(28S, 18S, and 5.8S). To control for overall ploidy, 3 different single copy reference genes were used for normalization (*HBB*, *PTGS2*, and *STK19*). Copy numbers were determined using the using the relative standard curve method. Genomic DNA was isolated from frozen tissue sections of radical prostatectomy specimens from n = 21 patients (Gleason scores 6-9). Relative normalized target gene quantity in tumor DNA was determined from the standard curves and divided by the relative normalized target gene quantity of the calibrator, which consisted of DNA from each patient's matched normal prostate tissue.

Results: The median ratio of rDNA copies in tumor vs. normal was 1.45 for 5.8S, 1.43 for 18S, and 1.56 for 28S. The difference between these values and the expected tumor/normal ratio of 1.0 (null hypothesis) was significant for each target (5.8S p=0.0055, 18S p=0.0072, 28S p=0.0129; Wilcoxon Sign Rank test). Overall tumor/normal ratios were increased in 16 of the 21 cases. There was a high level of concordance in median relative copy number (tumor/normal) using different reference genes (e.g. *HBB*, *PTGS2*, *RP1*) with the same target (e.g. 18S rDNA), and using the different target genes in the locus (e.g. for a given patient if 5.8S rDNA was amplified then the 18S and 28S genes were also amplified).

Conclusions: In the majority of cases of prostate cancer there was an increase in copy number of the entire rDNA locus (relative increase ~1.5 fold). Since there are approximately 400 copies of this repeat unit in normal cells, these increases are on the order of hundreds of extra copies. These results raise the possibility that rDNA gene copy number may: i) influence nucleolar size expansion in cancer cells, and/or ii) play an important role in the development and/or progression prostate cancer.

1035 The Study of Xp11 Translocation Renal Cell Carcinoma (RCC) in Adults by TMA, IHC and FISH

M Zhong, L Osborne, MJ Merino, M Hameed, S Aisner. University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark; National Cancer Institute, Bethesda; Memorial Sloan-Kettering Cancer Center, New York.

Background: Xp11 translocation renal cell carcinomas (RCC), a distinctive entity in the 2004 WHO renal tumor classification, are rare neoplasms and are often encountered in the pediatric and young adult population. The incidence is probably underestimated partly due to unavailable genetic studies and overlapping histopathological morphology with clear cell and papillary RCC. TFE3 immunohistochemical assay has been used for the diagnosis of the Xp11.2 translocation RCC. However, for a definitive diagnosis, one has to rely on identifying the translocation by genetic and molecular studies.

Design: A total of 120 consecutive adult (>18Y) RCC patient specimens (FFPE during the period 2001-2008) from our institute, were collected to construct a tissue micro array(TMA). IHC was performed on TMA using TFE3 antibody. The 2nd TMA was constructed from all the TFE3 positive specimens, 5 TFE3 negative and 5 TFE3 weak positive specimens. A dual-color, break-apart FISH assay, which can detect the gene rearrangement at Xp11 region, was applied to the 2nd TMA.

Results: Among the 120 RCC, 11(9.2%) cases were TFE3 positive . Among these 11 cases, FISH assay showed split signal, which confirmed chromosome translocations involving *TFE3*, in 5(4.2%) cases; fusion signal in 1 case; non-conclusive result in the rest 5 cases(most likely due to the ages of the blocks); no split signal in all tested TFE3 negative and weak positive cases. Further more, no *TFE3* fusion transcripts (ASPL, PRCC, CLTC, PSF and Nono), but *TFE3* wide type were detected by RT-PCR in the tested case with weak TFE3 nuclear stain.

Conclusions: 1) Xp11.2 translocation RCC is not an uncommon neoplasm. The incidence in this group of 120 RCC patients is at least 4.2% in adults (confirmed by FISH). 2) TFE3 IHC is a relatively sensitive and specific assay for the diagnosis Xp11.2 translocation RCC. The strong nuclear TFE3 stain is most likely indicative of Xp11.2 translocation. The weaker nuclear stain appears to be due to expression of full length TFE3 protein, rather than chimeric fusion protein due to translocation. 3) FISH assay is the most reliable method to detect Xp11 translocation RCC, when only FFPE material is available.

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1036 Inter-Observer Agreement among Pathologists for Assessing Invasion in Early Vulvar Squamous Cell Carcinoma: Still a Diagnostic Challenge

A Abdel-Mesih, D Daya, K Onuma, N Akhtar-Danesh, O Boutross-Tadross, K Ceballos, W Chapman, T Colgan, P Deb, MR Nucci, E Oliva, M Sur, S Tang, A Lytwyn. McMaster University, Hamilton, ON, Canada; University of British Columbia, Vancouver, BC, Canada; University of Toronto, Toronto, ON, Canada; Harvard University, Boston, MA.

Background: Despite published criteria, measuring depth of invasion to identify early squamous cell carcinoma of the vulva remains difficult, yet critical for surgical management. Tumours with ≤1mm depth of invasion have minimal risk of nodal involvement, and therefore surgery is limited to local excision without lymphadenectomy. Interobserver agreement for determining the presence of invasion and its measurement has not been studied. Our aim was to assess agreement among pathologists for (1) determining whether invasion is present, and (2) measuring the depth of invasion.

Design: We searched the pathology database from 2000-2008, and identified 72 vulvar squamous cell carcinomas diagnosed as showing up to 5mm depth of invasion. From these, 45 slides were selected for this study, with preferential selection of challenging examples. Eleven gynecologic pathologists, from both Canadian and American institutions, independently reviewed the slides. Each slide was categorized as: (1) not invasive, (2) invasive with depth of invasion ≤1 mm, (3) invasive >1 mm, (4) invasive but depth cannot be measured, or (5) cannot determine if invasive or not invasive.

Results: There was only fair agreement (mean κ =0.24) among pathologists for diagnosing a vulvar carcinoma as being invasive. Of the 45 cases, only 13 (29%) were unanimously diagnosed by all 11 pathologists as being invasive. There was not a single case where all 11 pathologists agreed that there was no invasion. In 32 cases (71%), the pathologists did not unanimously agree on the presence or absence of invasion. Mean agreement for depth of invasion was only moderate (mean κ =0.50), and ranged from poor (κ= 0.12) to excellent (κ =0.92).

Conclusions: There was only fair agreement among gynecologic pathologists in determining the presence of invasion in vulvar carcinoma. In cases where pathologists agreed there was invasion, agreement on depth was only moderate. The fair to moderate agreement in this study may, in part, be due to the preferential selection of a larger proportion of diagnostically challenging cases. This study highlights that assessing whether or not there is invasion, and ascertaining the critical ≤ 1mm depth, still poses a diagnostic challenge for pathologists.

1037 Endometrial Adenosarcomas: Diagnostic Use of Ki-67 Proliferation Marker as an Adjunct to Morphologic Diagnosis

N Aggarwal, R Bhargava, E Elishaev. University of Pittsburgh School of Medicine, Pittsburgh, PA.

Background: Endometrial adenosarcomas (AS) are rare biphasic neoplasms of the female genital tract containing benign glands and a low grade sarcomatous mesenchymal component. Diagnostic criteria include increased stromal cellularity in periglandular distribution (periglandular stromal cuffing) accompanied by variable degrees of cytologic atypia and mitotic activity. When not all the criteria are fulfilled a diagnosis of benign lesions are given and AS is considered in retrospect upon recurrence of the tumor.

Design: Eight cases of AS were identified at Magee-Womens Hospital between 2004 and 2008 and were compared to 15 cases of endometrial polyps (EP) and 14 cases of atypical polypoid adenomyomas (APA). Pertinent 1-3 blocks/case were selected and stained for Ki-67 proliferation marker, caldesmon, smooth muscle actin (SMA), desmin, and CD10 with appropriate negative and positive controls.

Results: All cases of AS had a polypoid growth pattern with round or slit-like glands with or without papillary projections into gland lumen. Increased periglandular stromal cellularity was observed focally or diffusely in all cases (8/8) of adenosarcoma, which also demonstrated variable degrees of stromal nuclear atypia. The mitotic activity ranged from 1/10 high power fields (HPF) to 15/10HPF. All cases of AS demonstrated distinct increase in Ki-67 positive nuclei in the peri-glandular zone compared to adjacent stroma, regardless of the mitotic count. The average Ki-67 labeling index in peri-glandular zones of AS was 20 % compared to less than 5 % in adjacent stroma. This zonation was not observed in any case of APA or EP all of which showed scattered positive cells with less than 5% Ki 67 proliferation index. The AS stroma was positive for CD10, with variable focal positive staining for SMA and desmin, and was negative for caldesmon. APA showed positivity for all muscle markers with SMA being the most consistent and diffuse. EPs were negative for caldesmon, variably positive for desmin and SMA and diffusely positive for CD10.

Conclusions: Distinct increase in peri-glandular Ki-67 staining pattern is very helpful and Ki 67 should be considered as an adjunct to the routine morphologic diagnosis of AS. This could be especially useful in curettage specimens and other challenging lesions that lack some of the classic criteria of an AS.

1038 New Antibodies for Clear Cell Carcinoma (Pax-8, HNF-α, vHL) Are Also Positive in Arias-Stella Reaction

CE Aguilar, E Silva. Cedars-Sinai Medical Center, Los Angeles, CA.

Background: Recent studies have shown clear cell carcinoma of müllerian origin to be positive for Pax-8, hepatocyte nuclear factor- α (HNF-α), and von Hippel Lindau (vHL). Clear cell carcinoma of müllerian origin can present a diagnostic challenge in endometrial specimens showing areas of Arias-Stella. Therefore, Pax-8, HNF-α, vHL were applied to products of conception showing areas of Arias-Stella reaction.

Design: Ten products of conception specimens showing Arias-Stella reaction and ten cases of clear cell carcinoma were stained with Pax-8, HNF-α, vHL.

Results: In all ten cases, Arias-Stella cells and secretory glands displayed Pax-8 and HNF-α nuclear positivity and vHL cytoplasmic staining. All three immunostains were positive in each case of clear cell carcinoma with the expected pattern of staining.

Conclusions: Clear cell carcinoma antibodies, Pax-8, HNF-α, and vHL, are positive in Arias-Stella reaction. These antibodies therefore cannot be applied to endometrial specimens in which the differential diagnosis includes clear cell carcinoma and Arias-Stella reaction. Future immunohistochemical studies to determine whether other molecular targets involved in the clear cell carcinoma pathway(s) can also be detected in Arias-Stella reaction would be useful in this unusual but challenging and important distinction.

1039 Prognostic Factors of Adenocarcinoma of the Endocervix: Pattern of Invasion vs Depth of Invasion

I Aguilera-Barrantes, EG Silva. M.D.Anderson Cancer Center, Houston, TX.

Background: The treatment of endocervical adenocarcinoma (EA) depends primarily on the depth of invasion. The grade of the tumor is typically considered a less important prognostic factor and the pattern of invasion has not been evaluated. The purpose of our study is to evaluate whether these features impact prognosis.

Design: We reviewed 43 cases of invasive adenocarcinoma of the endocervix seen at our institution. For each case 1 to 49 slides (median 9) of cone/ LEEP and/or hysterectomy specimens were available for review. Follow up (FU) was obtained in all cases. These cases were divided in two groups. One group with pushing border (PB) of invasion or PB with focal infiltration (defined as 1 or 2 foci of infiltration) and the other group with diffuse infiltration (DI) of the stroma. Tumors were graded observing only architectural features and were classified as well differentiated (WD) if composed only of glands, moderated differentiated (MD) composed of glands and solid tumor and poorly differentiated (PD) predominantly solid.

Results: Patient ages ranged 25 to 58 (mean 39). The follow up for those who survived ranged from 37 to 250 months (median 94 months). A summary of the results for all patients is in table 1.

Table 1

Pattern of invasion	n	Depth mm	Grade	LVI	LN Mets	FU	TX
PB	22	1 to 14 (median 5.5)	21/22 WD; 1/21 MD	1/22	0/15	22/22 NED	5/22
DI	21	2 to 14 (median 5)	10/21 WD; 8/21 MD; 3/21 PD	13/21	6/18	8/21 DOD; 2/21 AWD; 11/21 NED	11/21
P value			0.0006 (WD)	<0.0001	0.0213	0.0002 (NED)	

LVI: Lymphovascular invasion; LN Mets: lymph node metastasis (on cases with lymph node dissection); Depth: Depth of invasion; TX: Adjuvant treatment after surgery; NED: no evidence of disease; AWD: Alive with disease; DOD: died of disease; P value: calculated with Fisher's exact test

Within the group with pushing margin there is a subgroup (4 cases) with "canalicular" pattern that appears to have excellent prognosis (NED with a FU 86 to 232m) in spite of deep invasion (5 to 14mm invasion).

Conclusions: The pattern of invasion should be considered a very important prognostic indicator in adenocarcinoma of the cervix. In this study it was a better prognostic indicator than depth of invasion. Tumor grade also correlates well with prognosis. LVI is more frequently seen in cases with DI. The significance of classical histological parameters should be re-evaluated in the decision making regarding the treatment of these frequently young patients.

1040 Loss of E-Cadherin Is a Shared Feature of Both Sarcomatous and Undifferentiated Components of Uterine Carcinosarcomas and Dedifferentiated Carcinomas

OM Al-Agha, K Garg, LJ Tafe, D Huntsman, RA Soslow. British Columbia Cancer Agency, Vancouver, BC, Canada; Memorial Sloan-Kettering Cancer Center, New York, NY.

Background: Uterine carcinosarcomas can resemble dedifferentiated endometrial carcinomas (i.e. combined differentiated and undifferentiated endometrial carcinomas). The distinction between carcinosarcoma and dedifferentiated carcinoma essentially depends on the presence of a sarcomatous component in the former even when undifferentiated foci are present in carcinosarcoma. We sought to study mechanisms responsible for mesenchymal differentiation in carcinosarcoma and loss of overt epithelial differentiation in dedifferentiated carcinomas. We hypothesized that loss of expression of the cell adhesion molecule, E-cadherin, would be a shared feature of the undifferentiated regions of these tumors and the sarcomatous regions of carcinosarcomas.

Design: The study comprised of 17 cases of carcinosarcoma and 14 endometrial carcinomas with undifferentiated components (either dedifferentiated or pure undifferentiated carcinomas). E-cadherin expression was examined by immunohistochemistry using an avidin conjugated monoclonal anti-E-cadherin antibody (DAKO, 1:125 dilution).

Results: E-cadherin expression was absent in undifferentiated and sarcomatous regions of 15 of 17 carcinosarcomas (88%) and 12 of 14 de-/undifferentiated carcinomas (86%). E-cadherin was strongly expressed in the carcinomatous components of all 17 carcinosarcomas and in the well-differentiated regions present in 6 of 14 dedifferentiated carcinomas.

Conclusions: Loss of E-cadherin might be linked to alteration of cell morphology from epithelial to sarcomatous and undifferentiated phenotypes in carcinosarcoma and from differentiated to undifferentiated phenotypes in dedifferentiated endometrial carcinoma. The common loss of E-cadherin between the two tumors may also explain their highly aggressive clinical behavior. The mechanisms of E-cadherin loss in these tumors remain unknown, but overt cellular dyshesion in undifferentiated carcinoma and preservation of adhesion in carcinosarcoma suggest that different mechanisms underlie E-cadherin loss in these tumors.

1041 Variable Pathogenesis of Clear Cell Carcinoma of the Mullerian System Based on Nuclear Morphology and Their Association with Endometriosis: An Immunohistochemical Analysis

A Alduaij, MR Quddus, C Zhang, K Hansen, M Lomme, WD Lawrence. Women & Infants Hospital and The Alpert Medical School of Brown University, Providence, RI.

Background: Clear cell carcinoma (CCC) arising in endometriosis or associated with endometrioid carcinoma often has a favorable prognosis, compared CCC associated with serous carcinomas. Although by convention, all clear cell carcinomas of the mullerian system are regarded as high grade tumors. The pathogenesis of various types of CCCs may be different. Morphologically the tumor cells of CCCs typically show low- and/or high-grade nuclear grade (LNG & HNG) features. We have categorized pure clear cell carcinomas into LNGs and HNGs and investigated their pathogenesis and correlated them with associated endometriosis, if any.

Design: 45 pure mullerian CCCs (18 endometrial and 27 ovarian) were retrieved after IRB approval and H&E slides were reviewed to assign nuclear morphologic grading of the tumor cells, e.g., LNGs, HNGs grade and mixed (MNG). CCC with greater than 80% of one type of nuclei was assigned that particular type. 4 micron sections were cut and p53 and Ki67 IHC were run using automated Dako Immunostainer. Positive and negative control slides were run with each batch. The nuclear grading of CCCs was correlated with p53 and ki67 expression and also with endometriosis.

Results: Morphologically 19 (42%) CCCs had predominant LNG, 20 (44%) HNG and 6 (13%) MNG. 7 (37%) LNG CCCs, 5 (25%) HNG CCCs and 3 (50%) MNG CCCs were associated with endometriosis. p53 and Ki67 IHC were done on 17 (10 ovarian and 7 endometrial). 7 of 10 (70%) ovarian CCCs and 5 of 7 (71%) endometrial cases showed p53 alteration. Of the 7 ovarian cases, 3 were LNG, 2 HNG and 2 MNG and among the 5 endometrial CCCs, 1 LNG, 3 HNG and 1 MNG tumors. Endometriosis was present in 2 of 3 (66%) LNG and 2 of 4 (50%) HNG/MNG ovarian CCCs. In endometrial CCCs 2 of 3 (66%) HNG p53 altered cases had associated endometriosis elsewhere. Ki67 was positive in all 17 cases.

Table 1: Summary of findings

	LNG		MNG		MNG	
	Endomet +	Endomet -	Endomet +	Endomet -	Endomet +	Endomet -
p53+ (N=10)	2	1	3	2	1	1

Endomet: endometriosis

Conclusions: Morphologically LNG ovarian CCCs arising in endometriosis show p53 alteration in a significant percentage of cases. Similarly endometrial CCCs with HNG are more frequently associated with underlying p53 alteration. Our study reveals that mullerian CCCs have variable pathogenesis irrespective of its nuclear grade and association with endometriosis.

1042 FISH for Diagnosis of Endometrial Stromal Sarcoma

R Ali, CH Lee, A Zayed, A Dastranj, A Elomami, E Mehl, J Senz, CB Gilks, D Huntsman. Vancouver General Hospital, Vancouver, BC, Canada; BC Cancer Agency, Vancouver, BC, Canada; Genetic Pathology Evaluation Centre (GPEC), Vancouver, BC, Canada; Tabriz University of Medical Science, Tabriz, Islamic Republic of Iran.

Background: Endometrial stromal sarcoma (ESS) account for 20% of all uterine sarcomas. t(7;17) (p15;q21) that results in the fusion gene JAZF1-JJAZ1 represents the most common type of translocations in ESS. This rearrangement has been demonstrated in about 50% of ESS studied to date and it has not been found in other uterine sarcomas. More recently, other types of translocations have been reported in ESS including JAZF1-PHF1 and EPC1-PHF1.

Design: The aim of our study is to determine the frequency of JAZF1-JJAZ1, JAZF1-PHF1 and EPC1-PHF1 in ESS and morphologically similar tumors by fluorescence in situ hybridization (FISH). A total of 58 formalin-fixed paraffin-embedded tissue samples were studied by FISH. These included 23 ESS, 6 undifferentiated endometrial sarcomas (UES), 16 adenosarcomas, 2 malignant mixed Mullerian tumors (MMMT), 2 uterine tumors resembling ovarian sex cord tumors (UTROSCT), 1 cellular leiomyoma, 1 leiomyosarcoma, and 7 polypoid endometriosis. For FISH, a combination of break-apart and fusion probe sets was used to interrogate for the presence of the different gene rearrangements/fusions involving JAZF1, JJAZ1, EPC1, PHF1.

Results: Of the 21 cases of ESS with interpretable FISH results, a total of 13 (62%) ESS showed evidence for gene rearrangements, with 7 (33%) cases showing JAZF1/JJAZ1 fusion, 3 (14%) cases showing JAZF1/PHF1 fusion, and 1 case showing EPC1/PHF1 fusion. In addition, one ESS demonstrated only JAZF1 rearrangement and another demonstrated only PHF1 rearrangement, with no fusion partner identified by the current probe sets. No rearrangements were present in the 8 remaining ESS and other non-ESS cases examined.

Conclusions: Our study provides further confirmation for the specificity of these genetic rearrangement events for ESS and shows that FISH assays with multiple combination probe sets for JAZF1, JJAZ1, EPC1, and PHF1 can achieve increased sensitivity (62%) for the detection of ESS. However, further studies to fully characterize the genetic spectrum of ESS will be necessary to improve the sensitivity of these ancillary diagnostic methods.

1043 A 10 Year Review of Primary Fallopian Tube Carcinoma (PFTC) at an Oncology Hospital. A Clinicopathological Study of 58 Cases with Evaluation of Staging and Prognostic Factors

I Alvarado-Cabrero, G Picon-Coronel, FO Quijano-Castro. Mexican Oncology Hospital, Mexico, DF, Mexico.

Background: PFTC represents only 0.3-1.9% of all gynecological tumors, this relative low number of patients do not permit any conclusive statement with regard to the prognostic value of factors affecting PFTC. On the other hand, the current staging system of the FIGO for PFTC does not consider two subsets of tubal carcinoma: non-invasive intraluminal carcinoma and carcinoma confined to the fimbriated end of the tube. To accommodate those subsets of tumors, a modification of the FIGO staging system was proposed by Alvarado-Cabrero et al (Gyn Oncol, 1999). The goals of this study were to assess the association between the "modified staging system" (**mss**) and survival and stage and carcinoma subtype.

Design: Patients diagnosed with PFTC between 1999-2009 were identified from the files of our Institution. Clinical and follow-up information was retrieved from patient´s (pts) charts. The tumors were staged in accordance with the **mss**. Tumor grade according with the FIGO-grading system and carcinoma subtype were also recorded. Statistical comparison was made using the chi-square test or Fisher´s Exact test.

Results: Pts ranged in age from 46 to 89 years (median:62): The most common complaints were abnormal vaginal bleeding and abdominal pain (> 80%). The symptom complex of hidrops tubae profluens was found in 8(13.7%) pts. Fourteen(24%) pts also had breast cancer. The PFTC were staged as follows: Stage IA-0:9, Stage IA-1:8, Stage IA-2:11, Stage IB-0:2, Stage IC: 20, Stage IF:2, Stage II: 2, Stage III: 2, and Stage IV: 2. There was a statistically significant difference in length of survival between pts with Stage I(A,B) -0 and 1 and those with Stage IA-2 tumors, 96.5 months vs 60.2 months, respectively (P: 0.006). The cell types were as follows: serous 41%, endometrioid 31%, transitional 10%, clear cell 8.6% and mucinous 5%. Only 2 carcinomas were undifferentiated and they were limited to the fimbrial end of the tube. Most serous carcinomas (85%) were associated with stage IC or higher, high grade and poor outcome. In contrast, 78.5% of patients with endometrioid carcinoma were low grade and associated with Stage I (A,B)-0 and 1 and with prolonged survival. (P:<0.0001)

Conclusions: Substaging Stage IA tumors into IA-1 and IA-2 indicates a decreasing survival with increasing depth of invasion Serous Carcinomas of the fallopian tube are associated with high grade, higher stage of the disease and poor prognosis.

1044 *JAZF1* and *JJAZ1* Gene Fusion in Primary Extrauterine Endometrial Stromal Sarcoma

C Amador-Ortiz, A Roma, XZ Zhu, N Becker, A Chiesa, JD Pfeifer. Washington University, St Louis, MO; Cleveland Clinic, Cleveland, OH.

Background: Endometrial stromal sarcoma (ESS) predominantly occurs as a primary tumor of the uterus. The most common cytogenetic abnormality in ESS is t(7;17)(p15;q21), which occurs in 37-80% of cases and results in a *JAZF1-JJAZ1* gene fusion. Rare cases of primary extrauterine ESS have been reported, but it remains uncertain whether the genetic features of uterine ESS are also characteristic of extrauterine ESS. The present study evaluates the prevalence of the t(7;17) and *JAZF1-JJAZ1* gene fusion in a series of primary extrauterine ESS by both reverse transcriptase polymerase chain reaction (RT-PCR) and fluorescence in situ hybridization (FISH).

Design: Cases of primary extrauterine ESS were identified from departmental files. Conventional nested RT-PCR was performed on RNA extracted from paraffin embedded tumor tissue using primers complementary to sense and antisense *JAZF1* and *JJAZ1* sequences; RNA integrity was confirmed by RT-PCR for *GAPDH*. Interphase FISH was performed to detect t(7;17)(p15;q21) using a break-apart strategy for both *JAZF1* and *JJAZ1*; for each case, 200 nuclei were analyzed. Cases of uterine ESS with known translocation status served as positive and negative controls for RT-PCR and FISH.

Results: The series of extrauterine ESS included three cases arising in the ovary and three arising in the abdominopelvic cavity unassociated with the gynecologic tract. All cases had morphologic features of conventional low grade ESS; a focal sex cord-like pattern was present in one case. None of the cases had evidence of intrauterine pathology. *JAZF1-JJAZ1* fusion transcripts were not detected by RT-PCR in any of the cases of extrauterine ESS, although all contained amplifiable RNA. None of the cases showed evidence of a rearrangement involving either *JAZF1* or *JJAZ1* by interphase FISH. Expected results of RT-PCR and FISH assays were observed in all controls.

Conclusions: Our findings demonstrate that the t(7;17)(p15;q21) and associated *JAZF1-JJAZ1* fusion transcripts are not universally present in primary extrauterine ESS. When our results are pooled with published case reports of primary extrauterine ESS, the t(7;17) and/or *JAZF1-JJAZ1* are present in 25% of cases, a prevalence somewhat below that reported for uterine ESS. While molecular testing for the t(7;17) and associated gene fusion may be useful for confirming primary extrauterine ESS, the low prevalence of the genetic aberration limits the clinical utility of the testing.

1045 Race Is an Independent Risk Factor for Poor Survival among Women with Late Stage, Type II Endometrial Cancers

S Bandyopadhyay, ML Cote, DS Schultz, AR Gaba, M Elshaikh, M Ibrahim, A Munkarah, R Ali-Fehmi. Wayne State Univ, Detroit; Karmanos Cancer Institute, Detroit; Henry Ford Hospital, Detroit.

Background: Studies have divided endometrial carcinoma into Type I (endometrioid) and Type II (serous and clear cell) based on epidemiologic and prognostic differences, and it has been noted that African American (AA) women are more likely to have Type II cancers. Clinically, FIGO III endometrioid carcinoma is aggressive with outcomes similar to Type II carcinomas. In our study, we analyzed clinical-pathologic parameters and survival outcome of FIGO III endometrioid to those of Type II in Caucasian (C) and AA patients from two large hospitals in Detroit.

Design: All cases (slides and reports) of high grade endometrial carcinoma from 1988-2008 were retrieved from the pathology databases of two urban hospitals. There were 196 C and 222 AA women (total of 418 patients). Age, stage, depth of invasion, angiolymphatic invasion and survival were recorded. Univariate and multivariate models were used to test for survival differences by tumor type and to assess the impact of race.

Results: In univariate analyses, stage, angiovascular invasion and depth of myometrial invasion were significant predictors of survival. Race and type (I/II) were not associated with survival in univariate models (P=0.06 and 0.24, respectively). In multivariate models, stage, race and age at diagnosis were significantly associated with survival (Table 1). Also, AA women with Type II, late stage (III and IV) cancers had a greater risk of death than C women with Type II, late stage cancers, (HR=1.74, 95% CI=1.06-2.84, p-value=0.03) after adjusting for other variables.

Table 1: Cox Regression Model for Endometrial Cancer Cases

	Hazard Ratio (95% CI), p-value
Type	1.27 (0.92-1.75), 0.15
Stage	2.39 (1.70-3.37),<0.0001
Angiovascular invasion	1.36 (0.98-1.89),0.07
Depth of Invasion	1.15 (1.00-1.32), 0.05
Race	1.43 (1.06-1.92), 0.02
Age at Dx	1.03 (1.01-1.04), 0.0003

Conclusions: We confirm various reports of similar survival among women diagnosed with FIGO III endometrioid and Type II carcinoma of the uterus. In our population we find that AA patients have poorer survival in Type II, later stage endometrial carcinoma group. The potential reasons for this, including differences in treatment decisions, tumor biology, response to chemotherapy and socio-economic factors should be explored further.

1046 Evaluation of the Combined Utility of p16 and ProEx C Immunohistochemistry in Distinguishing Endocervical Glandular Neoplasia from Its Benign Glandular Mimics

N Banet, C Livasy. Univ of North Carolina, Chapel Hill, NC.

Background: Cervical adenocarcinoma accounts for 15-25% of all cervical cancers. Identification of endocervical glandular neoplasia (EGN) in cervical specimens may be difficult due to small sample size and/or the presence of benign glandular mimics. Proteins p16, topoisomerase II alpha, and MMP2 have been shown to be upregulated in HPV-associated neoplasia. The aim of this study was to evaluate the combined utility of p16 and ProEx C in distinguishing EGN from its benign glandular mimics.

Design: Patients were selected retrospectively including those with endocervical adenocarcinoma in situ (AIS), usual-type invasive endocervical adenocarcinoma and several benign glandular mimics. Representative sections from each case were selected for immunohistochemical (IHC) studies for p16 and ProEx C. The IHC slides were reviewed by two pathologists and scored for intensity (0-3) and percent staining.

Results:

ProEx C and p16 results

	0%	1-25%	26-49%	50-75%	>75%
p16					
Normal endocervix (n=8)	2	6	0	0	0
Tubal metaplasia (n=10)	2	2	0	5	1
Endometriosis (n=8)	0	3	2	3	0
Inflammatory atypia (n=8)	5	3	0	0	0
Microglandular hyperplasia (n=8)	8	0	0	0	0
AIS (n= 11)	0	0	0	0	11
Invasive adenocarcinoma (n= 9)	1	0	0	0	8
ProEx C					
Normal endocervix (n= 8)	1	7	0	0	0
Tubal metaplasia (n=10)	1	7	1	1	0
Endometriosis (n=8)	2	3	3	0	0
Inflammatory atypia (n=8)	1	7	0	0	0
Microglandular hyperplasia (n=8)	2	5	0	1	0
AIS (n=11)	0	0	0	0	11
Invasive adenocarcinoma (n=9)	0	0	0	1	8

Both p16 and ProExC showed diffuse staining in essentially 100% of neoplastic cells in all 11 AIS lesions and in 8 of 9 invasive endocervical adenocarcinomas. Both biomarkers correlated strongly with EGN ($p<0.001$). There were a total of 9 benign glandular mimics showing ≥ 50% staining for p16, such that interpretation of result could be difficult in a small biopsy specimen. In 8 of these cases the corresponding ProEx C showed <50% staining suggesting a potential improvement in specificity from 79% to 97% in this study if ProEx C added.

Conclusions: Only diffuse staining for p16 or ProEx C should be considered as strongly supportive of the diagnosis of endocervical glandular neoplasia. In small samples where percent staining may be difficult to assess, a combined p16/ProEx C panel is likely to improve specificity as compared to p16 alone.

1047 Histopathologic Follow-Up of Women with Post-Hysterectomy LSIL Vaginal Paps and High Risk HPV Testing

M Bansal, RM Austin, CZ Zhao. University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: High risk (hr) HPV infection is now recognized as the dominant etiologic factor responsible in cervical carcinogenesis. Data on the prevalence of hrHPV infection and histopathological follow-up in women with SIL vaginal cytology results are very limited.

Design: A computer-based search was carried for a study period of 49 months between July 1, 2005 and July 30, 2009 to identify women with LSIL vaginal cytology results who also were tested for hrHPV DNA. All study Pap tests were Thin Prep Pap Tests (TPPT) imaged using the ThinPrep Imaging System (TIS). hrHPV DNA detection was by the Hybrid Capture 2 method. hrHPV test and histopathological follow-up results were documented and analyzed.

Results: During the study period, 148 vaginal LSIL TPPT samples had hrHPV testing. 113 of 148 (76.4%) tested HPV positive. 59 post-hysterectomy patients with vaginal LSIL cytology results and hrHPV DNA testing and had at least one follow-up biopsy were included in this study. 14.6% of hrHPV positive women had follow-up VAIN2/3; however none of the 11 patients who were hrHPV negative had VAIN 2/3 (Table 1). The time interval between LSIL cytology results and an initial histopathological diagnosis of VAIN 2/3 or VAIN 1 ranged from 0.2 to 26 months (mean 8.6 months) and 0.2 to 21 months (mean 4.1 months) respectively. Study subjects were an average age of 57 yrs (27-92). There was no statistically significant difference in detection rates of VAIN for women ≤ 54 and ≥ 55 years of age.

Histopathological VAIN Diagnoses in LSIL Vaginal Pap Tests and correlation with hrHPV Test

Total (59)		hrHPV Positive (48)		hrHPV Negative (11)	
VAIN 2/3	VAIN1	VAIN 2/3	VAIN1	VAIN 2/3	VAIN1
7 (11.9%)	41 (69.5%)	7 (14.6%)	34 (70.8%)	0	7 (63.6%)

Conclusions: 1. The prevalence of hrHPV detection in vaginal LSIL Paps is similar to that reported with cervical LSIL Pap results. 2. The incidence of underlying histopathological high grade dysplasia is higher in hrHPV positive LSIL patients than in hrHPV negative LSIL patients; this difference however does not reach statistical significance due to small case numbers. 3. 81% of women with vaginal LSIL Paps had VAIN documented on in histopathological follow-up. 4. Follow-up vaginal colposcopic and tissue examinations are appropriate for women with vaginal LSIL Pap results.

1048 Genotypic Analyses of Serous Ovarian Borderline Tumors and Peritoneal Implants Provide Evidence for Their Independent Origin

F Bartel, E Gerono, K Balschun, E Gradhand, K Kriese, H Taubert, S Hauptmann. University of Halle-Wittenberg, Halle/Saale, Germany; University of Kiel, Kiel, Germany; Vivantes Netzwerk für Gesundheit, Berlin, Germany.

Background: Patients with serous borderline tumors of the ovary (sBOTs) often present with multiple peritoneal implants (PIs), whereby the origin of the PIs is still unknown.

Design: We analyzed various genomic alterations – loss of heterozygosity (LOH), microsatellite instability (MSI), KRAS, BRAF, p53 mutations – in 19 sBOTs and in 43 corresponding PIs from 12 patients.

Results: Twenty-six out of 43 PIs carried 42 changes (MSI+LOH) whereas only five out of 19 sBOTs showed chromosomal aberrations. There was no concordance of genetic changes in the bilateral tumors of two patients. Furthermore, in six cases a concordant MSI and/or LOH was found in at least two PIs but not in the corresponding sBOT. Interestingly, the number of MSI was higher in non-invasive desmoplastic implants

(92%) than in non-invasive epithelial implants (29%). Mutations of the KRAS gene were detected in 21% of the sBOTs (4/19) but in only 5% of the PIs (2/43). The frequency of p53 mutations was 37% (7/19) in sBOTs and 28% (12/43) in the PIs. Most intriguingly, these p53 mutations showed almost no concordance in any patient; not even in cases where the primary tumor and at least a subset of implants shared the same LOH or KRAS mutation.

Conclusions: In summary, the data presented here strongly suggest a multiclonal origin for nearly all cases of sBOTs and PIs.

1049 Histologic and Clinical Response Characteristics in Ovarian Serous Papillary Carcinomas in Women Less Than 40 Years of Age

E Boeding, K Atkins, J Lyman, J Young, K Hanley. University of Virginia, Charlottesville, VA; Emory, Atlanta, GA.

Background: Without predisposing factors such as BRCA mutations and Lynch syndrome, serous ovarian carcinomas rarely occur in women <40 years old. In older patients, the 2- and 5-year survival rates are low, but the few studies of young women have shown a more favorable prognosis. It is unclear whether age is an independent prognostic factor or if the reported improved outcome is due to early stage, low-grade tumors. Many studies included borderline tumors, which skewed the outcomes. This study was designed to determine response to treatment and survival with respect to grade and stage of serous carcinomas in women < 40. Genetic predisposition was also assessed.

Design: Pathology archives, institutional data warehouse, and chart reviews yielded age at diagnosis, stage of disease, treatment and response, family and personal history of malignancies, and referrals to genetic counseling. Slides were reviewed and tumors graded. Exclusion criteria included borderline histology or ambiguous primary source. Survival data for the study group were compared to previously published studies for pre and postmenopausal women.

Results: 23 patients met inclusion criteria (age at diagnosis 18-40). Tumor stage at diagnosis was: 3 stage I, 2 stage II, 10 stage III, and 8 stage IV. 21 patients received cytoreductive surgery (optimal in 14). Tumor grades were: 7 grade 1, 13 grade 2, and 3 grade 3. 17/18 patients survived at least 2 years (5 additional women alive but still less than 2 years since diagnosis). 4/12 patients with 5- year survival data are alive.

Survival by stage

	2 year (N= 18)	5 year (N=12)
S1	100% (3/3)	
S2	100% (2/2)	0% (0/1)
S3	88% (7/8)	43% (3/8)
S4	100% (5/5)	0% (0/3)

S= stage

Survival by grade

	2 year (N=18)	5 year (N=12)
G1	100% (5/5)	25% (1/4)
G2	86% (8/9)	50% (2/4)
G3	100% (4/4)	25% (1/4)

G=grade

3 patients had other malignancies: (breast, endometrial and spinal astrocytoma). 2/23 patients had documented genetic counseling discussions.

Conclusions: In women <40 with ovarian serous carcinoma: 2-year-survival rate is excellent regardless of stage and grade. 5-year-survival rate in advanced staged patients is similar to postmenopausal women. Genetic counseling was markedly underutilized.

1050 Fatty Acid Binding Protein 4 (FABP4) Plays an Important Role in Ovarian Cancer Metastasis

R Buell-Gutbrod, S Dogan, K Gwin, E Lengyel. University of Chicago, Chicago, IL.

Background: Ovarian carcinomas reveal a strong preference for metastasizing to the omentum. FABPs are a family of cytoplasmic proteins involved in fatty acid uptake, transport and metabolism. FABP4 is expressed in adipocytes, macrophages and dendritic cells. We hypothesize that FABP4 plays a significant role in fatty acid transport from adipocytes to metastatic ovarian tumor cells providing an energy source as the disseminating tumor cells engraft within the omentum.

Design: Archival paraffin embedded material of 18 serous ovarian carcinomas with corresponding omental metastases were examined by IHC for the expression and localization of FABP4. Staining was graded for intensity in the primary carcinoma, ovarian stroma, omental fat, omental tumor interface, and established tumor within the omentum distant from the adipocyte interface.

Results: The primary ovarian tumor was negative for FABP4 expression in 16/18 cases with 2 cases showing focal, weak staining. No FABP4 was expressed in ovarian stroma. The omental adipocytes showed strong FABP4 expression in all cases. Strong cytoplasmic expression was also observed in the metastatic tumor cells at the adipocyte interface in 15/18 cases, with moderate staining in 3/18 cases. Metastatic omental tumor cells, distant from the adipocyte interface, were negative in 17/18 cases and showed focal weak staining in 1 case.

Conclusions: Omental metastases of ovarian carcinomas reveal a consistent histologic growth pattern with an interface between omental adipocytes and invading metastatic tumor cells. Following adhesion and extravasation, survival and proliferation of ovarian tumor cells must take place for successful metastatic growth in the omentum. FABP content is proportional to the rate of fatty-acid metabolism in the cells. The expression of FABP4 at the tumor omental adipocyte interface supports the role of FABP4 in fatty acid trafficking during tumor cell engraftment.

1051 EGFR Is Overexpressed in Uterine Carcinomas of Patients with Antecedent Tamoxifen Exposure

R Buell-Gutbrod, A Tergas, S Yamada, A Fefferman, K Gwin. University of Chicago, Chicago, IL.

Background: Epidermal growth factor receptor family members, EGFR and HER-2/neu, have been shown to be highly expressed in uterine cancers. In breast carcinomas, EGFR and HER-2/neu overexpression is associated with tamoxifen resistance. Recent data shows that EGFR activates downstream promoters, upregulating G protein coupled receptor 30 (GPR30) mRNA and protein levels in tamoxifen resistant breast cancer as well as in the endometrium . The role of these molecules in uterine cancers arising in patients treated with tamoxifen has not yet been explored. We compare EGFR and HER-2/neu expression in tamoxifen associated high grade uterine carcinomas and matched controls.

Design: Tissue microarrays were designed from paraffin embedded material of 14 high grade uterine cancers (8 MMMT, 4 serous, 1 endometrioid) of patients previously treated with tamoxifen for breast cancer, and histologic type, grade, age and stage matched controls group (n=27). Expression and localization of EGFR and HER-2/neu were evaluated by IHC.

Results: Results are summarized in Table I.

EGFR and HER-2/neu Expression

EGFR	Negative	Weak (1+)	Moderate (2+)	Strong (3+)
Tamoxifen (n=14)	0	2	4	8
Control (n=27)	4	8	10	5
HER-2/neu	Negative	Weak (1+)	Moderate (2+)	Strong (3+)
Tamoxifen (n=14)	12	0	1	1
Control (n=27)	25	0	1	1

In 33% of tamoxifen patients and 18.5% of controls, 3+ EGFR positivity was observed, and in 86% of tamoxifen associated tumors and 55% of control samples, 2+ immunoreactivity was seen, respectively. One case per group, making up 7% of the tamoxifen group and 3.7% in the control group, showed 3+ HER-2/neu positivity as defined by current CAP criteria for breast carcinomas.

Conclusions: Patients with antecedent tamoxifen exposure were more likely to show overexpression of EGFR compared to the matched control group. No significant difference was seen in Her-2/neu expression between the tamoxifen group and the controls. While we did not explore the underlying mechanism for EGFR overexpression in tamoxifen associated high grade uterine tumors in this study, this is an interesting finding as EGFR inhibitors might be a potential future treatment option.

1052 The IGF-1R/mTOR Pathway Is Differentially Activated in Tamoxifen Associated High Grade Uterine Tumors

R Buell-Gutbrod, A Tergas, S Yamada, A Fefferman, A Montag, K Gwin. University of Chicago, Chicago.

Background: Tamoxifen, a frequently used adjuvant therapy for breast cancer, has an estrogenic effect on the endometrium and a known association with low-grade endometrial cancers. Over the past years however, an increased number of high-grade uterine tumors arising from atrophic endometrium have been reported in patients with a history of tamoxifen use. The kinase mammalian target of rapamycin (mTOR) and its phosphorylated form phospho-mTOR (p-mTOR) are part of a molecular cascade linked to regulation of cell growth, and its activation has been demonstrated in multiple human cancers. Tamoxifen has been shown to interact with estrogen receptors in the endometrium to induce insulin like growth factor signaling via IGF-1R which is an upstream regulator of the P13K/AKT/mTOR pathway. In this study, we compared expression of ER, IGF-1R, mTOR and p-mTOR in patients with tamoxifen exposure and matched controls.

Design: Tissue microarrays were designed from paraffin embedded material of 14 high grade uterine cancers (8 MMMT, 4 serous, 1 endometrioid) of patients previously treated with tamoxifen for breast cancer, and histologic type, grade, age and stage matched controls group (n=27). Expression and localization of ER, IGF-1R, mTOR and p-mTOR were evaluated by IHC.

Results: Strong cytoplasmic staining for IGF-1R was observed in the tamoxifen treated group, with significantly less staining in the control group ($p < .01$). mTOR/p-mTOR both displayed strong staining in 43% of the tamoxifen cases and in 19/15% of the control group, respectively. Estrogen receptor expression varied among the cases; 64% of the tamoxifen group and 55% of the control group showing no or weak staining, and 28%/22% showing strong nuclear staining. The difference in ER expression of both groups was not statistically significant.

Conclusions: Our results show that IGF-1R is significantly upregulated in the tamoxifen group, indicating the upstream activation of the Akt/mTOR/4E-BP1 pathway in high grade uterine tumors developing after tamoxifen therapy. Expression of mTOR and p-mTOR was increased in the Tamoxifen group compared to the matched control group. No significant difference in the ER status between the tamoxifen group and matched controls was found in this study. Upregulation of IGF-1R and mTOR is of clinical interest as both are potential therapeutic targets.

1053 Algorithmic Approach to Intraoperative Consultation/Frozen Section May Reduce Discrepancies and Surgical Understaging in Different Types of Endometrial Cancer

A Cabrera, M Cibull, F Ueland, C DeSimone, RG Karabakhtsian. University of Kentucky, Lexington, KY.

Background: Intraoperative consultation (IOC) with frozen section (FS) for endometrial cancer (EC) is an important management tool for subtyping, staging and treatment. However, studies have shown discrepancies between tumor type and stage at FS and final diagnosis (FDx), mostly involving high-grade (HG) EC, including HG endometrioid (EMC), and most importantly, serous (SC) and clear cell (CCC). These so-called "type 2" EC often show atypical growth pattern, which may result in underestimated depth of

myometrial invasion (DMI) and understaging at IOC. Since the management for deeply invasive "type 2" tumors is the same as that for superficially invasive ones (due to high likelihood of early extrauterine spread), a legitimate question arises regarding the need to evaluate those for DMI at IOC.
Design: Pathology reports of 89 hysterectomies for EC at UK from 1/09 through 8/09 yielded 71 EMC (62 FIGO G1/2 and 9 G3), 6 SC, 5 CCC, 7 mixed. An algorithmic approach was attempted at IOC, partly on the basis of known diagnoses on prior biopsies, and partly based on gross evidence of advanced disease. Known FIGO G1/2 EMC were to be evaluated by FS for DMI and HG EC only for presence of myometrial invasion (MI). High-stage tumors as evidenced by gross adnexal, cervical, or serosal involvement, were to be excluded from FS. The diagnoses/stage at IOC/FS were compared to those at FDx.
Results: Of 62 FIGO G1/2 EMC, FS was performed in 81% (50/62). Of those only 4% (2/50) had discrepancies in DMI. Of 27 HG tumors FS was performed in 52% (14/27) and withheld in 48% (13/27). In 14% (2/14) of HG EC the final pathologic staging was discrepant from that at FS regarding the DMI. A total of 21 tumors were of high-stage, including 7 LG and 14 HG. Of the 21 high-stage tumors, FS was performed in 48% (10/21) with one discrepant case, and withheld in 52% (11/21).
Conclusions: 1) FS for EC may be avoided when gross evidence of advanced disease is present, regardless of tumor type or grade. 2) In HG EC, FS may be performed to determine only the absence (pelvic node sampling only) or presence (also paraaortic node sampling) of MI to minimize the potential for diagnostic underestimation and surgical understaging. 3) FS for DMI may be reserved for FIGO G1/2 EMC only. 4) Although the discrepancy rate between FS and FDx is higher for HG (14%) compared to LG (2%) EC, an algorithmic approach appears to lower the overall rate of discrepancy compared to those reported in the literature (up to 30%).

1054 Ovarian Clear Cell Carcinomas: RHO GTPases May Explain Their Singular Biologic Behavior

B Canet, ML Romero, I Espinosa, J Pena, J Prat. Hospital de la Santa Creu i Sant Pau. Autonomous University of Barcelona, Barcelona, Spain.
Background: The biologic behavior of clear cell carcinomas (CCCs) of the ovary differs from that of high-grade serous carcinomas(HGSCs). Although the former appear confined to the ovary (stage I) more often than the latter, their overall prognosis is poor and characterized by recurrence, metastasis, and poor response to chemotherapy. The RHO GTPase family of proteins is involved in tumor progression through regulation of the cytoskeleton.
Design: The expression of several RHO family genes, including *RHOA*, *RHOC*, *CDC42,* and *ARHGDIG* was studied by Real-Time PCR in 12 CCCs and 31 HGSCs.
Results: Four CCCs (4/12; 33%) were found at stage I. Postoperatively, all patients were treated with taxane and cisplatinum or carboplatinum.Of the 7 patients with advanced stage tumors and follow-up available, 3 died of tumor, 1 is alive with disease, and 3 are alive without evidence of disease after a short interval (mean 2.1 years). *ARHGDIG* (Rho GDP dissociation inhibitor gamma) mRNA expression was higher in CCCs than HGSCs (P=0.06). In contrast, *CDC42* mRNA levels were lower in CCCs than HGSCs (P=0.01). Stage I CCCs were associated with high *ARHGDIG* mRNA expression (P=0.08). In advanced stage tumors (stages II, III, and IV), *ARHGDIG* expression tended to be higher in patients who responded to chemotherapy (median ΔΔCt: 30 vs. 3; P=0.1).
Conclusions: *ARHGDIG* expression contributes to explain the clinicopathological features of CCCs; that is, earlier stage at diagnosis compared with serous carcinomas and variable response to chemotherapy.

1055 Post-Hysterectomy Vaginal Cuff Lesions: A Clinico-Pathologic Study, Emphasizing Diagnostic Challenges and Outcome

M Cascio, JT Rabban. UCSF, San Francisco, CA.
Background: Vaginal cuff nodules arise in a minority of patients following hysterectomy for either benign or malignant gynecologic disease. While polypoid granulation tissue is often the pathologic finding, atypical fibroblasts, atypical endometriosis or recurrent tumor may pose diagnostic challenges. The pathology of post-hysterectomy vaginal cuff lesions is not well-described, particularly with respect to significance of atypical findings and clinical outcome of recurrent tumor; this is the aim of this study.
Design: We identified 97 post-hysterectomy patients with a vaginal cuff nodule/polyp on speculum examination which was surgically excised. Clinical and pathologic features were evaluated, emphasizing morphologic features, atypical fibroblasts, atypical endometriosis and recurrent tumor morphology. Outcome data was obtained from our institutional cancer registry.
Results: Hysterectomy was via transabdominal approach in 82 while the remainder were laparoscopic. Among 13 patients with vaginal cuff lesions after hysterectomy for benign disease, the diagnosis was granulation tissue(3), scar (5), fibroepithelial polyp (1), endometriosis (1), or adenocarcinoma of colonic (1), breast (1) or Mullerian origin (1). Among 84 patients with vaginal cuff lesions after hysterectomy for malignant disease (Table 1), 36 (43%) represented recurrence of tumor, most of which were uterine or ovarian in origin. Benign polypoid granulation tissue composed 16/84 (19%); the remainder showed scar tissue, fibrosis, inflammation or fibroepithelial polyp. Atypical fibroblasts were noted in 1/84. Follow up of all vaginal cuff lesions diagnosed as benign revealed that 2 patients later presented with primary tumor recurrence at the vaginal cuff; 2 in the peritoneum and 6 had distant spread. Average survival after vaginal cuff recurrence was 23 months.

Pathology of Vaginal Cuff Lesions

Hysterectomy Indication	Granulation Tissue	Scar/Non-specific Changes	Recurrent Tumor
Ovarian/Tubal Cancer	4	8	10
Uterine Cancer	7	6	17
Uterine Sarcoma	0	4	0
Cervical Cancer	5	7	8
Vaginal Cancer	0	1	1
Metastatic Colon Cancer	0	1	0

Conclusions: Nearly half of vaginal cuff nodules excised following hysterectomy for malignancy contain recurrent tumor; this finding is associated with short survival time. While most vaginal cuff nodules presenting after benign hysterectomy are benign reactive or inflammatory lesions, a tumor rarely may occur and may be of metastatic origin.

1056 Immunohistochemical Study of DNA Repair Related Proteins in Sporadic Ovarian Cancer

J Castellvi, A Garcia, A Perez-Benavente, MC Ruiz-Marcellan, F Marginean, D Badia, C Parada, S Ramon y Cajal. Vall de Hebron Hospital. Universitat Autonoma de Barcelona, Barcelona, Spain.
Background: Molecules involved in DNA repair, like BRCA, are related with hereditary ovarian carcinoma development, and have also been found downregulated in sporadic ovarian cancers although only nuclear expression of BRCA1 has been evaluated. In the complex DNA repair machinery other molecules, like PARP1 and PML, have been less studied, and their implication in the response to therapy is unknown. The aim of our work is to analyze the level and the cellular pattern of expression of these molecules in sporadic ovarian cancers, correlating them with the pathological characteristics of the tumors and their clinical outcome.
Design: We selected 42 patients with sporadic ovarian carcinomas that recurred after cisplatin and taxol chemotherapy. Immunohistochemistry for BRCA1 (GLK-2), PARP (A6.4.12) and PML (PG-M3) was performed on paraffin sections. The percentage of positive cells, its subcellular location, and the intensity of the staining were scored by two independent pathologists.
Results: BRCA1 nuclear expression was lost in 35% of cases and was found in the cytoplasm of 59% of the tumors. In all cases, non-tumor cells showed only nuclear expression. Similarly, PARP was lost in tumor cells in 47% of the cases. PML showed a speckled nuclear pattern, and was partially lost in 49% of cases. None of the three markers correlated with histologic type, grade or stage, although PML showed a tendency to be more strongly expressed in advanced stages. No correlation was found between the three proteins. The cases with higher expression of PARP recurred before 6 months (p=0.023), while in the survival analysis only BRCA1 cytoplasmic expression was significant (p=0.0026).
Conclusions: These results show that cytoplasmic subcellular location of BRCA1 expression has prognostic significance in ovarian tumors. Moreover, higher levels of PARP were observed in the cases that recurred before 6 months, suggesting that DNA repair is involved in the mechanism of chemoresistance, and that these patients could be candidates to be treated with PARP inhibitors. Finally, in our series PML expression does not correlate with prognosis in ovarian tumors.

1057 STMN1 Expression Is Not Associated with FIGO Grade in Uterine Endometrioid Carcinoma

JSY Chan, LH Ellenson. NYP-Weill Cornell Medical Center, New York City.
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 PIK3CA mutations are common in uterine endometrioid carcinoma (UEC), they do not correlate with FIGO grade. Stathmin (STMN1) expression is associated with activation of the phosphatidylinositol 3-kinase (PI3K) pathway. Its expression has been shown to correlate with grade and other clinicopathologic features in UEC. In this study, we evaluated UEC for expression of STNM1, to further evaluate STMN1 as a possible prognostic marker.
Design: A tissue microarray was constructed using tumor tissue from 23 cases of UEC grade I, 20 cases of UEC grade II, and 10 cases of UEC grade III. Samples of proliferative and secretory endometrium were also included in the analysis. STMN1 expression was detected using standard immunohistochemical staining with polyclonal STMN1 antibody (#3352, Cell Signaling). A previously described staining index was calculated as the product of staining intensity (0-3) and extent of staining (0-absent, 1=focal, 2=diffuse) to grade each sample. Institutional IRB approval was obtained for this study.
Results:

STMN1 Expression in Uterine Endometrioid Carcinoma

FIGO Grade	Average STMN1 score	Range of Score
I (n=23)	2.3 ± 1.5	0-6
II (n=20)	3.2 ± 1.6	1-6
III (n=10)	2.9 ± 1.6	0-6

The average STNM1 staining score for grade I tumors was 2.3 (σ=1.5), grade II was 3.2 (σ=1.6), and grade III was 2.9 (σ=1.6). There was a diversity of staining patterns regardless of FIGO grade as indicated by the range of scores. In benign proliferative endometrium there was strong diffuse STNM1 expression, while secretory endometrium epithelium lacked STNM1 expression.
Conclusions: In this study STMN1 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 STMN1 was strongly expressed in non-neoplastic proliferative endometrium and was not expressed in the epithelium of secretory endometrium. Further studies to determine the association of STMN1 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.

1058 Mature Cystic Teratomas (MCT) of the Ovary Commonly Exhibit Anterior Embryonic Plate Development: An Analysis of 25 Cases

E Chen, CDM Fletcher, MR Nucci. Brigham and Women's Hospital, Boston, MA.
Background: MCT are typically composed of a cyst lined by neoplastic tumor cells recapitulating ectodermal differentiation including derivatives such as epidermis, dermis and appendages (sebaceous glands), although mesodermal and endodermal derivatives can also be seen. Following the observation of a meningothelial proliferation similar to

ectopic meningothelial hamartoma (EMH) in a consult case of MCT and subsequently within dermal tissue in two consecutive MCT, we wanted to assess the frequency of this finding to consider its possible significance.
Design: 25 consecutive cases of MCT diagnosed in 2008 were retrieved from our archives. The presence of meningothelial tissue was first identified by H&E and subsequently confirmed using immunostaining for EMA. The presence of glial tissue was confirmed using GFAP.
Results: Ten of twenty-five (40%) ovarian MCT (patient age ranging from 23 to 66 years, mean: 36.3 years; size ranging from 2.5 to 11.5 cm, mean: 6.1 cm) contained a meningothelial proliferation akin to EMH, morphologically characterized by a network of slit-like channels lined by flattened meningothelial cells. Eight and three of these cases had scattered pigmented dendritic cells and psammomatous calcifications, respectively, which have been described in EMH. Eight of twenty-five cases showed elements derived from all three germ layers, of which the endodermal component was respiratory in 6, thyroid in 1 and bowel in 1. Thirteen cases showed elements derived from ectoderm (skin and glial tissue) and mesoderm (bone, cartilage, adipose tissue). Four cases showed tissue derived from the ectoderm only. In all 10 cases, the proximity of the meningothelial element to ectoderm-derivatives, skin with pilosebaceous units and glial tissue, was noted. In all cases, the meningothelial differentiation was supported by positive EMA expression.
Conclusions: This study shows that the presence of meningothelial tissue in MCT of the ovary is not infrequent and its appearance is similar to that as described in ectopic meningothelial hamartomas, which frequently form mass lesions on the scalp in the pediatric population. The similar appearance of the meningothelial proliferation in MCT to EMH, its location in the dermis, and its frequent proximity to glial tissue supports the hypothesis that the neoplastic growth of MCT most closely parallels anterior embryonic plate development with the formation of primarily tissues of head and neck type (e.g. scalp skin, brain, upper respiratory, and less commonly thyroid).

1059 Secretory Cell Outgrowth (SCOUT) in the Distal Fallopian Tube: A Clonal Expansion of Secretory Cells Linked to Cancer Risk?

E Chen, M Mehrad, KK Mehra, CP Crum. Brigham and Women's Hospital, Boston, MA.

Background: Alterations in the fallopian tube mucosa have been described in association with ovarian cancer and more recently, secretory cell outgrowth (SCOUT) has been described in association with p53 mutations, and termed the "p53 signature". On the premise that p53 signatures are just one component of clonal expansion in the tube, we examined the relative frequency of p53-negative SCOUTs in the distal fallopian tubes from women with and without BRCA mutations and compared their frequency in these and women with pelvic serous carcinoma of tubal and ovarian origin.
Design: Normal appearing fallopian tubes from women with and without genetic risk (BRCA+) for pelvic serous cancer (PSC) and women with and without PSC were immunostained for secretory (bcl-2, pax-8) and ciliated (p73) epithelial cell phenotype. Selected cases were stained with H2AX (for evidence of DNA damage/repair). Secretory cell outgrowth (SCOUT) defined as 20 or more consecutive secretory cells without intervening ciliated cells, were scored, characterized by appearance and their frequency compared between the aforementioned groups by chi square analysis.
Results: SCOUTs were identified in both high (BRCA+) and low-risk women at equal frequency and in both proximal and distal tubal mucosa, at approximately 2.5 per 100x field of mucosa examined. SCOUTs consisted of either strongly bcl-2 positive pseudo-stratified or negative to weakly bcl-2 positive cuboidal epithelia. In all cases, p73 staining was negative, consistent with a secretory phenotype. SCOUTs displayed a greater trend in frequency in tubes from patients with presumed tubal carcinoma relative to other ovarian carcinomas (3.4 vs 1.3, $p = 0.12$). SCOUTs were typically H2AX negative.
Conclusions: SCOUTs are common in both proximal and distal fallopian tubes and fulfill histologic and histochemical criteria for a clonal expansion of secretory type cells, including positive staining for bcl-2 and pax-8, negative staining for p73 and an absence of intervening ciliated epithelium. The absence of H2AX staining suggests that this cellular expansion may result from milder genomic disturbances than the typical p53 signature. The concept of multiple clones of SCOUT linked to genotoxic stress in the distal tube is remarkable simlar to some pathways in the endometrium and merits further study as an additional intra or extra tubal pathway to pelvic epithelial malignancy.

1060 Comparison of Dysplasia Profiles in Stimulated Ovaries and in Those with a Genetic Risk for Ovarian Cancer

G Chene, MM Dauplat, N Lagarde, F Mishellany, I Raoelfils, P Dechelotte, J Dauplat, FM Penault-Llorca. Centre Jean Perrin, Clermont-Ferrand, France; CHU, Clermont-Ferrand, France.

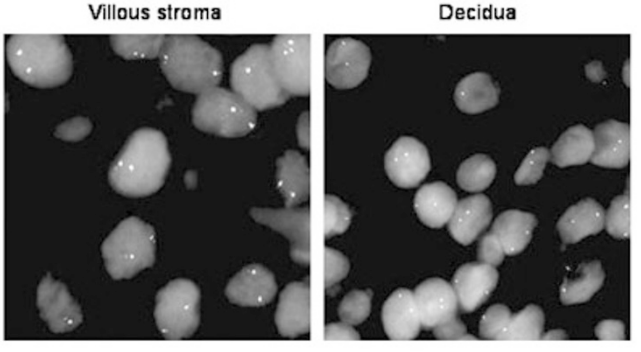
Background: Ovarian epithelial dysplasia (OED) was first described after prophylactic oophorectomy for genetic risk of ovarian cancer. In light of Fathalla's incessant ovulation theory, this study was set up to describe the presence of ovarian abnormalities (dysplasia) after ovulation induction, and to compare dysplasia profiles in stimulated and genetic risk ovaries.
Design: One-hundred and twenty-five oophorectomies or ovarian cystectomies performed between 1992 and 2005 were reviewed. These were divided into three groups: (1) previous in vitro fertilization (n=37); (2) prophylactic oophorectomies for genetic risk (n=28); and (3) fertile non-cancerous controls (n=62). Eleven cytological and architectural epithelial features were defined and a dysplasia score was calculated to quantify ovarian epithelial abnormalities.
Results: Mean ovarian dysplasia score was significantly higher in the genetic risk and stimulated ovary groups than in controls (9.67 vs. 3.62, $p<0.0001$; 7.64 vs. 3.62, $p<0.0002$, respectively). Cytological and architectural ovarian abnormalities were more frequent in the genetic risk group, while the profile of abnormalities was different in the genetic risk and stimulated groups.
Conclusions: These findings support a possible relationship between OED and the use of ovulation-stimulating drugs. The increasing dysplasia score in genetic risk ovaries might be consistent with progression towards neoplastic transformation, and may justify the use of the term dysplasia or intraepithelial ovarian neoplasia. On the other hand, the fact that the dysplasia profile after stimulation differs from that in genetic risk ovaries may suggest that ovarian stimulation may predispose to a different evolution.

1061 In Situ Genetic Analysis of Hydatidiform Moles by Polymorphic Deletion Probe Fluorescence In Situ Hybridization

S Chiang, L Fazlollahi, A Nguyen, DJ Roberts, AJ Iafrate. Massachusetts General Hospital, Boston.

Background: Molecular genotyping can aid in the diagnosis of complete (CHM) and partial hydatidiform moles (PHM) and nonmolar abortions (NMA). Polymorphic deletion probes (PDP), recently developed FISH probes based on copy number variants, are highly polymorphic and can genetically distinguish between cells from any 2 individuals in situ. Since products of conception (POC) contain maternal and villous tissue, the genotypes of mother and zygote based on deleted or expressed alleles can be assessed. We used a panel of 3 informative PDP (one probe each from chromosomes 2p, 4q and 8p) to determine the genetic identity of molar and nonmolar POC in situ with a focus on whether villous tissue lacked maternal contribution (indicating CHM) and on diploidy vs. triploidy (indicating PHM).
Design: FISH analysis using 3 PDP was performed on 40 archival POC, including 13 CHM, 13 PHM, 13 NMA, and 1 placental mesenchymal dysplasia (PMD), with supporting ancillary studies (p57 immunostaining, flow cytometry, cytogenetics). The following genotype pairs were considered informative for a definitive diagnosis of CHM: +/+ decidua and -/- villi; -/- decidua and +/+ villi. Combined 8p PDP-FISH and p57 immunofluorescence (IF) was performed on PMD.
Results: 3 probe PDP-FISH analysis was successful in all cases studied, with genotypes clearly identified for maternal and villous tissue. PDP-FISH permitted a definitive diagnosis of CHM in 5/13 known cases for which genotypes of maternal and villous tissue were mutually exclusive (Fig. 1). 13 CHM were diploid and homozygous for all PDP, consistent with monospermy. Based on a low rate of homozygosity for all probes in NMA (1/13), we calculated a 92.3% probability that any case is a CHM if all probes are homozygous. Triploidy was shown in 13 PHM, including 6 cases in which PDP-FISH allowed definitive determination of diandric triploidy. In PMD, FISH-IF showed p57 expression in heterozygous (+/-) cytotrophoblasts and syncitiotrophoblasts and no expression in homozygous villous stromal cells, confirming mosaicism in situ.
Conclusions: PDP-FISH analysis can be used in clinical practice and research studies to subtype hydatidiform moles and evaluate nonmolar POC with genetic abnormalities.

Figure 1. 2p PDP-FISH in CHM



1062 Loss of Heterozygosity (LOH) and Immunohistochemical Analysis (IHC) of a Subset of Primary Fallopian Tube Carcinomas (PFTC) Arising in the Background of Tubal Intraepithelial Carcinoma "TIC" from Primary Peritoneal Serous Carcinomas with/without Associated Tubal Intraepithelial Carcinoma "TIC"

M Chivukula, R Edwards, M Nikiforova, G Mantha, K McManus, LA Niemeier. Magee Women's Hospital of UPMC, Pittsburgh, PA; Magee Women's Hospital of UPMC, Pittsburgh.

Background: Recent literature is emerging on role of TIC as a precursor lesion of primary peritoneal serous carcinoma (PPSC) {Crum et al}.PAX2 is a developmental transcription factor expressed in wolffian and müllerian ducts has been demonstrated in serous carcinomas of the ovary (up to 60%). The role of PAX2 in PFTC /PPSC is yet to be defined. The aim of our study was to evaluate the expression of p53,PAX2, WT-1 both by LOH and IHC studies.
Design: Ten (10) cases of PFTC with TIC (**Group 1**), PPSC without associated TIC (PPSC/-) (**Group 2**) two (2) cases of PPSC with associated TIC (PPSC/+) (**Group 3**) were secured from the case files of MWH. p53 PAX2, WT-1 (nuclear stains) were scored as positive and negative. All tumors and corresponding normals were manually microdissected. A Panel of 6 polymorphic microsatellite markers corresponding to TP53, PTEN, and WT1 tumor suppressor genes were studied.

	PTEN/PAX 2		P53		WT1	
	LOH	IHC	LOH	IHC	LOH	IHC
Group 1	LOH 50% (5/10)	100% (10/10)	60% (6/10)	100% (10/10)	20% (2/10)	100% (10/10)
Adjacent TIC	LOH 30% (3/10)	100% (10/10)	20% (2/10)	20% (2/10)	No LOH 0% (10/10)	0% (0/10)
Group 3	No LOH 0% (0/2)	50% (5/10)	No LOH 0% (0/2)	100% (2/2)	No LOH 0% (0/2)	100% (2/2)
Associated TIC	No LOH 0% (0/2)	50% (5/10)	No LOH 0% (0/2)	100% (2/2)	No LOH 0% (0/2)	100% (2/2)
Group 2	LOH 50% (5/10)	80% (8/10)	60% (6/10)	100% (10/10)	20% (2/10)	100% (10/10)

Results: [Table1] shows a summary of LOH and IHC of all groups.
Conclusions: There was high frequency of LOH for the PTEN/PAX 2 and p53 genes in all groups. Group 2 and Group 3 exhibits different LOH patterns. Group 1 shows similar LOH patterns to Group 2. TIC of PFTC shows a different pattern in comparison to associated TIC.

1063 Lipolcalin-2/NGAL Overexpression in Endometrial Carcinoma

D Cho, J Torres-Mora, CE Sheehan, JS Ross. Albany Medical College, Albany, NY.
Background: Lipocalin-2, also known as neutrophil gelatinase associated lipocalin, is a 2k kDA glycoprotein that appears to function as a hydrophobic compound transporter. Upregulation of ipocalin-2 has been frequently described in a variety of inflammatory conditions involving numerous organs and tissues. Lipocalin-2 expression has not been widely studied in cancer and its role a prognostic biomarker for endometrial carcinoma has not been previously evaluated.
Design: Formalin-fixed, paraffin-embedded tissue sections from 124 endometrial carcinomas, including 101 endometrioid carcinoma (EC), 12 uterine papillary serous carcinoma (PSC) and 11 malignant mesoderm mixed tumor (MMMT) were immunostained by automated methods (Ventana Medical Systems Inc., Tucson, AZ) using rat monoclonal lipocalin-2/NGAL (R&D Systems, Minneapolis, MN). Cytoplasmic immunoreactivity was semiquantitatively scored based on staining intensity and distribution and the results were correlated with morphologic and prognostic variables.
Results: Cytoplasmic lipocalin-2 overexpression was observed in 47/124 (38%) tumors, including 37/101 (37%) EC, 6/12 (50%) PSC, and 4/11 (36%) MMMTs. Lipocalin-2 overexpression correlated with tumor grade (52% grade 3 vs. 43% grade 2 vs. 21% grade 1, p=0.045) and with ER negative tumors [52% ER negative vs 28% ER positive, p=0.049] overall and within the MMMT subgroup [100% ER negative vs 0% ER positive, p=0.046]. Within the ER negative subgroup, lipocalin-2 overexpression correlated with PR positive status [88% PR positive versus 31% PR negative, p=0.011] and showed a trend for association with depth of myometrial invasion (80% invading to more than 50% of the myometrium vs. 46% to less than 50% vs. 0% no invasion, p=0.088). There was no correlation with disease recurrence or overall survival.
Conclusions: Lipocalin-2 expression is associated with high grade endometrial carcinomas and may be a predictor of advanced tumor stage. Continued study of this biomarker in endometrial carcinoma cases appears warranted.

1064 Human Papillomavirus Type Distribution in 53 Neuroendocrine Tumors of the Cervix

O Clavero, B Lloveras, M Alejo, B Quiros, N Monfulleda, L Alemany, J Klaustermeier, W Quint, S de Sanjose, on Behalf of the RIS HPV TT Study Group. Catalan Institute of Oncology, IDIBEL, Hospitalet de Llobregat, Barcelona, Spain; Hospital del Mar, IMAS-IMIM, Barcelona, Spain; Hospital General de Hospitalet, Hospitalet de Llobregat, Barcelona, Spain; CIBER Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain; DDL Diagnostic Laboratory, Voorburg, Netherlands.
Background: Neuroendocrine carcinoma of the cervix is a rare entity with a very aggressive behaviour, frequent recurrences, distant metastasis and low survival rates, with little information regarding pathogenesis. The WHO classification includes under the term on Neuroendocrine Tumours (NET): carcinoid, atypical carcinoid, large cell carcinoma and small cell carcinoma, this last one accounting for the 0.5-1% of whole cervical carcinomas. Objective: To describe the Human Papillomavirus (HPV) type distribution in NET.
Design: In the RIS HPV TT study, among 10,365 cervical carcinomas, pathologists identified 53 carcinomas with neuroendocrine features based on morphological characteristics. WHO classification was applied taking into account cell size, nuclear features and architectural pattern. To determine the neuroendocrine differentiation, immunohistochemical staining included chromogranin A (Dako), synaptophysin (clone 5438 Dako), CD 56 (clone 123c3 DAKO) and p16ink4a (Clone E6H4, CINtec® Histology Kit) was carried out. HPV detection was done through amplification of HPV DNA by SPF-10 broad-spectrum primers PCR subsequently followed by DEIA and genotyping by $LiPA_{25}$ (version 1).
Results: Mean age of the patients was 48.8 y.o. Pathological classification showed 37 small cell NET, 11 large cell NET, 4 atypical carcinoid and 1 typical carcinoid. HPV detection yielded 46 HPV positive cases (86.8%) and 7 HPV negative. The types detected were HPV16 (n=22, 47.8%), HPV18 (n=20, 43.5%) and other HPV types in 4 cases (HPV35, HPV58, HPV18&52, HPV16&18&39). Overexpression of p16ink4a was observed in 86.1% of 43 cases.
Conclusions: The study confirms the association of cervical NET with high risk HPV. HPV 16 and 18 were identified in more than 90% of these tumors. We observed a four-fold increase detection of HPV18 when compared to that observed among all cervical cancer cases in this study, deserving further research.

1065 Villoglandular Adenocarcinoma of the Cervix Is Associated with Normal Expression of Mismatch Repair Proteins

NM Conlon, R Geraghty, L Kehoe, JJ O Leary, D Gibbons. Coombe Women's Hospital, Dublin 8, Ireland; St Vincent's University Hospital, Dublin 4, Ireland.
Background: Villoglandular adenocarcinoma of the cervix, a rare variant of cervical adenocarcinoma, has come to prominence in recent years due to an emerging consensus that the long-held belief in the indolent nature of this disease requires reassessment. Due to its relative rarity, there have been few investigations into the molecular genetics underlying the condition. Several previous studies have reported the presence of microsatellite instability (MSI) in 6-25% of all cervical carcinomas, but the number of cases of cervical adenocarcinoma included in these studies was low. The expression of mismatch repair proteins in the villoglandular subtype of cervical adenocarcinoma has not previously been assessed.
Design: We assembled a case series of eleven patients with villoglandular adenocarcinoma of the cervix who attended our centre over a ten year period. The epidemiological data and histopathological findings from the eleven patients were reviewed. We also undertook immunohistochemical analysis of the expression of four mismatch repair proteins (MLH1, PMS2, MSH2 and MSH6).
Results: Histological review confirmed the diagnosis of villoglandular adenocarcinoma of cervix in all cases. The average age at diagnosis was 32 years, with an age range from 26 years to 38 years. Eight patients underwent hysterectomy. At the time of review, no disease recurrence had been recorded in any case. All eleven cases demonstrated normal expression of mismatch repair proteins.
Conclusions: Villoglandular adenocarcinoma is a subtype of cervical adenocarcinoma which tends to present at a younger age than conventional endocervical adenocarcinoma. The genetic pathways underpinning the development of the disease remain to be elucidated. This series of eleven cases represents the first attempt to assess the expression of mismatch repair proteins in villoglandular cervical adenocarcinoma. The results suggest that there is no association between villoglandular adenocarcinoma of cervix and microsatellite instability.

1066 Differential Nuclear Expression of Aurora A Kinase in Benign, Borderline, and Malignant Serous Ovarian Tumors

JE Crane, DS Schultz, KV Inamdar, NL Lehman. Henry Ford Hospital, Detroit, MI.
Background: Aurora A is a serine/threonine kinase important in mitosis. It has been implicated in the tumorigenesis of several malignant neoplasms, including breast, colorectal, pancreas, and ovary (Sasayama T, *et al*. Genes Cells 2005;10:627). It has been suggested that aurora-A expression may also have prognostic value in ovarian carcinoma (Mendiola M, *et al.* Hum Pathol 2009;40:631).
Design: We investigated the expression of aurora A by immunohistochemistry in 10 benign serous cystadenomas, 8 serous borderline tumors, and 12 malignant serous ovarian tumors.
Results: All 10 benign neoplasms (100%) showed moderate to strong nuclear immunoreactivity for aurora A. No perinuclear cytoplasmic staining was demonstrated. Sixty-three percent (5/8) of the borderline tumors demonstrated weak to moderate nuclear staining. The remaining borderline tumors (38%, 3/8) were negative for aurora A. Twenty-five percent (3/12) of the malignant tumors demonstrated perinuclear cytoplasmic staining for aurora A, ranging from weak to strong. All malignant tumors lacked nuclear aurora A positivity (100%). Using Fisher's Exact Test the results were found to be statistically significant when the tumor grade (benign/borderline/malignant) was analyzed against the staining pattern (absent/cytoplasmic/nuclear) or staining intensity (weak, moderate, strong) ($p < 0.01$).
Conclusions: Our results show that benign serous ovarian tumors show strong nuclear staining, which is completely absent in malignant tumors. Borderline tumors tend to show nuclear staining like benign tumors, albeit generally weaker, but sometimes lacked nuclear staining like malignant tumors. This data may thus be of potential value in predicting the behavior of borderline tumors. The overall results of negative nuclear aurora A expression in malignant tumors may have important implications in the biology of ovarian serous tumors. Further studies of the possible clinical implications of the loss of nuclear aurora A expression in some borderline serous tumors, and the role of that loss in ovarian serous carcinogenesis are warranted.

1067 Examining the Relationship between Expression of Selected Tumour Associated Genes, Platelet Count and Survival in Ovarian Cancer

DJ Crowley, RJ Flavin, SA O'Toole, ST Aherne, PC Smyth, K Egan, E Dunne, D Kenny, JJ O'Leary, OM Sheils. Institute of Molecular Medicine, Trinity College Dublin, Dublin, Ireland; Harvard Medical School, Boston, MA; Trinity Centre for Health Sciences, Trinity College Dublin, Dublin, Ireland; Royal College of Surgeons, Dublin, Ireland.
Background: Ovarian cancer (OC) is the leading cause of death from gynaecological malignancy. It is a severe form of cancer with a mortality rate of ~75% and a long term (5 year) survival rate of ~20% associated with it. Up to half of patients diagnosed with ovarian cancer present with thrombocytosis – a blood platelet concentration of ≥ $450x10^9$/L. This may be associated with Tumour Cell Induced Platelet Aggregation, observed to protect platelet 'cloaked' cancer cells from TNF-α and natural killer cell mediated destruction, chemotheraputic agents and enhancing their capability for metastasis.
Design: FFPE tissues corresponding to normal ovary (19), grade 2 (32), grade 2/3 (6) and grade 3 (43) ovarian cancer were sectioned. Total RNA was extracted with using Ambion RecoverAll Kits, before being reverse transcribed. cDNA samples were then PreAmplified for a panel of genes (PDGFα, PDGFRα, PDGFβ, PDGFRβ, VEGF, CA125, THPO and endogenous controls) before TaqMan RT-PCR was used to investigate the samples' gene expression. Relative Quantitation (RQ) was determined by $2^{-(\Delta\Delta Ct)}$ method and significance by t-test using the Spotfire analysis suite and further analysis performed using SPSS.

Results: Significant ($p<0.05$) differences in expression of several target genes including CA125, VEGF, PDGFα+β, PDGFRα+β were observed. In cancer cases elevated PDGFβ expression was shown to correlate with preoperative thrombocytosis ($p<0.05$).
Conclusions: Variations in gene expression were observed with increasing tumour grades, with grades 2-3 displaying increased CA125 and VEGF expression ($P<0.05$) and a trend of reduced PDGFRα/β and increased PDGFβ expression ($p<0.05$) was observed. This supports previous histological observations in ovarian cancer. We propose that the ovarian tumour cells signal to encourage neovascularisation while remaining resistant to PDGF growth stimuli themselves, thus facilitating a microenvironment to sustain tumoural growth. In addition the correlation of elevated PDGFβ with elevated platelet levels suggests ovarian tumours may influence platelet production that may assist in their future metastasis.

1068 Prognostic Significance of Endoglin (CD105) as an Angiogenic Marker in Endocervical Adenocarcinoma

E Cullen, JF Silverman, MA Khalifa, N Ismiil, V Dube, S Nofech-Mozes, Z Ghorab, RS Saad. Allegheny General Hospital, Pittsburgh; Sunnybrook Health Sciences Centre, Toronto, Canada.
Background: There is growing evidence supporting the role of microvessel density as an important predictor of tumor behavior in a number of human malignancies. Endoglin (CD105), a member of transforming growth factor beta1 receptor complex, has been shown to be a more useful marker than panendothelial markers. However, there is limited data regarding the prognostic significance of angiogenesis evaluated with endoglin in cervical adenocarcinoma. In this study, we investigated endoglin as an endothelial marker of angiogenesis in endocervical adenocarcinoma.
Design: Surgical specimens from 45 consecutive patients with endocervical adenocarcinoma treated with radical hysterectomy and surgical staging were reviewed. Selected tumor blocks were immunostained for CD31 and endoglin. Positively stained microvessels (MV) were counted in densely vascular foci (hot spots) at x400 field in each specimen (=0.17 mm^2). Results were expressed as the highest number of MV count identified within any single field and correlated with other prognostic parameters and survival.
Results: Endoglin identified MV in all cases with a mean count of $15\pm7/0.17$ mm^2, while CD31 was positive in 43/45 (96%); with a mean count of $24\pm11/0.17$ mm^2 with significant correlation ($P< 0.05$). Both endoglin and CD31 MV counts correlated significantly with depth of invasion, circumferential involvement, lymphovascular invasion and tumor stage (r=0.63 and 0.41; 0.43 and 0.39; 0.56 and 0.40; 0.40 and 0.31; respectively, $P<0.05$). Only endoglin MV density was correlated with tumor size (r=0.47, $P< 0.01$), recurrence (r=0.40, $P< 0.001$) and distant metastases (r=0.44; $P< 0.001$). It was also associated with poor overall survival (Log Rank, P =0.032).
Conclusions: Our study shows that angiogenesis plays an important role in the progression of endocervical adenocarcinoma. By staining the proliferating MV, endoglin is a specific and a sensitive marker for tumor angiogenesis than commonly used pan-endothelial markers such as CD31.

1069 Functional Expression Analysis of TLR-4 and MYD88 in Epithelial Ovarian Neoplasia

CJ d'Adhemar, MF Gallagher, S O'Toole, C Murphy, PC Smyth, C Martin, O Sheils, JJ O'Leary. Trinity College Dublin, Dublin 2, Ireland; Coombe Women's Hospital, Dublin 8, Ireland.
Background: Toll-like receptors, key parts of the innate immune system, are being increasingly studied in cancer pathogenesis, influencing both tumour proliferation and chemoresistance. The expression of TLR-4 and its adaptor protein MyD88 have recently been analysed in epithelial ovarian neoplasia (EON). However, to date, this work has been limited to serous carcinomas. The aim of this study was to evaluate TLR-4/MyD88 expression in all common subtypes of EON (benign, borderline and malignant), to analyse its effect on patient survival and to assess its expression in ovarian cancer stem cells (CSCs).
Design: Archival tissue samples were obtained from 129 patients with EON and 50 with histologically normal ovaries, and were evaluated for TLR-4 and MyD88 by immunohistochemistry (IHC). Results were correlated with histologic subtype, grade and clinical disease progression. p53 and BRAF mutation analysis was performed on samples of borderline serous tumours (BSTs), using IHC and Taqman® SNP detection, respectively. TLR-4/MyD88 expression in CSC cell lines 2102Ep and NTera2 was determined using IHC, with and without differentiation in retinoic acid.
Results: While some degree of TLR-4 expression was observed in all ovarian epithelium, MyD88 expression was confined to serous neoplasms and was greatest in borderline and malignant tumours. MyD88 staining was positive in 46% of malignant tumours (n=40), all of which were serous carcinomas. No direct correlation was observed between tumour grade and TLR-4/MyD88 expression, or with p53/BRAF status in BSTs. MyD88 expression was associated with both shorter progression-free and overall survival (50 vs. 16 months and 78 vs. 36 months, respectively; $p < 0.05$). TLR-4 and MyD88 were expressed in both CSC lines in their undifferentiated state. However, MyD88 expression was significantly decreased in NTera2 cells following differentiation with retinoic acid.
Conclusions: Our findings suggest that MyD88 expression is restricted to serous ovarian neoplasms, independent of histologic grade and associated with significantly shorter patient survival. Furthermore ex vivo manipulation of ovarian CSC differentiation can significantly decrease MyD88 expression. These results suggest that MyD88 expression may be more important than histologic subtype or grade in epithelial ovarian cancers.

1070 Clinicopathological and Immunohistochemical Study in 148 Uterine Sarcomas: Bcl-2 and Proliferative Markers Identify Different Prognostic Groups of Uterine Leiomyosarcomas

E D'Angelo, R Ali, I Espinosa, CB Gilks, M Van de Rijn, C-G Lee, J Prat. Hospital de la Santa Creu i Sant Pau, Autonomous University of Barcelona, Barcelona, Spain; University of British Columbia, Vancouver, BC, Canada; Stanford University Medical Center, Stanford, CA.
Background: Other than stage, the prognostic parameters of uterine sarcomas are ill defined. Previously, we suggested that uterine leiomyosarcomas with strong bcl-2 immunoreaction and weak expression of proliferative markers are associated with good prognosis.
Design: To confirm this assumption, we studied the expression of bcl-2, Ki-67, TP53, and P16 in 148 uterine sarcomas (105 leiomyosarcomas, 32 endometrial stromal sarcomas, and 11 undifferentiated endometrial sarcomas) distributed over 3 TMAs.
Results: In leiomyosarcomas, strong bcl-2 staining was associated with good prognosis (P=0.009) whereas cases with high expression of Ki-67, TP53, and P16 had adverse prognosis (P=0.01, P=0.02, and P=0.03, respectively). Using the four markers, unsupervised hierarchical clustering analysis identified 3 groups with significant differences in survival (P=0.004). Leiomyosarcomas with strong bcl-2 expression were associated with good prognosis regardless of their immunoreactivity for P16 (groups 1 and 3), whereas the leiomyosarcomas lacking bcl-2 that reacted for P16 had worse prognosis (group 2). In a multivariate analysis, these cluster groups were of prognostic significance independent of age, tumor size, necrosis, mitosis, and nuclear atypia (hazard ratio, 3; 95% CI, 1.2 to 7.2; P=0.01). Almost all endometrial stromal sarcomas (28/32; 90%) clustered in the group with favorable prognosis (group 1). In contrast, most undifferentiated endometrial sarcomas (10/11; 91%) clustered in the group with poor prognosis (group 2).
Conclusions: Prognostically, leiomyosarcomas can be separated in different groups according to their expression of bcl-2 and P16 both of which may play a role in their pathogenesis. The distribution of endometrial stromal sarcomas and undifferentiated endometrial sarcomas paralleled that of leiomyosarcomas with favorable and unfavorable prognosis respectively.

1071 HPV DNA Testing: Is It Used Appropriately?

S Davion, E Lucas, A Kemp, R Nayar. Northwestern University, Chicago, IL.
Background: Triage of ASC-US by HPV DNA testing was endorsed by the ASCCP in 2001. In 2003 the FDA approved Pap plus HPV co-testing for primary screening of women ≥30 yrs. The high prevalence of HPV in women ≤ 20 (adolescents) lead to recommendations against any HPV testing in this age group. The 2006 ASCCP guidelines, and the "Statement on Human Papillomavirus DNA Test Utilization", a consensus of the NCI, ASCCP and ACS, endorsed the above recommendations. Since the 2006 ASCCP consensus, a few survey-based studies have evaluated compliance. We have anayzed the use of Pap and HPV DNA results, to screen, triage and manage, by reviewing *actual* HPV/Pap ordering and follow up behaviors at a large academic institution at 11 and 20 months after the 2006 ASCCP consensus publication.
Design: All Paps ordered in Sept 2008 and June 2009 were included (N = 6,365). HPV testing strategy, ordering clinician specialty, and follow up for September 2008 Paps were recorded.
Results:

HPV DNA TESTING; SCREENING,TRIAGE AND MANAGEMENT BY SPECIALTY

SCREENING	SEPTEMBER '08 (TOTAL / OBGYN / IM)	JUNE '09 (TOTAL/ OBGYN / IM)
PRIMARY PAP + HPV ≥ 30	18% / 23% /11%	19% /25% /10%
PRIMARY PAP + HPV < 30	3% / 3% / 3%	2% / 3% /1%
PAPS > 65 w/ CERVIX + NO ABNL PAP FOR 10 YRS.	40% / 28 % / 54%	37% / 34%/ 42%
REFLEX TESTING		
PAP + REFLEX HPV >20	79% / 73% / 88%	82% / 76% / 90%
PAP + REFLEX HPV ≤ 20	84% / 100% / 86%	84% /88% / 86%
MANAGEMENT		
NILM/HPV (-) W/ F/U PAP OR CX BX < 3 YEARS	9%/ 9%/ < 1%	
NILM/ HPV (+) W/ REPEAT PAP OR CX BX < 3 YRS.	45 % / 52% / 0%	
LSIL < 20 W/ CX BX < 1 YR.	75% / 100 % 25%	
HSIL >20 WITHOUT/ F/U LEEP OR CX BX AT 1 YR.	33% / 32% / 33%	

IM: INTERNAL MEDICINE, CX BX:CERVICAL BIOPSY

Conclusions: 1. Women ≥ 30 are not routinely screened with Pap+HPV co-testing (18%). 2. OBGYN are more likely than IM to screen women ≥ 30 with Pap+HPV co-testing (23% vs 11%). 3. NILM/HPV(-) results appear to prolong the screening interval to > 1 year. 4. NILM/HPV(+) women often proceed unnecessarily to repeat Pap or cervical biopsy in < 1 year (45%). 5. NILM/HPV(+) women are more likely to have repeat Pap, or cervical biopsy, in < 1 year in OBGYN vs IM practices (52% vs 0%). 6. Adolescents are overwhelmingly and inappropriately tested/triaged with HPV (84%). 7. Adolescents with LSIL often proceed unnecessarily to cervical biopsy in < 1 year (75%). 8. Approximately 40% of women >65 are screened for cervical cancer without indication. 9. Many HSILS are not followed by cervix biopsy or LEEP within 1 year (33%).

1072 Robotic Assisted Hysterectomies Increase Tubal Contaminants

DF DeLair, RA Soslow, MM Leitao, Jr. Memorial Sloan-Kettering Cancer Center, New York, NY.
Background: Robotic surgery has recently been incorporated into gynecologic surgery and is increasingly being performed for endometrial cancer. The amount of uterine manipulation appears to be greater in robotic assisted hysterectomies (RBT) compared to laparoscopic assisted vaginal hysterectomies (LSC). It has previously been shown

that uterine manipulation may increase the incidence of displaced tumor and subsequent contamination beyond the endometrium. The objective of this study was to determine if there is an association with RBT and tubal contamination.

Design: All RBT and LSC cases performed for endometrial cancer from 5/07 to 8/09 were reviewed. Of the cases not converted to laparotomy, 137 RBT and 184 LSC were identified. The patient's age, BMI, operative (OP) and hysterectomy (HYST) time, type and grade of tumor, stage, pelvic wash, and the presence of detached tumor fragments in the lumina of the fallopian tubes were recorded. Appropriate statistical tests were applied.

Results: The cases with tubal contaminants and their characteristics are listed below (tables 1&2). The majority of the patients with RBT and tubal contaminants had stage I disease (9/16, 56.2%) and grade 1 tumors (9/16, 56.2%). Four (4/16, 25%) of the patients had stage IIIa disease due to positive pelvic washes; they were otherwise stage I.

Table 1. Tubal contaminants

	LSC	RBT	p value
Completed cases N	184	137	<0.001
Contaminants N(%)	4 (2.2%)	16 (11.7%)	<0.001

Table 2. Tubal contaminants in robotic cases

	No Contaminants	Contaminants	p value
N	121	16	
BMI (kg/m2)/Median (range)	25.8 (18.6-60.6)	28.7 (23.3-47.9)	0.06
Age (yr)/Median (range)	60 (27-85)	59 (47-68)	0.2
OP time (min)	238 (115-497)	230.5 (153-533)	0.71
HYST time (min)	47.5 (16-230)	53 (27-283)	0.45

Conclusions: Tubal contaminants are much more frequent in RBT compared to LSC. The procedure duration does not appear to contribute to contamination. Those with contaminants seem to have a higher BMI, but this is not statistically significant, possibly due to small numbers. The majority of the patients had low grade and low stage disease. All four patients with stage IIIa disease were classified as such due to positive pelvic washes, which has previously been reported with uterine manipulation. In conclusion, these data demonstrate an association of RBT and tubal contamination. The clinical significance of this remains to be determined.

1073 Correlation of Immunohistochemistry and Mutation Status of PTEN in Endometrial Carcinoma

B Djordjevic, B Hennessy, R Broaddus. M.D. Anderson Cancer Center, Houston, TX.

Background: PTEN is a tumor suppressor that negatively regulates the PI3K signaling pathway, which has been implicated in the pathogenesis of endometrial carcinoma, with PTEN mutations occurring at high frequency. With the advent of targeted therapy and PI3K inhibitors entering clinical trials, gynecologic pathologists must accurately identify endometrial cancer patients that may benefit from these new treatments. However, PTEN mutations are highly variable in type, and their detection necessitates the use of full-length sequencing, which cannot be feasibly employed in routine clinical practice. The aim of this study was to evaluate the fidelity of immunohistochemistry (IHC) for PTEN as a surrogate for assessment of PTEN mutation status.

Design: A cohort of 156 tumors comprised of 101 endometrioid and 55 non-endometrioid cases was subjected to full-length PTEN sequencing to detect mutations as well as PTEN IHC analysis. The antibody was previously validated on cancer cell lines known to be PTEN positive or negative. IHC staining was scored as positive (>90% of tumor with diffuse cytoplasmic staining), negative (0% of tumor cells staining) and heterogeneous (distinct positive and negative foci). Adjacent normal tissue was used as an internal positive control.

Results: PTEN mutation status and immunohistochemistry are summarized in the table below.

IHC	Mutation Detected	Mutation Not Detected	Total
Negative	25 (68%)	45 (38%)	70 (45%)
Heterogenous	6 (16%)	21 (18%)	27 (17%)
Positive	6 (16%)	53 (44%)	59 (38%)
Total	37	119	156

In 56% of cases in which mutations were not detected, IHC identified PTEN protein loss (negative and heterogeneous groups). In the group with a detected mutation, IHC showed a strong PTEN signal in 16% of cases. All of these cases were heterozygous at the mutation locus. By histotype, IHC detected PTEN protein loss in a substantially higher percentage of non-endometrioid tumors than did PTEN mutational analysis (41% vs. 13%) and a dramatically higher percentage of endometrioid tumors (75% vs. 29%).

Conclusions: The assessment of PTEN status by sequencing may under-estimate the number of women with endometrial cancer potentially eligible for targeted therapy using PI3K inhibitors. IHC detects more cases with PTEN loss than does PTEN full-length sequencing in addition to being less costly and labor intensive. As such, PTEN IHC represents a useful tool for the gynecologic pathologist.

1074 p27 as a Predictor of Endometrial Cancer Risk

B Djordjevic, A McCampbell, J Burzawa, K Lu, R Broaddus. MD Anderson Cancer Center, Houston, TX.

Background: p27 is a nuclear protein that inhibits cell cycle progression and is controlled by insulin and adiponectin receptor signaling through the AKT signaling pathway. Activation of the adiponectin receptors 1 and 2 (AR1 and AR2) leads to inhibition of AKT and maintains p27–mediated cell cycle control. On the other hand, insulin-like growth factor 1 receptor (IGF 1R) is up-regulated in endometrial carcinomas and is thought to drive proliferation by activating AKT, which in turn targets p27 for degradation. We have shown in endometrial cancers that p27 either has decreased nuclear expression or changes its localization to the cytoplasm. The goal of this study was to assess p27 expression as potential biomarker of endometrial cancer risk in obese women, a group at risk for endometrial cancer.

Design: 51 timed endometrial biopsies were collected on days 5-10 of the menstrual cycle from asymptomatic lean (BMI<30; n=10) and obese (BMI>30; n=41) women. Presence of proliferative-phase endometrium was histologically verified. p27 immunohistochemistry (IHC) was scored semi-quantitatively. QPCR was used to asses levels of AR1, AR2 and IGF 1R. Statistical comparisons were performed by ANOVA with significance defined as $p<0.05$.

Results: There were four distinct categories of nuclear p27 expression depending on the number of glands with p27 expression and the number of cells per gland with p27 expression. Cytoplasmic staining for p27 did not correlate with loss of nuclear staining. In the lean women, there was a statistically significant correlation between decreasing AR1 and AR2 and decreasing p27. In the obese women, absolute levels of AR1 and AR2 were similar to those of lean women with decreased p27. The obese women showed a statistically significant correlation between increasing IGF 1R and decreasing p27 levels. Absolute IGR 1R levels were dramatically increased compared to the lean group. Importantly, the lowest p27 expression category was not identified in the lean group, but was present in 19% of cases in the obese group.

Conclusions: Abnormalities in adiponectin and insulin receptor signaling networks are present in proliferative-phase endometrium of asymptomatic obese women. Their combined effect results in inappropriately low p27 levels and potentially compromised cell cycle regulation. As such, p27 may represent a better marker of endometrial cancer risk than BMI alone and thus may assist in triaging asymptomatic obese women for increased endometrial cancer surveillance.

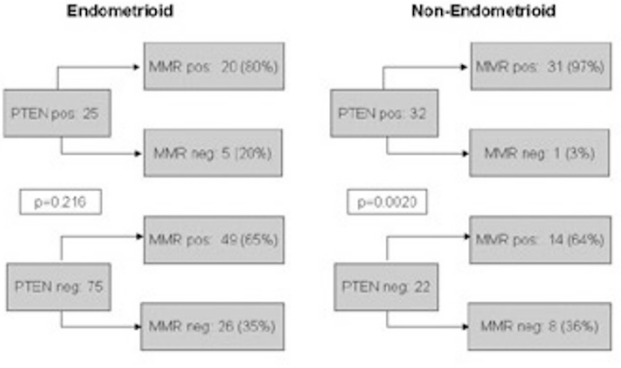
1075 Relationship between PTEN Protein Loss and Microsatellite Instability in Endometrial Carcinoma

B Djordjevic, R Broaddus. M.D. Anderson Cancer Center, Houston, TX.

Background: Mismatch repair (MMR) gene defects (especially MLH1, MSH2, and MSH6) are associated with microsatellite instability in endometrial carcinoma. Mutations in these genes occur in HNPCC, while MLH1 loss can also be found in sporadic tumors due to promoter methylation. PTEN is a lipid kinase tumor suppressor that negatively regulates the PI3K signaling pathway, which is an important driver of cell proliferation and survival. This pathway is implicated in the pathogenesis of endometrial carcinoma, with PTEN mutations occurring at high frequency. The aim of this study was to evaluate whether PTEN immunohistochemistry (IHC) could help identify endometrial cancers with microsatellite instability.

Design: IHC for PTEN and mismatch repair proteins (MLH1, MSH2 and MSH6) was performed in a cohort of 100 endometrioid and 54 non-endometrioid endometrial carcinoma cases. The patients, aged 28-92, had no known history of HNPCC. PTEN IHC was scored as positive (90% of tumor with diffuse cytoplasmic staining), negative (0% of tumor cells staining) and heterogeneous (distinct positive and negative foci). MMR IHC was scored as positive or negative. Adjacent normal tissue was used as an internal positive control.

Results: MMR loss was seen in 39 (25%) cases, of which 34 had MLH1 loss, 5 had MSH6 loss and 1 had combined MSH2/6 loss. PTEN was lost (negative and heterogeneous staining) more frequently in endometrioid tumors (75%) than their non-endometrioid counterparts (41%) ($p=0.0001$). A similar trend was observed with MMR IHC (31% and 17%, $p=0.0569$). In endometrioid carcinomas MMR loss occurred with a similar frequency in cases with PTEN loss (35%) compared to those without PTEN loss (20%) ($p=0.216$). However, in non-endometrioid tumors MMR loss was conspicuously absent in PTEN positive cases (3%) and appeared to be strongly associated with loss of PTEN (36%) ($p=0.002$).



Conclusions: For the first time, we report an interesting association of combined PTEN loss and microsatellite instability in non-endometrioid endometrial carcinomas. Therefore, positive PTEN IHC can be used to identify non-endometrioid endometrial cancer cases that do not require additional IHC for MLH1, MSH2, and MSH6.

1076 The Presence of Heterologous Elements Increases the Risk of Recurrence in Mullerian Adenosarcomas

V Dube, S Nofech-Mozes, N Ismiil, RS Saad, Z Ghorab, MA Khalifa. Sunnybrook Health Sciences Centre, Toronto, Canada.

Background: Mullerian adenosarcomas (AS) are biphasic tumors composed of benign epithelium admixed with malignant sarcomatous stroma. They can arise from the endometrium (EAS), cervix (CAS) or ovaries (OAS) and have a potential for recurrence. In this study, we summarized our experience with these tumors in a tertiary care setting.

Design: Cases of AS (1993-2009) were retrieved from our pathology archives. All available slides and patients' charts were reviewed and the following data were recorded: patients' age, symptoms, treatment, tumor size, atypia, sarcomatous overgrowth (SO),

heterologous elements, depth of invasion, lymphovascular invasion (LVI) and follow-up (FU). The association between various pathologic features and FU was analyzed using Fisher exact test.
Results: We identified 22 cases of AS (11 EAS, 8 CAS, 3 OAS) diagnosed in patients aged 33 to 66 years (median: 53 years). The main presenting symptom was abnormal bleeding for EAS and CAS and a pelvic mass for OAS. Twenty-one patients underwent hysterectomy and most of them also had BSO (19/22). One patient with OAS was treated with only salpingo-oophorectomy. Omental biopsy was performed in 10 patients (1 was positive for metastasis) and lymph nodes were biopsied in 7 (all were negative). Tumor size ranged from 1 to 16 cm (median = 6 cm). 9/19 (47.4%) cases of EAS and CAS showed no myoinvasion, 9/19 (47.4%) showed invasion limited to the inner half and only 1 (5.2%) showed invasion within the outer half. SO was present in 6 cases (27.3%) and heterologous elements in 7 cases (31.8%) (rhabdomyosarcoma, osteosarcoma, chondrosarcoma and liposarcoma). Ten cases showed mild stromal atypia, 8 moderate and 4 severe. Stromal mitotic activity ranged from 1 to 100/10HPFs (mean: 13, median: 3). Only 3 cases had LVI. FU information was available in all patients (1-117 months, median: 21.5); 17 patients were well with no evidence of disease, 5 recurred (3 EAS, 1 CAS, 1 OAS) at 7 to 42 months post-operatively. One EAS patient died of her disease. Patients with SO, myoinvasion and heterologous elements were at increased risk for recurrence, but only the presence of heterologous elements reached statistical significance (p=0.021).
Conclusions: Previous reports emphasized the presence of myoinvasion and SO as risk factors for recurrence in AS patients. Our results also show that the presence of heterologous elements is a significant histologic prognosticator for adverse outcome and therefore needs to be reported with adequate sampling of the resected specimen.

1077 HPV Is Not an Etiological Agent of Adenosarcomas of the Cervix: A PCR-Based Study of Formalin-Fixed, Paraffin-Embedded Tissue

V Dube, MA Khalifa, S Nofech-Mozes, Z Ghorab, N Ismiil, P Sun, G Koultchitski, G Yang, A Seth, RS Saad. Sunnybrook Health Sciences Centre, Toronto, Canada.
Background: Mullerian adenosarcomas are biphasic tumors composed of malignant stromal elements and benign epithelium that are mostly diagnosed in the endometrium. Rarely, adenosarcomas arise from the uterine cervix where little is known regarding their etiology. High-risk HPV is the etiological agents of most epithelial cervical cancers, but the association of HPV and cervical adenosarcomas remains unclear. We examined formalin-fixed paraffin-embedded tissue from primary cervical adenosarcomas for the presence of HPV viral DNA using PCR-based technology.
Design: In-house cases of cervical adenosarcomas accessioned between 2000 and 2009 were retrieved from the pathology archives. Adenosarcoma was diagnosed in the presence of periglandular stromal hypercellularity (cuffing), irregularly shaped glands with stromal budding (phyllodes-like pattern) and malignant stroma with atypia and mitoses (the range of atypia and mitotic activity was variable). HPV testing was performed with DNA extracted from deparaffinized sections of all cases using the Roche AMPLICOR® HPV Amplification Detection and Control Kits (Roche Molecular Systems, CA, USA), which enables detection of 13 high-risk HPV genotypes.
Results: Eight cases of cervical adenosarcomas were identified. One case showed sarcomatous overgrowth with subsequent recurrence and metastasis. In one case, the tumor involved both cervix and lower uterine segment; the case was included in the study because the bulk of the tumor was located in the cervix. PCR was successful in all 8 cases. High risk HPV DNA was not detected in any of the 8 cases.
Conclusions: Unlike in most cervical cancers, HPV infection does not appear to be a key risk factor in the carcinogenesis of cervical adenosarcomas, in keeping with the absence of epithelial dysplasia. We conclude that cervical adenosarcomas have a different carcinogenic pathway than that of most cervical cancers.

1078 Mammalian Target of Rapamycin Is Differentially Activated Leiomyosarcomas of Soft Tissue Locations Compared to Uterine Site and Benign Smooth Muscle Neoplasms

CT Elkins, T Scharschmidt, J Mayerson, OH Iwenofu. Ohio State University, Columbus, OH.
Background: The mammalian target of rapamycin (mTOR) is a protein kinase of the phosphatidylinositol 3-kinase (PI3K) signaling pathway that regulates cell growth and survival. Recent data suggest mTOR pathway is activated in leiomyosarcomas (LMS). Differences in uterine and soft issue LMS locations have not studied. In this study, we examined the differential pattern of activation of this pathway in uterine and soft tissue LMS vis-a-vis uterine and soft tissue leiomyomas using p-mTOR immunohistochemical expression.
Design: 34 cases of smooth muscle neoplasms including soft issue leiomyosarcomas (n=10), uterine leiomyosarcomas (n=10), soft tissue leiomyomas (n=4), and uterine leiomyomas (n=10) were retrieved from the OSU files. Cases were reviewed to confirm diagnoses and standard sections were stained with antibody to p-mTOR using the DAKO autostainer. Staining was evaluated semiquantatively for both quantity (%: 0,≤10%, ≤25%,≤50%, >50%) and intensity (0,1+ to 3+). Negative staining was defined as staining of 0 and 1+ <5%. Results are summarized below:

p-mTOR Expression in Soft Tissue & Uterine Leiomyosarcomas

Tumor Sites	Positve	Negative	Total
Soft tissue Leiomyosarcomas	8	2	10
Uterine Leiomyosarcomas	4	6	10

p-mTOR Expression in Benign Smooth Muscle Neoplasms of Uterine & Soft Tissue Sites.

Tumor Sites	Positive	Negative	Total
Uterine Leiomyoma (n=10)	10 (1+)	0	10
Soft Tissue Leiomyoma (n=4)	4 (1+)	0	4

Results: Soft tissue leiomyosarcomas (LMS) showed the highest expression, with 80% positivity and 50% of positive cases staining 2 or 3+ in up to 60% of tumor cells. Uterine LMS had a low expression of 40% positivity with 75% of positive cases with 1+ intensity. Chi-square analysis showed statistical significance between these two groups with p≤0.05. Weak staining was observed in the uterine leiomyoma group, with all 10 cases staining with 1+ intensity in 10-75% of tumor cells. The soft tissue leiomyomas stained similarly. There was no statistical significance between the latter two groups.
Conclusions: There is differential activation of the mTOR pathway in soft tissue LMS compared to uterine LMS's suggesting a better therapeutic benefit from mTOR inhibitor therapy for the former. Additional studies are needed to further to further define clinical subsets and molecular mechanisms.

1079 Intraoperative Cytology of Fallopian Tube

NN Esposito, PA Kruk, J Shutter, SV Nicosia. University of South Florida College of Medicine, Tampa, FL; Moffitt Cancer Center, Tampa, FL.
Background: Tubal intraepithelial carcinoma is a potential source of epithelial ovarian cancer. Consensus must be reached on the most reliable method to procure representative tubal tissue for biological studies without interference with pathological diagnosis. The aims of the current study study were to evaluate intraoperative cytology as a tool to harvest tubal tissue for pathobiological studies and to define the baseline cytology of the native tubal epithelium.
Design: Under IRB protocol, fallopian tube (FT) tissue was harvested from 12 postmenopausal women undergoing laparotomy for benign gynecologic disorders. FT tissue was procured by cytological imprints and RPMI washings of *fimbriae* and intraluminal lining, respectively. Cytological preparations were intraoperatively stained by Diff-Quik® and Papanicolaou as well as immunocytochemically for evaluation of cytokeratin (CAM 5.2), Ki67 (MIB1) and p53 expression. Cell harvests were also evaluated for total number of cells (number/FT ± SE) as well as for DNA, RNA and protein content (mean µg/FT).
Results: A total of 42,000 ± 8,500 cells/FT was harvested with greater recovery from washings (50,000 cells) than imprints (38,000 cells). Epithelial cells were uniformly cytokeratin+ with a Ki67 proliferation index of less than 5% and no p53 overexpression. Mean nuclear protein, nuclear DNA and cytoplasmic RNA contents were 1.05µg, 0.25 µg and 0.84µg/FT. Ciliated (12-30 µm) were more frequent than mucous (12-16 µm) cell populations while intercalary cells, fibroblasts and mesothelial cells were rare or absent. Epithelial cells were single or arranged in honeycombed sheets and pseudo-papillary groups (5-30/sample and 50-500 µm in size) and displayed fine chromatin, micronucleoli and rare nuclear grooves. The extracellular background was usually proteinaceous in cell imprints, watery in washings and with microcalcifications especially in fimbrial samples.
Conclusions: This study defines baseline cytological features of intraoperatively harvested fallopian tubes relevant to diagnostic pathologists and to researchers investigating the developmental biology of epithelial ovarian cancer. Initial laboratory studies show that tubal epithelial harvest is conducive to initiation of cell culture, cell immortalization and molecular biology studies. Development of further cytological criteria for tubal intraepithelial carcinoma and precursor lesions will facilitate intraoperative diagnosis and complement cancer detection by *in vivo* imaging falloposcopy.

1080 The Utility of PAX8 in Patients with Both Breast and Mullerian Carcinomas

ED Euscher, MT Deavers. The University of Texas MD Anderson Cancer Center, Houston, TX.
Background: The histologic and immunohistochemical features of breast and Mullerian carcinoma can overlap, and the distinction of metastatic carcinoma from a second primary may be problematic. PAX8, a transcription factor for organogenesis of the kidney, thyroid, and Mullerian system, has been described as a marker that distinguishes ovarian from breast carcinoma. In this study, tumors from patients with both breast carcinoma and Mullerian carcinoma (ovarian, peritoneal, or tubal) were stained with anti-PAX8 to determine its utility as a marker in a practical setting.
Design: Representative sections of the breast carcinoma and the Mullerian carcinoma from each patient were stained with anti-PAX8 (rabbit polyclonal, 1:100, Protein Tech Group, Inc Chicago, Il) using the BondMax autostainer. Staining was qualitatively assessed: negative, focal, or diffuse staining. Patient age at first cancer diagnosis, order of and interval between diagnoses of the breast and Mullerian carcinomas, and the histologic subtype of the tumors were recorded.
Results: Eight patients, 40 to 67 years old (mean 54 years) were identified. In 7 cases, the breast cancer diagnosis predated the Mullerian cancer by an interval of 2 to 10 years (mean 5.8 years). In one case, the patient presented with synchronous breast and ovarian carcinomas. Two patients had documented BRCA1 mutations, while BRCA status was unknown in 6 patients. In 3 of 7 patients treated at our institution, the primary Mullerian carcinoma had been misdiagnosed as metastatic breast carcinoma. In the eighth case, a consultation was sought for help in determining whether an ovarian tumor was metastatic breast carcinoma or a new primary. Six of the primary breast tumors were ductal carcinoma (5 invasive, 1 in-situ) and two were lobular carcinoma. All of the Mullerian carcinomas (5 ovarian, 2 peritoneal, 1 fallopian tube primary) were high grade serous carcinoma. All of the Mullerian carcinomas had moderate to strong diffuse staining for PAX8. None of the breast carcinomas had expression of PAX8.
Conclusions: The histologic distinction of metastatic breast carcinoma from primary Mullerian serous carcinoma remains problematic: nearly half of the cases in this series were initially misdiagnosed. The differential staining pattern of PAX8 in this series of patients with both serous carcinoma and breast carcinoma suggests that the marker can help distinguish a Mullerian primary from metastatic breast carcinoma. Consideration should be given to including this antibody in the evaluation of difficult cases.

1081 Papillary Endometrioid Carcinoma Is More Aggressive Than Endometrioid Adenocarcinoma FIGO Grade 2

ED Euscher, A Malpica. MD Anderson Cancer Center, Houston, TX.

Background: Papillary Endometrioid Carcinoma (PEC) is a variant endometrioid carcinoma characterized by short papillae lined by eosinophilic cells with at least grade 2 nuclear atypia and loss of nuclear polarity. Myoinvasion is often infiltrative wtih angulated, eosinophilic glands with attenuated epithelium and associated inflammation (neutrophils). Some have proposed that PEC may behave more aggressively than FIGO grade 2 endometrioid carcinoma (EC). This study compares the clinicopathologic features of PEC to a group of FIGO grade 2 EC to determine whether differences beyond morphologic appearance exist.

Design: 86 cases of PEC were retrieved (1993-2004) with slides reviewed by 2 pathologists for the following features: %PEC, depth of myometrial invasion (MI) and presence/absence of lymphovascular invasion (LVSI). Tumors with serous, clear cell or FIGO grade 3 endometrioid carcinoma were excluded. 92 consecutive ECs, FIGO grade 2, from the same period were evaluated for MI and LVSI and served as the control group. Cases with synchronous primary carcinoma or metachronous primary carcinoma within 5 years of treatment for EC were excluded. Patient (pt) age, FIGO stage, recurrence history and follow up (F/U) were obtained for both groups.

Results: Clinicopathologic features of pts with PEC and EC are summarized in Table 1 and % of PEC in relation to recurrence history is summarized in Table 2.

Comparsion of Clinicopathologic Data for Patients with PEC and EC

	Age (years)	Stage I/II	Stage III/IV	LVSI	Recurrence	F/U
PEC (n=86)	36-87 (mean, 60.8)	56 (68%)	30 (32%)	51 (59%)	21 (24%)	12 DOD (14%)
EC (n=92)	25-83 (mean, 62.5)	82 (89%)	10 (11%)	28 (30%)	10 (11%)	5 DOD (5%)

PEC, papillary endometrioid carcinoma; EC, endometrioid carcinoma; DOD, dead of disease

Percent PEC and Recurrent Disease

% PEC	Stage I/II, no recurrence	Stage I/II, recurred	Stage III/IV, no recurrence	Stage III/IV, recurred
≤25 (n=14)	7	3	2	2
26-50 (n=26)	12	3	4	7
51-75 (n=17)	9	2	5	1
>75 (n=29)	18	2	8	1

In pts with recurrent PEC, 9/21 pts had supradiaphragmatic recurrences while in pts with EC, 4/10 pts recurred had supradiaphragmatic sites.

Conclusions: PEC tends to present more frequently with advanced stage and has a higher percentage of pts with recurrent disease compared to EC. This suggests that tumors with PEC morphology may behave more aggressively than typical FIGO grade 2 EC. Because of the tendency towards advanced stage at presentation, recognition of PEC at the time of frozen section is essential in order to alert the clinican to the need for staging.

1082 Does P16 Expression in Undifferentiated Carcinoma of the Uterus Exclude Its Endometrial Origin?

H Faragalla, MA Khalifa, N Ismiil, S Nofech-Mozes, V Dube, Z Ghorab, RS Saad. Sunnybrook Health Sciences Centre, Toronto, Canada.

Background: Undifferentiated carcinoma of the endometrium is a rare neoplasm which, when involving the cervix, raises a question about tumor origin. Immunohistochemistry with p16 antibody plays a role in differentiating cervical from endometrial carcinomas since diffuse p16 positivity can be regarded as a surrogate marker of the presence of high-risk human papillomavirus (HPV) and favors the cervical origin. In this study, we investigated the expression of CK7, ER and p16 in undifferentiated endometrial carcinoma.

Design: Twenty eight cases of undifferentiated endometrial carcinoma were retrieved from the archives of anatomic pathology during the period between January 2000 and December 2007. In addition, 20 cases of high grade endometrial endometrioid adenocarcinoma and fifty cases of endocervical adenocarcinoma were included in the study as control groups. All cases were subjected to total hysterectomy and reviewed to confirm the diagnosis. Cases were stained for CK7, ER and p16. Staining was considered positive when it was cytoplasmic for CK7, nuclear for ER and both nuclear and cytoplasmic for p16. Percentage of cells staining was recorded as follow: Negative (0-5%), weak (1+, 6-25%), moderate (2+, 26-50%) and strong (3+, >50%). P16 was considered as positive if stained more 50% of the tumor cells.

Results: Majority of cervical adenocarcinomas (47/50, 94%) demonstrated diffuse/moderate-strong p16 expression with score 3+ (>50%). In undifferentiated endometrial carcinomas, 16/28 (57%) were strongly positive for p16 (score 3+, >50%). In high grade endometrioid adenocarcinoma, staining was weak or/and focal (score 1+). CK7 was positive in 10/28 (36%) of undifferentiated carcinoma, 20/20 (100%) in endometrioid carcinoma and 50/50 (100%) in endocervical adenocarcinoma. Estrogen receptors were positive in majority of undifferentiated carcinoma 24/28 (86%), all endometrioid carcinoma 20/20 (100%) and in 3/50 (6%) of endocervical carcinoma.

Conclusions: Our data indicate that p16 may play a role in the tumorigenesis of the subset of undifferentiated endometrial carcinoma. In the setting of p16 positivity, undifferentiated endometrial carcinomas are more likely to be ER positive when compared to endocervical adenocarcinomas. Distinction between undifferentiated endometrial carcinoma and endocervical adenocarcinoma, both of which can share diffuse p16 expression, should rely on detection of HPV in the latter.

1083 TTF1 Expression Is Increased with Progression to Endometrioid Carcinoma: A Tissue Microarray Analysis

RH Finck, O Dorigo, SM Dry, M Wadehra, J Braun, D Seligson, PS Sullivan. University of California, Los Angeles, Los Angeles, CA.

Background: Thyroid transcription factor-1 (TTF-1) is a transcription factor expressed in lung and thyroid tissues. Its expression by a tumor is often considered a reliable marker of origination from these tissues. However, its expression has been reported in various uterine neoplasms ranging from 22% in endometrioid carcinomas to 82% in carcinosarcomas (Zhang *et al*, 2009), suggesting its role as a marker of aberrancy with less differentiated neoplasms. We sought to determine the expression of TTF-1 in an "early" progression array of normal endometrium, hyperplasia, and endometrioid endometrial carcinoma.

Design: A tissue microarray was constructed from formalin-fixed paraffin-embedded endometrial specimens, composed of 119 endometrioid carcinomas (EC), 87 complex atypical hyperplasia (CAH), 50 complex hyperplasia (CH), 27 simple atypical hyperplasia (SAH), 138 simple hyperplasia (SH), and 336 normal endometrial controls. Specimens were sampled mostly in triplicate as 1.1 mm cores. 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 (a scale of 0-3) and percentage of endometrial glandular cells expressing TTF-1. A positive result was considered for cases with at least 1+ nuclear staining in 10% of cells.

Results: 132 (17.4%) of a total of 757 samples were considered positive. TTF-1 expression was generally nuclear and 1+ in intensity with several cases staining 2+ or more. The percentage of cases expressing TTF-1 was as follows: normal 11.3%, SH 10.8%, SAH 18.5%, CH 26%, CAH 26.4%, and carcinoma 31.9%. Statistically significant differences were found in TTF-1 expression between normal and SH to CH ($p=0.004$), CAH ($p=0.0003$), and EC ($p<0.0001$, all Chi-squared tests).

Conclusions: TTF-1 nuclear expression is seen in benign endometrium, hyperplasia, and endometrioid carcinoma. Its expression appears to increase with complex hyperplasia with and without atypia, and carcinoma, suggesting it may be associated with progression to hyperplasia and carcinoma. Nuclear expression is seen in up to 31.9% of endometrioid carcinomas (as supported by previous studies, Zhang 2009, Siami 2007, Alkushi 2003). This finding is significant as it may render the evaluation of possible metastatic lesions difficult, particularly in the case of a lung mass in a patient with a history of endometrioid adenocarcinoma.

1084 Well-Differentiated Mucinous "Adenoma Malignum" Adenocarcinoma of the Uterine Corpus: A Rare and Deceptively Bland Form of Endometrial Carcinoma

M Fujiwara, TA Longacre. Stanford University, Stanford, CA.

Background: Mucinous epithelial proliferations of the uterine corpus present diagnostic challenges due to their histologic similarity to cervical lesions. We present a distinctive endocervical-like mucinous carcinoma of the uterine corpus resembling adenoma malignum of the cervix that can be mistaken for a benign endometrial process.

Design: The clinical-pathologic features of 16 endometrial carcinomas exhibiting a pure endocervical-type mucinous proliferation, defined as mucin-containing columnar epithelial cells with basal nuclei were evaluated (9 consult and 7 in house files from 2003-2009). Hysterectomy and all available pre-hysterectomy specimens were assessed for architectural complexity (1-3; 3=carcinoma), nuclear pleomorphism (1-3; 3=carcinoma), nucleoli (1-3; 3=carcinoma), nuclear pseudostratification (simulating a villous adenoma), mitotic index, necrosis, and voluminous extracellular mucin (mucin encompassing >50% of a 40X field).

Results: Patients ranged in age from 45-70 years; 7/16 (44%) were postmenopausal. 11/16 (69%) had abnormal bleeding and 7/16 (44%) were receiving, or had been receiving hormonal therapy (1=tamoxifen, 6=estrogen and/or progesterone). Voluminous extracellular mucin was present in 9/16 (56%). Prominent neutrophils were present in 50%. The endometrial sampling specimens were cytologically bland with at most, moderate nuclear pleomorphism and moderate nuclear pseudostratification. With the exception of 2 cases (2/16; 13%), glandular architecture index was low (6/16, 38%) or borderline between low and high (8/16, 50%). A microglandular pattern was not present. Necrosis was seen in 1/16 (6%). On initial pathologic examination, 7/16 (44%) cases were misinterpreted as benign and 4/16 (25%) were classified as borderline. 5/16 (31%) cases were diagnosed as carcinoma prior to hysterectomy. 8/16 (50%) hysterectomies showed myoinvasive carcinoma (3 inner one-half, 5 outer one-half), with extensive lymphovascular involvement in 1/16 (6%) and lymph node metastasis in 1/16 (6%). Three cases had cervical stromal involvement, but a cervical primary was excluded on the basis of bulk of involvement of the corpus and/or negative p16 immunohistochemical stain.

Conclusions: Cytologically bland mucinous epithelial proliferations should be diagnosed with caution in endometrial samplings. The presence of an endocervical-like mucinous epithelial process in association with voluminous extracellular mucin should prompt consideration for a minimal deviation (adenoma malignum-type) mucinous adenocarcinoma of the uterine corpus.

1085 Early Complete Hydatidiform Mole: A Clinicopathologic and Flow Cytometric Study of 270 Cases

M Fukunaga. Jikei University Daisan Hospital, Komaeshi, Tokyo, Japan.

Background: With the increased use of ultrasound complete hydatidiform mole (CM) is being diagnosed at increasingly early stages of gestation. Thus, the classic features of CM may be lacking and CM can be easily misdiagnosed as partial mole (PM) or hydropic abortion (HA).

Design: Between 1981 and 2008, 270 cases of early CM (6-12 weeks' gestation, mean: 9.1 weeks) were identified at Jikei University Hospital and its affiliated hospitals. Clinicopathologic features of early CMs were examined. 210 cases were also analyzed by DNA flow cytometry (FC) using formalin-fixed, paraffin-embedded tissue blocks.

Results: Patients ages ranged from 18 to 49 years (mean: 29.1 years). Histologically, villi showed diffuse or focal stromal edema with bulbous or clubbed outlines and focal to circumferential trophoblastic hyperplasia. Villous stroma was hypercellular with capillary networks and karyorrhectic debris was observed. No stromal fibrosis was observed. Extravillous intermediate trophoblasts showed atypia. There were no fetal parts or amnion. Two patients had twins with CM. These cases were histologically characterized by two populations of villi, one with non-edematous stroma and another

with edema and moderate trophoblastic hyperplasia. No nucleated red blood cells were observed except in the two cases of twins. Substantial cases were initiallly diagnosed as PM. By DNA FC, 155 cases were diploid, 29 tetraploid and 26 (non-triploid or non-tetraploid) aneuploid. There were no histologic differences among the diploid, tetraploid and aneuploid CMs. In the follow-up information, 14 of 73 diploid CMs (19.2%) and 4 of 18 tetraploid CMs (22.2%) had invasive mole, and one each with diploid and tetraploid CMs developed choriocarcinoma. None of 18 patients with aneuploid CM had sequelae.

Conclusions: Early CMs have somewhat different histologic features than classical second-trimester CMs. The histologic features include: 1) bulbous or clubbed villi, 2) focal or diffuse villous edema, 3) focal to circumferential trophoblastic hyperplasia, 4) cellular villous stroma with capillary networks and karyorrhectic debris, 5) no stromal fibrosis, 6) atypia of extravillous intermediate trophoblasts. Early CMs can be easily misdiagnosed as PM because of less prominent villous edema and mild form of trophoblastic hyperplasia. There were no histologic differences among the diploid, tetraploid and aneuploid CMs. DNA FC is helpful in equivocal cases. Aneuploid CMs are associated with lower risk for persistent disease than diploid or tetraploid CMs.

1086 Squamous Cell Carcinoma of the Vulva: Clinicopathological Correlations and Prognostic Significance of HPV Infection and FIGO Staging

V Fuste, I Alonso, P Castillo, M del Pino, A Torne, J Ordi. Hospital Clinic, IDIBAPS, Barcelona, Spain.

Background: Invasive vulvar squamous cell carcinoma (VSCC) accounts for more than 90% of malignant vulvar neoplasms, but it represents only 3-5% of all cancers of the female genital tract. Based on their etiopathogenesis VSCCs have been divided into two groups with different clinical and pathological features. One group expresses sequences of high-risk human papillomavirus (HPV), whereas the other arises via independent-HPV pathways. The prognostic significance of these pathological groups is still controversial. In this study we evaluate the prognostic implications of HPV infection and surgical staging in a series of VSCCs, treated at a single institution.

Design: All VSCCs diagnosed at the Hospital Clinic of Barcelona from 1990 to 2007 were retrospectively evaluated (n=94). Clinical and pathological data were reviewed. HPV infection was determined and typed by amplification of HPV DNA by PCR using the SPF-10 primers. p16INK4a and p53 expression were determined by immunohistochemistry. Patients were followed-up 1-237 months (median 25 months). Overall and progression-free survival for HPV-positive and –negative patients and for stages I to IV were estimated by Kaplan-Meier analysis and by the use of a multivariate Cox proportional hazard's model.

Results: HPV DNA was detected in tumor tissue of 18/94 (19%) patients. HPV16 was the most prevalent viral type. HPV-negative women were significantly older than HPV-positive patients (77.4±11.2 vs. 65.0±22.3, p<.001) Immunohistochemistry for p16INK4a stained all HPV-positive and one of 76 HPV-negative tumors (100% vs. 1.3%, p<.001). p53 was positive in 1/18 HPV-positive tumors and 54/76 HPV-negative (5.5% vs. 71.0%, p<.001). No differences in FIGO staging between HPV-positive and –negative patients were observed (stage I: 22.2% vs. 31.6%; stage II: 27.6% vs. 33.3%; stage III: 34.2% vs. 22.2%; and stage IV: 6.6% vs. 22.2%; p=.320). Both progression-free and overall survival were associated with FIGO staging (p<.001, log-rank test). In contrast, no differences either in progression-free and overall survival were observed when comparing HPV-positive and –negative cases.

Conclusions: Both progression-free and overall survival are related to FIGO staging and not to HPV infection. Nevertheless, the results are limited due to the small number of HPV-positive tumors observed in most series.

1087 Does the LSIL-H Designation in Pap Tests Impact Patient Management?

SK Galfione, DA Smith, MJ Thrall. The Methodist Hospital, Houston, TX.

Background: There has been some controversy regarding the use of the interpretive category Low Grade Squamous Intraepithelial Lesion (LSIL) cannot exclude High Grade Squamous Intraepithelial Lesion (LSIL-H) in Papanicolaou (Pap) tests. Since ASCCP guidelines recommend biopsy for all LSIL (except in adolescents and menopausal women), the clinical significance of this category is unclear. LSIL-H is not currently endorsed by the Bethesda System and is only currently used in some laboratories. This study aims to determine whether the designation of LSIL-H changes the gynecologic management of patients.

Design: We identified all LSIL and LSIL-H Pap tests in women with no prior abnormal Pap tests or cervical biopsies from 5/1/2007 to 10/31/2008. The total Pap test volume for this period was 67,223 (80% ThinPrep and 20% SurePath), of which 4.3% were LSIL. Biopsy and Pap test follow-up was compiled from 5/1/2007 to 9/30/09.

Results:

Table 1: All Women

	total	biopsy as first follow-up	pap as first follow-up	CIN 2-3
LSIL-H	66	44 (66.7%)	7 (10.6%)	15
LSIL	1249	618 (49.5%)	208 (16.7%)	47

Table 2: Women Age 21-Menapause

	total	biopsy as first follow-up	Pap as first follow-up	CIN 2-3
LSIL-H	51	34 (66.7%)	5 (9.8%)	11
LSIL	854	470 (55.0%)	133 (15.6%)	36

Women with LSIL-H were significantly more likely to get a follow-up biopsy than women with LSIL (p=0.0094). They also had a much higher incidence of high grade dysplasia found (p<0.001).

Conclusions: The LSIL-H category helps to identify a subset of patients at greater risk of high grade dysplasia. Gynecologists at our institution are more likely to aggressively follow up a patient with LSIL-H, resulting in more biopsies.

1088 PAX2, a Sensitive and Specific "Negative" Marker To Differentiate Diffuse Malignant Mesotheliomas of Peritoneum from Serous Carcinomas of Müllerian Origin

FF Gao, AM Krasinskas, M Chivukula. University of Pittsburgh Medical Center, Pittsburgh, PA; Magee-Women's Hospital, University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: Mesotheliomas are rare neoplasms of serosal surfaces (pleura followed by the peritoneum, pericardium and tunica vaginalis of testis). Peritoneal mesotheliomas (PM) accounts for 10% of all mesotheliomas and are often disseminated in the peritoneal cavity as multiple nodules including localized masses in the ovaries that are clinically and histologically similar to serous adenocarcinomas of müllerian origin. It is important to differentiate these tumors given their diverse responses to the chemotherapy and/or radiotherapy. Several immunohistochemistry markers including calretinin and Wilm's tumor gene 1 (WT-1) are used in combination as positive/negative markers in a panel in distinguishing these tumors. PAX2 gene encodes a transcription factor and it was recently demonstrated in benign epithelial cells of the female genital tract as well as serous carcinomas of müllerian origin. The aim of our study is to determine the sensitivity/specificity of PAX 2 in definitely differentiating PM from serous carcinomas.

Design: Twenty-five (25) cases of PM were retrieved from our pathology case archives. The cases were represented on a single tissue microarray (TMA) with 3-fold redundancy (TMA-1). In comparison, forty-seven (47) cases of serous carcinomas of müllerian origin [fallopian tube (26), ovary (11), and peritoneum (10)] were retrieved. All cases were stained with rabbit polyclonal PAX2 antibody, WT-1 and calretinin. Only nuclear staining was considered positive for PAX 2 and WT-1. Cytoplasmic/nuclear staining is considered positive for calretinin.

Results: All PM were entirely negative for PAX2 (0%, 0/25) in contrast to 80% (38/47) of serous adenocarcinomas of müllerian origin expressed PAX2. WT-1 expression was seen in 92% (23/25) of PM and 100% (47/47) in serous carcinomas of müllerian origin. Calretinin positivity was seen in 88% (22/25) of PM while all serous carcinomas of müllerian origin were entirely negative (0%, 0/47).

Conclusions: Our results demonstrate that 1) PAX 2 is a "negative"marker for PM while Calretinin is a "negative" marker for serous carcinomas of müllerian origin. 2) Although other immunohistochemistry markers such as WT-1 are essential for making the diagnosis of PM or serous carcinomas of müllerian origin, the combination of PAX 2 and Calretinin is a valuable tool to differentiate this two histological similar diseases efficiently.

1089 Pathologic Correlation between Abnormal Endocervical Curettage (ECC) and the Follow-Up Loop Electrosurgical Excision Procedure (LEEP) and Cold Knife Conization

X Gao, JG Lake, G Birdsong. Emory University of Medicine, Grady Memorial Hospital, Atlanta, GA; Emory University School of Medicine, Atlanta, GA.

Background: Diagnosis of ECC specimens can be challenging for pathologists because specimens are typically small, poorly orientated tissue fragments. Nevertheless, positive results have significant clinical implications. This study compares the diagnoses of positive ECCs and the corresponding follow-up LEEP/conizations specimens.

Design: 170 specimens accessioned between 2004 and 2008 were selected. Selection criteria were positive ECC, no concurrent ectocervical biopsy during the colposcopy, and follow-up with LEEP/conization within 1 year of the ECC. ECC diagnoses include squamous dysplasia-cannot be graded, CIN I-III, and invasive lesions. Exactly matching ECC and LEEP were categorized as consistent. CIN I ECC followed by CIN II or higher LEEP was regarded as underscored; CIN II or higher ECC followed by a lower grade or negative LEEP was regarded as overscored. A diagnosis of "squamous dysplasia, cannot be graded" was arbitrarily interpreted as CIN II-III. Significant discordancy was designated as CIN I in the ECC with CIN II or higher LEEP, or CIN II or higher ECC with negative or CIN I LEEP. Bivariate significance tests were used to analyze the possible relationship between discordant results and patient age, time between ECC and LEEP, the grade of dysplasia in ECC, and whether the same pathologist reviewed the ECC and LEEP.

Results: 86 of 170 (51.6 %) patients had discordant results; of those, 54 were overscored and 32 underscored. Significantly discordant overscored diagnoses were found in 24 of 54 (44.4%) ECC; of those, the majority patients were 40 or older (16 of 24). Of 32 underscored ECC patients, significant discordancy was found in 24 (75%), and the majority were < 40 years old (18 of 24). An ECC with CIN I or II was more likely to be discordant than an ECC with CIN III. ($p < 0.05$). In addition, ECCs from patients 40 or younger were more likely to be discordant and to be underscored ($p < 0.05$). The other parameters motioned above were not significantly associated with discordant diagnoses.

Conclusions: Diagnoses for ECCs and subsequent LEEP/conization specimens are discordant in a substantial percentage of cases. ECCs with lower grade dysplasia or from younger patients are associated with higher rate of discordant diagnoses. ECCs from younger patients are also at higher risk to be underscored than those from older patients.

1090 PTEN Immunohistochemistry in Endometrial Carcinomas: Correlation with Mutation Analysis

K Garg, R Soslow, C Aghajanian, SU2C MSKCC Team, D Levine. Memorial Sloan Kettering Cancer Center, New York.

Background: With evolving knowledge about molecular pathways involved in endometrial carcinogenesis, new drugs, including mTOR inhibitors and PI3K inhibitors, are being explored as potential therapeutic targets in endometrial carcinomas (EC). Biomarker development that could predict response to such therapeutic agents is important. PTEN is frequently inactivated in ECs, but antibodies for PTEN IHC have been difficult to work with and have shown variable results. Our aim was to assess PTEN mutation and IHC status in different types of ECs.

Design: Only ECs that had both PTEN IHC and mutational analysis data were included in this study. PTEN IHC was performed using monoclonal PTEN antibody from DAKO (clone 6H2.1). PTEN staining was assessed in nuclei and cytoplasm. Complete absence of staining in tumor cells in the presence of internal positive control (stromal cells, lymphocytes) was interpreted as PTEN loss. Cases with any staining were interpreted as PTEN positive. PTEN mutational analysis was performed.
Results: 29 ECs with PTEN IHC and mutation analysis were included in this study. Of the 15 FIGO grade 1-2 endometrial endometrioid carcinomas (EECs), 8 showed PTEN loss by IHC and PTEN mutations were noted in 4 cases (of these 4, 3 showed PTEN IHC loss while 1 case had positive PTEN IHC). Of the 4 FIGO grade 3 EECs, 3 showed PTEN IHC loss, and all 3 of these tumors showed PTEN mutations. Of the 9 type 2 carcinomas (8 serous and 1 carcinosarcoma), 3 showed IHC loss and none showed mutations in PTEN. One EC with endometrioid and undifferentiated components showed IHC loss and PTEN mutation. Loss of PTEN by IHC did not correlate with the presence of PTEN mutations. Cases with retained PTEN IHC almost always lacked PTEN mutations.

PTEN IHC and mutation analysis

	FIGO 1-2 EEC (15)	FIGO 3 EEC (4)	SEROUS/ MMMT (9)	UNDIFFERENTIATED (1)
IHC PTEN loss	8	3	3	1
PTEN mutation (IHC status on cases with mutation)	4 (3 IHC loss, 1 IHC positive)	3 (3 with IHC loss)	0	1 (1 with IHC loss)

EEC: endometrial endometrioid carcinoma, MMMT: carcinosarcoma

Conclusions: PTEN mutations appear to be a common event in type 1 ECs, none of the type 2 ECs showed PTEN mutations. There is lack of correlation between PTEN IHC and mutation analysis. Some ECs with PTEN loss by IHC do not have detectable mutations while presence of PTEN IHC staining usually correlates with absence of mutations. PTEN IHC loss in cases without mutations may be due to epigenetic changes such as methylation, these mechanisms of PTEN inactivation should be explored further.

1091 Focal Glandular Crowding in Endometrial Specimens: What Does It Mean?

AS Godambe, V Zayat, P Rajan, A Salhadar, C Ersahin. Loyola University Medical Center, Maywood, IL.

Background: In the daily practice of gynecologic pathology, there are several instances where we find clusters of crowded glands without any atypia that do not fit into any diagnostic category. Many times in these instances, the pathologist makes a descriptive diagnosis of focal glandular crowding (FGC). This term is ambiguous, and leaves the gynecologist without any direction for decisive decision making. The significance of FGC is unknown.
Design: We performed an in-house search in endometrial biopsies or hysterectomy specimens with FGC in the final diagnosis. Sixty-two (n=62) such cases were found. We measured the cross sectional area and diameter of the glandular crowding. Since majority of FGC cases can be a feature of disordered proliferative endometrium (DPE) or simple hyperplasia (SH), the specimens were assessed for both DPE and SH. DPE usually shows focal cystic dilation of glands, and is diffuse in SH. In SH, ciliated cell change is common, while it is not a known feature of DPE. For each of the patients with a diagnosis of FGC, all subsequent gynecological specimens were reviewed.
Results: Out of the 62 cases, 10% of cases (6/62) showed progression in subsequent specimens, ranging from simple hyperplasia without atypia to adenocarcinoma. Retrospectively, SH without atypia is favored to DPE in initial biopsies. 34% (21/62) cases showed no progression to hyperplasia, and 56% (35/62) had no follow-up biopsies. Cystic dilation was focal (individual glands) in 30 cases, and more diffuse (with small to large variation in size) in 32 cases. Ciliated cell change was noted in 19 cases, and out of these 1 showed progression to simple hyperplasia. The surface area of FGC ranged from 0.16mm² to 4.97 mm², and the lesion diameter ranged from 0.12mm to 2.62mm.
Conclusions: Focal glandular crowding is a term used commonly by the pathologist, and interpreted widely by the gynecologist. FGC diagnosis alone cannot be interpreted as a marker of either benignity or malignancy. Pathologists should try to classify these lesions as DPE, SH or other. Diffuse cystic dilation of glands, ciliated cell change and larger lesions favor SH over DPE. Since significant number of FGC patients in our study received no follow-up biopsies, pathologists must clearly communicate with the clinician if SH is favored. The presence of subsequent hyperplasia and adenocarcinomas in 10% (6/62) of the patients with FGC stresses the need for clinical and histological follow-up on these patients.

1092 FIGO Grading and Binary Grading Systems for Endometrial Carcinoma in Biopsy and Hysterectomy

H Guan, S Bandyopadhyay, L Fathallah, J Feng, I Khalifeh, B Albashiti, M Ibrahim, K Hayek, A Munkarah, R Ali-Fehmi. Wayne State Univ, Detroit; Henry Ford Hospital, Detroit; St. John Hospital, Detroit; American Univ of Beirut, Beirut, Lebanon.

Background: The FIGO grading is the most widely accepted system for grading endometrial carcinoma, but has been critiqued for poor inter-observer reproducibility. Modified binary FIGO and other binary grading systems were proposed recently. The aim of this study is to evaluate prognostic power and correlation of FIGO, modified binary FIGO and Alkushi grading systems in biopsy and hysterectomy.
Design: Between 1995-2007, 271 patients (pts) underwent hysterectomy for endometrial carcinomas with matched survival information were identified. Only 58 of 271 pts had biopsy slides available for evaluation. The morphologic criteria used for Alkushi grading system were solid or papillary pattern, severe nuclear atypia and equal/more than 6 mitosis/10 HPF. A high grade tumor had at least two out of three features, while low grade had at most one. All tumors were also assigned a three-tier FIGO grade (I, II, III) and a modified binary FIGO grade (I&II low grade, III high grade). To evaluate Alkushi and FIGO grading systems, we used COX proportional hazards and chi-Square.
Results: Applied to all tumor cell types or endometrioid carcinomas only, Alkushi, FIGO and modified binary FIGO grading systems were independent predictors for survival ($P<0.05$) in both biopsy and hysterectomy. All three grading systems had suboptimal agreement rates between biopsy and hysterectomy specimens, 74% (43/58) for Alkushi, 69% (40/58) for modified binary FIGO, and 67% (39/58) for three-tier FIGO grading system. Among 15 pts who had discordant Alkushi grades in biopsy and hysterectomy, 5 pts were up-graded and 10 pts were down-graded in hysterectomy. Discrepancies in architectural pattern, nuclear atypia and mitosis all contributed similarly to the discordant Alkushi grades in biopsy and hysterectomy. Among 19 pts who had discordant three-tier FIGO grades in biopsy and hysterectomy, 18 of them had a grade change involving FIGO grade 2 (either down or up graded in hysterectomy).
Conclusions: FIGO grading system demonstrates unequivocal prognostic value for patients with endometrial carcinoma. However, poor correlation of grades in biopsy and hysterectomy specimens persists in three grading systems we tested. Ultimate Grade and risk estimates in patients with endometrial carcinoma should be determined in hysterectomy rather than biopsy.

1093 Predictors of Adverse Outcome in Smooth Muscle Tumors of Uncertain Malignant Potential (STUMP)

M Gupta, BJ Quade, MR Nucci. Brigham & Women's Hospital, Boston, MA.

Background: Current WHO classification defines STUMP as tumors that cannot be diagnosed reliably as benign or malignant on the basis of generally accepted criteria. This has led to different criteria being used; therefore, reliable outcome data is lacking. The aim of our study was: 1) to compare the frequency of adverse outcome in STUMP based on our interpretation of published criteria; and 2) to perform failure analysis to identify feature(s) helpful in predicting outcome.
Design: Cases of STUMP between 1983-2008 were retrieved and follow up data collected. Morphological parameters (procedure type, age, lesion number and size, margins, cellularity, degree of cellular atypia, maximum mitosis/10HPF, and presence of necrosis or atypical mitoses) were scored and correlated with outcome.
Results: 20 subjects with a median follow up of 56 (range 12- 139) months formed the final study group. Age ranged from 31-60 yrs (median 47). 12 subjects underwent hysterectomy and 8 had myomectomy. The degree of nuclear atypia ranged from absent (2) to severe (6). Maximum mitotic count ranged from 0-8/10 HPF ; 4 cases had atypical mitoses. Necrosis was present in 7 lesions (1"geographic", 2 ischemic, 4 indeterminate). Vascular intrusion was present in 1 case and 5 had infiltrative/ irregular borders. 15 (75%) subjects are alive with no evidence of disease (ANED). Adverse outcome was noted in 5 (25%) cases. Both cases with local recurrence (one as STUMP at 2 yrs and another as recurrent STUMP and then as LMS at 2.5 and 7 yrs, respectively) were treated with myomectomy and are ANED. 3 cases had metastasis and 2 were ANED at 5 and 3 years after metastasis, while the third died of disease. Of these cases with adverse outcome (n=5), notable features included moderate-severe nuclear atypia (5), focal epithelioid features (2), infiltrative/irregular margins (2), ≥5 mitoses/10HPF (1), atypical mitoses (1), and vascular intrusion (1).
Conclusions: The frequency of adverse outcome in our series was 25%, which is higher than previously published data (7-16%), suggesting that more stringent criteria can exclude some from further follow-up. Although "significant" nuclear atypia was not discriminatory in our series, its frequent association with adverse outcome has pathobiological implications. Ambiguous necrosis usually was associated with benign outcome, but was seen in the single fatal case. Atypical mitoses, epithelioid differentiation, vascular involvement and myometrial invasion appear to herald adverse outcomes, and therefore merit inclusion into the diagnostic regimen.

1094 Epithelial Membrane Protein-2 Expression Is an Early Predictor of Endometrial Cancer Development

O Habeeb, L Goodglick, RA Soslow, R Rao, O Schirripa, S Horvath, J Braun, DB Seligson, M Wadehra. David Geffen School of Medicine at UCLA, Los Angeles, CA; Memorial Sloan-Kettering Cancer Center, New York, NY.

Background: Endometrial cancer (EC) is a common malignancy worldwide. It is often preceded by endometrial hyperplasia, whose management and risk of neoplastic progression vary. Previously, we have shown that the tetraspan protein, Epithelial Membrane Protein-2 (EMP2) is a prognostic indicator for EC aggressiveness and survival (Wadehra M, Natarajan S, Seligson DB, *et al*. 2006). Here we examine whether EMP2 expression within preneoplastic lesions is a prognostic biomarker for EC development.
Design: A tissue microarray was constructed with specimens from 207 patients. Staining intensity of EMP2 levels by immunohistochemistry was scored from 0 to 3. Appropriate statistical analyses revealed differences in EMP2 expression by histology, along with its impact on the risk of endometrial cancer development. Progression-related criteria and analyses focused on cases whose clinical outcome was known after at least one year to exclude underlying carcinoma (Lacey JV, Jr., Ioffe OB, Ronnett BM, *et al.* 2008; Kurman RJ, Kaminski PF, Norris HJ 1985).
Results: A single, mean pooled value for EMP2 across multiple, informative spots within each histology of a case was generated. By doing so, we discovered a step-wise, significant increase in the average spot- and case-level expression of EMP2 (see Figure 1). Moreover, detailed analysis of EMP2 expression in premalignant cases demonstrated that EMP2 positivity was a strong predictor for EC development (see Figure 2).
Conclusions: Combined with our previous findings, these results suggest that EMP2 is a novel biomarker for EC development and progression.

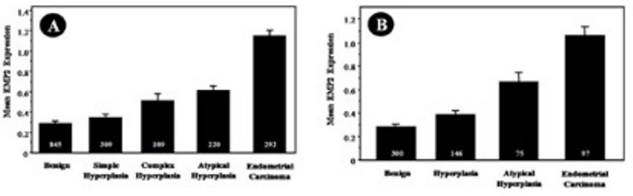


Figure 1. EMP2 expression stratified by histology. (A) EMP2 expression distribution as classified by spot-level histologies (Kruskal-Wallis P < 0.0001 across all histologies); Mann-Whitney P-values: benign versus simple hyperplasia, P = .11; benign versus complex hyperplasia, atypical hyperplasia, or endometrial cancer, P < 0.0001; simple hyperplasia versus complex and atypical hyperplasia, P = 0.0055 and P < 0.0001, respectively; simple hyperplasia, complex hyperplasia, or atypical hyperplasia versus endometrial cancer, P < 0.0001; complex hyperplasia versus atypical hyperplasia, P = .33). (B) EMP2 expression distribution as classified by ease-level histologies. "Hyperplasia" refers to both simple and complex variants (Kruskal-Wallis P < 0.0001 across all histologies; Spearman correlation = 0.480, P < 0.0001; Mann-Whitney P-values: benign versus hyperplasia, P = .0056 (benign versus simple hyperplasia, P = .09 & benign versus complex hyperplasia, P = .0013, data not shown); benign versus atypical hyperplasia or endometrial cancer, P < 0.0001; hyperplasia versus atypical hyperplasia, P = 0.014, hyperplasia or atypical hyperplasia versus endometrial cancer, P< 0.0001).

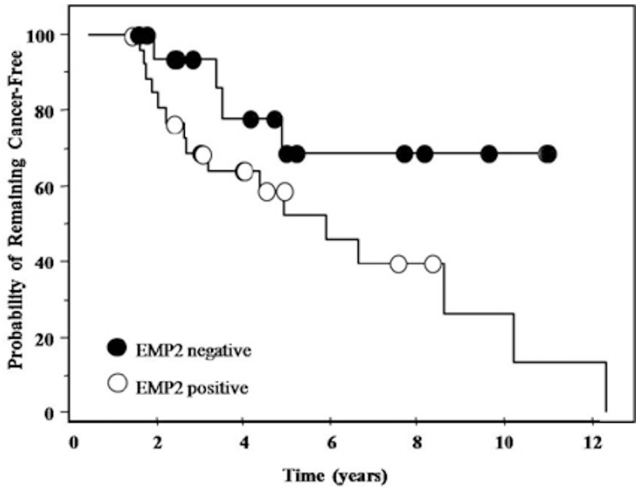


Figure 2. EMP2 Expression Levels in Hyperplastic Lesions Predict Future Tumor Development. Kaplan-Meier survival plot showing ○ positive EMP2 expression (n=26) versus expression which is ● negative or below the level of detection (n=19) in hyperplastic lesions. The breakdown of histologies for the positive EMP2 group was 16 simple hyperplasia, 6 complex hyperplasia, and 4 hyperplasia with atypia. The breakdown for the EMP2 negative group was 18 simple hyperplasia, 1 complex hyperplasia, and no hyperplasia with atypia. Note that women with positive EMP2 expression in preneoplastic lesions have a greater probability of developing EC compared to similar lesions in which EMP2 cannot be detected (Logrank P = 0.05). Similar results were obtained when comparing EMP2 expression as a continuous variable to EC development (Cox Model HR = 3.64, 95% CI 1.16-11.4, P = 0.03).

1095 Resolving the Predictive Ability of Simple Hyperplasia

O Habeeb, DB Seligson, CH Ersahin. Loyola University Medical Center, Maywood, IL; David Geffen School of Medicine at UCLA, Los Angeles, CA.

Background: Simple hyperplasia (SH) is an early, preneoplastic lesion of the endometrium. Previous studies have attempted to characterize the rate (Kurman RJ, Kaminski PF, Norris HJ, 1985) and risk (Lacey JV, Jr., Ioffe OB, Ronnett BM, *et al.,* 2008) of progression from SH to endometrial cancer (EC). However, studies like these have examined the progression of SH alone, as it directly relates to EC. In contrast, our study significantly refines the prediction of SH progression by first determining whether a woman's first SH regresses or not.

Design: We performed a metachronous, retrospective data analysis of selected cases from the David Geffen School of Medicine at UCLA (1982-2002) and Loyola University Medical Center (1988-2008). Included in the analysis were patients with at least three endometrial samplings during their follow-up at each institution, along with those who fulfilled established progression criteria (Lacey JV, Jr., *et al*., 2008). 'Benign' endometrium consisted of non-hyperplastic, non-neoplastic samples.

Results: Women within the UCLA group, whose first SH reverted to benign endometrium on the subsequent biopsy, were at lower risk of developing endometrial cancer (Log-rank P = .0342, N = 26). In addition, when the Loyola group was combined with the UCLA group, the significance of the relationship was maintained (Log-rank P = .0358, N = 44; see Figure 1).

Conclusions: Our predictive model suggests that women whose first SH regresses to benign endometrium are at lower risk of developing cancer over time. The preservation of the significant trend in the combined group suggests that the results are more likely to be robust and merit further investigation. Potentially confounding clinical factors are addressed and discussed.

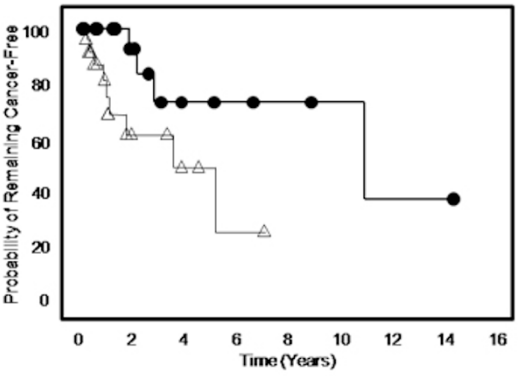


Figure 1. Regression from SH to benign helps predict lower likelihood of tumor development. Women from UCLA & Loyola were combined in this Kaplan-Meier analysis. Those whose first SH reverted to histologically benign endometrium ● tended to remain cancer-free (Log-rank P = .0358, N = 44), with nearly a 4-fold reduction in their relative likelihood of developing EC over women △ whose first SH persisted or progressed (Cox Model HR = 0.267, 95% CI = 0.071-0.998; P = .0497).

1096 Between-Site Variability in Immune Response to Metastatic Serous Ovarian Carcinoma

IS Hagemann, AR Hagemann, P Lal, G Coukos, MD Feldman. University of Pennsylvania, Philadelphia, PA.

Background: The presence of tumor-infiltrating lymphocytes (TILs) in epithelial ovarian carcinoma predicts significantly improved survival. Immunomodulation might thus offer important therapeutic opportunities in ovarian cancer. It is not well established, however, whether the presence, distribution, or activity of these TILs is different in metastatic tumor deposits as compared to the primary lesion. Between-site heterogeneity could explain the resistance of certain tumor sites to immunotherapy and provide guidance for treating residual disease.

Design: We retrieved a series of 53 cases of metastatic serous ovarian cancer (stages IIIC and IV) resected at our institution between 2005 and 2008. H&E-stained slides were reviewed and blocks were selected to construct a tissue microarray of primary and metastatic tumors. Immunostains for immune markers CD3 (pan T lymphocyte), CD8 (cytotoxic T lymphocyte), and FoxP3 (T regulatory lymphocyte) were performed. Staining in three cores from a primary tumor and two metastases (nine cores/case) was semi-quantitatively scored using a grading rubric.

Results: TIL counts were higher at metastatic sites than at primary sites (mean score 1.61 versus 1.42; p=0.015 by Mann-Whitney test). For tumor infiltration by CD3+ lymphocytes, there was significant variation between cases (accounting for 47% of the total variance in TIL counts; p<0.0001 by 2-way ANOVA), while the interaction between case and site accounted for a further 30% of the total variance (p<0.0001). Similar results were obtained for FoxP3 and for CD8. The degree of between-site heterogeneity, as assessed for each case by taking the SEM of the TIL scores at each site, was normally distributed by D'Agostino-Pearson test.

Conclusions: The density of TILs in all three populations (CD3+, CD8+, FoxP3+) differed significantly from case to case and from site to site within a given case. Some cases demonstrated greater between-site heterogeneity in the density of TILs. In terms of their propensity to exhibit between-site variation, the cases showed a continuum of behavior rather than a bimodal distribution. We conclude that assessments of tumor immune response derived from a single tumor sample may be inaccurate; further, tumors with greater between-site heterogeneity may be expected to display unique biology, potentially including resistance to immunomodulatory therapy.

1097 Endometrial Adenocarcinoma with Clear Cell Morphology: Interobserver Variability and Immunohistochemical Analysis Using Hepatocyte Nuclear Factor -1β

G Han, D Delair, CB Gilks, M Kobel, PB Clement, AD Tabrizi, RA Soslow. Memorial Sloan-Kettering Cancer Center, New York; University of British Columbia, Vancouver, Canada; Tabriz University of Medical Science, Tabriz, Islamic Republic of Iran.

Background: Accurate subtyping of endometrial adenocarcinoma remains a challenge. We investigated interobserver variability in diagnosing endometrial carcinoma with clear cell morphology and the usefulness of HNF-1β for diagnosis of endometrial clear cell carcinoma (CC).

Design: 44 cases of endometrial adenocarcinoma originally diagnosed as CC, mixed clear cell carcinoma (MEC), or non-clear cell carcinoma (NCC) with clear cell changes were reviewed by one gynecologic pathologist (the reviewer) and reclassified into 4 groups: CC (n=12), NCC (n=22), MEC (n=2), and not sure (NS, n=8). Cases were then reviewed by 5 pathologists (the panel) using WHO criteria, without knowing

original or reviewer diagnoses. Interobserver reproducibility was evaluated by kappa analysis. Immunohistochemistry for HNF-1β was performed. Results were recorded as the percentage of positive tumor cells multiplied by stain intensity (0-absent, 1-weak, 2-moderate, 3-strong).

Results: There was only moderate agreement among panelists in 3 of 4 diagnostic subgroups (kappa=0.56 for CC, 0.57 for NCC, 0.53 for NS, 0.65 for MEC). 10 cases (91%) with an HNF-1β score > 150 were diagnosed as CC by the reviewer or by a majority of panelists (5 of these cases were diagnosed as CC by both the reviewer and a majority of panelists). One case (9%) was diagnosed as NS by the reviewer and NCC by a majority of panelists. Of 31 cases with an HNF-1β score <150, 1 case (3%) was diagnosed as CC by the reviewer and a majority of panelists; 3 cases (10%) were diagnosed as NS by the reviewer and CC by a majority of panelists; 19 cases (61%) were diagnosed as NCC by both the reviewer and a majority of panelists; 8 cases (26%) were diagnosed as NCC by either the reviewer or a majority of panelists. The clear cell and non-clear cell areas in two MEC cases showed different HNF-1β scores, 160 and 0, respectively, in one case, and 290 and 90, respectively, in the other.

Conclusions: Only moderate interobserver agreement was noted in the evaluation of endometrial CC, unlike ovarian CC where interobserver agreement is reportedly substantial to almost perfect. Despite that, diffuse HNF-1β staining defines a homogeneous group of carcinomas with clear cells that with only rare exceptions represent pure CC. Weak or negative HNF-1β staining is encountered predominantly in NCCs.

1098 Hypoxia-Inducible Factor (HIF) and Mammalian Target of Rapamycin (mTOR) Pathway Markers in Ovarian Clear Cell Carcinoma

G Han, RA Soslow, VE Reuter, E Oliva, CB Gilks, H Asad, SK Tickoo. Memorial Sloan-Kettering Cancer Center, New York; Massachusetts General Hospital, Boston; Vancouver General Hospital, Vancouver, Canada.

Background: Studies report that ovarian clear cell carcinoma (OCC) have gene expression profiles similar to clear cell renal cell carcinoma (CRCC). Targeted therapies against HIF and mTOR pathway molecules have recently shown to be effective in metastatic CRCC. We studied the expression of HIF and mTOR pathway markers in OCC to investigate the justification for potential utility of similar therapies.

Design: Immunohistochemistry using antibodies against HIF pathway markers HIF-1α, vascular endothelial growth factor-receptor 2 (VEGF R2), carbonic anhydrase IX (CA IX), and activated mTOR pathway markers phospho-S6 (p-S6) and phospho-4E biding protein 1 (p-4E BP1) were performed on tissue microarrays consisting of OCC (n=64). Positivity was graded as 0-3 (0 = 0-5%, 1 = 6-25%, 2 = 26-50%, 3 >50% tumor cells positive) for p-S6 (cytoplasmic), p-4E BP1 (nuclear and/or cytoplasmic), HIF-1α (nuclear), and CA IX (membranous). VEGF R2 was graded on the intensity of cytoplasmic staining in tumor vessels (0 = absent, 1 = weak, 2 = intense).

Results: The percentage of cases showing ≥2 staining for HIF-1α, VEGF R2, p-S6, and p-4E BP1 are comparable to those previously reported in CRCC (Table 1). Similar to CRCC, VEGF R2 positivity was observed in OCC tumor vessels, not in tumor cells. CA IX positivity was observed only in a small number of cases. Spearman's correlation coefficient analyses indicated significant relationships ($p < 0.05$) between the expression of HIF1α and VEGF R2 (rho = 0.628), p-S6 and p-4E BP1 (rho = 0.779), HIF-1α and p-4E BP1 (rho = 0.354), VEGF R2 and p-4E BP1 (rho = 0.332), and VEGF R2 and p-S6 (rho = 0.266). There was no significant correlation of expression between HIF-1α and p-S6, or CA IX and any other marker.

Table 1. Immunohistochemistry Results

Grade	HIF-1α/%	VEGF R2/%	CA IX/%	p-S6/%	p-4E BP1/%
0	9/14	4/6	55/86	12/19	15/23
1	12/19	17/27	4/6	15/23	10/16
2	14/22	43/67	3/5	21/33	19/30
3	29/45	-	2/3	16/25	20/31

Conclusions: Like CRCC, HIF-1α, VEGF R2, p-S6 and p-4E BP1 are expressed in OCC in most cases. Expression of mTOR and HIF pathway markers show significant correlations in OCC. Unlike CRCC, only rare OCCs have CA IX expression, despite over-expression of HIF-1α. This discordance suggests that HIF-1α over-expression may not always result in activation of CA-IX. Other molecular mechanisms controlling their inter-relationship may exist.

1099 An Analysis of Histologic Features Associated with Occult Lymph Node Metastasis in Clinical Stage I FIGO Grade I Endometrial Carcinoma

G Han, MM Leitao, Jr, N Abu-Rustum, RA Soslow. Memorial Sloan-Kettering Cancer Center, New York.

Background: A previous study of microcystic, elongated and fragmented (MELF) invasion in endometrial endometrioid adenocarcinoma (EEC), including all FIGO grades and stages, failed to find associations with clinical outcome. We studied histologic features associated with occult lymph node (LN) metastasis in FIGO grade 1, clinical FIGO stage I EEC with occult LN metastasis.

Design: Clinical stage I FIGO grade 1 myoinvasive EEC with occult positive LN(s) (n=18) were identified in a clinical database. Deep myometrial invasion (DMI >50%), stromal reaction, lymphovascular invasion (LVI) (absent, mild - 1 focus, severe - diffuse or multifocal), metaplasia type, and adenomyosis (absent, mild - 1-3 foci, severe - 4 or more foci) were assessed. Controls were 36 node-negative, myoinvasive, EECs matched for grade, patient age, body mass index and surgery date. The number, size (isolated tumor cells (ITC) <0.2 mm, micrometastasis 0.2-2 mm, macrometastasis >2 mm) and histology of LN metastases were evaluated.

Results: LN-positive cases had significantly higher rates of LVI ($p<0.001$) and MELF invasion ($p=0.03$) than LN-negative cases on univariate analysis (Table 1). LVI was the only independently associated factor for LN metastasis (odds ratio 32.49, $p=0.002$) on multivariate analysis. Macrometastases were seen in only 7 cases (39%), with or without micrometastasis and/or ITC. ITCs were seen in 12 cases (67%), with 8 cases containing ITC only (44%). In 3 cases (17%), sentinel LN workup was required to detect ITCs or micrometastases. Histiocyte-like tumor cells in LNs were seen in 11 cases (61%). ITCs and micrometastases often failed to resemble the primary tumor.

Table 1. Summary of Histologic Features

	DMI	MELF	LVI	Mucinous Metaplasia	Myxoid Stroma	Adenomyosis
Node-positive (n=18)	7 (39%)	12 (67%)	A 2 (11%), M 3 (17%), S 13 (72%)	4 (22%)	12 (67%)	A 10 (56%), M 3 (17%), S 5 (28%)
Node-negative (n=36)	9 (25%)	13 (36%)	A 26 (72%), M 4 (11%), S 6 (17%)	9 (25%)	15 (42%)	A 20 (56%), M 7 (19%), S 9 (25%)

A- Absent M-Mild S-Severe

Conclusions: LVI and MELF invasion are more frequent in FIGO grade 1 EEC with occult LN metastasis than in those without, but only LVI is independently associated with LN metastasis. The tumor cells in these lymph nodes often present as ITCs. Identifying these cases will further our understanding of the clinical significance of ITCs and micrometastases in EEC and, perhaps, yield information regarding which patients with clinically low stage EEC benefit most from LN dissection.

1100 Histological and Immunohistochemical Findings of the Intermediate Trophoblasts in Normal Full Term and Pre-Eclamptic Placentas

SH Han, K-R Kim, SA Kim, Y Lee, JY Ro. University of Ulsan College of Medicine, Asan Medical Center, Seoul, Republic of Korea.

Background: Recent studies have suggested that extravillous intermediate trophoblasts can be divided into implantation site intermediate trophoblasts (ISITs) and chorionic type intermediate trophoblasts (CTITs) according to the histological and immunohistochemical findings and their distribution. Except for a presumed role of ISITs in the implantation of embryos, function of ITs has not been clearly defined. Unlike previous observation from which the name of ITs are derived, we observed that ISITs were not confined to the implantation sites and CTITs not to the chorionic membrane in normal placentas, and the number of both subtypes were increased around the degenerating villi in pre-eclamptic placentas, prompted us to consider their possible roles for compensating degenerated trophoblasts in pre-eclamptic placentas.

Design: In order to examine the composition of ITs in various extravillous compartments of normal placentas including fetal membranes, intervillous septa, subchorionic areas, basal plates, and to examine the changes of ITs, if any, in pre-eclamptic placentas, quantitative analyses of ISITs and CTITs were peroformed using immunohistochemical stainings for p63, CD146, PLAP, hPL, and α-inhibin in 13 normal full term and 10 pre-eclamptic placentas containing infarcts.

Results: Both CTITs and ISITs were closely associated in every anatomical compartment in normal and pre-eclamptic placentas. ISITs were predominant in the intervillous septum (approximate ratio 8:1) and basal plate over CTITs (12:1), while CTITs were predominant in the subchorionic area over ISITs.(5:1) Fetal membrane show equal distribution of both subtypes, and they formed two distinct layers. In pre-eclamptic placentas, both subtypes were proliferated with increased Ki-67 proliferating rates around the degenerating villi in areas of placental infarcts, extensive fibrinoid deposit, and fibrotic villi, and they formed two distinct layers at the periphery of placental infarcts.

Conclusions: The subtypes of ITs do not have site specificity in all extravillous compartments of the placentas. Increased proliferation and formation of distinct layers around the degenerating villi/trophoblasts suggests that CTITs and ISITs have close differentiation-associated relationship, and the proliferation and differentiation might be related to the microenvironment of placenta, such as intraplacental oxygen concentration.

1101 ER-α Transcript Levels in Normal and Atrophic Squamous Epithelium of the Uterine Cervix and the Detection of a Splicing Variant

J Hemminger, D Richardson, J Pinsonneault, A Papp, J Stephens, D Cohn, A Suarez, D O'Malley. The Ohio State University Medical Center, Columbus, OH.

Background: Description of the molecular mechanisms leading to genital atrophy, a common clinical problem, will produce a better understanding of aging conditions in women. The major estrogen receptor in the female genital tract is estrogen receptor α (ER-α). Little is known about the regulation of ER-α expression and the significance of splicing variants in normal cervical squamous epithelium (NCSE) and atrophic cervical squamous epithelium (ACSE). We determined the relative ER-α transcript levels in NCSE from young women and compared it to ACSE from older women. Using primers to amplify different exons, we also investigated the ratio of full transcripts vs a splicing variant missing exon 1.

Design: Ectocervical squamous cells were exfoliated from 25 fresh hysterectomy specimens from women <45 years and >55 years without known cervical pathology. Cells from one half of the ectocervix were placed in RNAProtect and cells from the other half were placed in a liquid cytology vial (Surepath) for cytologic examination. Eight specimens showing a pure population of squamous cells were chosen for the study: four NCSE and four ACSE. Preoperatively, serum hormone levels were measured using standard clinical laboratory methods. After RNA isolation, three different regions of the ER-α transcript were studied (Exon 1, Exon 4, and 3'UTR) using reverse transcription polymerase chain reaction (PCR) followed by real-time PCR.

Results: Lower estradiol levels and much higher FSH levels in older women (ave. age 68) with ACSE compared to younger women with NCSE (ave. age 42) was confirmatory of the postmenopausal/hypoestrogenic state in the former. However, NCSE and ACSE showed similar total ER-α transcript levels (NCSE: ave. 3.45 relative abundances, std dev 1.8; ACSE: ave. 5.25 relative abundances, std dev 4.6; p value = 0.34). A splicing variant lacking Exon 1 contributed 50% of the total ER-α RNA transcripts in both NCSE and ACSE.

Conclusions: ER-α RNA levels in NCSE and ACSE are similar and appear to be unaffected by the postmenopausal hormonal milieu. An ER-α splicing variant lacking Exon 1 contributed half of the transcripts in both NCSE and ACSE. This is likely representative of a recently described variant referred in the literature as ERα46. To date, ERα46 has been reported in bone, colon and MFC7 cells, but not in cervical squamous epithelium. The role of ER-α splicing variants in the atrophic phenotype is unknown.

1102 Proliferative Activity of Non-Fimbriated-End Fallopian Tube Epithelium in Women with and without High Grade Serous Carcinoma of Ovary/Peritoneum

J Hemminger, J Hurt, J Stephens, S Barsky, K Yearsley, A Suarez. The Ohio State University Medical Center, Columbus, OH.

Background: Until recently, the fallopian tube (FT) of women diagnosed with high grade serous carcinoma of the ovary/peritoneum (HGSC) has been superficially examined by pathologists and excluded from staging systems and models of HGSC carcinogenesis. But putative precursor lesions of HGSC have now been identified in the fimbriated end of FT from women with BRCA mutations undergoing risk reducing salpingo-oophorectomy. However, non-fimbriated-end FT archival material is much more abundant and includes histologically identical epithelium, presumably exposed to similar field effects. Hence, we compared, using immunohistochemistry and digital image analysis, the proliferative activity of non-fimbriated-end FT epithelium in women with and without HGSC.

Design: Tissue microarrays were constructed with 2mm cores of uninvolved FT from 72 patients with HGSC (group 1) and 72 age-matched controls (+/- 2 years) without gynecologic cancer (group 2). Immunohistochemistry for Ki67 was performed on both tissue microarrays and staining quantification was determined with digital image analysis (TissueMine ®). Results were confirmed by light microscopy and technically poor cores were excluded. 49 FT pairs were considered adequate and used for further analysis.

Results: Both groups had almost identical ages (group 1: mean 61.39, std dev 12.83; group 2: mean 60.78, std dev 12.07; p value=0.808). The fallopian tubes associated with HGSC demonstrated a mean Ki67 proliferative index of 1.38% (std dev 1, range 0.19-4.39). Age-matched controls demonstrated a mean Ki67 proliferative index of 1.7% (std dev 1.83, range 0.02-8.85%). Thus, the proliferative activity was similar between these two groups (p value=0.857). Also, women ≤55 years of age had a mean proliferative index of 1.34 (std dev 1.02, range 0.21-3.95) and women > 55 had a mean proliferative index of 1.4 (std dev 1.01, range 0.19-4.39), p= 0.832.

Conclusions: Proliferative activity, represented by Ki67 nuclear immunoreactivity, of non-fimbriated-end fallopian tube epithelium is similar in patients with HGSC and age-matched controls. The proliferative index in both groups was low (average, 1.54%) and was not affected by the patient age. Further studies, with molecular and immunophenotypic tools, are necessary to evaluate the merits of non-fimbriated-end FT material in HGSC research.

1103 Unusual Morphological Types of Cervical Adenocarcinoma Are Not Associated with Human Papillomavirus Infection

OP Houghton, J Jamison, R Wilson, J Carson, WG McCluggage. Royal Group of Hospitals Trust, Belfast, Ireland; Antrim Area Hospital, Antrim, Ireland.

Background: The association between human papillomavirus (HPV) and cervical carcinoma is well known with HPV being identifiable in almost all cervical squamous carcinomas and most adenocarcinomas. However, the prevalence of HPV in unusual morphological types of cervical adenocarcinoma has not been extensively investigated.

Design: We looked for HPV in a series of primary cervical adenocarcinomas, enriched for unusual morphological types. We also investigated the relationship between HPV and p16 immunoreactivity. 58 cervical adenocarcinomas, comprising those of usual type (n=42), minimal deviation type (n=4), gastric type (n=3), intestinal type (n=2), mesonephric type (n=2), clear cell type (n=4) and hepatoid type (n=1) underwent linear array HPV genotyping and immunohistochemical staining with p16.

Results: Overall, HPV was identified in 31 of 53 cases (58%) in which sufficient DNA was present for analysis. The most common HPV types were 16 and 18 with these being identified in 19 and 18 cases respectively, either alone or in combination. 78% of usual type adenocarcinomas were HPV positive. In contrast, all minimal deviation adenocarcinomas and those of gastric, intestinal, mesonephric and clear cell type were HPV negative, as was the single hepatoid carcinoma. All usual type adenocarcinomas exhibited p16 immunoreactivity (diffuse staining in all but one case), as did 9 of 15 of those of unusual morphological type (5 focal, 4 diffuse).

Conclusions: Most, but not all, cervical adenocarcinomas of usual type contain HPV but those of unusual morphological type are HPV negative. This has implications regarding the efficacy of HPV vaccination in the prevention of cervical adenocarcinoma. Although diffuse p16 staining in the cervix is regarded as a useful surrogate marker of the presence of high risk HPV, some adenocarcinomas of usual and unusual type are positive in the absence of HPV.

1104 Incidence of Positive Pelvic Washings Obtained during Hysterectomy for Endometrial Adenocarcinoma: A Comparison of Robotic-Assisted Hysterectomy Versus Total Abdominal Hysterectomy

S Hu, Y Sun, A Brown, S Mandavilli. Hartford Hospital, Hartford.

Background: Robotic-assisted hysterectomy (RH) is being increasingly used in patients undergoing treatment for endometrial adenocarcinomas (EACs). Pelvic washing specimens obtained during such procedures are evaluated for tumor cells and the presence of positive pelvic washings upstages the patients and may alter post-operative treatment. Having observed a case of grade 1 endometrial adenocarcinoma with no myometrial invasion that had positive washings, we wanted to examine the incidence of positive pelvic washings in RH when compared to total abdominal hysterectomy (TAH).

Design: 143 hysterectomy specimens with diagnosis of EAC were retrieved from the pathology files at a tertiary hospital for the period 2004 to the present time. This included 74 RH and 69 TAH specimens in which pelvic washing specimens were obtained. For purposes of analysis, all EACs were divided into low grade (LG) and high grade (HG) tumors. LG criteria included: EACs endometrioid type FIGO grade 1, nuclear grade 1-2, and < 50% myometrial invasion. HG criteria included: EACs endometrioid type FIGO grades 2-3, nuclear grade 3, serous & clear cell carcinomas and endometrioid carcinomas with > 50% myometrial invasion. All pelvic washing specimens with atypical cells were reviewed and compared with the primary EACs.

Results: 7 of 37 LG RH specimens and 0/26 LG TAH had positive pelvic washings. Of the 7 cases that were positive in the LG group undergoing RH, 5 cases had no myometrial invasion. The other two had <5% and 7% myometrial invasion respectively.

Positive Pelvic Washings in RH vs TAH

	RH	TAH
LG	7/37 (20.6%)	0/26 (0%)
HG	11/37 (29.7%)	8/43 (18.6%)

Conclusions: The incidence of positive pelvic washings in patients undergoing RH is higher compared to TAH. This was specially the case in LG EACs. This included cases in which there was no myometrial invasion and this raises the possibility that the positive pelvic washings are an artifact. Whether this represents a pre-operative spillage associated with hysterography, or direct seeding via the fallopian tube during hysterectomy warrants further investigation. The clinical significance of such "positive" pelvic washings is unclear but brings a possible procedural artifact to the attention of both surgeons and pathologists.

1105 Clinical Outcome in Diagnostically Ambiguous Foci of "Gland Crowding" in the Endometrium

EC Huang, GL Mutter, CP Crum, MR Nucci. Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Background: Premalignant endometrial lesions (Endometrial Intraepithelial Neoplasia or EIN) are clonal neoplasms that arise focally and can be diagnosed using specific criteria. We occasionally encounter localized groups of crowded endometrial glands which are subdiagnostic of EIN that are interpreted as ambiguous by the terms "focal gland crowding". The differential diagnosis includes artifacts or mimics, and tangentially or poorly sampled EIN lesions. It has been our practice to flag these in the pathology report by describing gland crowding, and requesting rebiopsy to resolve the issue. We here report clinical follow-up of these patients.

Design: We conducted a retrospective study in all gynecologic pathology reports since 2001 (introduction of EIN criteria). These reports were retrieved from our anatomic pathology information system using a free-text index search for the flag term "gland crowding" through the final anatomic diagnoses. The analysis included the age of the patients, number of subsequent specimens, the duration and the outcome of the follow-ups.

Results: Of the 71,579 gynecologic pathology reports searched, 206 (0.3%) had the term "gland crowding" within the primary diagnostic field or associated note. Of these, 69.4% (143/206 cases; age 18-80) had follow-up pathology reports. Among the cases with follow-up (number of subsequent biopsies: range=1 to 16, median=1, average=1.8), 33 (23.1%) had a subsequent diagnosis of EIN (27 cases; 18.9%; follow-up range=1 month to 7 years, median=1 year, average=1.5 year) or carcinoma (6 cases; 4.2%; follow-up range=1 month to 5 years, median=0.5 year, average=1.7 year). EIN cases that were diagnosed within the first year (14 cases, 42.4%) were presumed concurrent with the initial subdiagnostic biopsy. An additional 13 subsequent EIN cases occurred after one year and were interpreted as a later stage of disease or new events. Two of the EIN cases were subsequently diagnosed as carcinoma in the hysterectomy specimens.

Conclusions: The "crowded glands" in this study represent interpretively difficult samples rather than a discrete, or new, diagnostic class. It is worthwhile in these diagnostically ambiguous cases to consider the option of follow-up sampling to resolve the possibilities, as 23.1% of cases will be followed by a histologic outcome of neoplasia. The range of histologic changes falling into this category will be illustrated and contrasted with diagnostic (EIN) features.

1106 Reversal of Progesterone Receptor Modulator Associated Endometrial Changes upon Completion of a Withdrawal Bleed

OB Ioffe, RJ Zaino, GL Mutter. University of Maryland School of Medicine, Baltimore, MD; Hershey Medical Center, Hershey, PA; Brigham and Women's Hospital, Boston, MA.

Background: Selective progesterone receptor blockers such as CDB-4124 (Proellex™) administered for treatment of fibroids or endometriosis produce an altered endometrium including irregular cysts and non-physiologic combinations of glandular secretion, mitotic activity, and apoptosis that we have previously designated "PAEC"[1]. This study examined endometrial histology during and following treatment with oral CDB-4124 to determine if these changes are reversible.

Design: 19 premenopausal women taking 12.5 to 25mg daily CDB-4124 had endometrial biopsies during treatment, and post-treatment biopsy. Endometrial slides were blindly examined by each of 3 pathologists, and scored as "PAEC" when cysts, combined with one or more of the PAEC associated epithelial types (seroid differentiation, secretory with mitoses, mixed phase) were present.

Results: 16/19 patients had adequate biopsies, with a median interval of 91 days between last drug administration and followup biopsy (range 41-351, mean=135 days). Of these, 69% (11/16) showed PAEC changes while on therapy. PAEC changes resolved in 91% (10/11), showing secretory (6/10), proliferative (3/10) or atrophic (1/10) endometrium.

One patient (age 50) with PAEC changes did not menstruate upon agent withdrawal, and she had PAEC changes 102 days after discontinuing therapy.
Conclusions: Conclusion: Across the varied conditions studied, discontinuation of Proellex administration and completion of withdrawal endometrial shedding reversed pre-existing PAEC changes in the majority (91%) of cases. Failure to complete a withdrawal shedding may occur in older patients as they approach the perimenopause, and this can be associated with retention of PAEC changes. Reference List 1. Ioffe OB, Zaino RJ, Mutter GL. Endometrial changes from short term therapy with CDB-4124, a selective progesterone receptor modulator. Mod Path 2009; 22:450-459.

1107 Individual Tumor Cell Apoptoses in Uterine Smooth Muscle Tumors

PPC Ip, KY Tse, ANY Cheung. University of Hong Kong, Queen Mary Hospital, Hong Kong, SAR, Hong Kong.
Background: Individual tumor cell apoptosis (TCA) in uterine leiomyomas has not been specifically studied. There is relatively few information on their causative factors and the significance of their presence is uncertain. It has been suggested that tumors that have foci of TCA, but absence of atypia, mitotic activity or necrosis should be labeled as smooth muscle tumors of uncertain malignant potential (STUMP).
Design: Three hundred consecutive cases of uterine smooth muscle tumors other than leiomyosarcomas from the surgical pathology files of Queen Mary Hospital were screened for the presence of TCA. The clinicopathologic features and follow-up information were obtained. Immunohistochemical stains for p16, p53, MIB1 (ki-67) were performed on cases regarded as STUMP.
Results: Twenty-one cases (7%) of uterine smooth muscle tumors were found to possess TCA. Patient age ranges from 38 through 61 (median, 46). Twelve of the 21 patients (57%) had a history of drug usage (oral contraceptives and/or tranexamic acid). One patient was treated for ovarian endometriotic cyst and another for carcinoma of the cervix. The remainder all presented with symptoms related to uterine fibroids, including menorrhagia, urine frequency, abdominal mass or combinations thereof. Five were treated with myomectomy and the rest had total hysterectomy and bilateral salpingo-oophrectomy. Secondary changes found alongside the TCA include: infarct type necrosis (43%), early infarcts (19%), necrosis of an uncertain type (14%), myxoid/hyaline change (81%), hemorrhage (38%) and hydropic change (57%). Five cases (24%) had TCA unaccompanied by any necrosis. Three cases were diagnosed as STUMPs based on the presence of necrosis of an uncertain type and/or TCA, 1 was a leiomyoma with bizarre nuclei and 1 was a cellular leiomyoma. None of the STUMPs showed over-expression of p16, p53 or MIB1. Follow-up ranged from 1 to 64 months (median, 58). The follow-up for patients with STUMPs were 49, 60 and 67 months respectively. None of the patients had tumor recurrence.
Conclusions: TCA is an uncommon finding in uterine leiomyomas. Many of such cases also have secondary degenerative changes and are found in patients taking oral contraceptives and/or tranexamic acid. Although commonly coexists with infarct type necrosis, TCA found in isolation does not appear to be a worrisome microscopic feature.

1108 FOXL2 – A Novel Marker for Granulosa Cell Tumors

M Ivanovic, E Lengyel, A Montag, K Gwin. University of Chicago, Chicago, IL.
Background: FOXL2, a member of the forkhead-winged-helix family of transcription factors, is one of the earliest expressed genes during the development of the female gonad and is required for proper differentiation of granulosa cells (GC) during folliculogenesis. FOXL2 expression persists into adulthood. We hypothesized that FOXL2 could be a useful marker for granulosa cell differentiation in morphologic challenging cases.
Design: Paraffin embedded material of 38 granulosa (adult type =37, juvenile type =1) cell tumors (GCT) and 30 non-granulosa cell tumors (non-GCT) of the ovary were used to construct tissue microarrays (TMA). The control group of the non-granulosa cell tumors included various subtypes of sex cord-stromal tumors (other than GC), epithelial, germ cell and mixed germ cell-sex cord-stromal tumors. FOXL2 expression was evaluated by IHC for localization and intensity of immunoreactivity. Staining intensity was divided into three groups: no staining, low intensity staining and high intensity staining.
Results: All thirty-eight cases of GCT stained positive for FOXL2. 92% (35 cases) showed high-intensity staining and 8% (3 cases) showed low-intensity staining. In the control group of non-GCT tumors, 63.3% (19 cases) were negative for FOXL2, and 23.3% (7 cases) revealed low-intensity staining. Only 13.3%, 4 cases (one of each: fibrothecoma, thecoma,fibroma, and gonadoblastoma) exhibited high-intensity staining. In gonadoblastoma, only the sex cord derivatives exhibited staining. None of epithelial tumors stained positive for FOXL2.
Conclusions: All granulosa cell tumors exhibited immunoreavity for FOXL2, with 92% showing strong immunoreactivity. In contrast, the control group was mostly negative or revealed only weak staining. In addition, the sex cord derivatives resembling immature granulosa-cells of a gonadoblastoma expressed FOXL2. Our findings support that FOXL2 is a very useful marker to identify granulosa cell differentiation in ovarian tumors. This may be helpful in the differential diagnosis of the sex cord-stromal tumors group, especially between granulosa cell tumor and Sertoli cell tumor, that often display overlapping morphologic features.

1109 Expression of C-Kit, Platelet-Derived Growth Factor D and Platelet-Derived Growth Factor Receptor α in Ovarian Granulosa Cell Tumors

T Jazaerly, S Bandyopadhyay, A Semaan, M Ibrahim, B Albashiti, A Munkarah, M Raza, K Hayek, R Ali-Fehmi. Wayne State University, Detroit, MI; Henry Ford Health System, Detroit, MI; Karmanos Cancer Institute, Detroit, MI; St John Hospital, Detroit.
Background: Granulosa cell tumors (GCTs) generally carry a good prognosis but with a small subset exhibiting a more aggressive clinical course including a relative insensitivity to chemotherapy. The aim of this study was to determine the potential prognostic significance of the immunohistochemical (IHC) expression of C-Kit, Platelet-derived growth factor D (PDGFD) and platelet derived growth factor receptor-α (PDGFR-α), in ovarian GCTs.
Design: We identified 30 consecutive ovarian GCTs resected at the Karmanos Cancer Center between 1999 and 2005. Clinical/surgical and detailed pathological data were available on all patients including age, tumor stage, capsule integrity and histologic type (juvenile vs. adult). Representative tumor sections were stained with antibodies to C-Kit, PDGFD and PDGFR-α. IHC intensity (graded as 1+, 2+, and 3+) and percentage of positive cells (with a cut off 30%) were used to determine high and low expression and were correlated with the clinicopathological parameters.
Results: The mean patient age at diagnosis was 48.23 years (19-88 years). Twenty five had stage I disease with 7 patients with capsule involvement, 4 patients were stage II and 1 patient was stage III. Twenty two patients, (73%) had adult type GCT tumors. PDGFD and PDGFR- α were seen in 66.7% and 53.3% of the tumors respectively. Of all the parameters investigated, high expression of PDGFD and PDGFR- α showed significant correlation with tumor size. Tumors< 5 cm in diameter had high expression of PDGFD and PDGFR- α in 28.5% and 33.5% of the tumors, while tumors > 5 cm in diameter had a high expression in 48% and 54% of the tumors respectively (P =0.04 and =0.02 respectively). There was no association between the expression of the proteins and patients' age, tumor stage, tumor rupture or histologic type. There was no immunoreactivity with C-Kit in any of the samples studied.
Conclusions: Our data indicate a high frequency of PDGFD and PDGFR- α expression in GCTs. We believe our study offers one of the first evaluation of the expression of PDGFD in GCTs and demonstrates a significant correlation between the size of the tumor and the expression of PDGFD and PDGFR- α.

1110 Omental Icing: An Unusual Variant of Peritoneal Carcinomatosis Due to Metastatic Gastric or Breast Cancer

CA Jimenez, JT Rabban. UCSF, San Francisco, CA.
Background: Peritoneal carcinomatosis due to primary peritoneal cancer or metastasis often presents with radiologic omental caking. Morphologically, tumor cells typically infiltrate omental or peritoneal tissues in a destructive pattern. Some forms of cancer, such as gastric or breast cancer can spread with minimal destruction. This study reports an unusual variant of peritoneal carcinomatosis due to metastatic gastric or breast cancer in which sparse tumor cells line the peritoneal/omental surface/septae with minimal or no stromal invasion yet diffuse fibrosis is present. We report the pathologic features and diagnostic pitfalls of this variant pattern, which we term "omental icing".
Design: Peritoneal carcinomatosis cases due to gastric(36) or breast cancer(7) were evaluated. Tumor growth pattern was classified as: Pattern A= destructive invasion of fat or mass-like growth; Pattern B= non-destructive/infiltrating tumor cells confined to a thin layer at the peritoneal surface; Pattern C= Pattern B with sparse tumor cells along omental fat septae or minimal hypocellular infiltrate of fat. Omental fibrosis, atypical fibroblasts and stromal inflammation were graded. Tumor nuclear grade, signet ring morphology, LVI and mitosis were evaluated.
Results: Primary gastric/breast cancer was known prior to surgery in 34/43 cases; primary peritoneal origin was suspected in 9/43. Omental caking was seen radiologically in 14/38 and 14 had elevated serum CA125. Tumor morphology was: pattern A: 19 ;pattern B: 1;pattern C: 23. Among the 24 pattern B/C tumors: frozen sections were definitive in 21/24. In permanent sections tumor cells were visible only on high magnification and often obscured by fibrosis/inflammation. Tumor cells were purely single cells in 17/24; clusters/tubules in 2/24 or a mixed in 5/24. Signet rings were in 14/24. Nuclear atypia was mild (7), moderate (15) or severe (1). Mitoses were absent or sparse (avg 1.5/10 hpf). Despite sparse tumor cells, extensive diffuse omental fibrosis and inflammation was present in 15/24. Atypical stromal fibroblasts were in 3/24.
Conclusions: Peritoneal carcinomatosis from gastric or breast cancer may present with significant omental caking due to diffuse stromal fibrosis despite sparse tumor cells confined to the omental surface or septae. This so-called omental icing pattern may pose diagnostic challenges, particularly at frozen section since tumor cells often show only low grade atypia, scant mitoses and lack signet rings. Attention to omental surface and septae can prevent under-recognition of this unusual pattern of tumor growth.

1111 Immunohistochemical Classification of the 5 Major Subtypes of Ovarian Carcinoma and the Introduction of the Calculator for Ovarian Subtype Prediction (COSP)

SE Kalloger, M Kobel, S Leung, E Mehl, D Gao, K Masson, DG Huntsman, CB Gilks. Vancouver General Hospital and the British Columbia Cancer Agency, Vancouver, BC, Canada; University of Calgary/CLS, Calgary, AB, Canada.
Background: With the emerging evidence that the five major ovarian carcinoma subtypes comprise distinct entities, management of ovarian carcinoma will become subtype specific in the future. Although subtype assignment has been improved recently, it would be desirable to assist subtype assignment with objective molecular markers.
Design: Immunohistochemical expression data of 22 biomarkers were examined using nominal logistic regression in order to produce a subtype prediction model on a set of 322 archival ovarian carcinomas accrued from the British Columbia Cancer Agency between 1984 - 2000. This equation was validated on a cohort of 242 high quality ovarian carcinomas from the Gynaecologic Tissue Bank at Vancouver General Hospital collected between 2001 - 2006.
Results: Using a panel of 9 antibodies (CDKN2A, DKK1, HNF1B, MDM2, PGR, TFF3, TP53, VIM, WT1) morphological type can be predicted with relatively good sensitivity and specificity as shown in the table:

Receiver operator characteristic and kappa statistics for prediction and validation.

Subtype	ROC AUC Prediction	Kappa Prediction	ROC AUC Validation	Kappa Validation
Clear Cell	0.9944	0.876 +/-0.022	0.9401	0.699 +/- 0.044
Endometrioid	0.9776		0.9435	
Mucinous	0.9932		0.9723	
High-grade Serous	0.9811		0.9138	
Low-grade Serous	0.9997		0.9100	

Conclusions: These results indicate that this panel of 9 markers can be reliably used to differentiate the five major subtypes of ovarian carcinoma.

1112 Frozen Section Diagnosis of Low Grade (LG) Endometrial Adenocarcinoma (ECA): Is It a Predictor of Lymph Node Status? A Multi-Institutional Study of 110 Cases

S Karaburun, D Wagner, SM Lele, S Mandavilli. Harford Hospital, Hartford, CT; University of Nebraska Medical Center, Omaha, NV.

Background: Hysterectomy specimens are routinely submitted for frozen section (FS) evaluation for grade of ECA and depth of invasion. This information, in come cases, impacts the clinical decision of whether further staging is performed, but the benefit of lymphadenectomy remains unclear in LG ECA. The aim of this study was to assess the combined experience of two institutions to evaluate the accuracy of frozen section (FS) diagnosis of ECA and correlation with final diagnosis including lymph node status.

Design: Cases of ECA were retrieved from the pathology files of two institutions in which FS were performed during the time period 2005 to 2009. The FS diagnosis was compared with the final pathology report. For purposes of analysis, the tumors were divided into Low Grade (FIGO 1, nuclear grades 1 & 2, less than 50% myometrial invasion, and no lymphovascular invasion), and High Grade (FIGO 2 & 3, nuclear grade 3, more than 50% myometrial invasion, or presence of lymphovascular invasion). Additionally, the lymph node status in cases with lymph node dissections (LND) was summarized.

Results: There were 110 hysterectomy specimens in which FS were performed.74 cases were categorized as LG ECA. Of these 42 patients had LND done and all of these were negative for tumor. 6 cases with diagnosis of LG ECA in frozen section diagnosis had HG ECA on final diagnosis. 2 of these 6 patients did not have LN dissections. The 4 with LN dissections were all negative. 36 cases were categorized as HG on FS and remained HG on final diagnosis. Of these 25 patients had LN dissections done, of which 8 cases showed positive LN and 17 cases had negative LN.

Conclusions: There is a good correlation between FS categorization of tumors into LG and HG with only 6 cases (5.45 %) of discrepancy between FS and final diagnosis. Diagnosis of LG ECA on FS is a reliable predictor of nodal status and merits further investigation as to whether LND should be limited to cases with FS diagnosis of HG ECA.

1113 The Spectrum of Non-Cervical Neoplastic/Dysplastic Lesions of the Uterine Cervix with p16INK4a Immunohistochemical Positivity Encountered in the Uterine Cervical and Endometrial Biopsy Samples in the Daily Surgical Pathology Practice

HG Kaspar, XM Zhang. Geisinger Health System, Wilkes Barre, PA.

Background: Overexpression of p16 is described in cervical intraepithelial neoplasia associated with oncogenic human papilloma virus (HPV) infections and in several neoplastic and non-neoplastic gynecologic conditions. Immunohistochemical testing with p16 is therefore broadly used in the diagnosis of dysplastic and neoplastic cervical lesions. The spectrum of lesions with p16 positive staining encountered on the uterine cervical and endometrial biopsy samples in the daily surgical pathology practice is evaluated in this retrospective study.

Design: This is a retrospective review of 213 cervical and endometrial tissue samples where immunohistochemistry using p16, MIB-1 and in-situ hybridization for HPV was performed as a part of a diagnostic work-up differentiating neoplastic/dysplastic versus reactive/metaplastic epithelium, and cervical versus metastatic origin of a cervical lesion.

Results: There were 167 cases with all p16 and MIB-1 immunohistochemistry, and HPV in-situ hybridization available for evaluation. Of these 167 cases, 97 samples demonstrated p16 positive staining distributed as follows: 6 invasive squamous cell carcinomas and 4 invasive adenocarcinoma of the cervix, 36 cases of HGSIL, 8 in-situ adenocarcinoma, and 24 LGSIL. Other malignant conditions of non-cervical origin with strong and diffuse p16 staining, and a high MIB-1 proliferative index included 3 cases of endometrial adenocarcinomas and 2 cases of carcinosarcoma with direct extension to the cervix, two cases with fragments of endometrial mucinous carcinoma obtained on endocervical samples, two cases of metastatic tubal carcinoma, three cases of metastatic serous carcinoma of the ovary and peritoneum, and one case of papillary serous carcinoma with HPV inclusions on in-situ hybridization. The spectrum of the non-neoplastic conditions included 12 cases of endometrial squamous metaplasia with a mosaic pattern and predominant cytoplasmic staining, six tubal metaplasias and three cases with cervical endometriosis.

Conclusions: While it is now established that p16 immunohistochemical testing is a valuable tool in the diagnosis of cervical neoplasia/dysplasia, in particular when used in conjunction with MIB-1 and in-situ HPV testing, the current study demonstrates p16 positivity in several other neoplastic and non-neoplastic conditions encountered in the daily evaluation of cervical and endometrial tissue samples.

1114 miRNA Profiling in Cervical Cancer

L Kehoe, CD Spillane, M Gallagher, O Sheils, C Martin, JJ O'Leary. Trinity College Dublin, Dublin, Ireland; Coomne Womens and Infants University Hospital, Dublin, Ireland.

Background: MicroRNAs (miRNA) have emerged as important molecules involved in cancer development. They are thus potential biomarkers and in the future may be developed as potential therapeutic targets.

Design: Using miRNA TaqMan low density arrays [TLDAs], we have profiled the expression of 377 miRNAs in cervical cancer (CC) cell lines. This data has been compared in two ways: comparison of CC cell lines to normal cervix and comparison of HPV positive to HPV negative cell lines. This approach allows the identification of CC and HPV specific miRNA expression patterns.

Results: Comparison of CC cell lines, HeLa, SiHa (HPV positive) and C33A (HPV negative) to normal cervix, has identified 87 miRNAs which are putative biomarkers of CC. The majority of miRNAs were found to be down regulated on comparison to normal cervix. Only one up regulated miRNA, common to all cell lines, was found: miR-301b. Top common down regulated miRNAs identified were: miR-133a, miR-139-3p, miR-145 and miR-223. Several of these have been described in cervical cancer (miR-145, miR-133a and miR-223) previously. Five miRNAs were found significantly dysregulated in HPV positive cell lines on comparison to normal cervix (miR-200a, miR-381, miR-493, miR-548b-5p and miR-146b-3p). miRNA expression was found to be associated with the presence of HPV 16 and 18, expanding our current understanding of miRNA regulation in CC. These can be grouped into miRNAs associated with HPV or those specific to HPV 16 or 18. 50 miRNAs were found to be common between the CC cell lines and therefore are putative biomarkers of CC. miR-548b-5p was found to down regulated in a HPV specific manner. Examination of common miRNAs revealed miR-335 and miR-214 to be the top up and down regulated miRNAs respectively. Further analysis revealed miR-335 may be up regulated in a HPV 18 specific manner. 33 miRNAs (28↑, 5↓) were associated with HPV 16 and 66 miRNAs (38↑, 28↓) with HPV 18. We have also identified that 16 miRNAs cluster to a 'hot-spot' miRNA region on chromosome 14, which appears to be associated with HPV 18 induced CC.

Conclusions: We have generated a list of miRNAs specific to CC and HPV 16 and/or 18 associated CC; thus providing a list of miRNAs for validation in CC material and also for target prediction analysis. This data may determine additional mechanisms of HPV induced cellular dysregulation and may also identify future therapeutic targets. We also describe a novel miRNA cluster on chromosome 14 in HPV 18 infected HeLa cells.

1115 Pathologic Ultrastaging of Sentinel Lymph Nodes in Endometrial Cancer

F Khoury-Collado, RA Soslow, MP Murray, ML Gemignani, NR Abu Rustum. Memorial Sloan-Kettering Cancer Center, New York.

Background: Sentinel lymph node (SLN) evaluation in patients with endometrial cancer (EMC) has been proposed as an alternative to selective or reflexive lymphadenectomy.

Design: Patients undergoing primary surgery for EMC between 09/2005 and 09/2009 were studied prospectively. SLN mapping was performed using blue dye injection into the cervix in all cases. Additional injection methods included blue dye injection in the uterine findus, and cervical injection of 99m Tc sulfur colloid. SLN were removed followed by regional LN dissection. The pathology protocol for SLN evaluation follows: 2 adjacent 5-µm section were cut at each of two levels 50 µm apart from paraffin blocks lacking metastatic carcinoma appreciable in a routine hematoxylin and eosin (H&E) section. At each level, one slide was stained with H&E and the other with immunohistochemistry (IHC) using the anti-cytokeratin AE1:AE3, as well as one negative control slide, for a total of 5 slides per block. All other non-SLN nodes were examined only by routine H&E. Micrometastasis (MM) was defined as a focus of metastatic cancer ranging from 0.2-2mm. Isoltaed tumor cells (ITC) were defined as metastasis measuring ≤0.2 mm, including the presence of single non-cohesive cytokeratin positive tumor cells.

Results: 153 EMC patients were evaluated. The histologic subtypes were: endometrioid, 122 (80%); serous, 16 (10%); carcinosarcoma, 6 (4%); clear cell, 2 (1%); mixed, 7 (5%). The surgical stages were: stage I, 118 (77%); stage II, 3 (2%); stage III, 31 (205); stage IV, 1 (1%). SLN identification was possible in 131 (86%) cases. A median of 3 SLN (range, 1-14) and 10 non-SLN (range, 0-55) per patient were examined. The total number of patients with positive LNs was 20/153 (15%). In 6/20 (30%) cases with positive LNs, tumor cells were detected only by review of additional sections or IHC on the SLN required by the protocol (3 cases of MM and 3 cases of ITC). The SLN was negative for carcinoma in 3/20 (15%) cases with a positive non-SLN (false negative rate of 15%). All such cases occurred in the hands of surgeons new to the SLN procedure.

Conclusions: Pathologic ultrastaging of SLN removed during the lymphatic mapping for EMC is associated with a high (30%) detection rate of micrometastasis and ITCs that may have otherwise been undetected by routine LN evaluation. The clinical significance of ITCs in EMC requires longer follow-up.

1116 Defect of Pericyte Recruitment Can Induce Villous Hydropic Change in Complete Hydatidiform Moles

K-R Kim, T-J Kwon, JS Song, S-A Kim, SJ Robboy. University of Ulsan College of Medicine, Asan Medical Center, Seoul, Republic of Korea; National Institute of Scientific Investigation, Seoul, Republic of Korea; Duke University Medical Center, Durham, NC.

Background: Why villi become hydropic in complete hydatidiform mole (CHM) is poorly understood. Most stromal blood vessels in early gestational moles are immature, lacking distinct vascular lumina and hematopoietic components, similar to the angiogenic cell cords in the normal vasculogenetic stage of early placental development (gestational weeks 5-6). Although the histologic features of the immature villous stromal vessels resemble those of normally developing placenta, the villous stroma of early moles is

more frequently hydropic, raising the suspicion that leakage or permeability through the immature vessel wall is increased in early moles. We thought that a defect of pericyte recruitment around the immature blood vessels can be one possible cause of the villous hydropic change in CHMs.
Design: The structure of stromal vessels in 20 early CHMs were compared to immature stromal vessels in 61 normally developing placentas (gestational weeks 4-9) using immunohistochemical pericyte markers (a-smooth muscle actin, desmin, PDGF-b), Electron-microscopic examination was performed in 5 CHMs to examine if there is any defect of pericyte recruitment around the blood vessels in CHMs.
Results: PDGF-b was normally expressed in trophoblastic layers, villous stroma, angiogenic cell cords and mature stromal vessels both in normal placentas (from late week 5) and early moles. In normal placentas, a-smooth muscle actin was expressed only in the chorionic plate at week 4, but then gradually spread through the villous branches to form a reticular network in the villous stroma during weeks 5-8, and became accentuated around the blood vessels after week 7. In contrast, a-smooth muscle actin expression in the villous stroma was significantly decreased in the early moles, and perivascular cuffing was found only in a single instance. Desmin was not expressed around the stromal vessels both in normal placenta and CHMs.Ultrastructurally, molar immature vessels consisted of endothelial cells linearly arranged without pericyte attachment.
Conclusions: Pericyte recruitment around immature stromal vessels is defective in early moles, which may induce increased vascular leakage, and thus an important cause of villous hydropic change.

1117 The Utility of Circulating Tumor Cells in Monitoring Patients with Recurrent Ovarian Cancer

DW Kindelberger, U Matulonis. Brigham and Women's Hospital, Boston, MA; Dana-Farber Cancer Institute, Boston, MA.
Background: Introduction: Epithelial ovarian cancer is diagnosed in approximately 22,000 women every year in the United States. Over 16,000 deaths per year occur, making this cancer the most lethal gynecologic malignancy. The number of circulating tumor cells in the peripheral blood before treatment is an independent predictor of survival in patients with metastatic breast cancer, prostate cancer, and colorectal cancer. In patients with ovarian cancer, the prognostic significance of circulating tumor cells is unclear, and investigators report conflicting results. We examined the utility of immumagnetically isolated circulating tumor cells (CTCs), captured using the CellSearch System by Veridex, in predicting response to treatment in patients with recurrent ovarian cancer.
Design: Fifty consecutive patients with known recurrent ovarian cancer were selected as part of an IRB-approved study at the Dana Farber Cancer Institute. Circulating tumor cells were isolated according to standard protocols for the Veridex CellSearch System. Briefly, 7.5 ml of blood was collected in Cell Save Vacutainer tubes. Blood samples were processed within 36 hours of collection by dilution with buffer, followed by a low-speed (800xg) centrifugation in a Beckman table-top centrifuge. Samples were then immediately processed with the CellTracks Autoprep instrument using the Epithelial Cell Kit. Following processing, tumor cells were enumerated using the CellTracks Analyzer. Clinical data, including CA-125 levels, type of chemotherapy regimen, and clinical course were correlated with the number of CTCs.
Results: The average number of CTCs obtained using the CellSearch system was quite low (2 CTCs/sample). The number of CTCs failed to correlate with CA-125 levels, type or duration of chemotherapy, or clinical course.
Conclusions: In patients with recurrent ovarian cancers (predominantly high grade serous adenocarcinoma) the recovery of CTCs using the CellSearch system is very poor, possibly due to the low rates of hematogenous spread for these tumors. As a consequence of thses low numbers of recovered cells, the absolute CTC counts do not show any correlation with CA-125 levels, type or duration of chemotherapy, or disease progression. Unlike the scenario seen with some other epithelial tumor types, (breast, prostate, colorectal cancers), the number of CTCs determined by the Veridex CellSearch System does not seem to be clinically a useful marker for patients with recurrent ovarian cancers.

1118 Correlation of the Hybrid Capture II High-Risk HPV DNA Test Chemiluminescense Intensity from Cervical Samples with Follow-Up Histologic Results: A Cytologic/Histologic Review of 367 Cases

SM Knoepp, DL Kuebler, DC Wilbur. University of Michigan, Ann Arbor, MI; Massachusetts General Hospital/Harvard Medical School, Boston, MA.
Background: The Hybrid Capture II (Qiagen) high-risk human papilloma virus (hrHPV) DNA test is an in vitro nucleic acid hybridization assay that uses chemiluminescence for the qualitative and semiquantitative detection of hrHPV in cervical samples. Results, measured in relative light units (RLUs), are reported as positive or negative based on a ratio to cutoff value (CO) calculated from positive and negative controls. The resulting ratio (RLU/CO) is recorded as negative for hrHPV if less than 1.0, positive for hrHPV if greater than 2.5, or "equivocal" (*i.e.*, retested) if between 1.0 and 2.5.
Design: We examined the 2-year follow-up histology of cervical biopsies from cohorts of patients initially diagnosed with atypical squamous cells of undetermined significance (ASC-US) on cervical cytology: Group 1 consisted of 148 patients with "equivocal" results on the hrHPV assay, and Group 2 consisted of 148 patients with unequivocal positive results on the hrHPV assay. We next compared the chemiluminescence intensity of hrHPV tests from patients in Group 2 based on presence and severity of dysplasia found on follow-up histology. Finally, we stratified patients for risk of high-grade CIN (CIN 2/3) based on the chemiluminescence intensity of their hrHPV results.
Results: Follow-up histology was available for 85 patients in Group 1 and 98 patients in Group 2. Results of cervical biopsy, expressed as percentage of biopsied cohort, were (Group 1/Group 2): CIN 2 or 3 (16.5%/22.4%), CIN 1 (27%/23.5%) and negative or reactive (56.5%/54.1%). Subsequent high-grade (CIN 2 or 3) histologic results for patients in Group 2 based on chemiluminescence intensity were (quartile lowest to highest): 24%, 22.2%, 22.7%, and 20.8%. Patients whose hrHPV (RLU/CO) intensity were greater than 1000 (6% of cases) showed CIN 2 or 3 in 25% of cervical biopsies on follow-up.
Conclusions: While patients with "equivocal" hrHPV tests show fewer high-grade CIN lesions on follow-up biopsy compared to patients with unequivocal positive hrHPV tests, the percentage of high-grade CIN in this cohort is significant and therefore management should be similar to the unequivocal population. After an unequivocal positive hrHPV test, the hrHPV chemiluminescence intensity does not further predict the presence, absence, or degree of CIN, as patients with low RLU/CO values show similar CIN rates to those with high RLU/CO values.

1119 Diagnostic and Prognostic Value of TP53 Expression in Ovarian High-Grade Serous Carcinomas

M Kobel, A Reuss, A DuBois, S Kommoss, F Kommoss, D Gao, SE Kalloger, DG Huntsman, CB Gilks. University of Calgary/CLS, Calgary, AB, Canada; German Working Group Gynecologic Oncology, Ovary (AGO-OVAR), Wiesbaden, Germany; Vancouver General Hospital and British Columbia Cancer Agency, Vancouver, BC, Canada.
Background: Data on TP53 expression and their association with outcome are controversial. TP53 expression has either been associated with poor outcome or has not shown association with prognosis, depending on the cut-off used to define overexpression and the composition of the study cohorts.
Design: We aimed to examine the association between TP53 expression and outcome restricted to high-grade serous carcinomas (N=508) from two population based cohorts from British Columbia representing cases with or without residual tumor after initial surgery, and one clinical trial cohort from Germany (AGO-OVAR-3). After tissue microarray construction, TP53 expression was assessed by immunohistochemistry using the DO-7 antibody (dilution 1:400) as completely negative, focal expression and overexpression defined with a cut-off of > 50% positive tumor cell nuclei. Complete negativity and overexpression have been shown to correlate with the mutation status of TP53. Univariate and multivariate cox regression models were used to correlate TP53 expression status with recurrence free survival.
Results: TP53 was completely negative in 30.3%, focally expressed in 12.0% and overexpressed in 57.7% of high-grade serous carcinomas, which was an inverse pattern compared to other subtypes of ovarian carcinomas, where the majority of cases showed focal expression. High-grade serous carcinomas that were completely negative for TP53 were associated with an increased risk of recurrence compared to cases with overexpression for the British Columbia cohort with no residual tumor (HR = 1.41, 95% CI 1.01- 1.98) and for a combined British columbia and German cohort (HR = 1.43, 95% CI 1.12- 1.82) in multivariable analysis including age, stage residual tumor and stratified for cohort.
Conclusions: Focal TP53 expression is an uncommon finding in high-grade serous carcinomas. Complete absence of TP53 expression is associated with an increased risk of earlier recurrence in high-grade serous carcinomas, and conversely, TP53 overexpression appears to indicate a favorable outcome in high-grade serous carcinomas. The molecular basis for this difference remains unclear.

1120 Histopathologic Differences between Primary and Secondary Vestibulodynia Provide Insights into Pathophysiology

V Korcheva, MF Goetsch, R Anderson, CM LeClair, TK Morgan. OHSU, Portland, OR.
Background: Chronic vestibulitis (vestibulodynia) is severe localized introital pain elicited by vaginal entry. It is common, affecting 15% of women. The cause is unknown, but prior studies have provided mixed data regarding the potential role of ERa, neural hypertrophy, and/or chronic inflammation. We hypothesize that mixed results in the literature may be related to differences between "primary" and "secondary" disease. Primary sufferers report severe pain from the first introital touch. Secondary sufferers present later in life with no prior history of introital pain. Our objective was to compare the histopathologic features of both primary and secondary vestibulodynia.
Design: We performed a retrospective analysis of archived vestibular biopsies obtained at the OHSU Vestibulodynia Clinic from 2002-2008 (n=111). We also reviewed four negative control biopsies prospectively collected at our clinic. Patient records were reviewed by expert gynecologists (mfg, cml) to classify each case as either primary or secondary vestibulodynia. The vestibular biopsies were H&E stained and serial sections immunostained for nerves (S100), mast cells (CD117), and ERa. Sections were scored by two pathologists (tkm, vk) for chronic inflammation, neural hypertrophy/hyperplasia, mast cell density, and basal cell ERa expression using previously published criteria. Significance between groups was tested by Chi-square and the Mann-Whitney U test.
Results: Chart review identified 49 primary and 62 secondary vestibulitis cases. Histopathologic scoring showed excellent reproducibility between pathologists (kappa statistics 0.70-79) and multiple differences between groups were identified. Primary vestibulitis showed significant neural hypertrophy/hyperplasia compared to secondary vestibulitis ($P<0.0001$) and controls. Secondary vestibulitis had more chronic inflammation ($p<0.01$) and mast cells/hpf ($p<0.05$). There was also a trend towards less ERa in secondary vestibulitis ($P=0.08$), which may be related to inflammation.
Conclusions: Our data show for the first time that primary and secondary vestibulitis have different histologic features, which may explain the mixed results of prior studies that did not discriminate between these categories. Either primary and secondary vestibulitis are separate disease entities, or inflammation represents an early stage in the disease process that culminates in neural hypertrophy/hyperplasia.

1121 Artefactual Changes in Robot-Assisted and Laparoscopic Hysterectomies

A Krizova, BA Clarke, M Bernardini, S James, S Boerner, AM Mulligan. University of Toronto, Toronto, Canada; University Health Network (UHN), Toronto, Canada; UHN, Toronto, Canada; St. Micheal's Hospital, Toronto, Canada.

Background: Total laparoscopic hysterectomy is a minimally invasive technique resulting in reduced morbidity and better cosmesis. The literature is discrepant as to whether it results in a higher incidence of positive peritoneal cytology and associated artefactual changes, including vascular pseudoinvasion (VPI), have been described.

Design: We conducted a retrospective histopathologic review of 191 hysterectomy specimens (79 malignant, 112 benign) from two centers, blinded to the surgical technique. The hysterectomy types included total abdominal (63), vaginal (18),laparoscopic with intrauterine manipulator (IM) (47),laparoscopic without IM (6) and robot-assisted laparoscopic (43). We documented the following features: endometrial disruption (ED), nuclear crush, VPI, endomyometrial cleft artefact with or without epithelial displacement, inflammatory debris in clefts and vascular spaces and debris (inflammatory or tumor fragments) in fallopian tube (FT) lumen. Comparisons of findings between procedures using IM and those without IM were performed using $\chi 2$ or Fisher exact test with a significance of $P<0.05$. Subgroup analysis was performed based on benign or malignant endometrial disease.

Results: Artefactual changes of ED, nuclear crush, cleft formation, inflammatory debris within intravascular, artefactual spaces and FT lumen and VPI were significantly more associated with the use of IM, independent of whether the endometrial pathology was benign or malignant. Use of IM also resulted in significantly more detached tissue fragments in the endocervix. There was no significant difference in peritoneal washing cytology between the two groups. In two cases, misinterpretation of artefactual changes resulted in patients being incorrectly staged.

Artefactual changes according to use of IM

	Intrauterine Manipulator (%)	No Intrauterine Manipulator (%)
Malignant	55	35
Benign	45	65
ED	34	6
VPI	26	4
Clefts	23	3
Debris in spaces/vessels	34	5
Debris in FT lumen	33	4
Nuclear crush	34	7

Conclusions: Artefactual changes are significantly more common in laparoscopic procedures in which an IM is used. Pathology interpretation of cell type may be limited by nuclear crush and ED. Furthermore, displaced epithelial fragments present within vessels, cervix or artefactual clefts may result in misinterpretation of prognostic and staging parameters.

1122 Shorter Telomere Length in Most Serous Tubal Intraepithelial Carcinomas

E Kuhn, A Meeker, A Smith Sehdev, RJ Kurman, IM Shih. Johns Hopkins Medical Institutions, Baltimore, MD.

Background: Telomere length shortening has been well known as one of the main genetic manifestations in human cancer. The shortened telomere plays an important role in inducing genomic instability and propelling tumor progression. The purpose of this study is to determine if changes in telomere length occurs in serous tubal intraepithelial carcinoma (STIC), a putative precursor of "ovarian" high-grade serous carcinomas.

Design: A total of 23 STICs from 15 patients who had concurrent pelvic high-grade serous carcinoma were analyzed for telomere length. All STICs were discrete from the carcinomas. Paraffin-embedded tissue sections were prepared for telomere FISH. p53 immunofluorescence was also co-applied to confirm STICs, thus facilitating the scoring of telomere length. The concurrent high-grade serous carcinomas were also analyzed from the same patients. The telomere length in STICs and carcinoma was compared to normal fallopian tube epithelium and scored as abnormally short, no change or abnormally long using a quantitative imaging analysis system.

Results: We found that 16 (84.2%) of 19 STICs harbored significantly shorter telomeres, while only 1 (5.3%) showed no change and 2 (10.5%) had longer telomeres. In high-grade serous carcinomas, shortened telomeres occurred in 8 (66.7%) of 12 carcinomas while longer telomeres were found in 4 (33.3%) cases. The pattern of telomere length change was the same in both STICs and carcinoma from the same patients in 83.3% of cases. Among all 9 patients in which their STICs had shorter telomere, 8 of them also demonstrated shorter telomere in their corresponding carcinomas. In multifocal STICs from the same patients, all STICs showed the same pattern of telomere length change.

Conclusions: The above findings lend further support to the proposal that STIC is a precursor of high-grade serous carcinoma and suggests that telomere alteration is one of the earliest molecular changes in the development of high-grade serous carcinomas. This new finding is consistent with previous observations of increased DNA damage (as evidenced by γH2AX) and aneuploidy in STICs and has important implications for the further studies on pathogenesis of pelvic serous carcinoma.

1123 Alterations of Telomere Length in Different Histologic Subtypes of Ovarian Carcinoma

E Kuhn, RJ Kurman, A Meeker, IM Shih. Johns Hopkins Medical Institutions, Baltimore, MD.

Background: It has been well known that aberration of telomerase activity as reflected by telomere length is associated with most human solid tumors. Abnormally short telomeres lead to the aberrant fusion of chromosome ends and subsequently chromosome instability, especially when *TP53* is mutated. On the other hand, abnormally long telomeres indicate higher telomerase activity that protects tumor cells from telomere attrition due to excessive cellular proliferation. A comprehensive analysis of telomere length has not yet been studied in different histologic subtypes of ovarian carcinoma.

Design: We performed semi-quantitative telomere FISH on a total of 224 ovarian carcinomas including 109 high-grade serous carcinomas, 26 low-grade serous carcinomas, 57 clear cell carcinomas and 32 endometrioid carcinomas. All the carcinomas were arranged in tissue microarrays to minimize the potential technical inconsistency related to in situ hybridization. The telomere length was scored in a blinded fashion as abnormally short, no change and abnormally long as compared to adjacent normal stromal cells.

Results: The results of telomere length change in all specimens are shown in Table 1 . There is no statistically significant difference of telomere pattern in different subtypes of ovarian carcinoma.

Table 1: Telomere abnormality in Ovarian Carcinoma

Histologic subtype	Total number	Shorter Telomeres n (%)	Longer Telomeres n (%)	No Telomeres change n (%)
HGSC	109	87 (79.8)	11 (10.1)	11 (10.1)
LGSC	26	26 (100.0)	0 (0.0)	0 (0.0)
EMC	32	23 (79.8)	3 (9.4)	6 (18.8)
CCC	57	39 (68.4)	11 (19.3)	7 (12.3)

HGSC: high grade serous carcinoma, LGSC: low grade serous carcinoma, EMC: endometrioid carcinoma, CCC: clear cell carcinoma.

Conclusions: Our result clearly demonstrates that abnormally short or long telomeres were detected in the great majority of ovarian carcinomas irrespective of their histologic subtypes. This finding implies that ovarian carcinomas may utilize telomerase-related pathways for tumor development.

1124 Outcome and Survival in Women Diagnosed with Early Stage Endometrial Intraepithelial Carcinoma: The Role of Adjuvant Therapy

JG Lake, KZ Hanley, MB Mosunjac. Emory University School of Medicine, Atlanta, GA; Emory University Hospital, Atlanta, GA.

Background: Endometrial intraepithelial carcinoma (EIC) is characterized by surface glandular involvement of the endometrium and or endometrial polyp by tumor cells resembling papillary serous carcinoma. EIC often presents with extrauterine disease at the time of diagnosis, despite the absence of myoinvasion. Therefore full staging is recommended in women diagnosed with EIC. The overall survival of patients with early stage EIC (pT1a) is reported to range from 64-95%.The role of adjuvant treatment in setting of such limited disease is controversial. The aim of this retrospective study is to compare the outcome and survival of women receiving adjuvant therapy (control group) for early stage EIC with those treated with surgery alone (study group).

Design: The pathology electronic database was searched for uterine EIC (TNM stage pT1a, N0,M0). The tumor size extent and location was documented. Patients with adverse prognostic findings were excluded. Histologic type, grade and tumor stage were confirmed. Follow up clinical data was searched for tumor recurrence, type of adjuvant therapy if any, and survival. Results were analyzed using the Kaplan-Meier method.

Results: A total of 16 patients were identified (9 control, 7 study). EIC was limited to an endometrial polyp (10), involved an endometrial polyp and background endometrium (3), and the endometrial surface only (3). The adjuvant treatment in the control group consisted of chemotherapy (4), radiation (4) and chemoradiation (1). Follow-up was 10-62 months. The survival in both group was 100%, the disease free survival was 100% in the study group. One patient from the control group recurred, therefore the disease free survival was 93.7% in that group.

Conclusions: While complete surgical staging in patients diagnosed with EIC is essential, the role of adjuvant therapy in pT1a EIC is controversial. The current standard of care is administration of toxic adjuvant therapy and long-term surveillance. Even though the numbers in this study are small, they suggest a role for watchful waiting in pT1a EIC patients who have been fully staged. Patients with poor performance status, advanced age and small tumor size may be treated with surgery alone, if optimal cytoreduction and full staging can be accomplished.

1125 PAX8 Distinguishes Serous Ovarian Neoplasms from Malignant Mesothelioma with High Sensitivity and Specificity

AR Laury, JL Hornick, JM Corson, JF Krane, R Drapkin, MS Hirsch. Brigham and Womens Hospital, Boston, MA; Dana Farber Cancer Institute, Boston, MA.

Background: Ovarian serous neoplasms, whether primary in the peritoneum or metastatic to the pleura, can have morphologic overlap with mesothelioma. This distinction is critical clinically, yet most studies have failed to identify immunostains that reliably distinguish between these two tumor types. Recently, the transcription factor PAX8 has been shown to be sensitive and relatively specific for Mullerian tumors. Additionally, some studies suggest that h-caldesmon is sensitive and specific for mesothelioma when compared to serous ovarian tumors. The goal of this study was to evaluate whether PAX8 and h-caldesmon expression can successfully distinguish mesothelioma from serous ovarian tumors.

Design: Immunohistochemistry was performed after pressure cooker antigen retrieval using rabbit anti-PAX8 polyclonal (Proteintech; 1:800) and mouse anti-h-caldesmon monoclonal (DAKO; 1:300) antibodies on archival tissue from 254 ovarian serous tumors (152 high grade serous carcinomas, 10 low grade serous carcinomas, and 92 serous borderline tumors) and 54 mesothelial tumors (24 pleural malignant mesotheliomas, 27 peritoneal malignant mesotheliomas, 2 well-differentiated papillary mesotheliomas, and 1 multicystic mesothelioma). Only nuclear and cytoplasmic immunoreactions were considered positive for PAX8 and h-caldesmon, respectively.

Results: Diffuse and strong PAX8 staining was present in 151/152 (99%) high grade serous ovarian carcinomas, and all (100%) low grade ovarian carcinomas and serous borderline tumors. None of the pleural malignant mesotheliomas were reactive with PAX8. 3/27 (11%) peritoneal malignant mesotheliomas demonstrated focal, weak staining for PAX8; the remaining 24 cases were negative. The 2 well-differentiated

mesotheliomas and the 1 multicystic mesothelioma each demonstrated focal, weak staining for PAX8. h-caldesmon was negative (with appropriate positive internal controls) in all cases evaluated, including all mesotheliomas.
Conclusions: Strong PAX8 staining is highly specific (p<0.00001) for ovarian serous carcinoma and serous borderline tumors when compared to malignant mesotheliomas of the peritoneum and pleura. The presence of weak staining for PAX8 in the 3 "non-invasive" mesotheliomas raises questions about cell lineage of these tumors, as well as the utility for PAX8 in this differential diagnosis. Based on this study, h-caldesmon is not a useful marker for mesothelioma.

1126 Expression of SOX17: A Novel Marker in Ovarian Germ Cell Tumors

AM Leathersich, D Cao. Washington University School of Medicine, St. Louis, MO.
Background: Ovarian germ cell tumors (GCT) are rare and range from benign to malignant lesions. A novel marker, SOX17, has recently been shown to distinguish seminoma from embryonal carcinoma in the testis. The goal of this study is to investigate the diagnostic utility of the transcription factor SOX17 in ovarian GCT's and non-GCT's.
Design: One hundred and three ovarian GCT's were retrieved including 27 dysgerminomas, 35 yolk sac tumors (YST), 5 Embryonal carcinomas (EC), 13 immature teratomas (IM), 8 mature teratomas (MT), 5 gonadoblastomas (GB), 6 combined carcinoid and teratoma, 1 carcinoid, and 2 struma ovarii. We also stained 72 primary non-GCT's of the ovary including 9 endometrioid carcinomas, 11 high grade serous carcinomas, 9 mucinous carcinomas, 1 transitional cell carcinoma (TCC), 13 clear cell carcinomas, 2 benign Brenner tumors, 2 neuroendocrine tumors, 7 juvenile granulosa cell tumors (GCT), 6 adult GCTs, 7 steroid cell tumors, 2 Sertoli-Leydig cell tumors, and 3 fibrothecomas. Unstained slides generated from 1 to 2 paraffin embedded tissue blocks per case were stained with SOX17 monoclonal antibody. Only nuclear staining was counted as positive. The percentage of tumor cells stained was scored semiquantitatively as 0 (no tumor cells staining), 1+(<30% cells), 2+(31-60% cells), 3+(61-90% cells), 4+(>90% cells).
Results: Twenty-five out of 26 (96%) dysgerminomas and 28 of 35 (80%) YST showed 3+ to 4+ (>60%) nuclear staining for SOX17. 100% of GB cases showed staining. IT was negative in 62% of cases. EC and MT were negative for SOX17.

Sox17 Staining in Ovarian Germ Cell Tumors

Tumor Type	0	1+	2+	3+	4+
Dysgerminoma	0	0	1	1	25
YST	0	3	4	14	14
EC	5	0	0	0	0
Immature Teratoma	8	4	1	0	0
Mature Teratoma	8	0	0	0	0
Gonadoblastoma	0	1	0	0	4
Carcinoid and Teratoma	7	0	0	0	0
Struma Ovarii	2	0	0	0	0

All cases of endometrioid, high grade serous, and clear cell carcinoma showed at least 1+ nuclear staining, with 3+ to 4+ staining in 67, 55, and 38% of cases, respectively. Juvenile GCT, steroid cell tumor, Sertoli-Leydig cell tumor, fibrothecoma, choriocarcinoma, struma ovarii, and benign Brenner tumors showed no nuclear staining in 100% of the cases. One case in six of adult GCT showed 3+ staining, the remaining cases showed no nuclear staining. The TCC showed 4+ staining.
Conclusions: SOX17 is a novel marker that can distinguish dysgerminomas and YST from EC. SOX17 does not show nuclear staining in sex-cord stromal tumors and most teratomas.

1127 Prognostic Value of c-MYC Amplification by Fluorescence In Situ Hybridization in Cervical Cancer

K Lee, TJ Kim, A Lee, YJ Choi, KY Lee, JS Park, WS Lee. College of Medicine, the Catholic University, Seoul, Republic of Korea.
Background: We examined c-MYC amplification in cervical cancer and analyzed its clinico-pathologic implication.
Design: Archival tissue from 163 patients with cervical cancer was prepared for tissue microarray (TMA) blocks. Immunohistochemical staining including bcl-2 and Ki-67 was performed and c-MYC gene amplification was determined by the dual-probe fluorescence in situ hybridization using a centromere-specific probe for choromosome 8 (8CEP) and a region-specific probe for c-MYC (Vysis® LSI® MYC (8q24) Spectrum™ Probe, Abbott/Vysis).
Results: We identified 6.7% (11/163) c-MYC amplification in cervical cancer, of which comprises 8 sqamous cell carcinomas, 2 adenocarcinomas and 1 adenosquamous cell carcinoma. c-MYC amplification was not associated with age, tumor depth of invasion, lymph node metastasis, histologic type and histologic differentiation but was significantly associated with higher mitotic count (p=0.022) and adversely associated with bcl-2 expression (p=0.022). bcl-2 expression was significantly associated with higher proliferation index (ki67≥51%)(p=0.039). Univariate survival analysis revealed that MYC amplification was significantly associated with worse recurrent free survival (p=0.011) and worse overall survival (p=0.037). Multivariate survival analyses using Cox-regression model revealed that c-MYC amplification and high stage were independantly associated with worse recurrence-free survival (p=0.042 *and* p=0.001) and that high stage was independently associated with worse tumor specific overall survival (p=0.001).
Conclusions: c-MYC amplification correlates with worse recurrence free survival and is independent prognostic factor in cervical cancer.

1128 Complex Atypical Hyperplasia: Improving the Prediction of Associated Carcinoma

MM Leitao, Jr, G Han, NR Abu-Rustum, RR Barakat, RA Soslow. Memorial Sloan-Kettering Cancer Center, New York.
Background: The primary objective was to identify factors associated with an endometrial carcinoma (CA) diagnosis on hysterctomy after a diagnosis of complex atypical hyperplasia (CAH) on biopsy or curettage.
Design: We retrospectively identified all CAH cases diagnosed on biopsy (BX) or curettage (EMC) from March 1994-May 2008 with follow up hysterectomies. CAH cases were subclassified as: CAH-suspicious if features bordered on carcinoma or carcinoma could not be excluded; CAH-polypoid if CAH arose in or was associated with a polyp; CAH-focal; or CAH-NOS (not otherwise specified). The categories were not mutually exclusive. A subset analysis was performed for cases diagnosed by a gynecologic pathologist (subspecialist CAH) to determine whether any differences in CAH diagnostic criteria affected rates of CA in followup hysterectomy.
Results: We identified 197 CAH cases. The median age was 54 y (range 32-86 y). The median time from BX or EMC to hysterectomy was 47 days (5-572 days). CA was subsequently diagnosed on hysterectomy in 34% of cases. CA was diagnosed after: CAH-suspicious in 56% of cases, compared to 28% not suspicious; CAH-polypoid in 20% of cases, compared to 38% nonpolypoid; and CAH-focal in 19%, compared to 40% nonfocal. All comparisons were statistically significant (p-values=0.001, 0.02 and 0.005, respectively). Method of preoperative endometrial sampling, age, menopausal status and BMI were associated with CA on univariate analysis. On multivariate analysis, CAH-suspicious, CAH-nonfocal, CAH-nonpolypoid, BX compared to EMC, and older age were independently associated with an increased risk of CA (all p-values <0.001). 112 of the 197 cases were subspecialist CAH cases, and these were as likely to be followed by a CA diagnosis as other cases (34% versus 34%; p=NS). Subspecialist CAH cases were as frequently associated with myoinvasive CA as other cases (13% versus 15%; p=NS) and rates of deep myoinvasion (>50%) did not differ between groups (4% versus 5%). All hysterectomy diagnoses of CA were FIGO grade 1 with only rare exceptions, n=2 in the subspecialist CAH group and n=3 in the others.
Conclusions: The rate of CA on hysterectomy after a diagnosis of CAH is influenced by the method of sampling, age and CAH type. Subspecialty review of CAH cases by a gynecologic pathologist had no apparent impact on the ability to predict CA, including myoinvasive CA, on follow up.

1129 Immunohistochemical Expression of Estrogen and Progesterone Receptors and Outcomes in Patients with Newly Diagnosed Uterine Leiomyosarcoma

MM Leitao, Jr, ML Hensley, R O'Cearbhaill, RA Soslow. Memorial Sloan-Kettering Cancer Center, New York.
Background: The objective was to assess the immunohistochemical (IHC) expression of estrogen receptors (ER) and progesterone receptors (PR) in uterine leiomyosarcomas (LMSs) and the prognostic significance of ER and PR expression in LMS.
Design: We identified all patients with uterine LMS seen at our institution from 7/82 to 7/07 for whom ER and PR IHC analysis was performed at the time of initial diagnosis. We only included cases that were considered conventional LMS (i.e. "high grade") and in which IHC was performed at our institution. ER and PR IHC expression was considered positive (+) if more that 5% of cells were stained. Progression-free (PFS) and overal survival (OS) were estimated using Kaplan-Meier estimates and compared with log-rank test where indicated.
Results: We identified 43 patients with a median age of 52 years (range 26-73). Disease was confined to the uterine body in 20 (47%) cases. 18/43 (42%) cases were ER(+) and 17/42 (41%) cases were PR(+). At last follow-up, 33 (77%) tumors had recurred or progressed and 23 (54%) patients had died. The median follow-up for survivors was 48.4 months (range 4-142.4). PR(+) was associated with improved PFS (P=0.002) and OS (P=0.03) in the entire cohort but not ER(+). Adjusting for stage, ER expression was associated with PFS (P=0.01) but not OS (P=0.3). PR expression maintained its significant association with both PFS (P=0.002) and OS (P=0.05) after adjusting for stage. Neither ER nor PR expression was associated with outcome in patients with disease outside the uterine body. However, when disease was confined to the uterine body, the median PFS for ER(+) and PR(+) cases was not reached for either compared to 16.9 months (95%CI:8.1-25.7) for ER(-) cases (P=0.03) and 13.5 months (95%CI:5.9-21.1) for PR(-) cases (P=0.001). In the 10 PR(+) cases, only one patient experienced recurrence and died compared to the 10 PR(-) cases in which 9 recurred and 5 died.
Conclusions: ER and PR expression is associated with outcome in patients with uterine LMS confined to the uterine body. PR expression identifies tumors confined to the uterine body with the best outcomes.

1130 Carbonic Anhydrase IX, p16 and Human Papillomavirus Infection in Endocervical Glandular Neoplasia Associated with Lobular Endocervical Glandular Hyperplasia: A Gynecologic Oncology Group Study

SY Liao, WH Rodgers, J Kauderer, KM Darcy, R Carter, L Levine, NM Spirtos, N Susumu, K Fujiwara, JL Walker, M Hatae, EJ Stanbridge. UC Irvine, Irvine; Lenox Hill Hosp., NY; GOG Statistical and Data Center, Buffalo; Univ. of Taxas, Galveston; Univ. of Nevada School of Medicine, Las Vegas; Keio Univ. School of Medicine, Keio, Japan; Saitama Medical Univ. Int. Medical Center, Hidaka City, Japan; Univ. of Oklahoma, Oklahoma; Kagoshima City Hosp., Kagoshima, Japan.
Background: Lobular endocervical glandular hyperplasia (LEGH) is a rare lesion of the uterine cervix. Others propose that LEGH represents pre-cancerous minimal deviation adenocarcinoma (MDA) independent of high-risk human papillomavirus (H-HPV) infection. Carbonic anhydrase IX (CAIX), a hypoxic marker, is highly expressed in neoplastic cervical glandular lesions (GLs), including in-situ and invasive

adenocarcinoma. Expression of CAIX in LEGH and MDA has not been reported. We report a comparative study of CAIX, p16 expression and H-HPV infection in conventional GLs (CGLs) and LEGH.
Design: In 1998, the Gynecologic Oncology Group initiated an international trial to evaluate the diagnostic utility of CAIX expression and H-HPV in women with a cytologic diagnosis of atypical glandular cells from the United States (U.S.) and Japan. Pathological diagnoses were based on diagnostic criteria for LEGH proposed by Nucci et al. Immunostaining was used to detect CAIX and p16. Polymerase chain reaction was used to detect H-HPV in liquid based cytology specimens.
Results: No cases of LEGH were observed in the U.S. cohort. In the Japanese cohort, we identified 15 CGLs and 14 LEGHs (six had coexistent well-differentiated GLs and all but one had variable cytologic atypia). HPV testing was performed on 13 of 15 CGLs and 11 (85%) were infected with H-HPV. All of the HPV positive CGLs showed high levels of CAIX and p16 expression with the exception of one. The one p16 negative CGL was also negative for H-HPV. Among the 14 LEGH lesions, none of them were infected with H-HPV and all expressed CAIX. They were either negative for p16 expression or showed low levels of p16 expression.
Conclusions: The results support different mechanisms of carcinogenesis in conventional GLs verses GLs associated with LEGH. CAIX expression may serve as a specific marker for detection of LEGH in the absence of H-HPV.

1131 PAX-8 as a Marker for Müllerian Tumors: Comparison with PAX-2

N Liles, G Hamilton, SS Shen, B Krishnan, LD Truong. The Methodist Hospital, Houston, TX; Baylor College of Medicine, Houston, TX.
Background: Useful diagnostic markers for tumors of Müllerian origin remain in development. PAX-8 and PAX-2 are members of a transcription factor family instrumental for fetal development and probably neoplastic transformation of kidney, Müllerian organs, and thyroid. Expression of PAX-8 in Müllerian tumors is evaluated and compared with that of PAX-2.
Design: Consecutive tissue sections of Müllerian tumors of ovary (n=60), endometrium (n=79), omentum (n=18), and other locations (n= 5, lymph nodes and pleura) were submitted for immunostain for PAX-8 and PAX-2.
Results:

PAX-8 and PAX-2 Expression

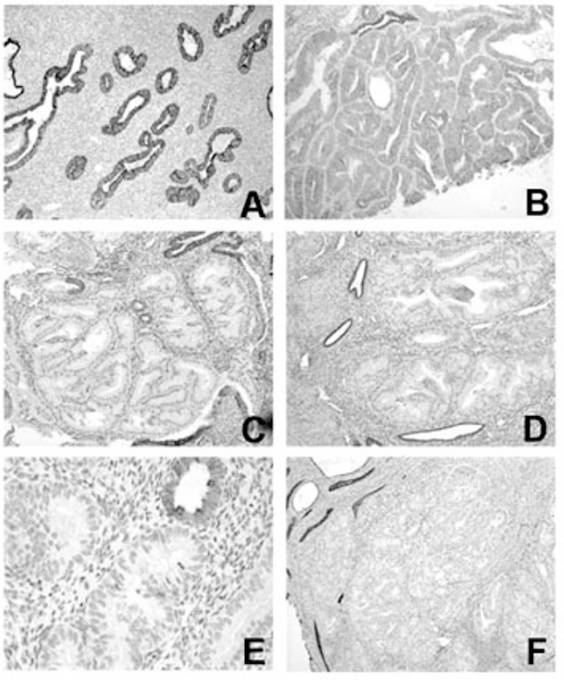
		Ovary (n=60)	Uterus (n=79)	Omentum (n=18)	Others (n=5)
PAX-8	Serous	44/44 (100%)	12/12 (100%)	18/18 (100%)	5/5 (100%)
	Endometriod	7/9 (77%)	56/59 (95%)	0/1 (0%)	1/1 (100%)
	Mucinous	1/1 (100%)	0/1 (0%)		
	Clear cell	6/6 (100%)	6/7 (86%)		
	Total	58/60 (97%)	74/79 (94%)	18/19 (95%)	6/6 (100%)
PAX-2	Serous	6/44 (14%)	2/12 (16%)	7/18 (39%)	3/5 (60%)
	Endometriod	1/9 (10%)	9/59 (15%)	0/1 (0%)	1/1 (100%)
	Mucinous	0/1 (0%)	0/1 (0%)		
	Clear cell	1/6 (17%)	1/7 (14%)		
	Total	8/60 (13%)	12/79 (15%)	7/19 (37%)	4/6 (67%)

Both PAX-8 and PAX-2 showed nuclear staining. The staining for PAX-8 was much stronger and involved much more tumor cells than PAX-2 (mean 86% vs 23%).
Conclusions: 1) Both PAX-8 and PAX-2 are expressed by tumors of Müllerian origin, regardless of histologic types; 2) The frequency of tumors stained, the staining intensity, and the percentage of tumor cells stained are significantly higher for PAX-8 than PAX-2; and 3) PAX-8 appears to be a sensitive marker for tumors of Müllerian origin, and it is much better than PAX-2 in this aspect.

1132 IMP2, a Promising Biomarker Identifies Precursor of Endometrioid Carcinoma

Y Liu, L Zhang, K Dresser, D Lu. UMass Memorial Medical Center, University of Massachusetts, Worcester, MA.
Background: Endometrioid carcinoma is preceded by precursors, of which the diagnosis is difficult and inconsistent. Biomarker, PTEN, has been proven of diagnostic utility. Here we present a new biomarker IMP2, an insulin-like growth factor II mRNA-binding protein. We demonstrate that loss of IMP2 expression can specifically identify the precursors of endometrioid carcinoma.
Design: 308 cases are selected from University of Massachusetts between 1999 and 2002; benign endometrium (n=92), simple or complex hyperplasia without atypia (n=51), complex hyperplasia with atypia (n=83), and low grade endometrioid carcinoma (n=82); including endometrial curettage/biopsy (n=144) and hysterectomy (n=178). H&E and IMP2 immunohistochemical stains are performed on consecutive tissue sections following routine method. Positive staining is defined as dark brown cytoplasmic staining.
Results:

Figure 1.



The glandular epithelium in both benign endometrium and complex hyperplasia without atypia have IMP2 staining (Figure 1A, 1B). The stroma has no staining, which provides a clean background. Complete loss of IMP2 expression is observed in complex hyperplasia with atypia alone (Figure 1C) or that adjacent to carcinoma (Figure 1D). The IMP2 negative gland is clearly demarcated from the adjacent positive gland (Figure 1 E). Low grade endometrioid carcinomas demonstrate loss of IMP2 expression (Figure 1F).

Figure 2

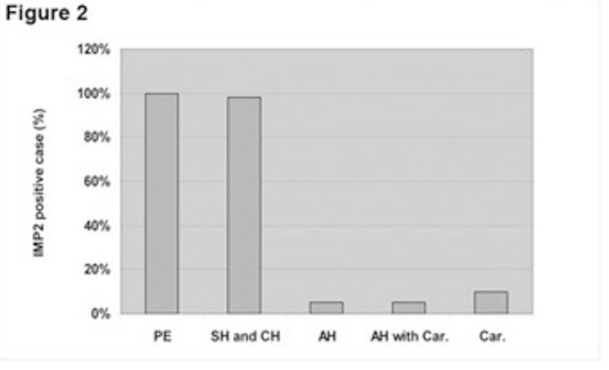


Figure 2 shows IMP2 is positive in 100% of benign endometrium and 98% of simple or complex hyperplasia cases, but only 5% of complex hyperplasia with atypia and 10% of the low grade carcinoma cases.
Conclusions: Atypical hyperplasia and low grade endometrioid adenocarcinoma, in a sharp contrast to benign emdometrium, showed a completely and persistent loss IMP2 expression. Therefore, IMP2 is a promising specific and sensitive marker for precursors of endometrioid adenocarcinoma, especially in the diagnostic challenging cases.

1133 Low Grade Vulvar Intraepithelial Lesions: A Correlation Study among Koilocytosis, Viral Load, Ki-67, and P16

S Loghavi, EG Silva. Cedars-Sinai Medical Center, Los Angeles, CA.
Background: In cervical intraepithelial neoplasms there is good correlation between Ki-67, p16 and the degree of dysplasia. Since in the vulva most intraepithelial lesions are low grade (LGVIL) and are associated with low risk HPV we decided to investigate the degree of correlation between koilocytosis, viral load, Ki-67, and p16 in these lesions.
Design: 13 vulvar biopsies from different patients diagnosed as LGVIL were reviewed and stained for Ki-67 and P16 by immunohistochemistry. Ki-67 was evaluated according to the level of the positive reaction as staining the lower third, lower and middle third and the entire full thickness of the epithelium. P16 positivity was defined as both nuclear and cytoplasmic staining in single cells or a band like pattern of staining. The presence of low and high risk HPV types were studied by in-situ hybridization and evaluated as the maximum number of positive cells in one high power field (HPF). 12 fibroepithelial polyps from the vulva were used as the controls for this study and were stained with the same methods.
Results: All cases of LGVIL were positive for HPV, 11 cases were positive for low risk (maximum 3-220 positive cells/HPF), 1 case was positive for high risk HPV (maximum 46 positive cells/HPF), and 1 case was positive for both low and high risk

HPV (maximum 15 positive cells/HPF). Koilocytosis was absent in 3 cases and ranged between 1+ up to 3+ in the other 10 cases. Ki-67 was positive in the lower third of the epithelium in 3 cases, in the lower and middle part of the epithelium in 5 cases, and in the entire thickness of the epithelium in 5 cases. P16 was positive in single scattered cells in 6 cases, in a band like pattern in 1 case and negative in 6 cases. In the control group none of the fibroepithelial polyps showed ki-67 staining beyond the lower third of the epithelium. Only 1 case showed single cell p16 immunoreactivity. All cases were negative for low and high risk HPV.
Conclusions: 1. In LGVILs there is no correlation between the degree of the koilocytosis and the viral load. 2. There is no correlation between Ki-67 and HPV; low or high risk. Ki-67 can be positive in all layers of the epithelium. 3. There is no correlation between P16 and HPV; low or high risk. Therefore polypoid lesions that are not fibroepithelial polyps should be stained for HPV regardless of the presence of koilocytosis. The HPV screening should include both low and high risk HPV because a small percentage of cases were positive for high risk HPV. Ki-67 and p16 do not provide additional information.

1134 Mucosal Carcinoma of the Fallopian Tube Coexists only with the Serous Subtype of Ovarian Cancer: The First SEE-FIM Study in Asia

D Maeda, Y Takazawa, S Ota, K Ohashi, M Mori, T Imamura, M Fukayama. Graduate School of Medicine, the University of Tokyo, Tokyo, Japan; Toranomon Hospital, Tokyo, Japan; Mitsui Memorial Hospital, Tokyo, Japan; Teikyo University Hospital, Tokyo, Japan.
Background: Previous studies in Western countries have revealed that mucosal carcinoma of the fallopian tube frequently coexists with pelvic (ovarian, tubal and peritoneal) high-grade serous carcinomas, and it is now regarded as a possible precursor of these lesions. The present study was performed to elucidate the relationship between mucosal carcinoma of the fallopian tube and ovarian cancers of various histological subtypes in the Japanese population.
Design: We prospectively submitted the fallopian tubes *in toto* for histological examination (SEE-FIM protocol) in 53 cases of ovarian carcinoma (serous, n=12; clear cell, n=23; endometrioid, n=9; mucinous, n=14; and others, n=5) and 3 cases of peritoneal serous carcinoma. The diagnosis of mucosal carcinoma of the fallopian tube was based solely on morphology. In serous adenocarcinoma cases, immunohistochemical analyses using antibodies to p53, WT-1, and Ki-67 were performed in the main ovarian/peritoneal tumors and coexisting tubal carcinomas when present.
Results: Mucosal carcinoma of the fallopian tube did not coexist with any of the non-serous adenocarcinomas (n=41). By contrast, tubal mucosal carcinoma was observed in 7 of the 15 serous adenocarcinomas. Of these seven cases, six had coexisting tubal carcinoma in the fimbria only, while in the remaining case, mucosal carcinoma was observed in both the fimbriated and non-fimbriated tubal mucosa. In all cases, the immunophenotypes for p53 and WT-1 were similar in the tubal mucosal carcinoma and invasive ovarian or peritoneal carcinoma. The tumors were negative for p53 in 4 out of 7 cases, and one of the p53-negative serous adenocarcinomas had a low-grade morphology.
Conclusions: Mucosal carcinoma of the fallopian tube frequently coexists with pelvic serous adenocarcinomas in the Japanese population. Our results suggest that mucosal carcinoma of the fallopian tube may be a precursor of some pelvic serous carcinomas. However, alterations in p53 may not necessarily play a role in its development. Tubal lesions appear to be unrelated to the carcinogenesis of non-serous ovarian cancers, such as clear cell adenocarcinomas.

1135 The Molecular Profile of Ovarian Transitional Cell Carcinoma

TL Mao, MI Hsieh, KT Kuo, MC Lin. National Taiwan University Hospital, Taipei, Taiwan.
Background: Ovarian transitional cell carcinoma (TCC) is a distinct type of ovarian surface epithelial carcinoma as defined by WHO. Some studies had demonstrated differences in pathological and immunohistochemical features from those of ovarian Brenner tumor. However, the distinction of ovarian TCC from other types of ovarian carcinoma can be subjective and the morphological features of TCC often coexist with other types of ovarian carcinoma, especially high-grade serous carcinoma.
Design: To better classify ovarian TCC, we retrospectively retrieved 24 ovarian carcinomas with pure or partial features of TCC for immunohistochemical study and mutational analysis of genes frequently mutated in ovarian carcinomas. High density (250K) single nucleotide polymorphism (SNP) array was also performed in 5 cases using affinity purified samples from fresh specimens. The DNA copy number variation profiles were compared to those of 9 high-grade serous carcinomas.
Results: The 24 cases included 6 pure TCC and 18 mixed TCC and high-grade serous carcinoma. Immunostaining of p53 was either positive (>60% nuclear staining, n=16) or totally negative (n=8). Mutation of TP53 was found in 100% of informative cases (n=16). No mutation was found in PIK3CA, PTEN, BRAF and β-catenin. Kras mutation was detected in two cases. The pattern of DNA copy number variations was similar to that of high-grade serous carcinoma, with frequent DNA gains and losses. No significant molecular difference was found between pure TCC and mixed TCC and high-grade serous carcinoma.
Conclusions: Our result demonstrated that pure TCC and mixed TCC and high-grade serous carcinoma have similar molecular alterations. In conjunction with the observation that focal morphological feature of TCC is not uncommon in high-grade serous carcinoma, ovarian TCC can be regarded as a variant of high-grade serous carcinoma when making diagnostic and treatment decisions.

1136 Genome-Wide Copy Number Alteration Profiles in Various Ovarian Carcinomas

TL Mao, HL Kao, JJ Sheu, KT Kuo. National Taiwan University Hospital, Taipei, Taiwan; Graduate Institute of Chinese Medical Science, Taichung, Taiwan.
Background: Ovarian carcinoma consists of several different types as defined by WHO, namely serous, endometrioid, clear cell, mucinous and transitional cell. Based on morphological features and molecular studies of ovarian carcinomas, dualistic pathogenetic pathways had been proposed and classified into low grade and high grade. High-throughput methods including expression profiling, comparative genomic hybridization and high-resolution single nucleotide polymorphism (SNP) array had been applied in an effort to search tumor suppressor genes and oncogenes in ovarian carcinoma. However, the results varied in different studies and the comparison among different types of ovarian carcinoma is not comprehensive.
Design: In this study, we compared the genome-wide copy number alterations among different types of ovarian carcinomas, including 9 high-grade serous carcinomas (HG), 13 endometrioid carcinomas (EM), 9 clear cell carcinomas (CC) and 5 transitional cell carcinomas (TC). High-resolution SNP array (250K) was performed using affinity-purified samples (>95% purity) from fresh specimens. The data was analyzed using dChip 2006 software. Probesets with an inferred $\log_2$ ratio > 0.3 or < -0.3 were classified as gain and loss, respectively. LOH analysis was carried out by the HMM-based method, using 60 normal human samples for reference genotypes.
Results: Among the four types of ovarian carcinoma analyzed, HG and TCC had the highest level of copy number alterations whereas CCC had the lowest level of copy number alterations. LOH is also most frequent in HG and TCC, while CCC and EM had relatively fewer LOH events. Amplification of Ch 1q is most frequent and is unique in EM. Amplification of 8q is most frequent in CCC and less common in other types of ovarian carcinoma. Candidate genes within the minimal amplicons include ZBTB10, KLF10 and AZIN1. Another specific region of amplification most common in CCC resides in Ch 20q, in which TPD52 and ZNF217 are candidate genes amplified. For HG and TCC, similar patterns of amplification of Ch 3q, 10p and deletion of Ch 3p, 5q and 8p were observed.
Conclusions: Our result demonstrated distinct patterns of copy number alterations in different types of ovarian carcinoma. CCC had the lowest level of copy number alterations, while HG and TCC had the highest level of copy number alterations. Interestingly, HG and TCC had similar molecular profiles, suggesting the wide morphological spectrum within a molecularly unique type of carcinoma.

1137 Internet-Based Assessment of Observer Variability for Diagnostically Challenging Endometrial Biopsies

JD Marotti, K Glatz, V Parkash, JL Hecht. Beth Israel Deaconess Medical Center, Boston, MA; University of Basel, Basel, Switzerland; Bridgeport Hospital, Bridgeport, CT.
Background: Endometrial Intraepithelial Neoplasia (EIN) is a diagnostic schema for premalignant endometrial lesions that has been developed by histopathologic correlation with clinical outcome, molecular changes, and objective computerized histomorphometry. However, several benign mimics of EIN continue to cause diagnostic confusion. Using an internet-based quiz we assessed observer variability in the diagnosis of EIN to better understand the diagnostic pitfalls.
Design: An online quiz consisting of 18 cases of endometrial biopsies considered difficult was prepared. Each case contained clinical history and at least 3 microscopic images. Answer choices included: 1) EIN, 2) Polyp, 3) Benign endometrium (proliferative, secretory, disordered, tubal metaplasia, lower uterine segment) and 4) Adenocarcinoma. For analysis, polyp and benign answers were combined into a single answer. Online EIN tutorial materials were offered to all participants prior to starting the quiz and the authors' diagnosis and clinical follow-up were provided at the end.
Results: The online quiz was completed by 51 participants with percentage agreement with the authors ranging from 22 to 100% (mean 55%). The mean percentage agreement was highest with benign/polyp cases: tubal metaplasia (86%) polyp (86%), and secretory -change (79%). Participants performed the worst with cases containing morular metaplasia (29%) and EIN arising in a polyp (52%). The mean percentage agreement for EIN without these features was 71%, and those participants who reviewed online tutorial performed better than those who did not (60% vs 49%).
Conclusions: Reproducibility of EIN criteria is good. A subset of biopsies with morular metaplasia and EIN in polyps are problematic and likely require consensus review. Limitations of this study include the limited format of selected case images.

1138 Synchronous and Metachronous Breast and Endometrial Cancer

RC Marques, F Silva, S Andre, A Felix. IPO Lisboa Francisco Gentil, Lisboa, Portugal.
Background: Breast cancer(BC) and endometrial cancer(EC) are very common neoplasms but synchronous(SC) and/or metachronous(MTC) tumours are rare. The purpose of our study was to characterize these tumors regarding clinical/morphological and immunohistochemical profile.
Design: We retrieved 55 cases of MTC/SC tumours with paraffin blocks from a group of 11378 cases treated for BC and EC in our Institution. We performed immunohistochemistry for Mlh1, Msh2, Msh6, ki67, ER, p53, Rb, ErbB2.
Results: Tumours were MTC in 42 cases. BC was the first tumour(GroupI) in 28 patients and EC (GroupII) in 14. In the remaining 13 patients, tumours were SC(GroupIII). Ten patients had bilateral BC. Seven patients (7,2%) had family history of breast/uterine cancer (FHBUC).The mean age of onset of the first tumour was 66,8yrs and of the second was 70,2yrs (mean time between: 3,4yrs; 1-15yrs) without difference between Groups I, II and III. FHBUC patients were younger (mean:60,7yrs) than patients from other groups. All were postmenopausal and the interval between menopause onset and first cancer was smaller in GroupII (mean:11,8yrs) than in GroupI and III (mean:18,3yrs

and 18,4yrs). The 5 and 10yrs survival was 75% and 31%, (identical for all Groups). In Group I, 20 women had tamoxifen therapy(Tx) before the appearance of EC. No differences were found regarding the time elapsed between tumors, age of patients and survival rates for patients with or without Tx. The prevalent histological type of BCwas invasive ductal carcinoma(81,8%) and of EC was endometrioid cancer(87,3%). The prevalent histological grade of BC was G2(58%) and of EC was G1(54,5%). No significant differences regarding histological type and grade, FHBUC and survival were found between Groups I, II and III. EC diagnosed before and after 5 years of Tx had a similar histological grade. Immunohistochemical results are shown in table 1. The loss of Rb and MSI-H was higher in Group III than other groups. FHBUC cases have high p53 expression.

		MSI-H(%)	Rb(%)	p53(%)	ki67(%)	ER(%)
GroupI	B	7.1	75	21.4	35,7	82.1
	E	37	61	37	70.4	77.8
GroupII	B	7.7	69	30.8	61.5	92
	E	38	46	38.5	69.2	92
GroupIII	B	35	85,7	14	57.1	78.6
	E	61.5	38.5	23	53.1	84

B-Breast cancer; E-Endometrial cancer.

ErbB2 was negative in all tumours.

Conclusions: Sychronous and metachronic tumours have good survival rate and good prognostic factors (RE+; erbB2-). No differences were found regarding age, family history, histological type and grade, survival and Tx therapy between groups. Synchronous tumors have different profile regarding instability and Rb expression.

1139 Endometrial Surveillance Outcomes for a Cohort of Breast Cancer Patients Treated with Tamoxifen

MS McLemore, A Malpica. UT MD Anderson Cancer Center, Houston, TX.

Background: Hormone therapy with tamoxifen for breast carcinoma (CA) is known to increase risk for endometrial cancer & polyps. However, only a few studies have evaluated these risks in tamoxifen patients (pts) undergoing regular endometrial surveillance. In this study, we present the outcomes of a cohort of breast cancer pts treated with tamoxifen who have been followed with serial endometrial sampling at a single institution.

Design: From 1998-2008, a cohort of 75 breast cancer pts treated with tamoxifen & evaluated with baseline endometrial biopsy was identified. Clinical follow-up was obtained for all pts. In cases where additional endometrial sampling was available, the diagnostic results were extracted from pathology reports.

Results: 81% of pts were evaluated by serial endometrial biopsies/curettings, & 19% underwent biopsy & eventual hysterectomy. The mean age was 50 years. 7% of pts were black, 9% Asian, 12% Hispanic, & 72% white. 69% of pts were treated for invasive ductal CA, 11% for invasive lobular CA, & 4% for mixed ductal & lobular CA; two pts were treated with tamoxifen for mucinous CA, & one for tubular CA. 4% of pts developed ductal CA in situ, & 3% had lobular CA in situ; 5% of pts received tamoxifen as chemoprevention for increased breast CA risk. In addition to tamoxifen, 39% of pts were treated with chemoradiation, 21% with chemotherapy, & 17% with radiation; 23% of pts received none. The mean duration of tamoxifen treatment was 3.6 years, & the mean follow-up duration was 3 years (range: 1 mo-9 years). Over the course of follow-up, 40% of pts maintained benign endometrium, 5% had inactive endometrium, & 4% showed disordered proliferative endometrium; one pt had insufficient endometrial tissue on her last biopsy. 39% of pts developed an endometrial polyp, & 7% developed a lower uterine segment polyp. 4% of pts showed complex hyperplasia without atypia, & 3% showed complex hyperplasia with atypia. One pt (1.3%) developed uterine serous CA in an endometrial polyp, & one (1.3%) developed well-differentiated endometrioid adenocarcinoma in an endometrial polyp. 18% of pts (8/45) showed an endocervical polyp on cervical biopsy with available results; 9% showed microglandular hyperplasia.

Conclusions: Although an endometrial polyp was the most common abnormality identified among pts, the risks for hyperplasia with atypia & for carcinoma while on tamoxifen remain real. Ongoing endometrial surveillance is warranted in breast cancer pts during treatment with tamoxifen. In addition, these pts can develop endocervical polyps & microglandular hyperplasia.

1140 Detection Frequency of p53 Signatures in Fallopian Tubes from Women with BRCA 1 or 2 Mutations (BRCA+) and Controls: The Role of Serial Sections

KK Mehra, M Chang, AK Folkins, C Raho, L Yuan, M Mehrad, SS Tworoger, CP Crum, A Saleemuddin. Brigham and Women's Hospital, Boston, MA; University of Toronto, Toronto, ON, Canada; Stanford, Palo Alto, CA.

Background: The p53 signature in the distal fallopian tube has been proposed as an early precursor to pelvic serous carcinoma, but its absolute and relative frequency in BRCA positive or negative women has been controversial. Precise ascertainment of the frequency is critical to determining the risk factors for this entity in either population.

Design: 24 BRCA+ and 40 controls (women with non-malignant disorders) were studied. In each case, three serial sections from every tissue block from each fallopian tube were removed from the block. Following sectioning through 100-200 microns, an additional three serial sections were placed on slides followed by an additional three serial sections following another 100-200 microns. One slide from each round was stained for p53. The number of cases, total blocks, proportion of blocks with signatures seen in each of the three rounds, and proportion of blocks with more than one signature detected following staining of the three sections were recorded. Comparisons were analyzed by chi-square.

Results: 17 (71%) and 20 (50%) of BRCA+ and control tubes harbored p53 signatures following the sectioning protocol outlined above (p = .12); 21 and 33% of all tissue blocks sectioned harbored signatures from the two groups (p = .07) respectively. In 49 and 32 per cent of p53 signature positive cases in the two groups, the p53 signatures were not discovered until the *second or third round of sectioning*. Thirty eight and 40% of BRCA+ and control subjects harbored p53 signatures in more than one focus in a single block.

Conclusions: p53 signatures are more common than previously reported and the frequency of detection increases as a function of sectioning through the tissue block, both in absolute frequency and in numbers of p53 signatures detected in a given block. There is a trend for a higher absolute frequency of p53 signatures (71 vs 50%; p = .12) in BRCA+ subjects, but this is not reflected in a greater *number* of p53 signatures or positive blocks *per case*. This study underscores the importance of systematic immunohistochemical examination of fallopian tubes when comparing the frequency of p53 signatures in different populations. Attention to this detail is critical when exploring risk factors germane to early serous carcinogenesis.

1141 Expression Profiling of a Candidate Precursor to Pelvic Serous Cancer in the Distal Fallopian Tube (p53 Signature)

KK Mehra, SW Tay, M Mehrad, CP Crum, W Xian. Brigham and Women's Hospital, Boston, MA; Institute of Molecular Biology, Singapore, Singapore.

Background: A link between many pelvic serous cancers and the distal fallopian tube has been strengthened by the discovery of a putative precursor that shares many features with serous carcinoma and resides in the distal fallopian tube – the p53 signature. This entity is phenotypically (secretory cell), immunophenotypically (p53 staining), genotypically (p53 mutations) and in some cases topographically linked to early (intraepithelial) carcinomas in the fimbria. The extremely small size of the p53 signatures has hampered efforts to assess its expression profile.

Design: Consecutively banked (frozen) fimbria from controls (benign conditions) and subjects with ovarian cancer were sectioned, immunostained for p53 and examined for the presence of p53 signatures. If p53 signatures were detected, serial sections from the tissue block were placed on membrane slides, the presence of the precursor confirmed by immunostaining of subsequent sections and RNA isolated by laser capture microdissection. Extracted RNA was amplified and hybridized to Affymetrix Human Exon 1.0 ST Array and the resulting data was resolved by Partek Genomics Suite software and Ingenuity Pathway Analysis software.

Results: Two p53 signatures were identified from analysis of 25 frozen tissue blocks. Hybridization efficiency of the amplified p53 signature RNA exceeded 80%. A supervised comparison of one p53 signature revealed deregulation of the p53 pathway compared to the normal epithelium. p53 was down-regulated by almost 2-fold in the p53 signature and was accompanied by down-regulation of MDM2; consistent with reduced proteasomal degradation of p53 and its accumulation in the secretory cell nuclei. PI3K and AKT, upstream of p53, were also down-regulated in the p53 signature, consistent with their role in the phosphorylation of MDM2 and potential loss of proteasomal degradation of p53. Disturbed expression in other genes participating in apoptosis, cell-cycle control and angiogenesis was observed.

Conclusions: This is the first report describing the gene expression profile of the p53 signature and the abnormalities of the p53 and related pathways that could contribute to the early phases of serous carcinogenesis. The relationship of these pathways to both the pathogenesis of the p53 signature and subsequent steps in serous carcinogenesis will be discussed.

1142 Cell Cycle Regulatory Markers in Uterine Atypical Leiomyoma, Cellular Leiomyoma, STUMP and Leiomyosarcoma: Immunohistochemical Study of 74 Cases with Clinical Follow-Up

AM Mills, A Ly, BL Balzer, MR Hendrickson, RL Kempson, JK McKenney, TA Longacre. Stanford University, Stanford, CA.

Background: Cell cycle regulatory protein expression may have diagnostic and prognostic value in distinguishing leiomyosarcoma from leiomyoma variants and in predicting recurrence for smooth muscle tumors of uncertain malignant potential (STUMP), but data - especially with clinical follow up - are limited.

Design: p16, p21, p27, p53, Ki67, and PHH3 protein expression was evaluated by immunohistochemistry (IHC) on full tissue sections of 44 atypical leiomyoma (AL), 8 cellular leiomyoma (CL), 6 STUMP, and 16 leiomyosarcoma (LMS). Nuclear staining was scored as absent (-), <33% (+), 33-66% (++), or >66% (+++). All results were correlated with clinical follow up, when available.

Results: Table 1 summarizes IHC staining for p16, p21, p27, and p53. Proliferation rate (Ki67) ranged from 0-25% in AL (mean, 2%), 0-10% in CL (mean, 3%), 0-10% in STUMP (mean,7.5%), and 6-50% in LMS (mean, 25%), while proliferation rate (PHH3) ranged from 0-3% in AL (mean, <1%), 0-2% in CL (mean, <1%), 0% in STUMP, and 0-10% in LMS (mean, 2%). Two of 35 AL had local recurrence following myomectomy at 24.9 and 25.7 mos (mean, 50.8). 7 of 8 LMS had local recurrence and/or distant metastasis (mean, 29.7 mos). There were no recurrences in 4 CL (mean, 22.6 mos) and 6 STUMP (mean, 16.2 mos) with follow up. The two AL with local recurrence showed only weak and focal (<33%) staining with all cell cycle markers aside from p21, which was diffusely positive in one case with recurrence.

Table 1

p16	AL	CL	LMS	STUMP
-	18%	0%	0%	0%
+	57%	75%	25%	66%
++	18%	25%	12%	17%
+++	7%	0%	63%	17%
p53				
-	67%	12%	19%	0%
+	14%	50%	44%	100%
++	12%	38%	12%	0%
+++	7%	0%	25%	0%
p21				
-	0%	0%	10%	0%
+	76%	50%	60%	80%
++	21%	50%	30%	20%
+++	3%	0%	0%	0%
p27				
-	10%	0%	20%	0%
+	52%	50%	20%	60%
++	31%	50%	20%	20%
+++	7%	0%	40%	20%

Conclusions: Uterine atypical leiomyoma, cellular leiomyoma, STUMP, and leiomyosarcoma demonstrate a heterogeneous pattern of cell cycle regulatory protein expression. Caution should be exercised in distinguishing leiomyosarcoma from atypical leiomyoma variants on the basis of cell cycle protein expression alone. In our study, cell cycle markers were not useful for predicting recurrence in AL or STUMP, but numbers are limited.

1143 Immunohistochemical (IHC) Characterization of Mullerian Adenosarcomas (AS)

L Mirham, MA Khalifa, S Nofech-Mozes, N Ismiil, RS Saad, Z Ghorab, V Dube. Sunnybrook Health Sciences Centre, Toronto, Canada.

Background: Mullerian AS are rare biphasic tumors composed of benign epithelium admixed with sarcomatous elements. Little is known regarding their IHC profile and its potential prognostic implication. In this study, we aim at characterizing the IHC expression of a panel of descriptive and potentially prognostic markers in AS, as well as markers linked to targeted therapies.

Design: In-house cases of AS accessioned between 1999-2008 were retrieved from the archives of Sunnybrook HSC. Slides and patients' charts were reviewed and the following data was recorded; tumor site, sarcomatous overgrowth (SO) and tumor recurrence. Original and recurrent tumors were studied using a panel of antibodies (ER, PR, CD10, p53, CD117, Her2/neu, EGFR) and IHC staining of the sarcomatous component was recorded semiquantitatively (0%; 1-25%; 26-50%; >50% of positive cells).

Results: 21 AS patients were identified (10 endometrial, 8 cervical and 3 ovarian). 4 patients had recurrrence but tissue was available for IHC study in only 3 of them (total: 24 tumors studied). SO was identified in 6 cases. IHC staining was positive at least focally (>1% of cells) in the following number of tumors: ER 24/24 (100%), EGFR 23/24 (95.8%), PR 21/24 (87.5%), p53 19/24 (79.2%), CD10 11/24 (45.8%) with only 4 cases showing positivity in >25% of cells, CD117 4/24 (16.7%) and Her2/neu 0/24 (0%). ER-PR were significantly underexpressed in cases with SO (Fisher exact test: p=0.014 and p=0.001, respectively). A trend toward underexpression of ER-PR in the original tumor of patients who subsequently experienced recurrence was observed, but it did not reach statistical significance. Interestingly, 20 cases showed staining for EGFR in >25% of the cells and 17 cases (including all cases with SO) showed diffuse positivity in >50% of cells.

Conclusions: All cases of AS were at least focally positive for ER and almost all cases for PR. The frequent expression of ER-PR in AS with significant underexpression in cases with SO confirms data from previous studies. The prognostic significance of ER-PR needs to be further assessed. A stromal origin doesn't seem to be a key feature in AS since CD10 was surprisingly expressed in less than half of our cases and consisted of only focal staining (1-25%) in most of them. We did not identify noteworthy expression for CD117 or Her2/neu, but the diffuse positivity for EGFR in most cases of AS is an interesting finding in the era of targeted therapy.

1144 Morphologic Criteria for Distinguishing Endometrial Adenocarcinoma from Complex Atypical Endometrial Hyperplasia

K Mittal, S Sasturkar, A Salem. New York University School of Medicine, New York.

Background: Morphologic criteria for distinguishing endometrial adenocarcinoma from complex atypical endometrial hyperplasia have been described previously, but have not been examined extensively for their individual predictive ability for finding endometrial adenocarcinoma in subsequent hysterectomy. We examined endometrial biopsies diagnosed in the spectrum of complex atypical endometrial hyperplasia to well differentiated endometrial adenocarcinoma for various morphologic features that may be predictive for presence of endometrial adenocarcinoma in subsequent hysterectomy.

Design: Cases diagnosed as FIGO grade I endometrial adenocarcinoma or complex atypical endometrial hyperplasia in endometrial biopsies seen at NYU Medical Center from 2003 to 2006 were analyzed for the presence of various morphologic features without the knowledge of hysterectomy findings. Only those cases with subsequent hysterectomy were included in the study. The data was analyzed to identify features with high specificity for finding of endometrial adenocarcinoma in subsequent hysterectomy.

Results: There were a total of 95 cases in the study, with 50 cases previously diagnosed as endometrial adenocarcinoma and 45 cases previously diagnosed as complex atypical endometrial hyperplasia. The following features were found to have high specificity for the finding of endometrial adenocarcinoma in subsequent hysterectomy (feature, specificity, sensitivity): A. Greater than 95% of area occupied by glands, with or without cribriforming, in single or multiple fragments with combined diameter of 2 mm or more, 83%, 81.6% B. Cribriforming in a fragment of tissue at least 2 mm in length, 83.3%, 16.6%. C. At least 2 mm combined diameter of all foci of cribriforming, 84%, 35%. Fibroblastic stroma, masses of squamous cells, or extensive papillary pattern were not found to be specific or sensitive predictive markers for endometrial adenocarcinoma.

Conclusions: The criteria generated in this study can be used to provide a more reliable diagnosis of endometrial adenocarcinoma than is possible with the currently used criteria. Dilated glands, secretory endometrium, gestational endometrium, and atypical polypoid adenomyoma should be excluded when making the evaluations of glandular crowding for endometrial hyperplasia and carcinoma.

1145 Expression of BDNF, TrkB and p53 in Uterine Cervical Cancer

A Moon, JY Lee, I Kang, SK Lee, YW Kim, YK Park, J Lee. Kyung Hee University, Seoul, Republic of Korea.

Background: Cervical cancer is the 4th most common cancer in Korean women. The neurotrophic receptor tyrosine kinase B (TrkB) and its high affinity ligand brain-derived neurotrophic factor (BDNF) play critical role in the central and peripheral neural system. Recent reports showed that higher levels of BDNF and TrkB generally correlate with more aggressive tumor behavior and they are involved in tumor proliferation, invasion and metastasis in a variety of cancers. The tumor suppressor gene p53 is frequently mutated gene in human cancer. P53 has important role in cell cycle arrest, DNA repair activation and regulation of apoptosis. The aim of the present study was to examine the potential role of BDNF, TrkB and p53 in cervix cancer focusing on the correlation between the expression of each protein and clinicopathologic parameters.

Design: RT-PCR analysis for BDNF and TrkB mRNA expression was performed on not only cervix cancer cell lines (SiHa, CaSki, HeLa, ME180) but also on human tissues of squamous cell carcinoma and normal tissue of the uterine cervix. We also analyzed TrkB, BDNF and p53 expression using immunohistochemistry in 80 patients with invasive squamous cell carcinoma of cervix.

Results: Consistent over-expression of BDNF and TrkB mRNA was found in all of the cervix cancer cell lines and squamous cell carcinoma tissues of cervix, compared to the normal cervical tissues. In immunohistochemical stain, high expression of BDNF, TrkB and p53 was observed in 54 (67.5%), 35 (43.8%) and 31 (38.8%) cases in total 80 cases, respectively. High expression of BDNF was inversely correlated to depth of invasion (p=0.04), lymphatic invasion (p=0.026) and lymph node metastasis (p=0.009). High TrkB expression was associated with keratinizing squamous cell carcinoma (p=0.001). P53 expression was significantly related to the tumor stage (p=0.001), greatest dimension (p=0.002) and depth of invasion (p=0.005).

Conclusions: Our data demonstrated that BDNF and TrkB expression might have a significant role in tumorigenesis of uterine cervical squamous cell carcinoma. BDNF predicted a better prognostic value but, TrkB did not correlate with patient outcome. As expected, p53 was a useful poor prognostic marker in uterine cervical cancer.

1146 Interaction of PAX2 and PTEN Drives Emergence of Endometrial Precancers from a Preclinical Latent Phase

GL Mutter, NM Monte, KA Webster, D Neuberg, GR Dressler. Brigham and Women's Hospital, Boston, MA; Dana Farber Cancer Institute, Boston, MA; Univ. Michigan, Ann Arbor, MI.

Background: Latent endometrial precancers are small numbers of normal appearing glands with sporadic inactivation of tumor suppressor genes such as PTEN. These advance to cancer at low efficiency only upon acquisition of additional genetic damage. We here present evidence that inactivation of the transcription factor PAX2, which is required for embryonic development of a uterus, behaves as a latent precancer, and study its association with PTEN during endometrial carcinogenesis.

Design: Normal (premenopausal proliferative n=191), premalignant (EIN, n=52), and malignant (endometrial adenocarcinoma, n=62) endometrial biopsy and curetting tissues were immunostained for PTEN and PAX2. Proliferative samples with discrete loss of PAX2 or PTEN protein in at least one gland were scored as latent precancers, and the number of affected glands counted. EIN and cancer lesions were scored overall. Latent precancer prevalence and number of affected proliferative glands was analyzed by biopsy indication and age. Overlapping PAX2 and PTEN co-inactivation was examined in each tissue type.

Results: The prevalence of PAX2 inactivation in the sequence of normal (latent precancer) to EIN to cancer was 36%, 71%, and 77% respectively, and for PTEN 49%, 44%, and 68%. Amongst normal proliferative endometria, the prevalence of both PAX2 and PTEN defined latent precancers was unaffected by biopsy indication but increased significantly with age. Coincident inactivation of PAX2 and PTEN in an individual normal endometrium was seen in 21% (40) of patients, but these usually involved different subsets of glands. Of a total of 1281 null glands (449 PAX2 null and 847 PTEN null) seen in these 40 patients, only 15 individual glands (1.2%) had inactivation of both. Coincident inactivation was more common in EIN (31%) and carcinoma (55%), with both genes co-inactivated in the majority (>90%) of glands.

Conclusions: PAX2 and PTEN are biomarkers for latent precancers which accumulate independently with increasing age in normal premenopausal endometrium. Independent inactivation of either may occur in normal tissues, whereas inactivation of both genes in an overlapping distribution characterizes premalignant EIN lesions and carcinoma. This suggests that PAX2 acts as a tumor suppressor in the endometrium, but other events such as PTEN inactivation are required to develop clinical disease.

1147 Uterine Involvement by Colorectal Adenocarcinoma: Morphologic and Immunohistochemical Patterns That May Mimic Primary Endometrial, Endocervical or Vaginal Adenocarcinoma

A Naujokas, JT Rabban. UCSF, San Francisco, CA.

Background: Although spread of colorectal adenocarcinoma to the ovaries is well recognized, spread to the uterus/vagina is rare and may potentially be misclassified as primary gynecologic in origin; this may also lead to under-recognition of Lynch syndrome. This study reports the morphology and immunohistochemistry of colorectal cancer involving the uterus/vagina, emphasizing features that may be confused with those of primary endometrial, endocervical or vaginal adenocarcinoma.

Design: Cases of colorectal adenocarcinoma involving the uterus/vagina were identified from our institutional cancer registry (1985 to 2009). Clinical history was obtained from electronic records; slides were reviewed and confirmatory immunostaining was performed with CK7, CK20, CDX2, estrogen receptor, and p16. MLH1, PMS2, MSH2 and MSH6 staining was performed in a subset. Tumor growth pattern was classified by whether the classic features of metastatic colon cancer were present: dirty necrosis, segmental necrosis and garlanding. Tumor was also classified by whether the growth pattern simulated a primary endometrial, endocervical or vaginal adenocarcinoma.

Results: Among 1,734 women with colon cancer, 15 (age 28-75 years) had pathologically diagnosed involvement of uterine body, uterine cervix or vagina. History of primary colon origin was known in 14/15 prior to undergoing uterine surgery and 12/15 had prior chemotherapy and or radiotherapy. Tumor distribution included: endometrium(6), myometrium(6), endocervix(7), and/or vagina(3). Tumor type was conventional adenocarcinoma(11), pure mucinous adenocarcinoma(4) or a mixed(1). Histology revealed low grade architecture in 12/15, low grade cytology in 7/15, goblet cells in 4/15, dirty necrosis in 10/15, segmental necrosis in 10/15, garlanding in 6/15; none had squamous differentiation. All were CDX2 positive but 9/11 were p16 positive (7 strong/diffuse, 2 weak/patchy); 11/12 were CK20+CK7-. H&E morphology was clearly colonic in origin in only 5/15; the remainder had growth simulating primary uterine/vaginal cancer. Mismatch repair was defective in 2/5 cases. One patient had Lynch syndrome.

Conclusions: Uterine/vaginal involvement by colorectal cancer may mimic primary gynecologic cancer for several reasons: 1.) classic colonic morphology is not always present 2.) p16 expression may be present 3.) goblet cells may mimic intestinal type endocervical cancer and 4.) growth patterns may resemble primary endometrial, endocervical or vaginal cancer.

1148 CDX2 Expression in Gynecologic Yolk Sac Tumors

MY Ngae, MT Deavers. The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Yolk sac tumor (YST) has a variety of microsopic patterns that can mimic carcinomas. In the gynecologic tract, the glandular variant of YST can be confused with endometrioid adenocarcinoma; papillary, glandular, solid or hepatoid features may be difficult to distinguish from clear cell carcinoma. As such, alpha-fetoprotein (AFP) has traditionally been used to help identify YST. However, AFP can be focal or negative in YSTs and lacks adequate sensitivity and specificity to be used in isolation, with documented expression in other gynecologic and non-gynecologic malignancies. Immunohistochemical reactivity for CDX2 is often used to demonstrate intestinal differentiation in adenocarcinomas. Recently, CDX2 expression has also been found in YSTs of the testis and even lung, but to our knowledge has not been reported in YSTs of the gynecologic tract.

Design: In this study, we evaluate 13 cases of YST collected over an 18 year period for CDX2 expression. Immunohistochemical staining for CDX2 (CDX2-88, 1:50, Biogenics) was performed on BondMax automated immunostainers (ER2 20 minutes). The IHC slides were reviewed in conjunction with the H&E-stained sections. Nuclear staining was graded as follows: 1+ (1-25%), 2+ (26-50%), 3+ (51-75%), 4+ (76-100%). Controls reacted appropriately. The majority of the tumors were located in the ovary, with the exception of 1 endometrial primary, and 1 metastasis to a retroperitoneal lymph node. Of the ovarian tumors, 6 cases were pure YST, 3 cases were mixed YST and teratoma, 1 was a case of YST with endometrioid adenocarcinoma, and the last was a YST with clear cell carcinoma. The endometrial tumor was composed of YST with serous and endometrioid adenocarcinoma. The metastatic YST in the lymph node was associated with an ovarian dysgerminoma.

Results: All the YSTs (and different patterns) had at least 1+ staining for CDX2. There was 2+ staining in the YST associated with clear cell carcinoma of the ovary, and 2-3+ staining in an ovarian mixed germ cell tumor with teratoma and YST components. The different patterns of tumor that demonstrated staining included reticular, polyvesicular-vitelline, solid and papillary forms. Focal staining of the teratomatous intestinal glandular components of the mixed germ cell tumors was noted.

Conclusions: CDX2 can be used as an adjunct to AFP in support of the diagnosis of gynecologic YST. Caution should be exercised in interpreting any positivity for CDX2 in the setting of a teratoma containing components with intestinal differentiation.

1149 Expression of DPC4 in Primary Mucinous Neoplasms of the Ovary and Breast

LA Niemeier, K McManus, A Krasinskas, M Chivukula. Magee Women's Hospital, Pittsburgh, PA; UPMC-Presbyterian, Pittsburgh, PA.

Background: DPC4 tumor suppressor pathways are genetically inactivated in up to 40% of pancreatic carcinomas and was shown by few studies to be highly correlated with the presence of widespread metastasis. DPC4 antibody has utility in the diagnosis of primary pancreatic mucinous neoplasms. Positive immunohistochemical staining has been shown in pancreatic intraductal mucinous tumors (100%) and pancreatic mucinous cystic neoplasms (85%) and in 52% of cholangiocarcinomas. The aim of our study was to evaluate the DPC4 expression in primary mucinous neoplasms of the ovary and breast and also to determine if DPC4 can be utilized to differentiate primary ovarian mucinous neoplasms from metastatic mucinous breast and pancreatico-biliary tract neoplasms to ovary.

Design: Cases were collected from histology at our institution as follows: 14 primary breast cancers (9 invasive ductal carcinomas with mucinous features(BR-MIXED) and 5 invasive mucinous cancers(BR-MUC), 33 primary mucinous ovarian lesions (20 mucinous adenocarcinomas(OV-CA) and 13 mucinous borderline tumors(OV-LMP), 22 cholangiocarcinomas (13 extrahepatic(C-EH) and 9 intrahepatic(C-IH), and 5 metastatic(MET) lesions to the ovary (3 gastric (GA)and 2 pancreatic(PAN). Immunohistochemical staining for DPC4 was performed on all cases and interpreted as a combined score (CS) of intensity (I) of staining (0=negative,1+=weak,2+=moderate,3+=strong) and percentage(%) (0=negative, 1-20=weak, 21-80=moderate,81<=strong). The median CS was calculated for each group.

Results: Similar CS staining was identified in each of subgroups of the primary breast (CS=6.0) and ovarian lesions (CS=6.0). On the other hand, extra/intra hepatic cholangiocarcinomas showed a lower CS (2.0) compared to all other groups.

Combined Score of Immunohistochemical Staining of DPC4

BR-MIXED (n=9)	BR-MUC (n=5)	C-EH (n=13)	C-IH (n=9)	OV-CA (n=20)	OV-LMP (n=13)	MET-GA (n=3)	MET-PAN (n=2)
6.0	6.0	2.0	2.0	6.0	6.0	5.0	4.5

Conclusions: DPC4 staining is similar among all mucinous breast cancers. DPC4 staining is also similar in ovarian mucinous cancers and borderline tumors. Due to a lower expression in both groups of cholangiocarcinomas, DPC4 may be utilized in a panel to differentiate between metastatic lesions of cholangiocarcinomas versus ovarian primary. DPC4 expression to differentiate between metastatic mucinous gastrointestinal/pancreatic /breast origin from primary ovarian mucinous lesions is being further evaluated.

1150 A Non-Infectious Mechanism for Pre-Term Delivery: Free Fetal DNA Sensed by Maternal TLR-9

AS Nugent, S Daly, S Corr, JJ O'Leary, LAJ O'Neill. The Coombe Women and Infants University Hospital, Dublin, Ireland; Trinity College Dublin, Dublin, Ireland.

Background: Preterm delivery (PTD) is the largest contributor to the modern day perinatal mortality rates in developed countries. Although much research has gone into the causation of preterm labour, the rate of PTD has remained the same. In this study, we have investigated the potential for fetal DNA, which has been shown to be elevated in maternal blood of women who deliver premature, to cause an inflammatory response.

Design: Purified fetal DNA was used to stimulate Namalwa cells and female peripheral blood mononuclear cells(PBMCs) to measure NFK-B activation by immune blotting. IL-6 concentrations were also measured using ELISA. To test induction of TLR-9, choloroquin and synthetic inhibitory oligodinucleotides were used to block the action of TLR-9. Fetal DNA was then applied to TLR-9 deficient bone marrow derived macrophages. Findings were then applied to an *in vivo* mouse model.

Results: We show through that purified fetal DNA induces IκB degradation in female peripheral blood mononuclear cells (PBMC). The effect was inhibited by chloroquin and inhibitory oligodinucleotides which block CpG DNA. Fetal DNA induced IL-6 production for PBMC and bone marrow derived macrophages. This effect was not evident in TLR-9 deficient macrophages. When Fetal DNA was injected into mice at increasing concentrations, this was found to have a negative effect on carrying the pregnancy to term.

Conclusions: These results indicate that fetal DNA is an agonist for TLR-9. TLR-9 is present in maternal blood cells and free Fetal DNA is seen in elevated concentrations in mothers who deliver preterm. Fetal DNA induces the production of IL-6 which a key cytokine in the pathway to PTD. Studies *in vivo* also demonstrate that increasing doses of free Fetal DNA have a negative effect on pregnancy. Taken together, it is possible that Fetal DNA might provoke inflammation seen in PTD via TLR-9.

1151 Primary Squamous Cell Carcinoma of the Vagina: HPV Detection, p16 Immunostaining and Clinicopathological Correlations

J Ordi, V Fuste, M del Pino, I Alonso, A Garcia, A Perez, A Torne, J Pahisa. Hospital Clinic, IDIBAPS, University of Barcelona, Barcelona, Spain; Hospital Vall d'Hebron, Barcelona, Spain.

Background: Primary squamous cell carcinoma of the vagina (SCCVa) is rare. Human papillomavirus (HPV) has been implicated in its pathogenesis in a percentage of cases. Nevertheless, there is scant information on the role of HPV in SCCVa, and the clinico-pathological significance of HPV involvement in this tumor has not been clearly defined.

Design: All cases of SCCVa diagnosed between 1995 and August 2008, were retrieved from the surgical pathology files of two Hospitals from Barcelona. The clinical charts and the pathological materials were reviewed. Patients with a previous history of carcinoma of the uterine cervix diagnosed less than 5 years before were excluded from the study. HPV was detected and typed by PCR using the SPF10 primers In all cases immunohistochemical staining for p16 and p53 was performed.

Results: We retrieved 32 cases of SCCVa. HPV was detected in 25 cases (78.1%). HPV 16 was the most prevalent type identified (18 out of 25 [72.0%] HPV-positive cases). Patients with HPV-positive tumors were significantly younger than patients with HPV-negative neoplasms (62.6±13.8 vs. 74.0±8.5; p=0.049). A previous history of carcinoma or intraepithelial neoplasia of the uterine cervix or vulva diagnosed more than 5 years before the diagnosis of SCCVa was identified in 56.0% (14/25) of women with HPV-positive tumors and none of HPV-negative tumors (p=0.01). The upper third of the vagina was exclusively involved in 8/25 (32%) HPV-positive and 0/7 (0%) HPV-negative tumors (p=0.14). Histologically, 21/25 (84%) HPV-positive tumors and 1/7 (14.3%) of HPV-negative tumors were non-keratinizing, basaloid or warty, whereas 4/25 (16%) and 6/7 (85.7%) were of keratinizing type (p=0.006). Diffuse staining for p16 was observed in 24/25 (96%) of HPV-positive and 1/7 (14.3%) of HPV-negative

tumors (p<0.001). Positive staining for p53 was observed in 12.0% HPV-positive and 57.1% HPV-negative tumors (p=0.02).
Conclusions: 1) A high proportion of SCCVa are related to HPV infection; (2) Immunostaining for p16 may be helpful in the identification of tumors associated with HPV infection; and (3) HPV-positive tumors tend to affect younger women with previous history of carcinoma of the uterine cervix and to involve more frequently the upper third of the vagina.

1152 Pathological Features of Endometrial Cancer in Patients with Lynch Syndrome

J Palacios, C Alenda, D Lujan, C Cordero, E Mendoza, P Trillo, JL Soto. Hospital Universitario Virgen del Rocio, Seville, Spain; Hospital Universitario de Alicante, Alicante, Spain; Hospital General Universitario de Elche, Elche, Spain.
Background: The incidence of endometrial cancer (EC) in women with Lynch syndrome (LS) equals or exceeds that of colorectal cancer (CRC) and in more than 50% of cases these women present with a gynaecological cancer as the first or sentinel malignancy. Although pathological characteristics of CRC in LS patients are well documented, there are few studies analyzing pathological features in LS patients with documented germline mutations.
Design: The pathological features of 16 ECs found in patients with LS (mean age 44.9 years) with documented germline mutation in *MLH1* (8 patients), *MSH2* (6 patients) or *MSH6* (2 patients) genes were compared with those observed in a series of 28 EC found in patients 50 years old or younger (mean age 46.2 years). Histological type and grade, deep of myometrial invasion, presence of vascular invasion, peritumoral lymphocytes, tumor infiltrating lymphocytes (TILs), and the presence of Cronh-like aggregates were compared between both groups.
Results: Amomg LS-ECs, 6 (37%) had a non-endometrioid (serous or clear cell) component, 7 (46%) extensive peritumoral lymphocyte infiltration and 8 (50%) more than 40 TILs/10 HPFs, but these characteristic were only found in 4 (14%), 3 (10.7%) and 5 (17.8%) control ECs (p<0.05 for all comparisons). There were no statistical significant differences in the frequency of all other variables analyzed, although LS-ECs were more frequently grade 3 (20% vs 8%) and showed more frequently Cronh-like aggregates (27% vs 18%). Immnohistochemistry analysis of MLH1, PMS2, MSH2 and MSH6 was concordant with the genetic alteration in LS-ECs. In addition, we found 5 MSH1/PMS2-negative and 2 MSH2/MSH6-negative cases (presumptive LS patients) among control ECs.
Conclusions: Tumour pathological features together with age and familial history of LS-associated cancers can help in selecting tumors for the study of expression of MLH1, PMS2, MSH2 and MSH6 by immunohistochemistry and to select women with EC for LS genetic testing.

1153 Vitamin D Receptor Expression in Normal Endometrium and Endometrial Carcinoma. A Tissue-Microarray Study

J Pallares, S Lopez, M Santacana, X Dolcet, JM Valdivielso, J Valls, X Matias-Guiu. Hospital Universitari Armau de Vilanova-IRB, Lleida, Spain.
Background: Vitamin D insufficiency has been involved as an etiological agent in different types of tumors, because of its effects in cellular proliferation, apoptosis and angiogenesis. Recent studies have demonstrated an inhibitory effect of Vitamin D in cell proliferation endometrial carcinoma (EC) cell lines with an induction of cell differentiation.
Design: We analyzed the immunohistochemical expression of the Vitamin D receptor (VDR) in two tissue microarrays (TMA). One constructed from 80 samples of normal endometrium in different phases of the menstrual cycle and a second TMA containing 62 ECs with different histological types, FIGO grades and pathological stages.
Results: In normal endometrium (NE) VDR expression was very significantly reduced in the proliferative phase (mean: 190.76) compared with the secretory phase (mean: 214.39) (p = 0.006). EC showed a significant reduction in VDR expression compared with its expression in normal endometrium (mean EC: 181.7, mean NE: 207.7) (p = 0.001). No differences were observed in the VDR expression in different types of EC (mean endometrioid type: 181.30, mean non-endometrioid type: 183.88) (p = 0.99), histological grade (mean grade I: 177.06, grade II: 189.55 and grade III: 176.33) (p = 0.43) or with pathological stage (p = 0.18).
Conclusions: VDR expression is significantly reduced in the proliferative endometrium and endometrial carcinoma and could have a role in endometrial cell carcinogenesis by controlling mechanisms involved in cell proliferation.

1154 Androgen Receptor Coactivator p44/Mep50 in Ovarian Cancer

LY Pan, JJ Wei, RR Patwa, M Ligr, S Jain, XY Zou, G Daniels, YR Li, RL Xu, W Sun, ZX Wang, P Lee, F Chen. New York University, New York, NY; NY Harbor, New York, NY; MD Anderson, Houston, TX.
Background: Hormones, including estrogen and progesterone, and their receptors play an important role in the development and progression of ovarian carcinoma. Androgens and androgen receptors have also been implicated. The methylosome complex, with a p44 as a subunit, has lately been characterized as an androgen receptor coactivator, which enhances androgen receptor- and estrogen receptor-mediated transcriptional activation in a ligand-dependent manner. We previously examined the expression and function of p44 in prostate, testis and breast cancers and observed organ specific p44 subcellular location and function. In this report, we examined the expression of p44 in ovarian cancer.
Design: Immunohistochemistry was performed using a p44/Mep50 antibody on a tissue microarray of ovarian cancer (n = 56): 14 mucinous (MUC), 13 clear cell (CCC), 10 endometrioid (EMC), 8 serous borderline (SBT), and 11 fallopian tube high grade serious carcinoma (FT). The levels of p44 cytoplasmic and nuclear expression were scored semi-quantitatively: 0 as negative, 1+ as faint, 2+ as weak, 3+ as moderate and 4+ as strong expression. The percentage of cells was given: 1 as 0-10%, 2 as 10-50%, 3 as 50-75% and 4 as 75-100%. Statistical analyses were performed by t-test.
Results: All 5 types of ovarian carcinoma revealed both nuclear and cytoplasmic p44 at various levels. For cytoplasmic staining, EMC showed the strongest intensity (2.85 ± 0.06), while MUC showed the weakest of both intensity (1.35 ± 0.06) and percentage (3.5 ± 0.09). In contrast to normal ovarian tissue, all types of tumors showed positive nuclear staining in greater than 50% of cells. Again, MUC showed the weakest staining, in intensity (2.04 ± 0.04) and percentage (3.13 ± 0.17). SBT showed the strongest intensity, (3.0 ± 0). FT and CCC tumors showed the next highest intensity, (respectively 2.52 ± 0.16 and 2.5 ± 0.13). This nuclear p44 expression is consistent with its growth stimulatory effects.
Conclusions: The expression of p44 shows strong cytoplasmic expression in morphologically normal ovarian surface epithelium and fallopian tubes, while cytoplasmic and nuclear p44 localization is observed in invasive ovarian carcinoma. Since MUC has lower ER cytoplasmic p44 expression, these findings suggest that nuclear p44 may play a role as an estrogen receptor mediator in the tumorigenesis of serous and clear cell ovarian carcinoma.

1155 Smooth Muscle Actin Immunostaining as a Marker of Invasion in Cervical Adenocarcinoma

VJA Panico, Y-F Liu, EC Pirog. Faculdade de Ciências Médicas de Santos - FCMS, Santos, SP, Brazil; Weill Medical College of Cornell University, New York, NY.
Background: Differentiation of adenocarcinoma in-situ (AIS) from invasive adenocarcinoma (INV) of the cervix is difficult in some cases. Lack of objective markers of invasion may result in discrepant measurements of depth of invasion rendered by different pathologists. The goal of the study was to investigate if the expression of smooth muscle actin (SMA) in the cervical stromal cells surrounding invasive glands of adenocarcinoma may highlight the invasive foci and help to standardize the diagnosis.
Design: The study included cases of unequivocal invasive adenocarcinoma (n=21) as determined in a consensus review. The negative control group consisted of cases of unequivocal adenocarcinoma in-situ (n=9) and normal cervix (n=11). All cases were immunostained with smooth muscle actin antibodies (Dako).
Results: The stromal cells surrounding benign endocervical glands were negative for SMA staining in all of the normal cases; however, in 2 cases faint positive staining was identified surrounding deep Nabothian cysts. In addition, there was a positive reaction in the stroma of an endocervical polyp. In 6 of 9 cases of AIS the stromal SMA staining was negative, but in 3 cases focal faint positivity was identified. A strong stromal SMA positivity surrounding invasive tumor glands was present in 15 of 21 adenocarcinomas. In 11 cases the staining was seen throughout the entire tumor and in 4 cases the staining was present around majority of the glands. In 4 of 21 adenocarcinomas there was a marked inflammatory infiltrate surrounding the invasive glands and in these cases no SMA staining was identified in the stromal cells. Finally, two cases of adenocarcinoma had an exophytic growth pattern and these cases showed no stromal SMA staining in the papillary fibro-vascular cores.
Conclusions: Positive stromal SMA immunostaining may be useful to identify invasive glands in cervical adenocarcinoma, however, the staining may not be present in tumors surrounded by marked inflammation or in polypoid exophytic tumors.

1156 Acquired Vulvar Lymphangioma Circumscriptum: A Comparison between Crohn's Associated and Radiation Therapy Induced Tumors

JA Papalas, SJ Robboy, MA Selim. Duke University Medical Center, Durham, NC.
Background: Lymphangioma circumscriptum is a tumor of lymphatic origin which occurs throughout the body. Vulvar involvement may occur in a variety of clinical settings.
Design: We review 11 cases, with an average follow-up time of 6 years with an emphasis on comparison between cases that occur in patients with Crohn's disease and those with a history of pelvic radiation exposure.
Results: The average age at presentation was 52 years old. 64% of patients had lesions in multiple anatomically distinct regions. 27% of patients had a history of Crohn's disease, 64% had a history of radiation therapy for abdomino-pelvic malignancy, and 10% had no significant past medical history. 4/11 patients had lower extremity edema and 4/11 patients had fistula tracts. Average time to onset after first documentation of Crohn's disease or after undergoing radiation therapy was 17.8 years. Common presenting complaints were vulvar puritus, wetness, and edema. Clinically, the tumors ranged from tan-white pedunculated papules to sessile nodules (2.0 cm on average) or were weeping vesicular patches, most often found on the labia majora followed by the mons pubis. The tumors were composed of subcutaneous dilated lymphatics located at the junction of the reticular and papillary dermis with some demonstrating focal extension into the epidermis. No lesions involved the subcutaneous tissues. Cytologically, cells protruded into the dilated lymphatic channels in a hobnail pattern with minimal cytologic atypia and mitotic activity. All lesions so examined were positive for D240. Patients were most often treated with surgical excision followed by excisional biopsy and laser ablation. 4/11 patients would experience disease progression necessitating additional excisions.
Conclusions: When comparing patients with a history radiation therapy to those with Crohn's disease, those treated with radiation present almost 20 years sooner, are more likely to have associated co-morbidities, and are more likely to have disease progression requiring additional surgeries.

Comparison of patients with a history of Crohn's disease versus radiation therapy induced tumors

Clinical parameters	Crohn's	Radiation
Number of patients	3	7
Average age	58	53
Years to onset	32	15
Associated lower extremity edema	0	4
History of fistula tract formation	3	1
Progression requiring additional surgery	0	4
Multifocal disease	1	5

Acquired vulvar LC may have numerous causes. We show that the overall prognosis may be different depending on the etiologic factors leading up to the disease.

1157 Can We Accurately Predict High Risk Endometrial Carcinoma Preoperatively?

V Parkash, N Rassaei, GL Keeney, CN Otis, JL Hecht, O Fadare. Yale University, New Haven, CT; Mayo Clinic, Rochester, MN; Baystate Health Systems, Springfield, MA; Beth Israel Deaconess Medical Center, Boston, MA; Vanderbilt University, Nashville, TN.

Background: The management of endometrial carcinoma (EMCA) requires accurate staging. Most patients present with stage 1 disease, but prediction of stage is inaccurate in 15-20% of cases when using clinical parameters and FS evaluation. Therefore, the NCCN guidelines recommend complete staging of ALL cases of EMCA. This, however, means that 15-20% of patients get staging for low stage, low grade disease, which may be associated with some morbidity. The Mayo Clinic has proposed criteria that may allow more accurate identification of such low risk patients with endometrial biopsy (EMB) and intraoperative assessments (IO), and avoid staging in these cases. This study was carried out to determine if these triage criteria worked at an institution other than the Mayo Clinic.

Design: Pathology reports on patients with EMCA, who had IO and staging were reviewed over a one year period and patients were classified as high risk based on EMB and IO. Patients were classified as high risk if they had any of the following -- grade 3 carcinoma on EMB or IO, tumor size > 2cm, myoinvasion of > 50% and lymphovascular invasion (LVSI) at IO. The final reports of the remainder of patients (putative "low risk") were reviewed to determine if any "high risk cases" were inaccurately identified using this protocol.

Results: Of a total of 85 patients who underwent staging for EMCA, 52 patients had EMB's reported as low grade EMCA. Of these, 5 had ovarian enlargement that on frozen section (FS) revealed carcinoma (4 synchronous ovarian carcinoma). 23 patients had bulky endometrial tumors, > 2 cm grossly. Of the remaining 24 cases, 3 had depth of invasion > 50%, while1 had a high grade carcinoma at FS (a minimal serous carcinoma). All the remaining 20 cases remained low stage (16 stage 1A, of which 3 had only complex hyperplasia on hysterectomy). Of the four stage 1B cases, 1 had a single focus of LVSI. In this case, the depth of invasion was 30%.

Conclusions: Using a strategy of exclusion of patients with negative predictive parameters, "low risk patients" were identified accurately in > 95% of cases. Application of this strategy may save an estimated 20% of patients an expensive and potentially morbid staging procedure.

1158 Morphologic and Immunohistochemical Correlation of Ovarian and Tubal Dysplasia in Prophylactic Oophorectomies from BRCA1/2 Mutation Carriers

FM Penault-Llorca, G Chene, MM Dauplat, I Raoelfils, N Lagarde, YJ Bignon, J Dauplat. Centre Jean Perrin, Clermont-Ferrand, France.

Background: Histopathological examination of material from prophylactic salpingo-oophorectomies (pBSO) performed in patients at genetic risk has revealed frequent abnormalities interpreted as possible pre-cancerous " ovarian dysplasia " lesions. We sought to study the morphologic features and immunohistochemical expression patterns of neoplasia-associated markers in prophylactically removed ovaries.

Design: Morphologic features and immunohistochemical expression patterns of Ki-67, p53 and ALDH1 (an enzyme significantly associated with early-stage ovarian cancer) were evaluated in 35 pBSO from BRCA1/BRCA2 carriers and 30 normal salpingo-oophorectomies (nBSO). Representative slides from formalin-fixed, paraffin-embedded tissue blocks were all read blindly by two gynecological pathologists (FPL & NL). Immunohistochemical staining results were correlated with morphologic findings.

Results: Mean ovarian and tubal dysplasia score were significantly higher in the genetic risk group than in controls (respectively 9.29 vs 3.17, $p< 0.0001$ for ovaries and 6.54 vs 1.37, $p<0.0009$ for tubes). Increased ALDH1 expression was observed in pBSO compared with nBSO whereas expression patterns of Ki67 and p53 were low in both groups. Interestingly, ALDH1 expression was low in non dysplastic epithelium, high in dysplasia and constantly low in the carcinoma found incidentally on pBSO.

Conclusions: The increased dysplasia score and ALDH1 expression in ovaries from BRCA1/2 carriers might be consistent with progression towards neoplastic transformation and could justify the use of the term "dysplasia" or intraepithelial ovarian neoplasia. Ovarian and tubal dysplasia may be a pre-malignant, non-invasive histopathological abnormality that could be an important step in early neoplasia, especially in ovaries from BRCA1/2 carriers. The ALDH1 activation in pBSO should be considered as a target for prevention.

1159 A Tissue Microarray Immunohistochemical Study of Gynecologic Versus Breast Primary

TC Pereira, SM Share, KM Jasnosz, AV Magalhaes, JF Silverman. Allegheny General Hospital, Pittsburgh, PA; Universidade de Brasilia, Brasilia, Brazil.

Background: In the work-up of metastatic carcinoma of unknown primary, some of the immunohistochemical (IHC) markers commonly used for a gynecologic (GYN) primary such as estrogen (ER) and progesterone receptor (PR) proteins are also reactive in breast primaries. Therefore, an expanded panel can be of value in indeterminate cases in the work-up of metastases of unknown primary when the differential diagnosis is a GYN versus a breast primary and/or a patient with either a known GYN or breast primary and a second primary needs to be excluded. For this differential, we evaluated a panel including ER, PR, GCDFP-15, CDX2, villin, Pax-2, mammaglobin and WT1 in a tissue microarray of breast carcinomas and GYN carcinomas including ovary and endometrium.

Design: We performed immunostains in a total of 171 cases: 40 endometrium, 45 ovary, and 86 breast, including different histologic types on each. We scored as positive if at least 5% of the cells were staining, with nuclear staining for ER, PR, CDX-2, Pax-2 and WT-1; cytoplasmic staining for GCDFP-15 and mamaglobin; and membranous staining for villin. We calculated the percentage of positive cases after excluding cases in which the microarray "dot" did not contain tumor cells or was not present in that particular slide.

Results:

Percentage of positive cases

	Breast	Ovary	Endometrium
CDX-2	0	18.2%	15.4%
Villin	0	9.3%	10.3%
GCDFP-15	67.1%	2.3%	0
Mammaglobin	61.3%	52.3%	69.2%
ER	89.2%	83.7%	80.0%
PR	62.5%	85.7%	97.3%
WT-1	10.1%	59.1%	7.9%
Pax-2	6.3%	18.6%	46.2%

Conclusions: In the differential diagnosis of GYN versus breast metastatic carcinomas, the most useful markers are Pax-2 for endometrium, WT-1 for ovary, and GCDFP for breast. The markers ER, PR, and mammaglobin were not helpful due to lack of specificity. CDX-2 and villin were not very sensitive, but when positive could be helpful in excluding breast carcinoma.

1160 Immunolocalisation of Key Spindle Assembly Checkpoint Proteins – Correlation with Cellular Prolifaration and Chemotherapeutic Response

SM Phelan, B McGrogan, M Prencipe, P Fitzpatrick, D Brennan, C O'Herlihy, M Foley, E Doyle, J Harford, T O'Grady, E Kay. University College Dublin, Dublin, Ireland; National Maternity Hospital, Holles St., Dublin, Ireland; St. Vincent's University Hosptial, Dublin, Ireland; Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland.

Background: Epithelial ovarian cancer (EOC) is the most lethal gynaecological malignancy, often presenting at an advanced stage. Treatment for EOC is hampered by high levels of intrinsic and acquired drug resistance. The taxanes are microtubule stabilizing agents, used as first-line agents in the treatment of EOC. The spindle assembly checkpoint (SAC) is a regulatory mechanism, preventing chromosome segregation during mitosis. It forms the molecular machinery through which the taxanes exert their effect. Studies analyzing the immunohistochemical localization of these markers in clinical material are limited. BUBR1 transcription is controlled by p53 and silencing of BUBR1 reduces phosphorylation and stability of p53, with higher levels of BUBR1-p53 interaction in mitotic cells. Overexpression of BUBR1 and MAD2, have been associated with high cellular proliferation, as measured by Ki-67 expression, in a number of human tissues but this has not been studied in EOC.

Design: We performed IHC for BUBR1, MAD2, Ki-67 and p53 on a tissue microarray (TMA) constructed from a cohort of EOC (n=61), including a variety of histological subtypes, in order to characterize the immunolocalisation patterns of key SAC proteins in EOC.

Results: Both of the SAC proteins, MAD2 and BUBR1 were overexpressed in this tumour type, with cytoplasmic and nuclear staining patterns observed. Cytoplasmic BUBR1 and nuclear MAD2 both associated with cellular proliferation in our cohort ($p=0.009$, $p=0.008$ respectively). Importantly, increased nuclear MAD2 expression was associated with an improved response to combined platinum-taxane based chemotherapy ($p=0.044$).

Conclusions: Both BUBR1 and MAD2 are commonly overexpressed in EOC, with MAD2 most commonly localizing to the nucleus and BUBR1 to the cytoplasm in this tumour type. BUBR1 did not associate with treatment response, however tumours with strong nuclear MAD2 expression showed a significant association with a positive response to chemotherapy and this may be a useful marker in predicting patient response in the future.

1161 Comparative Analysis of HPV DNA Test Utilization in Community and Colposcopy Clinics

A Plotkin, MA Khalifa, N Ismiil, V Dube, RS Saad, Z Ghorab, J Wong, G Desai, S Nofech-Mozes. Sunnybrook Health Sciences Centre, Toronto, Canada.

Background: American Society for Colposcopy and Cervical Pathology (ASCCP) recommends testing for high-risk Human Papilloma Virus (HPV) DNA in women age 30 and older with an ASCUS result on their Pap smear. Accordingly, women are triaged for either colposcopy (positive HPV) or follow up (negative HPV). The purpose of this study was to compare trends in utilization of HPV testing between colposcopy and community clinic referrals from the time the test was implemented in our lab.

Design: All HPV DNA tests ordered between September '05 and December '08 in the department of Cytopathology were retrieved. HPV DNA was assessed on liquid base Pap smears using either Hybrid Capture II or PCR-based methodology (AMPLICOR® Roche) both directed to identify 13 high risk HPV types. Pertinent data regarding age, status of concurrent Pap smear and type of referral were collected from the medical records. The significance of the difference between the two independent proportions was tested using chi square test.

Results: Overall, 1,551 HPV DNA tests were performed during the study period, 699 (45%) of cases were requested by community clinics and 852 (55%) by colposcopy clinics. The proportion of women ≥30 was significantly higher in community clinic referrals 97.4% vs. 77% in colposcopy clinic referrals (p<0.0002). The most common setting for HPV testing in community clinic referrals was women ≥ 30 with ASCUS, accounting for 91.5% vs. 45.6% in colposcopy clinic referrals (p<0.0002). The proportion of SIL was significantly higher in colposcopy clinic referrals accounting for 30.6% of the requests compared with 4.1% in community referrals (p<0.0002). The proportion of positive cases was 27.7% in community clinic referral compared with 43.1% in colposcopy clinic referral (p<0.0002).
Conclusions: As expected from ASCCP practice recommendations, ASCUS in women ≥ 30 was the most common reason for the HPV DNA test requests in all referrals, but the nature of requests from colposcopy clinics were significantly different; characterized by higher proportion of women with evidence of dysplasia or younger than 30 and significantly higher positive tests. These findings are useful for health services planning as they uncover the trend of the broader test utilization beyond the existing recommendations.

1162 Notch1 and HPV Genotyping in Cervical Adenocarcinomas

AC Policiano, P Alves, L Martins, A Felix. Faculdade de Medicina, Lisboa, Portugal; Instituto Portugues de Oncologia, IPOLFG, Lisboa, Portugal; IPOLFG e Faculdade de Ciências Médicas, Lisboa, Portugal.
Background: The link between HPV infection and cervical carcinogenesis is well established. However, additional events are needed for tumor development. Several reports suggest that Notch signaling may be involved in neoplastic transformation and it can function as a promotor or suppressor of tumor growth. It is believed that in cervical carcinomas Notch signalling might contribute to the process of tumorigenesis in association with HPV. In this study we aim to examine Notch1 and HES1 immunoexpression and to genotype HPV in a series of adenocarcinomas of the uterine cervix.
Design: Notch1C-20(receptor); Notch1EC(extracellular domain); Notch IC(intracellular domain); Notch1-S3(cleaved) and HES1(Notch1 signaling transcription target) immunoexpression was evaluated in a tissue microarray constructed with representative regions from 41 endocervical adenocarcinomas and from 6 normal endocervical specimens. Patients files were reviewed and morphological features characterized. HPV detection was done using SPF-10 broad-spectrum primers PCR, subsequently followed by DEIA and genotyping by LiPA25. Samples for both techniques were obtained from formalin fixed and paraffin embedded tissues.
Results: The Notch proteins were highly expressed in adenocarcinomas of the cervix (Table 1) in contrast to normal endocervical glands (all 6 were negative with all antibodies and had no HPV DNA).

Table 1

	nº	Notch1C20	EC	IC	S3	HES1	HPV(+ve)
invasive adc	37	19(51%)	11(30%)	11(30%)	21(57%)	19(51%)	33(89%)
in situ adc	4	2(50%)	1(25%)	4(100%)	4(100%)	4(100%)	2(50%)

adc - adenocarcinoma; (+ve) - positive

An association was found between the presence of the NOTCH1-S3 and HES1 (p=0.02). No correlation was found between Notch activation and outcome or other morphological features evaluated (invasion, differentiation or metastatic behaviour).The presence of DNA HPV in carcinomas was detected in 35 cases. Multiple types were identified in a 4 cases and DNA HPV types were HPV16 (60%); 18(49%) and HPV45 (3%). No correlation was found between the presence of HPV and Notch1 immunoexpression.
Conclusions: Notch1 proteins and HES1 are highly expressed in invasive and *in situ* cervical adenocarcinomas indicating that Notch signaling is activated in cervical adenocarcinomas. This suggests that Notch1 may play a permissive or promoting role in cancerigenesis of cervical adenocarcinomas.

1163 Epigenetic Inactivation of Clusterin Gene May Predict for Mesenteric Metastasis of Primary Ovarian Cancer

CM Quick, HH Zhang, GF Yang, CY Fan. University of Arkansas for Medical Sciences, Little Rock, AR; John L. McClellan Memorial Veterans Hospital and UAMS, Little Rock, AR; The First Affiliated Hospital, Sun Yat-Sen Univ Med Sciences, Guangzhou, China.
Background: Clusterin, a multifunctional glycoprotein, is ubiquitously produced in mammalian tissues. Clusterin has been shown to play significant roles in many aspects of human tumor biology, such as cell-cell adhesion, cell proliferation, apoptosis, chemoresistance and angiogenesis. While clusterin is shown to be overexpressed in many types of human malignant tumors, its exact roles in the biologic behavior in ovarian epithelial cancer have not been fully investigated.
Design: Immunohistochemical (IHC) staining for clusterin was performed on a Tissue Microarray (TMA) containing 50 primary ovarian epithelial cancer (39 serous; 11 mucinous) and 50 matched mesenteric metastasis. Methylation-specific PCR for clusterin gene promoter was performed on all 100 tumors to determine whether clusterin protein expression is affected by promoter hypermethylation.
Results: High levels of clusterin protein were seen in 32 of 50 (64%) primary ovarian epithelial carcinoma and in 17 of 50 (34%) matched mesenteric metastasis. Decreased clusterin protein expression was significantly correlated with mesenteric metastasis ($p = 0.03$). Clusterin promoter hypermethylation was seen in 3/50 (6%) primary cancer and in 8/50 (16%) metastatic lesions. Among 8 metastatic lesions that displayed clusterin promoter hypermethylation, 7 were seen in metastatic lesions but not in their corresponding primary tumors. Among these 7 cases, 5 demonstrated significant reduction of clusterin protein expression in metastatic lesions as compared to that in their corresponding primary tumors.
Conclusions: Decreased clusterin protein expression is significantly correlated with mesenteric metastasis of ovarian cancer and promoter hypermethylation of the clusterin gene appears to play important roles in silencing the clusterin gene expression in these metastatic lesions.

1164 HPV Genotyping and HPV16 Variant Analysis in Glandular and Squamous Neoplastic Lesions of the Uterine Cervix

KD Quint, MNC de Koning, L-J van Doorn, WGV Quint, EC Pirog. DDL Diagnostic Laboratory, Voorburg, Netherlands; Weill Medical College of Cornell University, New York.
Background: The goal of the study was to compare the distribution of HPV16 genotypic variants in glandular and squamous neoplasia of the uterine cervix. The incidence of cervical adenocarcinoma is increasing and since the reason for this phenomenon is not known, a detailed HPV variant analysis in cervical neoplasia is of interest.
Design: The study consisted of 278 cases including endocervical adenocarcinoma in-situ (AIS, n=33) invasive adenocarcinoma (ADCA, n=55), cervical intraepithelial neoplasia-3 (CIN3, n=130) and squamous cell carcinoma (SCC, n=60) collected at the New York - Presbyterian Hospital in New York. All cases were tested for HPV by SPF10PCR-LIPA25 (version1) genotyping assay and cases positive for HPV16 were further tested for intratypic variants using a multiplex PCR and reverse hybridization assay.
Results: HPV DNA was detected in all cases with the exception of one case of CIN3 and three cases of ADCA. There was a significant difference in the spectrum of HPV genotypes identified in the glandular and squamous neoplastic lesions: while as much as 13 different HPV genotypes were detected in CIN3 as single infections and 11 types were found in SCC, only 4 genotypes were detected in AIS and 3 in ADCA. The most common single HPV types in CIN3 were HPV16, 31, and 52 (56.9%, 10%, 8.4%, respectively). In SCC the most common were HPV16, 18 and 31 (70%, 6.5%, 4.9%). In AIS, HPV16, 18, 45 and 35 accounted for 69.7%, 27.2%, 3%, 3% of cases. The 3 single types in ADCA were HPV16 (43.6%), HPV18 (41.8%) and HPV45 (10.9%). There were significant differences in prevalence of HPV16 variants between the squamous and glandular neoplastic lesions. The European variants of HPV16 were the most common in CIN3 (83.8%), SCC (71.4%) and AIS (73.9%). In ADCA the Asian American (AA) variant of HPV16 was the most common (41.7%) followed by the European variants (33.3%). Overall, AA variant of HPV16 was detected in 2.3% of all CIN3, 1.6% of all SCC, 12.1% of all AIS and 18.2% of all ADCA.
Conclusions: Asian American variant of HPV16, HPV18 and HPV45 are preferentially associated with in-situ and invasive cervical adenocarcinoma.

1165 Lichen Sclerosus Is a Risk Factor for De Novo Recurrence of HPV Negative Vulvar Squamous Cell Carcinoma

S Regauer. Medical University Graz, Graz, Austria.
Background: There is some debate if lichen sclerosus (LS) should be considered a risk factor for HPV-negative vulvar squamous cell carcinomas (SCC), since more than 50% of patients with vulvar cancer suffer from LS of many years duration. Treatment is a complete wide resection of the SCC. Despite clear margins, some patients develop de novo recurrent SCC in residual LS-affected skin / mucosa. The pattern and time course of recurrences is presented.
Design: 60 patients with a complete surgical excision of LS-associated SCC were evaluated for recurrences occuring >3 months after intitial surgery.
Results: 37/ 60 patients (62%) had no recurrences in an average follow-up of 61 months. 1/60 patients developed a low-grade VIN 18 months after the primary resection, but no invasive SCC during further 48 months follow-up. A total of 21/60 patients (36%; 1 pT3 SCC, 11 pT2 SCC, 8 pT1b SCC and 1 pT1a SCC) developed recurrent invasive SCC in the residual anogenital LS. 18/21 patients with recurrences (86%; average age 66 years, range 43 - 85 years at initial presentation) developed one de-novo SCC in the residual LS. Four / 21 patients (3 pT2, 1pT1b) had multiple recurrences: a 62-yr-old patient had 2 recurrences after 36 months and 24 months later; a 77-yr-old woman had 3 recurrences after 13, 11 and 45 months; a 60-yr-old woman with a pT1b primary SCC had 4 recurrences during 12 years of follow-up after 15, 14, 14, 8 and 4 months resp; a 66-yr-old woman had 5 recurrences after 17, 8, 4, 3 and 3 months. The first recurrence was observed on average 30 months (range 4 – 84 months) after the primary diagnosis of a LS-associated vulvar SCC. There was, however, a bimodal distribution in the pattern of recurrences: 48% (10/21) of recurrent de-novo SCCs were observed within the first 18 months, mostly in less than 10 months or after a long latency of typically more than 60 months (11/21patients ;52%). The inflammatory infiltrate of 4 evaluated primary SCC contained T-lymphocytes with monoclonally re-arranged γ-chain gene of the T-cell receptor.
Conclusions: Once patients have developed an LS-associated SCC**,** the entire residual LS is at risk for de-novo development of SCC. Recurrences were either rapid within 12 months or after a long latency of several years. The etiology of LS-associated carcinogenensis may be explained by an underlying immune dysregulation with increased numbers of T-cells with monoclonally rearranged γ-gene chain of the T-cell receptor resulting in reduced T-cell receptor diversity with ineffective immune response of tumor infiltrating lymphocytes.

1166 The Role of HuR in Gemcitabine Efficacy in Ovarian Cancer

W Rizzo, R Gogoi, C Constantino, J Sawicki, J Brody, A Witkiewicz. Thomas Jefferson University Hospital, Philadelphia, PA; Lankenau Institute for Medical Research, Wynnewood, PA.
Background: Ovarian cancer recurs in 50% of women who respond to first line chemotherapy. In patients with platinum-sensitive disease, standard second line chemotherapy agents include gemcitabine (GCB) in combination with platinum. Most second line agents however have only a 15-20% efficacy. The vast majority of patients eventually succumb to the disease. We recently demonstrated that HuR, a stress response protein, is a key mediator of GCB sensitivity through its ability to enhance translation

of the drug metabolizing enzyme, deoxycytidine kinase (dCK). In pancreatic carcinoma patients treated with GCB, we reported a 7-fold increased mortality risk in patients with low cytoplasmic HuR levels as compared to those with elevated cytoplamic HuR levels. Thus, the present study investigated the cytoplasmic expression of HuR in ovarian carcinomas and its correlation with response to GCB-based therapy.

Design: Immunohistochemistochemistry for HuR (SCBT, Santa Cruz, CA) was performed on 19 cases of ovarian carcinoma (15 papillary serous, 2 clear cell, and 2 carcinosarcomas). All patients received GCB as part of their second line chemotherapy. Cytoplasmic and nuclear HuR expression was scored as low (< 30% cells staining) or high (>30% of cells staining). Associations between clinicopathologic variables and protein expression were evaluated using a chi-square test. Survival was calculated using the Kaplan–Meier method. Utilizing an RNP-immunoprecipatation assay, we checked the association of deoxycytidine kinase mRNA with HuR in an ovarian cancer cell line, A2780, after GCB treatment. Whether HuR translocated to cytoplasm after GCB treatment in ovarian cancer cells was examined by immunofluoresence.

Results: Median survival was 27 months with low cytoplasmic HuR and 45 months with high HuR tumors (p=0.0081). Abundant cytoplasmic HuR was associated with more advanced disease. An increase in the association of HuR protein bound to dCK mRNA was observed in ovarian cancer cells treated with GCB and HuR translocated to the cytoplasm after exposure to GCM.

Conclusions: Cytoplasmic HuR levels were found to predict GCB response in ovarian carcinoma. This work points to HuR as the regulator of dCK, the key metabolic enzyme of GCB. Thus, our data support the hypothesis that HuR is a key regulator and predictor of GCB efficacy in ovarian cancer.

1167 Endometrial Sarcomas, How Many Categories Are Out There? A Tissue Microarray and Immunohistochemistry Study of 33 Cases

M Rouzbahman, G Rasty, F Azimi, BA Clarke, H Begley, PA Shaw. University Health Network, Toronto, ON, Canada.

Background: The current WHO classification divides endometrial sarcomas into endometrial stromal sarcoma (ESS) representing low-grade tumors and undifferentiated endometrial sarcoma (UES). Yet it is not clear whether UESs are a heterogenous group or all represent undifferentiated tumors. Recent studies suggest that a subgroup of UES with uniform nuclei (UES-U) have similar morphology, immunoprofile or molecular genetics to low grade tumors. The objective of this study was to determine the morphology and immunoprofile of endometrial sarcomas in a series of cases from our institution and compare low grade and non low-grade sarcomas.

Design: 33 endometrial sarcomas with material available for study were retrieved in a 15 year period (1994- 2008). A tissue microarray (TMA) block, including duplicate 0.6 mm cores of each tumor, was prepared. The H&E slides of UES group were examined by 3 gynecologic pathologists separately and 4 cases were selected as having marked pleomorphic nuclei (UES-P). 9 cases were classified as UES-U. Immunohistochemistry for CD10, estrogen receptor (ER), progesterone receptor (PR), p53, p16, βCatenin, Ki67 was performed using standard techniques on TMA sections and protein expression was scored according to percentage tumor cells positive and staining intensity.

Results: The mean age of patients was ESS:51 and UES:61 yrs. Follow up was available in 10 of 13 UES and 15 of 20 ESS pateints. All UES-P, 3 of 6 UES-U and 13 of 15 ESS patients were alive with or without disease at the time of study. 1 UES-U and all UES-P patients had received adjuvant chemotherapy. 4 of 9 UES-U patients had received adjuvant radiotherapy.

Immunohistochemistry results in ESS & UES

	CD10	Np16	ER	PR	Nβcatenin	p53	ki67 (>10%)
ESS	80%	28%	90%	80%	27%	0%	5%
UES	61%	15%	15%	15%	15%	23%	61%

N: nuclear

Immunohistochemistry results in UES-U & UES-P

	CD10	Np16	ER	PR	Nβcatenin	p53	Ki67(>10%)
UES-U	55%	66%	11%	22%	11%	0%	66%
UES-P	75%	50%	0%	0%	0%	75%	50%

N: nuclear

Conclusions: Our data suggests that there is a difference in expression of ER, PR and Ki67 (>10%) between ESS and UES groups. A significant percentage of UES tumors (both UES-U and UES-P) express CD10. Within UES group none of UES-P tumors showed ER, PR or nuclear βcatenin expression and diffuse strong staining for p53 was seen only in 3 of 4 UES-P and none of UES-U or ESS patients.

1168 Endometrial Carcinoma in Lynch Syndrome: Genotype-Phenotype Correlation

P Ryan, AM Mulligan, M Aronson, A Jennings, S Ferguson, S Gallinger, P Shaw, A Pollett, BA Clarke. Mount Sinai Hospital, Toronto, Canada; St Michael's Hospital, Toronto, Canada; University of Toronto, Toronto, Canada; University Health Network, Toronto, Canada.

Background: The endometrium is the commonest site of extraintestinal malignancy in Lynch syndrome. To date there have been limited data on genotype-phenotype correlation in these tumors, a question examined in this study.

Design: Endometrial cancer cases meeting Amsterdam II criteria for mismatch repair (MMR) screening were identified from the records of the Familial GI Cancer Registry in Mount Sinai Hospital and pathology departments of participating institutions. Microsatellite instability (MSI), MMR gene product immunohistochemistry (IHC), and gene sequencing results were correlated retrospectively with cell type and specific gross and microscopic features that have been found useful in screening for Lynch syndrome-associated tumors including: tumor location, type and grade; areas of dedifferentiation; tumor-infiltrating lymphocytes (TILs); and peritumoral lymphoid aggregates.

Results: Of 31 cases identified (mean age 49 years, 12 patients > 50 years old, 23 type 1 and 8 type II histology) 15 had germline mismatch repair gene mutations confirmed by sequence analysis (mean 48.8, 5 > 50 years) - 8 *MSH2*, 4 *MLH1*, 3 *MSH6* - with the other 16 cases being either MSI high, IHC deficient, or both. Detected pathologic features suspicious for Lynch syndrome were: lower uterine segment tumors - 2/31 (1/15 mutation positive); mixed histologic type - 5/31 (2/15); peritumoral lymphocytes - 17/31 (6/15); TILs - 8/31 (2/15). Suspicious pathologic features were seen in 7 of 15 mutation positive cases, and in 3 of 5 patients older than 50. LUS location and tumor heterogeneity were seen only in association with *MSH2* mutations, whereas peritumoral lymphoid aggregates were seen in all mutation groups. Six of 8 *MSH2* mutations and all 3 *MSH6* mutations are predicted to cause protein truncation, with 2 (non-related) *MSH6* cases having the same exon 5 mutation (c.1104delT).

Conclusions: This study confirms the preponderance of *MSH2* mutations in Lynch syndrome-associated endometrial carcinoma. Pathologic features may identify a large proportion of cases, including those over 50 years old, and should be incorporated along with age and family cancer history into future guidelines for the identification of sentinel tumors.

1169 Can Tumor Necrosis and Lymphocytic Infiltration Be Used as Predictors of Local Recurrence in Endometrial Adenocarcinoma?

RS Saad, M El-Rabbany, N Ismiil, S Nofech-Mozes, V Dube, Z Ghorab, MA Khalifa. Sunnybrook Health Sciences Centre, Toronto, Canada.

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 prognostic significance of these histopathologic characteristics in endometrial adenocarcinoma. In this study, we investigated their prognostic significance in a series of endometrial endometrioid adenocarcinoma.

Design: Endometrial endometrioid adenocarcinoma cases with total hysterectomy and bilateral salpingo-oophorectomy were retrieved from the archives during the period from 2000 to 2008. Cases with missing slides, tumors with non-endometrioid components or carcinosarcoma were excluded. We identified 419 cases of endometrial endometrioid adenocarcinoma; 37/419 (9%) of which had local recurrence. All cases were reviewed under multiheaded microscope for 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 was analyzed using Cox regression and Spearman correlation test.

Results: Of the 419 patients, 43/419 (10%) had 1+ necrosis, 38/419 (9%) had 2+ necrosis and 60/419 (14%) had 3+ necrosis. There was a significant correlation between the degree of necrosis and local recurrence (Cox regression, P< 0.001). In addition, there was a strong significant correlation between the degree of necrosis, depth of myometrial invasion and cervical involvement (Spearman correlation, P< 0.001). Severe tumor lymphocytic infiltration (3+) was identified in 45/419 (11%) cases and did not show any significant correlation with other histopathologic prognostic parameters.

Conclusions: Our study shows a significant correlation between necrosis and local recurrence, depth of myometrial invasion and cervical involvement. A similar correlation is not identified with the degree of lymphocytic infiltration. Documenting the presence and degree of necrosis in pathology reports of endometrial curettage may add insight for deciding the next management modality and needs to be further investigated.

1170 Clustering of Ovarian High-Grade Serous Carcinoma Based on Estrogen-Induced Gene Expression Predicts Poor Survival

M Schlumbrecht, D Urbauer, R Broaddus. M.D. Anderson Cancer Center, Houston, TX.

Background: Assessment of estrogen receptor (ER) expression has yielded mixed results as a prognostic indicator in epithelial ovarian carcinoma. In breast and endometrial cancers, the use of panels of estrogen-induced genes has improved prognostic capability over the use of ER alone. For both breast and endometrial cancer, over-expression of estrogen-induced genes is associated with better prognosis. We hypothesized that estrogen-induced gene expression can predict outcome in ovarian carcinoma and differentiate between tumors of varying estrogen sensitivities.

Design: qRT-PCR was used to quantify the expression of six genes known to be induced by estrogen in the female reproductive tract (EIG121, sFRP1, sFRP4, RALDH2, PR, and IGF-1) and ER in 83 patients with advanced stage, high-grade serous carcinoma of the ovary or peritoneum who subsequently received adjuvant treatment with platinum and taxane agents. Clinical data was collected by retrospective chart review. Unsupervised cluster analyses in multiple permutations were used to categorize patients as high or low estrogen-induced gene expressors. Statistical analyses were performed using Fisher's exact test, Cox proportional hazards models, and the Kaplan-Meier method.

Results: Median follow-up time was 38.7 months (range 1-68 months). In a multivariate Cox model, overall survival was predicted by optimal debulking (HR 0.37 [CI 0.15-0.95], p=0.038), sFRP1 expression (HR 1.04 [CI 1.00-1.07], p=0.0274), and EIG121 expression (HR 1.19 [CI 1.06-1.33], p=0.0026). EIG121 expression was also associated with recurrence-free survival (HR 1.15 [CI 1.03-1.24], p=0.011). A cluster defined by EIG121 and ER best segregated tumors into groups of high and low estrogen-induced gene expressors. High expressors demonstrated significantly shorter overall survival compared to low expressors, even when adjusting for race, body mass index, and residual disease at debulking (HR 2.73, p=0.037). Nonwhite women also represented a greater proportion of high expressors compared to low expressors (p=0.044).

Conclusions: In sharp contrast to breast and endometrial cancers, high estrogen-related gene expression negatively predicts overall survival in patients with high-grade serous ovarian carcinoma. An estrogen-induced gene biomarker panel may have utility both as an indicator of prognosis and as a guide to management with estrogen antagonists.

1171 BRCA 1 or 2-Associated (BRCA+) Pelvic Serous Carcinomas Arise from Both the Ovaries and Fallopian Tubes: The Contrast between Symptomatic and Asymptomatic Women

S Schulte, M Roh, AK Folkins, EA Jarboe, J Garber, J Hecht, CP Crum. University of Pennsylvania, Philadelphia, PA; University of Michigan Medical Center, Ann Arbor, MI; Stanford University Medical Center, Palo Alto, CA; University of Utah Medical Center, Salt Lake City, UT; Dana Farber Cancer Institute, Boston, MA; Beth Israel Deaconess Medical Center, Boston, MA; Brigham and Women's Hospital, Boston, MA.

Background: With greater emphasis on the distal fallopian tube as a source of pelvic serous carcinoma (PSC), the possibility that bilateral salpingectomy alone will prevent this disease in both BRCA+ women and the general population is being debated. This study was designed to compare PSCs in the two populations to determine if PSCs in the BRCA+ group are more likely to arise from the fimbria.

Design: Pathology reports from 55 symptomatic BRCA+ PSCs, including histologic review of 16, and 15 asymptomatic (early) carcinomas discovered at prophylactic salpingo-oophorectomy were reviewed and compared to 85 consecutively accessioned PSCs. The goal was to estimate the frequency of primary ovarian vs tubal carcinomas in these groups relative to consecutively accessioned PSCs.

Results: Of 15 asymptomatic BRCA+ women with early PSCs, 13 (87%) were documented in the distal fallopian tube. Of 55 symptomatic BRCA+ women, 49 were high grade PSCs. Review of the pathology in 15 revealed a probable source in the fallopian tube in 6 (40%) and in the ovary in five (33%), with 4 of unclear origin. In 31 of 85 PSCs in the general population (36%) the primary site was assigned to the tube. The difference in frequency of probable tubal origin between the BRCA+ and general population was not significant. The differences in frequency of probable tubal origin between symptomatic (either group) and asymptomatic PSCs (BRCA+) was significant ($p<0.001$).

Conclusions: The proportion of asymptomatic BRCA+ women whose tumors arise in the fallopian tube is substantial, but this group is only a subset of of women at risk for PSC. Tumors in the symptomatic BRCA+ women and the general population are indisinguishable, underscoring multiple origins for PSC. Without data to the contrary, prophylactic salpingectomy cannot be expected to prevent all PSC and further epidemiologic and pathologic comparisons of high and low risk women with PSC are needed to ascertain the risk reduction expected by salpingectomy alone.

1172 High-Grade Serous Carcinoma of the Ovary: Negative but Highly Significant Correlation of PI3K Pathway Activation with Specific Chromosomal Aberrations

M Schumann, B Karberg, P Kuhlmann, J Behm, O Buchweitz, A Staebler. University of Tuebingen, Tuebingen, Germany; Ludwigsburg Hospital, Ludwigsburg, Germany; University of Muenster, Muenster, Germany.

Background: The Phosphatidyl-inositol 3-kinase (PI3K)-p-Akt signal transduction pathway has been implicated in ovarian carcinogenesis by several studies and elements of this pathway serve as potential therapeutic targets. To elucidate the mechanism of activation and obtain information on potential alternative pathways, this study aims at correlating the activation of different components with specific chromosomal alterations in high-grade serous carcinoma.

Design: 114 cases of high grade serous carcinoma were analyzed by immunohistochemistry in a tissue microarray for expression of p110alpha PI3K, phospho-Akt, pmTOR and pFKHR. Corresponding chromosomal analysis by CGH was available for 85 cases.

Results: Unexpected results showed a significant but negative correlation between the activation of individual components of the PI3K-Pathway and the average number of chromosomal alteration (for example Pearson correlation: -0.278, p=0.009 for detection of pmTOR and -0.239 with p=0.028 for expression of PI3K(p110 alpha). Similar results were found with individual chromosomal alterations, that were negatively correlated with pathway activation: for example detection of pmTOR showed a significant inverse correlation with amplifications at chromosome 19q, losses of 4q and Xq (p= 0.004, 0.006 and 0.007). Interestingly, there were no positive correlations.

Conclusions: Expression and activation of individual components of the PI3K-pathway shows a negative but highly significant correlation with specific chromosomal alterations and is also reflected in a significantly lower number of overall aberrations. This finding suggests, that the entity of high-grade serous carcinomas consists of a broad spectrum of tumors, which are characterized by activation of signal-transduction pathways at one end, and increasing chromosomal instability at the other end.

1173 Morcellation Leading to Peritoneal Dissemination of Gynecologic Lesions

MA Seidman, AL Pelletier, MG Muto, CP Crum, P Dal Cin, MR Nucci, BJ Quade. Brigham and Women's Hospital, Boston, MA.

Background: Uterine morcellation is a technique that allows for laparoscopic removal of large lesions, e.g. leiomyomata, by fragmentation. Although decreasing recovery time for the patient, it recently has been suggested that morcellation can result in microscopic seeding of the primary lesion throughout the peritoneum. Additionally, although recommended for lesions with low suspicion for malignancy, some tumors assessed clinically as benign may be revealed to have unexpected malignant potential upon histologic examination.

Design: Over the past 3 years, a series of 8 cases of both benign and malignant gynecologic smooth muscle tumors evaluated for iatrogenic dissemination following morcellation were culled from our pathology files. Follow-up was obtained by reviewing the electronic medical record.

Results: Two cases were morcellations of benign leiomyomata, one associated 7-years later with disseminated peritoneal leiomyomatosis (DPL) with cytogenetic clonality, the other with DPL 1-year post morcellation and disseminated leiomyosarcoma (LMS) 2-years post morcellation. One case of uterine stromal sarcoma revealed no dissemination 7-weeks post morcellation. A case of atypical smooth muscle tumor was associated with foci of dissemination as well as endometriosis just over 4-years post morcellation. Two cases of smooth muscle tumor of uncertain malignant potential (STUMP) were associated 2-months later with apparent dissemination. Two LMS cases were identified, one apparently associated with a benign peritoneal leiomyoma 1-month post morcellation, the other with overt dissemination with cytogenetic clonality 2-weeks post morcellation. Peritoneal cytology at the time of re-exploration was negative in all cases. All patients are alive at this time, most without therapy; both cases of disseminated LMS have received chemotherapy and are currently asymptomatic.

Conclusions: These findings underscore the potential risk of intraperitoneal dissemination following morcellation, emphasizing the need for appropriate patient consent procedures to explain the procedural risks weighed against the benefits of reduced recovery time, and, more importantly, for strict guidelines for selecting individuals for morcellation procedures. Further analysis of these tumors may identify pathological factors required for survival in distant sites.

1174 Paget Disease of the Vulva – A Study of 56 Cases

R Shaco-Levy, SM Bean, RC Bentley, RT Vollmer, JA Papalas, MA Selim, SJ Robboy. Soroka Medical Center, Beer-Sheva, Israel; Duke University Medical Center, Durham, NC; VA Medical Center, Durham, NC.

Background: Vulvar Paget Disease (VPD) is a rare locally recurrent chronic disease, accounting for < 1% of vulvar neoplasms. This study of 56 patients examines numerous clinical and pathological features as they relate to recurrence and survival rates.

Design: The medical records and pathology slides from all patients with VPD seen over a 30 year period in one institution were analyzed.

Results: The mean age at diagnosis was 69 years. The average length of follow-up was 5.6 years. Substantial delay between appearance of symptoms and diagnosis (mean 20 months), was significantly associated with larger lesions (p=0.001), leading to more radical resections. Recurrence rate after surgical management was 32%, with interval of 13-131 months from diagnosis. The two statistically significant risk factors for recurrence were disease in the perineum (p=0.044) and epidermal acantholysis (p=0.035). Patients with involved surgical margins had an increased recurrence rate, but not of statistical significance (p=0.14). Intra-operative frozen section analysis of the margins as well as radical surgery as initial treatment did not reduce recurrence rate. Stromal invasion occurred in 10 patients (18%), and was not a statistically significant adverse prognostic indicator. Radiation therapy given to five patients resulted in complete response with no further recurrences. On the last day of follow-up 24 patients (43%) had no evidence of disease, 24 patients (43%) were dead of other causes, five patients (9%) were alive with disease, two patients (3%) were lost to follow-up, and one (2%) died due to VPD with invasive adenocarcinoma.

Conclusions: Significant delay in diagnosis, leading to larger lesion size, is common in VPD patients, emphasizing the importance of awareness to this disease. Surgical treatment should aim to completely excise the lesion with the smallest possible resection. Intra-operative frozen section analysis is not useful unless invasion is suspected. Recurrences are common with disease in perineum and presence of acantholysis being adverse risk factors. Invasion is not a statistically significant poor prognostic indicator. Radiation therapy may serve as an alternative to surgery in selected patients. VPD only rarely results in patient's death, but as recurrences are common and may occur greater than a decade after initial treatment, long term follow-up is required.

1175 Natural History of Biologically Malignant Struma Ovarii: Analysis of 27 Cases with Extra-Ovarian Spread

R Shaco-Levy, SM Bean, RC Bentley, SJ Robboy. Soroka Medical Center, Beer-Sheva, Israel; Duke University Medical Center, Durham, NC.

Background: Biologically malignant struma ovarii is exceedingly rare. Most histologically malignant cases reported had a benign clinical course. This study analyzes 27 biologically aggressive cases and reports clinical courses, histological features and prognosis based on long-term follow-up in many cases exceeding 20 years.

Design: Cases were considered biologically malignant if there was: (1) extra-ovarian spread at presentation, (2) infiltration of the ovarian serosal surface, or (3) recurrence after initial surgery. Strumas composed of proliferative hypercellular thyroid tissue were classified as follicular adenoma. The diagnosis of follicular carcinoma required the presence of vascular invasion. Tumors with true papillary structures along with optically clear "Orphan Annie" nuclei, nuclear overlap, grooves and pseudo-inclusions were classified as papillary carcinoma.

Results: Extra-ovarian spread was evident at presentation in 17 patients. The malignant nature of the other 10 tumors became apparent only after they recurred. The tumors measured 5-24.5 cm and were >50% thyroid tissue in all but 2 cases. The microscopic diagnosis was follicular adenoma in 17 cases (63%), papillary carcinoma in seven (26%), unremarkable thyroid tissue in two (7%), and follicular carcinoma in one (4%). Generally, the clinical course was protracted, with long-term survival documented in most patients. Factors predictive of poor prognosis were large size (≥10 cm), strumal component > 80%, and extensive papillary carcinoma, especially with solid areas, necrosis and ≥5 mitoses per 10 HPF. Follow-up for all patients was 1.5-33 years (mean=13.5 years). On last follow-up three patients (11%) had no evidence of disease, nine (33%) were alive with disease, five (19%) died of other causes, and 10 (37%) died of disease. Death from disease occurred 1.5-32 years after diagnosis (mean 14 years). Recurrence was seen 2 months to 29 years after initial surgery (mean=7 years).

Conclusions: Biologically malignant struma ovarii generally has a protracted clinical course. Long term survival may occur even with metastatic disease. Some patients experience rapid disease course with death shortly after diagnosis. Factors indicative of aggressive clinical course are large tumor size, >80% strumal tissue, and extensive papillary carcinoma histology, especially with anaplastic features. As recurrences may occur more than a decade following diagnosis, long-term follow-up required.

1176 Risk Factors for Candidate Precursors of Serous Cancer in Tubal Epithelium of BRCA Mutation Carriers

PA Shaw, D Vicus, A Finch, P Sun, S Armel, SA Narod. University Health Network, Toronto, ON, Canada; Women's College Hospital, Toronto, ON, Canada.

Background: Candidate precursors of high grade serous carcinoma in tubal epithelium have been reported, and include the p53 signature and the Tubal Intraepithelial Carcinoma (TIC). Both lesions have been identified in prophylactic salpingectomy specimens from BRCA1/2 mutation carriers. In support of the theory that these lesions may play a role in the development of serous cancer, we sought to identify risk factors for these non-invasive tubal lesions, and to compare these risk factors with the traditional risk factors for ovarian cancer.

Design: We have previously reported the incidence of pre-invasive lesions in prophylactic salpingectomy specimens from 173 BRCA mutation carriers. Each participant from that study was asked to complete a risk-factor questionnaire, which included information on their reproductive and medical history and various lifestyle factors. Women for whom a tubal lesion was found (cases) were compared to women with no lesion (controls). Student's *t* test was used to test for statistical significance for continuous variables and the Fisher exact was used for categorical variables.

Results: Of the 173 patients, 43 (25%) were found to have a tubal abnormality, including 23% of the BRCA1 mutation carriers and 27% of the BRCA2 mutation carriers. The prevalence of a non-invasive tubal lesion increased with age; an abnormality was present in 5% of women who had surgery before the age of 40 (1 of 12) and in 56% of women who underwent surgery at age 60 or above (6 of 13; $p = 0.004$). The prevalence of either non-invasive lesion was lower for women who had used an oral contraceptive for ten or more years (2.9%), compared to women who had one to nine years of use (29.2%). A non-invasive lesion of either type was found in 31.2% of women with a BMI > 25 kg/m^2) compared to 18.0% of patients with a BMI <25 kg/m^2 at the time of surgery ($p = 0.05$). Additional variables evaluated included parity, age at first birth, breastfeeding, hormone replacement therapy use, previous breast cancer. None of these variables was associated with the presence of p53 signature or TIC.

Conclusions: We have demonstrated that some but not all known ovarian cancer risk factors are also risk factors for putative cancer precursors in tubal epithelium. The prevalence of p53 signature and TIC increases with age at salpingectomy and with BMI. Long-term oral contraceptive use is associated with a decrease in the prevalence of both lesions.

1177 Synchronous Endometrioid Adenocarcinomas in the Ovary and Uterus Are Less Likely Two Independent Primaries

N Sherwood, JF Silverman, SD Finkelstein, MA Khalifa, N Ismiil, V Dube, S Nofech-Mozes, RS Saad. Allegheny General Hospital, Pittsburgh, PA; RedPath Integrated Pathology,, Pittsburgh, PA; Sunnybrook Health Sciences Center/University of Toronto, Toronto, ON, Canada.

Background: Histologic criteria have a limited role in determining whether synchronous endometrioid adenocarcinomas in the ovary and uterus represent two independent primaries or a metastatic event. Each of the two scenarios has its own staging and therapeutic implication. We carried out a molecular analysis of synchronous tumors to determine whether they are originating from a single (metastatic) or different clones (two primaries).

Design: We studied 28 patients with synchronous endometrioid tumors. The mutational profile for each neoplasm and time course of mutation acquisition was determined using PCR and quantitative genotyping for a broad panel of LOH markers targeting 1p,3p,5q,9p,10q,17p,17q,21q,22q and k-ras-2 sequencing. Extent of concordant mutational markers, specific alleles affected and temporal sequence of mutation acquisition was correlated, with clinical, pathologic and outcome features when available to validate relatedness determination. Cancers were considered to be one tumor with metastasis when over 50% of the mutational markers were concordant and temporal sequence of mutation acquisition was preserved. Independent primaries were determined to be present when these criteria were not met.

Results: Molecular analysis showed discordant mutations in 6/28 cases (21%), supporting the diagnosis of de novo primaries. All six cases showed low FIGO grade, no/superficial myometrial invasion and no cervical involvement, lymphovascular invasion (LVI) or lymph node metastasis. Both ovarian and endometrial endometrioid adenocarcinomas shared the same mutations, revealing a metastatic lesion in 22/28 cases (79%). These cases showed low FIGO grade in all cases (22/22, 100%), no/superficial invasion in 16/22 (73%), no LVI in 19/22 (86%), and negative lymph node metastases in 18/22 (82%). Ovarian tumors were grade 1 in 9/22 (41%); grade 2 in 10/22 (45%) and grade 3 in 3/22 (14%) cases.

Conclusions: In the majority of cases, clinicopathological features of synchronous ovarian and endometrial endometrioid adenocarcinomas are not helpful to differentiate between primary versus metastatic tumors. In this study, molecular testing shows that the majority of synchronous ovarian and endometrial endometrioid carcinomas represent metastatic events rather than independent primaries.

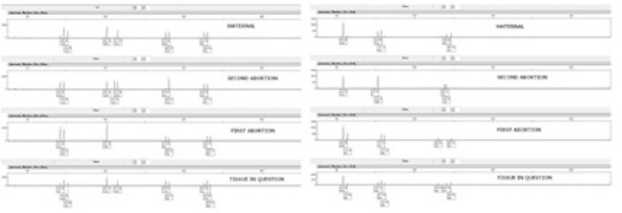
1178 Glial Heterotopia of the Uterine Cervix: DNA Genotyping Confirmation of Its Fetal Origin

AJ Siddon, P Hui. Yale University School of Medicine, New Haven, CT.

Background: Uterine glial heterotopia is a rare, yet biologically intriguing lesion, mostly involving the cervix. Although an implantation of the fetal brain tissue is widely accepted as the etiology, there has been no confirming evidence to support such a hypothesis.

Design: We investigated a case of polypoid glial heterotopia of the uterine cervix in a 42-year old woman who underwent an elective termination of pregnancy of a Down syndrome fetus. One-year prior, the patient had a pregnancy termination of a fetus with Klinefelter's syndrome.

Results: Gross and microscopic examination revealed a 2.5 cm polypoid cervical lesion consisting of lobulated mature glial tissue covered by endocervical glandular epithelium. The neural nature of the lesion was confirmed by glial fibrillary acidic protein (GFAP) and S100 immunohistochemistry. DNA genotyping of the cervical polyp, the maternal, the first and second fetal tissue samples demonstrated an identical genetic profile between the cervical glial tissue and the first aborted fetus.



Genotyping also attested the presence of Klinefelter's syndrome of the first gestation and Down syndrome of the second gestation.

Conclusions: This molecular case study confirmed the fetal origin of uterine glial heterotopia.

1179 PTEN Status in Untreated and Progestin-Treated 'Complex Endometrial Hyperplasia' and Its Correlation with Disease Outcome

RA Simon, CJ Sung, WD Lawrence, MM Steinhoff, K Hansen, MR Quddus. Alpert Medical School of Brown University, Women & Infants Hospital of Rhode Island, Providence, RI.

Background: Complex endometrial hyperplasia without atypia (CEH) according to the WHO is defined by architecturally complex glands without significant cytologic atypia and a relatively low chance of progression to atypical endometrial hyperplasia (AH) or endometrioid carcinoma (EMCA). Other classifications, such as endometrial intraepithelial neoplasia (EIN), maintain that marked glandular crowding is a cardinal feature and that ~44% of CEHs correspond to, and represent 29% of, EINs. *PTEN,* a tumor suppressor gene, is reported to be mutated (PTEN-null) in 55-83% of endometrial precancers and EMCAs, respectively. We studied 23 cases of CEH treated with progestins i) to assess the PTEN status before the start of treatment and any change in PTEN status post progestin therapy and ii) to assess any association between post treatment PTEN status and disease resolution, persistence, or progression to AH or EMCA.

Design: From our institutional archives we identified 23 cases of CEH that had been treated with progestin and had available post-treatment samples. The initial biopsy and post-treatment samples (varied from 2-12) were retrieved and immunostained for PTEN (Cascade Bioscience; 1:100 dilutions). Histological changes of progression, regression and persistence were noted. The median follow-up was 35 months (range: 1-114 months).

Results:

Tabke 1: PTEN status before and after progestin therapy

PRE-progestin PTEN status	6 PTEN-null	9 Mixed PTEN-null and PTEN+	8 PTEN+
POST-progestin PTEN status	4 PTEN-null; 0 Mixed; 2 PTEN+	1 PTEN-null; 3 Mixed; 5 PTEN+	0 PTEN-null; 2 Mixed; 6 PTEN+

Table 2: Post-progestin therapy follow-up

POST-progestin PTEN status	5 PTEN-null	5 Mixed PTEN-null and PTEN+	13 PTEN+
Post-progestin follow-up	2 Progressed; 3 Regressed; 0 No change	3 Progressed; 1 Regressed; 1 No change	3 Progressed; 10 Regressed; 0 No change
Final pathology	2 EMCA	3 AH	2 AH; 1 EMCA

Conclusions: 65% of CEH exhibited PTEN mutation prior to progestin therapy, whereas 43% showed PTEN mutation after therapy. In 13 cases (57%) of CEH the PTEN status remained unchanged after progestin treatment. Half of progestin-treated PTEN-null cases of CEH progressed to AH/EMCA while 23% of progestin-treated PTEN+ cases progressed to AH/EMCA. These suggest that the PTEN status of CEH may be used in predicting its biologic behavior after progestational therapy and in determining the frequency of clinical follow-up studies.

1180 Significance of E-Cadherin and p16 Advanced Endometrial Cancer: A Gynecologic Oncology Group Phase II Study

M Singh, KM Darcy, WE Brady, Z Weber, A Akalin, CW Whitney, R Zaino, N Ramirez, KK Leslie. SUNY at Stony Brook & SBUMC, Stony Brook, NY; Gynecologic Oncology Group, Buffalo; University of Colorado Denver, Aurora; University of Massachusetts, Worcester; Christiana Gynecology Group, Newark, DE; Hershey Medical Center, Hershey; Nationwide Children's Hospital, Columbus, OH; University of Iowa, Iowa City.

Background: Biomarkers in advanced endometrial cancers have the potential for guiding treatment and improving patient survival. Our aim was to evaluate the cadherin-catenin complex biomarkers in advanced endometrial carcinoma patients treated with tamoxifen and medroxyprogesterone acetate (T+M) in a multi-center phase II trial (GOG protocol # 119).

Design: Tissue microarrays with multiple replicate cores of endometrial carcinoma tissue from 42 patients with stage IV or recurrent tumor were evaluated immunohistochemically for expression of the proteins of the cadherin-catenin pathway, proliferation markers, cell cycle inhibitors & p53. Expression in epithelium (E) was categorized into tertiles (T1, T2, T3) for E-cadherin, N-cadherin, alpha-catenin, beta-catenin, gamma-catenin, p120-catenin and Ki-67; as negative, below median or above median for p16 and p27; and as negative or positive for p53. Expression in stroma (S) was categorized as negative or positive for Ki-67. Clinicopathologic correlations were analyzed.

Results: Relationships were observed between race and E-cadherin^E ($p=0.003$) and p16^E ($p=0.024$); histologic type of cancer and N-cadherin^E ($p=0.015$), Ki-67^E ($p=0.011$), p16^E ($p=0.005$) and p27^E ($p=0.021$); and between patient age and p16^E ($p=0.036$). E-cadherin^E

expression (T2 or T3 versus T1) was associated with a reduced risk of progression (p=0.008 or p=0.006, respectively) or death (p<0.001 or p<0.001, respectively) and was an independent prognostic factor for better progression free survival (PFS) (p=0.025 or p=0.112, respectively) and overall survival (OS) (p=0.004 or p=0.01, respectively). In addition, expression above the mean versus no expression of $p16^{E}$ was associated with an increased risk of death (p<0.001) and was an independent prognostic factor for worse OS (p=0.018) while positive versus no expression of $p53^{E}$ was associated with worse OS (p=0.018).
Conclusions: E-cadherin^{E} and $p16^{E}$ appear to be clinically relevant and of prognostic value in stage IV or recurrent endometrial cancer treated with T+M.

1181 HPV Genotype Distribution According to Severity of Cervical Neoplasia

KD Sjoeborg, AK Lie, A Trope, CM Jonassen, C Cuschieri, A Eskild. Oestfold Hospital Trust, Fredrikstad, Norway; Akershus University Hospital, Loerenskog, Norway; Norwegian Radium Hospital, Oslo, Norway; Akershus University Hospital, Loersenskog, Norway; Royal Infirmary, Edinburgh, United Kingdom; University of Oslo, Oslo, Norway.
Background: Knowledge of the HPVgenotype distribution on a population based level is important to estimate the potential effect of the HPV vaccines. The aims of our study were to investigate the HPV genotype profile and the prevalence of multiple infections according to severity of cervical neoplasia.
Design: Cross sectional study including women with CIN2+ in Health Region East Norway during 2005 and 2006 (N=643). Median age was 35 years (range, 17-76 years). Histology revealed CIN2 in 135, CIN3/ACIS in 495 and invasive carcinoma in13 women. HPV genotypes were detected by the L1 based PCR test Linear Array, which differentiates 37 HPV genotypes. Logistic regression adjusted for age was used to evaluate the role of the different HPV genotypes according to severity of lesion. Single HPV infections except HPV16, 18, 31 and 33 were used as a reference.
Results: HPV was detected in 98% of the women of whom 53 % had multiple infections. HPV16 was the most common genotype detected in 51%, followed by HPV31, 33, 52, 18, and 51. HPV16/18 were detected in 58% of whom 35% without concurrence of other high-risk genotypes. HPV16 and HPV33 as single infections were more common in CIN3+ as compared to CIN2, age adjusted odds ratio5.93 (95% CI: 2.73-12.87) and 4.57 (95% CI: 1.43-14.60) respectively. Also in combination with other genotypes, HPV16 and HPV33 were associated with CIN3+, OR 2.30 (95% CI: 1.16-4.58) and 3.37 (95% CI: 1.10-10.34) respectively. Multiple HPV infections, not including HPV16 or HPV33, did not seem to be associated with severity of lesion, OR 1.37 (95% CI 0.72-2.59).
Conclusions: HPV16 and HPV33 as single or multiple infections were associated with CIN3+. Multiple infections with other genotypes were not associated with severity of the lesion. Based on our analyses, prophylactic vaccines against HPV16/18 seem to have the potential to prevent at least 35% of high-grade preinvasive lesions. If HPV16/18 is the causal agent even in the presence of other high-risk genotypes, the preventive potential could be 58%.

1182 GADD45a Protein Expression in Endometrial and Ovarian Tumors

EA Slodkowsak, J Torres-Mora, CE Sheehan, JS Ross. Albany Medical College, Albany, NY.
Background: GADD45a (growth arrest and DNA-damage-inducible 45 alpha) expression is increased by growth arrest conditions and exposure to DNA-damaging agents mediated by the activation of the p38/JNK pathway via MTK1/MEKK4 kinase. The prognostic significance of GADD45a expression in endometrial and ovarian epithelial tumors has not been previously studied.
Design: Formalin-fixed paraffin-embedded tissue sections from 82 endometrioid carcinomas (EC), 17 uterine papillary serous carcinomas (PSC) and 80 primary ovarian epithelial tumors including benign tumors (BN), tumors of low malignant potential (LMP), and primary ovarian carcinomas (OC) were immunostained by an automated method (Ventana Medical Systems Inc., Tucson, AZ) using rabbit polyclonal GADD45a antibody (sc-792; Santa Cruz Biotechnology, Santa Cruz, CA). Cytoplasmic immunoreactivity was semiquantitatively scored based on staining intensity and distribution and the results were correlated with morphologic and prognostic variables.
Results: Overexpression of GADD45a was observed in 49% EC and 59% PSC and showed an overall trend for high expression in early stage tumors (55% early vs. 33% advanced stage, p=0.08). Within the endometrioid carcinoma subtype, GADD45a overexpression was higher in early stage tumors (54% early vs. 23% advanced stage, p=0.038) and correlated with the depth of myometrial invasion (p=0.039). GADD45a overexpression was noted in 29% BN, 20% tumors of LMP, and 46% OC. A trend for a higher expression rate in OC versus BN and LMP was noted, but did not reach statistical significance (p=0.09). In BN, LMP and OC, 0% mucinous tumors overexpressed GADD45a, compared to 60% clear cell, 48% papillary serous and 25% endometrioid tumors (p=0.04). GADD45a expression did not correlate with tumor grade, stage or survival in either EC or OC.
Conclusions: GADD45a overexpression correlates with tumor stage and the depth of myometrial invasion in EC, subtypes of ovarian carcinoma and trends toward an association with invasive versus benign or borderline status in ovarian epithelial tumors. Further study of GADD45a expression in gynecologic malignancies appears warranted.

1183 Upregulation of Potential Oncogenes in Serous Tubal Intraepithelial Carcinoma (STIC)

AE Smith Sehdev, RJ Kurman, E Kuhn, IM Shih. Cascade Pathology, Portland, OR; Johns Hopkins Medical Institution, Baltimore.
Background: STIC has been proposed as a precursor for many pelvic high-grade serous carcinomas (HGSC). Our previous analysis of the ovarian cancer genome identified several genes with oncogenic potential that are amplified and/or overexpressed in the majority of HGSC. Determining whether these genes are upregulated in STIC is important in further elucidating the relationship of STIC to HGSC and is fundamental in understanding the molecular pathogenesis of HGSC.
Design: 35 morphologically defined STICs were obtained from 24 patients with stage IIIC/IV HGSC. All STICs were discrete from the HGSC. Both STIC and the HGSC were analyzed for expression of ovarian cancer-associated markers including Rsf-1 (chromatin remodeling gene), fatty acid synthase (FASN, enzyme involved in fatty acid synthase), cyclin E (cell cycle protein) and mucin-4 (CA125 binding protein and signal transducer). These four proteins were selected because they have been shown to participate in the pathogenesis of HGSC. In addition, STICs and HGSC were examined for expression of conventional markers including p53, Ki-67 and p16. The percentage of intensely immunoreactive nuclei was determined for p53, Ki-67, p16 and cyclin E while a three-tier intensity score was used to compare the immunoreactivity of Rsf-1, FASN and mucin-4 in STIC to adjacent normal-appearing tubal epithelium. In a few STICs the lesions were not present in deeper sections for some markers.
Results: Diffuse nuclear p53 and p16 immunoreactivity (>50% of nuclei) was observed in 25 (69%) of 36 and 14 (45%) of 31 STICs, respectively, while an elevated Ki-67 labeling index (≥5%) was detected in 34 (94%) of 36 STICs. Among the STICs with diffuse p53 immunoreactivity, 23 (92%) of 25 STICs displayed an elevated Ki-67 labeling index (≥5%). Cyclin E nuclear staining was seen in 25 (71%) of 35 STICs while normal tubal epithelial cells were all negative. Increased Rsf-1, FASN and mucin-4 immunoreactivity as compared to adjacent normal-appearing tubal epithelium occurred in 69%, 66% and 54% of STICs, respectively. Almost all HGSC showed the same staining patterns as the STICs.
Conclusions: STICs express several potential oncogenes that are frequently amplified and/or upregulated in HGSC. Their frequency of overexpression is similar to that found in a large series of HGSC previously reported. These results provide additional evidence that STICs are possible precursors of HGSC and suggest that overexpression of Rsf-1, cyclin E, FASN and mucin-4 occurs early in tumor progression of HGSC.

1184 MicroRNAs Differentiate Low and High-Risk Endometrial Carcinomas

JF Snowdon, X Zhang, TJ Childs, VA Tron, H Feilotter. Queen's University, Kingston, ON, Canada.
Background: Endometrial carcinoma is the most common gynecologic malignancy in industrialized nations and is clinically classified as low-, intermediate-, or high-risk based on clinico-pathologic staging criteria. Treatment decisions are based on this risk assessment and accurate prediction of patients who are likely to recur can reduce unnecessary adjuvant treatment. Currently, there are few molecular biomarkers that assist in the differentiation of these categories. MicroRNAs (miRNAs, miRs) are small non-coding RNAs that negatively regulate gene expression at the post-transcriptional level. MicroRNA expression can be dysregulated in cancer and this may impact tumorigenesis through altered regulation of cell proliferation, apoptosis, invasion/metastasis, and angiogenesis. Additionally, miRNAs have been shown to have prognostic and diagnostic value in certain types of cancer.
Design: We compared the expression profiles of 723 human miRNAs from low (n=5), intermediate (n=17), and high (n=4) risk formalin-fixed paraffin-embedded (FFPE) endometrial carcinomas using Agilent Human miRNA arrays. Differentially expressed miRNAs were identified and potential mRNA targets were identified using miRBase.
Results: We identified 5 miRNAs whose expression pattern was significantly different between the low and high-risk tumors (p<0.05). Four of the microRNAs (miR-106a, miR-130a, miR-18a, and miR-885-5p) were down-regulated, while miR-424 was up-regulated in the low-risk tumors compared to the high-risk tumors. Four of these dysregulated miRNAs (miR-106a, miR130a, miR-18a, and miR424) have predicted or validated targets related to tumorigenesis or poor prognosis in other tumor types.
Conclusions: These preliminary results show that miRNAs can be used to stratify endometrial carcinomas into low- and high-risk groups. This information can be used to identify important steps in tumour progression that could be exploited for cancer treatment. Additionally, these results show that miRNAs could be useful for prognostication or pre-operative risk assessment in this tumor type.

1185 Morphological Changes Induced by Neoadjuvant Chemotherapy in the Invasive Squamous Cell Carcinoma of Uterine Cervix

JS Song, SA Kim, JY Ro, K-R Kim. University of Ulsan College of Medicine, Seoul, Republic of Korea.
Background: Neoadjuvant chemotherapy (NAC) has emerged as a possible alternative, which may improve a survival rate in patients with locally advanced malignant disease. In the squamous cell carcinoma of the uterine cervix, we observed that some of the histologic changes after NAC mimic prognostically important histologic variants of cervical carcinoma, or produce histologic mimicry of prognostically significant histologic parameters. Nevertheless, histological changes after NAC have rarely been described and underrecognized among pathologists.
Design: This study comprised 88 radical hysterectomy specimens including 44 invasive squamous cell carcinomas in advanced stage after receiving a platinum-based NAC, and 44 age matched controls without NAC. We compared the H&E findings to recognize the histologic changes induced by NAC.
Results: Cytoplasmic vacuolization and microcystic change mimicking adenoid cystic carcinoma, pseudopapillary arrangement simulating papillary serous adenocarcinoma,

peritumoral cleft mimicking lymphovascular tumor emboli, were far more frequent in NAC group, although they were not statistically significant. Moreover, histiocytic infiltration ($p<0.001$), thick walled blood vessel ($p<0.001$), single isolated tumor cell in the stroma ($p=0.003$), and abrupt keratinization ($p=0.007$) were significantly more frequent in NAC group, suggesting that those findings were induced by NAC.
Conclusions: The pathologist's knowledge regarding chemotherapy-induced morphological changes is important to avoid a possible misdiagnosis.

1186 "Low Grade Uterine LMS" a Clinically Favorable, but Heterogeneous Group of Mesenchymal Tumors

R Soslow, O Zivanovic, L Jacks, M Hensley, E Veras. Memorial Sloan-Kettering Cancer Center, New York, NY.
Background: Reliable pathologic predictors of clinical outcome have yet to be determined for uterine leiomyosarcoma (LMS). The literature suggests a correlation between tumor grade and clinical behavior. In this study, we reviewed LMS cases and focused on the clinicopathologic features of LMSs considered to be "low grade."
Design: The Murray Brennan Sarcoma Database was used to retrieve "low-grade" LMSs diagnosed between 1986 and 2009 (n=14). All available slides from hysterectomies were reviewed by two pathologists using Stanford criteria. "STUMP" was used as an abbreviation for "atypical leiomyoma with low recurrence rate" in this abstract.
Results: Only 29% (4/14) of all cases originally diagnosed as "low-grade" LMS met Stanford criteria for LMS. Four tumors were STUMPs, 2 of which were known to be recurrent and 1 high stage at presentation. 2 were leiomyoma variants. 3 were cellular spindle cell neoplasms requiring immunohistochemistry for confirmation of smooth muscle differentiation (two were suspected of being endometrial stromal sarcoma variants or malignant solitary fibrous tumor) and one was a cellular, benign endometrial polyp. 43% (6/14) of patients were NED, 21% (3/14) were AWD (2 STUMPs and one leiomyoma; range 59-295 months to recurrence) and 14% (2/14) were DOD (1 LMS and 1 probable endometrial stromal sarcoma; 15 and 18 months from diagnosis). Three patients died of unknown causes.
Conclusions: "Low grade LMS" is a heterogeneous group of mesenchymal tumors with favorable outcomes. When endometrial stromal tumor variants, STUMPs and other leiomyomas are excluded from this group, only rare LMSs remain, all of which are histologically indistinguishable from conventional LMS. The number of such tumors in this group precludes a statistical comparison of outcomes versus tumors diagnosed as conventional LMS. Whether patients diagnosed with recurrent STUMPs have low grade LMS is debatable.

1187 Small (≤ 5 cm) Uterine Leiomyosarcomas Are Rare, Frequently Misdiagnosed and as Clinically Aggressive as Larger Tumors

R Soslow, M Leitao, O Zivanovic, L Jacks, M Hensley, E Veras. Memorial Sloan-Kettering Cancer Center, New York, NY.
Background: The literature suggests that low grade, young age (≤ 50 years) and small tumor size (≤ 5 cm) are all associated with improved outcomes in patients with uterine leiomyosarcoma (LMS). The FIGO 2009 staging system considers organ confined LMS < 5 cm as stage IA and implies more favorable outcomes.
Design: We identified all cases of LMS seen at our institution from 7/82-7/07. Tumor size was dichotomized into 2 groups (≤ 5 cm and >5 cm). Cases were staged according to FIGO 1988 after adaptation for use in sarcoma. Two pathologists reviewed all available hysterectomy specimen slides from tumors in the former group.
Results: We identified 268 cases. The median age was 51 y (range 23-81 y). 55% were stage I, 6% stage II, 18% stage III and 21% stage IV. Primary tumor size was available in 95% of cases (n=254). The median follow-up was 39.7 months. After excluding cases diagnosed as "low grade LMS" (n=5), there were 123 FIGO stage I cases, 23 of which were ≤ 5 cm. The overall survival for ≤ 5 cm tumors was 77 months (95% CI: 0-169.2 months) compared to 73.9 months (95% CI: 55.9-91.9 months) for larger tumors (p=0.3). Slides were available for re-review in 15 of 23 cases. An LMS diagnosis was upheld in 13 of 15 cases; 1 case was considered a STUMP and the other an undifferentiated sarcoma.
Conclusions: LMS measuring ≤ 5 cm constitute less than 10% of all LMSs. Excluding cases diagnosed as "low grade LMS," many of which are not LMSs, yields an even smaller cohort. The remaining cohort resembles the much more common group of LMSs >5 cm and had similar clinical outcomes. These data do not support substaging for small LMS in FIGO 2009.

1188 Global Hypoxic Patterns of Placental Injury: Clinicopathologic Associations

J Stanek. Cincinnati Children's Hospital Medical Center, Cincinnati, OH.
Background: There is no universally accepted system of histological classification of global hypoxic patterns of placental injury. This retrospective analysis has been designed to validate the Kingdom-Kaufmann classification system of patterns of placental hypoxia as preuterine (PR), uterine (UH) and postuterine (PU), based on selected histological features of chorionic villi: maturation, extracellular matrix, cytotrophoblasts, vascularity, macrophages, and syncytial knotting.
Design: Of ≥20weeks 5097 placentas consecutively signed by the author, 4413 did not feature histological patterns of global hypoxia while 684 did, of which 289 placentas showed PR, 237 placentas showed UH, and 158 placentas showed PU. Several maternal, fetal and neonatal, and gross and microscopic placental features were statistically compared among PR, UH, and PU using analysis of variance or Yates chi-square.
Results: . There were statistically significant differences (P≤0.001) between PR, UH and PU, respectively: average gestational age 35, 33 and 34 weeks, preeclampsia 12, 45, and 33%, cesarean sections 32, 50, and 58%, intrauterine growth restriction 10, 23, and 28%, umbilical cord compromise 6, 0, and 9%, complications of 3rd stage of labor 5, 2, and 12%, placental weight 437, 328, and 318g, meconium staining 39, 22, and 25%, infarction 12, 37, and 29%, hypertrophic decidual arteriolopathy 18, 44, and 28%, atherosis of spiral arterioles 4, 23, and 12%, membrane laminar necrosis 11, 24, and 21%, membrane microscopic chorionic pseudocysts 5, 14, and 13%, increased extravillous trophoblasts 3, 20, and 13%, multinucleated trophoblastic giant cells in maternal floor 7, 24, and 20%, and chorangiosis 35, 13, and 2%.
Conclusions: Despite a significant overlap of associated clinical conditions and placental histological features, the Kingdom-Kaufmann classification of hypoxic patterns of placental injury helps to clarify the etiopathogenesis of a significant proportion of complications of pregnancy and abnormal fetal or neonatal outcomes. PR seems to portend the best prognosis to the fetus, and tends to occur later in 3rd trimester, typically featuring chorangiosis and meconium staining. UH is strongly associated with preeclampsia, infarctions, decidual arteriolopathy, laminar necrosis and microscopic chorionic pseudocysts of membranes, multinucleated trophoblastic giant cells of the maternal floor, and increased amount of extravillous trophoblasts. PU features the smallest placentas and most strongly correlates with the umbilical cord compromise and complications of 3rd stage of labor.

1189 Analysis of Human Papillomavirus 16E6 Oncogene Mutation in Cervical Cancer

F Sun, X Ha, C Xing, X Cao. The 2nd People's Hospital of Zhuhai, Zhuhai, Guangdong, China; General Hospital of PLA Lanzhou, Lanzhou, Gansu, China; General Hospital of PLA Lanshou, Lanzhou, Gansu, China.
Background: Cervical cancer (CC) is the second most common type of cancer in women worldwide, after breast cancer. High-risk human papillomaviruses (HR-HPVs) are considered to be major causes of cervical cancer. HPV16 is the most common type of HR-HPVs and HPV16 E6 gene is one of major oncogenes. Specific mutations are considered as dangerous factors causing CC. In recent studies the mutations of HPV16 E6 gene and the protein coded by it are considered to have a direct relationship with the development of CC. The mutation of HPV16 E6 is different with respect to the different geographical regions over the globe. This study was designed to find mutations of HPV16 E6 and the relationship between the mutations and the happening of Chinese CC population.Current work aimed at finding this gene mutations and its relationship between mutations and happening of CC.
Design: The tissue DNA was extracted from 15 CC biopsies. Part of HPV16 E6 gene (nucleotide 201-523) was amplified by polymerase chain reaction (PCR) from the CC tissue DNA. The PCR fragments were sequenced and analyzed. Collect 15 CC specimens (age range, 24-65 years; mean age, 41 years) from a Maternal and Child Health Hospital from 2007 to 2008. All patients didn't receive radiotherapy or chemiotherapy. All cases were reviewed and diagnosed as CC by 3 associate chief physicians of pathology department. And the specimens were stored in -60° C freezer.
Results: The result of PCR showed that the positive rate of HPV16 E6 was 93.33% (14/15). After sequencing and analyzing the 13 of 14 PCR fragments we found that 4 of them maintained prototype (30.77%), 8 have a same 350G mutation (61.54%), and 1 has a 249G mutation (7.69%).
Conclusions: Our findings suggested that HPV16 E6 is the highly occurring gene mutation. The mutations of it may shed light on the development of cervical cancer. Further work is needed to disclose the new found 249G mutation.

1190 Mammaglobin Expression in Primary Ovarian Carcinoma and Its Utility in the Differential Diagnosis of Metastatic Mammary Carcinoma to Ovary

Y Sun, P Newcomb, D Mandich, R Cartun, S Mandavilli. Hartford Hospital, Hartford, CT.
Background: Metastatic mammary carcinoma to ovary (MMCA) can mimic primary ovarian carcinoma and pose a diagnostic dilemma. Mammaglobin (MG) has been suggested as a breast-specific marker, but there is limited literature evaluating MG expression in primary ovarian tumors. We wanted to evaluate the utility of MG and a panel of other IHC markers including breast-associated markers (GCDFP-15 and ER) along with markers seen in ovarian carcinomas (WT1 and CA125).
Design: 19 cases of primary ovarian tumors including 11 cases of endometrioid carcinoma (ECA), 5 papillary serous carcinoma (PSCA), 4 clear cell carcinoma (CCCA), 2 cases of undifferentiated carcinoma (UDCA) and 5 cases of MMCA including 3 cases of invasive ductal carcinoma and 2 cases of invasive lobular carcinoma were retrieved from the tertiary hospital pathology files. MG, GCDFP-15, ER, WT1, and CA125 immunostain were performed in all cases.
Results: MG was positive in 4 cases of MMCA (80%), but was also positive in 50% of ovarian ECA. GCDFP-15 was expressed in 3 cases of MCA (60%) and none of the primary ovarian tumors. ER was present in both MCA and primary ovarian tumors except CCCA. CA125 was seen in the majority of MMCA and one case of MMCA was WT-1 positive. The IHC results are summarized below:

IHC profile in MCCA and primary ovarian carcinoma

Diagnosis/case #	MG	GCDFP15	CA125	ER	WT-1
MCCA (5)	4 (80%)	3 (60%)	3 (60%)	5 (100%)	1 (20%)
ECA (8)	4 (50%)	0	7 (86%)	8 (100%)	3 (38%)
PSCA (5)	0	0	5 (100%)	5 (100%)	5 (100%)
CCCA (4)	0	0	3 (75%)	0	0
UDCA (2)	1 (50%)	0	1 (50%)	1 (50%)	0

Conclusions: MG was expressed in the majority of MMCA, but was also expressed in a significant proportion of ovarian ECA. GCDFP-15 was highly breast-specific and was not expressed in primary ovarian carcinomas. WT1 was seen in all ovarian serous carcinomas and can occasionally be expressed in MMCA. ER was not useful in this differential diagnosis. These results favor the use of a panel of IHC stains with careful evaluation of other clinical findings in distinguishing primary ovarian carcinoma from MMCA.

1191 CDX-2 Expression in Ovarian Endometrioid Tumor and Its Utility in the Differential Diagnosis of Metastatic Colonic Adenocarcinoma to the Ovary

Y Sun, P Newcomb, D Mandich, RW Cartun, S Mandavilli. Hartford Hospital, Hartford, CT.

Background: Metastatic carcinomas to the ovary can mimic primary ovarian tumors. In particular endometrioid tumors (OET) including endometrioid adenocarcinoma (EAC) and endometrioid borderline tumor of ovary (EBTO) can be very difficult to separate from a metastatic colonic adenocarcinoma (MCA). CDX-2 (a transcription factor which regulates the differentiation of intestinal epithelial cells) is consistently expressed in colorectal adenocarcinomas. Additionally, mucinous adenocarcinomas (from a variety of primary sites) can also show CDX2 expression. There is limited literature regarding CDX-2 expression in OET. The aim of this study was to evaluate CDX-2 expression in OET and its utility in the differential diagnosis of MCA from primary OET.

Design: 12 cases of primary OET (3 EBTO and 9 EAC) and 5 cases of MCA were retrieved from tertiary hospital pathology files. A panel of immunohistochemical stains was performed on all cases including CDX-2, CA125, and ER. The IHC stains were scored as: 0-no expression; 1+ < 10% cells staining; 2+ 10-25% of cells staining; 3+ 25-50% of cells staining; and 4+ > 50% of cells staining.

Results: CDX-2 was positive in 100% of MCA and 67% of OET. CA125 and ER were positive in the majority of OET, but may be seen rarely in MCA. The results are summarized in Table 1 and Table 2.

Table 1. CDX2 Expression in MCA and OET

Diagnosis/case #	1+ to 2+	3+ to 4+	Total Positive
MCA (5)	0	5	5 (100%)
OCT (12)	5	3	8 (67%)

Table 2. Immuno Expression Profile in MCA and OET

Diagnoses/case #	CDX2	CA125	ER
MCA (5)	5 (100%)	1 (20%)	1 (20%)
OET(12)	8 (67%)	9 (82%)	11 (92%)

Conclusions: CDX-2 is expressed in a significant proportion of ovarian endometrioid tumors. Therefore, CDX-2 IHC results should be interpreted in the context of other IHC stains (CA125 and ER) and clinical findings when evaluating primary endometrioid tumors versus metastatic colonic tumors to the ovary.

1192 Multiple Molecular Pathways in the Development of Uterine Serous Carcinoma

AD Tabrizi, CB Gilks. Tabriz University of Medical Science, Tabriz, East Azarbyjan, Islamic Republic of Iran; University of British Columbia, Vancouver, BC, Canada.

Background: A pathway for development of frankly invasive uterine serous carcinoma through endometrial glandular dysplasia (EmGD) to intraepithelial and invasive serous carcinoma has been proposed.

Design: We present histologic and immunohistochemical findings in 26 cases of endometrial serous carcinoma and their putative precursor lesions, based on a study of cases of serous carcinoma of the endometrium involving endometrial polyps. Immunostaining for six markers (p53, PTEN, Ki67, p16, ER, and PR) were performed in selected sections of these cases.

Results: In five cases EmGD, characterised by glands lined by atypical cells but not showing a sufficient degree of cytological atypia or mitotic activity to warrant a diagnosis of endometrial intraepithelial carcinoma (EIC), was present adjacent to the EIC and invasive serous carcinoma, and in these cases all three components showed identical immunostaining patterns for, PTEN, p16, ER and PR for a given case, and the results for P53 and Ki 67 showed increasing score from EmGD to EIC. In four cases, hyperplastic glands showing crowded architecture, and lined by cells with low grade atypia, similar to that seen in atypical hyperplasia of the endometrium, were present adjacent to the serous carcinoma. These cases showed ER positivity and PTEN negativity in the hyperplasitic areas, with at least focal p53 positivity. In the remaining 17 cases no EmGD or atypical hyperplastic lesions were identified. As a control group we examined 8 cases of atypical endometrial hyperplasia with no associated carcinoma. All 8 these cases were negative for P53 and positive for ER, PR and showed at least one focus of PTEN null glands.

Conclusions: This study suggests that there are different molecular pathways leading to development of uterine serous carcinoma, i.e. through EmGD, with p53 mutation in nonhyperplastic glands, in cells that lack expression of hormone receptors and do not have loss of PTEN expression, or through atypical hyperplasia of the endometrium, with p53 mutation occurring in cells that express hormone receptors and show loss of PTEN expression.

1193 Frequency of Serous Tubal Intraepithelial Carcinoma in Various Gynecological Malignancies – A Study of 290 Consecutive Cases

S Tang, K Onuma, P Deb, E Wang, A Lytwyn, M Sur, D Daya. McMaster University, Hamilton, ON, Canada.

Background: Serous tubal intraepithelial carcinoma (STIC) has been reported in association with 32%-46% of pelvic serous carcinoma, suggesting that the tubal fimbria is the primary source of ovarian and peritoneal serous carcinoma. We hypothesized that if this is the case, the frequency of STIC should be substantially lower in endometrial serous carcinomas, in non-serous gynecologic malignancies, and in benign gynecologic neoplasms than in ovarian or peritoneal serous carcinomas.

Design: From 2007-9 the fallopian tubes of 342 consecutive gynecologic cases (269 TAH-BSO, 21 BSO) were entirely submitted for histology using the Sectioning and Extensively Examining the FIMbriated end (SEE-FIM) protocol. This study included 290 of these cases (slides from 16 cases were not available for review and 36 cases only had 1 fallopian tube removed). The slides from the fallopian tubes were independently reviewed by two gynecologic pathologists who were blinded to all other findings; disagreements were resolved by a third pathologist.

Results:

FREQUENCY OF STIC IN SEROUS AND NON-SEROUS MALIGNANCIES

Primary Site	Carcinoma Type	Mean Age	No. Cases	No. (%) STIC
Ovary	Serous	66	33	6(18)
	Non-serous	56	14	0(0)
Endometrium	Serous	68	27	4(15)
	Non-serous	66	70	0(0)
Cervix	Serous	Not available	1	0(0)
	Non-serous	46	12	0(0)
Peritoneum	Serous	70	7	2(29)

STIC IN NON-GYNECOLOGICAL MALIGNANCIES AND OTHER CONDITIONS

Groups	Mean Age	No. Cases	No. (%) STIC
Non-gyn malignancies	54	15	0(0)
Benign conditions	53	87	0(0)
Other pathology*	50	24	0(0)

*4 cervical AIS and CIN III; 8 endometrial atypical complex hyperplasia; 12 ovarian borderline tumor.

Conclusions: STIC was present in 18%, 29%, and 15% of ovarian, peritoneal, and endometrial serous carcinomas, respectively. STIC was **NOT** identified in non-serous ovarian, endometrial and cervical malignancies, or other conditions. The fallopian tube may be the origin of some pelvic serous carcinomas. However, given that STIC coexisted with 15% of endometrial serous carcinomas, a more unifying theory may be that gynecologic serous carcinomas and STIC are multifocal lesions. Further studies are needed to elucidate the clinical significance of STIC and define the role of STIC in endometrial **serous** carcinoma.

1194 Differential Genome-Wide DNA Methylation Patterns between Low Grade and High Grade Papillary Serous Carcinoma of the Ovary

A Ting, B Yang. Cleveland Clinic, Cleveland, OH.

Background: It has been clear that low grade and high grade ovarian papillary serous carcinoma (PSC) have distinct pathologic features, clinical presentations and prognosis. A line of studies indicates that different genetic pathways exist between low grade and high grade PSC. However, epigenetic alterations, such as DNA methylation, between low grade and high grade PSC have not been well characterized. Utilizing MBD-isolated Genome Sequencing technique, we studied genome-wide DNA methylation patterns of low grade and high grade PSC.

Design: Twenty cases of stage IIIc ovarian PSC, including 10 cases of low grade PSC and 10 cases of high grade PSC, were included in the study. Genomic DNA was extracted from FFPE tissue. Genome-wide DNA methylation was characterized using Methy-CpG binding domain (MBD)-isolated genome sequencing (MiGS) technique.

Results: Among 27 millions of methylated sequences, we observed that globally, DNA methylation levels are similar in low grade and high grade PSC, with a slight increase of 5' promoter methylation observed in high grade PSC (3249 promoters in low grade vs. 3623 gene promoters in high grade). In addition to identifying the majority of reported promoter DNA methylation in the two groups, we detected differential methylation at over 1,000 loci between low grade and high grade PSC. These differential DNA methylation sites include RefSeq gene promoters, miRNAs, and intergenic sequences. Specifically, 306 genes are at least 10-fold hypermethylated in high grade PSC relative to low grade PSC. Only two genes show at least 10-fold hypomethylated in high grade than low grade PSC. Although chromosome 1 has, not surprisingly, the greatest number of differences because chromosome 1 is the largest chromosome in the genome, chromosome 19 harbored disproportional numbers of differences (55 genes out of 308 genes) between the two groups.

Conclusions: This is the first report of full genome-wide methylation sequencing between low grade and high grade PSC of the ovary. We found there were more than 300 gene promoters significantly hypermethylated in high grade than low grade PSC. Functional studies of these differential DNA methylation patterns could provide insights into the pathogenesis of ovarian PSC. Furthermore, these distinct methylation differences could be used as biomarkers to precisely distinguish, at a molecular level, between low grade and high grade PSC.

1195 E2F-1 Expression Correlates with p16 in Cervical Dysplasias

WU Todd, JF Silverman. Allegheny General Hospital, Pittsburgh, PA.

Background: E2F-1 is a nuclear transcription factor that binds with pRB during the regulation of several genes needed for cell cycle entry and DNA synthesis. The expression of E2F-1 has not been reported in cervical dysplasia.

Design: 72 cases of normal cervix and cervical dysplasia were retrieved for immunohistochemical analysis. The cases encompassed 8 normal, 24 cervical intraepithelial neoplasia (CIN) I, 21 CIN II and 22 CIN III. Immunohistochemical analysis was performed using antibodies directed against p16 and E2F-1. Positive staining was quantitatively and qualitatively recorded. The fisher exact test was used to determine significance.

Results: Normal biopsies of cervical squamous epithelium predominantly showed a uniform staining pattern along the basal layers and more superficial layers of the epithelium. Higher grade dysplastic epithelium (CIN II and III) showed a loss of this uniform pattern with E2F-1 staining became weaker and more sporadic ($P<0.01$). The loss of the uniform pattern also correlated with p16 expression ($P<0.001$).

Conclusions: There are qualitative differences between higher grade cervical dysplasias (CIN II and CIN III) and normal/CIN I cervical biopsies. This change in staining pattern also correlated well with p16 expression in cervical biopsies. These findings may indicate a role for E2F-1 in the progression to higher grade cervical dysplasias and help in the differentiation of CIN I from higher grade dysplasias.

1196 Heparin-Binding EGF-Like Growth Factor (HB-EGF) Overexpression Is Associated with Aggressive Disease in Endometrial Carcinoma

J Torres-Mora, CE Sheehan, JS Ross. Albany Medical College, Albany, NY.

Background: HB-EGF is a cell proliferation associated growth factor which is also known to stimulate angiogenesis. Although linked to adverse disease outcome in several tumors, HB-EGF expression in endometrial cancer has not been previously studied as a potential clinically useful biomarker for endometrial carcinoma.

Design: Formalin-fixed, paraffin-embedded tissue sections from 126 endometrial carcinomas, including 102 endometrioid carcinoma (EC), 12 uterine papillary serous carcinoma (PSC) and 12 malignant mesoderm mixed tumor (MMMT) were immunostained by automated methods (Ventana Medical Systems Inc., Tucson, AZ) using mouse monoclonal HB-EGF (Abcam, Cambridge, MA). Cytoplasmic immunoreactivity was semiquantitatively scored based on staining intensity and distribution and the results were correlated with morphologic and prognostic variables.

Results: Cytoplasmic HB-EGF overexpression was observed in 66/126 (52%) tumors, including 53/102 (52%) EC, 8/12 (67%) PSC, and 5/12 (42%) MMMTs. HB-EGF overexpression correlated with tumor grade (61% grade 3 vs. 59% grade 2 vs. 31% grade 1, p=0.035), advanced tumor stage (74% stage III/IV vs. 45% stage I/II, p=0.007), depth of myometrial invasion (74% invading to more than 50% of myometrium vs. 47% to less than 50% vs. 40% no invasion, p=0.023). A trend for correlation with disease recurrence was noted overall [70% recurrent vs 49% non-recurrent, p=0.079] and within the PSC subgroup [100% recurrent vs 50% non-recurrent, p=0.083]. There was no correlation with overall survival.

Conclusions: HB-EGF expression is associated with aggressive forms of endometrial carcinoma and significantly correlates with tumor grade and pathologic stage and may be a predictor of overall disease recurrence after primary treatment. Continued study of this biomarker in endometrial malignancies appears warranted.

1197 Identification of a mRNA Protease Profile Associated with Early Relapse in Serous Ovarian Cancer

D Trudel, I Bairati, CM Overall, R Kappelhoff, M Plante, B Tetu. Centre Hospitalier Universitaire de Québec, Québec, Canada; University of British Columbia, Vancouver, Canada.

Background: Mostly diagnosed at an advanced stage (FIGO III or more), patients with OC usually respond well to chemotherapy. However, at 18 months, about 60% of women with complete response to treatment relapse. Expression of proteases (such as kallikreins) have been associated with OC prognosis. The proteins are mostly secreted by stromal cells but some are mainly secreted by tumour cells. Our objective is to test the hypothesis that a specific mRNA protease profile allows the identification of women with serous OC who experience early relapse after a complete response to standard treatment.

Design: Ten stage III tumours from cytoreductive surgical specimen were selected from our tissue bank. After treatment with platinum-paclitaxel, these women were in complete response as defined by serum CA-125 levels and they were divided in two groups of 5 women according to their time to relapse as early relapse (ER) or late relapse (LR). Snap-frozen tissue was micro-dissected to isolate stromal cells from tumour cells. mRNA was extracted from stromal cells and from tumour cells. A microarray dedicated to proteases study (CLIP-CHIP) was used to determine gene expression in both cell types. For each gene, expression in ER group was compared to expression in LR group. P-values were estimated using the empirical bayes method of Smyth within the limma package in Bioconductor and then adjusted following the Benjamini-Hochberg procedure.

Results: mRNA was available from tumors of all women and from stroma of 8 women, 4 with ER and 4 with LR. Mean time to relapse was 356.2 days in the ER group and 1137.4 days in the late relapse (LR) group. In the ER group, among 27 significant genes, WAP four-disulfide core-2 (WDFC2, HE4) was overexpressed 5.1 times (adjusted p= 0.047), ADAM-10 was overexpressed 8.7 times (adjusted p = 0.028) and proteasome beta-1 subunit (PSMB1) was underexpressed 4.1 times (adjusted p = 0.015).

Conclusions: To our knowledge, this is the first study aimed at identifying a set of proteases associated with prognosis in women with OC and complete response after standard treatment. These findings may help to better understand the biology of OC and to develop targeted treatment strategies.

1198 HPV Is Associated with Many Types of Rare Cervical Cancer

SM Tucker, JA Hanson, V Rajendran, Z Peng, CS-C Adamson, K Cooper, MF Evans. Fletcher Allen Health Care, Burlington, VT; University of Vermont, Burlington, VT.

Background: The role of human papillomaviruses (HPV) in the pathogenesis of squamous cell carcinoma (SCC) of the cervix is well-established. However, the significance of HPV in the etiology of rare cervical cancer types is largely unknown; the aim of this study was to investigate HPV in these tumors by combining polymerase chain reaction (PCR), chromogenic *in situ* hybridization (CISH) and immunohistochemical (IHC) assays.

Design: Thirty-one archival (1995-2008) formalin-fixed, paraffin-embedded samples of adenoid basal carcinoma (AB, n=6), adenoid cystic carcinoma (AC, n=4), adenosquamous carcinoma (AS, n=9), basaloid squamous carcinoma (BS, n=1), mesonephric adenocarcinoma (MA, n=1), mucinous adenocarcinoma (MuA, n=7), small cell carcinoma (SC, n=2), and transitional cell carcinoma (TC, n=1) were collected from 23 patients. PCR was performed using GP5+/6+ primers, CISH using biotinyl-tyramide amplification, and IHC for $p16^{INK4a}$.

Results: HPV was detectable by PCR in all types of rare cervical tumors. High-risk (hr) types (HPV16 [n=8], 18 [n=6], 31 [n=3], 33 [n=2], 45 [n=1], 59 [n=4], 66 [n=1]) low-risk (lr) types (HPV11 [n=1]) and an unidentified type or types [n=1] were detected. Four individual samples were PCR negative for HPV (2 AC, 2 MuA). CISH signals (punctate [integrated HPV] or both punctate and diffuse [integrated and episomal HPV]) were detected in 1/2 AC, 6/9 AS, 1/1 BS, 1/2 SC and 1/1 TC samples. CISH signals were not detected in any of the AB or MuA samples. $p16^{INK4a}$ staining demonstrated no identifiable pattern for any tumor type. $p16^{INK4a}$ staining was identified in samples that were negative for HPV by PCR/CISH and was absent in PCR positive samples; all CISH positive samples demonstrated some $p16^{INK4a}$ staining.

Conclusions: This study is the first to combine PCR, CISH and $p16^{INK4a}$ IHC in the investigation of the role of HPV in rare types of cervical cancer. The data indicate that HPV is associated with at least a subset of adenoid cystic, adenosquamous, basaloid squamous, small cell and transitional cell carcinomas and for a broad range of hrHPV types. The non-detection of HPV by CISH in PCR positive adenoid basal carcinomas and mucinous adenocarcinomas allows for the possibility that in these tumors the HPV detected is incidental to tumor pathogenesis. $p16^{INK4a}$ staining does not appear to be a surrogate marker for HPV across the variety of rare cervical tumor forms.

1199 Study of Epithelial-Mesenchymal Transition in 70 Endometrial Carcinosarcomas (ECS)

KK Van De Vijver, MA Castilla, L Romero, M Biscuola, G Moreno-Bueno, J Palacios, E Oliva. Maastricht University Medical Centre, Maastricht, Netherlands; Hospital Universitario Virgen del Rocio, Sevilla, Spain; Instituto de Investigaciones Biomedicas, Madrid, Spain; Massachusetts General Hospital, Boston.

Background: Epithelial-mesenchymal transition (EMT) is a process of cellular trans-differentiation by which epithelial cells lose polarity and cell-cell contacts, reorganize their cytoskeleton, acquire expression of mesenchymal markers and manifest a migratory phenotype. EMT occurs during embryogenesis and although implicated in tumor progression, its occurrence in human neoplasias is controversial. The goal of this study was to compare cadherin switching, expression of mesenchymal markers and snail in the epithelial component of ECS and endometrial endometrioid carcinomas (EEC) in order to know if ECS can be considered a true example of EMT in human neoplasias.

Design: Expression of E-cadherin, N-cadherin and mesenchymal markers (SPARC and fascin) was analyzed by immunohistochemistry on the epithelial component of 70 ECS and 44 grade I and II EECs on tissue microarray sections. Expression for all markers was scored as absent, mild or moderate/intense according to intensity and extension. In addition, mRNA *SNAI1* expression was analyzed by real time quantitative RT-PCR in both the epithelial and sarcomatous components of 22 ECS.

Results: Of the 70 ECS, 65 showed an intermingled distribution of carcinoma and sarcomatous components while in 5 the two components appeared to collide. Absent or mild E-cadherin expression was found in 43% ECS and 16% EEC (p= 0.005). Moderate/intense expression of N-cadherin, SPARC and fascin was observed in 38%, 35%, and 33% ECS, but in only 7%, 12%, and 0 EEC (p< 0.01 for all comparisons). E-cadherin was absent but SPARC and fascin were expressed at different levels in the sarcomatous component of all ECS. 90% ECS expressed detectable *SNAI1* mRNA in the sarcomatous component but only 50% in the epithelial component. In addition, mean *SNAI1* mRNA level was ten-fold higher in the sarcomatous than in the epithelial component.

Conclusions: Switching of cadherin subtypes and expression of snail and mesenchymal markers in the epithelial component of ECS indicate that this tumor represent a true example of stable EMT. Cadherin switching and expression of mesenchymal markers by epithelial cancer cells probably signal EMT capabilities in ECS. Once EMT is established, snail expression probably is necessary to maintain the mesenchymal phenotype in the sarcomatous component.

1200 Tissue Array Analysis of Apoptotic and Cell Cycle Regulatory Markers in Benign and Malignant Uterine Smooth Muscle Tumours: A Clinicopathologic Study of 52 Cases

P Vasudev, M Farag, C Salib, D Browning, W Foster, O Boutross-Tadross. McMaster University, Hamilton, Canada.

Background: This study was undertaken to investigate the expression of apoptotic and cell cycle regulatory markers in uterine smooth muscle tumors (USMTs), in order to evaluate the utility of immunohistochemistry in differentiating benign from malignant tumors, and to identify signalling pathways underlying the development of leiomyosarcoma (LMS).

Design: Fifty two cases of USMTs including LMS (n=15), undetermined malignant potential (STUMP, n=4), leiomyoma variants (LV, n=14) and benign leiomyomas (BL, n=19) were reviewed light microscopically to measure apoptotic count. Tissue arrays were created with 2 representative cores from each case. Immunohistochemical staining was performed for bcl-2, Ki-67, p53 and p16 and each case was given a value of 0% to 100% positive cells. Comparison of numerical data was performed by the Kruskal-Wallis test. Findings were correlated with pathological and clinical parameters.

Results: The mean apoptotic count was significantly higher in LMS (13/10HPF) compared to BL (<1/10HPF) (p<0.001). While the mean percentage expression of p53 (22 % vs. <1%; p=0.017), p16 (46 % vs. 2 %; p=0.002) and Ki-67 (22 % vs. 3%; p=0.001) was significantly higher in LMS than in BL; the bcl-2 expression (71 % vs. 81%; p=0.48) was not significantly different.

Table 1 : Comparative expression of cell cycle and apoptotic markers.

Marker	LMS	STUMP	LV	BL	p-value*
P53(%)	22 (0-80)	22 (0-80)	9 (0-30)	1 (0-10)	0.017
P16(%)	46 (0-100)	10 (0-40)	3 (0-20)	2 (0-20)	0.002
Bcl-2(%)	71 (0-100)	88 (50-100)	95 (70-100)	81 (40-100)	0.48
Apoptosis (/10HPF)	13 (1-36)	4 (1-8)	2 (1-4)	<1 (0-1)	<0.001
Ki67(%)	22 (0-60)	14 (0-40)	4 (0-20)	3 (0-25)	0.001

Results are expressed as mean (range). *p-value vs. LMS and BL.

Conclusions: The significantly higher apoptotic count in LMS combined with lack of significantly lower expression of bcl2 suggests that other anti/pro-apoptotic proteins may be involved in increased apoptosis in LMS. p16 positivity was found to be the most useful marker for LMS showing both nuclear and cytoplasmic positivity in up to 100% of cells. The differential expression of p53 and p16 indicates that these proteins may have a role in the pathogenesis of LMS.

1201 Late Recurring Uterine Smooth Muscle Tumors Constitute a Heterogeneous Collection of Leiomyosarcomas and STUMPs

E Veras, O Zivanovic, L Jacks, M Hensley, R Soslow. Memorial Sloan-Kettering Cancer Center, New York, NY.

Background: Reliable pathologic predictors of clinical outcome have yet to be determined for uterine leiomyosarcoma (LMS). The literature suggests a correlation between tumor grade and clinical behavior. We studied the pathologic features of uterine smooth muscle tumors with late recurrences, a surrogate for clinically low grade behavior, in an attempt to learn which histologic features correlated with a protracted clinical course.

Design: The Murray Brennan Sarcoma Database and pathology records were reviewed to access STUMPs and LMSs with recurrence and long disease-free intervals, defined as recurrence ≥ 5 years of diagnosis. Stanford diagnostic criteria were used.

Results: 21 cases were retrieved from the database. Slides were available for review in only 9 cases, only 5 of which were LMSs. The cases from the sarcoma database represented approximately 3% of all LMSs.

Initial Diagnosis	Mitosis / 10 HPFs	Cytologic Atypia / Necrosis	Review Diagnosis (2009)	Size (cm)	Status	Time to Recurrence (Years)
Atypical cellular LM of uncertain malignant potential with epithelioid features	5	P (mild/diffuse) / A	Atypical LM	8	AWD	9.188
Atypical smooth muscle tumor	2	(moderate/diffuse) / A	ALMLRR	7	AWD	16.229
Infarcted smooth muscle tumor of uncertain malignant potential	2	P (mild/diffuse) / A	ALMLRR	15	AWD	13.103
LMS, low grade	8	P (mild/diffuse) / A	ALMLRR	10	AWD	9.798
LMS, high grade	>20	P (marked/diffuse) / P	LMS	5	AWD	5.486
LMS, low grade	12 (including atypical)	P (marked/diffuse) / P	LMS	N/A	AWD	7.978
LMS, high grade	11	P (marked/diffuse) / P	LMS with epithelioid features	20	AWD	6.822
LMS	7	P (marked/diffuse) / P	LMS	8	DOD	5.092
LMS arising in LM	28	P (marked/diffuse) / P	LMS	8	DOD	5.905

A: absent; ALMLRR: atypical leiomyoma with low recurrence rate; AWD: alive with disease; DOD: dead of disease; LM: leiomyoma; LMS: leiomyosarcoma; P: present

Conclusions: Late recurring smooth muscle tumors are extremely rare and only approximately half of them meet Stanford criteria for LMS. Late recurring LMSs exhibit high grade histologic features that are similar to other conventional LMSs. Late recurring STUMPs may represent low grade leiomyosarcomas, but additional work is needed to define diagnostic criteria that can be used prospectively.

1202 Pathological Diagnosis of Grade in Primary Mucinous Ovarian Tumor

K Wakasa, T Wakasa, M Ohsawa. Osaka City University Graduate School of Medicine, Osaka, Japan; Osaka Red Cross Hospital, Osaka, Japan.

Background: Pathological diagnosis of the grade in mucinous tumors is often difficult, because malignant tumors often appear benign, and the judgment of invasion is often complicated. Furthermore, differentiation from the metastatic ovarian tumors is complex.

Design: We studied 196 cases of ovarian mucinous tumor surgically resected in our hospital. Of these 196, benign cases numbered 113, borderline 57, malignant 26. Intraoperative consultation was carried out in 155 of the 196 cases. We verified the accuracy of the pathological diagnoses of frozen sections from these 155 cases by a comparison with the final pathological diagnoses. In 13 borderline and 17 malignant tumors we carried out the immunohistochemical staining of cytokeratin 7 and 20.

Results: In 11 out of the 155 frozen sections of mucinous ovarian tumor, pathological diagnoses of frozen sections were underdiagnoses in comparison with the final pathological diagnoses. Nine out of 48 borderline mucinous tumors were diagnosed as benign, and 2 out of 18 malignant cases were diagnosed as borderline on intraoperative consultation. There was no case of overdiagnosis. The discordance rate was about 7%. In all 11 cases, the reason for the underdiagnoses was the absence of the most atypical area in the frozen sections. Two out of 30 borderline and malignant mucinous tumors were negative for cytokeratin 7. In one of the two cases, autopsy was performed, and no other primary site was demonstrated. In the other case, no other primary site was identified during a two-year follow-up. There was no metastatic tumor in the 57 cases of borderline tumor and 26 cases of malignant tumor. The results of immunohistochemistry for cytokeratin 20 were variable.

Conclusions: The pathological diagnosis of mucinous tumor is difficult because areas showing a variable degree of atypicality are present in the same tumor. Underdiagnosis in intraoperative consultation is inevitable because the number of specimens that can be examined is only one or two. There was no metastatic tumor in the 57 cases of borderline tumor and 26 cases of malignant tumor. Immunohistochemically, most of the ovarian tumors were positive for cytokeratin 7. Cytokeratin 20 was variable in the ovarian mucinous tumors.

1203 UTF-1 Is a Novel Sensitive Marker for Ovarian Germ Cell Tumors

P Wang, J Li, Y Peng, D Cao. Beijing Ditan Hospital, Beijing, China; Washington University School of Medicine, Saint Louis; The University of Texas-Southwestern Medical Center, Dallas.

Background: The diagnosis of ovarian germ cell tumors (GCTs) and distinguishing them from non-GCTs sometimes can be challenging. In difficult cases, immunohistochemical markers often needed to achieve this goal. Here we investigated the diagnostic utility of a novel marker UTF-1.

Design: Seventy-one (71) primary (42 pure and 29 mixed) ovarian GCTs were included for this study and included the following tumor and tumor components: 28 dysgerminomas, 5 gonadoblastomas (GBs), 5 embryonal carcinomas (ECs), 24 yolk sac tumors (YSTs), 14 imamture teratomas, 9 mature teratomas, 3 carcinoids, and 2 struma carcinoids. Unstained slides were prepared from paraffin blocks for immunohistochemical staining with an UTF-1 antibody. Only nuclear staining was counted as positive. The staining intensity was scored as weak, moderate or strong. The percentage of tumor cells stained was scored semiquantitatively as: 0 (no tumor cell staining), 1+ (<=30%), 2+ (31-60%), 3+ (61-90%), and 4+ (>90%).

Results: The UTF-1 staining results in these GCTs were summarized in table 1.

Immunostaining of UTF-1 in ovarian GCTs

Tumor type and component	Staining Pattern				
	0	1+	2+	3+	4+
Dysgerminoma (N=28)	0	3	4	4	17
Gonadoblastoma (N=5)	0	0	0	2	3
Embryonal carcinoma (N=5)	0	0	0	0	5
Yolk sac tumor (N=24)	16	2	1	4	1
Immature teratoma (N=14)	11	3	0	0	0
Mature teratoma (N=9)	5	4	0	0	0
Carcinoid (N=3)					
Strumal carcinoid (N=2)					

Of the 28 germinomas, weak staining was seen in 9, moderate in 11, and strong in 8. All 5 GBs demonstrated either weak (n=5) and moderate (n=4). The staining was strong in all 5 ECs. The staining in YSTs and immature and mature teratomas was very weak to weak.

Conclusions: UTF-1 is expressed in ovarin GCTs but its expression is variable in different types of tumors as measured by semiquantative immunostaining. UTF-1 is a sensitive marker for EC, GB, and dysgerminoma. Study is in progress to test its specificity in ovarian non-GCTs.

1204 HMGA2 in Early-Stage High-Grade Papillary Serous Carcinoma in Fallopian Tubes

J-J Wei. Northwestern University, Chicago, IL.

Background: Before high-grade papillary serous carcinoma (HG-PSC) becomes invasive, it is believed to be a poorly defined short-lived precursor lesion. A recent characterization of serous tubal intraepithelial carcinoma (STIC) and of the p53 signature suggested that HG-PSC may follow a stepwise progression on cellular and molecular levels. HMGA2, an oncofetal protein, is overexpressed in ovarian cancer. The relationship between HMGA2 expression and p53 in the various stages of tumor progression in HG-PSC has never been tested.

Design: To test whether HMGA2 can be another valuable marker for STIC, we examined HMGA2 expression in 3 groups of patients: (1) 24 patients with STIC and its invasive counterpart, HG-PSC of the fallopian tubes, (2) 24 patients with HG-PSC of the ovaries but without STIC (positive control), and (3) 30 patients with cancer and normal fallopian tubes (negative control). The purpose of the study is to characterize whether HMGA2 can be a valuable marker complementary to p53 in detection of precursor or early serous carcinoma arising from fallopian tube. Our goal is to establish a link of HMGA2 with tumorigenesis of high grade serous carcinoma and identify a tool for the diagnosis of early ovarian cancer.

Results: We found that HMGA2 was overexpressed in 75% of patients with STIC, was coexpressed with p53 in more than 50% of patients, and was completely negative in the secretory cells of the 30 patients with normal fallopian tubes. Among 7 patients with cells negative for p53 staining, HMGA2 was positive in 5; among 6 patients whose tumor cells were negative for HMGA2 in STIC, 3 were positive for HMGA2 in the invasive component; about 70% of invasive HG-PSC tumor cells were immunoreactive for both HMGA2 and TP53. In invasive carcinoma, HMGA2 overexpression was correlated with p53 ($r = 0.45$), indicating the role of HMGA2 in p53 mediated tumor progression.

Conclusions: Our findings of immunoreactivity for HMGA2 in both STIC and invasive PSC may 1) lead to a novel, useful biomarker to complement p53 in the detection of early-stage serous carcinoma; 2) provide a new avenue for the study of the role of HMGA2 in the tumorigenesis of HG-PSC.

1205 Diagnostic Markers in the Differentiation of Gynecologic and Urological Clear Cell Carcinoma

RJ Wolsky, E Lengyel, AG Montag. University of Chicago, Chicago.

Background: The clear cell carcinoma (CCC) phenotype includes tumors from various sites with optically clear cytoplasm containing glycogen or lipid. CCC of the gynecologic tract is relatively common, including ovary, endometrium, cervix and vagina in descending order. Although CCC of the bladder and urethra are rare, differentiation from direct extension of a gynecologic tumor is important for appropriate staging and therapy. The present study analyzes markers of potential utility in differentiating CCC of the gynecologic and genitourinary tract.

Design: With IRB approval, archival slides and blocks were retrieved and tissue microarrays were designed containing CCC tumors from ovary (20), endometrium (21), cervix (15), vagina (4) bladder (3), urethra (3), kidney (17) and control tissues. Five micron sections were stained using monoclonal antibodies to E cadherin, alpha methyl co-A racemase (P504S), mesothelin, PLAP, uroplakin, CD10, and cytokeratins (CK) 7, 19 and 20. Staining intensity was visually scored by two observers on a scale of 0 to 3. Statistical analysis was performed using the Fisher exact test.

Results: All 6 bladder and urethral tumors had strong expression of E cadherin (see table), significantly greater than gynecologic sites or kidney ($p<.01$ for all comparisons). P504S was absent or weak in all cervical and vaginal tumors, while strong in 4 of 6 bladder/urethral lesions ($p<.01$). Only 1 of 6 bladder and urethral tumors was strongly positive for CK19, significantly less than gynecologic sites ($p<.05$ for all comparisons).

CK7 (2/17) was less commonly and CD10 (12/17) more commonly expressed in renal CCC than in all other sites (p<.001 and p<.005 respectively). Few tumors of any type expressed CK20. Urologic tumors rarely expressed uroplakin (1/6 and 1/16 respectively) and PLAP and mesothelin showed no significant difference among any of the comparisons.

Staining intensity (number of cases)

	E cadherin		P504S		CK19	
Stain intensity	0 to 1	2 to 3	0 to 1	2 to 3	0 to 1	2 to 3
Ovarian	12	7	13	6	9	10
Endometrial	15	4	12	7	9	10
Cervical/vaginal	14	4	18	0	5	13
Bladder/urethral	0	6	2	4	5	1
Renal	15	2	10	7	16	1

Conclusions: E cadherin, P504S and CK19 are useful in the differentiation of clear cell carcinoma of bladder or urethra from gynecologic sites, particularly cervical and vaginal lesions. CK7 and CD 10 have utility in differentiating renal cell carcinoma from other clear cell carcinomas.

1206 The Li Fraumeni Syndrome: A New Paradigm for p53 Signature Initiation in the Distal Fallopian Tube and *In Vitro* Modeling

W Xian, KK Mehra, A Miron, E Oliva, J Garber, M Roh, BJ Quade, CP Crum. Institute of Molecular Biology, Singapore, Singapore; Brigham and Women's Hospital, Boston, MA; Dana Farber Cancer Institute, Boston, MA; Massachusetts General Hospital, Boston, MA; University of Michigan Medical Center, Ann Arbor, MI.

Background: Serous carcinogenesis in the distal fallopian tube may develop via p53 mutation and loss of heterozygosity, leading to stabilized p53 protein in secretory cells (p53 signatures). We examined tubes from women with pre-existing (germ-line) mutations in *p53* (Li Fraumeni Syndrome or LFS) to assess the interplay between a constitutive p53 mutation, the tubal mucosa, and p53 signature development.

Design: Benign fallopian tubes from three women with LFS were stained for p53, Ki-67 (proliferation) and H2AX (DNA damage response) and analyzed for *p53* mutations and p53 LOH by laser capture micro-dissection (LCM) and *p53* genomic sequencing (exons 2-11). Tubal epithelia from one case was established in tissue culture.

Results: Tubal epithelium from all three subjects contained abundant (10-20 per section) p53 signatures with evidence of DNA damage and low proliferative activity. 6/11 LFS microdissected p53 signatures (55%) and 15/21 serous carcinomas (71%) revealed LOH at the *p53* locus. Growth i*n vitro* of the LFS tubal epithelium revealed a population relative to control tubes with unique morphology that could be identified by nuclear localization of p53 protein.

Conclusions: The LFS model confirms that the fimbria are prone to *p53* gene inactivation in the absence of malignancy and that loss of p53 function via LOH occurs frequently in the fimbria and in the setting of a prior (or inborn) mutation. This supports a model of carcinogenesis in which an initial occult event (p53 mutation) is at significant risk for a followup genetic "hit" (LOH) in a specific target area (fimbria). Established cell cultures of the LFS salpingeal epithelium – specifically of those with the properties of p53 signatures - offer the opportunity to construct an *in vitro* model of sequential carcinogenic events.

1207 Ovarian Cancer Risk Assessment Via Expression Profiling of the Distal Fallopian Tube Epithelium

W Xian, KK Mehra, V Vathipadiekal, SW Tay, M Birrer, CP Crum. Brigham and Women's Hospital, Boston, MA; Massachusetts General Hospital, Boston, MA; Institute of Molecular Biology, Singapore, Singapore.

Background: Risk of ovarian cancer is associated with inherited mutations in the BRCA1 or BRCA2 genes; however, this group accounts for only 10% of the ovarian cancer population and the factors that would distinguish the remaining 90% of women are not known. Recently, the distal fallopian tube has been proposed as a site of origin for a significant subset of ovarian cancers, specifically high grade serous carcinomas, and a serous carcinogenic sequence has been described in this site. This study addressed the hypothesis that the *benign appearing* distal fallopian tube epithelium of women with high grade serous carcinomas is fundamentally different from the background population of lower risk individuals.

Design: Ten cases each of high-grade pelvic serous carcinoma and benign disorders (ovarian fibroma, mucinous cystadenoma) were selected. Frozen fimbrial sections from each case were sectioned and RNA isolated by laser capture microdissection. RNA was amplified, hybridized to Affymetrix U133 genechips and the resulting data resolved by Partek Genomics Suite software and Ingenuity Pathway Analysis software.

Results: A supervised comparison using Partek software identified 558 genes expressed at more than or equal to 2 fold different levels (P<0.05) in benign fimbrial epithelium associated with high-grade pelvic serous carcinoma vs benign disorders. Moreover, analysis revealed important pathways that might cooperate with the p53 pathway to regulate serous carcinoma development and progression. Currently, pathway specific PCR array is being used to validate this finding in larger pool of samples and will be discussed.

Conclusions: This study has revealed, for the first time, that differences in gene expression might distinguish normal distal fallopian tubes from women with serous cancer and controls. This approach offers the potential for identifying unique gene pathways that can be employed, singly or in aggregate, to identify greater ovarian cancer risk and underscores the distal fallopian tube as a potential surrogate for ovarian cancer risk assessment.

1208 Overexpression of Clusterin Is Predictive of Aggressive Biology of Primary Ovarian Cancer

GF Yang, D Xie, Y Liu, YR Lia, WP He, B Fu, CY Fan. The First Affiliated Hospital, Sun Yat-Sen Univ Med Sciences, Guangzhou, China; Cancer Center, Sun Yat-Sen Univ Med Sciences, Guangzhou, China; Guangdong Specialty Hospital of Fertility, Guangzhou, China; Xu Wen Hospitail of Traditional Chinese Medicine, Zhanjiang, Guangdong, China; John L. McClellan Memorial Veterans Hospital and UAMS, Little Rock, AR.

Background: Clusterin, a multifunctional glycoprotein, is ubiquitously produced in mammalian tissues. Clusterin has been shown to play significant roles in many aspects of human tumor biology, such as cell proliferation, apoptosis, chemoresistance and angiogenesis. While clusterin is shown to be overexpressed in many types of human malignant tumors, its exact roles in the biologic behavior in ovarian epithelial cancer have not been fully investigated.

Design: Immunohistochemical (IHC) staining for clusterin was performed on a Tissue Microarray (TMA) containing 181 primary ovarian epithelial cancers. 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). The levels of clusterin expression was scored based on staining intensity and percentage of immunopositive cells and were correlated with various clinicopathologic parameters by Chi-square test.

Results: High levels of clusterin protein were seen in 102 of 181 (56%) ovarian epithelial carcinoma. Overexpressed clusterin was significantly correlated with the histologic type of serous carcinoma ($p = 0.02$), high-grade histology ($p = 0.002$), and high FIGO stages ($p = 0.05$).

Conclusions: Overexpression of clusterin gene is commonly detected in primary ovarian cancer and can be used to predict more aggressive biologic behavior of these malignant tumors.

1209 Pax8 Expression in Uterine Adenocarcinoma: Immunohistochemical Analysis of 94 Cases

A Yemelyanova, AM Gown, BM Ronnett, R Vang. Johns Hopkins University, Baltimore, MD; PhenoPath Laboratories, Seattle, WA.

Background: Pax8 has recently emerged as a potentially useful immunohistochemical marker for the diagnosis of gynecologic tract malignancies. The few studies published to date, however, have focused only on tumors in the ovary and fallopian tube. The goal of this study is to evaluate Pax8 expression in various types of uterine adenocarcinomas.

Design: Ninety four cases were studied including 21 HPV-related endocervical carcinomas, 21 endometrial serous carcinomas, and 52 endometrial endometrioid adenocarcinomas (17- FIGO grade 1, 22- FIGO grade 2, 13- FIGO grade 3). Immunohistochemical staining was performed with Anti-PAX8 rabbit polyclonal antibody from Proteintech Group, Inc. (Chicago, IL).

Results: All groups demonstrated high variability in extent of Pax8 expression. Serous carcinoma displayed highest level of expression with the mean/median percent of positive cells - 73%/80% (range 0-100%), followed by endometrial endometrioid adenocarcinoma (mean/median – 55%/60%, range 0-100%). The lowest level of expression was observed in endocervical adenocarcinoma (mean/median 28%/20%, range 0-90%).

Table 1. Pax8 expression in uterine adenocarcinomas

Histologic type	N	Staining intensity	Extent of staining				
		(mean)	**0**	**1+**	**2+**	**3+**	**4+**
Endocervical adenocarcinoma	21	1.5	3 (14%)	9 (42%)	5 (24%)	3 (14%)	1 (5%)
Serous carcinoma	21	2.5	1 (5%)	1 (5%)	2 (10%)	5 (24%)	12 (57%)
Endometrioid carcinoma	52	1.5	2 (4%)	5 (10%)	17 (33%)	15 (29%)	13 (25%)
FIGO grade 1	17	2.1	0	2 (12%)	4 (24%)	4(24%)	7 (41%)
FIGO grade 2	22	1.7	2 (9%)	1 (5%)	5 (22%)	9 (41%)	5 (22%)
FIGO grade 3	13	2.0	0	2 (15%)	8 (62%)	2 (15%)	1 (8%)
Total	94	-	6 (6%)	15 (16%)	24 (26%)	23 (24%)	26 (28%)

Staining intensity: 0 – no staining; 1 – weak; 2 – moderate; 3 – strong. Extent of staining based on % of positive cells: 0 - negative (0%); 1+ - 1-25%; 2+ - 26-50%; 3+ - 51-75%; 4+ - 76-100%.

Conclusions: Pax8 is expressed in the vast majority of uterine adenocarcinomas, including both those of endometrial and endocervical origin. The highest level of pax8 expression is observed in endometrial serous carcinoma; the lowest level - in endocervical adenocarcinoma. This high prevalence of immunopositivity precludes use of this marker for distinguishing the various kinds of uterine adenocarcinomas (or various grades of endometrioid carcinoma) from one another. In extrauterine sites, Pax8 can serve as a marker to distinguish metastatic uterine adenocarcinomas from non-gynecologic adenocarcinomas.

1210 Immunohistochemical Profile of Endometrial Clear Cell Carcinoma

A Yemelyanova, R Vang, BM Ronnett. Johns Hopkins University, Baltimore, MD.

Background: Clear cell carcinoma (CCC) of the endometrium is uncommon (~5% of endometrial cancers), yet is not infrequently considered in the differential diagnosis of more common endometrioid and serous carcinomas when cells with clear cytoplasm or hobnail cells are present. These latter changes can reflect squamous and metaplastic-type differentiation in endometrioid carcinomas which are typically low-grade tumors, so identification of a true high-grade CCC component is important. The goal of this study is to characterize the immunoprofile of CCC for markers potentially useful to distinguish CCCs from other types of endometrial carcinomas.

Design: Immunohistochemical analysis of a panel of markers, including p53, p16, Pax8, HNF1B, WT-1, estrogen (ER) and progesterone receptors (PR), HSD3B1, and Ki-67 was performed on 20 cases of CCC (13 pure and 7 mixed tumors [3 with serous and

4 with endometrioid components]). For mixed tumors, expression was assessed in the CCC component. Percentage of positive cells was estimated (nearest 10%).

Results:

Table 1. Immunohistochemical Profile of Endometrial CCC

Marker (# cases)	Number of negative cases	Number of positive cases	% positive cells (in positive cases)		Staining intensity
			Mean/Median	Range	
Pax8 (n=19)	1 (5%)	18 (95%)	84/100	40-100	Moderate to strong
HNF1B (n=18)	3 (17%)	15 (83%)	79/90	30-100	Moderate to strong
HSD3B1 (n=18)	3 (17%)	15 (83%)	79/90	30-100	Weak to strong
P16 (n=20)	1 (5%)	19 (95%)	52/40	10-100	Weak to strong
P53 (n=20)	6 (30%)	14 (70%)	60/70	10-100	Weak to strong
ER (n=20)	15 (75%)	5 (25%)	58/80	20-80	Weak to moderate
PR (n=20)	17 (85%)	3 (15%)	40/30	10-80	Moderate
WT-1 (n=19)	19 (100%)	0	-	-	-
Ki-67 (n=19)	-	-	42/40	10-70	-

Conclusions: CCC is characterized by high frequencies and extent of expression of Pax8, HNF1B, and HSD3B1; p53 and p16 expression are also common but with more variable extent and intensity. Hormone receptor and WT-1 expression are infrequent or absent. Proliferative activity is variable. HNF1B and HSD3B1 have the potential to distinguish CCCs from other endometrial carcinomas, provided formal analysis of the latter demonstrates limited or absent expression in the other types. Variable expression of p53 and p16, most often overlapping with patterns seen in endometrioid carcinomas but occasionally sharing the pattern seen in serous carcinomas, limits the utility of these markers.

1211 Histologic Effects of Short Term Progestin Therapy on Endometrioid Adenocarcinoma

RJ Zaino, WE Brady, W Todd, K Leslie, E Fischer, H Ball. Hershey Med Ctr, Hershey; GOG, Buffalo; U Iowa Med Ctr, Iowa City; UNM, Albuquerque; U Mass Med Ctr, Worcester.

Background: Some recurrent endometrial carcinomas respond to hormonal therapy, but prediction of response is imperfect, its duration is usually short, and the mechanism is unknown. We wished to determine the efficacy of progestins to induce a histologic response in endometrioid carcinomas and explore its effects on immunohistochemical measures of growth and apoptosis.

Design: The Gynecologic Oncology Group initiated a study of 75 women with endometrioid endometrial adenocarcinoma, 60 of whom received medroxyprogesterone acetate for 21-24 days immediately prior to hysterectomy and have available slides. Initial biopsies and hysterectomies were H&E stained and immunostained for estrogen receptor (ER) and progesterone receptor (PR) (38 cases), Bcl-2, Mib-1, and cleaved caspase-3 (Casp3) (56 cases). Histologic response, including regression of characteristics of neoplasia, and presence of metaplasia, secretion, and decidual change was assessed, and semi-quantitative scores (0-3+) of immunohistologic variables of initial biopsies were compared to post treatment slides.

Results: Only 1 complete histologic response was seen, but 37 tumors had a partial response (more often in tumors of initial lower grade, $p<0.05$), reflected by increased eosinophilic cytoplasm and luminal secretion. Decidual change was sometimes prominent but limited to the stroma surrounding benign glands. About 90% of tumors were initially ER and PR positive. ER and PR were significantly down-regulated as was Mib-1. Although Bcl-2 decreased following therapy, Casp3 did not change significantly.

Table 1 immunohistochemical response

parameter	pre Rx (mean)	post Rx (mean)	significance
ER	2.25	1.62	p<0.05
PR	1.94	0.79	p<0.05
Mib-1 (nucl/HPF)	209	136	p<0.05
Bcl-2	0.60	0.24	p<0.05
Casp3	10.55	8.19	p=0.52

Conclusions: Short term progestin therapy induces partial histologic responses in most endometrioid adenocarcinomas, which is quantitatively and qualitatively different from that of benign endometrium. Based on observations at 21 days, the mechanism appears to reflect increased differentiation of tumor and a diminished growth rate rather than tumor cell death. Stromal decidulaization was confined to areas surrounding benign glands suggesting a paracrine effect. Down regulation of PR by the progestin may limit its efficacy and duration of response.

1212 IMP2, an Adjunct Marker in the Diagnosis and Grading of Cervical Intraepithelial Neoplasia

L Zhang, Y Liu, D Lu. University of Massachusetts, Worcester, MA.

Background: Accurate diagnosis and grading of cervical intraepithelial neoplasia (CIN) play a key role in the cervical cancer prevention. But it has met with difficulty and inconsistency in pathology practice. Biomarkers, such as p16 and ki-67, have shown to be very helpful. Here we present data of a new biomarker, IMP2 (insulin-like growth factor II mRNA-binding protein 2), and its immunohistochemical stain in CIN lesions. IMP2 is a member of family of three components, IMP1, 2, and 3. In this article, we demonstrate that IMP2 staining identifies CIN, and the staining pattern correlates with CIN's grade.

Design: The total 254 biopsies are selected from the specimen database at Department of Pathology, University of Massachusetts between 1997 and 1998, including benign (n=76), CIN-1 (n=25), CIN-2 (n=43), and CIN-3 (n=186). All cases had follow up LEEP or hysterectomy to confirm the diagnoses. The conventional H&E and IMP2 immunohistochemical stains are performed on consecutive tissue sections. The staining method is used routinely in our lab and published elsewhere. Positive is defined as dark brown cytoplasmic staining and the patterns are defined in concordance with CIN grading. Scant fine granular background staining, or no staining at all is considered negative.

Results: 1. IMP2 immunohistochemical stains of benign and CIN lesions:

Figure 1. IMP2 immunohistochemical stains of benign and CIN lesions

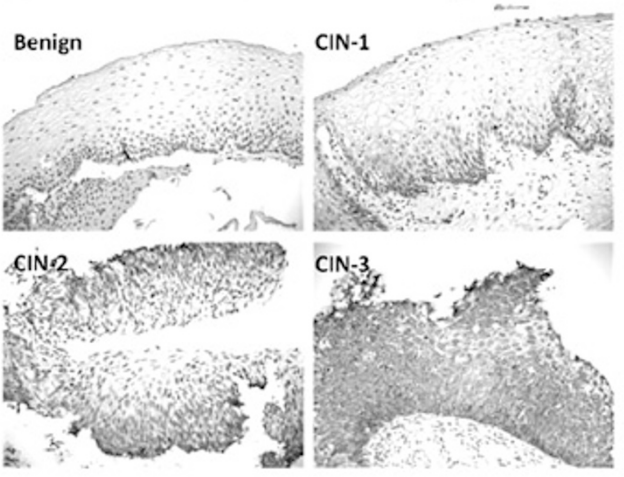


Figure 1 demonstrate the different IMP2 staining patterns in benign, CIN-1, -2 and -3 lesions. 2. IMP2 staining pattern is adjunct tool in CIN grading:

Figure 2. IMP2 staining pattern is adjunct tool in CIN grading

Pathology diagnosis and # of cases	IMP2 staining pattern			
	Positive	<1/3	<2/3	>2/3
Benign: 76	1	1	0	0
CIN-1: 25	25	25	0	0
CIN-2:43	43	7	36	0
CIN-3: 186	186	0	17	169

Figure 2 illustrate the positivity of IMP2 staining in CIN lesions (in comparision to benign lesions); it also illustrate the positive correlation between the IMP2 staining and the patterns of dysplasia among different CIN lesions.

Conclusions: Our study indicates positive IMP2 stain identifies CIN. Its staining pattern correlates with CIN's grades. IMP2 can be a useful adjunct in diagnosis and grading of CIN lesions.

1213 IMP2, an Adjunct Marker That Differentiates Endometrial Serous from Endometrioid Carcinomas

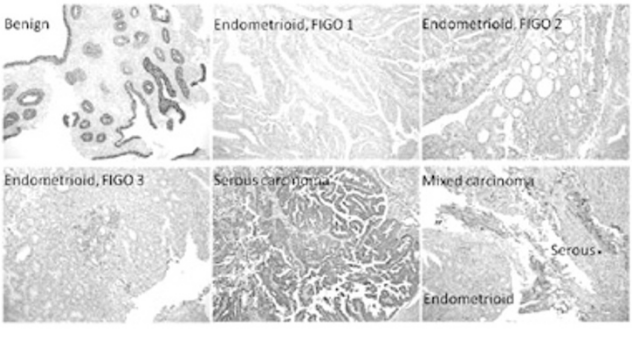
L Zhang, Y Liu, D Lu. University of Massachusetts, Worcester, MA.

Background: Differential diagnosis of endometrial serous from endometrioid carcinoma can be difficult especially when the endometrioid carcinoma is poorly differentiated. Accurate diagnosis however is important since their clinical course can be different and serous carcinoma may receive chemotherapy in addition. Biomarker p53, has shown to be very helpful. Here we present data of a new biomarker, IMP2, and its immunohistochemical stain for such differential diagnosis. IMP2, insulin-like growth factor II (IGF-II) mRNA-binding protein 2, is a member of family of three components, IMP1, 2, and 3. In this article, we demonstrate that IMP2 staining is always diffuse and strong in serous carcinomas, but negative, or focal in endometrioid carcinomas.

Design: The total 320 cases are selected from the specimen database at Department of Pathology, University of Massachusetts between 1997 and 2002, including benign endometrium (n-93), endometrioid carcinoma (n=178), serous carcinoma (n=27), and mixed endometrioid and serous carcinoma (n=22). The pure endometrioid carcinomas are divided into FIGO grade 1 (n=89), grade 2 (n=57), and grade 3 (n=32). For the study, the conventional H&E and IMP2 immunohistochemical stains are performed on consecutive tissue sections. The staining method is those used routinely in our lab and published elsewhere. Positive staining was defined as dark brown cytoplasmic staining pattern. Scant fine granular background staining, or no staining at all was considered negative.

Results: 1. IMP2 immunohistochemical stains of benign, endometrioid and serous carcinomas:

Figure 1. IMP2 stains of benign, endometrioid and serous carcinomas



2. IMP2 staining differentiates serous from endometrioid carcinomas:

Figure 2. IMP2 differentiates serous from endometrioid carcinoma

Pathology diagnosis	IMP2 staining pattern				
# of cases	<5%	<25%	<50%	<90%	>90%
Benign: 93	0	0	0	0	93
Endometrioid					
FIGO-1: 89	54	27	8	0	0
FIGO-2: 57	3	28	26	0	0
FIGO-3: 32	0	8	21	3	0
Serous: 27	0	0	0	0	27
Mixed: 22					
Endomotrioid: 22	0	16	5	1	0
Serous: 22	0	0	0	0	22

Conclusions: Our study indicates serous carcinomas are diffusely and strongly IMP2 positive. Endometrioid carcinomas however are always partially positive or negative. IMP2 staining pattern therefore can differentiate these two carcinomas.

1214 Cytological Results and Clinical Findings Associated with 265 Histopathological Diagnoses of Cervical Glandular Neoplasia: Results of 10 Years

C Zhao, RM Austin. Magee Womens Hospital, UPMC, Pittsburgh.

Background: The effects of population screening on cervical glandular neoplasia (CGN) have been limited. The incidence of invasive cervical adenocarcinoma (ICA) has continued to increase during the same period an accelerated decline in cervical squamous carcinoma has been documented, both trends coinciding with the widespread introduction of liquid-based cytology.

Design: A computer-based search of our databases was conducted over a study period of almost 10 years between January 2000 and September 2009 to identify the cases diagnosed surgically as cervical AIS, ICA, or invasive cervical adenosquamous carcinoma. Pathological findings, clinical history, and previous Pap test results were documented.

Results: 265 patients were identified with cervical glandular neoplasia from our databases, including 81 cases of ICA, 17 cases of invasive adenosquamous carcinoma, and 167 cases of AIS. Of these cases, 60% had associated CIN (126 CIN2/3, 34 CIN1).Among 98 women with invasive carcinoma, 85 (86.7%) had associated AIS in histology. The Pap tests or clinical findings leading directly to histologc diagnoses of CGN included 105 (39.6%) AGC/AEC/AIS, 35 (13.2%) HSIL, 16 (6.0%) LSIL, 29 ASC (10.9%), 20 (7.6%) AGC/SIL, 8 (3.0%)AGC/ASC-H, 44 (16.6%) unknown, and 9 (3.4%) with clinical symptom/sign. 23 patients with CIN2/3 cervical biopsy results had AIS diagnosed on subsequent LEEP/Cone biopsies. 14 women with a Pap test history of AGC had initially negative ECC follow-up, not accompanied by cervical biopsies, resulting in delayed diagnoses of CGN. High percentage of women had abnormal and normal Pap test history.

Pap Test History Preceding Abnormal Pap Tests Directly Resulting in Histopathological Diagnoses of Cervical Glandular Neoplasia

Time interval preceding histologic dx	No. Patients	At least one abnormal Pap test (%)	At least one normal Pap test (%)	Both normal and abnormal Pap tests (%)	AGC Pap test (%)
0-1 yr	80	26 (32.5)	54 (67.5)	0	11 (13.8)
>1-3 yr	114	41 (36.0)	96 (84.2)	23 (20.2)	18 (15.8)
>3-5 yr	121	46 (38)	109 (90.1)	34 (28.1)	20 (16.5)

Conclusions: Early diagnosis of cervical glandular neoplasia remains challenging. Preceding negative cytological and histologic results reflect the special challenges associated with both endocervical cytological sampling and with identification and sampling of CGN during colposcopic examinations. Women with AGC Pap should remain classified as high risk patients even after negative/benign histologic follow-up results.

Head & Neck

1215 Salivary Mucoepidermoid Carcinoma: Clinicopathologic Study of 53 Patients

E Adler, C Sarta, RV Smith, R Owen, J Smith, B Schiff, D Gitler, M Brandwein-Gensler. Montefiore Medical Center, Bronx, NY.

Background: Mucoepidermoid carcinoma (MEC) is one of the most common salivary malignancies. We previously published a grading system which modified the AFIP schema by adding the variables of tumor pattern of invasion, lymphovascular invasion, bony invasion and increasing the weighted value for perineural invasion. Our goal is to 1) review the clinicopathologic features of a new cohort and 2) compare the two grading systems.

Design: We searched MMC pathology files (1978-2009) and reviewed cases diagnosed as "MEC". Tumors were graded according to both published criteria. Charts were reviewed for tumor site, stage, treatment, and outcome.

Results: We reviewed 67 tumors; 14 were excluded after reclassification as squamous carcinoma (SCC) (3), high-grade salivary duct carcinoma (HGSDC) (3), low-grade salivary duct carcinoma (1), carcinoma-ex-BMT (1), sebaceous carcinoma (1), carcinoma-not-otherwise-specified (3), cystadenoma (1), and MEC metaplasia in BMT (1). The diagnosis of MEC was confirmed on 53 tumors. Ages ranged from 18-77 (mean 50), female: male ratio was 3:1. Most common sites were parotid (55%), oral cavity (32%), and submandibular gland (9%). Unusual presentations in 2 patients merit mention. One grade I MEC was diagnosed as a benign cyst. This tumor was inadeqately excised and its persistence became manifest 9 years later. This woman is disease-free 3 years after definitive surgery. One woman with grade II parotid MEC presented with a draining cutaneous fistula. The frequencies of grades I, II, & III MEC by our criteria are 36%, 34%, & 30%, respectively. Our criteria upgraded 30% of MEC: 20% from grades I to II, 8% from grades II to III, and 2% from grades I to III. Information on outcome was available on 35 patients. Two patients (grade III, both schemas) died of disease at 12 and 16 months, respectively. One patient is alive with persistent disease. Thirty two patients are disease-free (mean 44 months).

Conclusions: MEC is usually associated with good outcome when appropriately treated. The diagnosis of MEC is unlikely for tumors with extreme pleomorphism, abundant keratinization, or hyalinization. SCC and HGSDC comprised the most common tumors misdiagnosed as MEC. Our proposed grading criteria upgraded 30% of MEC compared to the AFIP criteria, however the limited number of disease-progression events did not allow for comparison of the performance of the two grading classifications.

1216 Expression of the GLUT1 Glucose Transporter in Adenoid Cystic Carcinomas Transformed into Adenocarcinoma or Undifferentiated Carcinoma

A Altemani, VL Bonfitto, AP Demasi, AF Costa, JF Bonfitto, VC Araujo. University of Campinas (UNICAMP), Campinas, São Paulo, Brazil; CPO São Leopoldo Mandic, Campinas, São Paulo, Brazil.

Background: In neoplasia, enhanced expression of GLUT1 has been interpreted as increased glucose uptake as well as response to tissue hypoxia. The latter has been implicated in the pathogenesis of dedifferentiated phenotype in certain types of cancer. GLUT1 expression has also been reported to correlate with poor prognosis, tumor aggressiveness and lymph node metastases. Transformation of adenoid cystic carcinoma to adenocarcinoma or undifferentiated carcinoma (ACC-Ad/UC) has been linked to accelerated clinical course and high propensity for lymph node metastases. In order to analyze whether a) hypoxia could be involved in the pathogenesis of ACC-Ad/UC and b) GLUT1 expression could be used as a predictor of clinical outcome, we looked at the expression of this marker in conventional and transformed areas of ACC-Ad/UC.

Design: In six cases of ACC-Ad/UC and in 18 ordinary ACC the immunohistochemical expression of GLUT1 was assessed using a three-tiered scale: >10% - 25%, and > 25- 50% and >50% of positive cells. α-SMA was used for detection of myoepithelial cells and the proliferation index was evaluated by Ki-67. Demographic and clinical information was obtained from the patients' medical records.

Results: The transformed areas were classified according to histological patterns as adenocarcinoma in 4 cases and solid undifferentiated carcinoma in 2. Loss of myoepithelial layer was found only in the transformed component, which also showed higher Ki-67 index. In ACC-Ad/UC, only one patient died of disease and presented lymph node metastases. Three did not show recurrence (median follow-up 54 months), including one long-term survivor (131 months). Both conventional areas of ACC-Ad/UC and ordinary ACC were negative for GLUT1 in most cases (83.3% and 81.3%, respectively), whereas the Ad/UC component presented increased expression of GLUT1+ in 50% of cases. However, the degree of GLUT1 expression correlated neither with clinical outcome nor with the histological subtype of the transformed component.

Conclusions: The scanty expression of GLUT1 in conventional areas of ACC-Ad\UC as well as in ordinary ACC suggests that hypoxia may not play a crucial role in the development of the Ad/UC phenotype. The enhanced metabolic demand leading to increased glucose uptake could explain higher GLUT1 expression in the Ad/UC areas, but GLUT1 cannot be considered useful as prognostic marker.

1217 Does Tall Cell Histology Affect the Clinical Behavior of Papillary Thyroid Microcarcinoma?

ZW Baloch, K Shafique, KT Montone, VA LiVolsi. UPENN Medical Center, Philadelphia, PA.

Background: Papillary thyroid microcarcinoma (PTMC) is an indolent tumor with favorable long term prognosis, however, recurrences in the neck and distant metastases have been reported. Tall cell variant of papillary thyroid carcinoma (TCV-PTC) is an aggressive variant of papillary thyroid, which can show increased propensity towards lymph node metastases, tumor recurrences and distant metastasis as compared to classic variant of papillary thyroid carcinoma. In this study we evaluated the effect of tall cell histology on the biologic behavior of PTMC.

Design: The computerized pathology files at our institution were searched for cases diagnosed as papillary thyroid carcinoma and TCV-PTC. Fourteen cases of PTMC with tall cell histology (PTMC-TCV) were identified. Clinicopathologic data and follow-up (serum thyroglobulin measurements, radiologic studies and/or additional tissue sampling) through present date were extracted from medical records and was compared with similar data from 10cases of PTMC classic variant (PTMC-CV) and 16 cases of PTMC follicular variant (PTMC-FV).

Results: PTMC-TCV presented as a single focus in 8 (57%) and as multiple foci in 6 (43%) cases(size range 0.2-1.0 cm), whereas PTMC-CV and PTMC-FV presented as single focus in 7 and 13 and as multiple foci in 3 cases each respectively (size range 0.2-1.0 cm). A higher rate of lymphovascular invasion(LVI), extrathyroidal extension (ETE) and lymph node metastases (LN-mets) was seen in PTMC-TCV as compared to PTMC-CV and PTMC-FV; LVI 28% vs. 10% and 6%, ETE 50% vs. 10% and 0% and LN-mets 28%% vs. 10% and 6%. Regional tumor recurrence was seen in 5 (5/14 36%) cases of PTMC-TCV, in 1 (1/10 10%) of PTMC-CV and in 1 (1/16-6%) case of PTMC-FV. Distant metastases were not seen in any three variants of PTMC at an average mean follow-up of 9-years.

Conclusions: In our experience, tall cell histology portends a more aggressive clinical course in PTMC cases. Therefore, this should be mentioned in the pathology report as these cases may require close clinical follow-up as compared to the cases of PTMC-CV and PTMC-FV.