and immunohistochemical parameters exist to aid in this distinction; however, they are not entirely specific. Cytokeratin (CK) 17 is a basal-type cytokeratin expressed in pancreaticobiliary carcinomas, whereas DPC4 (SMAD4) is a tumor suppressor that is inactivated in up to 40% of pancreatic adenocarcinomas. This study aims to determine the expression of CK17 and DPC4 in primary and metastatic mucinous ovarian tumors.
Design: DPC4 and CK17 expression were determined by immunohistochemistry (IHC) on primary low malignant potential (LMP) and invasive ovarian mucinous tumors (IMT-O), and metastatic mucinous ovarian tumors (MMT-O) (upper gastrointestinal in origin). Primary cholangiocarcinomas (CC), primary breast mucinous carcinomas

(MT-B), and primary pancreatic neoplasms (PMT) were also stained. The intensity of staining (IS) was graded as negative-0, weak-1, moderate-2, and strong-3, and the proportion of positive staining cells (PS) was recorded as none (0), 1-5% (1), 6-20% (2), 21-80% (3), and 81-100% (4). A cumulative score (CS) was derived from PS and IS, and ranged from 0 to 7. CS was divided into negative (0), weak (1-2), moderate (3-5), and strong (6-7).

Results:

	DPC4 (SMAD4) N(%)			Cytokeratin 17 N(%)		
Cumulative Score (CS)	Negative/weak (0-2)	Moderate (3-5)	Strong (6-7)	Negative/weak (0-2)	Moderate (3-5)	Strong (6-7)
LMP-O* (n=15)	0	4 (27)	11 (73)	4/13 (31)	9/13 (69)	0
IMT-O (n=19)	0	6 (32)	13 (68)	9 (47)	9 (47)	1 (5)
MMT-O (n=12)	2 (17)	1 (8)	9 (75)	6 (50)	3 (25)	3 (25)
MT-B (n=13)	0	1 (8)	12 (92)	13 (100)	0	0
Primary CC* (n=30)	26 (87)	4 (13)	0	9/16 (56)	5/16 (31)	2/16 (13)
PMT* (n=10)	3 (30)	2 (20)	5 (50)	2/9 (22)	4/9 (44)	3/9 (33)

*Cytokeratin 17 was performed on a subset of cases.

Conclusions: 1. Moderate-strong DPC4 expression is seen in primary LMP and invasive mucinous carcinomas. **2.** Our study is the first to evaluate CK17 in ovarian neoplasms; moderate staining is seen in more than half of primary ovarian LMP and invasive mucinous tumors. **3.** All primary mucinous carcinomas of breast demonstrate strong DPC4 expression (100%) and all are negative for CK17. **4.** Though metastatic tumors to ovary show stronger DPC4 than CK17 expression, in determining the origin of a mucinous tumor of ovary, a combination of both marker expression favors metastatic disease from a pancreaticobiliary primary over an ovarian or breast primary.

1091 CDX2 Is a Useful Marker To Distinguish Ovarian Primary Carcinoid from Gastrointestinal Neuroendocrine Tumors (NET).

JM Lloyd, H Xu, C Zhao. UPMC, Pittsburgh, PA; University of Rochester, NY.

Background: Primary ovarian carcinoids are the second most frequent monodermal teratomas. They occur in a pure form (15%) or combined with other teratomatous components (85%). Primary ovarian carcinoids and metastatic tumors share similar morphologic features. They can be designated as primary with relative ease when they occur in association with other teratomatous components. However, when they occur in a pure form, metastatic disease must be excluded. Gastrointestinal (GI) NET (carcinoid) are more common with the majority arising from small intestine (SI) (29%) and appendix (25%). CDX2 is a nuclear transcription factor critical for intestinal differentiation, and is a relatively specific marker of intestinal epithelium. To the best of our knowledge, CDX2 expression has not been assessed in primary ovarian carcinoids (and is known to be expressed in a subset of GI NET). The aim of this study is to evaluate the expression of CDX2 by immunohistochemistry (IHC) in a large series of primary ovarian carcinoids and primary GI NET.

Design: CDX2 expression was determined by IHC on 28 primary pure ovarian carcinoids, 13 primary ovarian carcinoids arising in association with benign teratomas, 2 ovarian carcinoids metastatic from SI and 70 GI NET (11 stomach, 10 duodenum, 20 SI, 12 appendix, 17 colorectal). Nuclear staining in >5% of cells was considered positive.

Results: None of the 28 primary ovarian carcinoid tumors are positive for CDX2, whereas 49/70 (70%) cases of GI NET and 2/2 (100%) SI NET metastatic to ovary showed strong nuclear staining (diffuse or focal) (**Table 1**). Two primary carcinoids mixed with benign teratomas showed only weak positivity.

Table 1.

		CDX2+ N/total (%)
Ovarian Carcinoid Tumors	Pure Primary	0/28 (0)
	Primary Mixed with Benign Teratoma	2/13 (15)
	Metastatic from Small Intestine	2/2 (100)
GI NET	Stomach	0/11 (0)
	Duodenum	8/10 (80)*
	Small Intestine	19/20 (95)*
	Appendix	11/12 (92)*
	Colorectal	2/17 (12)

* p<0.0001 as compared to pure primary ovarian carcinoids, Fisher exact test

Conclusions: CDX2 positive carcinoid tumors involving the ovary are unlikely to represent primary ovarian carcinoids and are more likely to be metastatic from the GI tract in the absence of other associated teratomatous elements. CDX2 positivity can be seen in primary ovarian carcinoids that occur in association with benign teratoma suggesting that some of these tumors may arise from GI derived epithelium within the teratoma.

1092 Validation of Diagnostic Criteria for Endometrial Adenocarcinoma.

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Background: We previously identified morphologic criteria for endometrial biopsies which showed increased sensitivity and specificity for distinguishing endometrial adenocarcinoma (EA) from complex atypical hyperplasia (CAH). In the current study we evaluated the application of these criteria to endometrial biopsies received at our institution between 2007 and 2010.

Design: We searched the pathology database at NYULMC for all endometrial biopsies with subsequent hysterectomy received between January 2007 and August 2010 which were diagnosed as either CAH or EA, FIGO grade I. Slides for each identified case were retrieved, and were reassessed for the presence or absence of adenocarcinoma without the knowledge of hysterectomy findings. A diagnosis of EA was made if the biopsy possessed either 1)areas of ≥95% glandular crowding that were 3 mm or larger in aggregate diameter or 2) any cribiform architecture. We compared our diagnosis with that of the resection.

Results: We identified 95 patients that fulfilled all of the criteria for inclusion. On assessment of the slides using the previously discussed criteria, we diagnosed 50 of these cases as EA. On resection, 42 of these patients had EA (42/50, 84%); the remainder had CAH or contained no residual atypical lesion. In 45 cases we diagnosed CAH; 36 of these cases had CAH on resection. The remaining 10 cases were diagnosed as adenocarcinoma and adenocarcinoma in situ on resection (7/45, 15.5% and 3/45,6.6% respectively). These figures produced a sensitivity of 84% and a specificity of 80% for the diagnosis of endometrial adenocarcinoma (p<.0001).

Conclusions: The findings of this study validated previously derived criteria for diagnosing endometrial adenocarcinoma. The criteria used in this study had a higher sensitivity and specificity for predicting endometrial adenocarcinoma in hysterectomy specimens than any of the previously published criteria. The probability of finding endometrial adenocarcinoma in cases diagnosed as CAH was lower than that reported in other published series. Use of these criteria should lead to more accurate classification of cases in the spectrum of complex atypical endometrial hyperplasia to well differentiated endometrial adenocarcinoma.

1093 Class III Beta-Tubulin Expression in Endometrial Tumors.

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Background: Tubulin, the major building block of microtubules, functions as structural and mobile elements in mitosis, intracellular transport, and in the cytoskeleton. Recent studies have shown that increased tumor expression of the class III beta-tubulin isotype (b3T), correlates with poor clinical outcomes in patients with various malignancies. These include breast, ovarian, gastric, and non-small cell lung cancers. Studies suggest that these poor clinical outcomes, in the setting of b3T overexpression, are related to resistance to tubulin-binding chemotherapeutic agents, such as taxanes and vinca alkaloids, used in the treatment of these malignancies. Treatment of endometrial cancer may also be treated with tubulin-binding agents. The purpose of this study is to investigate b3T expression in endometrial tumors.

Design: Endometrial cancer cases were identifed from the hospital database for a 3 year period. General information, including patient demographics, tumor characteristics, chemotherapeutic agents used, and survivor data, was collected using pathology reports, clinical records, and tumor registry data. Residual formalin fixed paraffin-embedded tissue, archived in the Department of Pathology, was retrieved for each tumor and subjected to immunohistochemical staining for b3T. Slides were evaluated for the presence of staining and scored semiquantitatively on a 4-tiered scale based on percentage of total tumor area staining and intensity of staining.

Results: Ninety-six uterine cancer cases were stained for b3T. The majority (n=64) were adenocarcinomas (45 endometrioid, 10 serous, 9 endometrioid with mucinous differentiation). Approximately half (46.8%) of adenocarcinomas showed b3T over expression. The majority of sarcomas (85.7%) were negative for b3T. Among carcinosarcomas, 64.3% showed b3T over expression, almost exclusively in the carcinomatous component. When comparing b3T positive to b3T negative tumors, no statistically significant differences were seen among demographic characteristics, stage or grade of tumors, or among chemotherapeutic modalities. However, tumors showing b3T over expression were more likely to have lymphovascular invasion (47.9% vs. 14.6%, p=0.001) and a worsened uterine cancer specific survival.

Conclusions: Differential class III beta-tubulin staining patterns are seen in endometrial cancers across all histologic subtypes. Further investigation is warranted to further characterize the correlation between class III beta-tubulin positivity and clinical outcomes.

1094 Prevalence of DNA Mismatch Repair Protein Loss in 342 Primary Malignant Epithelial Ovarian Tumors.

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Background: Lynch syndrome is an autosomal dominant condition caused by germline mutation in one of the DNA mismatch repair genes (hMLH1, hMSH2, hMSH6 and hPMS2), and is associated with increased risk for multiple malignancies, with colorectal carcinoma and endometrial carcinoma being the most common. Patients with Lynch syndrome are also at increased risk for ovarian malignancies, with a lifetime risk estimated at 10-12%. We analyzed the prevalence of DNA mismatch repair gene defects amongst primary malignant epithelial tumors of the ovary, using immunohistochemical staining for DNA mismatch repair (MMR) proteins.

Design: Tissue microarray (TMA) consisting of 342 primary malignant epithelial tumors of the ovary collected at Vancouver General Hospital were stained for hMLH1, hMSH2, hMSH6 and hPMS2. Positive staining for MMR proteins on TMA were verified on whole section slides for 25 randomly selected cases.

Results: Of the 342 primary malignant epithelial tumors of the ovary, loss of expression of MMR proteins was more commonly seen in the non-serous carcinomas, specifically: mucinous (1/8 cases), endometrioid (3/29 cases), clear cell (3/29 cases) and undifferentiated (1/9 cases) carcinomas, and mixed carcinomas with an endometrioid, clear cell and/or undifferentiated component (3/5 cases). The frequency of MMR protein loss was significantly higher in non-serous cases versus high-grade serous carcinomas (11/80 cases or 13.8% vs. 9/217 cases or 4.1%, p=0.007). No loss of MMR protein expression was identified in borderline tumors (22 cases), low-grade invasive serous carcinoma (9 cases) or malignant Brenner tumor (3 cases). All 25 cases positive for MMR gene products on TMA also stained positive when retested on whole section slides.

Conclusions: Our study demonstrated loss of expression of MMR proteins in 13.8% of ovarian carcinomas of non-serous types. These results raise the possibility of selective genetic screening for Lynch syndrome in patients with these types of ovarian carcinoma.

1095 Immunohistochemical Differentiation of Ovarian Clear Cell Adenocarcinomas and Yolk Sac Tumors Using CK7, EMA, CA125, SALL4, and AFP Antibodies.

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Background: The distinction between ovarian clear cell adenocarcinomas (CCAs) and yolk sac tumors (YSTs) is of significant clinical importance, because treatment options for CCAs ans YSTs are completely different. However, it is often challenging to make a histological distinction between these tumors. Among the variety of histological patterns of ovarian YSTs, hepatoid and glandular histological patterns are known to closely resemble CCAs. In this study, we aimed to identify a useful panel of immunohistochemical markers for differential diagnoses of ovarian CCAs and YSTs.

Design: We studied 94 ovarian CCAs and 14 ovarian YSTs that had been surgically removed in four major hospitals in Tokyo between 1986 and 2010. Immunohistochemical analyses were performed on a representative section from each case, using antibodies against the following: CK7, EMA, CA125, SALL4, AFP, GPC3, and Hep Par 1. Immunostaining was interpreted as positive when at least 5% of the cells were immunoreactive. Positive expression was further classified as 1+ (5–14%), 2+ (15–49%), or 3+ (≥50%).

Results: All 94 CCAs (100%) were positive for CK7 and EMA, with more than 90% of the cases showing diffuse (3+) immunoreactivity for each antibody. Of the 14YSTs, three each (21%) showed focal (1+) positivity for CK7 and EMA, respectively. Most (63/94; 67%) CCAs, but only one YST (7%), showed positive immunostaining for CA125. One CCA (1%) was positive for SALL4, a newly introduced germ-cell tumor marker. In contrast, all 14 YSTs (100%) showed diffuse (3+) nuclear immunoreactivity for SALL4. All of the YSTs, but none of the CCAs, were AFP-positive. However, the extent of AFP reactivity varied among the YSTs (1+: 2 cases, 2+: 5 cases, 3+: 7 cases). Positive staining for GPC3, another marker for hepatic tumors, was observed in 41 of 94 CCAs (44%) and in all 14 YSTs (100%), while Hep Par 1 immunoreactivity was occasionally observed in CCAs (27%) and YSTs (43%).

Conclusions: Our results suggest that positive immunostaining for CK7, EMA, and CA125, and negative immunostaining for SALL4 and AFP support a diagnosis of CCA, whereas positive reactions for SALL4 and AFP, and negative reactions for CK7 and EMA favor a diagnosis of YST. GPC3 immunostaining appears to be of limited value in distinguishing CCAs and YSTs, as a significant number of CCAs were GPC3-positive. Similarly, Hep Par 1 is not useful for differentiation, although it is interesting that approximately 30% of CCAs and 40% of YSTs expressed this hepatic marker.

1096 Clinicopathological Significance of ARID1A Immunoreactivity in Ovarian Clear Cell Carcinoma.

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Background: Recent genome-wide analysis has demonstrated that somatic mutations in ARID1A are the most common molecular genetic changes in ovarian clear cell carcinoma (CCC). ARID1A mutations which occurred in approximately half of CCC cases, lead to deletion of the encoded protein and inactivation of the putative tumor suppressor.

Design: In this study, we first correlated ARID1A mutation status and ARID1A immunoreactivity then applied immunohistochemistry to determine if there is any clinicopathological features associated with the loss of ARID1A expression in a total of 149 CCC cases collected from the University of Tokyo Hospital, National Taiwan University Hospital and the Johns Hopkins Hospital.

Results: We demonstrated that loss of ARID1A expression was associated with ARID1A inactivating mutations in CCC (p= 0.0073). Specifically, all 9 CCCs harboring ARID1A mutations showed undetectable or very weak immunoreactivity while negative staining could also be observed in one of 3 CCCs with wild-type ARID1A. ARID1A immunoreactivity was undetectable or very week in 88 (59%) of 149 CCCs. There was no statistically significant difference of ARID1A negative and positive cases in terms of histopathological features (structural patterns, nuclear atypia, cystic vs. adenofibromatous), age, clinical sage, overall survival, and frequency of lymph node metastasis. However, we observed that loss of ARID1A expression correlated with lower chance of peritoneal dissemination (p<0.05).

Conclusions: In conclusion, this study provides the first analysis of ARID1A mutations and clinicopathological features and we demonstrate that mutations in ARID1A resulted in loss of ARID1A protein expression in CCC and there was no significant difference of ARID1A positive and negative cases in all the clinical parameters examined except for frequency of peritoneal dissemination.

1097 Hypoxia Potentiates the Inverse Relationship between Resistance to Cisplatin and Paclitaxel in Ovarian Cancer Cell Lines.

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Background: Long term survival for ovarian cancer is poor (<30%). Patients undergo debulking surgery followed by platinum-taxane based chemotherapy. Most patients respond well initially, however relapse with chemoresistance is frequent. Tumour hypoxia is a factor in chemoresistance via HIF-1 (hypoxia inducible factor-1) pathway activation. Recent reports indicate an inverse relationship between resistance to cisplatin and paclitaxel across a variety of tumour cell lines. This study aimed to examine whether hypoxia could play a role in this inverse relationship in two ovarian cancer cell lines, A2780 and its cisplatin-resistant derivative, A2780cis.

Design: Cell lines were grown in RPMI 1640 media (10% FBS, 1mM glutamax and 1% penicillin/streptomycin) at 37°C, 5% CO_2. Hypoxic exposure was carried out in an *Invivo* hypoxic chamber at 0.5% O_2. Drug resistance following hypoxic exposure was determined using MTT assays. Dose response curves, IC_{50} values and Student *t*-test values were determined by GraphPad software. Experiments were performed in triplicate with significance at p<0.05.

Results: A2780cis were 5-fold more resistant to cisplatin than A2780s (IC_{50} 10μM vs. 2μM, p<0.001). Conversely A2780cis were more than 10-fold more sensitive to paclitaxel than A2780s (IC_{50} 250pM vs. 3.4nM, p<0.01). Exposure to acute hypoxia (4 h) prior to drug treatment significantly increased resistance to cisplatin in both cell lines. However, acute hypoxia before paclitaxel treatment increased sensitivity to the drug in A2780s, while increasing resistance in A2780cis. Chronic hypoxia (5 days) before treatment increased sensitivity to both cisplatin and paclitaxel in A2780s.

Table 1: IC_{50} values and fold-changes relative to normoxia for cisplatin and paclitaxel when A2780 and A2780cis cells were exposed to hypoxia prior to drug treatment.

Cell Line	Acute Hypoxia Before Drug Treatment	Chronic Hypoxia Before Drug Treamtent	Cisplatin IC_{50} (fold change)	Paclitaxel IC_{50} (fold change)
A2780	+	-	2.9μM (1.6)**	1nM (0.3)*
A2780	-	+	1.2μM (0.6)*	765pM (0.2)**
A2780cis	+	-	14μM (1.35)**	1.9nM (7.6)**
A2780cis	-	+	10.9μM (1.04)	600pM (2.4)

*p<0.05 **p<0.01

Conclusions: A2780 and A2780cis have an inverse relationship between resistance to cisplatin and paclitaxel. This relationship can be further potentiated by exposing the cells to hypoxia prior to drug treatment. Genes involved in this process may include HIF-1, p53 and BRCA; known to play a role in cisplatin and paclitaxel resistance whilst also regulated by hypoxia.

1098 Expression Analysis of Newly Identified EMT Genes in High Grade Serous Carcinoma of the Ovary and Uterus.

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Background: The pathobiology of high-grade serous carcinoma (HGSC) of the ovary is currently not well understood, and is an area of active investigation. In a recent study, we identified a group of epithelial-mesenchymal transition (EMT) genes that are directly or indirectly regulated by the oncogene *HMGA2* in ovarian surface epithelial (OSE) cells. To test whether HMGA2-mediated aggressive tumor growth in HGSC acts through regulation of EMT genes, we decided to examine whether these EMT genes are expressed in HGSC. These genes have never been studied in depth regarding the pathogenesis of ovarian tumors.

Design: Our study includes 96 serous tumors, including 56 ovarian high grade serous carcinomas (OSC), 18 serous borderline tumors (LMP) and 22 uterine serous carcinomas (USC). We examined HMGA2 and its target EMT gene expression, including ID1, LUM, POSTN, and STC2 by immunohistochemistry. Associated markers p53, ER, PR and Ki-67 were also included.

Results: In ovarian surface epithelial cells (OSE), four EMT associated genes (ID1, LUM, POSTN, and STC2) are regulated by HMGA2. In this study, we found that HMGA2 positive HGSC had significant down regulation of ID1, LUM and POSTN, and up regulation of STC2 in comparison to HMGA2 negative HGSC. The findings are consistent with our predictions based on gene expression in OSE cell lines. Importantly, we found HMGA2 and its EMT genes are positively associated with lymph node metastasis, suggesting an increased propensity towards aggressive behavior in tumors expressing these genes. There was significant difference of EMT gene expression between tumor cells and surrounding stroma. Lastly, HGSC from both ovary and uterus showed a very similar gene expression pattern.

Conclusions: HMGA2 and its associated EMT genes are significantly dysregulated in HGSC and they are positively correlated with lymph node metastases. Differential expression of these EMT genes between tumor cells and surrounding stroma suggests a functional role in aggressive tumor growth in HGSC. In particular, distinct expression patterns of these EMT genes in HGSC may provide novel markers for the study of HGSC.

1099 Ovarian Metastases of Pancreaticobiliary Tract Adenocarcinomas: Analysis of 35 Cases, with Emphasis on the Ability of Metastases To Simulate Primary Ovarian Mucinous Tumors.

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Background: Metastatic mucinous carcinomas in the ovary are readily recognized when they display characteristic features (bilateral involvement, moderate tumor size, surface involvement, nodular growth, an infiltrative pattern) but are capable of simulating primary ovarian mucinous tumors. Metastatic pancreaticobiliary tract adenocarcinomas present a particular diagnostic challenge due to their ability to exhibit borderline-like and cystadenomatous growth patterns which can be misinterpreted as underlying primary ovarian precursor tumors.

Design: Clinicopathologic features of 35 metastatic pancreaticobiliary tract adenocarcinomas in the ovary were analyzed. Immunohistochemical analysis of Dpc4 expression was performed on 33 cases and clinical follow-up was obtained.

Results: Mean patient age was 58 years (median, 59; range, 33-78). In 15 cases (43%), the pancreaticobiliary tract and ovarian tumors presented synchronously and in 2 cases (6%) the ovarian tumors presented prior as the first manifestation of disease. Ovarian tumors were bilateral in 31 cases (89%). Mean and median sizes were 10.6 and 9.5 cm, respectively (range, 2.5-21 cm). Nodularity was present in 22 cases (63%) and surface involvement was identified in 14 cases (40%). An infiltrative pattern was present in 28 cases (80%), most often admixed with borderline-like and cystadenomatous areas but as the exclusive pattern in 11 cases (31%). Conversely, borderline-like and cystadenomatous patterns were identified in 24 cases (69%) and as the exclusive patterns (either pure or combined with one another) in 7 (20%). Dpc4

expression was lost in 20 of 33 tumors analyzed (61%). Of 25 patients with follow-up, 23 died of disease (mean/median time, 9/6 months; range, 1-39) and 2 were alive with disease (at 1 and 25 months).
Conclusions: Frequent bilateral ovarian involvement, moderate tumor size, nodularity, and infiltrative patterns are useful features for identifying these ovarian tumors as metastatic; however, many also exhibit borderline-like and cystadenomatous patterns which, when dominant and combined with synchronous presentation, make recognition as metastases challenging. Loss of Dpc4 expression provides the most useful immunohistochemical evidence for establishing the pancreaticobiliary tract as the most likely source of these metastases.

1100 Uterine Leiomyosarcoma with an Adipocytic Differentiation (Lipoleiomyosarcoma): A Clinicopathologic Study of Seven Cases.

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Background: Uterine leiomyosarcoma may rarely have heterologous differentiation, resulting in the presence of components of rhabdomyosarcoma, osteosarcoma, or liposarcoma. Those with adipocytic differentiation have been described in single case reports under a variety of names including lipoleiomyosarcoma, and thus morphologic diversity and clinicopathologic features remain undetermined.
Design: Seven cases of uterine leiomyosarcoma with adipocytic components ("lipoleiomyosarcoma") were reviewed to determine clinicopathologic features and morphologic characteristics.
Results: The patient age ranged from 41 to 63 years (mean; 50.3, median: 44). All cases had features of ordinary leiomyosarcoma, but five also showed epithelioid features and/or myxoid changes. Three cases showed a mixture of spindle cells and mature adipocytes with bland nuclear morphology, and thus in areas resembled lipoleiomyoma or spindle cell lipoma. In addition, two cases harbored components resembling pleomorphic lipoma, one of which was associated with lipolieomyoma-like component. Adipocytic component was well-differentiated and/or cytologically pleomorphic, and in two cases showed myxoid changes. In these two cases mature adipocyte-like or epithelioid cells with multiple intracytoplasmic vacuoles were embedded in the myxoid matrix. Features of prototypical myxoid/round cell liposarcoma were not identified. Immunohistochemically the tumors in all four cases were positive for alpha-smooth muscle actin, h-caldesmon**,** and/or desmin. CD34 and S-100 protein were negative in leiomyosarcomatous component but CD34 were positive in areas of adipocytic component in three tumors**,** and S-100 protein in two. Adipocytic component was confirmed to be positive for CDK4 and MDM2 in four and two tumors, respectively. All four patients with follow-up information had recurrent disease within 2 years (interval; 2-16 months).
Conclusions: Adipocytic differentiation in leiomyosarcoma is frequently associated with myxoid and epithelioid features, and may harbor lipoleiomyoma- and/or bizarre leiomyoma-like component, resulting in diagnostic difficulty, particularly with limited sampling.

1101 Lynch Syndrome Screening Should Be Considered for All Patients with Newly Diagnosed Endometrial Cancer.

AM Mills, S Liou, JM Ford, JS Berek, RK Pai, TA Longacre. Stanford University, CA.
Background: Lynch syndrome (LS) is characterized by a high lifetime incidence of colorectal cancer and endometrial cancer. Given recent recommendations for universal, cost effective screening of all patients with newly diagnosed colorectal cancer using mismatch (MMR) protein immunohistochemistry (IHC), we evaluated for LS endometrial cancer in the general population.
Design: 387 consecutive cases of primary endometrial cancer at a single institution (1997-2010) were evaluated for LS regardless of age, family history or histologic features. Evaluation methods consisted of IHC for the MMR proteins MLH1, MSH2, MSH6 and PMS2, followed by real-time PCR (Methylight) DNA methylation analysis for cases with MLH1/PMS2 deficiency. A subset of tumors also had microsatellite instability and/or gene sequencing data, but this was not part of the study design.
Results: Twenty-six LS-associated endometrial cancers were identified: 2 MLH1/PMS2, and 24 with MSH6 and/or MSH2. Only 26% occurred in women <50 years of age (range, 39-88 years), one of which was in a prophylactic hysterectomy specimen for prior diagnosis of LS. Two had associated colorectal carcinoma, but there were no simultaneous ovarian carcinomas. 48% were grade 1; 26% grade 2; and 26% high grade. Most were endometrioid (n=21), with 2 mixed endometrioid/mucinous, 1 mucinous, 1 serous, 1 clear cell, and 1 carcinosarcoma. 3 were in the lower uterine segment; 9 had tumor infiltrating lymphocytes.
Conclusions: Screening using current Bethesda guidelines and recently suggested pathologic guidelines misses a substantial proportion of patients with MMR protein deficient endometrial cancer and many patients with LS. Based on recent recommendations for universal screening of newly diagnosed colorectal carcinomas, these data also support universal screening of all newly diagnosed endometrial cancer. A cost effective algorithm using only 2 MMR antibodies as an initial screen is proposed.

1102 Expression of Mismatch Repair Proteins in Endocervical Adenocarcinomas: A Review of 79 Cases Including Minimal Deviation Adenocarcinomas and Problematic Lower Uterine Segment Tumors.

AM Mills, S Liou, CS Kong, TA Longacre. Stanford University, CA.
Background: Endometrial carcinoma is one of the most common malignancies seen in Lynch Syndrome (LS). Recently, the prevalence of LS has been noted to be much higher (29%) in patients with endometrial tumors localized to the lower uterine segment (LUS) when compared to the general endometrial cancer population (1.8%) (Westin et al, J Clin Oncol, 2008). In this location, distinction from endocervical primary may be difficult, particularly when atypical morphology is present, immunohistochemical stains are inconclusive, and evidence of HPV-infection is lacking. While clinical studies do not suggest an association between LS and endocervical carcinomas, mismatch repair protein (MMR) testing has not been performed in large numbers of these tumors. In this study, we examined expression of MMR proteins in endocervical adenocarcinomas and problematic LUS tumors of uncertain origin. In addition, we focused on cases of minimal deviation adenocarcinomas (adenoma malignum) which may present diagnostic difficulties due to their HPV-negative status and unconventional morphologies.
Design: Expression of MMR proteins (MSH2, MSH6, MLH1, PM2) was assessed on a tissue microarray containing 33 endocervical adenocarcinomas and 36 problematic LUS cases. The endocervical adenocarcinomas consisted of 20 invasive and 13 in situ cases. Problematic LUS cases consisted of uterine tumors for which a site of origin could not be assigned due to discordance among radiographic, gross, and morphologic findings. Representative whole slides from 10 cases of minimal deviation adenocarcinoma were also stained with all 4 MMR proteins
Results: All 33 endocervical adenocarcinomas and all 10 minimal deviation adenocarcinomas demonstrated intact expression of the 4 MMR proteins. A single problematic LUS case showed loss of MSH2 and MSH6. Although this tumor was clinically thought to represent an endocervical primary based on its location in the cervix and lower uterine segment, the tumor's immunohistochemical profile (p16 negative, vimentin negative, hormone receptor positive) was more compatible with endometrial origin.
Conclusions: Conventional endocervical adenocarcinomas and minimal deviation adenocarcinomas do not exhibit MMR protein deficiency by immunohistochemistry, indicating that these mismatch repair genes are intact in these tumors. These findings suggest that endocervical adenocarcinoma is almost certainly not a feature of LS and that loss of MMR proteins in a LUS tumor argues strongly against endocervical origin.

1103 Clinicopathologic Features of Sporadic Endometrial Carcinomas with *MLH1* Promoter Hypermethylation: A Study of 54 Cases.

AM Mills, RK Pai, S Liou, TA Longacre. Stanford University, CA.
Background: MLH1 protein deficiency due to epigenetic silencing of *MLH1* by promoter hypermethylation occurs frequently in endometrial carcinomas, but the clinicopathologic features of these tumors have not been well characterized.
Design: Fifty-four endometrial carcinomas with deficient MLH1 immunohistochemical expression resulting from *MLH1* promoter methylation identified by real-time PCR (Methylight) DNA methylation analysis were studied. Tumors were assessed for histologic type, grade, lymphovascular invasion, tumor infiltrating lymphocytes, depth of invasion, concurrent hyperplasia, tumor location, and stage.
Results: The average patient age was 64.8 years (range: 42-88). The majority (86%, 44/51) of tumors were located in the uterine fundus. Four tumors were located in the fundus with extension into the lower uterine segment (LUS) while 3 tumors were limited to the LUS. While most tumors (81%, 44/54) showed pure endometrioid histology, 19% (10/54) also demonstrated mucinous differentiation. No serous, clear cell, or carcinosarcoma histologies were identified. Most tumors were either grade 1 (41%, 22/54) or grade 2 (42%, 23/54); only 9 cases were high grade. One endometrioid case showed predominantly grade 1 tumor immediately juxtaposed with a focus of undifferentiated carcinoma. Advanced FIGO stage was seen in 13% of grade 1, 24% of grade 2, and 44% of grade 3 carcinomas. Tumor-infiltrating lymphocytes were present in 33%. At least 35% had concurrent hyperplasia and in no instance was the hyperplastic component associated with loss of MLH1.
Conclusions: Sporadic endometrial carcinoma with *MLH1* promoter hypermethylation occurs predominantly in the uterine fundus, is associated with endometrioid or mucinous histology – often with associated hyperplasia, and exhibits a similar grade distribution to non-*MLH1* methylated endometrial cancer. Despite decreased high-risk histology, *MLH1*-methylation appears to be associated with an increased rate of advanced-stage disease.

1104 Size of Endometrial Adenocarcinoma and Non-Cancerous Tissue in Biopsy Have Predictive Value for Finding Endometrial Adenocarcinoma in Hysterectomy.

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Background: In a significant number of cases, a hysterectomy following a diagnosis of endometrial adenocarcinoma in endometrial biopsy does not show carcinoma. In this study we evaluated if the amount of carcinoma in biopsy or the presence and extent of non-cancerous tissue in biopsy correlates with the likelihood of finding endometrial adenocarcinoma in hysterectomy. This is of particular interest in Grade I Endometrial adenocarcinoma where conservative management with progestins may be a choice.
Design: Endometrial adenocarcinoma grade I cases diagnosed at NYU Medical center from 2003 to 2006 were reviewed without the knowledge of subsequent hysterectomy findings. The size of tissue with endometrial carcinoma was semi-quantitatively assessed using a ruler placed directly on the slide. The measurements were expressed as approx. aggregate diameter of cancerous tissue present. Similar measurements were taken for the non-cncerous tissue in each biopsy.

Results: The aggregate size of the carcinoma in biopsy was predictive of presence of endometrial carcinoma in hysterectomy specimens. There were a total of 49 cases of endometrial adenocarcinoma. Carcinoma was seen in hysterectomy in 33 of 34 (97%) patients with tumor diameter of 7 mm or greater versus 10 of 15 (66.6%) patients with tumor diameter of less than 7 mm in biopsy (p=0.007).
The extent of benign tissue present in the biopsy also correlated with the likelihood of finding endometrial carcinoma in hysterectomy. Carcinoma was seen in hysterectomy in 30 of 31 patients where only carcinoma was seen in biopsy versus 13 of 18 patients where non-cancerous endometrial tissue was also present (p=0.02). Carcinoma was seen at hysterectomy in only 1 of the 4 cases with non-cancerous tissue with aggregate diameter greater than 15 mm.
Conclusions: The findings of this study suggest that absence of endometrial carcinoma in some hysterectomy specimens where biopsy had previously shown carcinoma may be due to the focal nature and small size of the carcinoma in these particular patients. In endometrial biopsy specimens, the small size of the carcinoma tissue (less than 7 mm in diameter) and the presence of non-cancerous endometrial tissue may be used to identify such cases. The findings of this study may be useful in counseling individual patients with biopsy diagnosis of endometrial carcinoma about the likelihood of finding endometrial carcinoma in hysterectomy.

1105 Hedgehog Pathway Expression and Clinical Outcome in Endometrial Carcinoma (EC).

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Background: The expression of hedgehog pathway proteins including including Sonic (Shh) and Desert (Dhh) have been associated with embryonic development and human neoplasia. Hedgehog pathway signaling has not been linked to clinical outcome in EC.
Design: Formalin-fixed, paraffin-embedded tissue sections from 128 endometrial carcinoma, including 104 endometrioid carcinoma (EC), 12 uterine papillary serous carcinoma (PSC) and 12 malignant mesoderm mixed tumor (MMMT), were immunostained by automated methods (Ventana Medical System, Tucson, AZ) using goat polyclonal Shh and Dhh antibodies (Santa Cruz Biotechnology, Santa Cruz, CA). Cytoplasmic immunoreactivity was scored based on staining intensity (weak, moderate, strong) and percentage of positive cells (focal <= 10%, regional 11-50%, diffuse >50%). Results were correlated with clinicopathologic variables.
Results: Immunoreactivity for Shh and Dhh were predominately cytoplasmic. Strong diffuse Shh and Dhh overexpression was observed in 20% and 19% of tumors overall; and 21% and 23% ECs, 17% and 0% PSCs, and 17% and 0% MMMTs, respectively. Shh overexpression correlated with advanced stage (34% advanced vs 15% early, p=0.024) and depth of myometrial invasion (38% invading to more than 50% of the myometrium vs. 17% to less than 50% vs. 0% no invasion, p=0.024). Dhh overexpression also correlated with advanced stage (37% advanced vs 17% early, p=0.033) and depth of myometrial invasion (33% invading to more than 50% of the myometrium vs. 19% to less than 50% vs. 0% no invasion, p=0.04). A significant co-expression was identified between Shh and Dhh (p=0.012). On multivariate analysis, disease recurrence (p=0.004) and advanced stage (p=0.03) independently predict shortened survival.
Conclusions: Overexpression of both Shh and Dhh occurs in EC and is associated with depth of tumor invasion and pathologic stage. Further studies of the hedgehog signaling pathway in EC appears warranted.

1106 Clusterin Overexpression Promotes Angiogenesis in Primary Ovarian Cancer.

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Background: Clusterin, a multifunctional glycoprotein, is ubiquitously produced in mammalian tissues. While clusterin has been shown to play significant roles in many aspects of human tumor biology, such as cell proliferation, apoptosis, chemoresistance and angiogenesis, the relationship of clusterin expression with angiogenesis in ovarian cancer has not been studied.
Design: Immunohistochemical (IHC) staining for clusterin, VEGF and VEGF-R were performed on a Tissue Microarray (TMA) containing 181 primary ovarian epithelial cancer. These 181 tumors consisted of 119 serous carcinoma, 23 mucinous carcinomas and 39 other types of carcinoma (endometroid, mixed mullerian, clear cell, Brenner, and undifferentiated). A total of 170 cases were available for evaluation for all 3 markers. The levels of protein expression for these 3 genes were scored based on staining intensity and percentage of immunopositive cells and were correlated with one another.
Results: Among these 170 cases, clusterin, VEGF and VEGF-R were overexpressed in 94 (55%), 97 (57%) and 48 (28%) of the cases respectively. Among 94 tumors that overexpressed the clusterin, VEGF and VEGF-R were increased in expression in 66 (70%) and 29 (30%) cases respectively. By contrast, among 76 tumors without significant clusterin expression, overexpression of VEGF and VEGF-R were seen in only 31 (40%) and 19 (25%) of the cases.
Conclusions: Overexpression of clusterin in epithelial ovarian cancer appears to be correlated with increased tumor angiogenesis, consistent with the established role of clusterin as an oncogene in the biology of ovarian cancer.

1107 Mucinous Adenocarcinoma of the Endometrium Confers Increased Risk of Lymph Node Metastases.

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Background: Mucinous adenocarcinoma of the endometrium (MUC) is a rare histological variant of endometrial carcinoma accounting for less than 10% of endometrial tumors. The tumor develops in a background of hyperplasia and mucinous metaplasia of the endometrium. Few studies have characterized its clinical behavior. The aim of this study is to compare the risk factors and clinical course of MUC relative to endometrioid adenocarcinoma.
Design: A case-control study was performed which included patients treated for endometrial cancer at the New York Hospital – Cornell Medical Center between 1996 and 2006. Mucinous adenocarcinoma was defined as tumor with more than 50% of mucinous-type epithelium. 41 cases of were identified. Each case was matched by age and histological grade with two controls of endometrioid histology. Cases and controls were compared with regard to known risk factors for endometrioid carcinoma and the extent of disease at diagnosis. Chi-square tests were used to compare proportions and student T-tests for the comparison of means. Multivariate regression was used to identify the independent predictors of lymph node metastases. Overall survival was calculated using the Kaplan-Meier method and compared with the Log-rank test. $P<.05$ was considered significant for all tests.
Results: No significant difference was found between cases and controls with regard to ethnicity, body mass index, history of diabetes mellitus, hypertension, prior hormone replacement, and tamoxifen use. Prior oral contraceptive use was significantly less common among women with MUC compared to controls (6.5% vs 32.3%, $P= .01$). No significant differences in myometrial invasion (MI) > 50% or the presence of lymph-vascular space invasion was found between cases and controls, however 17% of patients with MUC had lymph node metastases compared to 3% of controls ($P= .01$). Multivariate analysis controlling for both tumor grade and depth of MI identified mucinous histology as an independent predictor of lymph node metastasis ($P =.02$). Adjuvant radiation or chemotherapy was used in 60% of cases compared to 42% of controls ($P =.05$). No difference in recurrence or survival was identified between the two groups.
Conclusions: Mucinous adenocarcinoma of the endometrium is associated with an increased risk of lymph node metastases that is independent of tumor grade and depth of MI. Comprehensive surgical staging including retroperitoneal node dissection should be considered in all patients with this diagnosis.

1108 Absence of V600E B-RAF Mutation in MLH1-Negative Endometrial Carcinoma.

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Background: Lynch syndrome (LS) is caused by germline mutations in the mismatch repair (MMR) system genes, of which those involving the *MLH1*, *MSH2* and *MSH6* genes are the most frequent. Among LS-associated neoplasms, colorectal carcinoma (CRC) is the most common and endometrial carcinoma (EC) the second most common. To compare the pathogenesis of these two neoplasms, we have prospectively studied a population screening EC series for the molecular alterations known to occur in LS-associated CRC.
Design: Seventy-eight surgically resected primary ECs were prospectively studied. Immunohistochemistry for MMR gene proteins (MLH1, MSH2, and MSH6) was performed in all cases. This was followed by microsatellite instability (MSI) analysis, with amplification of five different microsatellites (NR21, NR24, NR27, bat25, and bat26), and V600E *B-RAF* gene sequencing study in MLH1 (-) cases. Bethesda criteria were evaluated and MMR genes mutational analysis was performed in every case.
Results: Of the 78 EC patients studied, 6 were under 50 years of age, 30 were between 51 and 60, and 42 were older than 61. Twenty EC cases were found to be unstable. Of them, 14 were MLH1 (-) and showed wild-type (WT) *B-RAF*; 3 were MSH2 (-) and MSH6 (-); and 3 were MSH6 (-). No V600E *B-RAF* mutation was detected in any of the cases studied.
Conclusions: In conclusion, the absence of V600E *B-RAF* mutation in MLH1 (-) EC cases would indicate that in EC this mutation is not related to hypermethylation in the same way as it is in CRC. Therefore, algorithms for molecular studies of these two neoplasms should reflect this difference.

1109 Immunohistochemical Panel of p16, p63 and Ki-67 Is Useful in Distinguishing Mucinous Adenocarcinomas of Endometrium (MUC-ADs) from Microglandular Hyperplasia (MGH) of Endocervix.

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Background: MUC-ADs of endometrium represent 10% of all endometrial carcinomas. Owing to their overall bland cytology and frequent microglandular architecture, these lesions are difficult to differentiate from benign MGH of endocervix, particularly in the small volume endometrial curettings/biopsies. This study aims to determine a useful immunohistochemistry (IHC) panel for distinguishing MUC-AD of endometrium from MGH of endocervix.
Design: A total of 23 cases of MUC-AD of endometrium were selected from our institution's pathology database between the years 2006 to 2010. Eleven cases of MGH of endocervix were used as control group. All cases were stained for p63, p16, vimentin, CEA, CD10, and Ki-67 antigens. Staining intensity and the percentage positivity for each stain was evaluated.
Results: None (0%) of the MGH endocervix cases were positive for p16 whereas, 21/23(91.3%) MUC-ADs of endometrium cases showed moderate to strong nuclear and cytoplasmic staining for p16 in ≥50% of tumor (avg. staining, 76.4%). Nuclear staining

for p63 was noted in all(100%) the MGH cases (avg. staining, 93.2%) in a distinct continuous, linear and basal pattern. Although, 20/23(87%) MUC-ADs of endometrium showed nuclear positivity for p63, the pattern was focal, random and scattered (avg. staining 16.1%). CEA highlighted the surface and apical cytoplasm of the mucinous epithelium in all (100%) the MUC-ADs of endometrium (avg. staining, 62.8%). However, 4 of 11 MGH cases (36.4%) also expressed positivity for CEA (avg. staining, 17%). Ki-67 staining index was >5% in only 1/11(9%) cases of MGH of endocervix, in contrast to 17/23(73.9%) cases of MUC-ADs of endometrium. All 23(100%) cases of MUC-ADs showed basal cytoplasmic/membranous epithelial staining for vimentin (avg. staining, 70.2%). However, 4 of 11(36.4%) MGH cases also showed positivity in ≥50% of epithelial cells. CD10 was positive in stromal cells of 23/23(100%) cases of MUC-ADs of endometrium and 7/11(63.6%) cases of MGH.

Conclusions: A combined IHC panel of p16, p63 and Ki-67 is useful in distinguishing MUC-ADs of endometrium from MGH of endocervix. Diffuse and strong expression of p16, lack of continuous, linear and basal staining for p63, and increased Ki-67 index (>5%) strongly favors a diagnosis of MUC-ADs of endometrium over MGH of endocervix. CD10, CEA and Vimentin are non-contributory in this context. The exact mechanism of diffuse p16 expression in MUC-ADs of endometrium is unclear.

1110 Co-Ordinated Dysregulation of PAX2, ALDH1 and EZH2 in Tubal Serous Carcinogenesis.

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Background: The role of the fallopian tube in high-grade pelvic serous carcinoma (HGSCA) includes a benign precursor (p53 signature) with altered expression of p53, HMGA2 and PAX2. A second entity, secretory cell outgrowth (SCOUT), shares loss of PAX2 with p53 signatures and serous tubal intraepithelial carcinoma (STIC), which in turn have increased expression of Cyclin E, Ki-67, p16, FAS and RSF-1. This study expanded the list of genes altered in HGSCA development to more precisely define the components of this sequence.

Design: Whole-genome transcriptome analysis of laser micro-dissected paired cancer and tubal epithelium revealed loss of ALDH1 and upregulation of EZH2 (Xian unpublished). To determine their tissue specificity, tissue sections from HGSCA (n=16) and controls (n=65) were studied, and secretory cell outgrowths (SCOUTs; 30), p53 signatures (21), and STICS (33) were selectively stained with antibodies to p53, p16, PAX2, ALDH1 and EZH2.

Results: Fallopian tubes from HGSCA and controls averaged loss of PAX2 at frequencies of .56 and .11 per section respectively ($p < .001$). Coordinated loss of PAX2 and ALDH1 was seen in 70%; ALDH1 was also independently down-regulated in other SCOUTs. Concordant loss of PAX2 and ALDH1 was seen in 80% of p53 signatures exceeding 30-50 cells; expression was normal in very small p53 signatures (less than 20 cells). Loss of ALDH/PAX2 persisted in 80% of STICs but foci of staining were common in STICS and HGSCA. EZH2 stained scattered rare normal cells and a small number in SCOUTs and p53 signatures, and was increased in 33/33 TICS. p16 staining was positive in 12 of 14 TICs but patchy in 7/12.

Conclusions: The fallopian tubes of HGSCA exhibit two independent but related phenomena that may signify disruption of both genetic and epigenetic pathways. First, a globally distributed multifocal loss of PAX2 and ALDH1 expression is significantly associated with HGSCA and persists through the carcinogenic spectrum. Second, these two gene perturbations intersect in the fimbria with p53 mutations, where the transition to malignancy involves up-regulation of EZH2 and other biomarkers. Elevated EZH2 staining is a useful parameter for confirming STIC. Very small p53 signatures may not belong in the serous carcinogenic sequence.

1111 Mesonephric Adenocarcinoma of the Uterine Cervix: An Update of the Immunohistochemical Profile.

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Background: Mesonephric adenocarcinoma (MA) is a rare subtype of uterine cervical carcinoma (CA).Secondary to overlapping histologic features with other subtypes of cervical CA (endometrioid and clear cell carcinoma), proper recognition can be challenging.Immunohistochemistry (IHC) is frequently utilized to assist with proper classification.It has been suggested that the use of PAX-2 may distinguish benign and mesonephric cervical lesions from cervical CA on the basis that PAX-2 was lost in usual type cervical CA.This study evaluates the expression of PAX-8 and PAX-2 as well as other traditional IHC in a series of MA.In situ hybridization (ISH) for high risk HPV is also evaluated.

Design: Six cases of MA were retrieved from our institutional files spanning 1991-2008. Pathology material was reviewed in all cases to confirm diagnosis.All cases were investigated by IHC for PAX2, PAX8, and p16. ISH for high risk HPV was performed on all cases.Previously performed IHC was also reviewed as part of the study.Clinical information was obtained from patients' (pts) charts.

Results: Pts ages ranged from 41-57 years (median 46).The most common presenting symptoms included: vaginal bleeding, 4 cases; menorrhagia, 1 case; and cervical polyp, 1 case. Five pts underwent hysterectomy; in cases where the stage was known (n=4), all were stage 1.Follow up ranging from 12 to 60 mos was available in 5 pts.All are currently free of disease.The immunoperoxidase results are summarized in table 1.

Summary of Immunoperoxidase Findings in MA

Stain	Positive	Negative
PAX-2	5	1
PAX-8	5	1
p16	4	2
Calretinin	3	3
CD10	2	4
Vimentin	5	1
EMA (n=5)	4	1
CEA	2	4
ER (n=3)	2	1

For p16, 2 cases had patchy and 2 cases had focal staining.Staining for calretinin was focal in 2 cases and diffuse in 1.Vimentin staining was focal in 2 cases and diffuse in 3 cases.Staining for CEA was focal in the 2 positive cases. Staining for estrogen receptor was weak in the 2 positive cases.ISH for high risk HPV was negative in all 6 cases.

Conclusions: MA expresses both PAX-2 and PAX-8 suggesting that these markers could be useful to distinguish MA from usual types of cervical CA.The tendency of MA to express vimentin in conjunction with patchy or focal p16 can be misinterpreted as an indication of endometrial origin.In contrast to previous reports, the majority of the cases (66%) in this study were negative for CD10 and only 50% of cases were positive for calretinin.Therefore, these markers may have a limited role in the evaluation of MA.

1112 Clusterin Overexpression Promotes Cell Proliferation and Inhibits Apoptosis in Primary Ovarian Cancer.

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Background: Clusterin, a multifunctional glycoprotein, is ubiquitously produced in mammalian tissues. While clusterin has been shown to play significant roles in many aspects of human tumor biology, such as cell proliferation, apoptosis, chemoresistance and angiogenesis, the relationship of clusterin expression with cell proliferation and apoptosis in ovarian cancer has not been studied.

Design: Immunohistochemical (IHC) staining for clusterin, Ki-67 and Bcl-2 were performed on a Tissue Microarray (TMA) containing 181 primary ovarian epithelial cancer. These 181 tumors consisted of 119 serous carcinoma, 23 mucinous carcinoma and 39 other types of carcinoma (endometroid, mixed mullerian, clear cell, Brenner, and undifferentiated). A total of 158 cases were available for evaluation for all 3 markers. The levels of protein expression for these 3 genes were scored based on staining intensity and percentage of immunopositive cells and were correlated with one another.

Results: Among these 158 cases, clusterin, Ki-67 and Bcl-2 were overexpressed in 95 (60%), 60 (37%) and 26 (16%) of the cases respectively. Among 95 tumors that overexpressed the clusterin, Ki-67 and Bcl-2 were increased in expression in 40 (43%) and 18 (18%) cases respectively. By contrast, among 63 tumors without significant clusterin expression, overexpression of Ki-67 and Bcl-2 were seen in only 20 (31%) and 8 (12%) of the cases.

Conclusions: Overexpression of clusterin in epithelial ovarian cancer appears to be correlated with increased cancer cell proliferation and decreased cancer cell apoptosis, consistent with the established role of clusterin as an oncogene in the biology of ovarian cancer.

1113 Cross-Sectional Study of Cervical Cancer among Hispanic Versus Non-Hispanic White Women Living in the United States.

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Background: Despite advances in early detection and prevention modalities such as the HPV vaccine, cervical cancer continues to be a significant health problem particularly in underdeveloped countries. Changing demographics in the United States underscore the need to delineate variation in cervical cancer outcomes among racial/ethnic groups.

Design: The 1973-2006 National Cancer Institute, Surveillance, Epidemiology, and End Results (SEER) dataset was used to analyze clinicopathologic differences in cervical cancer between Hispanic and non-Hispanic white women.

Results: Hispanic women with cervical cancer were younger (mean age 48.1 yrs.) than non-Hispanic white women (mean age 51.1 yrs.)($P<0.001$) and less likely to present with advanced stage disease (OR=0.88, $P=0.030$). Correspondingly, regional lymph node involvement (OR=0.63, $P<0.001$) and/or distant metastasis (OR=0.68, $P=0.001$) occurred less frequently among Hispanic women. Whereas squamous cell carcinoma accounted for the most common histology within Hispanic (82%) and non-Hispanic white women (76%), adenocarcinoma was less prevalent among Hispanic women (OR=0.67, $P<0.001$). Primary-directed surgery was similar between groups ($P=0.223$). Survival analysis revealed a 23% reduction in risk of dying from cervical cancer ($P<0.001$) among Hispanic women, whereas multivariate Cox analysis adjusted for age and stage showed Hispanic ethnicity to be an independent predictor of survival (HR=0.79, $P<0.001$).

Conclusions: Hispanic women with cervical cancer present at an earlier age and have better outcomes than non-Hispanic white women; decreased prevalence of adenocarcinoma among Hispanic women may account for differences in survival. A detailed study will be necessary to elucidate the frequency of aggressive HPV types among Hispanic women.

1114 The Significance of the Pattern of Invasion in High Grade Serous Carcinoma of the Ovary.

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Background: Most high grade serous carcinomas (HGSCa) of the ovaries are advanced stage tumors, which respond to chemotherapy but frequently recur quickly with fatal

outcomes. However, there are some unusual cases that do not recur and have excellent survival. In this study, we compared cases that showed rapid recurrences with cases that did not recur.

Design: Out of a group of 216 cases of high stage (HGSCa), 45 cases did not recur. From this group, we studied 16 cases that had clinical and pathological material available and a minimum follow-up of 60 months. These cases were compared to 17 cases matched for comparable amounts of residual tumor and treatment that recurred in less than 18 months. A pattern of invasion characterized by small groups of cells in clefts or non-epithelial lined spaces (NELS) was evaluated in the primary and metastatic tumors. The volume of invasive tumor cells present within NELS was quantified as greater than or less than 50%. In addition, ER, PR, and p53 immunostains were performed in 29 cases. Significant p53 staining was defined as p53 staining greater than or equal to 90% of tumor cells or complete negativity (mutation without protein expression).

Results: Greater than 50% of tumor in NELS was identified in 14 of 17 cases (82%) that recurred, compared to only 4 of 16 cases (25%) that did not recur ($p = 0.0016$). ER was positive and PR was negative in most cases in both groups. Significant p53 staining was found in 12 of 15 cases (80%) that recurred and in 7 of 14 cases (50%) that did not recur ($p = 0.128$). Of the 10 cases that had greater than 50% of tumor in NELS and significant p53 staining, 9 cases (90%) recurred. Of the 4 cases that had less than 50% of tumor in NELS and p53 staining of less than 90%, all 4 cases (100%) did not recur. Of the 15 cases with discordant results, less than 50% of tumor in NELS but significant p53 staining or greater than 50% of tumor in NELS but p53 staining less than 90%, the pattern of invasion was more predictive than p53 staining (60% vs 40%).

Conclusions: 1. HGSCa with greater than 50% of tumor in NELS recur in a short time.

2. Significant p53 staining is found in cases that recur but without statistical significance.

3. When the amount of tumor in NELS and p53 expression agree, there is at least a 90% predictive value.

4. In cases that lack agreement between the amount of tumor in NELS and p53 expression, there is a slightly increased predictive value of the amount of tumor in NELS.

1115 Number of Lymph Nodes in Staging for Endometrialo Cancer: Is the Minimum Required by Some GOG Protocols Feasible?

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Background: GOG protocol 0210 is a molecular and surgico-pathological staging study of endometrial carcinoma. A requirement to enroll patients in this protocol is the presence of at least 8 pelvic lymph nodes (4 right, and 4 left), and 4 para-aortic nodes (2 right and 2 left). The aim of our study was to see the feasibility of obtaining these minimum number of lymph nodes in patients with endometrial carcinoma (many of whom are obese), and the impact that the surgeon may have in the number of nodes obtained.

Design: We retrospectively reviewed the pathology report of 146 consecutive patients with endometrial carcinoma surgically staged at our Institution from 1/1/2000 to 12/31/2005. The surgeons were six board certified gynecological oncologists with multiple years of experience. The patients underwent abdominal hysterectomy, bilateral salpingo-oophorectomy and pelvic and para-aortic lymphadenectomy. None of the cases were done laparoscopically.

Results: The diagnoses included: 100 type 1 endometrial carcinomas (endometrioid), and 46 type 2 endometrial carcinomas (serous, clear cell, undifferentiated, or mixed). The total number of pelvic nodes per patient ranged from 0 to 43 (mean 14.13), including a mean of 7.33 in the right and a mean of 6.80 in the left. The total number of para-aortic nodes ranged from 0 to 33 (mean 5.10). The table shows the mean number of nodes found per surgeon and location.

Surgeon	Mean Rt pelvic	Mean Lt pelvic	Mean para-aortic	Total # cases
A	8.43	7.71	8.50	42
B	7.70	7.40	4.60	10
C	4.92	4.85	2.85	13
D	8.96	6.65	5.27	26
E	6.33	6.59	3.04	51
F	4.75	5.75	3.00	4
Total Average	7.33	6.80	5.10	

There were no cases which did not identify any pelvic lymph nodes (left or right), and only one case where no para-aortic nodes were identified. 78 cases (53.42%) had less than the minimum number of pelvic and para-aortic lymph nodes required in the GOG 0210. Of the 78 cases, 54 were type 1 endometrial carcinoma, and 24 were type 2 endometrial carcinoma.

Conclusions: The minimum number of lymph nodes required by the GOG to accept patients into Protocol 0210 is often difficult to attain. In our study the majority of cases that failed to reach the minimum number were obese patients with type 1 endometrial carcinoma, in which dissection of para-aortic lymph nodes may have been technically challenging.

1116 Progesterone Receptors A and B, pAkt, and p4EBP1 Expression Patterns in Grade 1 Endometrial Adenocarcinoma(G1EAC) and Complex Atypical Hyperplasia(CAH) and Prediction of Response to Progestin Therapy.

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Background: Progestin therapy is a treatment option for CAH and G1EAC in women who desire fertility preservation.No markers that reliably predict response to treatment have been identified.Progesterone receptor status and activation of the pAkt/PTEN pathway have each been hypothesized to influence the effectiveness of progestin treatment.Progesterone receptor B(PRB)activation is known to alter the activity of this pathway.We investigated whether expression of progesterone receptor A(PRA) and B(PRB) as well as markers in the pAkt/PTEN pathway (pAkt and p4EBP1) change in response to progestin therapy and if any of these markers could be used to predict therapeutic response.

Design: Premenopausal patients with CAH or G1EAC who underwent progestin therapy for at least 8 weeks between 1998-2007 were retrospectively identified from three institutions.Patients had an initial diagnostic endometrial biopsy and a follow-up biopsy during the first nine months of treatment.Immunohistochemical(IHC) staining for PRA, PRB, pAkt and p4EBP1 was performed on all tissue.The H score system was used by a pathologist to quantify the IHC staining.H score>50 is considered positive with 50-100 weakly positive, 100-200 moderately positive, and 200-300 strongly positive. Tumor tissue was scored separately from stroma.

Results: 38 subjects were identified with a median age of 36 years(range 23-48). Moderate staining(H score >100)for PRA, PRB and pAkt was seen in 79%, 84%,and 62% of initial biopsies,respectively.Expression levels of all markers(PRA, PRB, pAkt, p4EBP1)were greater in the initial biopsy than after treatment ($p<0.05$).Strong(H score >200) expression of PRB in the initial biopsy was significantly associated with resolution(83% vs. 44% resolution,$p=.035$).

Conclusions: Our data suggests that progestin therapy appears to reduce expression of PRA, PRB, pAkt, and p4EBP1 in CAH and G1EAC, and hence,may influence activation of the pAkt/PTEN pathway.Strong PRB expression in both the initial and first follow-up biopsies with CAH or Grade 1 EA has the potential to predict response to progestin treatment.Staining for PRB in a patient's initial diagnostic biopsy therefore may provide additional information to assist in counseling patients regarding their individual likelihood of resolution of G1EAC and CAH with progestin therapy.

1117 Impact of DNA Mismatch Repair (MMR) in Endometrial Cancer in a Single Reference Center in Mexico.

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Background: To describe DNA mismatch repair (MMR) proteins in a Mexican cohort of patients with sporadic endometrial cancer and its impact on disease-free survival and overall survival.

Design: From the cohort of patients with sporadic endometrial cancer at the Instituto Nacional de Cancerología de México charts were reviewed for clinical and pathologic characteristics. Immunohistochemistry for DNA MMR proteins MLH1, MSH2, MSH6, and PMS2 was performed on tissue microarray of primary tumors from formalin-fixed, paraffin embed blocks (3 cores per block, predominantly from areas with different grades in the same tumor). Expression of all proteins reflected intact MMR system and lack of expression of one or more proteins was considered deficient. Comparisons were made using Chi-square test. Survival curves were constructed using Kaplan-Meier method and log-rank test for comparisons.

Results: One hundred sixty-four Mexican patients were included, 131 were older than 45 years, 41 cases (25%) were FIGO clinical stages III/IV, 152 (93%) were obese and 87 (56%) cases had deficient DNA MMR.(25 cases with defective MSH6 only; 22 cases with defective MLH1/PMS2, 18 cases with defective MLH1/PMS2/MSH6; 18 cases with defective MLH1/PMS2/MSH2/MSH6 and 4 cases with defective MSH2/MSH6). Overall and progression-free survival were not different between patients whose tumors had intact or defective MMR; mean follow up was 27.5 months. Subgroup analyses stratified by histology (non-endometrioid versus endometrioid) showed a higher frequency of defective MMR in endometrioid carcinomas ($p=0.013$) although this difference did not have impact on survival or recurrence. Others parameters as age, tumor grade, stage and P53 expression were not statistically significant.

Conclusions: Loss of DNA MMR proteins is a frequent event in sporadic endometrial cancer in Mexican population. It is also related to endometrioid histology but it does not have impact on disease-free survival or overall survival. Compared with other factors (histopathological grading, TNM stage and FIGO classification) the status of DNA MMR continues to be controversial as a prognostic factor.

1118 The Utility of HPV Testing in Predicting Disease Recurrence Post Colposcopic Treatment for Cervical Intraepithelial Neoplasia.

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Background: The risk of disease recurrence post treatment with LLETZ for high grade CIN ranges from 5-35%. Women with evidence of persistent HPV infection following treatment have a higher incidence of disease recurrence than those who clear their HPV post-treatment. The aim of this study is to evaluate the utility of HPV DNA and mRNA testing in the follow-up of women post treatment.

Design: To date, 800 women presenting for their first visit to the colposcopy clinics at the Coombe Women and Infants University Hospital have been prospectively enrolled in the study. Cervical cytology specimens are taken at first visit prior to colposcopic procedure and at regular intervals during follow up. High risk HPV DNA is detected using Hybrid Capture II assay (Qiagen) and HPV mRNA is detected using HPV PreTect Proofer (Norchip)

Results: 522/800 women recruited, have been treated by LLETZ/LOOP. The data presented in this abstract relates to 294 patients treated by LLETZ for low grade (n=53) and high-grade disease (n=241) on cytology/colposcopy. The prevalence of high risk HPV DNA and mRNA in these women prior to treatment was 94% and 85% respectively. HPV 16 was the most predominant HPV type representing 65% of the cohort. Histological examination revealed 83% had CIN2+ disease, 13 % CIN1, 1%

cGIN and 3% Normal. Post colposcopic follow up of these women, at 6-12 months post treatment, indicated HPV DNA persistence in up to 20% of cases, of which 13% had abnormal cytology.
Conclusions: HPV DNA/mRNA testing is useful for predicting recurrence of CIN in women treated for high grade CIN and can be used as test of cure in the colposcopy setting.
This study is funded under the CERVIVA Programme by the Health Research Board, Ireland.

1119 Low Grade, Low Stage Endometrioid Adenocarcinoma of the Endometrium: Clinical Implications of the Pattern of Myoinvasion.

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Background: Endometrioid adenocarcinoma of the endometrium (EEC) is the most common histologic type of endometrial cancer with stage being the most important prognostic factor. While the majority of patients with low grade, low stage disease have a favorable prognosis (95% Stage 1, 70% Stage II, 5 year disease free survival), a subset of patients have a risk of recurrence and death. We were interested in evaluating patterns of myometrial invasion and correlating it with clinical outcome in order to potentially identify patients at increased risk.
Design: 230 cases of low grade EEC, from November 1998 to June 2004 were identified from our archives and reviewed to collect cases of low grade, low stage ECC with myoinvasion with at least 2 years of clinical followup. The myoinvasive cases were subclassified based on the type of invasion present: infiltrating glands, broad front, adenomyosis-like, microcystic elongated and fragmented glands (MELF), and adenoma-malignum. The depth of invasion and presence or absence of lymphovascular invasion were confirmed, and clinical follow up data was obtained.
Results: 71 (31%) cases of invasive low grade EEC were identified of which 55 had greater than 2 years of followup and formed the basis of this study. Of these, 24 (45%) were superficially invasive (<10% myoinvasion), 27 (48%) invaded 10-49%, and 4 (7%) invaded >50% into the myometrium. 6 (10%) cases contained cervical stromal invasion. The invasive patterns consisted of infiltrative glands (34; 56%), broad front (14; 32%), MELF (4; 6%), adenomyosis-like (2; 4%), and adenoma malignum like (1 case with 90% invasion). Lymphovascular invasion was noted in 3 cases (7%). Twenty-seven (48%) patients had a hysterectomy and bilateral salpingo-oophorectomy while 28 (52%) also underwent a lymphadenectomy. Twenty percent (13 patients) received adjuvant therapy which consisted of chemotherapy, radiation therapy, or vaginal brachytherapy. None of the cases with myoinvasion recurred (follow-up from 24-141 months; mean 83 months).
Conclusions: In this series, only a small percentage of patients with low grade, low stage EEC had myometrial invasion (71/230; 31%). Pattern of myoinvasion does not appear to impact the rate of recurrence. Regardless of the pattern of myoinvasion, overall prognosis for women with low grade EEC remains excellent.

1120 Utility of PAX2 as a Marker for Diagnosis of Endometrial Intraepithelial Neoplasia (EIN).

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Background: Accurate diagnosis of premalignant endometrial disease, EIN, requires learning new diagnostic criteria and resolving its many mimics. PAX2, a gene clonally inactivated in three quarters of EIN lesions, is a potential tool for educational and/or diagnostic use. When informative, PAX2 can precisely delimit lesion extent and facilitate appreciation of lesion characteristics relative to background. We had two trainees diagnose routinely stained ("H&E") biopsies and then measured the effect of subsequently viewing a PAX2 stain on their interpretation.
Design: H&E recuts and PAX2 immunostained sections from 52 endometrial biopsies originally diagnosed as EIN were assembled (by GLM and NM) as a study set, and presented to two pathology fellows (MQ and AL, "subjects") for review. 71% (37/52) of the EINs were known to be PAX2 null. The subjects first diagnosed H&E slides using standard EIN criteria, recording those features that complicated diagnosis. PAX2 stains were then reviewed in all cases and problems and benefits of their interpretation noted.
Results: Results of 52 cases and two reviewers totalled 104 diagnostic passes. H&E diagnoses included EIN (82%), and crowded glands subdiagnostic of EIN (12%). Diagnostic areas seen on the original slides were depleted in some recuts. The most common features confounding H&E EIN diagnosis were altered differentiation ("metaplasia", 14%), separation of EIN and background glands within different tissue pieces (13%), large lesions lacking comparison normal endometrium (11%), and secretory background (8%). Trainees were more likely to consider PAX2 staining helpful when there was a secretory background, or foci of EIN lacked adjacent normal tissue in the diagnostic fragment. PAX2 staining confused the subjects where interpretation of PAX2 immunoreactivity was unclear (14%), and when non-diagnostic tissue fragments contained PAX2 null glands (11%).
Conclusions: PAX2 staining is not a panacea for resolution of all, or even most, EIN lesions. Some do not bear abnormalities of this marker, and others are difficult to interpret. There is, however, educational value in using small training sets of dual stained EIN lesions to illustrate patterns of lesion spread and variable presentations of EIN cytology and architecture. PAX2 immunostaining may have clinical value in carefully selected cases where contrast between the lesion and background are represented in different areas of the sample, or obscured by a secretory background.

1121 An Immunohistochemically Defined Correlate (PAX2-null SCOUTs) of Concurrent High-Grade Serous Carcinoma That Resides in the Fallopian Tube.

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Background: Secretory cell outgrowths (SCOUTS) are discrete expansions of tubal epithelium that share loss of PAX2 expression with high-grade serous carcinoma; and, in one recent study (Chen 2010), were observed more frequently in cases of serous carcinoma. This study computed the frequency of PAX2-null SCOUTs (PAX2NS) in tubes of a range of conditions, including prepubertal, reproductive age (tubal ligation and benign disorders), endometriomas and low-grade endometrioid carcinomas of the ovary as well as high-grade serous carcinomas (HGSCA).
Design: A series of 167 cases (65 benign \ non-lesional controls, 14 ovarian HGSCAs, 41 low grade ovarian endometrioid adenocarcinomas, and 47 ovarian endometriomas) were culled from divisional files. Immunohistochemical staining for PAX2 was performed on all sections of fallopian tube, and null areas with traditional SCOUT morphology were counted. The PAX2-null SCOUTS (PAX2NS) were scored as events per cross section of fallopian tube (n=1200 cross sections), and data compiled as an average per histologic cross section.
Results: Patient age ranged from 26 to 84 (average 49 years). The frequency of sections with PAX2NS ranged from 0 – 0.11 in pre-adolescents and benign controls to 0.042-0.066 in endometrioid lesions. In contrast, the percentage of PAX2NS sections in HGSCA was 0.314, a highly significant difference relative to the other groups by chi square analysis ($p < .001$). Moreover, the disparity in average frequency of PAX2NS per section was greatest when comparing benign samples (0.0-0.11), endometrioid lesions (0.045-0.085) and HGSCA (0.562) ($p < 0.001$). The patient groups with a higher frequency of PAX2NS were older on average, and SCOUTs tended to cluster at greater frequency around 50 and 65 years of age. However, at any age, the frequency was always higher in the group with HGSCA.
Conclusions: This study reveals, for the first time, an immunohistochemically detectible entity (PAX2-null SCOUT) in benign tubal mucosa that is strongly associated with high-grade serous carcinoma relative to benign conditions and endometrioid neoplasia. This supports further the concept that the fallopian tube is not only a site of origin for HGSCA but harbors alterations in gene function in the benign epithelium that could signal either the presence, or greater risk, of pelvic serous cancer.

1122 Significance of Gynecologic Vasculitis.

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Background: Vasculitis includes a heterogeneous group of disorders with the common histologic findings of vascular wall inflammation. Systemic or localized disease (e.g., renal vasculitis) has serious consequences. The incidence of gynecologic vasculitis (GynV) and its significance is little known.
Design: We performed a retrospective review of vasculitis cases affecting the female genital tract accessioned in our institution between 1990- 2010. Fifty-two cases were identified. Histopathologic evaluation, clinicopathologic review and follow-up was performed.
Results: Age of the patients ranged from 37 to 85 years (mean 55.2 years, median 51 years). None had prior symptoms or were diagnosed with generalized vasculitis, while one patient had prior diagnosis of fibromyalgia. Most patients presented with abnormal bleeding and were treated for conditions unrelated to vasculitis, including patients with hysterectomy for uterine tumors (n=34); ovarian tumors (n=15), cervical dysplasia (n=2), uterine prolapse (n=1), cesarean section complications (n=1), and unknown reasons (n=1). All patients had hysterectomy while 5 in addition had unilateral salpingo-oophorectomy and 37 had bilateral salpingo-oophorectomy. Vasculitis was confined to the cervix in 23 cases, endo-myometrium in 6 cases, ovaries 7 cases, fallopian tubes 3 cases, adnexal soft tissue 3 cases and diffuse (vasculitis involving more than one organ) 10 cases. The different types of vasculitis were: necrotizing 16 cases, predominantly lymphocytic 14 cases, granulomatous 5 cases, and non-specific in the remaining 17 cases. Only 2 patients had serologic tests: ESR, ANA, Anti-double stranded DNA and Anti-centromere. Only the patient with fibromyalgia was ANA +, but negative for the remaining tests. None of the patients with GynV received corticosteroids or additional treatment. Fourteen patients were lost to follow-up less than 1 month after surgery. Follow-up available for the remaining patients ranged from 2 month to 19.5 years (mean, 5.5 years). Seventeen patients had malignant tumors; two died of disease. During follow-up, two patients developed invasive ductal carcinoma (breast), one patient developed MGUS, and another was diagnosed with Hashimoto disease and ulcerative colitis. None of the patients developed systemic vasculitis.
Conclusions: GynV is rarely associated with systemic vasculitis. Potential GynV causes include: previous surgical interventions and vascular inflammation secondary to local malignancy. In the absence of symptoms of systemic vasculitis, serologic screening and systemic therapy is not required.

1123 Pathologic Abnormalities of Uteri Removed by Morcellation. A Review of 889 Cases.

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Background: Minimally invasive surgery has become an accepted alternative to traditional open surgical techniques. Pelviscopic hysterectomy by morcellation (with or without adnexa) is now routinely performed at Baystate Health Systems. The purpose of this study was to review such specimens to determine the presence of abnormal findings which would traditionally be approached without morcellation.

Design: A search of the anatomic laboratory information system was performed to identify morcellation procedures on gynecologic specimens from 1-1-01 to 1-1-10. All findings were recorded. Pre and postoperative information was obtained from the electronic medical records when available (IRB# BH-10-077).

Results: A total of 889 cases were reviewed (64 had non-morcellated components). The two most common diagnoses were leiomyoma (719 cases, 81%) and adenomyosis (327 cases, 37%). There were 30 (4.1%) atypical smooth muscle tumors, including 2 leiomyosarcomas. 75 cases (8.4%) were considered to have unexpected and significant diagnoses (see table). One case of epithelioid STUMP recurred 6 years after original diagnosis as low grade leiomyosarcoma.

Selected Significant Diagnoses in Morcellated Specimens

Epithelioid smooth muscle tumor of undetermined malignant potential	3
Leiomyosarcoma	2
Endometrial stromal tumor with muscle differentiation	1
Low grade endometrial stromal sarcoma	1
Endometrioid adenocarcinoma	3
Atypical endometrial hyperplasia (simple, complex, polyp)/atypical polypoid adenomyoma	10
Ovarian brenner tumor	1
Ovarian mature cystic teratoma	2
Ovarian adult granulosa cell tumor	1
Ovarian mullerian mucinous borderline tumor	1
Ovarian serous borderline tumor	1
Endometriosis	23

Documented preoperative evaluation was limited, and when available did not indicate the presence of neoplastic disease.

Conclusions: This review confirms the presence of unexpected significant lesions of the endometrium, uterine stroma and smooth muscle, and ovary. The local recurrence of one smooth muscle tumor 6 years following diagnosis may reflect seeding of the operative field following morcellation. In summary, unexpected neoplastic lesions of the gynecologic tract occur following morcellation, which may adversely affect staging, patient management and potentially patient outcome.

1124 Ovarian Tumors in Lynch Syndrome: Genotype-Phenotype Correlation.

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Background: The ovary is the second commonest site of gynecologic malignancy in Lynch syndrome. To date there have been limited data on genotype-phenotype correlation in these tumors, or on the sensitivity of clinical screening schemas – Amsterdam II (AmII), revised Bethesda (rBethesda), and Society of Gynecologic Oncologists (SGO) genetic risk assessment schemas – questions examined in this study.

Design: Ovarian cancer cases were identified retrospectively from the records of the cancer registries in Toronto, Vancouver and Montreal. Detailed patient family pedigrees, tumor microsatellite instability (MSI), MMR gene product immunohistochemistry (MMR-IHC), and pathology were correlated with genotype.

Results: Of 15 cancer registry cases identified (mean age 42.8 years, range 31-53 yrs, 1 > 50 years old) – 12 *MSH2*, 3 *MLH1*mutations – ovarian cancer was the sentinel tumor in 12 patients (all aged 50 or under), with 3 others having antecedent colorectal cancer. Tumors were unilateral, ovary-confined in 7 cases with 4 of these having synchronous endometrial carcinoma. Histologic types included endometrioid (3), clear cell (2), serous (2), mucinous (1), squamous cell (1) and mixed (6) tumors. Analysis of family pedigrees showed clinical schemas selected the following number of cases: AmII – 7/15 (47%); rBethesda – 5/15 (33%); SGO 20-25% risk – 9/15 (60%); SGO 5-10% risk – 14/15 (93%).

Conclusions: The newer SGO criteria have better sensitivity for detection of Lynch syndrome-related ovarian carcinomas than colorectal cancer-centred schemas such as AmII and rBethesda. The wide spectrum of histologic types and age profile of patients with sentinel tumors suggests any proposed screening algorithm for Lynch syndrome in ovarian carcinoma should consider starting with all patients 50 and under.

1125 Presence of Tumor Necrosis in Endometrial Biopsy Predicts Advanced Stage of Endometrial Adenocarcinoma.

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Background: Tumor necrosis and lymphocytic infiltration have been recognized as predictors for tumor behavior and chemotherapy response in some solid tumors. There is no available data regarding the clinical implication of these histopathologic features in endometrial adenocarcinoma. In this study, we investigated the value of the degree of tumor necrosis in a series of endometrial biopsies to predict advanced tumor stage on resection.

Design: Endometrial biopsies with endometrioid adenocarcinoma followed by total hysterectomy and bilateral salpingo-oophorectomy were retrieved from the archives during the period between 2000 and 2008. Cases with missing slides, tumors with non-endometrioid components or carcinosarcoma were excluded. We identified 198 endometrial biopsies eligible for the study. All cases were reviewed at the multiheaded microscope for histologic type and nuclear grades, presence of tumor necrosis and lymphocytic infiltration. The percentage of necrosis and lymphocytic infiltration were graded as follows: 1+ (0-25%), 2+ (26-50%) and 3+ (>50%). Data were analyzed using Cox regression and Spearman correlation test.

Results: Of the 198 biopsies, 88/198 (44%) had 1+ necrosis, 61/198 (31%) had 2+ necrosis and 49/198 (25%) had 3+ necrosis. Both the degree of necrosis and FIGO grade in endometrial biopsies showed a significant correlation with the depth of myometrial invasion, cervical involvement and lymphovascular invasion (Spearman correlation, P< 0.01) in the subsequent resection. While the degree of necrosis was associated with lower uterine segment involvement, FIGO grade was associated with presence of lymph node metastasis. There was a significant correlation among degree of necrosis, FIGO grade and nuclear grade in endometrial biopsies. Tumor lymphocytic infiltration and nuclear grade did not show any significant correlation with other histopathologic prognostic parameters in the hysterectomy.

Conclusions: Degree of tumor necrosis in endometrial biopsy is a predictor for deep myometrial invasion, cervical involvement and presence of lymphovascular invasion in the subsequent resection. Documenting the presence and degree of tumor necrosis in pathology reports of endometrial biopsy in addition to FIGO grade may add insight for the surgical management decision.

1126 Malignant Struma Ovarii: A Study of 86 Cases Demonstrating No Correlation between Pathological Features and Disease Course.

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Background: Struma ovarii that exhibit malignant histology is very uncommon, and aggressive clinical course in the form of initial extra-ovarian spread or later recurrence is even more exceptional for these tumors. This study analyzes in detail the morphological features of 86 histologically proliferative (adenoma-like) or histologically malignant struma ovarii cases that with many years of follow-up proved to be biologically benign or malignant, in an attempt to define if specific histological features have predictive value.

Design: Microscopic analysis in two categories (absence or presence ≤10% vs. presence >10% tumor) was performed for the following criteria: papillary architecture, pseudo-papillae, psammoma bodies, nuclear grooves, nuclear overlap, "orphan Annie" nuclei, nuclear pseudo-inclusions, nucleoli, hypercellularity, colloid scalloping, and nuclear pleomorphism. A qualitative analysis was performed for the absence or presence of vascular and capsular invasion. Fibrosis was categorized to four groups: absent, peripheral, central, or peripheral and central. Cell size was divided to three groups: large, normal, and small. Three cell types were recognized: regular, oxyphilic, and clear. Tumor architecture was classified according to the prevalent pattern as microfollicular, macrofollicular, and trabecular. For mitotic activity the number of mitoses per 10 high power fields (HPF) was stated. We examined if specific histological features are associated with malignant clinical course.

Results: The patients were 4 to 61 years old (median=41 years). Twenty-six cases were biologically malignant; among them 8 (31%) were histologically malignant as well, and 18 (69%) were histologically proliferative. Of the 60 biologically benign cases, 17 (28%) displayed malignant histology, and 43 (72%) were histologically benign. Furthermore, the probability of a malignant clinical course was around 30% in cases with either benign or malignant histology. The presence of all the histological features examined was similar in the biologically benign and malignant tumors. No specific histological feature was found to be predictive of an aggressive clinical course.

Conclusions: The clinical outcome of struma ovarii cannot be predicted based on the microscopic diagnosis of the thyroid tissue or on specific histological features. The lack of correlation between morphology and outcome in proliferative and histologically malignant struma ovarii is striking, making the behavior of these tumors particularly unpredictable.

1127 Relative Survival of Ovarian Surface Carcinoma Subtypes Depends upon Time after Diagnosis.

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Background: The major subtypes of surface ovarian carcinoma have been compared in multiple studies with respect to survival. Posited was the notion that survival differences of the subtypes might be shown to change as time passed after diagnosis.

Design: The Surveillance, Epidemiology, and End Results Program was queried for women at least 18 years old, with histologically confirmed serous, clear cell, endometrioid, or mucinous carcinoma of the ovary diagnosed between 1988 and 2003, and with at least one month of follow-up, without a prior cancer diagnosis or extra-peritoneal spread at presentation.

Results: Of 10,528 patients, 3,430 (32.6%) experienced cancer related deaths over the first decade. Adjusted for age and race, compared with serous carcinoma, clear cell carcinoma did not prognostically differ during year 1 (Time Ratio=0.84,99% c.i. 0.64-1.14) or years 2-5 (0.84, 0.66-1.1), but did impart a better prognosis during years 6-10 (2.06, 1.27-3.69). Endometrioid carcinoma imparted a better prognosis during year 1 (1.3, 1.01-1.7), years 2-5 (1.92, 1.55-2.44), and years 6-10 (2.28, 1.54-3.37). Mucinous carcinoma did not impart a prognostic difference during year 1 (0.92, 0.69-1.28), but did impart a better prognosis during years 2-5 (2.41, 1.74-3.52) and years 6-10 (8.12, 4.39-19.2). Compared with the clear cell: serous tiime ratio (TR) of year 1, whereas that of years 2-5 (Ratio of TR=1,99% c.i. 0.69-1.5) did not differ, that of years 6-10 (0.41, 0.22-0.7) did. Compared with the endometrioid: serous TR of year 1, those of years 2-5 (0.68, 0.48-0.96) and years 6-10 (0.57, 0.36-0.91) differed. Compared with the mucinous: serous TR for year 1, those of years 2-5 (0.38, 0.24-0.61) and years 6=10 (0.11, 0.05-0.23) differed. By contrast, differences with respect to age and race were not show to vary with respect to years after diagnosis.

Conclusions: Survival differences among surface ovarian carcinoma subtypes depend on time after diagnosis; general statements concerning survival differences are inapt.

1128 Increased Expression of HIF-1α in Invasive Endocervical Adenocarcinoma.

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Background: Tumor hypoxia is a well-known microenvironmental factor that causes cancer progression and resistance to treatment. The proposed mechanisms are complex but believed to involve mediation through transcriptional gene activation by the hypoxia-inducible factors (HIFs). HIFs in turn are known to upregulate GLUT receptors which are also known to be associated with tumor progression. Our objective was to characterize the expression of HIF-1α, GLUT-1 and Ki-67 in in-situ (AIS) and invasive endocervical adenocarcinoma.

Design: Seventy-nine consecutive patients diagnosed with AIS and invasive endocervical adenocarcinoma in our institute from 1994 to 2010 were included in the study. Of these 43 were AIS and 36 were invasive adenocarcinoma. Tissue microarrays were constructed and immunohistochemical staining was performed using antibodies against HIF-1α, GLUT-1 and Ki-67. Semi-quantitative scoring of immunoreactivity was based on intensity and percentage of tumor staining and grouped into low and high expression for statistical analysis.

Results: A significantly larger proportion of cases within the invasive cancer group showed high expression of all three markers (HIF-1α, GLUT-1 and Ki-67) when compared to the in situ cohort.

	Expression	HIF-1α	GLUT-1[a]	Ki-67
Invasive Carcinoma N=43	High	24(57%)	15(68%)	27(63%)
	Low	19(43%)	7(32%)	16(37%)
Adenocarcinoma In Situ N=36	High	12(33%)	2(6%)	10(28%)
	Low	24(67%)	34(94%)	26(72%)

$p<0.05$

[a]Some cases missing due to processing

Conclusions: The high expression of hypoxic markers including HIF-1α and GLUT-1 in invasive endocervical adenocarcinoma compared to AIS may provide insight into the pathway of tumor progression from the pre-invasive into the invasive phase of the endocervical adenocarcinoma. These data suggest that HIF-1α-mediated pathway may be a candidate for a targeted therapy.

1129 The Incidence of Microsatellite Instability in Synchronous Endometrial and Ovarian Endometrioid Adenocarcinomas: A Dilemma Revisited.

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Background: Because Lynch Syndrome more frequently affects patients with synchronous (rather than single) colorectal cancer, intuitively, the same principle would seem to apply to women with synchronous gynecologic primaries. Current literature is inconsistent regarding the prevalence of microsatellite instability (MSI) in this population, with reported rates between 0 and 47%. Moreover, it is unknown whether it is appropriate to test one or both tumors in synchronous cases. This study aims to answer these questions in an unselected series of women with presumed synchronous endometrial and ovarian endometrioid adenocarcinomas.

Design: Histopathologic review of 42 candidate synchronous endometrial and ovarian endometrioid adenocarcinomas over a 12-year-period produced 27 cases in which there was consensus among three pathologists regarding endometrioid histology and synchronicity based on published criteria. Bilateral ovarian tumors were tolerated in the absence of a parenchymal growth pattern (9 cases), in which cases, both were tested. All formalin-fixed, paraffin embedded tumors were tested by MSI PCR (Promega MSI Analysis System, v1.2, Promega Corporation, Madison, WI.) and immunohistochemistry (IHC) for DNA mismatch repair (MMR) proteins MLH1, PMS2, MSH2, and MSH6.

Results: All synchronous cases (tumor pairs/trios) had identical results for any one woman. Twenty-one cases were microsatellite stable with normal IHC. Six of 27 (22%) cases were microsatellite unstable (microsatellite instability-high, MSI-H). Five of these cases demonstrated loss of MLH1 and PMS2 expression; the remaining MSI-H case demonstrated normal MMR expression.

Conclusions: The incidence of MSI-H synchronous endometrioid adenocarcinomas closely matches the incidence of MSI in patients with single endometrial cancer (approximately 20%). Because IHC loss of MLH1 and PMS2 is often associated with somatic MLH1 promoter hypermethylation in the tumor, only a fraction of these patients are predicted to have a germline mutation (i.e., Lynch syndrome). The results suggest that screening patients with synchronous endometrioid tumors is no better or worse than screening a general population with only uterine cancer. In addition, the findings indicate that a single specimen may be acceptable for molecular screening in synchronous cases.

1130 Parametrial Invasion, an Important Landmark of Cervical Cancer Treatment; How Can We Recognize It?

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Background: According to the current treatment guideline of uterine cervical cancer, postoperative adjuvant treatment is indicated in patients having parametrial invasion in the radical hysterectomy specimens. Despite the importance of parametrial invasion, histological decision of parametrial invasion is often subjective because "parametrium" is a loosely defined structure and the definitive landmark has not been determined.

Design: To define a precise landmark of "parametrium", we scrutinized cervical wall and cervico-parametrial junctions on H&E and trichrome-stained sections of type III radical hysterectomy specimens from patients with FIGO stages IB/IIA (n=22), and IIB (n=66), and compared the applicability of 3 landmarks, 1) thick & large calibered blood vessels, 2) presence of adipose tissue/loose connective tissue on the H&E sections, and 3) compact muscle layers on trichrome stainings in the decision of parametrial invasion. We also compared the applicability of those 3 landmarks in the longuitudinal (n=37) and modified transverse sectioning (n=29).

Results: In the 22 FIGO stage IB/IIA cases, inner two thirds of cervical wall was composed of either compact/hapharzardly arranged muscle bundles (55%) or sparse/hapharzardly arranged smooth muscle bundles containing abundant interstitial connective tissue stroma (45%), while outer one third of the cervical wall was consistently composed of continuous and compact muscle layers, which were clearly distinguishable from parametrium. In the decision of parametrial invasion among 66 FIGO stage IIB cases, only a single case (2%) was difficult to decide by using smooth muscle layer on trichrome staining, while 30% and 8% were difficult to decise by using large calibered blood vessels and apipose tissue/loose connective tissue, respectively. In the decision using 3 different landmarks, modified transverse sectioning gave better tissue orientation for the relationship between cervical wall and parametrium.

Conclusions: Trichrome staining for identification of compact muscle layer of cervical wall is a simple and useful method for the decision of parametrial invasion status of cervical cancer in the difficult cases, and modified transverse sectioning was very helpful to obtain important pathological informations in the radical hysterectomy specimens.

1131 The Expression and Value of Aldolase in Endometrial Cancer and the Role of Clotrimazole in Endometrial Cancer Cell Viability and Morphology.

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Background: Previously, using cDNA microarray gene expression, we found that aldolaseC was among numerous genes upregulated in uterine serous carcinoma in comparison to endometrioid adenocarcinoma. Aldolase is a critical enzyme in the glycolytic pathway. Most cancer cells use this pathway for generation of ATP as a main source of their energy supply. Clotrimazole, an antifungal agent, has been proven to induce a dose-dependent detachment of aldolase from the cytoskeleton, leading to cell damage and destruction. Our aims are to evaluate the value of aldolaseC in endometrial carcinoma (EC) and to determine the impact of clotrimazole in inhibition of cell viability and alteration of cell structure in EC cell line.

Design: To accomplish the first aim, aldolaseC protein expression using immunohistochemistry analysis was performed on 350 paraffin-embedded EC tissues. Next, fresh frozen tissue from 70/350 samples were available to evaluate the aldolaseC mRNA level using Taqman RT-PCR. For the second aim, endometrial cancer cell line cell (HEC1) and colonic cancer cell line (CT26) (as a control) with and without treatment with clotrimazole were evaluated for cell viability by tryptan blue dye and for cell structure by transmission electron microscope at different times.

Results: AldolaseC protein was expressed in 78% EC cases. High aldolaseC mRNA levels were associated with low tumor grade (p=0.002), serous subtypes (p=0.006) and longer overall survival (p=0.051). Treatment of cancer cell lines with clotrimazole induced detachment of cancer cells from culture plates. Total cell death was seen 4 hrs post-treatment in CT26 and 24 hrs in HEC1. The effect of clotrimazole on HEC1 at 4 hrs manifested as small discontinuities of the cell membrane, swollen mitochondria with loss of cristae and very granular cytoplasm. At the 24 hrs time point, cell death had occurred evidenced by loss of integrity of the plasma membrane, leading to cell rupture.

Conclusions: Although preliminary**,** our data is the first to shed light on the value of aldolaseC in EC. We provided evidence of the effect of clotrimazole in EC cell viability and cell structure. We hope our results will open the door for future *in vivo* investigation and raise the option of clotrimazole as a new therapeutic agent in EC patients

1132 Morphologic Patterns Associated with *BRCA1* and *BRCA2* Genotype.

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Background: It has been hypothesized that ovarian tumors with *BRCA* dysfunction might have a distinctive histologic appearance, often referred to as "BRCAness." Data suggest that, like tumors with *BRCA1* mutation, those showing solid and/or transitional cell-like features may be prognostically superior to other high grade serous carcinomas; and tumors with prominent infiltrating lymphocytes have been reported in the setting of *BRCA* dysfunction.

Design: The existence of BRCAness was studied by reviewing H&E slides from 43 high grade serous ovarian carcinomas, included in The Cancer Genome Atlas (TCGA), with known BRCA1 and BRCA2 status. Genetic subgroups represented were: *BRCA1* germline mutation; *BRCA1* somatic mutation, *BRCA1* promoter methylation; *BRCA2* germline mutation; *BRCA2* somatic mutation; *BRCA* unaffected. Tumor architecture, tumor infiltrating lymphocytes (TILs), and the presence and extent of necrosis were recorded for each case. TILs were only evaluated in sections from the primary site (i.e. Fallopian tube or ovary). Histologic review was performed without knowlege of genotype.

Results: A combination of either solid or transitional cell-like architecture with tumor infiltrating lymphocytes was present in 13/31 cases (42%) with *BRCA1* or *BRCA2* abnormality, as compared with only 1/12 (8%) in the *BRCA* unaffected group. This phenotype was most commonly encountered in the *BRCA1* promoter methylation group (7/13; 54%). None of the 4 *BRCA1* somatic mutants showed this phenotype. TILs were found in 18/31 cases (58%) with *BRCA1* or *BRCA2* abnormality, as compared with only 2/12 (17%) in the *BRCA* unaffected group. TILs were found predominantly in cases with *BRCA1* abnormality (16/23; 70%), including *BRCA1* germline mutation (3/4). Geographic necrosis was also more common in cases with *BRCA1* or *BRCA2*

abnormality (14/31; 45%) as compared with 2/12 (17%) in cases with intact *BRCA1* and 2. Subgroups with the highest prevalence of geographic necrosis were *BRCA1* germline mutants (3/4) and *BRCA2* germline mutants (3/4).
Conclusions: BRCAness is not a specific trait, but it is characteristic of cases with *BRCA1* and 2 dysfunction. Solid and transitional-cell like architecture, along with TILs and geographic necrosis are seen in significant numbers of these cases. None of these findings is specific for either *BRCA1* or 2 germline mutation.

1133 BRCA1 Immunohistochemistry in a Genotypically Characterized Cohort.

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Background: Immunohistochemical evaluation for *BRCA1* dysfunction is not in widespread clinical use. If validated, this may provide an efficient and low cost initial screen to select patients for targeted therapies, specifically, PARP inhibition. This methodology will also narrow the pool of patients who may benefit from screening for hereditary cancer syndromes and might provide prognostically relevant information.
Design: One section from each of 43 high grade serous ovarian carcinomas included in The Cancer Genome Atlas (TCGA) with known BRCA1 and BRCA2 status was evaluated with a commercially available monoclonal antibody against BRCA1 (clone MS110) after optimization and IRB approval. Genetic subgroups represented were: *BRCA1* germline mutation; *BRCA1* somatic mutation, *BRCA1* promoter methylation; *BRCA2* germline mutation; *BRCA2* somatic mutation; *BRCA* unaffected. A semiquantitative estimate of the extent of tumor cell nuclear labeling was recorded and correlated with genetic status. Using a cutoff of 5% (negative versus positive) separated tumors into distinct groups.
Results: Negative results (loss of BRCA1 expression) were recorded in: all 4 *BRCA1* germline mutants; 3/6 cases with *BRCA1* somatic mutation; 11/13 cases with *BRCA1* promoter methylation; 1/4 *BRCA2* germline mutants; 0/4 *BRCA2* somatic mutants; and 0/12 *BRCA* unaffected cases. Equivocal results, generally stemming from weak or absent internal positive controls, were recorded in every category but *BRCA1* germine mutants, and totalled 6/43.
Conclusions: This study suggests that the use of a commercially available antibody against BRCA1 in a small series may have clinical utility.

1134 The NSAID Sulindac Inhibits Proliferation and Induces Apoptosis in Cervical Cancer Cell Lines.

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Background: Sulindac, a commonly used NSAID, has been investigated as a potential novel therapeutic for different forms of cancer, including colon and breast. A recent study looked at the molecular action of this drug on the HPV18 infected cervical cell line, HeLa, and demonstrated that not only could it induce apoptosis but also degrade one of the main oncoproteins, E7.
Design: This study aimed to validate the previous findings and to extend the analysis to other cervical carcinoma cell lines with differing origins, HPV status and viral DNA content. Three cervical cancer cell lines were examined, the adenocarcinoma derived HPV18 positive HeLa, the squamous cell carcinoma derived HPV16 positive SiHa and the HPV negative C33A.
Results: Sulindac had a time and dose dependent growth inhibitory effect on all three cell lines. However, the most potent response was observed in the HeLa cells, with the IC_{50} value approximately 200μM less than the other two cell lines. Analysis of the HeLa cells demonstrated that this activity occurred predominantly through induction of apoptosis but additionally by cell cycle arrest. We confirmed post-transcriptional degradation of the HPV18 viral oncogene E7. This decrease was dose dependent and appeared to correlate with an observed G1 arrest. In addition, it was demonstrated that a decrease in COX activity may be partially responsible for the anti-proliferative activity of sulindac.
Conclusions: This data indicates that the antineoplastic activities of sulindac are multifaceted. Since most cancers progress through the action of multiple pathways, drugs that simultaneously block several pathways might be particularly effective as therapeutic agents. Therefore, these results suggest that NSAIDs may offer potential as novel therapeutics for cervical cancer.

1135 Embryonic Stem Cell Factor Sox2 in Ovarian Carcinomas – Significant Association with More Poorly Differentiated Carcinomas and Longer Overall Survival in a Subgroup of Stage II-IV High-Grade Serous Carcinomas.

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Background: The transcription factor Sox2 is involved in the maintenance of embryonic stem cell pluripotency and is expressed in several carcinoma types such as adenocacinoma of the lung and squamous cell carcinomas (SCC) of various origins. The gene *SOX2* is located at chromosome 3q26, a region that is a frequently amplified in serous ovarian carcinoma. This study aims to explore the potential role of Sox2 in ovarian carcinogenesis by correlating Sox2 protein expression and gene amplification with different histological types of ovarian carcinomas, tumor grade and clinical outcome.
Design: 209 consecutive cases of ovarian carcinoma (149 serous, 38 endometrioid, 11 clear cell, 5 mucinous and 6 transitional cell carcinomas) were analyzed by immunohistochemistry in a tissue microarray for nuclear expression of Sox2. FISH analysis for gene amplification of *SOX2* was performed for the serous carcinomas. Overall survival was compared by Kaplan-Meier Analysis
Results: 57% of all carcinomas showed detectable Sox2 positive cells with no significant difference between the major histological types (serous 58.4%, endometrioid 57.9%, clear cell 45.5%). Overall, Sox2 expression was more frequent in less differentiated tumors (G1: 38.9%, G3: 63.4%, p= 0.04). Low level gene amplification was detected in 22% of cases and did not correlate with gene expression levels. Surprisingly, analysis of the largest homogenous group (high-grade serous, stage II-IV) showed a favourable effect of Sox2 expression on overall survival (median 40 months vs. 25 months, p= 0.008).
Conclusions: Sox2-positive cells can be detected by immunohistochemistry in a majority of ovarian carcinomas, with increasing frequency in high-grade tumors. However, within the group of high grade serous carcinomas Sox2 expression is associated with a significantly better prognosis, suggesting that in this specific tumor entity activation of certain stemness-pathways may unexpectedly predict favourable outcome.

1136 CYP11A1, STAR and SULT1E1 Expression in Ovarian Leydig Cell Tumors.

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Background: Leydig cell tumor (LCT) of the ovary is a rare sex cord-stromal tumor composed of cells recapitulating normal Leydig cell development, being composed of cells with abundant granular eosinophilic cytoplasm, distinct cell borders and, in 50% of cases, pathognomonic Reinke crystals. LCTs can have overlapping histologic features with granulosa cell tumors, clear cell carcinomas, vascular tumors or lipid-rich Sertoli-Leydig cell tumors (SLCT). Although vimentin and inhibin are commonly used as markers for LCTs, these are non-specific; there are currently no specific and sensitive IHC markers for ovarian LCTs. Based on the steroidogenesis pathway, we identified three potential new LC IHC markers: CYP11A1, STAR and SULT1E1. The aim of our study was to further characterize the expression of these enzymes in ovarian LCTs.
Design: Paraffin embedded material from 3 LCTs was examined by IHC for the expression and localization of CYP11A1, STAR and SULT1E1. Normal testicular tissue, 7 LCT of the testis and 10 Sertoli Leydig cell tumors (SLCT) were used as controls. Staining intensity was scored on a scale of 0-3 with 0 being no staining and 3 being the most intense staining.
Results: All 3 LCTs revealed cytoplasmic staining for SULT1E1 (3=33%, 2=67%, 1=0%, 0=0%), CYP11A1 (3= 67%, 2=0%, 1=33%, 0=0%), and STAR (3 =0%, 2=67%, 1=33%, 0=0%). In the control group, Leydig cells in both testicular LCTs and normal testis revealed cytoplasmic staining for CYP11A1, STAR and SULT1E1. Sertoli cells and germ cells in normal testis and Sertoli cells in SLCT were negative for all three markers. The Leydig cell component of SLCT expressed all three markers.
Conclusions: Our findings support that the three markers CYP11A1, STAR and SULT1E1, components of the biosynthetic pathway of testosterone production, are useful to identify ovarian LCTs and are more specific than the currently used markers inhibin and vimentin. As none of the new markers are expressed in either Sertoli cells or germ cells, this panel can help to distinguish LCTs from other sex cord-stromal tumors as well as from germ cell tumors.

1137 Her2-Neu Over-Expression in Serous and Clear Cell Endometrial Carcinoma.

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Background: Type II endometrial carcinoma includes high grade, non-estrogen driven tumors with a molecular pathway distinct from Type I (endometrioid type). Histologically, it consists of serous and clear cell carcinomas with some morphologic overlap. Although Type II represents 9-14% of endometrial cancers it is associated with aggressive clinical course and accounts for 48% of deaths. Her2/neu oncoprotein is an established target for therapy in breast cancer and has an emerging role in the treatment of gastric cancer. The aim of this study is to determine the proportion of Her-2/neu overexpression in Type II endometrial carcinoma and the association between this receptor and stage at presentation.
Design: We identified a set of 36 clear cell endometrial carcinomas and 34 serous endometrial carcinomas accessioned from 2000-10. Cases were reviewed independently by two gynecological pathologists. Her-2/neu oncoprotein over-expression was determined by immunohistochemistry. Only a strong and complete membranous staining pattern was considered positive. Clinical information was retrieved from the electronic medical records.
Results: Overall Her-2/neu overexpression was found in 11/70 (15.7%) endometrial Type II cases; 6/34 (18%) of the serous carcinomas, and 5/36 (14%) of clear cell carcinomas. Typically the expression was focal, in less than half of the tumor cells. 61/70 patients underwent full surgical staging. In the remaining 9 cases, staging was based on a more limited surgical procedure. Of the fully staged patients, 12 had extrauterine involvement at time of surgery and 49 did not. Her-2/neu expression was not associated with extrauterine disease ($p > 0.05$).
Conclusions: Our study demonstrates that Her2/neu oncoprotein is overexpressed in about 16% of Type II endometrial cancer. The proportion of this subset is similar to that observed in breast cancer. This observation opens the door for inclusion of novel targeted therapies directed against Her2/neu in clinical trials for aggressive type of endometrial cancer.

1138 Absence of TTF-1 in Benign Endometrium and Simple Hyperplasia Is Associated with Progression to Cancer.

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Background: Thyroid transcription factor-1 (TTF-1) is thought to be specific for tissues of thyroid and lung origin. Its expression may be seen in Mullerian tissue; the significance of this is unknown. Recent studies show TTF-1 may inhibit the epithelial mesenchymal transition in lung adenocarcinomas (Saito, 2009) and that it may be a favorable prognostic factor in ovarian neoplasms (Fujiwara, 2010). We wanted to examine its role in early endometrioid carcinoma progression.

Design: Archived formalin fixed paraffin embedded endometrial tissues were obtained from 535 cases in 207 patients who either did or did not progress to carcinoma. Specimens were sampled mostly in triplicate as 1.1 mm cores for tissue microarray construction. Immunoperoxidase staining of TTF-1 antibody (Dako, Carpinteria, CA) was performed using a Dako autostainer. Scoring was performed by a single pathologist blinded to diagnosis and was recorded by intensity (0 to 3+) and percentage of glandular cells expressing TTF-1. A positive result was considered in any case with 1+, 1% or more cells with TTF-1 nuclear expression. Patients who developed non-endometrioid carcinoma, did not have a follow-up surgical case, and did not have informative TTF-1 information (e.g., no lesional epithelium for evaluation) were excluded from the analysis.

Results: 392 cases from 134 patients composed of benign endometrium (n=216), simple and complex hyperplasia (n=45), simple and complex atypical hyperplasia (n=25), and endometrioid carcinoma (n=106) were examined for TTF-1 nuclear expression. Significant differences of expression were noted in benign endometrium versus adenocarcinoma and non-atypical hyperplasia versus carcinoma (p=0.0007 and p=0.0530, respectively, Mann-Whitney U test). Absence of TTF-1 expression in benign endometrium and simple hyperplasia cases was associated with progression to cancer (p=0.00018, log-rank test). This association was not significant in hyperplasia alone, with or without atypia, although the numbers were small. TTF-1 was further shown to be an independent predictor of progression to cancer in multivariate analysis including cancer family history, diabetes, hypertension, body mass index, cancer diagnosis age, and age of menopause (p=0.0004, HR=0.089, 95% CI 0.023-0.339).

Conclusions: The absence of TTF-1 expression in benign endometrium and simple hyperplasia is an independent early predictor of endometrioid carcinoma suggesting that TTF-1 expression may play a protective role in benign and "early" endometrial lesions. The role of TTF-1 in advanced endometrial lesions remains to be elucidated.

1139 Differentially Expressed Genes in Early Stages Uterine Serous Carcinomas in Comparison to G3 and G1 Endometrioid Adenocarcinoma.

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Background: Clinical studies demonstrated that early stages uterine serous carcinoma (USC) had almost similar outcome in comparison to early stages high grade endometrioid adenocarcinoma (EAC)-G3. The aim of this study is to explore the genetic fingerprints of these tumors that may explain their similar outcome.

Design: A transcriptome analysis was performed using the human genome wide illumina bead microarrays carrying 48,000 genes to profile stage I USC (n=11) vs. stage I EAC-G3 (n=11) and vs. stage I EAC-G1 (n=11), respectively. The expressions of 15 genes were selected for validation and determined using Taqman RT-PCR gene expression.

Results: We identified 988 differentially expressed genes (DEGs) between USC and EAC-G3 with 522 genes specific to USC and 1,499 DEGs between USC vs. EAC-G1 with 1,063 genes specific to USC. The up-regulated genes specific to USC were genes involved in cell proliferation, invasion and metastasis (*SNCG),* cell adhesion *(MSN),* tumor progression (*LMNA),* cell cycle (*TBX2)* and tumor development, growth and angiogenesis (*IRS2).* The down regulated genes were involved in suppressing tumor metastasis (*NME5),* reduce cell growth *(ALCAM),* tumor suppressor *(CEACAM1),* and slow cancer progression *(TFF3).* Although, many of these genes were novel genes to USC, they were found in other cancer types such as liver, colon, breast and lung cancers. Over-regulation of some genes had been proven to predict poor prognosis such as *SNCG* and *IRS2* in colon cancer, and downregulation of *NME5* to be a predictor of metastatic potential in breast cancer. In addition, genes like *ALCAM and SNCG* were implicated to be associated with chemoresistance in pancreatic and uterine cancer.

Conclusions: We found that stage I USC has relatively similar gene fingerprints in comparison to EAC-G3 than to EAC-G1. Numerous novel DEGs and their transcripts found to be specific to USC might have potential prognostic and therapeutic impact on patients with uterine cancer. However, our results should be confirmed by larger studies to evaluate the usefulness of some genes as biomarkers in endometrial cancer. Nevertheless, we believe that our findings shed meaningful insights into the clinical study of endometrial cancer patients that warrant further investigation.

1140 Stem Cell Marker Expression in Endometrial Carcinoma.

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Background: It has been suggested that cancers contain minor populations of neoplastic stem-cells that are capable of tumor initiation. Cancer stem cells (CSC) are postulated to be responsible for resistance to hypoxia and have been associated with resistance to radiation therapy. CD133, CD44, CD59, CD9, Oct-4 and ALDH1 have been suggested as stem cell markers in different malignancies but only CD133 has been proposed as a marker for the stem cell population in endometrial carcinoma (EC). However, the immunohistochemical profile of CSC in EC has not been fully elucidated.

Design: CD133, CD44, CD59, CD9, Oct-4 and ALDH1 expression was assessed in three tissue microarrays (TMA) constructed from 70 paraffin-embedded blocks of normal endometrium (NE) in different phases of the menstrual cycle, 157 primary EC and 16 vaginal post- radiation recurrences. CD133, CD44, CD59, CD9, Oct-4 and ALDH1 expression was correlated with stage, histological type and grade of primary EC, and was also compared between primary EC and post-radiation recurrences.

Results: CD44, CD9 and CD59 immunostaining was higher in the secretory than in the proliferative phase (p=0.0007, p=0.01, p=0.02, respectively). CD44, ALDH1 and CD59 immunostaining was higher in EC compared to NE (p=0.01429, p<0.00001, p<0.00001, respectively). A decreasing trend of CD44 and CD9 expression was observed with higher histologic grade (CD44 p=0.05, CD9 p= 0.015). CD44 (mean=29.11), ALDH1 (mean=7.96), CD59 (mean=56.9) and Oct-4 (mean=11.8) expression was lower in post-radiation recurrences when compared to primary EC (mean=122.86, 63.73, 118.1, 65.98, respectively). There was no significant correlation between CD133, CD44, CD59, CD9, Oct-4 and ALDH1 expression and tumor stage.

Conclusions: Some stem cell markers (CD44, ALDH1 and CD59) show increased expression in EC when compared to NE, suggesting a role in tumor development and progression. Moreover, CD44, CD9, ALDH1, CD59 and Oct-4 might not be good predictors of radiation resistance in EC.

1141 Comparative Proteomic Analysis of Uterine Leiomyomas and Leiomyosarcomas.

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Background: Proteomics confirm the presence of different proteins in normal tissues and tumors and provide a direct measure of the quantity present. By identifying proteins associated with a malignant process proteomics provide the information how to interfere with the action of those proteins and how to find a drug that may inactivate that action.

We compared protein expression in uterine leiomyomas (LM) and leiomyosarcomas (LMS) in search for targets related to histologic features of malignancy and an aggressive behavior.

Design: The IRB approved study included 14 LMS and 14 LM, identified in our file between 2002 and 2006. The selected formalin-fixed paraffin-embedded tissues were processed using a heat-induced / enzyme-mediated digestion methodology and analyzed in triplicate by LC-MS/MS on a linear ion trap mass spectrometer. Tandem mass spectra were searched against the UniProt human protein database and differences in protein abundance between the samples were derived by summing the total CID events that resulted in a positively identified peptide for a given protein accession across all samples (spectral counting). The spectral count data were normalized for each protein accession by calculating the percent contribution of the spectral count values for each protein accession against the total number of peptides identified within a given sample.

Results: The tumor samples were tested for the presence of 216 proteins and 39 were detected using stringency criteria based on an 80% population of samples with two or more peptides for identifications. Out of 16 proteins that show statistically significant up-regulated expression in LMS compared to LM, the following are known to be associated with malignancies: 60S acidic ribosomal protein P1, Ezrin, Fructose-bisphosphate aldolase A, Isoform 1 of Heterogeneous nuclear ribonucleoprotein K, Lamin-B1, Protein disulfide-isomerase, Tubulin alpha-1A chain. Of the 11 proteins showing statistically significant down-regulated expression, some (Actin, Alpha-actinin-1, Isoform 1 of Filamin-A, Isoform 1 of Sorbin and SH3 domain-containing protein 1, Transgelin) appear also to be associated with malignancies.

Conclusions: The statistically significant difference in expression of various proteins between LMS and LM may aid the histologic evaluation of smooth muscle tumors and help to identify tumors with more aggressive behavior. In addition the proteomic analysis may lead to a potential identification of a new treatment.

1142 Fetal Vascular Lesions Are Increased in Placentas from Women Infected with Pandemic H1N1 Influenza.

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Background: During the 2009-2010 H1N1 influenza (H1N1) pandemic, the CDC categorized pregnant women as high risk. Complications associated with H1N1 infection included preterm labor, preterm birth, and pregnancy loss, potentially reflecting effects of the H1N1 virus on the placenta. We sought to determine if placentas from women infected with H1N1 during pregnancy showed evidence of increased inflammation, fetal-derived lesions or other placental pathologies.

Design: Women were enrolled under IRB approved protocols who had H1N1 infection during pregnancy confirmed by antigen swab test or PCR. None were acutely infected at the time of delivery. Control subjects were matched for gestational age at delivery, parity and delivery mode. All placentas underwent gross and microscopic pathologic examination and were scored for the presence or absence of inflammation, vascular lesions, meconium and other findings. Fisher's exact test was used for statistical analysis and a p-value of <0.05 was considered significant.

Results: Placentas from H1N1-infected women (n=16) and controls (n=17) were examined. Gestational age (GA) ranged from 35 2/7 to 41 5/7 weeks and placental weights ranged from 330 to 630 grams. To control for GA, the fetal to placental weight ratios were determined and were not different between H1N1 cases and controls (7.28±1.27 vs. 6.89±1.15; p=0.37). No significant differences in inflammation were observed. Acute inflammation of the membranes was seen in 4 H1N1 and 7 control placentas (p=0.46), while chronic membrane inflammation was seen in 2 H1N1 and 1 control placenta (p=0.6). Chronic villitis was observed in 2 H1N1 placentas and 5 controls (p=0.40), while umbilical cord or chorionic plate vascular inflammation was

seen in 4 H1N1 placentas and 6 controls (p=0.7). Interestingly, 6 H1N1 placentas had intervillous or subchorionic thrombi, compared to 2 controls (p=0.12) and 3 H1N1 placentas had intimal fibrin cushions compared to 1 control (p=0.11), for an overall occurrence of 9 fetal vascular lesions in H1N1 placentas compared to 3 in controls (p=0.03).

Conclusions: There were no significant differences in maternal inflammation (membranes) or fetal inflammation (umbilical cord and chorionic plate vessels) between the H1N1 and control groups. In contrast, placentas from women infected with H1N1 during pregnancy showed a higher incidence of fetal-derived vascular lesions, suggesting that fetuses exposed to H1N1 may undergo changes that increase risk for vascular lesions.

1143 Pathologic Characteristics of Endometrial Carcinoma in Women 40 Years of Age and Younger.

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Background: Endometrial carcinoma (EC) is the most common invasive gynecologic cancer. Arising mainly in postmenopausal women, it is uncommon before 40 years (y) of age. Previously considered to have favorable histology and excellent outcome, recent studies suggest that EC arising prior to age 40y (EC<40y) may have unfavorable histology and extrauterine disease. The etiology of EC<40y is considered to be primarily unopposed estrogen stimulation in the setting of obesity and anovulatory cycles with a smaller number of cases attributed to microsatellite instability (MSI) as an inherited risk factor. The aim of this series is to analyze the pathologic features of EC<40y in our academic center.

Design: All cases of EC<40y diagnosed at our center between January 2005 and September 2010 were retrieved and information from the pathology reports at the time of surgery was recorded. For patients who elected for uterine conservation/medical treatment, results from endometrial biopsy/curettage were used. Status of MSI, estrogen (ER) and progesterone (PR) by immunohistochemistry was retrieved. Body mass index (BMI) of each patient was recorded.

Results: 15 cases of EC<40 y were identified, representing 13.2% of all 113 EC diagnosed during this interval. Women ranged in age from 20 to 40y (mean=34.8). Table 1 summarizes the findings for 11 patients (73.3%) who had surgery.

Pathologic features of endometrial tumors at total hysterectomy

Histologic type	**Total number of cases**
Endometrioid	10(90.9%)
Non-endometrioid	1(9.1%)
FIGO histologic grade	
I	3(27.3%)
II and III	8(72.7%)
Size of tumor	
< 2.0 cm (0.3-0.7 cm)	3(27.3%)
> 2.0 cm (3.9-9.8 cm)	8(72.7%)
FIGO stage	
IA	3(27.3%)
IB	3(27.3%)
II	1(9.1%)
III	4(36.3%)
LVI	
Absent	6(54.5%)
Present	5(45.5%)
Pelvic washing cytology	
Negative	6(54.5%)
Positive	5(45.5%)

4 patients elected uterine conservation/medical treatment; 3 had FIGO I endometrioid adenocarcinoma and 1 had serous carcinoma in an endometrial polyp. 86.6% of women were obese (BMI>30), mean BMI=45 (range 25-61). Of the 7 cases tested, 4 were ER positive (57.1%) and 3 PR positive (42.8%). MSI testing in 3 cases showed positive staining in 2 cases and MSH2/MSH6 negative result in 1 case submitted for mutational analysis.

Conclusions: EC<40 y is uncommon (13.2% of all EC) and is not an indolent disease. In our series 45.4% of patients had advanced disease, LVI, positive pelvic cytology and 72.7% had large tumors. 86.6% of EC<40y were endometrioid type. The majority of patients were obese and MSI studies were initiated by the clinician in a minority of cases.

1144 Interobserver Diagnostic Concordance of Serous Tubal Intraepithelial Carcinoma and Related Lesions.

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Background: Serous tubal intraepithelial carcinoma (STIC) is a candidate precursor to pelvic high grade serous carcinoma (HGSC). There also exists a spectrum of lesions that are putative STIC precursors, namely "p53 signature," which lacks nuclear atypia, and tubal intraepithelial lesion in transition (TILT), which exhibits atypia, but falls short of STIC. A recent study (Carlson, et al.) reported suboptimal interobserver concordance when morphologic guidelines, but not immunohistochemistry (IHC), were used for categorization. The current study tested an algorithm to enhance interobserver concordance, with the ultimate goal of developing a classification scheme that can be used for diagnostic standardization.

Design: Empirically derived morphologic criteria were tested using a panel of 6 pathologists who independently examined 67 lesions in round 1. An around-the-scope training session and IHC (using p53 and Ki-67) were added to morphologic examination, followed by independent assessment of 42 cases in round 2.

Round 2. STIC: serous tubal intraepithelial carcinoma; STIL: serous tubal intraepithelial lesion

Results: From round 1 to 2, kappa values improved from 0.3 to 0.5 for normal/reactive lesions, from 0.1 to 0.35 for atypical/p53 signature/STIL [akin to TILT], and from 0.4 to 0.78 for STIC.

Conclusions: Substantial concordance was achieved for categorizing STIC using a combination of morphologic assessment and IHC. Further work is required to optimize reproducibility for lesions falling short of STIC.

1145 Array Comparative Genomic Hybridization and Fluorescence In Situ Hybridization Analysis of Endometrial Carcinoma Specimens.

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Background: Endometrial cancer (EC) is the most common female genital tract cancer in the United States. The goal of this study was to determine the frequency of chromosomal gains and losses by array comparative genomic hybridization (CGH) in EC and whether fluorescence in situ hybridization (FISH) can detect these abnormalities in paraffin-embedded EC specimens.

Design: Specimens were collected from 109 patients undergoing hysterectomy from 2005-2007. Forty specimens were analyzed by CGH with the Genosensor assay (Abbott Molecular Inc., Des Plaines, IL), 36 were analyzed by FISH and 33 by both methods. The study comprised 61 endometrioid (EM) carcinomas (32 grade 1, 15 grade 2 and 14 grade 3), 19 non-endometrioid (NE) carcinomas (11 serous, 2 clear cell, 6 carcinosarcoma), 3 atypical complex hyperplasias (ACH), 7 hyperplasias without atypia and 19 normal endometriums. FISH probes evaluated included 18q21 (*DCC*), CEP18, 8q24(*MYC*), 1q25(*LAMC2*), 20q13*(ZNF217)*, 2p24(*MYCN)*, 10q26(*FGFR2*), 2p26(*PIK3CA*), 10q23(*PTEN*), CEP 10 and 8p11(*FGFR1*) (Abbott Molecular Inc). Signal copies for each probe were enumerated in 50 cells from the tissue area of interest.

Results: By CGH, the most common regions of gain in all ECs were 1q21-q41, 1q telomere, 8q24, 8q telomere and 3q27-q29. The most frequent regions of loss were 19p telomere, 9q33.2-q34, 18q 21, 17p12 and 16q22-q24. Differences were observed in the overall frequency and specific regions of gains and losses between EM and NE tumors. A four probe combination of 1q25, 8q24, 8p11 and 20q13 provided the most optimal sensitivity and specificity for the detection of EC in paraffin-embedded tissue sections. Cutoffs for abnormality of each probe were: ≥14% of cells with gains of 1q25 or ≥10% of cells with gains of 8q24 or ≥6% of cells with gains of 20q13 or ≥4% of cells with gains of 8p11. These cutoffs detected of 45/52 cases with EC or ACH (sensitivity 87%) and were negative in all 17 specimens with hyperplasia without atypia or benign endometrium (specificity 100%). The probe set detected 2/3 (67%) of ACH, 10/15 (67%) EM grade 1 tumors, 9/10 (90%) grade 2 tumors and all of grade 3 (n = 10) and NE tumors (n = 14).

Conclusions: This study revealed specific regions of gain and loss that are common in EM and NE EC. A four probe FISH set appears to have high sensitivity and specificity for the detection of EC. However, prospective studies are needed to determine the analytical performance characteristics and clinical utility of this FISH assay.

1146 MicroRNA Signatures in Early and Later Stage of High Grade Serous Carcinoma in Fallopian Tube.

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Background: High grade papillary serous carcinoma (HG-PSC) of the ovaries/fallopian tubes is one of the most lethal gynecological malignancies. Recent studies have raised a compelling hypothesis that fallopian tube (FT) secretory epithelial cells may harbor a cell of origin for most HG-PSC. About 80% of HG-PSC precursor lesions, also known as serous tubal intraepithelial carcinoma (STIC), are found in the distal (fimbriated) ends of the fallopian tubes. Beyond p53 mutations, the molecular changes of STIC are largely unknown.

Design: To explore the molecular difference between normal FT, STIC and invasive HG-PSC, we conducted a global microRNA expression analysis. In our well characterized cases presenting with normal FT, STIC and HG-PSC, we selected five cases for miRNA profiling analysis and 12 cases for validation by RT-PCR and microRNA in situ hybridization (MISH). In each case, areas of FT, STIC and PSC were microdissected from 10 um sections in formalin fixed paraffin embedded tissue (FFPE). MTG platform

was used for global microRNA profiling analysis, including 700 well characterized microRNAs. ANOVA significance analysis was performed to identify microRNAs above 2 fold changes.

Results: MicroRNA expression profiles were successfully accomplished in FFPE tissues. All cases examined had reasonable clean microRNA data and had good agreement within each component. The net changes of microRNA expression and distinct microRNA signatures among different components were established. Table 1 summarized the numbers of microRNAs differentially expressed (>2 folds) between FT and STIC, STIC and PSC, FT and PSC. Top dysregulated microRNAs, including *miR182, miR200c, miR34s, miR210* and *miR93,* were confirmed by RT-PCR and some were further validated by MISH.

Differential miRNA expression in FT, STIC and PSC

net changes	STIC-PSC	FT-PSC	FT-STIC
downregulated miRNA	14	16	17
upregulated miRNA	33	26	4
fold ranges	2-9	2-50	2-6

FT: fallopian tube; STIC: serous tubal intraepithelial carcinoma; PSC: invasive high grade serous carcinoma

Conclusions: STIC is early stage of HG-PSC and it has distinct microRNA expression and it can be discriminated by its unique microRNA signature from FT and HG-PSC. Top dysregulated microRNAs in HG-PSC have a high agreement with published data. We were able to identify a subset of oncogenic microRNAs differentially expressed between FT, STIC and PSC. This novel finding will provide molecular tools for the further analysis of tumorigenesis of STIC and will benefit in searching for new biomarkers (microRNA target genes) for STIC.

1147 microRNAs and Their Target Gene Networks in Uterine Leiomyomas.

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Background: Human uterine leiomyomas (ULMs) are characterized by dysregulation of a large number of genes and microRNAs. It is thus important to examine the roles played by the highly dysregulated microRNAs through regulation of specific target genes in tumorigenesis of ULMs. To explore the broader relationship between other dysregulated microRNAs and their target genes exhibiting aberrant expression in ULMs, global analysis of microRNA and target gene expression in ULMs is helpful to identify the potential candidates of microRNAs for tumorigenesis and growth of ULMs.

Design: Among 70 cases with ULMs, 45 cases were conducted for global microRNA expression analysis (Ambion); 5 cases for global gene expression analysis (Affymetrix); 8 cases for comparative genomic hybridization (CGH); 36 cases for immunohistochemistry; 14 cases for senescence; and 24 cases for validation. Uterine leiomyoma and myometrial cell lines with stable overexpression of lentiviral *miR-29b, miR-296, miR-200a* and *let-7c* were prepared for analysis of predicted target gene and tumor growth. Relationship of selected mRNAs in ULMs and target gene functional pathways were analyzed by GeneSpring GX11, TM4 Microarray Software Suite, DAVID and Gene set enrichment analysis.

Results: miRNA and mRNA expression were examined in paired sets of ULMs and matched myometria. Patterns of inverse association of microRNA with mRNA expression in ULMs revealed an involvement of multiple candidate pathways, including transcriptional reprogramming, cell proliferation control, MAP kinase, TGF-β, WNT, JAK/STAT signaling, remodeling of cell adhesion, cell-cell and cell-matrix contacts. Two distinct microdeletion detected by GCH might be responsible for loss of *miR-15* and *miR-200a* clusters. These miRNAs directly regulated IGF signaling pathway, EMT pathway, and some oncogenes. These miRNAs are tumor suppressors and significantly inhibit leiomyoma cell growth *in vitro*. Some miRNAs may participate in the leiomyoma aging process. Those miRNAs that are associated with smooth muscle differentiation are significantly dysregulated in ULMs.

Conclusions: The levels of the most dysregulated microRNAs in ULMs show an inverse association with the expression levels of many predicted target genes, and that they may affect multiple homeostatic pathways and functions. Some but not all of them can be validated as functional targets of specific microRNAs *in vitro*. Dysregulated miRNAs are associated with leiomyoma growth, differentiation and aging process.

1148 Improving Identification of CIN2+ Disease by Combining MCM2 and MCM7 Biomarker Over-Expression with a Pap Counterstain on a Standard Cervical LBC Specimen.

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Background: Over-expression of MCM 2 and 7 have been shown to correlate with aberrant S-phase induction and persistent HPV infection. To further improve accuracy in identifying abnormal cells and true disease from BD SurePath liquid-based cervical (LBC) cytology specimens, a new automated test, that combines the morphology of a standard SurePath Pap with protein biomarker immunostaining on a single slide (hereafter referred to as SurePath Plus), was developed. This research study evaluated the performance of SurePath Plus in identifying CIN2+ disease.

Design: This study included 996 cytology specimens ranging from NILM to HSIL. All LSIL and HSIL cases had biopsy results. As biopsies were not obtainable for all NILM and ASCUS cases, a negative HPV test was used as a surrogate for disease negative status. For each sample, 2 slides were produced, one prepared as a SurePath Pap and a second SurePath Plus slide prepared using a BD PrepStain Plus instrument that combines cell deposition with optimized immunocytochemical processing and Pap counterstaining. All slides were scored using standard Bethesda 2001 criteria. The SurePath Plus slide was further evaluated for the presence of nuclear immunostaining in morphologically abnormal cells. The distribution of cases within the various morphologic categories and their biopsy status were compared.

Results: Comparison of the SurePath Plus slides to the SurePath Pap slides, using a cytology endpoint, resulted in a 153% increase in the HSIL+ detection rate (123 cases) and a corresponding decrease in detection rates for LSIL (23% decrease, 58 cases) and ASCUS (54 % decrease, 207 cases). The biopsy endpoint analysis resulted in a significant increase in the number of CIN2+ cases associated with HSIL+ cytology and a corresponding reduction in the amount of CIN2+ within the LSIL and ASCUS groups for the SurePath Plus Test when compared to the SurePath Pap. Specifically, the number of CIN2+ cases within the HSIL+ group increased 154% (77 cases), while the number of CIN2+ cases decreased by 62% (52 cases) within the LSIL and 62% (13 cases) for the ASCUS population.

Conclusions: This study reports the successful development of reagents, assay, and instrumentation that combine biomarker specific immunostaining with standard Pap counterstaining. The use of the SurePath Plus test leverages the advantages of both biomarker expression and morphologic assessment on a single LBC slide. Within this biopsy confirmed research cohort, the SurePath Plus test resulted in a more accurate detection of high grade disease.

1149 The Clinical Significance of "Squamous Intraepithelial Lesion (SIL) of Indeterminate Grade" as a Distinct Cytologic Category.

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Background: "Squamous intraepithelial lesion of indeterminate grade" (SIL) was introduced in the 2001 Bethesda System for cervical lesions that lie between low-grade or high-grade lesions without discussion of follow-up or its clinical significance. At our institution we use the SIL terminology for such borderline lesions, which is similar to "low grade squamous intraepithelial lesion cannot exclude high-grade squamous intraepithelial lesion (LSIL-H)" utilized by some studies. In this study, we evaluated the follow-up of these borderline lesions collected over a 3 year period and compared them to the follow-up results of low-grade or high-grade squamous intraepithelial lesions (LSIL or HSIL) on Pap smears.

Design: A computer-generated review of cervical pap smears (100% Surepath) diagnosed as SIL from 01/2007 through 01/2010 revealed 152 cases. Histologic outcomes (cervical biopsy, endocervical curettage, loop electrosurgical excision procedure) or follow-up pap smears with available high-risk HPV serologies were obtained for 127 (84%) of these cases. Control groups of 150 cases with a Pap smear diagnosis of LSIL or HSIL with histologic follow-up were selected sequentially from this same time period. Patients with <6 months follow-up and no follow-up were excluded. If multiple follow-up specimens were available the highest degree of dysplasia was used.

Results: On follow-up, HSIL (cervical intraepithelial neoplasia (CIN) grade 2 or 3) was identified in 21% (27/127) of SIL cases, 8.7% (13/150) of LSIL cases and 69% (104/150) of HSIL cases. LSIL (CIN 1) was identified in 30.7% (39/127) of SIL cases, 59% (89/150) of LSIL cases and 17% (26/150) of HSIL cases. ASC-US (atypical squamous cells of undetermined significance) was identified in 20.5% (26/127) of SIL cases with most positive for high-risk HPV at 61.5% (16/26). In comparison, the ASC-US rate was similar for both the LSIL and HSIL control groups (7.3% and 6.7% respectively). A diagnosis of benign was identified in 27.6% (35/127) of SIL cases, 24.7% (37/150) of LSIL cases, but only 6.7% (10/150) of HSIL cases. The histologic outcomes between the SIL, LSIL and HSIL groups are statistically different ($p<0.001$).

Conclusions: The results showed that patients with the cytologic diagnosis of "SIL of indeterminate grade" have histologic outcomes which are intermediate between and statistically significantly different from patients with either a LSIL or HSIL diagnosis. Our findings support prior studies evaluating the follow-up of patients with LSIL-H and support retaining SIL as a unique category in the Bethesda system.

1150 *K-ras* Mutations in Endometrial Carcinoma of Uterus – Molecular and Morphological Correlation.

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Background: *K-ras* gene product in the EGFR pathway is critical in the development of many types of malignancy including lung, colon and endometrial cancer. Often, *K-ras* mutation-associated lung, pancreatic, colon or ovarian cancer display mucinous differentiation. Previous studies showed that *K-ras* gene mutation can be found in 10-40% of endometrial carcinoma. Endometrial carcinoma includes many histological subtypes with variable pathogenesis. To our knowledge, the correlation of *K-ras* mutations with carcinoma with significant mucinous differentiation of uterus has not been reported before. The current study is aimed to investigate the prevalence of *K-ras* gene mutations in the mucinous carcinoma and endometrioid carcinoma with significant mucinous differentiation (MC&ECMD) and compare it with endometrioid carcinoma (EC).

Design: With IRB approval, specimens of MC&ECMDs and ECs were searched from the archival files of the Department of Pathology, W&I Hospital. Thirteen MC&ECMDs and eight ECs were selected for this study. Genomic DNA was extracted from formalin-fixed paraffin-embedded tissue sections that were macrodissected to ensure more than 80% of tumor cells. PCR amplification for *K-ras* codons 12 and 13 were performed (RIH, RI), followed by sequencing using capillary electrophoresis (Sequencing Facility, Yale University, CT). The sequencing results were analyzed by "Sequence Scanner v1.0" program.

Results: *K-ras* codons 12 and 13 mutations were detected in 10 of 13 (76.9%) MC&ECMDs with the most prevalent mutation being G12D (codon 12, GGC > GAC) (4/10, 40%). Only 2 of 8 ECs (25%) were positive for *K-ras* mutation, all being G12D. Statistically significant difference of *K-ras* mutation was noted between MC&ECMDs and ECs ($P< 0.05$, Fisher's exact test). Overall G12 D mutation is the most prevalent mutation detected in this study (6/12, 50%).

Conclusions: Significantly higher prevalence of *K-ras* mutations was found in the MC&ECMD group compared to the EC group in this study, indicating close association between *K-ras* mutation and mucinous differentiation, and suggesting possible unique pathogenic pathway for MC&ECMD. Further study of *K-ras* mutation in MC&ECMD with its clinical application appears warranted.

1151 Utility of LIN28 To Diagnose Primitive Ovarian Germ Cell Tumors.

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Background: Ovarian primitive germ cell tumors (GCTs) include gonadoblastoma, dysgerminoma, embryonal carcinoma (EC), yolk sac tumor (YST), and mixed GCT. Sometimes morphologically they pose diagnostic difficulty and require immunohistochemical study. In this study we investigated the diagnostic utility of RNA-binding protein LIN28 in these tumors.

Design: Immunohistochemical staining of LIN28 was performed in 68 primary ovarian primitive GCTs [4 gonadoblastomas, 30 dysgerminomas (pure 24 pure, 4 as a component in mixed GCTs, 2 arising in association with gonadoblastoma), 6 ECs (all as a component in mixed GCTs), and 40 YSTs (20 pure, 20 as a component of mixed GCTs)] and 11 metastatic ones (9 dysgerminomas, 1 EC, and 1 YST). For comparison, 14 immature teratomas, 17 mature teratomas, 5 primary carcinoid tumors, 2 strumal carcinoids, 2 struma ovarii, and 2 squamous cell carcinomas arising in association with dermoid cysts were also stained with LIN28. To delineate LIN28 specificity in ovarian tumors, LIN28 staining was performed in 119 ovarian non-GCTs including 37 clear cell carcinomas, 11 endometrioid carcinomas, 20 high grade and 2 low grade serous carcinomas, 12 mucinous carcinomas, 2 transitional cell carcinomas, 4 small cell carcinomas (1 pulmonary-type, 3 small cell hypercalcemic type), 8 Sertoli-Leydig cell tumors, 7 steroid cell tumors, 10 adult granulosa cell tumors, 8 juvenile granulosa cell tumors, 5 fibrothecomas, and 3 benign Brenner tumors. The percentage of tumor cells stained (cytoplasmic staining) was scored as 0, 1+ (1-30% cells), 2+ (31-60%), 3+ (61-90%), and 4+ (>90%).

Results: Strong 4+ Lin28 staining was seen in 4/4 (100%) gonadoblastomas, 7/7 (100%) embryonal carcinomas (ECs), and 41/41 (100%) yolk sac tumors (YSTs). Among 39 dysgerminomas, 4+ staining was seen in 37 and 3+ staining in 2 (strong in 37, mixed weak and strong in 2). Twelve of 14 immature teratomas showed variable Lin28 staining (1+ to 4+) in the immature elements (weak to strong staining) whereas mature teratomas, carcinoids, strumal ovarii, and stromal carcinoids were negative. Only 5 of 117 non-GCTs (including 1/37 clear cell carcinomas) showed weak to moderate 1-2+ staining.

Conclusions: LIN28 is a highly sensitive marker for gonadoblastomas, dysgerminomas, ECs and YSTs. LIN28 can be used for their diagnosis and to distinguish them from non-GCTs.

1152 Molecular Genetics of Uterine Malignant Mixed Mullerian Tumor.

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Background: The admixture of carcinomatous (CC) and sarcomatous components (SC) in uterine malignant mixed mullerian tumor (UMMMT) has raised questions regarding its histogenesis. Studies favor a monoclonal epithelial origin with metaplastic sarcomatous elements. However, its molecular pathogenesis remains largely unknown. Recent studies have shown that p16 and p53 are overexpressed concordantly in CC and SC, and microsatellite instability (MSI) has been found in 5- 23%. To date an analysis of *PIK3CA*, the most frequently mutated oncogene in uterine endometrioid carcinoma, has not been reported in UMMMT.

Design: Paraffin-embedded archival tissue of 35 UMMMT, 10 leiomyosarcoma (LMS), and 8 low grade stromal sarcoma (LGSS) was utilized in this study. Immunohistochemical (IHC) analysis of p16, p53, MLH1, PMS2, MSH2, and MSH6 was performed. For p16 and p53, a score of 0 to 12 was calculated as the product of intensity (0 to 3+) and percentage (1 = 1-10%, 2 = 11-50%, 3 = 51-80%, 4 = > 80%). For mutational analysis, neoplastic tissue was microdissected, and DNA was extracted. Exons 9 and 20 of *PIK3CA* were amplified and directly sequenced. In addition, 20 UMMMT cases were analyzed for PTEN mutations and MSI (10 of which were included in the 35 cases used in the IHC analysis).

Results: P16 expression was seen in 23 of 34 (68%) of CC and 30 of 33 (91%) of SC. 12 of 35 (34%) UMMMT showed significantly less p16 expression in CC (score = 3.33) than SC (score = 9.83) ($P = < 0.0001$), compared to LMS (7.8) and LGSS (3.38). P53 expression was seen in 19 of 35 (54%) of CC and 18 of 33 (55%) of SC in UMMMT with no difference between the two components. Out of the 12 cases with loss of p16 in CC, 9 (75%) had low p53 expression. 12 out of 35 cases (34%) showed complete loss of p53 expression in both CC and SC. 5 of 35 UMMMT (14%) showed loss of MLH1 and PMS2 expression, and no loss was seen in LMS or LGSS. 8 out of 20 (40%) cases showed PTEN mutations that were identical in CC and SC. Currently, no mutations have been identified in *PIK3CA*, but these studies are ongoing.

Conclusions: The high concordance rate between CC and SC with regards to PTEN mutations, p53 expression, MSI, and DNA MMR immunostaining provide further evidence that the two components are of monoclonal origin. Although previous studies have found concordance of p16 expression in the two components we found a statistically significant difference in p16 expression. This finding suggests that specific biomarkers may provide insight into the divergent nature of the two components and the molecular underpinnings of this aggressive malignancy.

1153 Prognostic Significance of Aurora-A and BRCA2 Expression in Endometrioid Ovarian Carcinoma.

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Background: Aurora-A, a serine/threonine kinase, has been shown to regulate the cell cycle checkpoint and maintain genomic integrity. Aurora-A is overexpressed in various carcinomas. Breast cancer susceptibility gene 2 (BRCA2) plays an important role in maintaining genomic stability and acts as a tumor suppressor. Our recent study suggested that Aurora-A regulates genomic instability and tumorigenesis through cell cycle dysregulation and suppression of BRCA2 expression. However, the expression of Aurora-A, BRCA2 and their clinical significance is unknown in endometrioid ovarian cancer.

Design: In this study, we determined Aurora-A and BRCA2 expression in endometrioid ovarian carcinoma and correlated them with clinicopathologic characteristics and patient survival. Immunohistochemical staining was performed in 51 primary endometrioid ovarian carcinoma tumor samples using tissue microarray. We then analyzed the associations between Aurora-A and BRCA2 expression and clinical factors (tumor grade, disease stage, surgical type, clinical response, and relapse) and overall and disease-free survival durations.

Results: Aurora-A and BRCA2 expression were found in 48% and 29% of samples, respectively. The results of Fisher's exact test suggested that Aurora-A expression was significantly associated with no family history of ovarian cancer (P=0.03) and that BRCA2 expression was associated with early-stage disease (P=0.03), low ascites incidence (P=0.03), younger age (<60) at diagnosis (P=0.03), and low-grade tumors (P<0.01). The nuclear BRCA2 score was negatively correlated with Aurora-A score (P=0.019, two-tailed Pearson correlation). A log-rank test demonstrated that Aurora-A expression was associated with shorter overall (P=0.001) and disease-free (P=0.009) survival durations and that BRCA2 expression was associated with longer overall (P=0.000) and disease-free (P=0.002) durations. Patients with BRCA2-positive and Aurora-A-negative tumors had higher overall (P=0.001) and disease-free (P=0.001) survival rates than did patients with Aurora-A-positive and BRCA2-negative tumors.

Conclusions: Our results demonstrate that a negative regualory loop exists between Aurora-A and BRCA2 expression in ovarian endometrioid carcinoma. Aurora-A expression is an unfavorable prognostic factor in patients with endometrioid ovarian cancer and BRCA2 is favorable, combination of these two markers may better predict the prognosis of patients with endometrioid ovarian carcinoma than individual marker alone.

1154 Clinicopathological Characterization of Endometrial Carcinomas Arising in Elderly Women Aged over 70 Years.

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Background: The incidence of type II endometrial carcinomas (EMCs) increases with increasing age, and mixed type EMCs composed of type I and type II are also more often encountered. Thus, many EMCs in elderly women tend to harbor histologically variable combinations.

Design: This study was conducted to clarify the profiles of EMCs in elderly women aged over 70 years from the histological and clinical standpoint. The 161 examined EMCs were surgically resected during 1995 to 2005 and at FIGO stage I to III. Histologically, the cases were categorized into type I EMCs (G1 or G2 endometrioid, mucinous), type II EMCs (G3 endometrioid, serous, clear, undifferentiated) and mixed type EMCs (composed of more than 90% of type I and less than 10% of type II). Invasive pattern (infiltrative or expansile) and frequency of vessel infiltration (-, 1+, 2+, 3+) were evaluated. Immunohistochemically, expressions of estrogen receptor (ER) and p53 were analyzed using representative sections. Survival ratios were calculated by the Kaplan-Meier method and analyzed by log-rank test.

Results: The 161 cases were divided into 96 cases (59%) for type I, 54 cases (34%) for type II and 11 cases (7%) for mixed type. Type II included 22 cases (14%) with one histology and 32 cases (20%) with more than one histology. There was no significant difference in invasive pattern between type I and type II, but vessel infiltration was more frequently observed in type II than type I ($p<0.05$). ER and p53 expression manner of mixed type was found to be intermediate between type I and type II, but very closer to that of type II. Expression of p53 was evident in the serous components compared to the clear cell components, but expression of ER was stronger in clear cell components than serous components. The prognosis showed the tendency towards becoming less favorable in the order of type I, mixed type, type II with one histology and type II with more than one histology ($p<0.05$).

Conclusions: In the elderly women, one-third of total EMCs were type II. The mixed type was relatively few in number, but the clinicopathological profiles were nearly same as those of type II, in spite of the minority (5-10%) of type II components. It was suggested that the serous component was more significant in the determination of clinical outcome compared to other histological types.

1155 10-Year Retrospective Study on High-Grade Endometrial Endometrioid Adenocarcinoma (HGEEA), FIGOIII: Morphology and Immunohistochemical Characterization.

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Background: HGEEA is focus of attention since morphology overlaps with more aggressive non-endometrioid carcinoma (CA) (type II tumors), such as undifferentiated CA, serous CA, clear cell CA, etc. HGEEA's biologic behavior, hormone receptor expression, and proliferative activity are controversial.

Design: All endometrial CA with HGEEA diagnosis from 1999-2010 were second-reviewed. Immunostains performed on TMA blocks were p53, p16, MIB1, cytokeratin (CK), EMA, vimentin, ER/PR. Scoring system was as follows: p53 overexpression: >70% nuclear stain; ER/PR and MIB1+: >20% nuclear stain; p16 +: >75% cytoplasmic and nuclear stain; CK, EMA, and vimentin+: >20% cytoplasmic stain. Follow-up data was collected for all cases (follow-up period: 2 m-10 years). The χ^2 test was used to determine the significance of outcome in marker expression.

Results: Total 32 patients with HGEEA diagnosis were retrieved with mean age 70 (37-88). 22 had classic morphology of HGEEA; cells in solid areas were similar to the cells forming glands. Immunophenotypes and outcomes for 22 HGEEA cases were: 5 p53 overexpression (22.7%), all dead of disease (DOD); 17 p53- (77.3%), 4 DOD; 7 ER/PR-/- (31.8%), 6 DOD; 15 ER/PR+/+ (68.2%), 3 DOD; all 22 cases were diffusely positive for CK, EMA, vimentin; increased mitotic activities (MIB1+) were observed in 21 cases (95.5%); p16+ in 17 cases (77.3%). 10 cases with HGEEA diagnosis were misclassified: 5 exhibited features of undifferentiated CA, characterized by pattern less solid growth of tumor cells with marked nuclear atypia, occasional rhabdoid forms, frequent mitoses, necrosis. CK and EMA were only focally +. Other 5 misclassified tumors were: 2 clear cell CA, 2 adenosquamous CA, and 1 serous CA. Immunophenotypes and outcomes for 10 misclassified tumors were: 8 DOD, 7 p53 overexpression, 7 ER/PR-/-, 10 p16+, and 9 vimentin+. 5 undifferentiated CA exhibited p53 over expression (4/5) and completely loss ER/PR expression (5/5).

Conclusions: 1) HGEEA immunophenotypical overlaps with type II tumors. HGEEA with p53 overexpression (22.7%) and loss of ER/PR expression (31.8%) were associated with adverse outcome ($p<0.01$). 2) Non-endometrioid CA (type II tumors), more frequently undifferentiated CA, could be misinterpreted as HGEEA and more often had p53 overexpression (70%) and loss of ER/PR expression (80%), and were associated with adverse outcome ($p<0.01$). 3) Increased proliferative activity (MIB-1+), p16 and vimentin expression were commonly seen in both HGEEA and type II tumors, not associated with adverse outcome.

1156 PAX-8 Expression in Gynecologic and Breast Carcinomas.

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Background: The differential diagnosis among breast carcinomas and gynecologic carcinomas, including ovarian, endometrial and endocervical carcinomas, can be challenging when distinct morphologic features are not present, because they often share the phenotypic profiles of the common immunohistochemical markers (e.g. CK7+/ER+/CK20 negative). PAX8, a transcription factor, has been recently shown to be expressed in ovarian carcinomas, but not breast carcinomas. The aim of this study is to examine the expression profile of PAX8 in a spectrum of gynecologic carcinomas and a variety of breast carcinomas.

Design: A total of 145 gynecologic carcinomas (including 55 endometrial carcinomas, 37 endocervical carcinomas, and 53 ovarian carcinomas) and 184 breast carcinomas (including 170 invasive ductal carcinomas, 12 invasive lobular carcinomas, and 2 mixed ductal and lobular carcinomas) were retrieved for the study. Tissue microarrays of the tumors were stained with PAX8 rabbit polyclonal antibody. All tumors were semi-quantitatively scored using H-score method where the score combining intensity and percentage of positive cells ranges from 0 (negative) to 300 (diffuse strong reactivity).

Results: All 184 breast carcinoma were negative for PAX8. Among the 145 gynecologic carcinomas, PAX8 expression (H-score > 10) was found in 33 of 39 endometrial endometrioid carcinomas, 14 of 16 endometrial non-endometrioid carcinomas (including 8/8 serous carcinomas, 3/3 clear cell carcinomas, 2/3 undifferentiated carcinomas, 1/1 mixed serous and clear cell carcinoma, and 0/1 MMMT), 20 of 37 endocervical carcinomas (including 0/4 adenoid basal, 9/15 endocervical, 6/9 endometrioid, 0/2 mesonephric, 2/3 serous, and 3/4 villoglandular variants), 12 of 14 ovarian serous carcinomas, 24 of 35 ovarian clear cell carcinomas, and 2 of 4 ovarian endometrioid carcinomas.

PAX 8 Expression in Gynecologic Tumors

Site and tumor type	% of (+) cases	Average H-score of (+) cases
Endometrial endometrioid	85	92
Endometrial non-endometrioid	88	96
Endocervical carcinoma	56	66
Ovarian clear cell	69	91
Ovarian endometrioid	50	90
Ovarian serous	86	86

Conclusions: PAX8 nuclear expression (even weak) in a CK7+/ER+/CK20 negative tumor supported a gynecologic primary tumor rather than a breast carcinoma. Among gynecologic tract tumors, PAX8 was more often positive in endometrial and ovarian tumors compared to cervical tumors (p=0.011). PAX8 expression was generally higher in endometrial tumors compared to cervical tumors (p=0.004). Unlike WT1 (specific for ovarian serous morphology), PAX8 expression was not specific to any morphology.

1157 Stage II Endometrial Carcinoma: Endocervical Gland Spread Is Not Reproducibly Distinguished from Endocervical Stromal Invasion.

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Background: FIGO stage is among the strongest predictors of survival for women with endometrial adenocarcinoma. For the past 20 years, cervical gland involvement and cervical stromal invasion have defined stage IIA and stage IIB disease respectively. In 2009, FIGO changed the criteria for stage II disease to include only those with cervical stromal invasion. Given the importance of this distinction, we wished to assess the ability of pathologists to distinguish various forms of cervical spread.

Design: A slide of endometrial adenocarcinoma paired with slides from the endocervix of 46 women with cervical spread of tumor were independently examined by 6 pathologists from 5 institutions, most of whom have a subspecialty interest in gynecologic pathology, with a range of 3 to 30 years of faculty experience. The pathologists were instructed to assess 5 features regarding the pattern of cervical spread according to their application of the FIGO rules in their routine practice. Fleiss' kappa statistic was used to examine the reproducibility of these assessments.

Results: The 6 pathologists agreed upon the various pattens of cervical spread statistically more often than by chance alone, but the frequency of disagreement was very high. While agreement on the presence of discontinuous spread was relatively good, agreement on all other patterns of spread varied from poor to fair.

Patterns of cervical spread - interobserver reproducibility

pattern of spread	range of pathologists' identification of feature	Kappa
gland involvement	63-91%	0.15
stromal invasion*	30-78%	0.28
vascular invasion only	0-9%	0.09
contiguous spread	56-80%	0.29
discontinuous spread	13-37%	0.49

*definition of FIGO stage II

Conclusions: The low level of diagnostic reproducibility among pathologists with an interest in gynecologic pathology to distinguish cervical gland spread from cervical stromal invasion of endometrial adenocarcinoma indicates that the current FIGO staging system needs modification. Specifically, depending upon the pathologist who examined the cases, from 30% of the women to 78% of the women would be diagnosed as having stage II endometrial adenocarcinoma. Either better criteria that distinguish stromal invasion from gland spread must be identified or the staging classification that relies on this distinction should be abandoned.

1158 Correlation of HB-EGF Paracrine and Autocrine with Advanced Stages of Malignant Mixed Mullerian Tumor.

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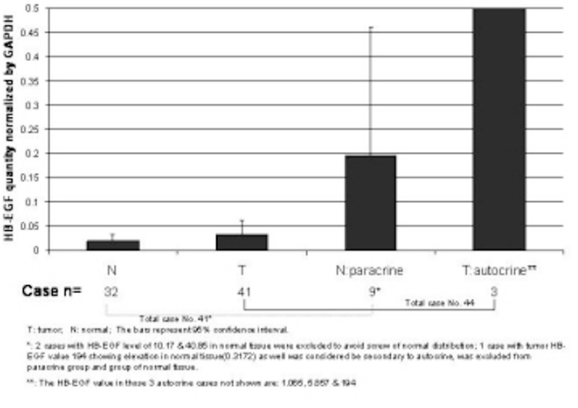
Background: HB-EGF(Heparin binding-epidermal growth factor like growth factor)is a secretary protein released by either target cells(autocrine) or cells nearby(paracrine) to promote epithelial-mesenchymal transition, tumor growth, invasion and metastasis. Malignant Mixed Mullerian tumor(MMMT) is a carcinoma with malignant mesenchymal transition. The aim of this study is to estimate HB-EGF secretion pattern in MMMT and how it is correlated to tumor staging.

Design: 44 cases of MMMT were selected including stage I (13), II(3), III(18), and IV(10). RNA was extracted from formalin fixed paraffin embedded blocks representing primary tumor and adjacent normal through macrodissection. HB-EGF expression was detected through RT-PCR and normalized by GAPDH(Glyceraldehyde 3-phosphate dehydrogenase).

Results: 1). Paracrine was defined as normalized HB-EGF ratio between normal and tumor higher than 25, e.g. N:T > 25. Although the absolute HB-EGF amount of adjacent normal was not defined in paracrine classification, the HB-EGF of normal tissue in paracrine (N:paracrine) showed an overall increase comparing with tumor(T) and normal(N) in non-autocrine, non-paracrine cases[Figure 1].

Fig 1. Quantitation of HB-EGF in normal, tumor, paracrine and autocrine



2). Autocrine was defined as normalized HB-EGF level more than 0.5 in tumor. This cutoff is higher than any 95% confidence interval of N, T and N:paracrine in [Figure 1].

3). By aforementioned criteria, the MMMT stage III/IV showed an increased percentile of combined autocrine and paracrine(39.3%) compared with MMMT stage I/II (12.5%), p=0.05. [Table 1]

Table 1. Correlation of HB-EGF expression with tumor stages

Tumor stages	Case No	Paracrine	Autocrine	Percentile of combined paracrine and autocrine
I & II	16	2	0	(2+0)/16 = 12.5%
III & IV	28	8	3	(8+3)/28 = 39.3%

Chi-Square test p=0.05

Conclusions: Paracrine and autocrine of HB-EGF contribute to the advanced stages of MMMT. The significant role of HB-EGF paracrine depicts the importance of tumor-microenvironmental interaction in tumor progression.

1159 Histopathologic Correlation Findings Associated with (Post-Hysterectomy) Vaginal Pap and HPV Test Results.

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Background: High risk (hr) HPV infection is recognized as the dominant etiology for cervical carcinoma. Data on prevalence of hrHPV DNA in patients with abnormal vaginal Pap results and the subsequent histopathologic findings are very limited. This is the largest study to date correlating histopathologic follow-up findings associated with (Post-Hysterectomy) vaginal Pap and hrHPV test results.

Design: A computer-based search from our Copath files was conducted over a study period of 49 months between July 1, 2005 and July 30, 2009 to identify vaginal ThinPrep Pap test (TPPT) cytology cases reported as ASC-US, ASC-H, LSIL, or HSIL for which Hybrid Capture 2 (HC2) hrHPV DNA test results were also reported. Vaginal Pap and HPV test results and histopathologic follow-up outcomes were analyzed.

Results: During the study period there were 1320 vaginal Pap tests reported as ASC-US, ASC-H, LSIL, or HSIL which also had HPV testing. The prevalence of hrHPV infection in women with abnormal vaginal Paps is shown in Table 1. The average age of patients with vaginal ASC-US results was 56.5 (17-91), with ASC-H 58.9 (22-87), with LSIL 56.9 (27-92), and with HSIL 63.4 (42-93). 86 women with vaginal LSIL, ASC-H, HSIL cytology and hrHPV DNA testing had at least one follow-up biopsy. 373 women with vaginal ASC-US and HPV test results had cytologic and/or biopsy follow up results. The follow-up results are shown in Table 2.

HPV results associated with abnormal vaginal Paps

	#Case	#Positive	%
ASC-US	1125	244	22
ASC-H	36	21	58
LSIL	148	113	76
HSIL	11	9	82
Total	1320	387	29

	HPV Pos F/U#	VAIN2/3 (%)	VAIN1 (%)	HPV Neg F/U#	VAIN2/3 (%)	VAIN1 (%)
ASC-US	138	7 (5)	59 (43)	235	1 (0.4)	10 (4)
ASC-H	16	1 (6)	14 (88)	8	1 (13)	2 (25)
LSIL	48	7 (15)	34 (71)	11	0 (0)	7 (64)
HSIL	2	2 (100)	0	1	1 (100)	0 (0)
Total	204	17 (8)	107 (53)	255	3 (1)	19 (8)

Conclusions: The prevalence of hrHPV detection in abnormal vaginal Pap tests reporting ASC-US, ASC-H, LSIL and HSIL results are similar to those reported in abnormal cervical Pap test specimens from older women.

Histopathologic diagnoses of VAIN1 and VAIN2/3 were significantly increased following hrHPV positive abnormal vaginal Pap tests when compared to follow-up findings for patients with hrHPV negative abnormal vaginal Pap tests.

Reflex hrHPV DNA testing is a useful tool for assessing risk of histopathologic VAIN and in considering follow-up management options for women with abnormal vaginal Pap test results.

Head & Neck

1160 Rearrangement of the EWSR1 Gene Is a Consistent Feature in Hyalinizing Clear Cell Carcinoma of Salivary Gland.

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Background: Hyalinizing clear cell carcinoma (HCCC) is a low grade salivary gland tumor with a characteristic nested and cordlike growth within hyalinized stroma. HCCC typically stains with squamous cell markers and shows occasional mucous cells making distinction from mucoepidermoid carcinoma (MEC) a challenge. We have observed that the characteristic clear cell nests of HCCC resemble those seen in soft tissue myoepithelial tumors (SMET). Up to 50% of MECs show a MAML2 gene rearrangement, while SMETs show EWSR1 rearrangement in 45% of cases. This has not been studied in HCCC to examine a possible link between these entities.

Design: 23 cases of HCCC with typical morphologic and immunohistochemical features were collected. FISH for EWSR1 and MAML2 were performed using custom BAC probes and 200 cells were scored per case. A case was called positive when ≥20% of cells had a break-apart signal.

Results: The 23 cases included 14 females & 9 males ranging from 25-87 yrs of age (avg. 59.4). Sites were 18 oral, 3 parotid, 1 nasal & 1 larynx. All cases showed nests and cords of clear cells in a hyalinized stroma. Follow up in 19 cases ranging from 2 to 195 mths (avg. 47.6) demonstrated 4 recurrences (21%). The remainder showed no evidence of disease (NED). There were no metastases or mortality in any case. Mucin was seen in 10 of 23 cases (44%) and varied from focal to diffuse. The tumors were positive for 34βE12 (16/17), p63 (19/20) and EMA (9/12). They were negative for S100, SMA and calponin. FISH showed a EWSR1 rearrangement in 18 of 22 cases (82%), while no MAML2 break-apart was detected in any of the cases (0/14), including all mucin containing tumors (0/7). No EWSR1 abnormality was present in any of the control cases tested, including 3 MEC with clear cells and 5 epithelial-myoepithelial carcinomas.

Conclusions: HCCC is a unique tumor entity that shares EWSR1 rearrangement with SMET, despite S100, SMA and calponin negativity. It is distinct from MEC, despite common mucinous differentiation. This critical distinction impacts on grading, since all mucin positive HCCC cases showed NED and would have been over graded (grade III) with conventional MEC grading schemes. FISH analysis for EWSR1 rearrangement can be used as a reliable tool when confronted with limited material or a challenging diagnosis.

1161 Differentiated Dysplasia Is a Frequent Precursor or Associated Lesion in Invasive Squamous Cell Carcinoma (SCC) of the Oropharynx.

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Background: The recognition of the spectrum of precursor lesions of oropharyngeal SCC, their classification and their grading have remainded controversial over the last decades. This contrasts to vulvar cancer precursor lesions which are related to either HPV or chronic inflammation and can manifest – under the VIN paradigm (VIN I-III) or as differentiated dysplasia (dVIN), respectively. Oropharyngeal SCC precursor lesions are etiologically more variable, being related to smoking, HPV or chronic inflammation, and the spectrum of lesions may thus admittedly be wider, but still no clinically useful international consensus exists on histological types of precursor lesions and on the significance of individual types.

Design: We reviewed all available histological slides of patients with oropharyngeal biopsies (excluding the tonsil) and subsequent resection specimens with SCC on file in the archives of Department of Pathology of our hospital from 1992 until 2009.

Results: Five basic patterns of precursor lesions or SSC associated lesions were identified: **Pleomorphic** similar to full thickness severe laryngeal squamous dysplasia (24/155), **basaloid** stratified similar to anal basaloid dysplasia of AIN III (6/155), **differentiated** similar to dVIN or lichenoid lesions with large cells with large nuclei and prominent eosinophilic nucleoli, abundant eosinophilic cytoplasm and promentent desmosomes, with additional minimal basal cell layer or suprabasal cell irregularities (63/155), **mixed differentiating** pleomorphic with superficial maturation (43/155) as well as **verrucous** with open often raisin like nuclei without prominent nucleoli (11/155). Keratinization was a common but variable feature in differentiated, mixed differentiating and verrucous dysplasia. In 8/155 no precusor lesion could be identified. Progression of isolated differentiated dysplasia was documented in 13% of patients (21/155) over variable time periods ranging from months to years.

Conclusions: Full thickness epithelial dysplasia of either pleomorphic or basaloid type is present in only 20% of oropharyngeal SCC. Differentiated dysplasia is a frequent precursor or associated in situ lesion in oropharyngeal SCC. Failure to recognise differentiated dysplasia results in underdiagnosis of a sizable proportion of patients at risk for invasive carcinoma. Our cases of documented progression of differentiated dysplasia call for efforts to refine criteria for separation of differentiated dysplasia from morphologically related lichenoid lesions.

1162 The Role of Postoperative Radiotherapy in the Management of Parotid Pleomorphic Salivary Adenomas: Is There Any Benefit?

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Background: Introduction: Pleomorphic Salivary Adenoma is the most common tumour of the Parotid Gland. Currently no national management guidelines exist. The objective of this study was to evaluate the role of adjuvant radiotherapy.

Design: A retrospective study of all patients with a histological diagnosis of PSA between 1981 and 2008 in Greater Glasgow and Clyde was undertaken. From intra- operative notes and pathology reports, adherence to facial nerve, excision margins, capsule status and postoperative radiotherapy were analysed. Two cohort groups were identified. The first cohort underwent surgery alone while the second received postoperative radiotherapy. Post-operative recurrence, short and long- term complications were compared in the two groups.

Results: 201 patients were identified. 167 (83%) had surgery alone and 34(17%) received adjuvant radiotherapy. Medical notes were retrievable in all patients receiving postoperative radiotherapy and in only 58 surgical patients .The rate of recurrence was 1.7% (1/58) in surgical patients and 2.9% (1/ 34) in patients receiving adjuvant radiotherapy. Short -term complications were significantly higher in the second cohort accounting for 100% compared to 38% in the first. While long- term complications 15/58 (25%) and 12/34 (32%) were observed in the first and second cohort respectively.

Conclusions: There was no significant difference in the recurrence rate between the two groups. Short term and long term complications were significantly higher in the postoperative radiotherapy cohort. Adjuvant radiotherapy is therefore not recommended in the treatment of PSA. As well as a higher long term complication rate, radiotherapy is less cost effective.

1163 Perivascular Epithelioid Cell Neoplasms of the Head and Neck: Report of 3 Cases.

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Background: Perivascular epithelioid cell tumors (PEComa) are a family of related mesenchymal tumors including angiomyolipoma, lymphangiomyomatosis, lung clear cell sugar tumor and rare clear cell tumors in visceral and soft tissue sites. PEComas share overlapping histology, immunohistochemistry, and ultrastructure. They are characterized by a female preponderance, coexpression of melanocytic and smooth muscle markers and an intimate relationship with blood vessels. The growing interest in PEComas has led to increasing reports of these tumors in unusual locations. We describe a series of PEComas arising in the head and neck of 3 female patients and discuss the behavior of these distinctive tumors.

Design: H&E slides from 3 cases of head and neck PEComa from the consultation files of the authors were reviewed. All 3 cases were stained with melanocytic markers (HMB-45/Melan-A), S-100, muscle markers, vimentin, synaptophysin and pancytokeratin. Follow-up information was obtained from 2 cases.

Results: All patients were adult women with a wide age distribution (18, 26 and 71 years). 2 arose in the nasal cavity and 1 in the larynx. The signs and symptoms of PEComa of the larynx and the nasal cavity revealed no specificity and clinically the