LI
LABORATORY INVESTIGATION
THE BASIC AND TRANSLATIONAL PATHOLOGY RESEARCH JOURNAL
VOLUME 98 | SUPPLEMENT 1 | MARCH 2018

USCAP 2018

ABSTRACTS

GYNECOLOGIC AND OBSTETRIC PATHOLOGY
(1127-1316)

107TH ANNUAL MEETING
GEARED TO LEARN

MARCH 17-23, 2018
Vancouver Convention Centre
Vancouver, BC, Canada

Published by
SPRINGER NATURE
www.ModernPathology.org

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AN OFFICIAL JOURNAL OF THE UNITED STATES AND CANADIAN ACADEMY OF PATHOLOGY

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1127 Does Loss of Heterozygosity Impact Prognosis in Uterine Serous Carcinoma?

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Background: While uterine serous carcinomas (USCs) are morphologically and immunophenotypically indistinguishable from their ovarian counterparts, questions remain on whether these tumors share similar molecular profile and therefore could benefit from similar therapies. Somatic and germline defects in genes of the homologous recombinant(HR) pathway(BRCA1, BRCA2 and others) have been implicated in ovarian cancer predisposition and are associated with high sensitivity to platinum-based and targeted therapies(PARP inhibitors). HR deficiency impairs normal DNA repair resulting in loss or duplication of chromosomal regions, termed genomic loss of heterozygosity(LOH). Whether this phenomenon applies to USCs is yet to be determined. In this study, we analyzed genome-wide LOH in USCs and correlated its extent with clinicopathological parameters and survival outcomes.

Design: 69 platinum treated USC patients diagnosed between 1998-2015 were included. DNA was isolated from FFPE and LOH analysis was performed querying over 220,000 SNPS using the Affymetrix OncoScan. Selecting the top quartile of LOH distribution as a cut point to separate patients into LOH "high" and LOH "low" groups, data was analyzed using KM survival analyses.

Results: Patients' ages ranged from 45 - 89(median 68) yrs. Stage distribution included: 11.5% I, 1.5% II, 62% III and 25% IV. Lymphadenectomy was performed in 74% and omentectomy in 62% of patients. 80% of patients received adjuvant radiotherapy. Median LOH was 1.4% (range 0-30.1%) and the top quartile threshold was 9.9%. 52 tumors showed LOH lower than the top quartile. In the top quartile of LOH, tumors were more common in African Americans(83% vs 56%), advanced stage(100% vs 82%) and more commonly associated with outer half MI(76.5% vs 47%) and LVI(88% vs 65%) when compared to tumors with LOH lower than the top quartile. In general, LOH did not significantly correlate with age(p=0.41), race(p=0.43), tumor size(p=0.72), stage(p=0.36), MI(p=0.52), LVI(p=0.40), LN metastases(p=0.43), or recurrence(p=0.34). KM analysis showed no survival benefit nor prolonged DFI for platinum treated patients with LOH in the top quartile compared to LOH lower than that.

Conclusions: In our cohort of USCs, median LOH was 1.4%, with 25% of tumors showing genomic LOH higher than 9.9%. In contrast to their ovarian counterparts, a high degree of genomic LOH does not correlate with survival benefit for platinum treated USC patients, suggesting that these tumors may have distinct biology and genomic profile.

1128 Molecular Classification of Endometrial Carcinoma Applied to Endometrial Biopsy Specimens: Towards Early Personalized Patient Management

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Background: The current risk stratification systems used to guide management of endometrial cancer(EC) are based on post surgical pathological information. However, pathologists are unable to reproducibly diagnose histotype and grade, hence the need for more reliable classification systems. TCGA recently identified four prognostically significant EC subtypes which subsequently proved reproducible using clinically applicable surrogate tests. Using these tests, we sought to determine the level of concordance between endometrial biopsies(Bx) and subsequent hysterectomy specimens(HS) in assessing the molecular classification of EC.

Design: 50 Bx with corresponding HS for EC patients(pts) were collected. Additionally, 10 cases of Bx proven complex atypical hyperplasia(CAH) and found to have EC on resection were included. DNA was isolated from FFPE. MSI analysis was performed with Promega Analysis System. MSI was interpreted as stable, low or high. IHC for mismatch repair(MMR) proteins and P53 was performed. MMR status was interpreted based on current protocol. P53 was abnormal(abn) if there was complete negative or diffuse expression in tumor cells. Sanger sequencing was performed to detect mutations in exons 9 and 13 of POLE gene. Level of concordance for tumor grade, histotype, IHC and molecular profile in both specimens was determined using kappa estimates. Kappa statistics of 0.86(95%CI) is consistent as "near perfect" level of agreement.

Results: Pts' ages ranged from 27-85(median 60)yrs. All pts underwent definite surgical treatment. Bx included 65% endometrioid carcinoma(EEC), 17% CAH, 15% serous carcinoma(SC), 1.5% clear cell and 1.5% mixed carcinoma. High level of concordance was achieved for MMR-loss; MLH1[1.0(0.0-7.74)], PMS2[0.91(0.61-7.08)], MSH2[1.0(0.0-7.74)] and MSH6[0.83(0.79-6.49)], MSI high[0.91(0.63-6.86)], P53 wild[1.0(0.0-7.74)] and abn[1.0(0.0-7.74)] types. In contrast, grade and histotype showed only moderate levels of agreement. Highest level of discrepancy in morphology was between SC and FIGO grade 3 EEC. POLE gene mutation was detected in 2 pts (3.5% of entire cohort and 14% of FIGO grade 3 EEC). For both pts, mutations were detected only in HS. When comparing CAH with subsequent HS tumor, the profile was identical to that of EC.

Conclusions: In our cohort of EC, high level of concordance was achieved between Bx and HS for IHC and molecular profile, superior to that of grade and histotype, providing earlier and more reliable prognostic information to inform management.

1129 Micropapillary Carcinoma of the Uterine Cervix. A Clinicopathologic Study of 25 Cases

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Background: Micropapillary carcinoma is a rare variant of endocervical adenocarcinoma. All nine cases reported in the literature have been associated with pelvic or para-aortic lymph node metastases. The behavior of this rare malignancy could be different from that of conventional endocervical adenocarcinoma. Hence, there is a need to highlight this histopathological entity.

The goal of this study is to analize the clinical, histopathologic findings and prognosis of micropapillary endocervical adenocarcinoma

Design: This was a retrospective multi-institutional study of patients who presented with endocervical adenocarcinoma (EAC) during the period from January 1, 2000 to December 31, 2016. The study was restricted to cases in which the micropapillary component constituied at least 95% of the tumor. Clinical data, including postoperative follow-up, were obtained from the medical records.

Results: Twenty-five cases of endocervical adenocarcinoma (EAC) containing a micropapillary component (MPC) are present. The patients´ mean age was 64 years (range, 49-84 years). Nineteen patients had pelvic or para-aortic lymph node metastases, each with a predominant (> or: 50%) MPC in the metastases; local recurrence was documented in 8 cases. Vascular-lymphatic invasion was consistently present. The cytologic features of the MPC were those of grade 3 EAC in 16 cases. A concurrent endocervical adenocarcinoma was identified in 12 cases. A total of 7(28%) patients were stage I, 11(44%) were stage II and 7 (28%) stage III. The mean tumor size was 3.61 cm (2-5cm). Depth of invasion ranged from 10 to 34mm (mean: 11.3mm). Tumor involvement in the lower uterine segment was present in 11 cases. Follow-up data (mean, 42.3 months; range 9-79 months) indicated that six patients were alive with disease, nine patients were dead of disease, and there was no evidence of disease in 10 patients.

Conclusions: 1. Micropapillary EAC is linked to higher frequencies of lympho-vascular invasion and lymph node metastases.

2. Micropapillary component associated to EAC could represent a poor prognostic histologic feature.

1130 Does Ki-67 Have a Role in the Diagnosis of Placental Molar Disease?

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Background: The use of p57 immunohistochemistry (IHC) can distinguish complete mole (CM) from partial mole (PM) and non-molar abortus (NMA). Molecular Genotyping (MG) is the gold standard method for the definitive diagnosis of PM and NMA. However, MG is expensive and not always available. Some data suggest Ki-67 IHC may be helpful in distinguishing NMAs from PMs and could be a substitute for MG. In this study, we examined the utility of p57 and Ki-67 IHC stains in the diagnosis of placental molar disease.

Design: The study cohort consisted of 60 cases of products of conception (20 CMs, 20 PMs and 20 NMAs). All CM cases showed absent (<10%) p57 IHC in chorionic villi. All PM and NMA cases had been subjected to MG and showed diandric triploid or biparental inheritance, respectively. Ki-67 and p57 IHC staining was done on formalin-fixed paraffin embedded sections from all 60 cases. Both

IHC stains were interpreted blinded to the diagnosis. On re-review, we recorded the percentage of cells with nuclear p57 staining in villous cytotrophoblast and stromal cells. Ki-67 proliferative index (%) was determined by manual count of at least 500 villous cytotrophoblastic cells in areas with highest Ki-67 reactivity. Any intensity of nuclear staining was considered positive.

Results: All CM cases had absent villous staining for p57 and all PM and NMA cases had retained p57 staining. The mean percentage of Ki-67 expression was 89.8% for CMs, 29.2% for PMs, and 16.0% for NMAs. (P<0.01 for CMs to both PMs and NMAs, P = 0.058 PMs to NMAs). Statistical analysis was performed by Kruskal Wallis H Test then Mann-Whitney test with Bonferroni correction.

Conclusions: The utility of p57 IHC is mainly to exclude or confirm CM. Although there is a significantly higher Ki-67 expression in CMs in comparison to PMs and NMAs, this did not add diagnostic utility. PMs tend to have higher Ki-67 expression than NMAs; however, the difference is not statistically significant. Our data suggest that the use of p57 and Ki-67 IHC cannot reliably distinguish PMs from NMAs.

1131 Papillary Proliferation of Endometrium (PPE): Clinicopathological Study

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Background: Papillary proliferation of endometrium (PPE) is associated with a spectrum of conditions from benign to neoplastic process. The majority of PPE patients are postmenopausal and some are seen with hormone therapy. PPE is classified as simple (short, non-branching papillae, focal) or complex (more than 3 foci, crowded intracystic). Complex PPE is reported to have a significantly association with concurrent or subsequent premalignant lesions or carcinoma, for which terminology of "complex papillary hyperplasia" has been suggested.

Design: Twelve cases of PPE in endometrial samplings with sufficient follow-up data were investigated. Based on the clinical course and sebsequent biopsy/hysterectomy, these cases were divided into two groups; PPE with regression (4) and PPE with progression [8 including premalignant (3) and malignant (5) condition]. Clinical informations were collected and histologic features of PPE were evaluated on the index EMS (endometrial sampling). The results were compared between these two groups.

Results: As shown in the Table, there are no definitive clinical characteristic and histopathologic features for distinguishing PPE with regression and PPE with progression.

	Regression (N=4)	Progression (N=8)
Average age (years)	42.3	49.5
Hormone Therapy	4/4	4/8
Diffuse vs focal	3 vs 1	6 vs 2
Nuclear size (>40 micron)	1/4	4/8
Prominent nucleolus	2/4	5/8
Mitotic counts per 10 HPF	0 to 4	0 to 14
Metaplasia (+)	4/4	5/8
Cribriform/intracystic pattern	4/4	8/8

Conclusions: PPE can be seen in patients with a wide range of age distribution (29-67 years old) and often, but not always, associated with hormone therapy. There seem to be no clearly identifiable histologic indicators for predicting clinical behavior. Since a considerable proportion of patients (8/12, 67% in our series) show disease progression, close clinical follow up should be highly recommended.

1132 Novel PLAG1 Gene Rearrangement Distinguishes Uterine Myxoid Leiomyosarcoma from Other Uterine Myxoid Mesenchymal Tumors

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Background: Myxoid leiomyosarcoma (mLMS) is a rare LMS variant that may be more clinically aggressive than its spindle cell counterpart. Its recognition remains difficult due to histologic and immunophenotypic overlap with other myxoid mesenchymal tumors, particularly *ZC3H7B-BCOR* high-grade endometrial stromal sarcoma (HGESS) and *ALK*-positive inflammatory myofibroblastic tumor (IMT). Genetic alterations in mLMS are currently unknown. Identifying novel gene fusions in mLMS may aid in tumor classification.

Design: 19 mLMS with available formalin-fixed paraffin-embedded tumor material was identified by a pathology database search from 1994-2017. Tumor RNA was extracted after macrodissection and subjected to next-generation targeted RNA sequencing using the Archer Anchored Multiplex PCR technology. Break-apart fluorescence in situ hybridization (FISH) for *PLAG1*, *BCOR*, *BCORL1*, *HMGA2* and *ALK*, and BCOR, PLAG1 and ALK immunohistochemistry were performed in cases which failed or lacked gene fusion by sequencing.

Results: Sequencing was successful in 13 cases; 3 failed, and 3 were not sequenced due to suboptimal RNA quality. Novel *TRPS1-PLAG1* and *RAD51B-PLAG1* fusions were detected in 3 and 1 mLMS, respectively. *ZC3H7B-BCOR* and *FN1-ALK* fusions were identified in 3 and 1 tumors and reclassified as HGESS and IMT, respectively. No fusions were detected in 5 tumors by sequencing. No *PLAG1*, *HMGA2*, *ALK*, *BCOR* or *BCORL1* rearrangements were detected in the 5 fusion-negative and 6 Archer failure cases. Strong PLAG1 expression in >95% of tumor cells was confirmed in all 3 *PLAG1*-rearranged mLMS with available material and 4 other fusion-negative tumors. Diffuse BCOR expression was seen in 2 of 3 *ZC3H7B-BCOR* HGESS. ALK expression was absent in all 15 tumors tested.

Conclusions: Novel *PLAG1* rearrangements resulting in PLAG1 expression underpin approximately 25% of mLMS and may serve as a useful diagnostic biomarker. *ZC3H7B-BCOR* HGESS and IMT are often misdiagnosed as mLMS. A panel of PLAG1, ALK and BCOR immunohistochemistry along with targeted RNA sequencing and/or FISH is helpful in the classification of myxoid uterine mesenchymal tumors.

1133 High-grade Endometrioid Carcinoma of the Ovary, a Clinicopathological Study of 30 Cases

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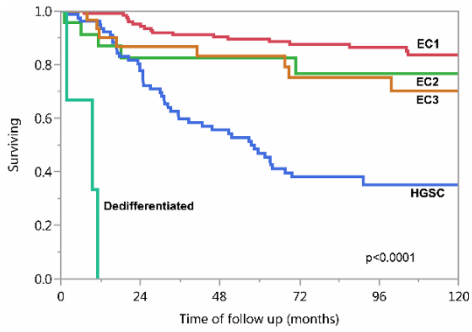
Background: Although infrequently encountered, the diagnosis of ovarian high-grade endometrioid carcinoma remains a diagnostic challenge with potential consequences for targeted therapies and genetic counselling. We studied the clinical and morphological features and the immunohistochemical profile of high-grade endometrioid ovarian carcinoma (EOC) as well as their diagnostic reproducibility compared to tubo-ovarian high-grade serous carcinomas (HGSC).

Design: Thirty cases confirmed as International Federation of Gynecology and Obstetrics (FIGO) grade 3 endometrioid carcinomas were identified from 207 EOCs diagnosed in Alberta, Canada between 1978 and 2010 from the population-based Alberta Ovarian Tumor Types cohort. Cases of lower grade endometrioid and HGSC served for comparison. 10 immunohistochemical markers were assessed on tissue microarrays. Clinical data were abstracted and survival analyses performed using Cox Regression. Interoberver reproducibility for histologic type was assessed using one representative hematoxylin and eosin stained slide from 25 randomly selected grade 3 EOCs and 25 HGSCs. Histotype was independently assigned by 5 pathologists initially blinded and subsequently unblinded to immunohistochemical WT1/p53 status.

Results: Patients diagnosed with grade 3 EOC had a significantly longer survival compared to HGSC in univariate (HR=0.34, 95% CI=0.16-0.67, p=0.0012, Fig.1) but not multivariate analysis adjusted for age, stage, treatment center and residual tumor (HR=1.01, 95% CI=0.43-2.16, p=0.98). Grade 3 EOCs (N=30) were identical to grade 2 EOCs (N=23) with respect to survival in univariate analysis (HR=1.07, 95% CI=0.39-3.21, p=0.89) and immunohistochemical profile. Using histomorphology alone, interobserver agreement on the diagnosis of grade 3 EOC or HGSC was 78%, which significantly (p<0.0001) increased to 96% agreement with the knowledge of the WT1/p53 status.



Conclusions: Our data support the diagnostic value of WT1/p53 status in differentiating between grade 3 EOC and HGSC. However, grade 3 and grade 2 EOCs showed no differences in immunophenotype or clinical parameters. Our findings suggest a two-tier classification system for EOC may be more appropriate, with grades 2 and 3 endometrioid ovarian carcinoma combined into a single high grade category.

1134 Tumor Oncogenes and Tumor Suppressor Genes May Play a Role in Predicting Rapid Recurrence of Stage III High Grade Ovarian Serous Carcinoma

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Background: High-grade ovarian serous carcinoma (HGOSC) is an aggressive cancer with overwhelming poor prognosis. The majority of patients present with advanced disease, rapid recurrence and chemoresistance. Detection of prognostic molecular markers and/or therapeutic targets is extremely important to manage this deadly malignancy. In this study, using next generation sequencing (NGS), we aimed to compare the molecular patterns of primary versus metastatic HGOSC.

Design: Eight paired tumor tissue samples from four patients with FIGO stage III HGOSC with similar histology and comparable residual disease, and at least 5 year follow up, were selected through an archival database search. These patients were selected because two of them had rapid recurrence (within less than 18 months) and the other two remained disease free. Histology slides were independently reviewed by three pathologists for accurate categorization and selection of appropriate tumor samples. Macro-dissection of tissue samples was performed and total nucleic acid was extracted from the tumor tissue. Multiplex PCR amplification using the Ion AmpliSeq 50 gene Cancer Hotspot Panel v2 followed by emulsion PCR and NGS on the Ion S5 platform<.

Results: All the ovarian tumor pairs shared common genetic alterations supporting their metastatic origin from the ovary, in accord with their clinical presentation. All samples had TP53 mutations, as previously described in HGOSC. Samples from the primary HGOSC group that rapidly recurred (group 1) additionally contained EGFR and FGFR3 mutations, whereas samples from primary HGSOC group with no recurrence (group 2) did not. Additionally, tumor pairs from group 1 had a homozygous TP53 mutation while those obtained from group 2 were only heterozygous for TP53 mutation. Group 2 samples had APC and FBXW7 mutations.

Conclusions: Our data from this pilot study suggest that aggressive tumor behavior in HGOSC in patients within the same clinical stage may be associated with activating oncogenic driver mutations, such as EGFR and FGFR3. Additionally, TP53 homozygous mutational status may also play a role in rapid recurrence of disease. Whereas patients who responded well to chemotherapy and had no recurrence had only tumor suppressor gene mutations in TP53, APC and FBXW7 in both the primary and metastatic tumors. Analysis of a larger number of samples, perhaps with a larger panel of genes, may allow further molecular characterization of these clinically important findings.

1135 POLE Mutations in Clear Cell Endometrial Carcinoma

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Background: Endometrial clear cell carcinoma (ECCC) is a rare histotype of endometrial carcinomas without unique identified molecular alterations. Its molecular relation to other histotypes (i.e. endometrioid and serous) is controversial. Herein, we assessed the TCGA molecular subtype in the largest sample of ECCC to date.

Design: ECCC cases were collected from 11 institutions. Diagnosis was confirmed by morphological review assisted by immunohistochemical markers (Napsin A, ER). Mismatch repair proteins (PMS2 and MSH6) and p53 expression was assessed by immunohistochemistry (IHC) on tissue microarrays. DNA was extracted and subjected to targeted next-generation sequencing for *POLE, TP53, KRAS,* and *PIK3CA*. Pathogenicity of mutations was determined using MutationTaster and PolyPhen online databases. For p53, IHC and sequencing were complimentarily used to assess the p53 status

Results: Of 57 cases collected, 46 were considered prototypical ECCC by morphology and IHC profile (Napsin-positive and ER negative). 3 cases were excluded because of insufficient sample for complete IHC analysis, and 6 had failed sequencing resulting in 37 cases for the study. Of the 37 cases, 7 (19%) had pathogenic mutations in the exonuclease domain of *POLE* with an allelic frequency greater than 10%, however, only 1 of which was a defined hot-spot mutation (S297F, 1/37, 3%) No cases were MMR abnormal/deficient. The gene most commonly affected was *TP53* (62%, 23/37), followed by *PIK3CA* (11%, 4/37), and *KRAS* (8%, 3/37).

Conclusions: When strict classification criteria are applied, prototypical ECCC seem to split over the *TP53* status. Other molecular subtypes are sparsely represented, which could be harvested in the differential diagnosis towards endometrial endometrioid carcinomas.

1136 Molecular Characterization of Recurrent Low Grade, Low-Stage Endometrioid Endometrial Carcinoma

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Background: The prognosis of low grade, low-stage endometrioid endometrial carcinoma (EEC) is favorable, with over 90% 5-year survivals. However, the reported recurrence risk has a wide range (7-23%), illustrating the lack of precision in predicting recurrence. Most studies have looked at morphological predictors of recurrence, with little data on the molecular nature of recurrent low-risk EEC. Furthermore, there are few studies differentiating vaginal versus distant recurrences. Recent molecular classification of endometrial cancer; *POLE*-mutated, Mismatch repair (MMR) deleted, p53 mutated (copy number high) and p53 wild-type has shown to be better predictor of prognosis, but it is unclear if it will aid in recurrence prediction. We molecularly analyzed 26 recurrent low-risk EECs and retrospectively assigned a PROMISE molecular classification.

Design: Primary cases of recurrent low-grade (G1-2 endometrioid), low-stage (pT1, pT2) (no lymphvascular invasion), EECs were collected from four institutions. Low-stage was defined as confined to the uterus to incorporate possible pT2 cases arising from the lower uterine segment. p53 and MMR immunohistochemistry (IHC) was completed. DNA was extracted and subjected to targeted next-generation sequencing for *POLE, TP53, KRAS,* and *PIK3CA*. Pathogenicity of mutations with greater than 10% allelic frequency was determined using MutationTaster and PolyPhen online databases.

Results: 26 recurrent cases (average patient age 63.8 years-old) represented 50% local versus 50% distant recurrences with an average interval time of 37.8 months (range 6 to 146). The cases split molecular subtypes as follows: 7/26 (27%) MMR-D by IHC, 2/26 (8%) *POLE* EDM mutated, 1/26 (4%) *TP53* mutated by IHC or sequencing, and 16/26 (61%) TP53 wild type. Within the latter group, 3/16 (19%) cases harbored a *PIK3CA* and 3/16 (19%) a *KRAS* mutation

Conclusions: Molecular classification of low-risk EECs reveals a third of patients would be reclassified as dMMR with most cases being hypermethylated (sporadic). Two patients would be reclassified as having a *POLE*-mutated tumour, one patient as p53 mutated, and the remainder falling into the p53 wildtype (copy number-low) group. Depending on the future of personalized medicine in gynecologic cancers, molecular classification would possibly intervene in deciding management for these patients with higher risk profiles that appear low risk. However, it remains to be determined if recurrences can be risk stratified by their location (local versus distal).

1137 Agreement for Ovarian Tumor Characteristics in the Nurses' Health Studies

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Background: Grade and histotype are often used as surrogates of molecular subtypes of ovarian epithelial carcinomas. We examined histologic factors that may affect grading agreement among in the context of two prospective studies.

Design: 459 women with ovarian cancer in the Nurses' Health Study (NHS) and NHSII were available for review. We described agreement on tumor grade and type. We used logistic regression, with disagreement as the outcome, to evaluate the contribution of slide and tumor characteristics to agreement between pathologist reviewers and with original pathology reports.

Results: Inter-rater agreement on tumor invasion was 95% (κ=0.81), on three-tier International Federation of Gynecology and Obstetrics (FIGO) grade was 89% (κ=0.58) and on Shimizu-Silverberg (SS) grade was 65% (κ=0.46). Agreement on histotype was 85% (κ=0.71). Inter-rater FIGO grading agreement was lower for non-serous than for serous ovarian cancer (OR=4.66, 95% CI 2.09-10.36) and higher for ovarian cancer with bizarre atypia than without (OR=0.13, 95% CI 0.04-0.38). Inter-rater Shimizu-Silverberg grading agreement was higher for ovarian cancer with solid, pseudoendometrioid, and transitional morphology (SET) architecture versus not (OR=0.35, 95%CI 0.15-0.78).

Agreement with original pathology reports for invasion was 94% (κ=0.73), for histotype was 78% (κ=0.60), and for FIGO grade was 79% (κ=0.23). FIGO grading agreement was significantly better for tumors with bizarre atypia (OR=0.09, 95%CI 0.03-0.27) and for tumors with SET architecture (OR=0.05, 95%CI 0.01-0.64). Tumor stage and age, number, and quality of slides did not affect agreement.

Conclusions: Overall, inter-rater agreement on the classification of ovarian tumor type and grade for archival tissue specimens in the Nurses' Health Studies was good. Agreement between the consensus review and original pathology reports was lower. Factors limiting grading agreement include non-serous histotype, absence of bizarre atypia, and absence of SET architecture. Our results are reassuring given a prior report from the Surveillance Epidemiology and End Results (SEER) tissue repository showing poor grading agreement between original reported diagnosis and pathologist reviewer (Matsuno *et. al.* Cancer Causes Control. 2013 April ; 24(4): 749–757). A small fraction of cases will always be difficult to classify based on morphology alone, but defining the scope and source of disagreement lends validity to previously published clinical and epidemiologic correlations.

1138 The Relationship of SET Morphology With BRCA1/2 Germline and Somatic Mutation Status in High Grade Serous Carcinoma

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Background: SET features (Solid/pseudoEndometrioid/Transitional cell carcinoma like) in high grade serous carcinoma (HGSC) have been described in association with BRCA1/2 germline mutations (GMs). For the first time, in this study we examined the presence and distribution of SET features in HGSC with somatic only mutations relative to HGSC from patients with GMs in BRCA1/2, in order to assess SET features as a potential triage tool toward patient genetic counselling and PARP inhibitor therapy eligibility.

Design: The study cohort included 24 chemotherapy naïve HGSC from patients who had undergone BRCA1/2 GM testing. A representative section of formalin-fixed paraffin embedded tumor was macrodissected for DNA extraction, followed by BRCA1/2 mutation analysis with next generation sequencing (NGS) (Ion Torrent S5XL, 500X average read depth). Presence of pure (SET in all tumor sections) or focal SET features (in tumor sections undergoing NGS) was assessed while blinded to BRCA1/2 mutation status.

Results: 11 (46%) cases had a BRCA1/2 GM. Of those, 9 were found to have additional BRCA1/2 mutations, which were presumed to be somatic. Among GM negative cases, 5 (21%) had BRCA1/2 mutations, again presumed to be somatic. 8 (33%) of cases were BRCA1/2 mutation negative. The pure SET pattern was most frequently associated with BRCA1/2 GMs, however, this pattern was also noted in some cases with somatic mutations or cases lacking BRCA1/2 mutations. For BRCA1/2 GMs, pure SET features had a specificity (sp) of 77% and sensitivity (sen) of 54%, in keeping with previous literature (sp 72-100% and sen 47-58%). For BRCA1/2 by NGS (somatic and GMs), pure SET features had a sp of 75% and sen of 44%, while presence of either pure or focal SET features had a sp of 63% and sen of 56%. BRCA 1 vs. 2 mutations did not show a particular predilection for tumor SET features.

Tumor morphology	BRCA1/2 mutation		
	Germline	Somatic only	Negative
SET pure (n=9)	6	1	2
SET focal in NGS block (n=3)	1	1	1
SET negative (n=12)	4	3	5
Total (n= 24)	11	5	8

Conclusions: Pure SET morphology was most preferentially associated with BRCA1/2 GMs. Tumors with somatic only mutations tended to be SET negative. However, the overall moderate sp and low sen of SET features for GMs alone, or a combination of either somatic or GMs in BRCA1/2, suggest that morphological analysis is insufficient to identify all HGSC patients who would potentially benefit from genetic counseling or PARP inhibitor therapy.

1139 Comparative Prognostic Value of FIGO and Silverberg Grading Systems in Ovarian Endometrioid Carcinoma

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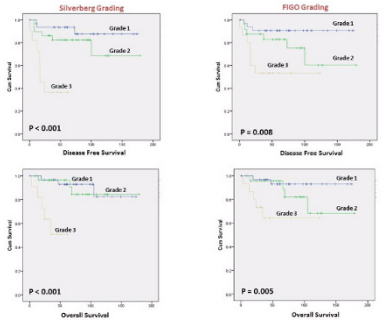
Background: The FIGO grading system for endometrial carcinoma is currently applied to ovarian endometrioid carcinoma (OEC) in many practices. However, previous reports claim superior prognostication by using the Silverberg grading system for ovarian carcinoma (SILV). A thorough comparison between FIGO and SILV in OEC is necessary.

Design: OECs diagnosed at our institution were independently graded using both systems. SILV grading was based on architecture (majority glandular=1, papillary=2, solid=3), nuclear atypia (mild=1, moderate=2, severe=3) and mitotic activity in 10 HPF (0-9=1, 10-24=2, ≥25=3); added score determined grade (G1:3-5, G2:6-7, G3:8-9). FIGO grading was based on the % of solid component (G1:<5%, G2:5-50%, G3:>50%); severe atypia warranted upgrade to the architectural FIGO grade (1 to 2 or 2 to 3). Case grouping by grade was correlated with disease-free (DFS) and overall survival (OS) measured in months (m).

Results: A total of 72 OECs were included. Table 1 shows the distribution rates of FIGO and SILV. 11 (15.3%) were bilateral, 26 (36.1%) had ovarian surface involvement, 12 (16.7%) had LVI. Distribution by stage was I 47 (65%), II 16 (22%) and III 9 (13%). Median follow-up period was 62 m (range 1-179); 16 (22%) OEC recurred and 9 (13%) died of disease. Median DFS was 60.5 m (1-179); median OS was 61 m (1-179). In univariate analysis, both FIGO and SILV correlated significantly with DFS (0.01 vs 0.001) and OS (0.02 vs 0.003). DFS and OS also correlated with tumor laterality (0.01 and 0.008) and stage (<0.001 and <0.001). SILV showed better survival prediction with higher R^2 for DFS and OS (0.17 and 0.15) compared to FIGO (0.12 and 0.1). G1 or G2 OEC by SILV had significantly better DFS and OS compared to G3; such separation was not seen with FIGO. In multivariate analysis, stage was the only significant factor.

Silverberg N (%)		1 32(44.4)	2 29(40.3)	3 11(15.3)	Total 72(100)
FIGO	1	26	7	0	33(45.8)
	2	5	18	1	24(33.3)
	3	1	4	10	15(20.8)



Conclusions: As currently defined, both FIGO and SILV grading systems for OEC carry prognostic value in univariate analysis. However, SILV appears to be a better predictor of survival than FIGO. Such difference may be explained by the G2 OEC groups, with G2 SILV having a more favorable behavior compared to G2 FIGO. G1 and G3 OECs show similar survival in both systems.

1140 Clinicopathological, Immunohistochemical, and Molecular Characterization of 30 Uterine Perivascular Epithelioid Neoplasms (PEComas)

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Background: Uterine PEComas are rare tumors with only five small series reported.

Design: We evaluated the clinicopathological and immunohistochemical features of 30 uterine PEComas and tested for *TFE3* and *RAD51B* rearrangements by FISH.

Results: Patients ranged from 28 to 77 (mean 50) years and 20% had tuberous sclerosis. Tumors were located in corpus (80%) or cervix (20%), ranged from 0.2 to 14 (mean 5.7) cm with extrauterine disease in 17%. Borders were infiltrative (20%), pushing (17%), well-circumscribed (13%), mixed (47%), or unable to be evaluated (3%), and all had thin delicate vessels. Most (77%) were epithelioid-predominant with mild/moderate nuclear atypia (73%) and lacked necrosis (67%). Focal stromal hyalinization was noted in 77%. Macronucleoli were seen in 37%, multinucleated cells in 30%, pseudoinclusions in 20%, Touton giant cells in 13%, and melanin in 3%. Clear/eosinophilic granular cytoplasm was present in all with a ganglion-like/rhabdoid appearance in 33%, florid glycogenization in 13%, and focal myxoid/chondroid change in 3%. Mitoses ranged from 0 to 36/10 HPF (mean 5) with atypical mitoses in 27%. Lymphovascular invasion was noted in 13%. Two tumors differed from the typical PEComa, consisting of a discrete nodule morphologically resembling lymphangioleiomyomatosis. HMB-45 was expressed in all tumors with MiTF in 78% and melan-A in 75% (Table 1). FISH (n=24) identified a *PSF-TFE3* rearrangement in one and a *RAD51B* in another. Follow-up was available in 28 patients, ranging from 2 to 171 (mean 40) months with 29% having recurrences. Most patients (64%) are alive and well, 21% died from disease, 11% are alive with disease, and 4% died from other causes. On univariate analysis, multiple clinicopathological features were predictive of adverse outcome (Table 2). Patients with an adverse prognosis had 4 to 10 of these features (mean 7), while those with a benign course had up to 4 (mean 1).

Table 1: Immunoprofile of Uterine PEComas

Immunostain	0 (<1%)	1+ (1-5%)	2+ (6-25%)	3+ (26-50%)	4+ (>50%)	Positive Per Report
HMB-45 (n=30)	0%	7%	10%	10%	50%	23%
Melan-A (n=28)	25%	25%	11%	11%	11%	18%
MiTF (n=9)	22%	0%	22%	22%	22%	11%
S-100 (n=17)	88%	0%	6%	6%	0%	0%
Desmin (n=25)	24%	12%	16%	4%	28%	16%
Smooth Muscle Actin (n=19)	16%	11%	5%	0%	42%	26%
Caldesmon (n=12)	17%	33%	0%	17%	33%	0%
TFE3 (n=8)	50%	0%	0%	13%	38%	0%
Cathepsin K (n=12)	0%	0%	0%	0%	92%	8%

(Note, remainder of IHC stains pending).

Table 2: Comparison of Clinicopathological Features with Associated P-Values

	Adverse Clinical Course* (n=10)	Benign Clinical Course (n=16)	P-value
Age (years)	48-67 (60)	32-77 (48)	0.005
Tuberous Sclerosis	0%	31%	0.121
Extrauterine Disease	40%	0%	0.014
Tumor Size (cm)	4-14 (8.6)	0.2-10 (3.4)	0.002
% Epithelioid	40-100 (77)	25-100 (90)	0.040
% Spindled	0-60 (23)	0-75 (10)	0.040
Necrosis	80%	6%	<0.001
Stromal Hyalinization	80%	75%	1.000
Multinucleated Cells	80%	6%	<0.001
Touton Giant Cells	30%	6%	0.264
Nuclear Atypia	Mild=20% Moderate=0% Severe=80%	Mild=81% Moderate=19% Severe=0%	<0.001
Macronucleoli	70%	19%	0.015
Mitoses / 10 HPF	0-36 (13)	0-7 (1)	0.003
Atypical Mitoses	80%	0%	<0.001
Lymphovascular Invasion	20%	0%	0.139

*Adverse clinical course defined by recurrence or death. (Note, only cases with follow-up were included in analyses and the 2 tumors that resembled lymphangioleiomyomatosis were excluded).

Conclusions: Uterine PEComas have a broad morphologic spectrum with consistent expression of HMB-45 and cathepsin, but rare *TFE3* or *RAD51B* rearrangements. Age ≥62 years, extrauterine disease, size ≥5 cm, ≥10% spindled component, necrosis, severe nuclear atypia, multinucleated cells, macronucleoli, mitoses ≥2/10 HPF, and atypical mitoses were predictive of an adverse outcome. PEComas can be diagnosed as malignant if >4 of these features are present. Additional molecular testing is necessary to identify mutations that can potentially be used for targeted therapy.

1141 Block-positive P16 Immunoreactivity as a Predictive Marker for Progression of Low-grade Squamous Intraepithelial Lesions of the Anal Canal

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Background: Human papillomavirus (HPV) infection of the anal mucosa causes low-grade squamous intraepithelial lesions (LSIL). Most LSILs are eradicated by the host's immune response. When the host is immunocompromised (e.g. by HIV infection), LSILs are prone to progress to high-grade squamous intraepithelial lesions (HSIL) and ultimately invasive cancer. Thus, identifying patients at higher risk for progression is paramount. We aimed to investigate (1) anal LSIL progression rates in a cohort of HIV-infected patients, and (2) biomarker p16's ability to identify high-risk patients who are likely to progress rapidly.

Design: 118 patients (100 HIV-infected, 18 HIV-uninfected) with anal LSIL were included. High-resolution anoscopy-guided biopsy was performed upon initial and follow-up visits. P16 expression of initial LSIL was assessed by IHC. P16 results were classified as negative (i.e. absent or weak staining), patchy-positive (strong cytoplasmic/nuclear staining < 50% of epithelium), or block-positive (strong staining ≥ 50% of epithelium). High-risk HPV strains were detected on initial LSIL lesions using real-time PCR.

Results: At follow-up, 48 (41%) patients progressed to HSIL (median 16 months, range 2-24). The progression group included one (6%) HIV-uninfected and 47 (47%) HIV-infected patients. P16 expression was block-positive in 30 LSILs, patchy-positive in 47, and absent in 41 lesions. 80% of block-p16 LSILs progressed, in contrast to 38% of patchy-p16 and 15% of negative-p16 lesions (p<0.001, Table 1). Among 60 AIN 1 lesions tested for HPV, 30 (50%) were negative for HR-HPV, 10 (16%) positive for HPV 16 or 18, and 20 (33%) were positive for non-16/18 HR-HPV (Table 2). Compared with patchy-p16 lesions, block-p16 ones showed a similar HPV 16/18 rate (16%), but a higher percentage of non-16/18 HR-HPV strains (44% vs. 24%), and fewer negative HPV results (40% vs. 60%). Multivariable analysis revealed that both block and patchy-p16 patterns were strongly associated with LSIL progression (odds ratio 19 and 5).

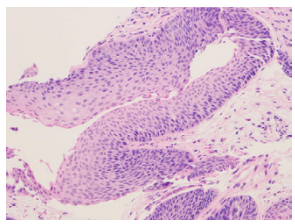
Table 1. P16 expression correlated with subsequent clinical outcomes

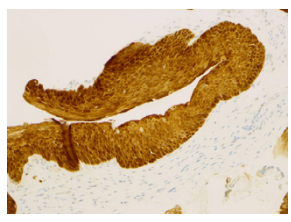
Initial LSIL's p16 expression (n=118)	Clinical outcomes	
	Progression to HSIL (n=48)	LSIL or benign (n=70)
Block-positive (n=30)	24 (80%)*	6 (20%)
Patchy-positive (n=47)	18 (38%)	29 (62%)
Absent (n=41)	6 (15%)	35 (85%)

P<0.001

Table 2. HR-HPV types detected from AIN 1 lesions with block or patchy p16 expression

P16 immunoreactivity	HR-HPV Types			
	16	18	Non-16/18	Negative
Block-positive (n=30)	4 (13%)	1 (3%)	13 (44%)	12 (40%)
Patchy-positive (n=30)	1 (3%)	4 (13%)	7 (24%)	18 (60%)
Total (n=60)	5 (8%)	5 (8%)	20 (33%)	30 (50%)





Conclusions: Progression of anal LSIL to HSIL occurred in less than half of cases, predominately among HIV-infected patients. While any p16 expression is capable of identifying patients with HR-HPV infection and progression, the block-positive pattern is a stronger predictor than the patchy-positive pattern. P16 IHC can play an important role in guiding clinical management of HIV-infected patients with anal LSIL lesions.

1142 Development of a Liquid Biopsy Platform for Detection of Endometrial Cancer Recurrence

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Background: Most endometrioid-type endometrial cancers (EEC) are diagnosed at early stage. Although the majority of these patients will have good survival, those patients who do recur tend to have poor outcomes. Determining which patients are at highest risk for a recurrence and would therefore benefit most from adjuvant treatment or more extensive surgical staging has been challenging. A blood-based assay to detect tumor recurrence earlier could be potentially helpful. The goal of this study was to determine the feasibility of peripheral blood-based EEC mutation testing.

Design: From our previous clinical next-generation sequencing (NGS) experiences, we determined that more than 90% of EEC is defined by mutation of at least one gene amongst *PTEN*, *PIK3CA, KRAS*, or *CTNNB1*. In collaboration with Swift Biosciences, a custom NGS panel containing 30 amplicons covering 2648 bases of different hotspots for these 4 genes was developed. This panel was applied to formalin-fixed, paraffin-embedded EEC and plasma cell free DNA from peripheral blood of 35 early stage (I or II) and 13 late stage (III or IV) patients. Both tumor and peripheral blood samples were collected at the time of surgery.

Results: At least one gene mutation was detected in the tumor in 45/48 patients (94%). For 30 patients, a mutation was only detected in the tumor, while in 15/45 patients (33%), the same mutation was detected in both tumor and plasma. The 4 gene NGS panel was sensitive, detecting mutations in the plasma at allelic frequencies ranging from 0.22 to 13%. For the cases in which plasma mutation allelic frequency was below 1%, no mutations were identified when the white blood cell buffy coat was also subjected to NGS analysis, demonstrating the assay's specificity. The presence of a gene mutation in the plasma was significantly associated with late EEC stage, deep myometrial invasion, presence of myometrial lymphatic/vascular invasion, and larger tumor size (Figure 1). Importantly, ability to detect a plasma mutation was not impacted by pre-analytic variables such as starting tumor cellularity in the primary tumor of cell free DNA yield in the plasma.

Characteristic	Plasma mutant	Plasma wildtype	p-value
Tumor stage, n(%)			0.023
Early	10 (28.6%)	25 (71.4%)	
Late	7 (70%)	3 (30%)	
Tumor grade, n(%)			0.06
1	0 (0.0%)	0 (0.0%)	
2	8 (27.6%)	21 (72.4%)	
3	9 (56.3%)	7 (43.7%)	
Myometrial invasion, n(%)			0.009
<50%	7 (23.3%)	23 (76.7%)	
≥ 50%	9 (64.3%)	5 (35.7%)	
LVSI, n(%)			0.01
yes	11 (57.9%)	8 (42.1%)	
no	5 (20%)	20 (80%)	
Tumor size in cm, mean(s.d)	7.09 (4.96)	4.24 (2.04)	0.019
Tumor percentage(%), mean(s.d)	70% (19.7)	60% (19)	0.119
cfDNA yield, mean(s.d)	16.07 (7.73)	14.21 (5.46)	0.463
cfDNA input for library preparation	0.83ng/uL (0.196)	0.81ng/uL (0.195)	0.818

Conclusions: This proof-of-concept study demonstrated that detection of EEC-associated mutations in the cell-free DNA of plasma is feasible. Nearly 30% of early stage EEC patients with small uterine tumors had plasma mutations at the time of surgery, suggesting that the presence of bulky disease outside the uterus is not necessary for plasma mutation detection.

1143 Evidence for a Unique Endometrioid Carcinogenic Sequence in the Fallopian Tube Associated with a type II (Endometrioid) Secretory Cell Outgrowth (SCOUT)

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Background: The most prominent carcinogenic sequence in the fallopian tube is derived from mutations in TP53 resulting in p53 signatures, serous tubal intraepithelial lesions of uncertain significance (STIL-US), and serous tubal intraepithelial carcinomas (STICs), which may predate extrauterine high grade serous carcinoma. Another entity, the secretory cell outgrowth (SCOUT) is not associated with Tp53 mutations and can manifest as Type I, with conspicuous ciliation and occasional association with low grade serous tumors of the ovary, and Type II, with an endometrioid phenotype and strong nuclear and cytoplasmic b-catenin expression (b-catenin+). Neither Type I nor Type II SCOUTs have been directly associated with malignancy in the fallopian tube.

Design: The consultation and surgical pathology files were surveyed for Type II SCOUTs or putative premalignant lesions with endometrioid differentiation. In addition, cases of endometrioid adenocarcinoma of the fallopian tube were identified. Where possible, cases were immunostained for b-catenin. Attention was paid to the possible co-existence of Type II SCOUTs and either a precursor (analogous to EIN/atypical hyperplasia) or endometrioid adenocarcinoma.

Results: Type II SCOUTs were identified as previously described, linear arrangements of b-catenin+ endometrioid epithelium in association with 12-35% of endometrial and ovarian endometrioid tumors . Six atypical endometrioid b-catenin+ clusters of glands similar to EIN, were identified, often with morular metaplasia. Two were associated with ovarian and/or endometrial endometrioid adenocarcinomas, one or more adjacent Type II SCOUTs, and endometrioid adenocarcinoma in the tube. All three - SCOUT, atypical hyperplasia and adenocarcinoma - were b-catenin+. In one, disparate b-catenin mutations and normal b-catenin staining were found in the concurrent ovarian and endometrial tumors. This case also contained a separate conventional p53+ STIC.

Conclusions: This study describes for the first time an endometrioid carcinogenic sequence in the fallopian tube that is closely associated with a Type II SCOUT, including atypical endometrioid hyperplasia and rarely, a well differentiated endometrioid adenocarcinoma sharing identical abnormal b-catenin staining. The fact that one case displayed multiple Type II SCOUTs as well as STIC underlines the possibility that the coexistence of disparate carcinogenic pathways may not be random but could signify shared risk factors across more than one tumor type.

1144 A Comparison of CK19, AFP and Glypican-3 in Distinguishing Yolk Sac Tumors from Other Germ Cell Tumors in the Ovary

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Background: Ovarian yolk sac tumors (YST) can exhibit a wide variety of morphologic patterns, ranging from reticular, micro/macrocystic, festoon/labyrinthine, solid, hepatoid, glandular/alveolar, papillary, endodermal-sinus or polyvesicular vitelline. Variant morphologic patterns can be particularly difficult to disintinguish from other germ cell tumors. Although immunohistochemical (IHC) stains for alpha-fetoprotein (AFP) and glypican-3 have traditionally been used to diagnose YST, staining for AFP is very often focal and glypican-3 is known to be less specific. It has recently been reported that CK19 is a very sensitive marker of YST in the testis, more sensitive than AFP. To date, the utility of CK19 for identifying YST has not yet been investigated in the ovary.

Design: We examined a total of 23 ovarian germ cell tumors (8 pure YST, 2 mixed germ cell tumors both with a significant YST component, 8 dysgerminoma, 4 immature teratoma and 1 gonadoblastoma). The expression of CK19, AFP and glypican-3 were assessed on tissue microarrays. Staining patterns were characterized by an intensity score (0 = no staining, 1 = weak, 2 = moderate, 3 = strong), proportion score in 10% increments and overall H-score (intensity score multiplied by proportion score). We included immature neuroepithelium in immature teratomas, as they can be confused with the glandular pattern of YST.

Results: Although CK19 demonstrated the most conspicious staining, with the highest overall intensity score, proportion score and H-score, it had a lower sensitivity than both AFP and glypican-3 (80% compared to 100% and 100% respectively). Specificity was highest for AFP (100%), intermediate for CK19 (92%) and lowest for glypican-3 (85%). None of the dysgerminomas or gonadoblastoma displayed any staining for CK19, AFP or glypican-3. Immature neuroepithelium however did exhibit focal weak staining for CK19 and glypican-3.

		Yolk Sac Tumor	Dysgerminoma	Gonadoblastoma	Immature Teratoma
CK19	Positive cases	8/10 (80%)	0/0 (0%)	0/0 (0%)	1/4 (25%)
	Intensity	2.55	0	0	1
	Proportion	82.2	0	0	10
	H-Score	223	0	0	10
AFP	Positive cases	10/10 (100%)	0/0 (0%)	0/0 (0%)	0/0 (0%)
	Intensity	2.36	0	0	0
	Proportion	55.5	0	0	0
	H-Score	133	0	0	0
Glypi-can-3	Positive cases	10/10 (100%)	0/0 (0%)	0/0 (0%)	2/4 (50%)
	Intensity	2.20	0	0	1.50
	Proportion	73.0	0	0	35.0
	H-Score	166	0	0	50

Conclusions: In contrast to the findings reported in the testis, CK19 is not a superior marker to AFP for detecting YST in the ovary. Although CK19 demonstrated the most conspicious staining (generally strong and diffuse), it had a slightly lower sensitivity than both AFP and glypican-3 and slightly lower specificity than AFP. Our study still highlights that CK19 a good marker for YST, and given that CK19 is widely available in many laboratories (used primarily to stain bile duct epithelium), CK19 can be used as an alternative marker for YST when AFP and/or glypican-3 are unavailable.

1145 Pleomorphic Squamous Intraepithelial Lesions of the Cervix: Association with Non-16/18 HPV and Squamous Metaplasia

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Background: Pleomorphic squamous intraepithelial lesions (PSIL) represent a rare, but distinct, morphologic subset of cervical SIL, which have been considered both low and high grade at different points in history. PSIL is characterized by markedly enlarged, bizarre nuclei with nuclear size variability of at least 3:1. Chromatin appears smudged with hyper- and polychromasia. Abundant cytoplasm results in a low N/C ratio. Atypical mitotic figures may be seen. P16 staining adjudicates difficult cases as LSIL or HSIL/CIN 2; however, p16 is positive in a subset of LSIL. CK7 is a biomarker for SIL arising from the squamocolumnar junction, demonstrating positive staining in HSIL and LSIL "progressors". This study examines the morphology of PSIL in conjunction with p16, CK7, HPV-PCR, and clinical follow-up to determine whether this SIL variant should be classified as LSIL or HSIL.

Design: 738 consecutive cervical biopsies (2009-11) were reviewed by 2 gynecologic pathologists and 1 resident for consensus diagnosis of PSIL. HPV-PCR, CK7 and p16 staining were performed on 30 most representative PSILs. P16 staining was scored as positive (block positivity) or negative. CK7 staining was interpreted as positive (gradient/top down or full thickness staining in at least 5-6 contiguous cells) or negative (patchy or no staining). High risk (hr) HPV typing was performed via PCR. The presence of atypical mitoses and squamous metaplasia were noted. Clinical follow-up data was collected.

Results: 45 (6%) PSILs were identified; 30 of which were selected for analysis. The original diagnosis for the 30 selected was LSIL (37%) and HSIL/CIN2 (63%) with patient age ranging from 22-43 years (mean 32.5). Immunohistochemistry was successful in 29/30. 26/29 (90%) of PSILs were p16 positive (full thickness: 13; lower third: 13). CK7 was positive in (19/29) 66% (full thickness: 13, gradient: 6). Co-expression of p16 and CK7 was seen in 17/29 (59%). 16 cases had follow-up data; the clinical-pathologic findings are outlined in Table 1.

Table 1: Clinical-Pathologic Parameters for Patients (n=16) with Follow-Up Data

Follow up Diagnosis	Age Range (mean)	P16	CK7	Hr-HPV	Squamous Metaplasia	Atypical Mitoses
Negative n=12 (75%)	28-43 (35)	Positive n=11 (91.7%) (6 FT, 5 BT1/3) Negative n=1 (8.3%)	Positive n=7 (58.3%) (5 FT, 2 Grad) Negative n=5 (41.7%)	Positive n= 5 (41.7%) 16/18: 3 other hrHPV: 2 Negative n=6 (50%) Inade-quate n=1 (8.3%)	Yes n=11 (91.7%) No n=1 (8.3%)	Yes n= 4 (33.3%) No n=8 (66.7%)
ASC-US n=2 (12.5%)	28-36 (32)	Positive n=2 (100%) (2 BT 1/3)	Negative n=2 (100%)	Positive n=1 (50%) other hrHPV: 1 Negative n= 1 (50%)	Yes n=2 (100%)	Yes n=2 (100%)
LSIL n=1 (6.25%)	31	Positive n=1 (100%) (1 BT 1/3)	Positive n=1 (100%) (1 Grad)	Inade-quate n=1 (100%)	Yes n=1 (100%)	Yes n=1 (100%)
HSIL n=1 (6.25%)	22	Positive n=1 (100%) (1 FT)	Positive n=1 (100%) (1 FT)	Positive n=1 (100%) other hrHPV: 1	Yes n=1 (100%)	Yes n=1 (100%)

FT: Full-thickness, BT 1/3: Bottom 1/3, Grad: Gradient, hr-HPV: high-risk Human Papilloma Virus

Conclusions: Awareness of this rare and difficult to classify variant of SIL is needed. Our study shows that PSIL exhibits a prominent association with:

- Hr-HPV negative or non-16/18 status despite high rate of p16 positivity and/or CK7 expression
- Strikingly benign follow-up
- Squamous metaplasia

Hence, pleomorphic-SIL should be classified as LSIL (CIN 1).

1146 Targeted Genomic Profiling of BRAF/KIT/NRAS Mutation-Negative Gynecologic Melanoma Reveals Recurrent NF1 Alterations and TP53 Mutations

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Disclosures:
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Background: Gynecologic melanomas (GM), including those involving the vulva, vagina, and/or cervix, are uncommon, aggressive tumors with poor long-term clinical outcome. Our group recently reported a relatively low rate of mutations (<30%) in conventional melanoma oncogenes (*BRAF*, *KIT*, and *NRAS*) in a large retrospective cohort of GM. In this study, we utilized targeted next-generation DNA sequencing (DNAseq) to characterize the genomic landscape of *BRAF*/*KIT*/*NRAS* mutation-negative ("triple-negative") GM.

Design: Four "triple-negative" GM at a single large academic institution were retrospectively identified from a previously published tumor cohort. Representative formalin-fixed paraffin-embedded tissue was selected for targeted DNAseq using the Oncomine Comprehensive Assay and an Ion Torrent Proton sequencer. Somatic variants and copy number alterations (CNA) were identified via in-house bioinformatics pipelines, and prioritized alterations were

nominated by manual curation using previously established criteria.

Results: Overall, a total of 7 prioritized somatic variants were identified (median per tumor = 2; range = 0-3), including *TP53* (*n* = 3), *NF1* (*n* = 1), and *NOTCH1* (*n* = 1) mutations; no *SF3B1* mutations were identified. A total of 20 CNA were identified (median per tumor = 3; range = 2-12), including recurrent *NF1* (*n* = 3) and *CDKN2A* (*n* = 2) deletions. One tumor showed focal 4q12 amplification (including *PDGFRA*, *KIT*, and *KDR*) with concurrent amplification of *MTOR*, while one tumor each demonstrated focal *CDK4* and *MYCL* amplification. Integrating somatic variant and CNA data, all tumors showed one or more *NF1* alteration, and half of tumors demonstrated *TP53* mutation with concurrent *CDKN2A* deletion.

Conclusions: "Triple-negative" GM show recurrent *NF1* alterations, including frequent deletion and occasional somatic mutation, indicating that altered NF1 activity likely plays a central pathogenic role in these tumors. Concurrent tumor suppressor alterations, including *TP53* mutations and/or *CDKN2A* deletions, and focal non-recurrent amplifications are also frequently observed. Overall, these data suggest that, despite the absence of conventional oncogenic mutations (*BRAF*, *KIT*, and *NRAS*), downstream activation of the RAS/RAF and/or PI3K/AKT pathways is common in "triple-negative" GM and may be therapeutically targetable.

1147 Patients with HLRCC have Multiple Leiomyomas with Heterogeneous Morphology that Often Show Retained Staining for Fumarate Hydratase

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Background: Hereditary leiomyomatosis and renal cell carcinoma (HLRCC), caused by germline mutation in fumarate hydratase (FH) gene predisposes patients to aggressive renal cell carcinomas and multiple cutaneous and uterine leiomyomas (ULM). FH deficient ULM can show characteristic morphologic features (FH-morph) including staghorn blood vessels, alveolar edema, eosinophilic cytoplasmic inclusions, nuclei with prominent pink macronucleoli with halos, although the sensitivity and specificity of these findings remains controversial. Immunohistochemistry (IHC) for FH appears to be specific (loss of staining correlates with presence of FH mutation) but not entirely sensitive (retained staining can be seen in the presence of FH mutation). The clinical, morphologic and immunohistochemical characteristics of ULM in patients with known germline mutations in *FH* have not been described.

Design: Five patients with proven FH germline mutations were included. All available slides were reviewed and FH stain was performed on multiple blocks when possible.

Results: All five patients presented with symptomatic ULM underwent myomectomy, followed by hysterectomy in 2 patients. None had RCC and 1 patient had skin nodules. Patients showed either conventional or atypical LM, FH-morph was present in all or only some of the LM. See Table 1. One notable finding in both hysterectomy specimens was that the background myometrium showed scattered irregular tongues and nodules of LM (leiomyomatosis like).

FOR TABLE DATA, SEE PAGE 466, FIG.1147

Conclusions: Patients with FH germline mutations often undergo surgery at young ages for multiple and highly symptomatic ULM. In our experience, FH-morph correlates with presence of FH mutation. Indeed all 5 patients were referred for genetic counselling and testing based on the pathologist's impression of FH-morph. ULM in this setting can show heterogeneous features and may not show FH-morph in every LM, which has implications for ULM sampling. FH stain can often be retained in the presence of FH mutation and therefore this result should not be used to exclude the possibility of HLRCC. Referral for genetic counselling and testing should be considered in a young patient with ULM showing FH-morph even if the FH stain is retained.

1148 Tumor infiltrating lymphocytes and PD-L1 expression in 162 endometrial carcinomas with deficient MMR: comparison between MLH1 methylation and Lynch syndrome

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Background: Tumor infiltrating lymphocytes (TILs) are suggestive of mismatch repair (MMR) protein deficiency in endometrial carcinomas (EC). We recently described a significant association between PD-L1 expression and MMR deficiency in EC. Herein TILs and PD-L1 expression are compared between main types of MMR deficient EC: those due to MLH1 methylation vs those due to germ line mutations (Lynch syndrome).

Design: 162 MMR deficient EC were identified, including 36 with Lynch syndrome and 126 with MLH1 promoter methylation. Immunohistochemistry (IHC) for PD-L1 was performed on TMA sections with all 162 cases in triplicate. Membranous PD-L1 staining in tumor cells or stromal cells was considered specific staining. TILs and peritumoral lymphocytes were evaluated on one to three digitally scanned whole slides with tumors from 150 cases including 34 with Lynch syndrome and 116 with MLH1 methylation.

Results: No statistically significant differences were identified in PD-L1 expression in tumor cells between MLH1 methylation and Lynch syndrome cases when using a cutoff value of 1%. However, a significantly higher percentage of PD-L1 expression in tumor cells was found in Lynch syndrome cases when using a 5% cutoff (p=0.015). PD-L1 expression in stromal cells did not show significant difference between these two groups using either 1% or 5% cutoff. Neither TILs nor peritumoral lymphocytes revealed significant difference between these two groups, although Lynch syndrome cases showed an average of 36.1% of TILs, slightly higher than that in cases with MLH1 methylation (33.0%).

Table 1. PD-L1 expression in tumor cells in endometrial carcinomas with deficient MMR.

Protein expression (IHC)	PD-L1 expression (≥1%) n (%)		PD-L1 expression (≥5%) n (%)		Total n (%)
	PDL1 (+)	PDL1 (-)	PDL1 (+)	PDL1 (-)	
Total Lynch syndrome	9(25.0)	27 (75.0)	9 (25.0)	27 (75.0)	36 (100)
PMS2 -	1(25.0)	3 (75.0)	1 (25.0)	3 (75.0)	4 (100)
MSH2-/MSH6-	6 (31.6)	13 (68.4)	4 (21.1)	15(48.9)	19 (100)
MSH6-	2 (15.4)	11 (84.6)	4 (30.8)	9 (69.2)	13 (100)
MLH1 methylation	24(19.0)	102 (81.0)	12 (9.5)	114(90.5)	126 (100)
Total	33(20.4)	129 (79.6)	21(13.0)	141 (87.0)	162 (100)
p value	NS	NS	0.015	NS	

Table 2. TILs and peritumoral lymphocytes in endometrial carcinomas with deficient MMR.

Protein expression (IHC)	Cases (n)	Stromal TILs (%) mean (range %)	Peritumoral lymphocytes by score comparison n (%)				
			0	+1	+2	+3	Total
Total Lynch syndrome	34	36.1 (3.3 -80)	6 (17.6)	10 (29.4)	7 (20.6)	11 (32.4)	34 (100)
PMS2 -	4	32.5 (5-80)	2 (50.0)	1 (25.0)	0 (0)	1 (25.0)	4 (100)
MSH2-/MSH6-	18	33.6 (3.3-70)	2 (11.1)	7 (38.9)	4 (22.2)	5 (27.8)	18 (100)
MSH6-	12	40.9 (5-80)	2 (16.7)	2 (16.7)	3 (25.0)	5 (41.7)	12 (100)
MLH1 methylation	116	33.0 (0-100)	11 (9.5)	42 (36.2)	37 (31.9)	26 (22.4)	116 (100)
Total	150	33.7 (0-100)	16 (10.7)	54 (36.0)	46 (30.7)	34 (22.7)	150 (100)
p value		NS	NS	NS	NS	NS	

Conclusions: MMR deficient EC in patients with Lynch syndrome may express PD-L1 more commonly than those with MLH1 methylation but the association is cutoff-dependent. TILs, a histologic feature suggestive of MMR deficiency, do not constitute a good marker to distinguish MMR deficient subtypes.

1149 Primary Ovarian Pregnancy: A case series and clinical implication

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Background: The preoperative diagnosis of primary ovarian pregnancy (POP) remains difficult, and the diagnosis of POP relies heavily on histological findings. In 1878, Spielgelberg established the diagnostic criteria for POP, which is mainly based on the identification of embryonic sac within the ovary. However, such strict diagnostic criteria for POP may significantly underestimate the prevalence of POP and mislead the clinical management.

Design: In this series, we present 7 cases, which showed no embryonic sac within the ovary thus not meeting the Spielgelberg criteria, yet unequivocally, evidence of ovarian pregnancy was apparent.

Results: At the time of diagnosis, the mean age of patients was 35.5 years, the mean gravidity was 2.53 and the mean parity was 1.63. Common chief complaints were lower abdominal pain (6/7), abnormal vaginal bleeding (5/7), and low-grade fever (1/7). Other findings included elevated serum β-hCG levels (6/7) and unilateral adnexal masses (5/7). One patient was diagnosed preoperatively as probable ovarian germ cell tumor. All patients underwent subsequently either laparoscopic or abdominal salpingo-oophorectomy. However, no embryonic sac was found in any of the 7 cases after extensive histologic examination (0/7), but with histologic finding of chorionic villi (7/7) and implantation site (4/7) within the affected ovaries with no evidence of products of conception (POC) identified within the ipsilateral fallopian tubes. Meanwhile, Immmunohistochemical staining of β-hCG and inhibin were used to identify or confirm the histologic findings of syncytial trophoblasts within chorionic villi and intermediate trophoblasts within implantation sites. Based on such findings, we concluded these 7 cases truly represent POP despite no embryonic sac being found.

Conclusions: We propose the following modified diagnostic criteria for POP: With no evidence of the ipsilateral fallopian tube involvement, any evidence of gestation including presence of chorionic villi and/or implantation site within the ovary with or without the presence of embryonic sac, is sufficient to make the diagnosis of ovarian pregnancy. We believe that the above proposed diagnostic criteria will not only lead to more accurate diagnosis of POP, it may also improve clinical awareness of the disease and optimize clinical management in a long run.

1150 Benign cytological features of Thinprep liquid based tubal cytology from patients Salpingo-Oophorectomy

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Background: Ovarian high-grade serous carcinoma (HGSC) remains a major threat to the public health. Two relevant issues need to be resolved: 1) lack of biomarkers for ovarian cancer early detection; 2) lack of tools to help clinician to determine if prophylactic salpingo-oophorectomy should be performed for high-risk patients without timing concern. It is widely accepted that the vast majority of HGSC cases arise from the fallopian tube instead of the ovary. Our previous studies show that 1) Tubal cytological diagnosis of HGSC and its precursor serous tubal intraepithelial carcinomas (STICs) is sensitive and specific. 2) HGSC has the following features from tubal brushing samples: three-dimensional cell cluster, large cherry red nucleoli and severe anisonucleosis. Here, we try to establish the baseline benign tubal cytology for the future clinical application.

Design: 45 benign fallopian tubes from 29 patients were studied. A standard procedure using cytobrush to collect tubal epithelia from freshly received salpingectomy specimens. Tubal samples were evaluated for cellularity, background, cytological features after Thinprep slides were made. Cytological variables of individual sample were recorded separately and subjected to statistical analysis.

Results: 43 samples (96%) yield sufficient cellularity. Among them, 17 (40%) showed cellularity of 20/HPF or less; 19 (44%) showed cellularity between 20-40/HPF; and 7 (16%) showed cellularity of more than 40/HPF. 36 cases (84%) presented with a clean background, while 7 cases (16%) the background was bloody/necrotic. Benign tubal epithelial clusters were angulated sheets with a mixture of cell types (small ciliated cells and large secretory cells with clear cytoplasm). Single small cells/nuclei and single large cells/nuclei had a ratio of approximately 3:1. Nucleoli were usually inconspicuous. However, small "cherry red nucleoli" were noted in 12 (28%) cases, while 13 (30%) had "non-cherry red nucleoli, and only 2 (4%) showed large "cherry red" nucleoli. Mesothelial-like cell sheets were noted in 20 (46%) cases. Spindle cell/stromal components were found in 21 (49%) cases.

Conclusions: Tubal ex vivo sampling is practical and feasible. Cytological findings are interpretable after a focused training. Clearly defined baseline cytological features of benign tubal mucosa is an essential step to practice tubal cytology in future. Tubal cytology may be a useful approach for ovarian cancer screening and early detection.

1151 Ovarian Low-grade Serous Carcinoma Likely Deriving from the Fallopian Tube

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Background: As a leading cause of cancer deaths among women, ovarian serous carcinoma can be further categorized into high-grade serous carcinomas (HGSC) and low-grade serous carcinomas (LGSC). While compiling data from past decades have suggested that the vast majority of HGSC arise from fallopian tube instead of the ovary itself, the cell of origin LGSC remains controversial. Our recent study based on the morphologic and immunophenotypic features of LGSCS suggests that most LGSCs may also be derived from the fallopian tube and likely derived from tubal secretory cells through a secretory cell expansion process. The current study is designed to explore the genetic basis of the cellular origin and oncogenesis of LGSC.

Design: Gene expression profiles were studied in 44 samples including 11 LGSCs, 7 Serous boarderline tumors (SBT), 6 serous cystadenomas (SC), 6 Ovarian epithelial inclusions (OEI), 7 Fallopian tubal epithelia (FTE), and 6 ovarian surface epithelia (OSE). Correlation analyses of ovarian serous tumors including its precursor OEIs with FTE and OSE samples were performed by unsupervised hierarchical clustering. Rank-sum analyses and Pearson correlation tests were then applied to determine the likelihood of cellular origin of LGSC and its precursors. Final validation was done on the selected genes and corresponding proteins.

Results: LGSC share gene expression profile significantly with FTE cells, as well as with OEI and serous tumors, yet differently from that of OSE. Furthermore, phylogenetic tree produced by unsupervised hierarchical clustering showed ovarian serous tumors are hierarchically clustered with OEIs and FTE, but separated from OSEs. After ascertaining the reliability of sequencing data, we found that OVGP1, WT-1, and FOM3 highly expressed in samples of the fallopian tube, OEI, and ovarian serous tumors, but not in OSE. In contrast, ARX and FNC1 were mainly expressed in OSE, but not in other studied samples.

Conclusions: This study provides molecular evidence that ovarian LGSC most likely originates from the fallopian tube rather than from ovarian surface epithelia. Similar gene expression profiles among FTE and OEI, and serous tumors further support that the majority of LGSC develops in a step-wise fashion. Such findings may have significant implications for "ovarian" cancer-prevention strategies.

1152 NTRK Fusions Define a Novel Uterine Sarcoma Subtype with Features of Fibrosarcoma

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Background: Neurotrophic tyrosine kinase receptor (*NTRK*) 1-3 genes encode for tropomyosin receptor kinases (TRK) involved in neuronal regulation. *NTRK* fusions have been found in diverse tumor types. TRK inhibitor clinical trials show high response rates in patients with tumors harboring *NTRK* fusion. We identified 3 *NTRK* fusion-positive uterine sarcomas that should be distinguished from leiomyosarcoma (LMS).

Design: *NTRK* rearrangements were detected by FISH or targeted RNA sequencing in the clinical work-up of 3 uterine high-grade sarcoma, NOS. Due to histologic overlap with LMS, NTRK1 and pan-NTRK immunohistochemistry was performed on tissue microarrays of 97 uterine LMS. *NTRK1-3* break-apart FISH was performed on tumors with NTRK1 or pan-NTRK staining. Whole transcriptome RNA sequencing was performed on frozen tissue from an LMS with NTRK1 expression.

Results: *TPM3-NTRK1*, *LMNA-NTRK1* and *RBPMS-NTRK3* fusions were detected by FISH and targeted RNA sequencing. Patients presented at 28, 46 and 47 years with FIGO stage I tumors that showed infiltrative or pushing myometrial invasion and consisted of fascicles of uniform spindle cells with mild nuclear atypia and eosinophilic cytoplasm arranged in a herringbone pattern. Mitotic index was 7-15 mitotic figures/10 high power fields. Tumor necrosis was seen in 2. SMA was focal in 2, while desmin, ER and PR were negative in all tumors. Pan-NTRK was expressed in all tumors with concurrent NTRK1 staining in 2. One patient was disease-free at 12 months; another recurred at 9 months; the third recurred at 16 months and died of disease 4 years later. NTRK1 and pan-NTRK staining was seen in 4 and 2 LMS, respectively, but lacked *NTRK* fusions or alternative isoforms by FISH or whole transcriptome sequencing.

Conclusions: *NTRK* fusion-positive uterine sarcomas constitute a novel tumor type with features of fibrosarcoma based on lack of myogenic differentiation and histologic resemblance to soft tissue fibrosarcoma. Recognition of its morphology and immunophenotype may prompt NTRK immunohistochemistry and confirmation of *NTRK* fusion. NTRK1 and pan-NTRK expression may be seen in some uterine LMS and does not correlate with *NTRK* rearrangement. Patients with *NTRK* fusion-positive uterine sarcomas may benefit from TRK inhibition.

1153 Expression of the Immunomodulatory Molecules IDO and PD-L1 in Cervical and Vulvar Invasive and Intraepithelial Neoplasias

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Background: The immunoregulatory enzyme indoleamine dioxygenase 2,3 (IDO) has been implicated in cervical squamous carcinomas (SCC) and may represent a mechanism of resistance to anti-PD-1/anti-PD-L1 checkpoint inhibitor therapy. Anti-IDO therapies are clinically available and may be of utility alone or in combination with drugs targeting the PD-1/PD-L1 axis. However, the relationship between IDO and PD-L1 has not been well investigated in cervical and vulvar neoplasias, nor have differences in HPV-related vs. HPV-independent lesions been well characterized.

Design: 65 cases of cervical SCC (n=16), HPV-associated vulvar SCC (n=18), differentiated vulvar intraepithelial neoplasia (dVIN)-associated vulvar SCC (n=3), dVIN (n=2), VIN 3 (n=12), and CIN3 (n=14) were collected from the archival pathology files and reviewed. IDO and PD-L1 immunohistochemical staining was performed and scored in the tumor cells based on staining extent as 0%, 1-5%, 6-10%, 11-25%, 26-50%, or >50%.

Results: Overall, IDO expression was seen in slightly more than half of all SCCs (51%, 19/37), while the majority (73%, 27/37) expressed PD-L1. IDO and PD-L1 co-expression was seen in 43% (16/37). At the >10% cutoff, cervical SCCs were more likely to be positive than HPV-related vulvar SCCs for IDO [44%, 7/16 vs 0%, 0/18, p=0.002] and PD-L1 [56%, 9/16 vs 17%, 3/18, p=0.03]. Furthermore, cervical SCC showed more co-expression compared to vulvar HPV-related SCC (69%,

11/16 vs 11%, 2/18). There were no significant differences in IDO and PD-L1 expression in dVIN-associated vs. HPV-associated vulvar SCC. A minority of SCCs showed >50% staining for IDO (25%, 4/16) and/or PD-L1 (19%, 3/16); these cases were often diffusely positive. SCCs showing <50% immunostaining typically showed expression concentrated at the invasive front. In contrast to invasive lesions, the majority of vulvar and cervical dysplasias were entirely negative for both PD-L1 (93%, 26/28) and IDO (75%, 21/28).

Conclusions: IDO and PD-L1 expression is common in cervical and vulvar SCCs, suggesting potential for synergistic immunotherapy in this setting. Furthermore, IDO and PD-L1 expression is more common in the cervical SCC compared to vulvar SCC irrespective of HPV status, suggesting differences in the immune milieu and potential for immunotherapeutic response; however, data on HPV-independent lesions is limited. Both IDO and PD-L1 expression are rare in intraepithelial lesions, providing no support for a role for immunotherapy in the absence of invasion.

1154 High-grade serous carcinoma with classic versus variant morphology; any immunophenotypic correlates of unusual patterns?

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Background: Numerous studies suggest that many high-grade ovarian tumors exhibiting unusual morphologic patterns actually represent ovarian high-grade serous carcinoma (OHGSC); however, limited data exists to address the utility of immunohistochemistry (IHC) in these cases. The aim of this study is to further investigate the value of IHC in these challenging cases.

Design: We retrieved 53 consecutive cases previously diagnosed as OHGSC without the use of IHC from 2002 to 2016. H&E-stained slides were reviewed by three pathologists and evaluated for the presence of classic architectural patterns of OHGSC (papillary, compressed papillary, solid/transitional cell-like, cribriform, and/or mixtures of). A panel of IHC stains (p53, p16, WT1, PAX8, ER, PR, and NapsinA) were performed on tissue microarrays of representative tumor sections and interpreted.

Results: Of the 53 cases, 34 (64%) displayed classic architectural patterns of OHGSC (group 1) versus 19 (36%) with variant morphology (group 2). The IHC results of these two groups is summarizes in Table. Within group 2, 12 of 19 (63.1%) cases expressed the expected p53-mutated/p16-positive/WT1-positive immunophenotype and four cases exhibited wild-type p53 expression. After a review of morphology and IHC findings, two of these cases were reclassified (one as endometrioid and the other as clear cell carcinoma). Studies are underway to further classify the two remaining wild-type p53 cases. Among group 1, the majority of cases showed aberrant p53. While the expected p53-mutated/p16-positive/WT1-positive immunophenotype was found in 25 (74%) cases in this group, three cases had wild-type p53 staining with absent p16 expression and were reclassified.

Morphologic Category	Aberrant p53	p16	WT1	PAX8	ER	PR	NapsinA
Group 1 (Classic Pattern)	31/34 (91%)	28/34 (71%)	29/34 (89%)	34/34 (100%)	25/34 (74%)	13/34 (38%)	0/34 (0%)
Group 2 (Variant Pattern)	15/19 (79%)	15/19 (84%)	16/19 (84%)	18/19 (95%)	12/19 (66%)	6/19 (32%)	1/19 (5%)

Conclusions: In the vast majority of our cases, consistent with contemporary cohort study findings, our data supports that IHC is a valuable tool for the correct diagnosis and classification of high-grade ovarian tumors with unusual morphologic patterns. Despite the limited number of cases, in our experience, IHC is useful even in tumors with classic architectural patterns to support the diagnosis of OHGSC.

1155 Mutational Analysis of Metachronous Ovarian Serous Borderline Tumor and Serous Carcinoma by Digital Droplet PCR

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Background: Patients with ovarian serous borderline tumor (SBT), particularly the micropapillary variant, are at increased risk for subsequent low-grade serous carcinoma (LGSC). SBT/LGSCs are genomically-stable tumors, of which around 60% harbor mutually exclusive activating mutations in *KRAS* or *BRAF*, in approximately equal proportions. We have previously presented Sanger sequencing data from a cohort of patients initially diagnosed with SBT who subsequently developed LGSC. We now report follow-up results obtained from the more sensitive technique of digital droplet PCR (ddPCR), which along with immunohistochemical staining for $BRAF^{V600E}$, reveals some unexpected new insights into the molecular features of these tumors.

Design: 33 matched FFPE tumor specimens from women initially diagnosed with SBT and subsequently developed serous carcinoma (32 LGSC, 1 high-grade) were obtained from a population-based study cohort. ddPCR was performed to analyze mutation hotspots in *KRAS* and *BRAF*. The threshold for detection of a mutant allele was set at an allelic frequency of ≥1.5%. $BRAF^{V600E}$ (clone VE1) immunohistochemistry was performed on selected cases.

Results: Of 33 matched SBT/serous carcinoma pairs, the mutation status for *KRAS* and *BRAF* was concordant in all (100%) cases. Of these, 20 (60.6%) were $KRAS^{mut}/BRAF^{wt}$ (including the high-grade case), 1 (3.0%) was $BRAF^{mut}/KRAS^{wt}$, 1 (3.0%) was $BRAF^{mut}/KRAS^{mut}$ and 9 (27.3%) were wildtype for both genes. In the 2 (6.1%) remaining cases, both SBT and LGSC were $BRAF^{mut}$, however a minor $KRAS^{mut}$ subpopulation was also detected exclusively in the LGSC. Immunohistochemical staining using the $BRAF^{V600E}$-mutant specific antibody revealed a heterogeneous pattern of staining in cases with co-existing *KRAS* and *BRAF* mutations.

Conclusions: *KRAS* and *BRAF* mutations are generally mutually exclusive, though may co-exist in rare cases. Metachronous SBT and LGSC show concordant mutation status in all cases, suggestive of a clonal relationship. There was an enrichment for *KRAS* mutations in this cohort.

1156 A Comparison of CTH, HNF-1β, Napsin and ER as Markers for Endometrial Clear Cell Carcinoma and its Mimics

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Disclosures:
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Background: Endometrial clear cell carcinoma (CCC) can be a challenging diagnosis, due to its morphologic overlap with other endometrial carcinomas (particularly in CCC that do not bear cells with clear cytoplasm) and other benign/malignant entities that can exhibit clear cell changes. Recently, through in-depth quantitative proteomics profiling, CTH (cystathionine γ-lyase) has been identified as a novel marker for CCC. In this study, we assess the performance of CTH against the well-known markers ER (estrogen receptor), HNF-1β (hepatocyte nuclear factor-1-beta) and Napsin-A as markers for endometrial CCC and the ability to distinguish CCC from its mimics.

Design: We studied a series of 58 uterine clear cell carcinomas (5 cervical, 52 endometrial, 1 pelvic recurrence), 4 mixed CCC/serous carcinoma (SC), 3 mixed CCC/endometrioid carcinoma (EC), 7 endometrioid carcinoma/atypical hyperplasia (EC/AH) with clear cell changes and 10 Arias-Stella reaction (ASR). Immunohistochemical stains for CTH, ER, HNF-1β and Napsin were assessed on tissue microarray, except for ASR which was done on whole sections due to their focality. All cases were given an intensity score (0=no staining, 1=weak, 2=moderate, 3=strong), proportion score for percentage of cells staining (scored in increments of 10%), and H-score (intensity score multiplied by proportion score).

Results: Results are summarized in Table 1.

Both CTH and HNF-1β had very high sensitivity for detecting CCC (98% and 90% respectively), and were slightly better than Napsin (78%). CTH had the highest overall intensity, proportion and H-scores compared to both HNF-1β and Napsin. However, all three markers suffered from low specificity (0-27%), showing staining (albeit weaker and in a lower proportion of cells) in mixed carcinomas and EC/AH with clear cell changes. Interestingly, ASR demonstrated fairly moderate-strong and diffuse staining for all of CTH, HNF-1β and Napsin. Ultimately, negative staining for ER ended up being the most specific marker for CCC (86%) and had the highest NPV (96%). All cases of ASR were positive for ER.

	Diagnosis	CTH	ER	HNF-1β	Napsin
Intensity	CCC	2.36	0.16	1.93	1.19
	Mixed CCC/SC	1.25	1.25	2.00	0.50
	Mixed CCC/EC	1.33	0	0.67	0
	EC/AH with clear cell change	1.86	1.43	1.00	0.57
	Arias Stella	2.70	1.71	2.00	2.00
Proportion	CCC	81.4	3.45	66.4	38.1
	Mixed CCC/SC	72.5	20.0	67.5	7.5
	Mixed CCC/EC	53.3	0	26.7	0
	EC/AH with clear cell change	41.4	38.6	34.3	11.4
	Arias Stella	93.0	58.6	77.0	57.5
H-Score	CCC	208	5	160	62
	Mixed CCC/SC	93	38	185	15
	Mixed CCC/EC	120	0	53	0
	EC/AH with clear cell change	80	77	63	11
	Arias Stella	251	101	182	128
Sensitivity*		98%	88%	90%	78%
Specificity*		0	86%	18%	27%
PPV*		77%	63%	79%	82%
NPV*		0	96%	33%	19%

Conclusions: Although CTH was the most sensitive marker for CCC and demonstrated the highest overall H-scores, it had suboptimal specificity. This predicament was also apparent in HNF-1β and Napsin. Overall, ER (negative staining pattern) was the most specific marker for CCC. Combining CTH, HNF-1β or Napsin with ER is needed to optimize the sensitivity/specificity of these markers as a diagnostic tool for uterine CCC.

1157 Morphological Features Distinguishing Metastatic versus Primary Mucinous Tumors of The Ovary: An Interobserver Variability Study.

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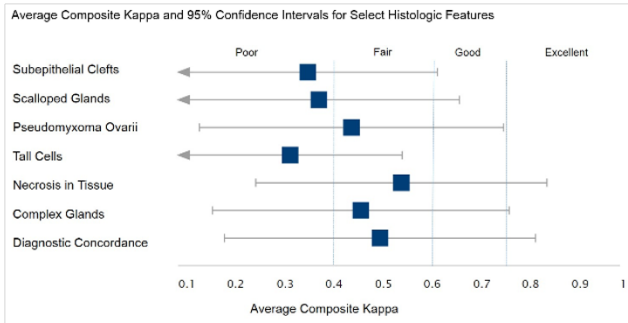
Background: Ovarian mucinous tumors (OMT) pose a diagnostic challenge, as morphologic features of primary versus metastatic tumors show significant overlap. Histologic findings of subepithelial clefts, scalloped glands, cellular stroma, and mucin pools have been identified to be helpful in distinguishing primary from metastatic tumors (Stewart CJR et al, Int J Gynecol Pathol. 2014). Our study assessed the reproducibility of these and other histologic features.

Design: 27 patients with unilateral (23) and bilateral (4) OMT were selected. Site of origin included: 14 primary borderline OMT, 8 metastases from appendiceal primaries and 5 others (metastases from endocervical and cecal primary). Representative H&E slides (1-4 slides/case) were reviewed by 5 pathologists (3 GYN and 2 GI focused) blinded to the original diagnosis. 7 histologic features were scored (subepithelial clefts, scalloped glands, tall mucinous cells, mucin pools, reactive ovarian stroma, necrosis, and complex highly cellular growth) and the tumor was classified as primary or metastatic. Interobserver variability using Cohen's Kappa statistics was calculated.

Results: Pathologists scored an overall "fair" agreement (diagnostic concordance, κ=0.512) when distinguishing primary OMT from metastasis. Necrosis had the best interobserver agreement among reviewers (κ=0.556). Complex highly cellular growth (κ=0.473) and mucin pools (κ=0.454) had "fair" agreements while subepithelial clefts (κ=0.326), scalloped glands (κ=0.336), and tall cell cells (κ=0.238) had "poor" overall agreement (Figure 1). Absence of mucin pools, presence of necrosis and complex highly cellular growth were the most helpful findings in differentiating primary versus metastatic OMT.



Conclusions: The use of morphologic features to distinguish primary versus metastatic OMT has only "fair" interobserver agreement and diagnostic concordance. The presence of necrosis, complex highly cellular growth and absence of mucin pools were the most helpful and reproducible morphologic features in establishing the diagnosis of primary OMT. Subepithelial clefts, scalloped glands, reactive ovarian stroma and tall cells were poorly reproducible and not helpful in differentiating primary versus metastatic OMT.

1158 Clinicopathologic and Immunohistochemical Correlates of CTNNB1 Mutated Endometrial Carcinoma

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Background: Endometrioid endometrial carcinomas (EECs) with exon 3 *CTNNB1* mutations characterize a more aggressive subset of tumors in patients with low-grade low-stage disease. The aim of this study was to evaluate 1) the clinicopathologic features of EECs with exon 3 *CTNNB1* mutations and 2) the feasibility of β-catenin immunohistochemistry to screen low-grade low-stage EECs to identify tumors at higher risk of recurrence.

Design: 42 *CTNNB1* mutated (MT) EECs and 28 *CTNNB1* wild-type (WT) EECs (determined by next-generation sequencing) were identified. Immunohistochemistry for β-catenin and CyclinD1, a putative target for β-catenin activation, was performed on all cases. Results were correlated with *CTNNB1* mutation status and clinicopathological parameters.

Results: The clinicopathologic and molecular findings are summarized in Table 1. Patients with *CTNNB1* MT EECs were younger than those with *CTNNB1* WT (57.2 vs. 63.6 years; p=0.02). *CTNNB1* MT correlated with β-catenin IHC positivity (p=<0.0001). Recurrence rate in patients with stage IA disease at diagnosis was significantly higher in patients whose tumors were *CTNNB1* MT compared to *CTNNB1* WT (28% vs. 0%; p=0.026); all recurrent tumors in this group harbored exon 3 mutations and were histologically low grade (5 grade 1, 2 grade 2). Nuclear β-catenin expression was 91.2% sensitive and 86.1% specific for exon 3 *CTNNB1* mutation. CyclinD1 expression correlated with overall *CTNNB1* MT status (p=0.002), but did not significantly correlate with exon 3 *CTNNB1* mutational status, β-catenin expression, or recurrence.

Table 1		CTNNB1 Mutated	CTNNB1 Wild-Type
		(n=42)	(n=28)
Age (years)	Mean	57.2	63.6
	Range	25-80	47-83
		[n](%)	[n](%)
Tumor type	EEC[a] grade 1	19 (45)	28 (100)
	EEC grade 2	7 (17)	0
	EEC grade 3	5 (12)	0
	EEC Metastasis	8 (19)	0
	Other	3 (7)	0
-catenin IHC	Positive[e]	32 (76)	2 (7)
	Negative	10 (24)	26 (93)
Cyclin D1 IHC	Positive[f]	13 (31)	0
	Negative	29 (69)	22* (100)
Exon	Exon 3	36[b] (86)	NA
	Other	13 (31)	NA
FIGO Stage	I-II	34 (81)	26 (93)
	III-IV	8 (19)	2 (7)
		Stage IA CTNNB1 MT[c]	Stage IA CTNNB1 WT[d]
		(n=25)	(n=21)
		[n](%)	[n](%)
Follow up available		23 (92)	21 (100)
Recurrence		7 (28)	0
		Exon 3 CTNNB1 MT	Non-exon 3 CTNNB1 MT
		(n=34)	(n=8)
Age (years)	Mean	60	66
	Range	25-80	53-79
		[n](%)	[n](%)
-catenin IHC	Positive[e]	31 (91)	1 (1)
	Negative	3 (8)	7 (9)
Cyclin D1 IHC	Positive[f]	10 (29)	3 (37)
	Negative	24 (71)	5 (63)

[a]Endometrioid Endometrial Carcinoma; [b]2 cases had 2 different exon 3 mutations each; [c]Mutated; [d]Wild type; [e] Any definitive nuclear positivity;[f] ≥50% nuclei positive; *material for cyclin D1 only available in 22 of 28 WT cases

Conclusions: Low-grade low-stage EECs with *CTNNB1* mutations demonstrated higher rates of recurrence when compared with *CTNNB1* WT EEC, in keeping with previous studies. Nuclear β-catenin expression is sensitive and specific for exon 3 *CTNNB1* mutation, and thus represents a potential low-cost method of screening for this aggressive subset of low-grade low-stage EEC.

1159 Correlation of Atypical Mitoses with Copy Number Variation and TP53 Mutations in Non-Serous Endometrial Carcinomas.

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Background: Endometrial carcinomas (ECs) can be stratified into 4 clinically significant molecular groups. The 'copy number high' (CN-H) molecular group has the poorest overall survival and exhibits predominantly serous morphology; however, a subset of CN-H ECs are endometrioid in histotype. To date little is known regarding atypical mitoses (ATMs) and their correlation with molecular groups in ECs. The aim of our study was 1) to ascertain the frequency and spectrum of ATMs in non-serous ECs, and 2) to establish whether ATMs can be used as a morphological correlate for *TP53* mutation and/or high copy number variation (CNVs) in the absence of serous histotype.

Design: 115 non-serous ECs with targeted next generation sequencing (NGS) data were evaluated for the presence and extent of ATMs. ATMs were divided into two morphological subtypes: Group A was defined as any bizarre mitotic figure with more than one spindle axis (as would be seen in tetrapolar or tripolar mitoses) or any obviously aneuploid metaphases, and Group B was defined as any mitotic figure in which individual sister chromatids lag behind or lack attachment to the spindle apparatus or in which single chromatids were tethered to both spindle poles. One representative tumor slide was examined from each case, with a second slide examined in the absence of ATMs on the first slide. Extent of ATMs was scored semi-quantitatively (focal, moderate, or extensive). Results were correlated with *TP53* status and CNVs.

Results: 103/115 (89.5%) of tumors contained at least focal ATMs (61 Group A and 42 group B). Of the entire cohort, the mean CNV was 35.5 (range 0-205) and 46 (40%) cases harbored at least one *TP53* mutation. The mean number of CNVs was significantly higher in ECs with any Group A ATM compared to ECs with no ATMs or only Group B ATMs present (35.5 vs. 7.8; p=<0.001). The sensitivity and specificity of any ATM for CNV-H or TP53 mutation was 96.0% and 11.8% respectively, with a negative predictive value (NPV) of 77.8%. The sensitivity and specificity of any group A ATM for CNV-H or TP53 mutation was 79.2% and 59.6% respectively, with a NPV of 75.6%. See Table 1 and 2 for summary of results.

Table 1	Group A ATM	Group B ATM	No ATM
N(%) of histotype in each ATM group			
Endometrioid (n=72)	36 (59)	28 (67)	8 (66)
Clear Cell (n=2)	1 (2)	1 (2)	0
Undiff/dediff* (n=5)	3(5)	2(5)	0
Mixed/ambiguous (n=36)	21 (34)	11 (26)	4 (33)
Total (all cases)	61	42	12

*Undifferentiated/dedifferentiated

Table 2		TP53[a]	CNV-H[b,c]	TP53 & CNV-H	Either CNV-H or TP53	No TP53 or CNV-H
Group A (61)***	N (%)	20	4	16	38	21
Group B (42)		3	3	3	10	31
No ATMs (12)		2	1	1	2	8

[a]tumor with any TP53 mutation regardless of CNV [b]CNV-High =>50 CNVs; [c] Tumor with any CNV-H regardless of TP53 mutation *** total number in each ATM group

Conclusions: ATMs are common in non-serous ECs. Group A ATMs are easily identified and are more likely to be found in association with *TP53* mutation and high level CNVs, thus offering a potential low-cost screening method for the CN-H subgroup in the absence of classic serous morphology.

1160 Undifferentiated Uterine Sarcomas Represent Underrecognized High-Grade Endometrial Stromal Sarcomas

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Background: Endometrial stromal sarcoma (ESS) represents 1% of all uterine malignant neoplasms and is classified as low- or high-grade (HG) ESS and undifferentiated uterine sarcoma (UUS). UUS is a diagnosis of exclusion, but shares morphologic and immunophenotypic features with HGESS. UUS may share similar genetic abnormalities with HGESS or harbor novel gene fusions. Fluorescence in situ hybridization (FISH) and next-generation targeted RNA sequencing of UUS may unveil gene fusions that allow accurate tumor classification.

Design: All available slides and archival formalin-fixed paraffin-embedded tumor tissue were obtained from 10 UUS (7 primary, 3 metastatic) in which 8 were monomorphic, and 2 pleomorphic. BCOR immunohistochemistry was performed to triage tumors with expression in >50% cells for FISH using break-apart probes flanking *BCOR*, *ZC3H7B*, *CCNB3*, *YWHAE*, *NUTM2*, *JAZF1* and *BCORL1*. RNA was extracted from macrodissected tumor tissue and subjected to targeted RNA sequencing using the Archer Anchored Multiplex PCR technology in all FISH-negative tumors. Morphology was correlated with fusion status.

Results: BCOR expression was moderate to strong in >50% cells in 8 tumors; 1 tumor showed weak staining in <5% cells, and another was negative. FISH detected *ZC3H7B-BCOR* fusion, *YWHAE-NUTM2* fusion and *YWHAE* rearrangement with no known partner in 2, 1 and 2 UUS, respectively. Tumors with *YWHAE* rearrangement and no known partner were pleomorphic. Targeted RNA sequencing of 5 UUS identified *BRD8-PHF1* fusion, *YWHAE-NUTM2B* fusion and *BCOR* internal tandem duplication (ITD) in 3 FISH-negative tumors. Tumors with *ZC3H7B-BCOR* and *YWHAE-NUTM2* fusions and *BCOR* ITD showed morphology characteristic of HGESS. The tumor harboring *BRD8-PHF1* fusion exhibited round cells arranged in nests and embedded in abundant myxoid stroma. Based of FISH and sequencing results, 80% of UUS were reclassified as HGESS.

Conclusions: Most UUS represent underrecognized HGESS with known gene fusions. BCOR immunohistochemistry with FISH and/or targeted RNA sequencing may aid in accurate classification of UUS. A second confirmatory assay is necessary in identifying gene fusions if FISH or sequencing is negative. *YWHAE* rearrangement may be seen in pleomorphic UUS. Targeted RNA sequencing can detect *BCOR* ITD. *BRD8-PHF1* ESS may represent a novel type HGESS based on morphologic features.

1161 Sentinel Lymph Node Ultrastaging in Endometrial Carcinoma: Compliance and Standardized Reporting

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Background: Sentinel lymph node (SLN) mapping benefits endometrial carcinoma patients by providing staging information with reduced morbidity. Ultrastaging or enhanced processing of SLN improves detection of lymph node (LN) metastasis, but protocols vary among institutions, and little is known regarding protocol compliance and reporting of results. Most studies to date include a predominant low risk population. In this study we present results of SLN ultrastaging and pathology reporting in a multi-center prospective trial that included 70% high grade endometrial cancers.

Design: SLN mapping and lymphadenectomy were performed on grade 2 and high grade endometrial cancer patients. The SLN ultrastaging protocol included 4 H&E sections at different levels for frozen section and routine processing, and 1 for AE1/AE3 immunohistochemistry (IHC). Positive SLN were classified as: isolated tumor cells (ITC) (single cells or small clusters <0.2mm), micrometastasis (0.2-2mm), and macrometastasis (>2mm). All pathology reports and positive SLN were reviewed.

Results: Patient and tumor characteristics are listed in *Table 1*. One or more SLN (mean 4.1; range 1-20) was removed in 87 cases, with 1 failed procedure. Additional review was available in 59 patients as presented in *Table 2*. For frozen section: sensitivity (Sn) was 22%, negative predictive value (NPV) was 87%, and false negative rate (FNR) was 78%. Ultrastaging alone detected low volume metastases in 5 (9.6%) patients. For those with positive SLN, 4 (50%) were diagnosed only on IHC. SLN status predicted overall lymph node status with a Sn of 89%, NPV of 98%, and FNR of 11%.

Table 1

Total number	Mean age (range)	Grade 2 Endometrioid	Grade 3 Endometrioid	Serous	Mixed	Clear cell	Carcinosarcoma	Undifferentiated
88	63.4 (40 - 77)	25 (28.4%)	16 (18.2%)	27 (30.7%)	8 (9.1%)	2 (2.3%)	9 (10%)	1 (1.1%)

Table 2

Total number	Frozen section performed	Ultrastaging protocol completed	SLN positive patients	Patients with multiple positive SLN	Total number positive SLN	Size of metastasis reported	Micrometastasis	ITC
59	56 (94.9%)	52 (88.1%)	8 (13.6%)	4 (50%)	11	5 (45.5%)	6 (54.5%)	5 (45.5%)

Conclusions: Compliance with ultrastaging protocol was high in this study. Frozen section diagnosis of SLN may be of limited clinical value. Classification of SLN was non-uniform and reproducibility may be improved by the use of standardized reporting templates. While the clinical significance of low volume metastases is uncertain in high grade endometrial cancers, the inclusion of IHC improves their detection, and therefore, ultrastaging of SLN is recommended. The findings add to data indicating that SLN ultrastaging in endometrial carcinoma accurately identifies LN metastasis. Adherence to protocol and standardized reporting is of importance in increasing the likelihood of detection and in effectively relaying this information.

1162 Integration of Massively Parallel Sequencing and Immunohistochemistry for the Classification of Prospectively Collected Endometrial Cancers into Prognostically Relevant Subgroups

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Background: The Cancer Genome Atlas (TCGA) employed whole-exome sequencing and microsatellite instability (MSI) data to define four molecular subgroups of endometrioid and serous ECs associated with distinct outcomes: 1) *POLE*-exonuclease domain mutated (EDM; ultramutated), 2) high levels of MSI (MSI-H; hypermutated), 3) copy number-low/endometrioid-like (CNL-EL) and 4) copy number-high/serous-like (CNH-SL). Here we sought to define whether targeted sequencing and immunohistochemistry (IHC) could be integrated to classify prospectively collected endometrial cancers (ECs) in the clinical setting, including various histologic subtypes not previously studied by TCGA.

Design: Primary ECs and corresponding normal tissue from patients who consented to the study between 05/2016 and 07/2017 were subjected to a massively parallel sequencing assay targeting all exons and select introns of 468 cancer genes. IHC for DNA mismatch repair (MMR) proteins and p53 was also performed in all cases. ECs were classified based on IHC and sequencing results into one of the four TCGA subgroups: 1) *POLE* EDM-mutant tumors were classified as *POLE*-EDM; 2) ECs with abnormal DNA MMR IHC, and/or hypermutated phenotype and/or high MSI score by sequencing (MSI sensor) as MSI-H, and 3) ECs displaying abnormal p53 IHC and/or *TP53* mutations and/or abundant gene copy number changes as CNH-SL. *TP53*/p53, *POLE* and MMR wild-type ECs (i.e. all remaining cases) were classified as CNL-EL. The concordance between IHC and sequencing results was also investigated.

Results: A total of 179 ECs of various histologic types, including carcinosarcomas, endometrioid, serous, de/undifferentiated, clear cell and mixed carcinomas, were included in this study . The distribution of molecular subgroups in this series was *POLE* 7.8%, MSI-H 28.4%, CNL-EL 38.5% and CNH-SL 25.1%, however distinct histologic subtypes displayed different distributions of molecular subgroups. The results for p53/*TP53* assessment between IHC and MSK-IMPACT were concordant in 88% of cases, and for MSI-H ECs in 93%.

Conclusions: The addition of genomics to IHC and *POLE* mutation status leads to a distinct classification of ECs in 12.3% of cases, suggesting that an approach integrating IHC and massively parallel sequencing may be most robust in defining prognostically relevant molecular subgroups of ECs.

1163 Loss of PERP as a Diagnostic Biomarker for Differentiated Vulvar Intraepithelial Neoplasia (dVIN)

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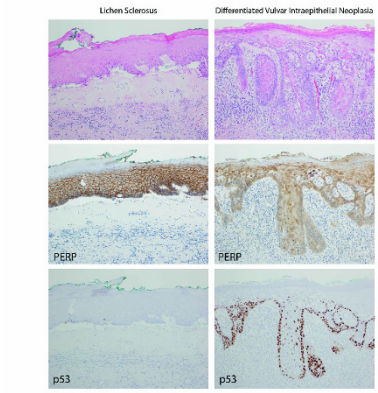
Background: Recognition of differentiated vulvar intraepithelial neoplasia (dVIN) continues to be a challenge as its features are often inconspicuous and may be difficult to distinguish from reactive conditions such as lichen sclerosus (LS) or lichen simplex chronicus (LSC). The majority of dVIN cases demonstrate p53 positivity; however, nonspecific patchy staining may also be present in reactive conditions. Identifying additional markers for the evaluation of atypical vulvar squamous proliferations is essential for diagnostic precision and appropriate clinical management. *PERP* (p53 apoptosis effector related to PMP-22) is a gene that plays a critical role in cell-cell adhesion and tumor suppression that is lost in a large proportion of oral cavity invasive and in situ squamous cell carcinomas. In this study, we investigate the utility of PERP as a biomarker for the diagnosis of dVIN.

Design: Immunohistochemical stains for PERP and p53 were performed on formalin-fixed, paraffin-embedded (FFPE) tissue sections from cases of dVIN (n=20), lichen sclerosus (n=21), lichen simplex chronicus (n=5) and usual (classic) VIN (n=5). Positive PERP staining was defined as distinct, membranous staining and scored based on the distribution of staining (intact = full epithelial thickness, loss = partial thickness or complete loss). P53 positivity was defined by strong continuous nuclear reactivity in the basal and parabasal layers.

Results: Loss of PERP staining was observed in 95% (19/20) of dVIN and 29% (6/21) of LS. P53 positivity was observed in 85% (17/20) of dVIN, and 0% (0/21) of LS (see Figure 1). No dVINs that had both intact PERP and negative p53 were identified. Similarly, no LS with both loss of PERP and positive p53 were identified. Of note, 6 cases of LS demonstrated patches of non-linear but elevated p53 staining that were considered equivocal for p53 staining; only one of these cases showed concurrent loss of PERP. All LSC demonstrated intact PERP and negative p53. All usual VIN demonstrated loss of PERP and negative p53.



Conclusions: Loss of PERP appears to represent an expansion of dysplastic basal/parabasal-type cells and is seen in the majority of dVIN and usual VIN cases. When combined with p53, PERP is 80% sensitive and 100% specific for dVIN and emerges as a promising biomarker to distinguish dVIN from histologic mimics.

1164 ALK-positive Tumors are Highly Enriched in the STUMP Subgroup of Uterine Tumors

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Background: ALK-positive tumors, such as inflammatory myofibroblastic tumors (IMTs), have recently become more widely recognized in the female genital tract and are important to identify given targeted therapeutic implications with ALK inhibitors. Smooth muscle tumors of uncertain malignant potential (STUMPs) are a heterogenous category of tumor with varying appearance and the potential for morphologic overlap with IMTs. Therefore, we predict that a significant number of uterine tumors previously classified as STUMP will exhibit ALK positivity and examine the diagnostic utility of screening tumors for ALK by immunohistochemistry (IHC) prior to rendering a diagnosis of STUMP.

Design: A total of fifty-one cases from 2007 to 2017 previously diagnosed as a STUMP or in which the diagnosis of STUMP was strongly considered with available formalin-fixed paraffin-embedded material were included in this retrospective study. Cases included tumors at uterine (n=34), extra-uterine (n=7), and non-gynecologic (n=10) sites. IHC for ALK was performed on whole sections. Positive cases underwent fluorescent in situ hybridization (FISH) to confirm ALK rearrangement.

Results: Four of the 34 uterine tumors (12%) were ALK IHC positive. All of the ALK-positive tumors showed myxoid features, and overall 36% of those with myxoid features were ALK-positive. FISH showed a classic ALK rearrangement break-apart pattern in ALK-positive tumors, except for one case which displayed an unusual fluorescence signal pattern characterized by numerous (3-8) single 3′ ALK signals. All tumors at extra-uterine or non-gynecologic sites were negative for ALK by IHC.

Conclusions: In our retrospective review of uterine tumors in which STUMP was a diagnostic consideration, 12% show aberrant expression and rearrangement of ALK. Importantly, all of the ALK-positive cases displayed myxoid features and were morphologically compatible with an IMT when re-reviewed. Interestingly, one of the ALK-positive cases showed multiple, isolated 3′ ALK signals, a pattern reported in non-small cell carcinomas of the lung, but not previously described in uterine IMTs. This study provides provisional data to support prospective ALK IHC screening of all uterine tumors being considered for the diagnosis of STUMP in order to more accurately identify patients with ALK-positive tumors that could potentially benefit from ALK-targeted therapy.

1165 Sporadic MLH1 Methylated Endometrial Adenocarcinoma is Frequently Association with Microcystic Elongated and Fragmented (MELF) Pattern of Myometrial Invasion

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Background: The microcystic elongated and fragmented (MELF) pattern of myometrial invasion in endometrial cancer (EC) can be a subtle feature and is associated with increased risk for lymph node metastasis. Previously, we observed in our own dataset that approximately 50% of EC cases with sentinel lymph nodes with isolated tumor cell metastasis also had sporadic MLH1 promotor methylation with frequent MELF pattern (unpublished data). We hypothesize that MELF pattern may be a common feature of *MLH1* methylated EC that has not been previously described.

Design: Mismatch repair immunohistochemistry (MMR IHC) is performed on EC hysterectomy specimens as part of our universal screening protocol. Tumors with loss of the heterodimer pair MLH1 and PMS2 were reflexed to *MLH1* promoter methylation analysis (methylation specific PCR). Retrospective review of clinicopathologic features including patient demographics, tumor stage, grade, histologic features and presence or absence of MELF pattern of invasion were recorded.

Results: Forty-seven consecutive sporadic EC 25 with dMMR and MLH1 methylation and 22 with intact MMR IHC, were included in this study. All cases were endometrioid histology, the majority low grade (FIGO grade I-II) with the exception of two grade III tumors in the unmethylated group. Fourteen of the 25 (56%) methylated cases showed MELF vs. two of the unmethylated group (P= 0.0008, chi square test). While the majority of cases were low stage (41 stage IA/IB), 5 patients in the methylated group had stage II disease or greater whereas, only one patient in the unmethylated group had stage II+ disease. Approximately 50% of tumors from each group showed a component of mucinous differentiation.

Conclusions: Our findings identified the MELF pattern of myometrial invasion as a frequent feature of EC with MLH1 promotor methylation; this is important to recognize as this patient population may be at increased risk of lymph node involvement. We will perform further analysis of our larger EC cohort to further corroborate these findings.

1166 Targeted Genomic Profiling of Progestin Treated Low Grade Endometrial Neoplasia Identifies Correlates of Therapy Response

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Background: Cellular mechanisms underlying lack of response to progestin therapy in low grade endometrial neoplasia are poorly understood. Using next generation sequencing (NGS), we surveyed the mutational landscape of atypical endometrial hyperplasia (AH) and low grade endometrioid carcinomas (ECs) that exhibited complete (CR), partial (PR) or no response (NR) to progestin therapy.

Design: 22 pre-treatment samples of AH (n=16) and FIGO grade 1 EC (n=6) from patients who subsequently underwent at least 6 months of oral progestin treatment were analyzed. CR (n=11), was characterized by resolution of architectural complexity and cytologic atypia, PR (n=3) by resolution of cytologic atypia only, and NR (n=8) by persistence of the original lesion. Formalin-fixed paraffin embedded lesional tissue was macrodissected for DNA extraction, followed by NGS survey of 146 genes using the Ion Torrent Oncomine comprehensive V3 assay (minimum 500X coverage). Immunohistochemistry (IHC) was performed for MLH1.

Results: 99 dbSNP/COSMIC mutations were observed. The most frequently altered genes were *PTEN* (15/22, 68%) and *CTNNB1* (β-catenin) (13/22, 59%), which were detected in CR, NR and PR samples. Of NR cases, 1 was *POLE* mutated and 2 displayed MLH1 protein loss by IHC (presumed MLH-1 methylation), suggesting microsatellite instability (MSI). Although there were no significant differences in overall mutational burden between CR and NR and PR samples (average of 4.7, 4.8 and 3.0 mutations/case, respectively), *PTEN* truncation mutations predominated in CR patients (10/11) compared to PR (1/3) and NR (1/8) samples. Notably, we identified mutations unique to CR in genes involved in DNA repair (*ATM, BRCA1 and RAD51*) and the PIK3CA pathway (*PTEN, PIK3R1* and *KRAS*), while mutations unique to NR primarily occurred in genes encoding for receptor tyrosine kinases (*MET, NF1, IGF1R, FGFR3, PTCH1 and NTRK1*).

Conclusions: For the first time, we present genomic profiling of a cohort of AH and low-grade ECs with a uniform minimum period of treatment with progestins and strictly defined histological outcomes. Mutations identified in CR versus NR cases arose within genes with different cellular functions. In particular, mutations in NR cases may be associated with constitutively activated pathways that drive cellular proliferation and/or POLE mutations and MSI. Further validation of these findings may lead to development of prognostic and predictive markers for management of low grade endometrial neoplasia with progestin therapy.

1167 Detection of p53 Abnormalities in High Grade Serous Carcinoma: IHC, NGS or Both?

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Background: Aberrant p53 function is characteristic of the

majority of high grade serous carcinomas (HGSCs). As such, p53 immunohistochemistry (IHC) is a useful diagnostic adjunct in histotype assignment of carcinomas of the upper Müllerian tract. As next generation sequencing (NGS) of tumor tissue begins to enter clinical diagnostic practice, this study aimed to explore the correlation between abnormal p53 IHC expression and p53 mutations.

Design: 45 HGSC were identified from our institutional database. H&E diagnosis was confirmed, and representative blocks of formalin-fixed paraffin embedded tissue were selected. Tissue microarrays were constructed (1mm cores in duplicates) and p53 IHC (DO7 mouse monoclonal antibody clone) was performed. p53 expression was scored as null (N) (no or very weak nuclear staining in < 5% of cells), overexpressed (OE) (+3 nuclear staining in > 75% of tumor cells) and wild type (WT). Tumor tissue was macrodissected and subjected to NGS (Ion Torrent S5XL, 500X average read depth). For cases with discrepant IHC and NGS results, IHC was performed on same whole tumor areas as those macrodissected for NGS.

Results: Aberrant p53 IHC was detected in 91% of cases, whereas 73% of cases exhibited a p53 mutation by NGS. The combination of IHC and NGS identified 98% of the cases. Two cases were reclassified from WT to OE IHC pattern based on whole section IHC review. Among the mutation positive and IHC aberrant cases, those with OE IHC pattern largely exhibited missense (ms) mutations, whereas nonsense (ns) and frameshift (fs) mutations predominated in the null IHC pattern group. 24% of cases were mutation negative, but had OE or null IHC patterns, suggesting that p53 protein in some HGSCs is dysregulated by post-transcriptional mechanisms. 7% of cases were IHC WT, but had p53 mutations. All cases with either WT IHC pattern or absence of p53 mutation demonstrated expression of Wt-1 and absence of Napsin-A expression by IHC.

Present (n=33, 73%)		p53 mutation (NGS)	
		Absent (n=12, 27%)	
p53 IHC pattern	Aberrant (n=41, 91%)	30 22 OE (19 ms, 2 fs, 1 fs and ms) 8 Null (4 ns, 1 ms, 2 fs, 1 fs and ms)	11 6 OE 5 Null
	Wild type (n=4, 9%)	3 (2 ns, 1 ms)	1

Conclusions: p53 dysregulation is the key pathogenetic abnormality in HGSC. In the majority of tumors, the pattern of aberrant p53 expression correlates with the type of p53 mutation. p53 IHC detects a greater proportion of p53 abnormalities than NGS, and should be considered as a first line diagnostic tool for histotype assignment purposes. In occasional cases when p53 IHC is WT and HGSC is strongly suspected on morphologic grounds, NGS may reveal a pathogenic mutation.

1168 Selective Versus Complete Sampling in Hysterectomy Specimens Performed for Atypical Hyperplasia—Balancing Limited Resources with Quality Diagnosis

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Background: Atypical hyperplasia (AH) of the endometrium is a significant risk factor for uterine endometrioid carcinoma (EC). A biopsy or curettage diagnosis of AH is often followed by a total hysterectomy. Standard sampling of these specimens includes histologic evaluation of the entire endometrium, with the goal of identifying possible EC and myometrial invasion. Given workforce and budget restraints across the health care industry, limitation of block submission is one method to optimize resources. We evaluated how selective versus complete sampling of the endometrium in hysterectomies performed for AH impacted the detection of EC, in an effort to reduce block submission while maintaining accurate diagnosis and staging.

Design: From 2008-present, 146 hysterectomies were performed at our institution for AH or had an incidental finding of AH. Cases in which the entire endomyometrial junction was submitted for histologic review were included in the study (141 cases). Fifty cases were analyzed for this abstract. Histologic diagnoses based on "selective sampling" (review of cervix, lower uterine segment, polyp(s), mass(es), frozen section controls, and every other block designated as "entire remaining endometrium") were compared to the original diagnosis.

Results: The initial 50 cases included diagnoses of EC in 30% (all stage T1a), serous carcinoma in 2%, AH in 62%, and benign usual hyperplasia (UH) in 6% of cases. Double-blinded review of these cases using the "selective sampling" method detected EC in 28%, serous carcinoma in 2%, AH in 56%, and UH in 14% of cases. Overall, selected sampling had 78% agreement with the final diagnoses. All cases with myometrial invasion (2), FIGO grade 2 (1), and serous carcinoma (1) were detected. Non-myoinvasive FIGO grade 1 EC was missed in one case, and diagnosed as AH. Selective sampling reduced the number of blocks by an average of 7.4 per case (range 2-14).

Conclusions: Selective sampling of the endomyometrial junction may be a reasonable method for evaluating hysterectomy specimens for EC in patients with a preoperative diagnosis of AH, detecting 93% of cases of EC, including all higher grade and myoinvasive tumors. While discordant diagnoses were seen in 22% of cases, the majority of these were attributable to interobserver variability in the diagnosis of AH vs minute foci of non-invasive EC or UH vs focal AH. Additional cases are being evaluated to determine if the clinical significance of these discrepancies justifies the use of additional resources.

1169 Evaluation of Lineage/Site of Origin Immunohistochemical (IHC) Markers (SATB2, CyclinD1, SALL4, PAX-8) in High Grade/Poorly Differentiated Neoplasms of the Gynecologic Tract

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Background: Poorly differentiated neoplasms of the gynecologic tract may be difficult to diagnose with regard to line of differentiation and/or anatomic site of origin. Diagnostic IHC with purported lineage or anatomic specificity are increasingly utilized when the differential diagnosis includes undifferentiated/dedifferentiated (undiff/dediff) carcinoma (Ca) or other high grade Ca, and include SATB2 (colorectal origin), Cyclin D1 (high grade endometrial stromal sarcoma), SALL4 (germ cell), and PAX8 (gynecologic carcinoma). Their specificity is not fully addressed in this setting.

Design: Our database was searched from 1/1/2000 to 6/24/2016 for undiff/dediff Ca, clear cell Ca, serous Ca, grade 3 (G3) endometrioid Ca, carcinosarcoma, and undiff uterine sarcoma. All cases were re-reviewed. Representative tumor blocks were selected for IHC studies on each case; SATB2, Cyclin D1, SALL4, and PAX8 were evaluated. Staining was graded as negative (<5%) or positive (5-100%), and the percentage of tumor cells staining was recorded.

Results: 127 cases were identified: clear cell Ca (13), G3 endometrioid (21), carcinosarcoma (21), undiff sarcoma (3), serous Ca of endometrium (27), serous Ca of ovary/fallopian tubes (11), and undiff/dediff Ca (31). SATB2 was positive in undiff sarcomas (100%, but with only 5% staining), carcinosarcomas (48%), G3 endometrioid Ca (38%), undiff/dediff endometrial Ca (32%), and endometrial serous Ca (30%), but was negative in clear cell Ca and ovarian serous Ca. SALL4 was positive in endometrial serous Ca (33%), carcinosarcoma (29%), undiff/dediff endometrial Ca (29%, high grade component), G3 endometrioid Ca (14%), ovarian serous Ca (9%), clear cell Ca (7%), and no sarcomas. CyclinD1 was expressed in all tumor types: G3 endometrioid Ca (90%), undiff/dediff endometrial Ca (87%), enendometrial serous Ca (81%), carcinosarcoma (71%), ovarian serous Ca (64%), clear cell Ca (53%), and undiff sarcoma (33%); however, only G3 endometrioid Ca and undiff/dediff endometrial Ca had strong and diffuse reactivity. PAX-8 expression was present in all clear cell Ca, endometrial serous Ca, and ovarian serous Ca, and in 90% of G3 endometrioid Ca, 81% of carcinosarcomas, 77% of undiff/dediff endometrial Ca (low grade component), and 33% of undiff sarcomas.

Conclusions: IHC markers that may be utilized in the differential diagnosis of gynecologic malignancies (SATB2, Cyclin D1, SALL4, and PAX8) have relatively low specificity for their intended utility in this setting.

1170 Endometrioid Adenocarcinoma Arising from Müllerian Adenosarcoma of the Uterus and Ovary: Clinico-Pathologic Characterization with Emphasis on its Distinction from Carcinosarcoma

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Background: Müllerian adenosarcoma is a mixed tumor histologically defined by the presence of benign epithelial elements and malignant stroma. Although focal glandular atypia has been recognized to occur in one-third of cases, only rare cases of coexisting endometrioid adenocarcinoma and adenosarcoma have been reported, interpreted as representing two independent tumors or a form of carcinosarcoma. The aim of this study is to document the occurrence of endometrioid adenocarcinoma arising in close association with Müllerian

adenosarcoma and identify characteristics that distinguish these rare tumors from carcinosarcoma.

Design: A multi-institutional search identified cases that met the following inclusion criteria: (1) adenosarcoma diagnosis supported by the presence of benign glandular epithelium and a malignant stromal component; (2) endometrioid adenocarcinoma intimately admixed with adenosarcoma. Cases of spatially separated adenocarcinoma and adenosarcoma were excluded. Pathological data were collected from the surgical pathology reports and reviewed by individual contributors.

Results: Twenty (n=20) cases meeting the criteria were identified. The tumors were located in the uterine corpus (16/20=80%), ovary (2/20=10%) and pelvis (2/20=10%). All the extra-uterine cases were associated with endometriosis at the tumor site. The Müllerian adenosarcoma component was classified as low-grade in 12/20 (60%) cases and high-grade in 8/20 (40%). Stromal overgrowth was present in 5/20 (25%) cases. Heterologous stromal elements were present in 7/20 (35%) cases. The adenocarcinoma component was low-grade endometrioid in all cases with FIGO grade 1 in 19/20 (95%) cases and grade 2 in 1/20 (5%). Background atypical hyperplasia/endometrial intraepithelial neoplasia was present in 7/20 (35%) cases. The majority of Müllerian adenosarcomas (14/16 cases; 87%) and endometrioid adenocarcinomas (15/16 cases; 94%) were FIGO stage I. Lymphovascular space invasion was present in 2/19 (10%) cases; lymph nodes were free of tumor involvement in 8/8 (100%) cases. There was no evidence of recurrent disease in 5/6 (83%) cases with a median follow-up of 13.5 months (range 6 to 180 months).

Conclusions: We describe the first series of endometrioid adenocarcinomas arising in close spatial association with Müllerian adenosarcomas. Unlike carcinosarcomas, these tumors are usually early-stage at presentation and the carcinomatous component is typically low grade. Extra-uterine tumors are strongly associated with endometriosis.

1171 Is There a Role for Exhaustive Ultrastaging of Sentinel Lymph Nodes in Vulvar Cancer?

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Background: Targeted sampling of regional lymph nodes, in the form of sentinel lymph nodes (SLNs), is becoming the standard of care for patients (pts) affected by vulvar cancer (CA) . The presence of metastasis (MET) in the SLN will usually trigger subsequent therapy on these pts. However, there is no universal SLN ultrastaging (US) protocol. This study compares the performance of an exhaustive ultrastaging method with other methods.

Design: We identified all cases of vulvar squamous cell CA with SLN (1993-2017) recording pt ages, tumor size, presence/absence lymphovascular invasion (LVI), #SLN/nonSLN, #positive SLN/nonSLN, MET size and how detected, and false negative rate. Pre 2007, negative SLNs on initial HE sections had either no (42 pts) or from 1-5 HE levels at 40 µm intervals +/- keratin immunohistochemistry (IHC) (31 pts). Post 2007, negative SLNs on initial HE sections had 5 additional HE levels cut at 250µm intervals with 2 unstained slides at each level; keratin IHC performed on level 1 in cases with negative HE levels (60 pts).

Results: For the entire study cohort, 24/133 pts (18%) had LN METS: 20 (15%) by initial HE; 3 (2.3%) detected by US; and 1 pt had a MET in a nonSLN (0.7%). Table 1 compares data for pre and post 2007 groups. Pre2007, a 0.9 mm MET was identified on the first level of a SLN that had US by 5 HE levels at 40µm intervals resulting in the identification of an additional pt with a positive LN. One pt in the pre 2007 group had a negative SLN but disease in a nonSLN (false negative). In this case, the negative SLN did not have US. Post 2007, US detected METs (0.2-1.0 mm) in 4 additional SLNs. Of these positive SLN, 3 had METs detected on HE level 2 or higher. Two were in pts who otherwise would not have been identified with a positive LN.

	Pre2007 n=73	Post2007 n=60
Median pt age (yr)	52.5 (20-88)	61 (33-89)
Median tu size (cm)	2 (.35-8.5)	2 (.2-6.6)
LVI present	12 (16.4%)	7 (11.7%)
#SLN (range per pt)	226 (1-10)	152 (1-9)
#nonSLN (range per pt)	646 (0-20)	167 (0-21)
#positive SLN	18 (15 pts, 20.5%)	10 (8 pts, 13.3%)
#SLN by US	1	4
Median SLN met size (mm)	7 (.3-45)	2.75 (.2-50)
#positive nonSLN	7	0
False negative	1 pt	0 pt

Conclusions: US increased detection of LN METs regardless of the protocol used and should be considered an essential step in the evaluation of SLNs from pts with vulvar squamous cell CA. The only false negative SLN in this series occurred in a case without US. Although the LN MET rate was similar between the two groups, the exhaustive protocol initiated in 2007 detected smaller metastases in more SLNs including METs present deep within the tissue block. While a universal recommendation for an exhaustive SLN protocol may be premature based on these findings, it is noted that even in the face of low volume disease these pts are nearly all treated.

1172 The Boundaries of the Lower Uterine Segment and its Assessment by Pathologists.

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Background: The prognostic significance of lower uterine segment involvement (LUSI) in endometrial carcinoma is unclear, with some studies demonstrating that LUSI confers a negative prognostic significance while others do not. However, most studies do not define the terms "involvement" or "lower uterine segment", which may perhaps explain the reasons for this difference. In this study, we attempted to assess the understanding of these terms among 16 gynecological pathologists/trainees.

Design: A 10 question brief survey was shared with participants, asking them to define "lower uterine segment" and "involvement". 3 questions included low magnification images from vertically sectioned uteri were also provided to assess definitional boundaries of the lower uterine segment.

Results: Respondents were predominantly gynecological pathologists (85%) in academic practice (85%). The lower uterine segment was variably described by respondents as "unsure", "1-1.5 cm", "no specific length even approximately", and "defined histologically by glandular morphology". Assessment of the submitted images showed marked variability among the 48 observations (3 slides x 16 respondents), with 53% of responses suggesting that the lower uterine segment was approximately 1 cm above the highest endocervical gland, 15% of responses suggesting that it was approximately 2 cm in length, and 35% indicating that they were unsure. Interpretation of the term "involvement" also showed variability among pathologists, with 31% of respondents using the term for non-invasive carcinoma and 54% for invasive carcinoma.

Conclusions: The lower uterine segment does not have anatomically well-defined landmarks, and there is lack of agreement amongst pathologists as to its boundaries. Biologically the LUS is well-defined only during labor. There is additional disagreement among pathologists on how to assess "involvement"- with at least a portion labeling endometrium-limited tumor as "involvement". The absence of consensus on definitions, may explain the differences between studies on the significance of endometrial carcinoma extension into the LUS.

1173 Clinical Outcomes of Patients with Tumoral Displacement into Fallopian Tubes in Patients Treated by Robotically Assisted Hysterectomy for Newly Diagnosed Endometrial Cancer

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Background: Robotic hysterectomies (RH) and laparoscopic hysterectomies (LH) are common surgical procedures performed for the treatment of endometrial cancer, which involve a significant amount of uterine manipulation. We previously described an association between RH and the presence of detached tumor fragments in the fallopian tubes and proposed that it represented artifactual displacement due to uterine manipulation, however, few long term studies have been performed to assess the clinical significance of this finding. The objective of this study was to examine the long term follow up in these cases to determine whether detached tumor present in the fallopian tube lumina is associated with recurrence and adverse clinical outcome.

Design: The cohort consisted of previously reported RH (n=145), LH (n=181) and total abdominal hysterectomies (TAH) (n=76) performed for endometrial cancer from May 2007 to August 2009. Clinical follow-up was retrieved from the medical record.

Results: A total of 13.6%, 2.4%, and 0% of the RH, LH, and TAH showed detached tumor in the lumina of the fallopian tubes respectively (p=<.001) . The majority of the patients with RH and tumor present in the tubes had FIGO (1988) Stage I disease (9/16, 56.2%) and Grade 1 or 2 tumors (9/16, 56.2%). Four (4/16, 25%) patients had Stage IIIa disease detected by pelvic wash. The remaining 2 patients were

stage IIIa. None of the cases with tumor fragments in the tubes were clinically upstaged to IIIa and if the patient received adjuvant therapy it was due to other clinicopathologic factors. Follow up ranged from 12 to 108 months with a median 67 months. At the time of follow-up none of the patients with tumor fragments in the fallopian tube have experienced recurrence.

Surgery Type	% with tumor in fallopian tubes	Adjuvant Therapy (Radiation, chemotherapy, and/or hormonal therapy)	Recurrence rate	Recurrence rate of tumors with tumor fragments in fallopian tube
RH (n=145)	11% (n=16)	50 % (n=73)	11% (n=16)	0
LH (n=181)	2% (n=4)	56% (n=101)	2% (n=4)	0
TAH (n=76)	0 % (n = 0)	34.9 % (n=27)	6.2 % (n=5)	N/A

Conclusions: In conclusion, our data suggest that tumoral displacement into the fallopian tubes does not increase the rate of recurrence in patients who are treated with RH or LH.

1174 PREVIOUSLY PUBLISHED

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1176 Analysis of Gene Expression and Mutations Involved in Epigenetic Regulation of Endometrial Carcinoma

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Background: The role of epigenetic changes is increasingly known in Endometrial Cancer (EC) pathogenesis and some epimutations are associated with clinical outcomes. We explored our institutional Total Cancer Care database for gene expression levels (81 genes) and mutational results (29 genes) related to DNA methylation, histone modification and chromatin remodeling in 81 EC.

Design: A custom Affymetrix microarray was used for the gene expression assays. Targeted sequencing of 1,321 genes was performed using custom Agilent SureSelect hybrid capture followed by paired-end sequencing on Illumina GAIIx sequencer. After pathology slide review, gene expression and mutational data were correlated with clinicopathologic features including tumor subtype, lymphovascular involvement, grade and stage of disease, and survival outcomes.

Results: The cancer subtypes included 65 endometrioid, 9 serous, 4 clear cell carcinomas and 3 carcinosarcomas. The cohort was divided into two groups: low-grade tumors including FIGO grade 1&2 endometrioid cancers (n= 51) and high-grade cancers with all other categories (n=30). The median clinical follow up was 86.5 months. Microscopic grade was associated with survival outcomes in multivariate analysis (p=0.014).The expression levels of *ARID2, ATRX, CHD2, CHD3, CREBBP, HDAC9, IDH2, KDM4C, KDM5A, KMT2C, NSD3, PBMRM1, SMARCE1* and *TET2* genes were decreased and *BRD4, CHD6, DAXX, DNMT3B, EZH2, HIST1H3B, NSD2* and *TET3* increased in high-grade cancers compared to low-grade lesions. When Bonferroni correction was applied only low *KMT2C* expression levels remained significant (p=0.00011). Total number of *KMT2C* non-synonymous filtered mutations was also higher in high-grade tumors compared to low-grade cancers (p=0.037). *CHD6* and *KDM5A* expression levels were associated with poor survival outcomes in univariate analysis.

Conclusions: *KMT2C* is a tumor suppressor and member of COMPASS family that regulates/enhances spatial structure of DNA. *KMT2B/KMT2C* serves as co-activator for *TP53* to induce target genes involved in DNA repair. In our data, *KMT2C* expression levels are decreased and total mutation numbers increased in high-grade tumors. *KDM5A* is required for activation of proliferative cell cycle genes and also promotes epithelial-mesenchymal transition in correlation with drug-resistance. The prognostic role of *KDM5A* and *CHD6* in endometrial cancer and the association of *KMT2C* with high-grade tumors should be validated in a larger cohort.

1177 NF-kB Expression is Increased in MMR-Deficient Endometrial Endometrioid Adenocarcinoma and is Associated With Increased Macrophage Infiltration at the Tumor Periphery

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Background: Mismatch repair (MMR) deficiency is one of the most common molecular events in endometrial endometrioid adenocarcinomas (EEC). MMR deficiency is associated with increased inflammatory infiltrate, typically lymphocytic, but the molecular mechanisms by which this occurs is not completely understood. The transcription factor NF-kB is known to be an important regulator of the tumor immune microenvironment. In this study, we explored the relationship between NF-kB expression, MMR deficiency, and tumor immune infiltrate.

Design: 336 EECs were screened for MMR deficiency using immunohistochemistry (IHC) for MMR proteins. To minimize effects of patient age and tumor histotype and grade, age-matched MMR-intact (n=49) and MMR-deficient (n=47) grade 2 EEC were studied. NF-kB IHC expression was evaluated using CAP and Allred scoring systems. In the Allred system, the proportion of positive staining is scored 0-5 and the intensity 0-3. The final score adds both together to yield a total score (negative: 0-2; positive: 3-8). CD3, CD8, and CD68 were evaluated using computer-assisted image analysis software (Aperio ImageScope) that measures the number of positive cells within a designated area. The positive cells were assessed at the tumor periphery, tumor center, and in a tumor hot spot. The data were analyzed using the Fisher exact test and Chi-Square analysis to compare NF-kB expression by MMR status and to determine if there was a correlation between NF-kB expression and inflammatory infiltrate.

Results: In the MMR-deficient group, NF-kB nuclear expression was present in 74.5% (CAP) and 70.2% (Allred) of tumor cells, which was significantly higher than the 52.1% (CAP) and 47.9% (Allred) in the MMR-intact group. The mean expression of NF-kB was not significantly different in positive cases between the MMR-deficient and –intact groups. In the MMR intact tumors, there was significantly higher number of CD68-positive macrophages at the tumor periphery in NF-kB positive cases compared to NF-kB negative cases. The number of CD3- or CD8-positive lymphocytes was not significantly different between the NF-kB positive and negative cases.

Conclusions: MMR deficiency is associated with increased NF-kB expression in EEC. NF-kB expression is associated with higher macrophage infiltration at the tumor periphery in the MMR intact group. Tumor-associated macrophages are typically thought to promote tumorigenesis, so activation of NF-kB signaling may be a mediator of more aggressive EEC clinical behavior.

1178 Human Papillomavirus is Identified in Majority of Biopsy Samples with High Grade Squamous Cervical Lesions in Women with Preceding Negative Cobas HPV Tests

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Background: High-risk human papillomavirus (hrHPV) testing has been increasingly used in clinical practice in recent years for triaging equivocal cytology or co-testing with cytology. It has been observed in several large series that a considerable portion of patients with high grade cervical lesions had preceding negative HPV tests. This study attempted to elucidate the factors potentially contributing to the phenomenon by testing biopsy samples from this group of patients.

Design: We retrospectively reviewed the correlation of cytology, histology and hrHPV testing from our Cytology Laboratory database of 130,648 Papanicolaou (Pap) tests between March 1, 2013 and June 30, 2014. Patients with negative HPV tests and high grade squamous lesion or worse (≥HSIL) on follow-up biopsy were identified, and the corresponding paraffin blocks were tested for hrHPV on the Cobas system, a DNA microarray against 40 HPV genotypes, and DNA sequencing. Immunohistochemical stains for p16 were performed on paraffin-embedded sections.

Results: Twenty-one of 252 patients (8.3%) with ≥HSIL (CIN2=9, CIN3=12) on follow-up biopsies had preceding negative HPV tests. These patients ranged from 19 to 49 years with an average age of 32 years, which was younger than the average age of 38 years of this screening population. Cobas HPV tests were positive in 11 biopsies (11/21, 52%) with HPV16 in one and non-16/18 hrHPV in 10. Among the remaining 10 cases either negative or insufficient on Cobas test, 6 had hrHPV and 3 had non-hrHPV on microarray testing. HPV DNA sequencing assay confirmed the microarray results. Only one sample was negative for all three tests (1/21, 5%). Block-like p16 immunohistochemical staining pattern was observed on all cases.

Conclusions: Our study demonstrated that women who had ≥HSIL lesions on biopsy with preceding negative HPV tests were on average much younger than those in screening population. HPV was detected in the vast majority (95%) of these biopsy tissue including hrHPV (81%) and non-hrHPV (14%). Cobas HPV test was less sensitive than HPV microarray and sequencing assays and detected 65% of hrHPV in these samples. True HPV-negative HSIL cases are rare (5%). The causes for false negative results in preceding HPV tests may be multifactorial including sampling variances, technical errors, interference material,

low viral titer, or responsible genotypes not included in detection panel.

1179 PREVIOUSLY PUBLISHED

1180 Androgen Receptor Expression in Epithelial Ovarian Carcinoma

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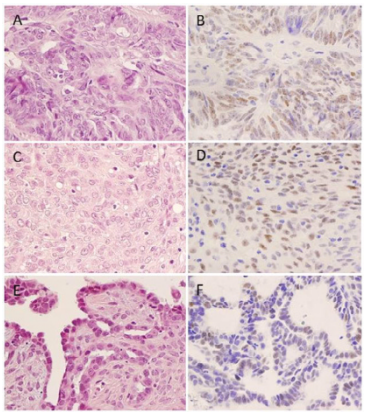
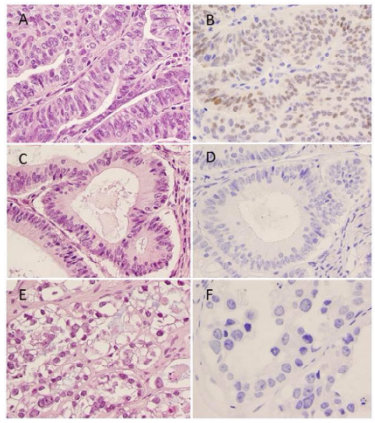
Background: The standard of care for the treatment of epithelial ovarian carcinomas (EOC) includes surgery and chemotherapy. However, the majority of women with advanced disease will experience disease recurrence. Estrogen receptor and progesterone receptor expression is common in some types of EOC but therapies targeting these hormones have shown limited efficacy. New generations of clinically available androgen antagonists have shown promise in some androgen receptor (AR)-positive breast and endometrial cancers, but little is known about the vulnerability of ovarian cancers to anti-androgen therapy. We herein assess AR expression across a range of EOC to assess for possible anti-androgen treatment candidacy.

Design: AR IHC staining was performed on a tissue microarray containing 100 EOCs including 10 low-grade serous carcinomas, 31 high-grade serous carcinomas, 19 mucinous carcinomas, 20 endometrioid carcinomas, and 20 clear cell carcinomas. AR IHC was scored using an adapted Liverpool endometrial steroid quick scoring system, with a final score out of 12 determined by multiplying the percentage of positive tumor cell nuclei (1%-10% = 1, 11%-20% = 2, 21%-40% = 3, >40% = 4) by the intensity of positive cells (0 = no staining, 1 = weak, 2 = moderate, 3 = strong). Scores of 1-4 = low, 5-8 = moderate, and 9 -12 = high.

Results: AR expression was most common in high-grade serous carcinomas (**Table, Figure 1:** AR expression was common in high-grade (A/B and C/D) and low-grade (E/F) serous carcinomas.). The only case in this series with high level staining was also found in this group. A subset of both low-grade serous and endometrioid carcinomas was also positive. Staining was uncommon in clear cell and mucinous cancers, and no cases from these subtypes showed more than low level staining (**Figure 2:** A subset of endometrioid carcinomas were at least focally AR-positive (A/B), whereas the majority of mucinous (C/D) and clear cell carcinomas were (E/F) were entirely AR-negative.).

Histologic Subtype	% Positive	% Low	% Moderate	% High
Low-Grade Serous	40.0	20.0	20.0	0.0
High-Grade Serous	64.5	45.2	16.1	3.2
Endometrioid	40.0	15.0	25.0	0.0
Mucinous	10.5	10.5	0.0	0.0
Clear Cell	5.0	5.0	0.0	0.0





Conclusions: EOC subtypes show different AR staining profiles, with high-grade serous carcinoma showing the highest proportion of positive cases. These results indicate that anti-androgen therapy may represent a treatment option for at least a subset of these malignancies.

1181 Molecular Analysis of Hydropic Leiomyoma: A Variant of Leiomyoma Closely Related to HMGA2 Overexpression

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Background: Hydropic leiomyoma (HL), originally described by R. Scully [1992], is a variant of uterine leiomyoma included in the 2014 WHO tumor classification. This entity characteristically shows edema, increased vascularity, and tumor cells arranged in nodules or cords all in zonal distributions, and thus can cause diagnostic difficulty. Patient management is further complicated by a lack of studies and unknown cause of disease. Identification of several driver gene mutations in usual-type leiomyomas (ULM) provided a concept and tools for investigating HL, and ultimately may serve a role in diagnostic assistance and disease management.

Design: We retrospectively searched our surgical pathology database of the prior 15 years for cases of uterine leiomyoma with increased vascularity (diagnoses hydropic, angio/vascular, cotyledon, and intravascular leiomyoma). Among 200 cases reviewed, we identified 24 that fit the diagnostic features of HL described by Scully. All cases were subjected to gene mutation analysis including MED12, HMGA2, and FH immunohistochemistry (IHC). Further evaluation for gene rearrangement using *HMGA2* (12q14.3) break apart FISH probe is currently ongoing. We also examined CD34, pAKT, p16, ER and Ki-67 IHC.

Results: Diagnosis of HL in our series was largely based on Scully criteria. Those tumors with only focal edema or increased vasculature were excluded. Clinical and histologic features of HL included: average age 43.8 years (range 18-71); average tumor size 14.4 cm (range 3-29); tumor cells with round-oval nuclei and pinpoint nucleoli, occasionally arranged in cords and perinodular growth around vessels; increased vascular density (average 17.5 thick-walled vessels /10x-lpf (range 7-40)); as well as edema, patchy hyalinization, and rarely ischemic necrosis (2 cases). Data of select IHC are summarized in Table 1, with ULM as controls.

Table 1. Select molecular marker analysis and additional immunohistochemistry in hydropic leiomyoma and usual-type leiomyoma

	n	MED12	FH	HMGA2	CD34 (average total vessels /three 20x-mpfs)	pAKT	Ki-67 (average index)
HL	24	1+: 3/24 (12%) 2+: 10/24 (42%) 3+: 11/24 (46%)	Positive: 23/24 (96%) Negative: 1/24 (4%)	Positive: 18/24 (76%) Negative: 6/24 (24%)	47 (range 5-106)	1+: 6/24 (25%) 2+: 11/24 (46%) 3+: 7/24 (29%)	4% (range <1-20)
ULM	21	1+: 2/21 (10%) 2+: 15/21 (71%) 3+: 4/21 (19%)	Positive: 21/21 (100%) Negative: 0/21 (0%)	Positive: 2/21 (10%) Negative: 19/21 (90%)	22 (range 6-80)	1+ 12/21 (57%) 2+: 6/21 (29%) 3+: 3/21 (12%)	1% (range <1-5)

Conclusions: 1. In addition to histologic features of HL described by Scully, we also noted some characteristic nuclear features and perivascular growth pattern differing from ULM.

2. 76% of HL gain HMGA2 overexpression by IHC in comparison to 10% of ULM. In particular, cells for thick-walled vessels are HMGA2 positive, indicating a neoplastic nature of involved vasculature. HL is a specific tumor variant likely driven by HMGA2 overexpression, and those HMGA2 negative cases are under further investigation.

3. Though FISH evaluation for *HMGA2* rearrangement is ongoing, we anticipate chromosomal abberation in contribution to the HMGA2 overexpression.

1182 Incidence of Partial Hydatidiform Mole when Fetal Triploidy is Detected in Prenatal Screening: Implications for Evaluation of Abnormal Products of Conception

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Background: Morphologic distinction of first trimester partial hydatidiform mole (PHM), defined by a diandric triploid genotype, from a non-molar gestation with abnormal villous morphology (AVM) due to a cytogenetic disorder is a well-known diagnostic challenge. Furthermore, distinction of AVM from first trimester normal chorionic villi can be subjective. Fetal triploidy can be detected by prenatal screening using chromosomal microarray analysis (CMA), but CMA cannot distinguish diandric versus digynic origin and therefore is not sufficient to diagnose PHM; neither is flow cytometry-based (FCB) DNA ploidy testing on formalin-fixed products of conception (POC) specimens. In contrast, DNA genotyping distinguishes diandric versus digynic triploidy, thereby distinguishing PHM from non-molar triploid gestation. This study employed DNA genotyping to define the incidence of PHM in the setting of fetal triploidy.

Design: POC from 16 patients with fetal triploidy detected by CMA for prenatal screening or evaluation of miscarriage were reviewed for morphology, ancillary diagnostic tests, and original diagnosis. DNA genotype testing (PCR amplification of 15 short tandem repeat markers) was retrospectively performed on all cases for which it was not performed in the original diagnosis.

Results: Median gestational age was 8.5 weeks. Prenatal fetal triploidy status was not conveyed to the pathologist at time of original POC evaluation in 12/16 cases. PHM was confirmed by DNA genotype in 8/16 cases of fetal triploidy; all but 1 were dispermic. Morphology suspicious for PHM was present in 5/8 PHM but was normal in 3/8 PHM; the original diagnosis was non-molar in those 3 cases. Among 8 cases deemed non-molar by DNA genotype, 7 were digynic triploid, correlating morphologically to normal findings (n=5) or findings suspicious for PHM (n=2). The latter 2 cases were originally shown to be triploid by FCB-DNA ploidy testing and originally interpreted as PHM. The remaining 1 non-molar case exhibited biparental diploid genotype and normal morphology. None had subsequent gestational trophoblastic disease.

Conclusions: Half of gestations with fetal triploidy on prenatal screening were PHM by genotype, though the POC morphology was not always abnormal. Clinicians should inform pathologists if prenatal testing shows triploidy and DNA genotyping should be performed even if the villous morphology is not suspicious. Reliance on triploid results from FCB-DNA ploidy testing alone may result in over-diagnosis of PHM.

1183 Molecular Characterization of Ovarian Carcinosarcoma Reveals Heterogeneous Oncogenic Mechanisms

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Background: Carcinosarcoma can occur anywhere along the gynecologic tract, most frequently in the uterus followed by the ovary. Like its uterine counterpart, ovarian carcinosarcoma is a biphasic tumor composed of a mix a high-grade carcinoma and sarcoma, and is generally regarded as a type of metaplastic carcinoma. There is currently little known about the oncogenesis of ovarian carcinosarcoma.

Design: We examined 30 ovarian carcinosarcomas by immunohistochemistry (IHC) and by targeted sequencing using a custom Illumina TruSeq amplicon panel that interrogated 26 gynecologic cancer genes.

Results: The average age of patients in this study cohort was 65 years. Nearly all patients (97%) presented with stage 2 or 3 disease. 26 of 30 tumors exhibited mutated pattern of p53 staining (16 diffuse strong nuclear staining and 10 "Null" pattern) with the patterns being concordant between the carcinoma and sarcoma components, while 4 tumors showed a wild-type pattern of p53 staining. Targeted sequencing identified *TP53* mutations in 24 tumors (61% missense, 39% non-sense/frame-shift). All 24 *TP53*-mutated tumors exhibited mutated p53 immunostaining, including 8 with "Null" p53 pattern harboring either frameshift or non-sense mutations. The results of TP53 IHC and sequencing analysis were concordant except for 2 tumors showing "Null" staining pattern and no *TP53* mutations. Among the 30 tumors, 4 showed wild-type TP53 by IHC and sequencing, and 3 of these 4 harbored mutations in *KRAS, CTNNB1, RPL22* and/or *ARID1A* (genes more frequently mutated in non-serous carcinomas). 5 tumors harbored exon 9 or 20 activating *PIK3CA* mutations. All tumors showed intact expression of mismatch repair protein with 86%, 67% and 66% showing PAX8, WT1 and ER nuclear expression respectively in the carcinoma components. All 4 tumors with wild-type TP53 by IHC and sequencing lacked WT1 nuclear expression. Among 20 patients with available clinical follow-up, 18 died from their disease (median survival of 15.5 months); there was no difference in survival based on *TP53* mutation status.

Conclusions: Our results show that while TP53 mutation appears to be an oncogenic driver in the majority of ovarian carcinosarcoma (akin to that seen in high-grade serous carcinoma), a minor subset (13%) develop without TP53 molecular alterations. The mutation profile and the consistent lack of WT1 immunoreactivity suggests that this subset likely arise through alternative oncogenic pathway(s).

1184 Primary Endometrial Carcinoma with Signet Ring Features in Polypoid Tumors

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Background: Primary endometrial carcinoma with signet ring cell morphology is rare. Since signet ring morphology is not commonly associated with a primary endometrial origin, it warrants exclusion of gastrointestinal metastases intraoperatively. At our institution we encountered 2 such problematic cases of polypoid endometrial tumors with signet ring cells at frozen section, which increased the surgical time during laparoscopic hysterectomy and were primary endometrial carcinomas at final diagnosis. We report morphologic features of a series of cases of primary endometrial carcinoma with signet ring cells.

Design: A 10 year retrospective search of the databases at our institutions was performed, for endometrial carcinomas with mention of signet ring cells either in the diagnosis or report. H&E slides for cases that met the search criteria were reviewed. The clinical findings, morphologic features, and TNM staging were recorded.

Results: 19 total cases met search criteria of which 4 were primary endometrial carcinomas in hysterectomy specimens. All 4 cases were polypoid and 3 cases had signet ring cells as a prominent finding of the polypoid component at frozen section. Of these, 2 tumors were limited to endometrial polyps and 2 tumors had myometrial invasion by signet ring cells. The immunohistochemical and clinical workup excluded metastases. Permanent sections of all 4 cases had other histologic types of endometrial carcinomas admixed with the signet ring cells. Extensive tumor sampling of non-polypoid areas when indicated, aided identification of other histologic types. The findings are summarized in the table.

Case	Age	Signet ring cell compo-nent	Associated primary endometrial carcinomas	Pathologic Staging
1	52	30%	Undifferentiated Carcinoma- 60% Serous Carcinoma- 5% Endometrioid Carcinoma- 5%	pT1A pN0
2	58	10%	Undifferentiated Carcinoma 90%	pT2 pN1
3	79	20%	Endometrioid with Mucinous Differentiation- 80%	pT1A pN0
4	77	20%	Clear Cell Carcinoma- 80%	pT1A pNX pM1

Conclusions: Signet ring cells in endometrial carcinomas pose a diagnostic and management challenge at frozen section. When signet ring cells are seen at frozen section, it is advisable to submit additional frozens from different and non-polypoid areas of the tumor, to identify associated primary endometrial carcinomas. Recognition of signet ring cells as a variant of endometrial carcinoma, at the time of frozen section can prevent a misdiagnosis of metastases and decrease surgical time during laparoscopic procedures. Signet ring cells are an underrecognized variant of endometrial carcinomas and can be found not only in mucinous endometrial carcinomas but are also seen in polypoid, high grade, and non-mucinous primary endometrial carcinomas.

1185 Clinico-Pathologic Characterization of Endocervical Adenocarcinoma: Biologic Differences in Favour of an Updated International Criteria and Classification Model

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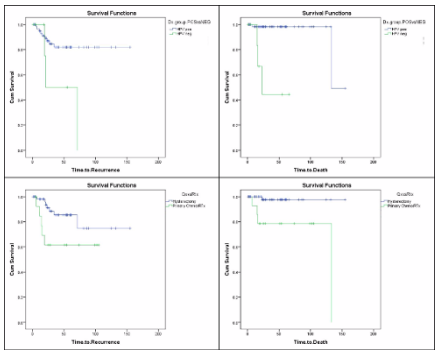
Background: Based on histogenesis, endocervical adenocarcinoma (EA) can be divided into HPV-related (HPV+) and HPV-independent (HPV-) groups, as recently proposed in the International EA Criteria and Classification (IECC). The prognostic value of distinguishing between these two groups has been addressed by some authors; however a comprehensive clinico-pathologic review of these subtypes is needed to validate the IECC model. In addition, advanced stage EA patients, who undergo primary chemoradiation (PCRTx), are usually underrepresented in previous studies, and the relevance of histotype in this subset requires further study.

Design: 82 cases of EA from a single institution were identified. The histological diagnosis for each case was confirmed by expert review using the IECC. Diagnoses were grouped as HPV+ or HPV-. Clinical and pathologic features for each case were recorded including patient outcome, and correlation with HPV subgroup was explored using inferential statistical analysis.

Results: HPV+ tumours comprised 87% (71/82) of our cohort; 10% (8/82) were HPV- and 3% (3/82) were unclassifiable (see Table 1). 83% (68/82) patients were treated by surgical resection while the remaining 17% (14/82) underwent upfront PCRTx. HPV+ and HPV- EAs were similar in age, tumor size and primary treatment modality. However, HPV- EA had significantly higher rates of destructive invasive pattern (p=0.009), LVI (p=0.02) and advanced stage (p<0.001). In univariate analysis, significantly worse recurrence free survival (RFS) and disease specific survival (DSS) were observed in HPV- compared to HPV+ EA (RFS p=0.03, HR 4.3, 95%CI 1.1-16; DSS p=0.003, HR 32.5, 95%CI 3.3-318, see Figure 1) and in EA patients treated with PCRTx versus surgery (RFS p=0.06, HR 3.1, 95% CI 0.97-9.8 and DSS p=0.02, HR 13.1, 95% CI 1.4-120). LVI (p=0.008), invasive depth (p=0.009) and stage (p=0.04) were also significant. In multivariate analysis, HPV EA type (p=0.005, HR 43.9 95%CI 3.2-609) and primary treatment modality (p=0.02, HR 17.9 95%CI 1.4-227) remained significant for DSS.

IECC Diagnosis	Primary surgery [n]	PCRTx [n]	Total [n]
HPV+ usual	46 (68%)	5 (36%)	51 (61%)
HPV+ villoglandular	0	0	0
HPV+ mucinous	6 (9%)	4 (29%)	10 (12%)
HPV+ intestinal	2 (3%)	0	2 (2%)
HPV+ signet ring cell	0	0	0
Adenosquamous	0	1 (7%)	1 (1%)
HPV+ i-SMILE	6 (9%)	1 (7%)	7 (8%)
HPV- endometrioid	0	0	0
HPV- gastric	4 (6%)	2 (14%)	6 (7%)
HPV- serous	0	0	0
HPV- clear cell	1(1%)	0	1 (1%)
HPV- mesonephric	1 (1%)	0	1 (1%)
EA, NOS	2 (3%)	1 (7%)	3 (4%)
Total [n]	68	14	82



Conclusions: HPV+ and HPV- EA, as defined by the IECC, are biologically different neoplasms. HPV- EA represents a high-risk group with aggressive behavior. Conversely, HPV+ EA has significantly better outcomes. These observations support the adoption of the IECC as a more biologically-congruent system to classify EA. Next generation sequencing is being performed on this cohort, aiming to identify and correlate diagnostic and prognostic molecular correlates.

1186 International Endocervical Adenocarcinoma Criteria and Classification: Validation and Interobserver Reproducibility

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Background: The International Endocervical Adenocarcinoma Criteria and Classification (IECC) categorizes endocervical adenocarcinoma (EA) based on histogenesis and biologic behavior, and is being proposed as a superior system compared to the WHO classification. This multi-institutional study aims to validate the IECC by assessing interobserver agreement.

Design: Original slides from 83 EAs were independently reviewed by 7 gynecologic pathologists and classified using the IECC system. A diagnosis was provided based on H&E material alone, and subsequently taking immunohistochemistry (IHC) results into consideration. IHC was performed in 76 EA represented in tissue microarrays, and included p16, vimentin, PR, p53, CDX2, NapsinA and GATA3. Interobserver agreement was calculated. For subgroup analysis, the 13 IECC categories were grouped as HPV-EA, non-HPV-EA and unclassifiable EA (EA-NOS). HPV in situ hybridization (ISH) was performed and correlated with p16 results.

Results: Overall, IECC classification showed fair interobserver agreement on H&E evaluation (K=0.33), with modest improvement after IHC (K=0.36, Table 1). Majority agreement (≥4 reviewers) was achieved in 61 cases (74%, Table 2). Highest levels of agreement were observed in non-HPV categories (K=0.62 for gastric EA, K=0.8 for clear cell EA). In contrast, grouping of HPV-EA into WHO subcategories (usual, mucinous, intestinal, villoglandular) had lower levels of agreement (K=0.26, range 0.17-0.28 for individual types, Table 3). Remarkably, reproducibility was higher when distinction was restricted to HPV vs non-HPV-EA vs EA-NOS: 81 (98%) cases had majority agreement (Table 2). HPV and non-HPV diagnoses showed K=0.4 and 0.5 respectively, which improved to 0.54 and 0.61 with IHC. As expected, EA-NOS showed poor agreement (Table 1). Concordance between p16 IHC and HPV-ISH was high (70/75 cases, 93%).

Table 1. Kappa among IECC categories

All EA categories	H&E only	H&E + IHC
Overall	0.33	0.36
HPV vs non-HPV	0.38	0.51
HPV-EA	0.4	0.54
Non-HPV-EA	0.5	0.61
EA-NOS	0.16	0.27

Table 2. Levels of agreement in IECC diagnosis

	Overall	HPV vs non-HPV vs EA-NOS
Level of agreement (# reviewers)	# cases (%)	# cases (%)
None (2 or less)	12 (14)	1 (1)
3	10 (12)	1 (1)
4	19 (23)	3 (4)
5	19 (23)	16 (19)
6	14 (17)	16 (19)
Perfect (7)	9 (11)	46 (55)

Table 3. Kappa among HPV-EA categories

HPV EA	H&E only	H&E + IHC
Overall	0.26	0.24
Usual	0.28	0.3
Villoglandular	0.2	0.22
Mucinous	0.17	0.14
Intestinal	0.28	0.27
Invasive SMILE	0.41	0.35

Conclusions: Despite the broad spectrum of EA histologic types, the IECC demonstrates acceptable levels of agreement among pathologists, especially in the distinction between HPV and non-HPV-EA and when a selected panel of IHC is used for interpretation. Sub-classification of HPV-EA into WHO morphologic subtypes is less reproducible and carries no significant biologic importance. On the other hand, agreement is high among important high-risk EA subtypes such as gastric and clear cell carcinoma. p16/HPV-ISH correlation is high in our cohort. This validation effort supports replacing the current WHO classification with the IECC.

1187 Prognostic Significance of Lymph Node Metastasis Patterns in Endometrial Cancer

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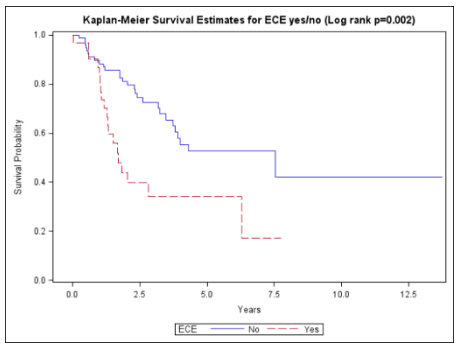
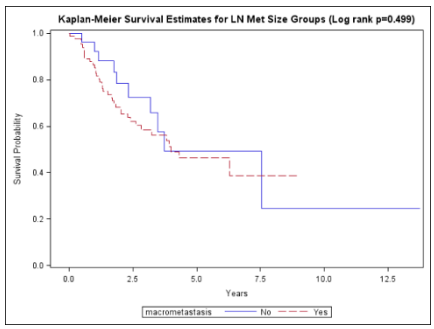
Background: The clinical significance of the size and extracapsular extension (ECE) of lymph node (LN) metastasis is not clear. With the advent of sentinel LN biopsies in endometrial cancer, prognostic significance of lymph node metastasis pattern is critical and may guide further management.

Design: This is a retrospective study of patients with endometrial cancer metastatic to regional LN, who underwent treatment at our institution between 1986 and 2007. After IRB approval, we assessed histologic dimension of largest LN metastasis, and presence of ECE. LN metastases are classified as macrometastases (when => 2mm) and micrometastasis (when <2mm). Clinical data were collected from the medical records. Descriptive analysis and Cox regression models were produced.

Results: 126 cases fit our inclusion criteria. Mean age at diagnosis was 65yrs (37-85). Of those, 65 (52%) were considered type 1. LN metastasis was classified as macrometastasis in 92 (73%) cases, including 42 (46%) type 1 cancers. ECE was identified in 33 (26%) cases, including 11 (30%) type 1 cancers. Within the entire sample, LN macrometastasis was significantly associated with recurrence (HR, 3.3; p=0.02), but not overall survival (HR, 1.27; p=0.5). This effect is not significant when adjusting for histologic type, and adjuvant treatment received (HR, 0.78; p=0.519). ECE was associated with worse overall survival and recurrence free survival, within the entire sample (HR, 2.5; p=0.003 and HR, 3.5; p<0.001, respectively) and within type 1 endometrial cancers (HR, 5.4; p=0.001, and HR, 7.23; p<0.001, respectively) but not within type 2 endometrial cancers, where ECE was more common (HR, 1.28; p=0.515). After adjusting to tumor type and adjuvant treatment received, ECE is still associated with worse overall and recurrence-free survival (HR, 2.65; p=0.003 and HR, 4.41; p<0.001, respectively).





Conclusions: As the size of lymph node metastasis does not significantly influence survival, lymph node micrometastasis should be carefully searched for within the surgical specimens. This, in addition to the adverse outcome related to ECE, extensive pathologic lymph node sampling, using protocols that detect <0.2 cm metastasis and preserve perinodal fat, may be implemented. Routine reporting of ECE is also recommended.

1188 A Proposed Panel of Immunohistochemical Stains to Distinguish Between Clear Cell Carcinoma of Renal Versus Gynecologic Origin

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Background: A combination of clinical history, imaging and histologic findings are often reliable to distinguish between gynecologic and renal clear cell carcinomas. However, in a subset of these tumors, it can be challenging to identify the site of origin due to overlap of histologic features and immunohistochemical (IHC) profile, especially in the setting of metastatic disease. The aim of this study is to evaluate a panel of select IHC stains and determine their applicability in establishing a primary site of origin in these problematic clear cell carcinomas of the abdomen and pelvis.

Design: Tissue microarrays (TMAs) were constructed from primary clear cell carcinomas of the ovary (n=21), endometrium (n=17) and kidney (n=25), with each tumor represented in triplicate. IHC for renal cell carcinoma marker (RCC), CD10, alpha-methylacyl-CoA racemase (AMACR), β-catenin, mesothelin, carbonic anhydrase-IX (CA-IX) and cytokeratin-7 (CK-7) were scored for extent as described in prior studies, strength and localization of staining. The extent of staining was averaged across the three cores for a case. Cases with varying strengths were deemed "patchy" and all localizations within a case were noted.

Results: Positivity rates, as well as details regarding strength and extent for each stain within the different tumors, are summarized in Table 1. Notably, CK-7 was positive in 100% of gynecologic and 32.0% of kidney cases. Additionally, mesothelin was positive in only 12% of renal cases, but 85.7% and 94.1% of ovarian and endometrial cases, respectively. All mesothelin positive renal cases showed strong diffuse RCC, >10% CD10 and negative CK-7 staining. In contrast, 6 of 7 gynecologic cases with negative or rare mesothelin staining were CD10, RCC negative and CK-7 positive. All CK-7 positive renal cases were negative for mesothelin and showed some degree of staining for CD10 or RCC or both. AMACR is positive in 68% of renal cases. Ovarian and endometrial cases demonstrated a distinctive dot-like cytoplasmic pattern for β-catenin in 57.1% and 64.7% cases, respectively. This pattern was not observed in any renal cases.

FOR TABLE DATA, SEE PAGE 467, FIG.1188

Conclusions: This study highlights that AMACR, which is a good marker of gynecologic clear cell carcinoma, is not reliable when the differential diagnosis is renal cell carcinoma. A panel including mesothelin, CK-7, CD10, RCC and β-catenin may serve as a diagnostic aid in challenging cases. Larger studies are warranted to further evaluate the trends noted here.

1189 Genomic Characterization of Uterine Low-grade Endometrioid Adenocarcinoma with MELF Pattern

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Background: Low-grade endometrioid adenocarcinoma (LGEA) is the most common cancer of the uterine body. It is known that its prognosis is favorable and the risk of postoperative recurrence is low in LGEA. Recent studies showed that cases with acronym MELF (microcystic, elongated and fragmented) pattern have more risk of lymph node metastasis and trend toward decreased time to recurrence. The Cancer Genome Atlas Research Network (TCGA) identified four genomic subgroups in the uterine body carcinoma: POLE, MSI, copy-number low (CNL) and copy-number high (CNH). In this study, we examined genomic classification of LGEA with MELF pattern.

Design: Using JFCR pathology database we enrolled LGEA cases with hysterectomy between January 1, 2005 and December 31, 2009. The HE-stained slides of each hysterectomy specimen were reviewed without clinical information. Each case was categorized by invasive pattern into MELF type and usual type. Genomic classification was performed by following method: MMR immunohistochemistry (IHC) testing (MLH1, MSH2, MSH6, PMS2), POLE mutation study using DNA extracted by FFPE materials, and p53 status determination by IHC.

Results: A total of 384 consecutive hysterectomy cases were diagnosed as FIGO grade 1 and 2 endometrioid adenocarcinoma. 52 cases (13.5%) were classified as MELF type. Relapse rate of MELF type (11.5%, 6 out of 52) was significantly higher compared with that (3.6%, 12 out of 332) of usual type (p=0.0119). Kaplan-Meier disease free survival (DFS) analysis demonstrated that MELF type was associated with a lower cumulative DFS than usual type (p=0.0430). MELF type was classified into 4 genomic subgroups as following, 1.9% in POLE , 21.2% in MSI, 5.8% in CNH, 71.2% in CNL. Usual type was classified as 12.5% in POLE, 20.9% in MSI, 3.2% in CNH, 63.3% in CNL.

Conclusions: There were no significant differences in genomic characterization between MELF type and usual type. Metastasis or recurrence of MELF type LGEA might be relevant to genes associated with invasion, metastasis and recurrence.

1190 Molecular Characterization of Endometrial Neuroendocrine Carcinoma

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Background: High grade neuroendocrine carcinomas (NEC) (small cell and large cell NEC) of the endometrium are rare but aggressive tumors that have not been fully characterized at the molecular level.

Design: Endometrial carcinomas (ECs) diagnosed as NEC (pure or mixed) with available material for DNA extraction were included in this study. DNA was extracted from the NEC and any associated non-neuroendocrine component, if feasible, and all samples underwent targeted next generation sequencing of exonic regions of 447 genes to identify single nucleotide variations (SNVs) and copy number variations (CNVs). Cases were categorized using TCGA classification as *POLE*-mutated, microsatellite instability (MSI), copy number high (CN-H), or copy number low (CN-L); clinicopathologic and follow-up data were obtained when available.

Results: 14 cases diagnosed as NEC, either pure (n=4) or mixed with endometrioid adenocarcinoma (9) or carcinosarcoma (1) were sequenced. In 8 of the mixed cases, individual components could be dissected for analysis. The NECs were predominantly of large cell type (8), with 6 cases representing small cell carcinoma. In mixed cases, correlation between NEC and non-neuroendocrine alterations was high, though in general the non-neuroendocrine component harbored higher numbers of mutations than the NEC component. In 7/14 tumors, the mutational pattern was indicative of MSI, and in 1 case a *POLE* P286R mutation was present, associated with ultramutation (643 SNVs). The 6 remaining cases were divided into CN-H(n=2) and CN-L (n=4). The CN-H cases harbored *TP53* mutations, *RB1* deletions, and were associated with high numbers of CNVs. The CN-L cases lacked *TP53* and *RB1* mutations, had low CNVs, and had SNVs typical of endometrioid adenocarcinomas (summarized in Table 1). One CN-H tumor also had high level *NKX2-1* (encoding TTF1) gene amplification, while the other CN-H tumor contained high level amplification of *MYC*. Follow-up data was limited but showed no clear association with clinical outcome or any molecular subgroup. Of the 8 cases with available follow-up data, 4 died of disease, including one patient who was diagnosed at stage IA.

FOR TABLE DATA, SEE PAGE 468, FIG.1190

Conclusions: NEC of the endometrium, like other high grade ECs, are heterogeneous at the molecular level and the molecular profiles demonstrate frequent hypermutation and more closely resemble those found in ECs rather than small cell carcinomas of other anatomic locations.

1191 PD-L1 and DNA Mismatch Repair Protein Expression in Clear Cell Carcinoma of the Ovary: Clinicopathologic Analysis and A Comparison of Two International Cohorts

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Background: Prior reports have identified a correlation between the expression of PD-L1 (CD274) and DNA mismatch repair proteins (MMR) in endometrial and colorectal cancers, among others. This raises the possibility of an increased susceptibility to immune checkpoint inhibitors in MMR-deficient tumors. Since a non-negligible subset of ovarian clear cell carcinomas (CCC) are known to display MMR deficiency, we analyzed the frequency of PD-L1 expression in CCC as well as its relationship to MMR expression. Additionally, we compared the findings between 2 international cohorts, to determine whether any geography-related differences are discernible regarding PD-L1 and/or MMR expression.

Design: 82 cases of CCC, comprised of 54 cases from the USA (US Cohort) and 28 cases from Hong-Kong (HK cohort) were assessed by immunohistochemistry on whole tissue sections using the SP263 antibody (Roche) and Ventana Automated platforms. A 25% cut off for positivity was used for both tumor and immune cells. Results in the combined cohort were correlated with clinicopathologic parameters, and the individual cohorts were compared

Results: PD-L1 expression was present in tumor and immune cells in 26.8% (22/82) and 28% (19/67) of cases respectively. Loss of at least one MMR was present in 8.5% (7/82) of cases: MLH1/PMS2 (n=3); MLH1 (n=1); MSH6 (n=3). Patients with MMR-deficient tumors were somewhat younger than their counterparts with MMR-proficient tumors (46 vs 54 years, p=0.07). PD-L1 and MMR-status showed no significant positive or negative correlation. Neither PD-L1 nor MMR were associated with clinicopathologic parameters, including FIGO stage, gross (cystic vs solid), endometriosis, mitotic index, architectural pattern, and peri-/intratumoral lymphocytes. Interestingly, there were significant differences between the US and HK cohorts regarding PD-L1 expression, with the HK cohorts showing a higher frequency in both tumor cells (57.1% vs 11.1%, p<0.001) and immune cells (53.6% vs 10.3%, p<0.001). Endometriosis was more common in the HK cohort (89 vs 46%, p<0.001). Otherwise, the 2 cohorts were not distinguishable.

Conclusions: PD-L1 expression is present in tumor and/or immune cells in approximately 30% of CCC, and PD-L1 status is not correlated with MMR status. There were significant differences between the USA and HK cohorts regarding the frequency of PD-L1 expression, with more frequent expression in the latter. Whether this finding represents an analytic artifact or true biologic phenomenon, requires further study

1192 Female Genital Melanomas: A Clinicopathologic and PD-L1 Expression Study

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Background: Genital melanomas (GMs) account for 3 to 7 % of female malignant melanomas. Unlike cutaneous melanomas (CMs), GMs are not developed on sun-exposed skin and have different pathogenesis that is less well understood. GM is treated similarly as CM, but overall response is poor. Immune checkpoint inhibitors have been found useful in treating patients with advanced CMs but limited data are available for GMs.

Design: Clinicopathologic features were analyzed and immunohistochemical studies were performed on whole tissue sections of 54 genital melanomas using 2 clones of PD-L1 antibodies (Roche SP263 and Dako 28-8 by using automated platforms). Seventeen cutaneous melanomas were also included for comparison. Tumor cells staining was evaluated using cutoff scores of 0%, <5%, 5-24% and ≥25%.

Results: Median age of patients was 65 years. The tumors consisted of 20 vulval, 31 vaginal and 3 cervical melanomas with median tumor size of 20 mm (range 2 - 50). Median Breslow thickness was 6.45 mm (range 2.4 – 130). Tumors were composed of spindle cells in 50% of cases (27/54), epithelioid cells in 33.3% (18/54) and 16.7% of cases (9/54) were mixed. Median mitotic count was 13.1/10 high power fields. Peritumoral lymphocytes were present in 72.2 % (39/54) and tumor infiltrating lymphocytes (TILs) were present in 79.6% (43/54) (median TILs, 40.5/10 high power fields). For GMs, PD-L1 cutoff scores of 5-24% and ≥25% were 29.6% and 14.8% respectively when SP263 clone was used, and were 25.9% and 9.3% respectively when 28-8 clone was used. For CMs, the frequencies of PD-L1 expression at each cutoff score were similar to GMs (Table). The vulval tumors were resected with wide local excision and 13 vaginal/cervical tumors were treated by radical surgery. Fourteen were inoperable. Adjuvant/palliative radiation therapy was administered to 28. Three had adjuvant chemotherapy (including cisplatin, dacarbazine, carboplatin and paclitaxel). One had C-KIT inhibitor imatinib. Median follow-up was 15 months (range 2 to 197). Forty-three patients (79.6%) died of disease, 5 (9.3%) were alive with tumor and 2 (3.7%) were alive with no tumor.

	Genital melanomas (n=54)		Cutaneous melanomas (n=17)	
	Roche SP263	Dako 28-8	Roche SP263	Dako 28-8
Negative	3	3	1	1
<5%	27 (50%)	32 (59.3%)	9 (52.9%)	9 (52.9%)
5-24%	16 (29.6%)	14 (25.9%)	5 (29.4%)	4 (23.5%)
≥25%	8 (14.8%)	5 (9.3%)	2 (11.8%)	3 (17.6%)

Conclusions: GMs are aggressive, with high mortality and the management of these patients using conventional therapy is largely ineffective. The frequent expression of PD-L1 supports the use of PD-L1 inhibitors in selected patients.

1193 BRCA 1/2 Mutation Tumor Testing in High Grade Serous Carcinoma: An Alternative First Line Triage Strategy

Nadia Ismiil[1], *Bojana Djordjevic*[2], *Dina Bassiouny*[1], *Andrea Eisen*[3], *Matthew Cesari*[1], *Yutaka Amemiya*[4], *Arun Seth*[1], *Sharon Nofech-Mozes*[1]. [1]Sunnybrook Health Sciences Centre - University of Toronto, Toronto, ON, [2]Sunnybrook Health Sciences Centre - University of Toronto, [3]Sunnybrook Health Sciences Centre - Medical Oncology, Odette Cancer Centre, Toronto, ON, Canada, [4]Sunnybrook Research Institute

Background: BRCA1/2 germline mutation (GM) testing for patients (pts) with upper Müllerian high-grade serous carcinomas (HGSC) is

available in developed countries, however, may be difficult to obtain in a timely fashion due to difficulties in accessing genetics counselling clinics. In addition, PARP inhibitor (PARPi) therapies have recently been approved for pts with platinum sensitive HGSC with GMs or somatic mutations (SMs) in BRCA1/2. Tumor testing for BRCA1/2 status upfront, followed by GM analysis only in positive pts may expedite their access to counselling and potentially identify more patients for PARPi therapy. The objective of this study was to assess whether next generation sequencing (NGS) for BRCA1/2 in a cohort of HGSCs from patients with a previously known GM BRCA1/2 mutation status could identify all GM positive patients.

Design: DNA from FFPE samples of 41 HGSC was extracted and analyzed by NGS using a customized targeted Ion AmpliSeq gene panel. Variants found with the Ion Reporter Software were further analyzed to determine the likelihood that they were deleterious. NGS BRCA1/2 results were compared with results of paired GM-BRCA1/2 analyzed between 2000-2013 for the Familial Cancer Program.

Results: 15 (36.5%) of cases contained pathogenic GMs or unclassified variants (unc) in BRCA1/2. NGS analysis revealed at least one BRCA1/2 mutation in 23 (56%) pts. NGS detected *all* BRCA1/2 mutations identified by clinical GM testing (Table 1). Among the 15 pts with GMs, NGS uncovered 9 pts with additional, presumed BRCA1/2 SMs (Table 2) and 8 (19.5%) pts with presumed SMs only.

Table 1.

Test for BRCA1/2 mut (n)	GM + (15) (11 BRCA1, 2 BRCA2, 2 Unc)	GM – (26)
NGS + (23)	15 (6 BRCA1, 1BRCA2, 8 BRCA1 and 2)	8 (3 BRCA1, 5 BRCA2)
NGS – (18)	0	18

Table 2.

	Additional M detected by NGS			
GM	BRCA1	BRCA2	BRCA 1 and 2	none
BRCA1 (11)	1	3	3	4
BRCA2 (2)	1	0	0	1
Unc (2)	1	0	0	1

Conclusions: NGS BRCA1/2 analysis reliably identified all HGSC patients with GMs, as well as a significant number of pts with presumed SMs. Therefore, upfront HGSC testing with NGS may be an effective first line testing tool to improve pt access to genetics clinics and identify pts who can benefit from PARPi therapy.

1194 Mutation Profile of High Grade Ovarian Serous Carcinoma

Nadia Ismiil[1], *Bojana Djordjevic*[2], *Dina Bassiouny*[1], *Matthew Cesari*[1], *Yutaka Amemiya*[3], *Arun Seth*[1], *Sharon Nofech-Mozes*[1]. [1]Sunnybrook Health Sciences Centre - University of Toronto, Toronto, ON, [2]Sunnybrook Health Sciences Centre - University of Toronto, [3]Sunnybrook Research Institute

Background: Tubo-ovarian high grade serous carcinoma (HGSC) is the most common ovarian carcinoma characterized by aggressive behavior. Understanding the spectrum of molecular alterations in HGSC may explain the diversity in disease biology and variability in response to available therapies even among cases with the same histologic subtype.

Design: Next-generation sequencing analysis of 41 HGSC- FFPE samples was performed on the Ion S5XL system with a custom Ion AmpliSeq Breast and Ovarian Cancer Predisposing (BrOCaP) Panel that targets 395 coding exons of 23 genes. The bioinformatics analysis was performed with Ion Torrent platform-specific software, Ion Reporter v5.2. The AmpliSeq analysis workflow detects SNPs, INDELs and CNVs and also annotates the variants with information from dbSNP, 1000 genomes, OMIM, COSMIC, Polyphen and ClinVar.

Results: Mutations (M) were observed in 40 (95.2%) samples, with 9 (21.9%), 7(17.1%) and 23 (56.1%) of these having 1, 2 or ≥3 M respectively. TP53 was the most frequently mutated gene observed in 29 (70.1%). M in BRCA1/2 and its related genes were detected in 26 (63.4%) patients [22 (53.6%) ≥1 BRCA1/2; 6 (14.6%) BARD1; 2 (4.9%) BALB2; 2 (4.9%) BRIP1]. M in genes related to DNA repair were detected in 26 (63.4%) patients [4 (10%) MUTYH; 6 (14.6%) ATM; 3 (7.3%) MRE11A; 3 (7.3%) RAD51D; 1 (2.4%) RAD51C; 5 (12.2%) STK11; 3 (7.3%) MSH2; 7(17.1%) MSH6; 5 (12.2%) MLH1; 6 PMS2 (14.6%); 3 (7.3%) RAD50; 1 (2.4%) FAM175A; and 1 (2.4%) NBN]. M in tumor suppressors were detected in 5 (12.2%) CDH1; 1 (2.4%) EPCAM and 2 (4.9%) CHECK2, only one of those occurred in TP53-wild type. No significant correlation was observed between the BRCA1/2 status and M in any of the gene groups.

Conclusions: These findings demonstrate that HGSC are a genetically heterogenous group and highlight the role of different molecular pathways namely tumor suppressors, BRCA1/2-associated and DNA repair. The role of mutational signatures as prognostic and predictive biomarkers or potential directed therapies should be tested in future clinical trials.

1195 Ovarian Carcinomas Associated with Seromucinous Borderline Tumor (SMBT)

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Background: The majority of ovarian seromucinous tumors belong to the borderline category while seromucinous carcinomas (SMC) are rare. Occasional ovarian carcinomas associated with SMBT have been reported. This study was carried out to explore the pathological features of ovarian carcinomas coexisting with SMBT.

Design: The pathology archives of our hospital were searched for ovarian carcinoma between January 2014 and August 2017. All H-E slides were reviewed to evaluate: histological type of carcinoma, pattern of invasion (infiltrative and/or expansile), and the presence of a SMBT component. Histologic criteria were based on 2014 WHO classification. Carcinomas composed of two or more histological types were designated as mixed carcinoma if the minor component was > 10%. Other features recorded included: tumor size, presence of endometriosis, and presence/type of synchronous endometrial carcinoma in patients who also underwent hysterectomy.

Results: A total of 13 patients with ovarian carcinoma with coexisting SMBT were identified, 3 being bilateral (total = 16 carcinomas). Patients ranged in age from 39 to 64 (median 51; mean 53) years. 11 out of 13 patients were diagnosed with FIGO stage I disease, and one each with stage II and III. Tumors ranged from 5.0 to 12 (median 8.3; mean 8.8) cm, and at least one section per cm of tumor had been sampled in all cases. 15 out of the 16 carcinomas were EMCs (Grade 1 in 12 and Grade 2 in 3), and one was a mixed clear cell carcinoma (CCC) and SMC. Mucinous differentiation was appreciated in 13 of 15 EMCs. The amount of intracellular mucin within the carcinoma cells varied from slide to slide within individual tumors in both EMC and SMC. Excluding the CCC component, 9 out of 16 carcinomas (8 EMCs and 1 SMC) had infiltrative-type invasion. Endometriosis was identified in the same ovary in 13 out of 16 carcinomas. 5 out of 11 patients with concomitant hysterectomy, had synchronous endometrial EMC (Grade 1 in 4, and Grade 2 in 1), 4 with variable mucinous differentiation.

Conclusions: Our findings suggest that most of ovarian carcinomas that coexist with a SMBT component are low-grade EMCs often with mucinous differentiation and that infiltrative-type invasion is not rare. As the amount of intracellular mucin within the carcinoma cells may vary from area to area within individual tumors in both EMC with mucinous differentiation and SMC, the distinction between these two should be made with generous sampling and thorough microscopic evaluation.

1196 Does APOL1 Status Correlate with a Distinct Histopathologic Pattern in the Placenta?

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Background: Apolipoprotein L-1 is a member of a class of apolipoproteins, known to be expressed in cells of multiple organs including the kidney and placenta. Recently, expression of high risk (HR) genetic variants of APOL1 has been shown to be associated with chronic kidney disease in African American (AA) patients. At the same time, recent transgenic mouse models implicate the same APOL1 HR genotype in pregnancy related changes such as pre-eclampsia, eclampsia, fetal/neonatal deaths, and small litter sizes.

Although the severity of pre-eclampsia is not affected by APOL1 expression, mothers with the HR variant are more likely to have cerebral or visual disturbances. Additionally, fetuses are more likely to have lower APGAR scores at 5 minutes. We therefore examined whether pre-eclampsia in the setting of the APO-L1 HR versus the LR genotype has distinct placental pathologic features.

Design: We identified AA mothers with births complicated by pre-eclampsia and who had previously been classified into HR and LR APOL1 genotype groups. Slides were scanned and converted to electronic format and cases were evaluated by three blinded observers, two of them practicing pathologists and one a pathologist in training. We looked for overall decidual vasculopathy (DV), DV of the membrane roll and basal plate, thrombosis, hypertrophic changes, atherosis, fibrinoid necrosis, retroplacental hemorrhage, chronic villitis, infarction, intervillous thrombosis, distal villous hypoplasia, intervillous fibrin, syncytial maturation, and villous edema/dysmaturity. Statistical analysis was performed via the Mann

Whitney Test using Excel 2017.

Results: We evaluated 36 placental cases of which 17 were of the HR genotype and 19 were of the LR genotype. After considering all the pathologic features listed above, no single or group of variables significantly predicted HR or LR APOL1 patient status.

Conclusions: While conferring a greater risk of pre-eclampsia, the APOL1 HR variant is not associated with a distinct histopathologic phenotype when compared to the LR group. Therefore, while the HR variant yields a worse clinical picture, one must wonder if the mechanism of worse clinical outcome is independent of placenta pathology.

1197 Evaluating the Use of the Intraoperative Frozen Section for Endometrioid Endometrial Adenocarcinoma

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Background: Endometrial adenocarcinoma is the most common gynecologic malignancy in the U.S. Surgical resection is the standard of care; however, determining when to stage these patients with lymph node dissections is controversial. SOG recommends staging for FIGO 3 and DMI as these confer a higher risk of metastases. Intraoperative frozen section to assess myometrial depth of invasion (DOI) is often used to guide this decision. We evaluated the use of frozen sections at our institution to see if these diagnoses correlate with the decision to stage.

Design: A retrospective database search identified 93 hysterectomy specimens for endometrioid endometrial adenocarcinoma between 01/2016 and 07/2017. The use of frozen section for staging and pre-operative, frozen, and final diagnosis were evaluated for these cases.

Results: Fifty-nine out of 93 total cases (63%) had a frozen section while thirty-four did not (37%). Of those that were frozen, DOI was assessed in 50 cases (85%). Forty-one (69%) were non-invasive (nl) or invasive to a depth of <50% (non-deep myometrial invasion [nDMI]). Nine (15%) cases were invasive to a depth of >50% (deep myometrial invasion [DMI]). The frozen DOI remained the same in 98% of cases at final diagnosis. All of the DMI cases (100%) and seven (12%) of the nl/nDMI cases were staged. Of the staged nl/nDMI cases, the pre-operative diagnoses were FIGO 1 (36%), FIGO 2 (33%), FIGO 3 (20%), and other (13%). A gross-only evaluation was reported in one case with no appreciable lesion and the final diagnosis was FIGO 2 with superficial invasion. Pre-operative biopsies graded FIGO 1 were predominantly nDMI (87%) on final diagnosis, whereas those graded FIGO 2-3 were more likely to have DMI (28%).

Conclusions: Staging of endometrioid adenocarcinoma is controversial among gynecologic oncologists. In our institution, we found that 12% of cases diagnosed nDMI by frozen section, regardless of pre-operative grade, were staged. In addition, many pre-operative FIGO 3 cases still had frozen sections to assess DOI. There does not appear to be a clear consensus in our institution on staging for endometrioid adenocarcinoma grades 1-2 or nDMI, and intraoperative assessment is often over-used. We recommend performing a gross-only evaluation in conjunction with a pre-operative FIGO 1 diagnosis if there is no gross evidence of invasion. This will decrease the number of unnecessary frozen sections performed on hysterectomy specimens, while also minimizing morbidity and cost to the patient.

1198 Clinicopathologic Characteristics of Selective Estrogen Receptor Modulator (SERM) Associated Uterine Malignant Mixed Mullerian Tumor After Breast Carcinoma

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Background: Treatment with long-term selective estrogen receptor modulator (SERM) including Tamoxifen and Toremifen for breast cancer is known to cause uterine malignant mixed mullerian tumor (uMMMT), however, the incidence and clinicopathologic features of the SERM-associated uMMMT have not been clearly defined. We compared a clinicopathologic characteristics of SERM-associated (n=13) and unassociated uMMMT (n=3) in breast cancer patients and also compared SERM-associated (n=13) uMMMT after breast cancer and uMMMT without clinical history of breast cancer to know if there is any histopathological or prognostic difference.

Design: Of 113 patients diagnosed with uMMMT during 2006-2016, 16 patients whose tumor occurred after breast cancer treatment were selected, and divided into the SERM treated (n=13) and non-treated (n=3) groups. The incidence of the uMMMT in 12,204 breast cancer patients who had a SERM treatment and that of uMMMT among 20,280 breast cancer patients without SERM treatment were calculated during the same period. Histopathologic features and the immunophenotypes of the uMMMT for myogenin, desmin, P53, WT-1, ER, PR and GATA-3 were compared between the two groups.

Results: Patients with breast cancer treated with SERM have an increased risk of uMMMT (0.11%) than those without SERM treatment (0.01%) (odds ratio: 7.208). However, Kaplan-Meier survival curves comparing overall and recurrence-free survival rates were not significantly different between the patients with SERM associated and unassociated uMMMT in breast cancer patients, and also between the uMMMT with and without breast cancer. There is no significant histopathological and immunophenotypic differences including myogenic differentiation of the tumor cells. Both groups showed immunoexpression patterns suggesting the P53 mutation.

Conclusions: Patients with breast cancer treated with SERM have an increased risk of uMMMT than those without SERM treatment, but both tumors have similar clinical outcomes, histopathologic features, and probably similar pathogenetic mechanism through the P53 mutation.

1199 PD-L1 is Expressed in Undifferentiated and Dedifferentiated Endometrial Carcinomas with Mismatch Repair Proteins deficiency

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Background: Uterine undifferentiated (UC)/dedifferentiated (DEAC) endometrial carcinomas are rare neoplasms with aggressive clinical course and advanced stage at presentation. The use of immunotherapeutic drugs targeting programmed cell death protein 1 (PD1)/programmed death-ligand 1 (PD-L1) axis is associated with improved survival in several types of cancers, and particularly in patients with impaired DNA mismatch-repair (MMR) proteins. The aim of this study is to evaluate the expression of PD-L1/PD-1 axis and MMR status in uterine UC/DEAC.

Design: Review of endometrial carcinoma (EC) diagnoses rendered in the period of 2011 to 2017 in our institution identified 15 UC /DEAC cases (n=15). All cases had immunohistochemistry performed on MMR antibodies (MLH1, PMS2, MSH2 and MSH6), PD-L1 antibody (Clone SP142 from Ventana) and PD-1 antibody (Clone NAT 105 from Ventana). Protein expression was examined in both undifferentiated component and low-grade component, where applicable. Positivity of PD-L1 and PD-1 was defined as membranous staining; Expression was scored in both the tumor and the peri-tumoral lymphocytes. MMR proteins were scored retained (+) if > 10% of tumor cells were positive.

Results: Loss of MMR expression was present in 8/15 (53.3%) patients. In the MMR deficient group, 6/8 (75%) had PD-L1 expression, while in MMR proficient group 1/7 (14.2%) had PD-L1 expression ($p<0.05$). The overall proportion of PD-L1 positivity was less than 5%. Regarding PD-1 expression, 6/8 (75%) had expression in peri-tumoral lymphocytes in the MMR deficient group; And 5/7 (71.4%) patients had positivity in MMR proficient group ($p>0.05$). None of the 15 cases presented PD-1 positivity in tumoral components. In the MMR deficient group, 5/6 (83.3%) PD-L1 positive cases also presented PD-1 positivity.

Conclusions: PD-L1 and PD-1 are expressed in the majority of MMR-deficient UC /DEAC cases. PD-L1 is not expressed in MMR-proficient carcinomas. These findings might help guide the potential immunotherapy in UC /DEAC.

1200 189 Consecutive Female Genital Skin and Mucosa Biopsies Submitted to UCSF Dermatopathology: A Learning Opportunity for General Surgical and Gynecologic Subspecialty Pathologists

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Background: For the non-dermatopathologist signing out skin biopsies from the female genital area, the threshold for a dermatopathology consult is low on even the simplest cases. Experience and education in this arena should result in a more efficient sign out for both the gynecologic subspecialist and the general surgical pathologist.

Design: We reviewed 189 consecutive tissue biopsies from women from the vulva, vagina, perineum, pubic area, groin, or inguinal fold. We classified diagnoses as squamous intraepithelial neoplasia, melanocytic proliferations, lichenoid dermatoses, non-lichenoid dermatoses, infectious processes, reparative processes, or miscellaneous. The clinical impression was also compared to the pathologic diagnosis.

Results: Most biopsy specimens could be classified as squamous intraepithelial neoplasia (n=42, 22.2%), melanocytic proliferations (n=33, 17.5%), lichenoid dermatoses (n=29, 15.3%), non-lichenoid dermatoses (n=16, 8.5%), or infectious (n=12, 6.3%) or reparative etiologies (n=8, 4.2%). The remaining cases (n=53, 28.0%) had

miscellaneous diagnoses ranging from common entities such as polyps, cysts, and seborrheic keratoses to more rare entities in the genital region, such as calcinosis cutis, adnexal neoplasms, or basal cell carcinoma. Additionally within the miscellaneous category were a number of descriptive diagnoses where the clinicopathologic information was not specific for a single diagnosis. Five cases had multiple diagnoses that fell into two separate categories.

Clinicians most often included the actual diagnosis in their differential diagnosis for melanocytic lesions (85% of the time) and least often included the actual diagnosis in their differential diagnosis for inflammatory lesions (65% of the time). However, there were a number of cases for which either a clinical description without a differential diagnosis was included (n=27, 14% of the time) or no helpful clinical information was submitted (n=7, 4% of the time). The distribution of whether correct diagnoses were included in the clinical differential was similar between submitting physicians (dermatologists/gynecologists) and mid-level providers (physician assistants/nurse practitioners).

Conclusions: Reviewing melanocytic pathology, as well as harnessing various lichenoid and other inflammatory diagnoses, is critical for female genital tract skin pathology. Often, the provided clinical differential can be useful, but diagnoses are frequently missed and/or differential diagnoses are not provided.

1201 Clinicopathologic Features of 97 Consecutive FIGO Grade 3 Endometrial Endometrioid Adenocarcinomas

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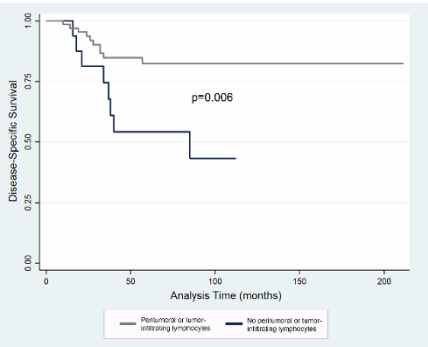
Background: FIGO grade 3 endometrial endometrioid carcinomas (EEC) are a heterogeneous and often diagnostically irreproducible cohort of tumors with variable morphology, immunophenotype and prognosis. In this study, we aim to evaluate the morphologic features of FIGO grade 3 EEC and correlate them with clinical outcomes and molecular profiles.

Design: We reviewed 126 hysterectomies diagnosed as FIGO grade 3 EEC. We recorded clinicopathologic, morphologic, and outcome details using slide review, electronic medical records and the California Cancer Registry. Immunohistochemical staining (IHC) and molecular analysis are in progress.

Results: Of 126 cases originally diagnosed as FIGO grade 3 EEC, 97 had components of FIGO grade 3 EEC on review. The 10 cases that were not purely FIGO grade 3 EEC had areas that were suspicious for undifferentiated carcinoma and are being evaluated by IHC. The remaining cases were reclassified as FIGO grade 1 or 2 EEC (n=15), serous carcinoma or serous mixed epithelial carcinomas (n=8), or carcinoma not further classifiable (n=3). Most cases showed squamous differentiation (n=61, 63%); fewer showed mucinous differentiation (n=23, 24%). Tumor necrosis was common (n=84, 87%), as was lymphovascular invasion (n=51, 53%). Overall, most cases could be described as uniform (n=92, 95%), but many had serous-like features (n=51, 53%), such as scattered anisonucleosis, markedly hyperchromatic nuclei, macronucleoli, or micropapillary growth. Additionally, many cases demonstrated tumor infiltrating lymphocytes (TILs) (n=62, 64%) or peritumoral lymphocytes (PTLs) (n=60, 62%), with 76 cases demonstrating either or both (78%).

Clinical follow up time ranged from 1 to 211 months (median 45). Most patients remained alive with no evidence of disease (n=56, 58%), but a few (n=5, 5%) were alive with disease, and 18 women (19%) had died of their disease, from 10 to 85 months (median 30) after presentation. The remaining women died of other causes (n=11) or unknown causes (n=7). Of note, the presence of TILs and/or PTLs correlates with better disease specific survival by a two-sided log-rank test comparing Kaplan-Meier survivor functions (Figure 1).



Conclusions: Our study confirms that FIGO grade 3 EEC is a heterogeneous entity with low diagnostic reproducibility. Our cohort of 97 women showed variable clinical outcomes but about a quarter of them had persistent or fatal disease. The presence of TILs/PTLs was associated with significantly improved disease-specific survival.

1202 Genomic Profiling of Undifferentiated Endometrial Carcinomas Reveals Frequent Aberrations in SWI/SNF Chromatin Remodeling Genes, Extensive Microsatellite Instability, and Novel Recurrent Mutations in Epigenetic Regulatory Genes

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Background: Undifferentiated endometrial carcinoma (UEC) is a highly aggressive form of endometrial cancer (EC), whether pure or admixed with well-differentiated endometrioid adenocarcinoma (so-called dedifferentiated endometrial carcinoma (DDEC)). Genetic abnormalities in subunits of the SWI/SNF chromatin remodeling complex (*SMARCA2, SMARCA4, SMARCB1, ARID1A, ARID1B)* have recently been described in UEC/DDEC, but the complete spectrum of genetic alterations underlying these tumors has not been elucidated.

Design: We performed targeted-capture next-generation sequencing of approximately 500 cancer-associated genes in nine UEC (3 pure, 6 DDEC) from hysterectomy specimens, along with paired normal tissue, to assess for mutations, structural variants, and copy number changes. In DDEC cases, both endometrioid and undifferentiated tumor were sequenced individually.

Results: Somatic hypermutation and microsatellite instability was present in 8/9 cases. Of these, germline MSH2 intragenic deletion with somatic loss of heterozygosity was present in 1 case and MLH1 promoter hypermethylation was detected in the other 7 cases. Biallelic SMARCA4 mutations were present in the UEC component of 4 DDEC cases; one of these showed the same mutations in the endometrioid component. Biallelic ARID1A and ARID1B mutations were detected in 3 cases: two were in UEC without an endometrioid component that could be separately analyzed and one was present in the undifferentiated component of DDEC only. Homozygous deletion of SMARCB1 was found in 1 UEC. Heterozygous SMARCA2 missense mutation was present in both tumor components of 1 DDEC.

Recurrently mutated genes not previously associated with EC included *FAT1* (5/9), *KMT2B* (5/9), *NIPBL* (4/9), and *PBRM1* (3/9). Additionally, well-established drivers of EC were identified, such as *PTEN* (8/9), *ARID1A* (8/9), *PIK3CA* (6/9), *PIK3R1* (5/9), and *KRAS* (4/9), as well as less-established but previously reported mutations such as *KMT2D* (7/9), *CTCF* (6/9), *TP53* (5/9), *BCOR/BCORL1* (4/9), and *ARID5B* (3/9).

Conclusions: This comprehensive analysis identifies a high incidence of microsatellite instability and lends support that disruption of the SWI/SNF chromatin remodeling complex is a driver of carcinoma dedifferentiation. While many of the recurrently mutated genes are well recognized drivers of endometrial cancers, a few novel recurrently mutated genes were identified including in epigenetic regulators such as *KMT2B* and *NIPBL*.

1203 Hedgehog Signaling Pathway Status in Serous Ovarian Carcinomas

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Background: Hedgehog (Hh) signaling pathway is a highly conserved signaling pathway involved in cell proliferation and differentiation as well in cell polarity in early embryogenesis and morphogenesis. In adults, it is usually inactive while its hyperactivation is associated with carcinogenesis. Hh signaling pathway is activated via binding of the Hh ligands with PTCH transmembrane protein resulting in increased activity of the GLI transcription factors that subsequently activate targeted genes. PTCH1 acts as a tumor suppressor while GLI1 and GLI3 factors have the opposite transcription activities (former has activating and later mainly inhibiting activity).

Design: Formalin-fixed paraffin-embedded samples of 52 ovarian serous carcinomas (low-grade [LGSC, n=11] and high-grade [HGSC, n=41]) were explored for PTCH1, GLI1 and GLI3 expression using immunohistochemistry. *PTCH1* DNA methylation status was assessed using methylation-specific PCR assay (MSP). Five normal/benign ovarian tissues served as controls.

Results: PTCH1 nuclear expression was significantly higher in both LGSC and HGSC compared with normal/benign ovarian tissue ($p<0.001$ and $p<0.001$, respectively) while cytoplasmic PTCH1 protein expression was significantly higher in normal ovaries compared with both LGSC and HGSC ($p=0.003$ and $p<0.001$, respectively). *PTCH1* DNA methylation was exclusively observed in HGSC (6/41, 15%). GLI1 protein expression was significantly higher in HGSC compared with normal ovaries ($p=0.032$) while GLI3 protein exhibited significantly higher expression in both LGSC and HGSC in comparison with normal ovaries ($p<0.001$ and $p<0.001$, respectively). Notably, GLI3 protein had

a higher expression in LGSC compared with HGSC (p=0.043).

Conclusions: Hedgehog signaling pathway components PTCH1, GLI1 and GLI3 appear to be actively involved in pathogenesis of serous ovarian carcinomas. A significant proportion of serous ovarian carcinomas exhibits aberrant (nuclear) PTCH1 protein expression. Therefore, PTCH1 may play an active transcriptional role in LGSC and HGSC. A subset of HGSC may have *PTCH1* gene deregulation caused by its promoter methylation. Further studies involving more samples should confirm these findings and their clinical relevance.

1204 Prognostic Value of p53 and p16 Expression in Vulvar Squamous Cell Carcinoma

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Background: Two distinct pathways, HPV-related and non HPV-related, exist during tumorigenesis of vulvar squamous cell carcinoma (SCC) and its precursor vulvar intraepithelial neoplasm (VIN). Overexpression of p16 is a hallmark of HPV-related usual type VIN and SCC whereas aberrant p53 expression is characteristic of differentiated VIN and related SCC. This study aims to correlate p53 and p16 immunostaining patterns in vulvar SCC with disease recurrence and patient survival.

Design: The study includes 101 women with invasive SCC of the vulva. All patients had at least 5 years clinical follow up since the initial diagnosis. Detailed clinical information was documented. Immunostaining results were categorized as negative, focally positive (+) and strong diffusely positive (++) for both p53 and p16 antibodies. Total loss of p53 immunostaining, representing p53 allelic deletion, was included in p53(++) for its aberrant expression.

Results: p53(++) staining pattern was seen in 53 cases of vulvar SCC that were mostly associated with differentiated VIN. Strong and diffuse p16(++) staining pattern was observed in 39 cases of vulvar SCC and all cases associated with usual type VIN. 68 (67.3%) cases had mutually exclusive p53 or p16 staining patterns. Disease recurrence was noted in 40 (39.6%) patients and death of disease (DOD) was seen in 25 (24.8%) patients. Tables 1 and 2 correlate p53 and p16 staining patterns with recurrence and DOD, respectively. In patients with p53(++) staining pattern, recurrence and DOD occurred in 62.3% and 41.6% of patients, respectively. In patients with p16(++) staining pattern, recurrence and DOD occurred in 12.8% and 5.1% of patients respectively. Disease recurrence and DOD correlates significantly (p<0.001) with p53(++) and p16(++) staining patterns. Increased nuclear staining of p53(+) was seen in 53.8% (21/39) of vulvar SCC cases with diffuse p16(++) pattern. However, there is no significant difference (p>0.1) of recurrence and DOD between p53(-)/p16(++) and p53(+)/p16(++) groups.

Table 1. Correlation of p53 Staining Patterns in Vulvar SCC with Recurrence and DOD

	p16 neg	p16 (+)	p16 (++)	Recurrence (%)	DOD (%)
p53 neg	1	1	17	2/19 (10.5%)	1/19 (5.3%)
p53 (+)	4	4	21	5/29 (17.2%)	2/29 (6.9%)
p53 (++)	46	6	1	33/53 (62.3%)	22/53 (41.6%)
Total cases	51	11	39	40/101 (39.6%)	25/101 (24.8%)

Table 2. Correlation of p16 Staining Patterns in Vulvar SCC with Recurrence and DOD

	p53 neg	p53 (+)	p53 (++)	Recurrence (%)	DOD (%)
p16 neg	1	4	46	27/51 (52.9%)	19/51 (37.3%)
p16 (+)	1	4	6	8/11 (72.7%)	4/11 (36.4%)
p16 (++)	17	21	1	5/39 (12.8%)	2/39 (5.1%)
Total cases	19	29	53	40/101 (39.6%)	25/101 (24.8%)

Conclusions: Expression of p53 and p16 in vulvar SCC correlates significantly with disease recurrence and disease-related death. Patients with SCC harboring aberrant p53 expression have the worst prognosis and patients with diffuse p16 staining have the most favorable prognosis. Our data suggests that application of p53 and p16 immunostains may provide prognostic information and aid in the management of patients with vulvar SCC.

1205 Loss of Claudin-4 is Expression Is Common in Undifferentiated/Dedifferentiated Endometrial Carcinomas

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Background: Undifferentiated/dedifferentiated endometrial carcinomas (UEC/DDECs) are aggressive endometrial cancers with frequent genomic inactivation of switch/sucrose non-fermentable (SWI/SNF) complex proteins. A recent study suggests that claudin-4 may be used to separate SWI/SNF-deficient undifferentiated carcinomas from sarcomas. The aim of this study is to examine claudin-4 expression in UEC/DDEC and other high-grade uterine carcinomas/carcinosarcomas.

Design: We examined claudin-4 expression by immunohistochemistry (clone 3E2C1; Invitrogen) on tissue microarrays that contained 45 UEC/DDEC (24 SWI/SNF-deficient), 50 carcinosarcomas, 164 grade 3 endometrioid carcinomas, 57 serous carcinomas and 20 clear cell carcinomas. Tumors with less than 5% claudin-4 expression were considered to be negative.

Results: The results are summarized in Table 1. Nearly all SWI/SNF-deficient UEC/DDEC showed a complete absence of claudin-4 in the undifferentiated tumor, while the differentiated component in DDEC showed consistent and diffuse claudin-4 expression. Only one SWI/SNF-deficient DDEC showed focal (20%) expression for claudin-4 in the undifferentiated component but diffuse expression in the corresponding differentiated component. These findings indicate consistent loss or downregulation of claudin-4 during cellular dedifferentiation.

Table 1: Claudin-4 expression in uterine malignancies

Tumor type	Claudin-4 positivity
Uterine carcinosarcoma (n=50)	
Carcinoma component	46/50 (92%)
Sarcoma component	4/49 (8%)
UEC/DDEC (n=45)	
SWI/SNF-proficient (n=21)	5 (24%)*
SWI/SNF-deficient (n=24)	1 (4%)*
Clear cell carcinoma (n=20)	11 (55%)
Grade 3 uterine endometrioid carcinoma (n=164)	125 (76%)
Uterine serous carcinoma (n=57)	42 (74%)

* result for undifferentiated tumor

Conclusions: Our results show that claudin-4 expression can be absent or very focal in a number of carcinoma types and carcinosarcoma of the uterus and is almost always lost in SWI/SNF-deficient DDEC, despite the apparent epithelial origin. Claudin-4 expression therefore cannot be used to infer mesenchymal or epithelial origin in the upper gynecologic tract. The consistent loss or downregulation of claudin-4, a tight junction protein, in SWI/SNF-deficient UEC/DDEC further supports the undifferentiated nature of these tumors.

1206 SMARCA4-Deficient Undifferentiated Uterine Sarcoma (Malignant Rhabdoid Tumor of the Uterus): A Clinicopathologic Entity Distinct from Undifferentiated Carcinoma

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Background: Small cell carcinoma of the ovary, hypercalcemic type (SCCOHT) is a rare, aggressive ovarian tumor that occurs in young females. With the discovery of the sole underlying molecular alteration, *SMARCA4* mutation, some have proposed a shift in nomenclature to "malignant rhabdoid tumor (MRT) of the ovary" given its similarity to MRTs associated with loss of SMARCB1, a related protein. SMARCA4-deficient sarcomas have been reported in other anatomic sites, but a uterine variant has not been previously characterized. Undifferentiated carcinomas of many organs, including endometrial, may have loss of SMARCA4 expression; however, their clinicopathologic and genetic features are dissimilar to SCCOHT.

Design: Pathology archives were searched to identify undifferentiated uterine tumors in patients <40 yrs. Morphologic review and immunohistochemistry for SMARCA4, claudin-4, WT-1, and mismatch repair (MMR) proteins were performed.

Results: 3 cases of SMARCA4-deficient uterine sarcoma (SDUS) were identified, diagnosed either on endometrial biopsy (n=1, 34 yrs) or hysterectomy/bilateral salpingo-oophorectomy (n=2, 25 and 33 yrs) and were composed of sheets of large atypical epithelioid cells with prominent rhabdoid morphology. Immunohistochemically, all 3 cases showed loss of SMARCA4, and were negative for claudin-4 with intact MMR expression. One of three cases was positive for WT-1. In 2 cases, the ovaries were pathologically examined to exclude a primary ovarian SCCOHT. Both cases diagnosed on hysterectomy had extensive lymphovascular invasion, extra-uterine spread, and marked infiltrative growth. In the 2 cases with followup, the patients died of disease at 7 and 9 months after hysterectomy.

Conclusions: SDUS share the clinicopathologic features of SCCOHT, including young age, aggressive clinical behavior, morphologic resemblance to the large cell variant of SCCOHT, and loss of SMARCA4 expression. In contrast to SCCOHT, they arise in the uterus and may be negative for WT-1. Compared to undifferentiated carcinoma, SDUS occurs in a younger age range and lacks microsatellite instability. We propose that these tumors be called "SMARCA4-deficient undifferentiated uterine sarcoma (malignant rhabdoid tumor of the uterus)." Differentiating them from undifferentiated carcinoma is important, as SDUS/MRT have a dismal prognosis, but may merit trials with targeted therapy, such as EZH2 inhibitors. Association with germline mutation in *SMARCA4* is unknown but should be investigated.

1207 High Grade Serous Carcinoma: Is There a Correlation Between Putative Site of Origin, Hormonal Status and Talc Usage?

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Background: Although most early extra-uterine high grade serous carcinomas (HGSCs) discovered incidentally involve the distal fallopian tube in the form of an intraepithelial carcinoma (STIC), up to 60% of symptomatic HGSCs are not associated with a clear-cut STIC. This leaves open the possibility that HGSCs could emerge in the proximity of a serosal surface. Genital use of talc has been associated with a 40% increase in risk of HGSC especially in premenopausal or postmenopausal women who use hormone replacement therapy (HRT). Since it is known that inert particles the size of talc, if present in the vagina, promptly reach the upper tract and exit into the pelvis, we postulated that talc users might be more likely to present with HGSCs that did not involve the endosalpinx.

Design: Cases of HGSC were obtained from the gynecologic pathology files. All had been subjected to the SEE-FIM protocol, which provided for complete examination of the fallopian tube and all patients had completed a questionnaire regarding talc use, among other factors, including menopausal status and hormonal replacement therapy (HRT). The pathology reports were reviewed and the status of the fallopian tubes was classified as 1) the presence of STIC; 2) no STIC identified but endosalpingeal involvement or replacement by HGSC, and 3) no evidence of endosalpingeal involvment, either normal tubes or tubes with serosal metastases only. Association with talc use was determined by statistical analysis.

Results: 183 eligible cases were surveyed; 30 reported talc use. For premenopausal women and postmenopausal women who had not used HRT (n=105), there was no significant association between talc use and tumor distribution. However, in the postmenopausal group who had used HRT (n=46) STIC or endosalpinx involvement was absent in 85% of talc users versus those reporting no history of talc exposure ($p = 0.03$).

Conclusions: The prior use of talc was associated with a significantly lower frequency of endosalpingeal involvement in women with HGSC who had previously used HRT. This partially addresses the hypothesis that talc could be a risk factor for extra tubal HGSC and the argument could be made that if talc influences HGSC risk, prolonged exposure of serosal surfaces to talc would be more likely than tubal epithelium. However, the significance of HRT is unclear and interpretation of this association with analysis of a larger cohort of subjects both to address the question of talc and the possibility of a dual pathogenesis for HGSC.

1208 The Molecular and Morphologic Features of KRAS-Mutated Endometrial Carcinomas

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Background: Molecular profiling of endometrial carcinomas (ECs) has led to categorization into 4 molecular groups (*POLE*-mutated, microsatellite instability (MSI), copy number low and high). *KRAS* mutations are seen in ~20% of all ECs, usually associated with MSI and/or other common alterations seen in EC, such as *PTEN* mutations. An unusual subset of ECs, mesonephric-like adenocarcinomas (MLAs) are characterized by *KRAS* mutation, lack of MSI, and lack of *PTEN* mutations. This study examined the histopathologic features and molecular context of *KRAS*-mutated ECs, with a specific focus on the morphology and molecular features of *KRAS*-mutated microsatellite stable (MSS) ECs lacking histologic features of endometrioid differentiation (i.e. squamous/mucinous differentiation).

Design: ECs with canonical *KRAS* mutations were identified from a database of 570 ECs that had undergone next generation sequencing. Histopathologic parameters (tumor subtype, grade, and squamous/mucinous differentiation) and molecular characteristics (MSI/MSS status and *PTEN* status) were noted. Tumors with canonical *KRAS* mutations that were MSS, lacked squamous and mucinous differentiation, and lacked *PTEN* mutations were re-reviewed for morphologic features of MLA.

Results: 98/570 ECs (17%) harbored canonical *KRAS* mutations. Tumor histotypes, and MSI status are shown in Table 1. Of the *KRAS*-mutated, MSS cases with morphology review, 39/61 (64%) had squamous and/or mucinous differentiation while 22 (36%) lacked these histotype-defining features. 8 of these 22 had *PTEN* mutations, leaving 14 cases with a possible MLA-like molecular profile that underwent morphologic re-review. 9/14 had morphology typical of serous (3), carcinosarcoma (4), or endometrioid (2) carcinoma. In 5 cases, there was striking resemblance to mesonephric carcinoma.

	MSI n=18 (16%)		MSS n = 80 (69%)	
Canonical activating KRAS mutations (codons 12,13,61)	EEC 17 (94%) Serous 0 (0%) CS 0 (0%) Other 1 (6%)	64% mucinous diff 60% squamous diff 20% neither squamous nor mucinous	EEC 58 (72%) Serous 7 (9%) CS 10 (12%) Other 5 (6%)	48% mucinous diff 37% squamous diff 36% neither squamous or mucinous

EEC=endometrial endometrioid adenocarcinoma; CS=carcinosarcoma; MSI=microsatellite instability-present; MSS=microsatellite stable

Conclusions: *KRAS*-mutated ECs are most commonly of endometrioid morphology. They frequently have mucinous or squamous differentiation, and rarely show mesonephric carcinoma-like morphology. Identifying *KRAS*-mutated, MSS ECs without squamous/mucinous differentiation enriches for mesonephric-like morphology.

1209 L1CAM Expression in a Large Population Based Cohort of Molecular Classified (ProMisE) Endometrial Cancer

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Background: The newly developed and validated Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) has been shown to be reproducible and prognostic in endometrial carcinomas (EC). In a recent study, we confirmed L1CAM to be a significant independent prognostic factor in EC. In the current study, we wished to determine the potential of L1CAM in the context of molecular classification a large single center EC cohort.

Design: A large population-based cohort of EC was assessed using ProMisE. In addition, L1CAM expression was studied by immunohistochemistry (IHC). A tumor was considered L1CAM positive if ³ 10% of epithelial tumor cells stained positive. Association between ProMisE molecular subtypes and L1CAM expression was evaluated using the log-rank test, survival analyses were calculated using cox-regression models.

Results: Of all 452 EC, 127 (28%) were found to have mismatch repair abnormality (MMR-D), 42 (9%) presented with a polymerase-E exonuclease domain mutation (POLE EDM) and 55 (12%) displayed mutant p53 (p53 abn). Respectively 228 EC (50%) were classified as p53 wildtype (p53 wt). 97/452 (21%) of EC were L1CAM positive. The distribution of L1CAM positive tumors among ProMisE subtypes was as follows: 45/55 (80%) p53 abn, 26/127 (21%) of MMR-D, 7/42 (17%) POLE EDM and 20/229 (8%) p53 wt. In multivariate survival analyses including L1CAM and ProMisE, L1CAM positive tumors showed a Hazard Ratio of 2.25 [Confidence Intervall (CI) 1.14 - 4.37] for disease specific survival (DSS). L1CAM positivity among p53 wt tumors was highly predictive of worse DSS with a HR of 6.53 (CI 2.26 – 16.85; $p < 0.0001$) and associated with high FIGO grade and FIGO stage. In a multivariate model including preoperative risk factors within the p53 wt subtype subgroup, L1CAM remained a significant prognosticator for DSS [HR 3.75 (CI 1.11 – 11.63)] alongside FIGO grade [HR 4.57 (CI 1.24 – 14.85)].

Conclusions: Our study confirms a distinct correlation between mutant p53 and high L1CAM expression levels in EC. L1CAM could add additional value to molecular classification systems in EC, especially within the p53 wt subgroup. In cases of EC with p53 wt and positive L1CAM IHC at preoperative evaluation, a high-risk therapeutic

strategy could be considered.

1210 Infiltrative Growth Pattern: Significant Prognosticator in a Large Molecularly Classified Cohort of Endometrioid Endometrial Carcinoma.

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Background: We previously showed that an infiltrative growth pattern is able to predict adverse prognosis in endometrioid (type I) endometrial carcinoma. The aim of our current study was to investigate the prognostic impact of invasion pattern in a molecular classified cohort with L1CAM expression and TCGA-based molecular subtype available.

Design: 325 endometrioid (type I) endometrial carcinomas (EEC) treated at a single academic center were available. Previous gynecopathological case review had focused on histological type, FIGO grade and depth of myometrial invasion. Type of invasion was categorized as either expansile or infiltrative based on the arrangement of tumor glands and nests, tumor borders and degree of desmoplastic stromal reaction. TCGA-based molecular subtypes were analyzed according to ProMisE molecular classifier (MLH1, MSH2, MSH6, PMS2, p53 immunhistochemistry and POLE sequencing). L1CAM status was analyszed by immunohistochemistry, the threshold for positivity being >10% of epithelial tumor cells. Clinical follow-up data including disease-specific survival and ESMO risk classification (low, intermediate, intermediate-high, high) were available.

Results: EEC with an expansile growth pattern (n=238) were more frequently classified as ESMO low-risk (G1/2, FIGO I, LVSI negative; p<0.001) and were found to be L1CAM negative more often (p=0.004), as compared with EEC with an infiltrative growth pattern (n=87). EEC with expansile growth pattern also more frequently showed MMR deficiency (MMR-D), mutations in the exonuclease domain of POLE (POLE EDM) and p53 wild type (p53 wt), however without statistical significance (p=0.08). Univariate survival analysis showed a significantly better outcome for the expansile compared to the infiltrative growth pattern for all stages as well as for stage I only (p<0.001). After multivariate analysis, L1CAM status remained a significant prognosticator both for stage I (p=0.007) and all stages (p=0.005), and growth pattern remained significant for all stages (p=0.002).

Conclusions: Growth pattern analysis is able to predict prognosis in EEC, particularly if used in combination with L1CAM Immunohistochemistry. TCGA-based molecular classification differs between infiltrative and expansile growth patterns, however, in this study of type I tumors only, molecular subtyping showed only a prognostic trend in multivariate analysis.

1211 Frequent Presence of β-Catenin Signature in the Tubal Epithelium

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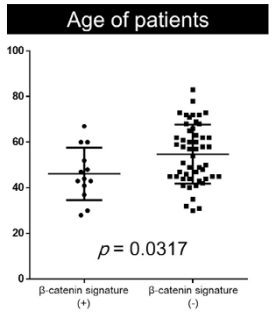
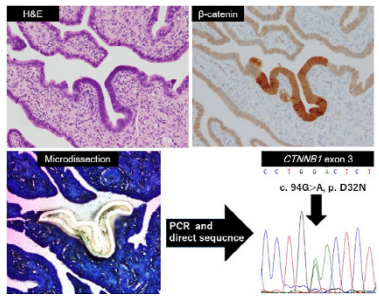
Background: Recently, *TP53* alterations in the tubal epithelium have been investigated extensively. This led to identification of the "p53 signature", a linear expansion of morphologically bland p53-immunopositive cells. A subset of the p53 signatures harbors *TP53* mutations, and is now presumed to be a precursor of serous tubal intraepithelial carcinoma. Clonal expansion of morphologically bland cells with cancer-driver-gene mutations or alterations is a peculiar phenomenon. It is of interest whether such tubal events occur specifically in association with *TP53*, or if similar lesions exist for other cancer-associated genes. In this study, we focused on β-catenin (*CTNNB1*), because its alteration can be evaluated by immunohistochemistry. We aimed to identify linear expansion of tubal cells showing β-catenin alteration.

Design: We performed *in toto* sectioning of the bilateral fallopian tubes of 64 patients. Immunohistochemistry for β-catenin was performed in all sections containing tubal epithelium. We defined "β-catenin signature" as a linear expansion of more than 12 tubal epithelial cells showing benign morphology and aberrant (cytoplasmic and/or nuclear) β-catenin expression. First, the frequency and location of β-catenin signature were investigated, together with its clinicopathological significance. Second, Ki-67 immunostaining was performed in the sections with β-catenin signatures. Third, four representative β-catenin signatures were microdissected and subjected to Sanger sequencing of exon 3 of *CTNNB1*.

Results: A total of 33 β-catenin signatures were identified in 13 cases (20.3%); only 6.1% of the signatures were located in the fimbria. None of the β-catenin signatures showed an increase in Ki-67 labelling index. The presence of β-catenin signature was not associated with coexistence of gynecological malignancies (p = 0.7512). Of note, the patients with β-catenin signatures were significantly younger than those without (p = 0.0317) (Figure 1). Sanger sequencing of *CTNNB1* revealed a hot-spot missense mutation (c. 94G>A, p.D32N) in one of the β-catenin signatures (Figure 2).





Conclusions: This is the first study to elucidate the incidence of β-catenin signatures in the fallopian tube. Our data suggest that the β-catenin signature does not accumulate with age, and its malignant potential seems to be extremely low. We speculate, based on the presence of β-catenin and p53 signatures, that diverse types of clonal expansion occur in the tubal epithelium, and that these may involve various genes.

1212 Grade 1, Stage I Uterine Endometrioid Adenocarcinoma with Early Recurrences: Clinicopathologic Features, β-catenin and p53 Immunohistochemistry in 32 Cases

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Background: Despite the generally favorable prognosis of patients with low-grade, low-stage endometrioid adenocarcinoma, a subset experience early recurrences and the pathologic and molecular risk factors are not entirely known. Recent studies demonstrated that mutational status of CTNNB1 (β-catenin) and TP53 can aid in identifying patients with low-grade (1 or 2), early stage (I-II) endometrioid adenocarcinoma at high risk of recurrence. The aim of the study was to evaluate the immunohistochemical correlates of these markers in patients with grade 1, stage I uterine endometrioid adenocarcinoma (G1S1EC).

Design: Thirty-two cases of G1S1EC diagnosed between 2003 and 2016 on hysterectomy with biopsy proven recurrences were included. Primary tumors were reviewed and clinical and histologic features were documented. Immunohistochemical stains for p53 (>70% strong nuclear positivity=mutant) and β-catenin (any nuclear staining=mutant) were performed.

Results: The median age at diagnosis was 64 (range 38-77) years. Patterns of myoinvasion included in order of frequency: adenomyotic-like, microcystic elongated and fragmented (MELF), infiltrative, broad front, and adenoma malignum, with several tumors demonstrating more than one pattern. Squamous differentiation, as squamous morules, was seen in 11 and mucinous differentiation in 4. The median time to recurrence was 24.5 months and occurred in the vagina in 47% (15/32, mean age 66 years) and extra-vaginally in 53% (17/32, mean age 59 years) of patients. No correlation was found between the pattern of invasion and recurrence site. Nuclear β-catenin positivity was seen in 43% (13/30), more commonly in tumors with squamous

differentiation (11/13). However, no correlation was seen with site of recurrence (7 vaginal versus 6 non-vaginal). p53 expression was wild type in all tumors (32/32) with a subset (5/32) demonstrating increased p53 staining (~30%).

Conclusions: In this series, a high percentage of G1S1EC demonstrated nuclear β-catenin positivity known to correlate with CTNNB1 mutations, which may represent a predictive biomarker for recurrence. This finding was seen more frequently in tumors with squamous differentiation. In this study, G1S1EC were not associated with mutant p53 expression, albeit some tumors demonstrated increased p53 staining. Additional molecular studies are necessary to corroborate our findings and further identify other potential markers associated with recurrence in these tumors.

1213 p16 Immunonegativity in Squamous Cell Carcinoma of the Uterine Cervix: Pathologic Study in Correlation with HPV DNA Status and CDKN2A (p16) FISH.

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Background: Negative immunostaining for p16 (CDKN2A gene product) is rare in uterine cervical squamous cell carcinoma (SqCC) and there are few reports about its pathologic features.

Design: This study included 107 patients who received hysterectomy for cervical SqCC in Kyoto university hospital between January 2010 and December 2015. p16 immunohistochemistry were performed in all cases. For p16-negative cases, standard HPV genotyping were performed. For HPV-genotyping negative cases, analysis of CDKN2A (p16) FISH and additional PCR assay using the L1 consensus HPV primers were added.

Results: Five cases (4.7%) were negative for p16 (mean age: 60.2 years old). All cases had lymph-vascular invasion. Homozygous CDKN2A deletion was observed only in one case. Only two cases were genotype positive for HPV. One HPV56-positive case was non-keratinizing SqCC, and the other was HPV6-positive showing non-keratinizing SqCC with extensive condylomatous area. One showed amplification of L1 gene of HPV. This L1 gene-positive case was non-keratinizing SqCC, and showed sarcomatous morphology at recurrence. The two HPV-negative cases were both keratinizing SqCC and one of them was the case with homozygous CDKN2A deletion.

Conclusions: Among the cases of p16-negative uterine cervical SqCCs, homozygous deletion of CDKN2A was uncommon, while failure of HPV genotyping and non-keratinizing morphology were more commonly observed. In non-keratinizing cases, p16-negativity with unusual morphology can be a pitfall for a diagnosis of cervical SqCC.

1214 Negative Cone Biopsy: Is p16 Helping Us?

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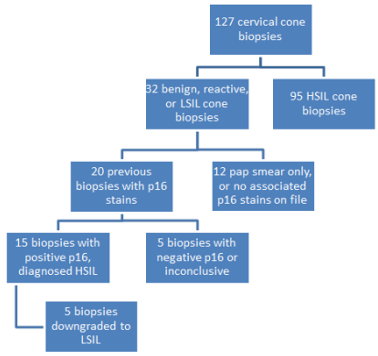
Background: To support the diagnosis of high-grade intraepithelial lesion (HSIL), the Lower Anogenital Squamous Terminology (LAST) project introduced the use of p16 immunohistochemical staining. Practical use of p16 has led to new challenges in interpretation and possible over diagnosis of high-grade lesions. The aim of this study is to assess the use of p16 in the diagnosis of HSIL on cervical biopsies in patients with a subsequent negative cone biopsy.

Design: We retrospectively studied 127 cervical conization specimens with available previous pap smears and/or biopsies, as well as p16 results of the biopsies and HPV status of the patients. We further analyzed only the biopsies that preceded the cones with no evidence of HSIL. We blindly reviewed the hematoxylin and eosin, as well as the p16 IHC slides of those biopsies and compared them with the original diagnoses.

Results: Of 127 cervical conization specimens, 32 (25%) cervical conization specimens were either benign, reactive, or showed a low grade squamous intraepithelial lesion (LSIL). Of these, 20 (17%) had previous biopsies with p16 immunohistochemistry stains performed. Of these 20 biopsies, 15 (75%) showed positive p16 staining and were diagnosed as HSIL. Upon review of these biopsies, 5 did not fully meet LAST criteria for block positivity of p16 and were therefore downgraded to LSIL. Two biopsies were initially inconclusive by p16 stain and three were negative for p16 and were subsequently diagnosed as LSIL. Review of these five biopsies confirmed the initial diagnosis.



Conclusions: Twenty-five percent of reviewed biopsies were over diagnosed as HSIL as a result of non-judicious use and challenges in interpretation of p16. Our study highlighted a few challenges in daily practice of appropriate usage and interpretation of p16: 1. overuse of p16 on unequivocal LSIL, 2. over interpretation of block positivity on small, poorly-oriented fragments of squamous epithelium, 3. discrepencies between areas suspected for HSIL and areas positive on immunohistochemistry stains. This study recommends adherence to the LAST criteria for use of p16 and careful morphologic correlation, especially with respect to small, poorly-oriented fragments of squamous epithelium.

1215 Somatic Cancer-Driver Alterations Among Benign Endometriosis Subtypes

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Disclosures:
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Leah Prentice: *Employee*, Contextual Genomics
Jas Khattra: *Employee*, Contextual Genomics
David Huntsman: *Employee*, Contextual Genomics

Background: Endometriosis rarely progresses to cancer, yet endometriosis lesions are characterized by cancer-like features such as angiogenesis and resistance to apoptosis. Our recent study further revealed the presence of somatic cancer-driver mutations in deep infiltrating endometriosis. Such mutations may be important in the growth and survival of endometrial cells outside of the uterine environment, however it is unclear whether these cancer-drivers also exist in other subtypes of endometriosis such as incisional endometriosis – an iatrogenic form of endometriosis resulting from gynecologic or obstetrics surgeries.

Design: To compare molecular alterations between endometriosis subtypes, we reviewed and sampled lesions from 22 women with deep filtrating endometriosis and 22 women with incisional endometriosis. A hypersensitive cancer hotspot sequencing panel was used to analyze macrodissected archival tissue samples and identified mutations were validated using droplet digital polymerase-chain-reaction (ddPCR). PTEN and ARID1A immunohistochemistry were performed as surrogates for mutational events resulting in functional loss of respective proteins and scored by two pathologists.

Results: Targeted sequencing revealed cancer-driver mutations in 10 of 22 cases of deep infiltrating endometriosis compared to 3 of 22 cases of incisional endometriosis. Loss of PTEN was observed in 6 cases of incisional endometriosis yielding a total of 9 of 22 cases with suspected somatic alterations. No abnormalities in ARID1A were observed. Somatic single nucleotide variants (SNV) mutations across our cohort included cancer hotspot mutations in *KRAS* (11), *ERBB2* (2), *PIK3CA* (2), and *CTNNB1* (1).

Conclusions: Despite the differing etiology of incisional and deep infiltrating endometriosis, a similar spectrum of somatic cancer-driver alterations was observed. The occurrence of cancer hotspot SNV appears higher in deep infiltrating endometriosis than incisional endometriosis: 45% versus 14% of cases respectively, while preliminary IHC data revealed additional changes. Our findings are consistent with

both naturally occurring and iatrogenic endometriosis arising through similar molecular mechanisms. Further study to examine the presence of such somatic alterations in eutopic endometrium is warranted. This data may provide valuable evidence on the contentious origins of endometriosis, mechanisms of endometriosis initiation and/or spread, and lead to a better understanding of pathways leading to malignant transformation.

1216 SOX2 Distinguishes Squamous Cell Carcinoma of the Lower Gynecologic Tract from Endometrioid Adenocarcinoma with Squamous Differentiation

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Background: The distinction of squamous cell carcinoma/high grade squamous intraepithelial lesion (SCC/HSIL) of the lower gynecologic tract from endometrioid adenocarcinoma with squamous differentiation is critical. While straightforward in resection specimens, detached fragments of squamous epithelium in small endometrial or cervical biopsies can raise the differential of SCC/HSIL versus squamous differentiation within an endometrioid adenocarcinoma (SqDiffEM). Traditional immunohistochemical (IHC) markers of squamous epithelium (p63, CK5/6) and endometrioid adenocarcinoma (ER) do not distinguish SCC/HSIL from SqDiffEM. Furthermore, the interpretation of p16 (block positive in SCC/HSIL) can be challenging in small fragments of squamous epithelium. The transcription factor *SOX2* is an oncogene involved in pluripotency of embryonic stem cells and is expressed in SCC in multiple sites. Recently, SCC/HSIL of the cervix and vulva have been reported to show full-thickness SOX2 expression, while benign squamous epithelium shows SOX2 expression confined to the parabasal layers. SOX2 expression in SqDiffEM has not been previously investigated.

Design: To determine if SOX2 IHC can reliably distinguish SqDiffEM from SCC/HSIL, SOX2 and p16 expression were examined in resection specimens of SqDiffEM (n=18) and cervical/vulvar SCC/HSIL (n=20). The intensity (weak, moderate, strong) and distribution (diffuse (D): >80%; patchy (P): 10-80%; focal (F): <10%; and negative (N)) were compared. Positive p16 expression was defined as block positive (nuclear +/- cytoplasmic) staining. Positive SOX2 expression was defined as patchy or diffuse nuclear staining.

Results: (See table)

	SOX2			p16		
	Negative	Positive	p-value	Wild-type	Positive	p-value
SCC	0	20	<0.05	4	16	<0.05
EMSq	16	2		13	5	

Conclusions: This is the first study to demonstrate that SOX2 IHC distinguishes cervical/vulvar SCC/HSIL from SqDiffEM. In resection specimens, SOX2 was more sensitive and specific (100%, 89% respectively) for SCC/HSIL than p16 (80%, 72%). When faced with small fragments of atypical squamous epithelium in endometrial or cervical biopsies, SOX2 could be useful in conjunction with p16 to distinguish cervical/vulvar SCC/HSIL from SqDiffEM.

1217 Uterine Inflammatory Myofibroblastic Tumor Arising During Pregnancy: Use of Short Tandem Repeat Genotyping to Determine Site of Origin

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Background: Inflammatory myofibroblastic tumor (IMT) is a neoplasm of intermediate malignant potential that rarely involves the gynecologic tract. Several cases of IMT have been reported in association with pregnancy, including three cases involving the placenta. Although these tumors were small and confined to the placenta, they showed direct continuity with the decidua basalis, suggesting that these "placental" IMTs are, in fact, uterine IMTs that secondarily infiltrate the placenta. However, no testing has been performed on these cases to confirm the site of origin.

Design: We identified two cases of IMT that arose during pregnancy in association with the placenta. We performed short tandem repeat (STR) analysis, a technique commonly used for forensic identity and paternity testing, on DNA extracted from the placental villous parenchyma, maternal decidua, and tumor to determine site of origin.

Results: Tumor #1 presented in a 44-year-old woman at 32 weeks gestation as a 6.8 cm mass and was thought to be a placental succenturiate lobe by ultrasound. The mass was delivered immediately following delivery of the infant and placenta and was entirely separate from both. H&E sections showed bland spindle cells within a myxoid stroma. FISH showed an ALK rearrangement, confirming a diagnosis of IMT. STR genotyping showed identical alleles at 15 of 15 loci between the IMT and the maternal decidua, consistent with an IMT of maternal origin. The IMT and the placental villi showed matching alleles at only 5 of 15 STR loci. Tumor #2 was a 1.5 cm well circumscribed mass entirely contained within the placenta, composed of bland spindle cells and myxoid stroma. FISH showed an ALK rearrangement, confirming a diagnosis of IMT. STR genotyping showed the tumor and placental villi shared alleles at only 4 of 15 loci, again confirming maternal origin.

Conclusions: We report two IMTs that arose during pregnancy in association with the placenta that were determined to be of maternal origin, confirming that IMTs can arise from the uterus during pregnancy and in some cases secondarily involve the placenta. We report a novel case of a uterine IMT that mimicked a placental succenturiate lobe by imaging and presented as a separate mass following delivery of the infant and placenta. We also demonstrate a novel application for STR analysis, demonstrating the utility of this technique in determining the origin of tumors presenting in association with the placenta.

1218 FOXL2 Immunoexpression in Endometrial Stromal Sarcoma and Non-Neoplastic Endometrial Stroma-Associated Lesions: A Potential Diagnostic Pitfall with Ovarian Sex Cord-Stromal Lesions

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Background: Immunoexpression of FOXL2, a transcription factor of the forkhead box family that regulates ovarian sex cord-stromal development and differentiation, is a highly sensitive diagnostic marker for ovarian sex cord-stromal proliferations and tumors that is also reported to have high diagnostic specificity. However, recent studies show that FOXL2 mRNA and immunoexpression can be detected in endometrial stroma (ES) of endometrium and endometriosis. We hypothesize that endometrial stromal sarcoma (ESS) may also express FOXL2 and thereby present a potential diagnostic pitfall, particularly in cases of extra-uterine ESS. This study evaluates FOXL2 staining in ESS and its main diagnostic mimics, as well as in normal ES and in ES-associated benign lesions.

Design: FOXL2 staining was performed on low grade (LG) ESS, including extra-uterine sites of involvement, high grade (HG) ESS, undifferentiated uterine sarcoma, adenosarcoma, non-neoplastic endometrium, endometriosis, and adenomyosis. Stroma of normal endocervix and fallopian tube was also evaluated. Leiomyomas and leiomyosarcomas were stained for comparison. A positive result for FOXL2 was defined as nuclear staining. Distribution was diffuse, patchy or focal. Intensity was classified as weak, moderate or strong.

Results: Diffuse strong FOXL2 staining was present in the stroma of 32/37 normal endometrium, 13/14 normal fallopian tube, and 4/12 normal cervix. Diffuse strong FOXL2 was present in ES of 6/14 adenomyosis and 25/30 endometriosis. FOXL2 was positive in 19/21 LG-ESS (47% diffuse, 47% patchy, 5% focal), including 12/13 extra-uterine sites of LG-ESS (25% diffuse, 67% patchy, 8% focal); 3/6 HG-ESS (33% diffuse, 33% patchy, 33% focal), 2/2 UUS (patchy only), and 0/3 adenosarcoma. None of the leiomyomas or leiomyosarcomas showed diffuse strong FOXL2 though focal/patchy staining was present in 38% and 21% respectively.

Conclusions: FOXL2 staining is common in endometrial stromal neoplasms and in endometrial stroma of endometriosis, adenomyosis and normal endometrium. This may pose a diagnostic pitfall in the rare setting of extra-uterine lesions and neoplasms derived from endometrial stroma that may mimic an ovarian sex cord-stromal lesion or tumor.

1219 Utility of Single Nucleotide Polymorphism Microarray (SNPM) and Targeted Mutation Analysis in Resolving the Dilemma of Synchronous vs. Metastatic Carcinomas of the Endometrium and Ovary/Fallopian tube

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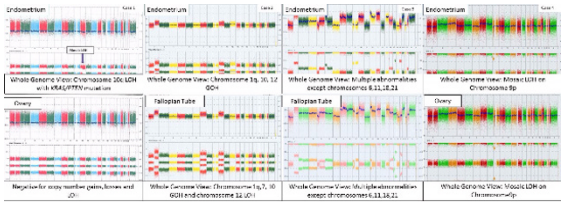
Disclosures:
Alka Chaubey: *Speaker,* Affymetrix/Thermo Fisher Scientific
Ravindra Kolhe: *Speaker*, Illumina Inc, Affymetrix Inc, BMS Inc, Asuragen Inc; *Consultant*, BMS Inc, Asuragen, Inc

Background: Distinguishing between synchronous independent primary endometrial and ovarian/fallopian tumors and metastatic lesions has been diagnostically challenging in the past. Historically, a set of clinical and histopathologic criteria was utilized. Utilization of immunohistochemical stains, ploidy analysis, gene mutational analysis, and whole genome sequencing has also been attempted. Recent literature utilizing massively paralleled sequencing has demonstrated that the majority of synchronous endometrial and

ovarian cancers are clonally related, and that the clinicopathologic diagnosis was largely inconsistent with the molecular findings. Evaluation of the concurrent endometrial and fallopian/ovarian cancer cases at our facility was performed utilizing SNPM and targeted mutation analysis to determine the clonal relationship.

Design: Two pairs of synchronous endometrial and fallopian tube and two pairs of endometrial and ovarian endometrioid type cancer were evaluated utilizing SNPM. The whole genome SNPM was performed on the DNA isolated from FFPE specimens following manufacturer's protocol (OncoScan® FFPE Assay Kit, Affymetrix, Inc). The raw data was analyzed in Chromosome Analysis Suite 3.0 software and matched to in silico FFPE reference sets. This platform consists of 274,000 probes including 74 somatic mutations from 9 important oncogenes (BRAF, KRAS, EGFR, IDH1, IDH2, PTEN, PIK3CA, NRAS and TP53).

Results: Two cases demonstrated multiple independent copy abnormalities, suggestive of two independent primary cancers (Figure 1, cases 1 and 2). The other two cases demonstrated identical multiple copy abnormalities, suggestive of a single primary tumor with metastasis (Figure 1, cases 3 and 4).



Conclusions: Here we demonstrate the utility of a novel technique for confirming/ruling out synchronous vs metastatic lesions in histologically difficult endometrial and fallopian tube/ovarian cases. Our finding, which indicated that 50% of our cases were not clonally related via SNMP and targeted mutation analysis, is in contrast to the recent literature which has demonstrated the majority of concurrent endometrial and ovarian cancers represent single primary tumors with metastasis. The findings also emphasize the reliability and ease of obtaining high resolution data from a small FFPE sample at reduced cost. This improvement in facilitating accurate diagnoses, which were not previously possible, has tremendous prognostic and therapeutic implications for our patients.

1220 Clinicopathological Characterization of Endometrial Carcinomas with More Than One Molecular Classifying Feature

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Background: Molecular stratification of endometrial carcinomas (EC) into DNA polymerase epsilon mutant (*POLE*mut), mismatch repair deficient (MMRd), p53 mutant (p53abn) and no specific molecular profile has provided a novel classification with prognostic impact: POLEmut EC have an excellent prognosis while p53abn cases have an unfavourable outcome. This approach however leaves unclassifiable cases due to the presence of more than one classifier, referred to as "multiple classifiers". We aimed to describe the clinicopathological characteristics of these unclassifiable EC.

Design: Multiple classifiers were divided into four groups: *POLE*mut-p53abn, *POLE*mut-MMRd, MMRd-p53abn and *POLE*mut-MMRd-P53abn. These were derived from 2312 EC molecularly profiled in multi-institutional collaborative setting by sequencing of exons 9-14 or exons 9 and 13 of *POLE* and immunohistochemistry of p53 and MMR proteins, completed with TCGA cases. Univariable and multivariable Cox proportional-hazards models were used to evaluate overall survival (OS) and recurrence free survival (RFS) of multiple classifier groups alone and in combination with age, stage and grade. In addition Kaplan Meier curves with log-rank test were performed for RFS and OS.

Results: In total, 82 multiple classifiers were identified (3,5%) with median age 63.6 (range 27-87). FIGO stage (2009) was: 37 IA(45,1%), 32 IB(39%), 13 II-IV(15,9%). 66 (80,5%) cases were endometrioid and 16 (19,5%) non-endometrioid, with 19 cases grade 1 (23,2%), 11 grade 2 (13,4%) and 52 grade 3 (63,4%). There were 20 (24,4%) *POLE*mut-p53abn, 17 (20,7%) *POLE*mut-MMRd, 40 (48,8%) MMRd-p53abn and 5 (6,1%) *POLE-MMRd*-p53abn. There was no significant difference between multiple classifier groups both for RFS and OS, also when the analysis was limited to stage I patients (table 1). When including age, FIGO stage and grade, multivariable analysis did not reveal a multiple classifier group with distinct impact on RFS or OS (also when limited to stage I). FIGO stage II-IV was the strongest negative independent prognostic factor for RFS and OS. Age was independent prognostic for OS.

	All patients	Stage I	All patients	Stage I
	RFS (5years)	RFS (5years)	OS (5years)	OS (5years)
POLEmut-p53abn	78,7	95,8	81,1	85,8
POLEmut-MMRd	95,0	100	100	100
MMRd-p53abn	93,8	93,3	77,4	83,0
POLE-mut-MMRd-p53abn	80,0	80,0	75,0	75,0
Log-rank p-value	0,75	0,23	0,84	0,98

Conclusions: This is the first study on ECs with multiple classifying features. These ECs are rare (3,5%) and can be identified in all histotypes and grades. Our data suggests that *POLE* mutations drive the biological behavior of EC even in the presence of MMRd or p53 mutations. Further characterisation will be required to determine the optimal treatment of these unusual ECs.

1221 ZC3H7B-BCOR High-Grade Endometrial Stromal Sarcomas: A Report of 17 Cases of a Newly Defined Entity

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Background: High-grade endometrial stromal sarcoma (ESS) likely includes underrecognized tumors with genetic alterations besides *YWHAE-NUTM2* fusion. Prompted by 3 initial ESS with *ZC3H7B-BCOR* fusion with high-grade morphology and aggressive clinical behavior, we evaluated the clinicopathologic features of 17 such tumors.

Design: *ZC3H7B-BCOR* ESS were identified through clinical work-up or pathology database search of undifferentiated uterine sarcoma (UUS) and myxoid leiomyosarcoma (mLMS) with the fusion confirmed by fluorescence in situ hybridization or targeted RNA or DNA next-generation sequencing performed on archival formalin-fixed paraffin-embedded tumor tissue. CD10, ER, PR, SMA, desmin, h-caldesmon, cyclin D1 and BCOR immunohistochemistry (IHC) was performed and/or interpreted from slides and pathology reports from referring institutions. Clinical data was obtained from electronic medical records.

Results: *ZC3H7B-BCOR* fusion was confirmed in 17 tumors, including 4 and 2 previously diagnosed as mLMS and UUS, respectively. All arose in adults (median age 54; range 28-71 years). Median tumor size was 9.7 (range 1.5-12.0) cm. Most were endomyometrial-based (5/6 tumors grossly, 11/13 histologically) with tongue-like (5/12) or pushing (2/12) myometrial invasion or a mixture of both (5/12). Most were uniformly cellular with haphazard fascicles of spindle cells with mild-moderate nuclear atypia. Myxoid matrix and collagen plaques were seen in 14/17 (82%) and 8/17 (47%) tumors, respectively. Mitotic index was ≥10 (median 14.5; range 1-50) per 10 high power fields in 14/17 (82%) tumors. Necrosis was seen in 10/17 (59%) tumors and was of infarct-type in 8, tumor-type in 1 and both in 1; vascular invasion was present in 11/17 (65%). No conventional or variant low-grade ESS foci were seen. All tumors expressed CD10 with only limited or absent desmin, SMA and/or h-caldesmon staining; 4/12 (33%) tumors expressed ER and PR in >5% of cells. Diffuse cyclin D1 and BCOR staining was seen in 7/8 (88%) and 7/14 (50%) tumors, respectively. Clinical data from 5 patients are detailed below (table).

Clinical characteristics of 5 patients with ZC3H7B-BCOR ESS

(C indicates chemotherapy; DOD, died of disease; H, hormonal therapy; NA, not available; NED, no evidence of disease; R, radiation; RFA, radiofrequency ablation; S, surgery)

Patient	FIGO (2009) stage	Time of pre-sentation	Time to recurrence (mo)	Treatment	Time to last fol-low-up (mo)	Outcome
1	III	Primary	3	S	15	NED
2	III	Primary	19	C, H, R	40	DOD
3	I	Recurrence	NA	C, H, R	34	DOD
4	I	Recurrence	NA	C, R	41	DOD
5	I	Recurrence	NA	C, H, R, RFA	80	DOD

Conclusions: *ZC3H7B-BCOR* ESS constitutes a distinct uterine sarcoma subtype with high-grade morphology that should be distinguished from other uterine mesenchymal neoplasms with myxoid morphology. Limited clinical data suggest patients present at higher stage and have worse prognosis compared to published outcomes in low-grade ESS. *ZC3H7B-BCOR* ESS should be included among high-grade ESS.

1222 Evaluation of the Mismatch Repair Status of Ovarian Clear Cell Carcinomas in an Asian Cohort

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Background: Ovarian clear cell carcinoma (OCCC) is a distinct subtype of epithelial ovarian cancer with a higher incidence in Asia. Studies from mostly Caucasian cohorts show that some tumors can arise in the setting of Lynch syndrome. However, the incidence of mismatch repair (MMR) deficiency in OCCC arising in the Asian population is not well characterized. We evaluated the incidence of MMR deficiency in an Asian cohort of OCCC and also assessed potential clinicopathological associations.

Design: Immunohistochemistry for MMR proteins (MLH1, MSH2, MSH6 and PMS2), ARID1a, CD3 and CD8 was performed on whole tumour sections from 99 OCCC. Immunoexpression profiles were correlated with histopathological features (tumour architecture, stromal and inflammatory response, presence of endometriosis) and clinical data [age at diagnosis, personal/family history of gynecologic and colonic cancer, disease stage and overall survival (OS)].

Results: Abnormal MMR expression was identified in 5% of OCCC [MLH1/PMS2(2), MSH2/MSH6(1), MSH6(2)] . Patients with MMR deficiency were younger than those without (mean of 48 versus 52 years). Two had concurrent diagnoses of endometrial endometrioid carcinoma, one of which had a family history of endometrial and colon cancers. Another patient also had a family history of colon cancer. OCCC with MMR deficiency had more prominent intratumoural chronic inflammatory infiltrate ($p = 0.0007$), including significantly higher numbers of CD3 and CD8 lymphocytes ($p<0.001$) but showed no association with the presence or severity of neutrophilic infiltrate or other histopathological features. There was also no association between MMR deficiency and disease stage or OS. 34% of OCCC showed absent ARID1a immunoexpression. This was associated with MMR protein loss ($p = 0.046$), but showed no correlation with the intensity of CD3/CD8 lymphocytic infiltrate, disease stage or OS.

Conclusions: The incidence of MMR deficiency is similar in Caucasian and Asian cohorts of OCCC. OCCC with MMR deficiency is associated with ARID1a loss and increased intratumoural chronic inflammatory infiltrate, particularly CD3 and CD8 lymphocytes.

1223 Immunohistochemical Survey of Mismatch Repair (MMR) Status in 1467 Solid Tumors – Searching Potential Targets for PD-1/PD-L1 Blockage Treatment

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Background: A recent study indicated that mismatch repair (MMR)-deficient tumors and tumors with high somatic mutations/high mutation burdens, including colorectal and non-colorectal cancers, are more responsive to PD-1 blockage treatment (*N Engl J Med* 2015; 372:2509-2520). It has been well documented that there is an excellent correlation between immunohistochemical (IHC) detection of MMR proteins and molecular testing for microsatellite instability-high (MSI-high). In this study, we re-evaluate the MMR status in a large series of solid tumors using IHC with the Ventana Ultra staining platform.

Design: Tissue microarray blocks containing 1467 solid tumors from various organs were constructed, including lung adenocarcinomas (ADC, N=114), mesothelioma (N=23), breast carcinoma (CA, N=34), prostatic ADC (N=132), renal clear cell carcinoma- low grade (CRCC, N=80), CRCC-high grade (N=86), renal papillary RCC (N=33), renal urothelial carcinoma (UC, N=46), hepatocellular CA (N=36), bladder UC (N=43), thyroid papillary CA (N=48), stomach ADC (N=21), seminoma (N=100), glioblastoma (N=23), leiomyosarcoma (N=50), and the cases listed in Table 1. IHC for MLH1, PMS2, MSH2 and MSH6 was performed on these cases. Only cases showing tumor cells with complete absence of nuclear staining and the presence of a strong internal positive control were regarded as positive (MMR-deficient tumors).

Results: The tumors with evidence of MMR deficiencies are summarized in Table 1. All tumors mentioned above showed intact expression of MMR proteins.

Table 1. Summary of MMR-Deficient Tumors

Diagnosis/ MMR Proteins	Loss of MLH1 and PMS2 (%)	Loss of PMS2 only (%)	Loss of MSH2 and MSH6 (%)	Loss of MSH6 only (%)	Total cases with loss of one or more mark-ers (%)
Colon ADC (N=86)	13 (15%)	13 (15%)	0	0	13 (15%)
EMAD- FIGO 1 (N=34)	8 (23.5%)	0	0	1 (3%)	9 (26.5%)
EMAD-FIGO 2 (N=59)	12 (20%)	5 (8.5%)	0	0	17 (29%)
EMAD- FIGO 3 (N=38)	13 (34%)	1 (2.6%)	0	2 (5.3%)	16 (42%)
OSC (N=41)	2 (4.9%)	0	0	0	2 (4.9%)
OCCC (N=26)	2 (7.7%)	2 (7.7%)	1 (3.9%)	1 (3.9%)	3 (11.5%))
Esophageal ADC (N=48)	1 (2%)	1 (2%)	0	0	1 (2%)
Lung SCC (N=171)	0	0	1 (1.4%)	1 (1.4%)	1 (0.6%)
Pancreatic ADC (N=95)	0	0	1 (1.1%)	1 (1.1%)	1 (1.1%)

Note: EMAD – endometrial adenocarcinoma; lung SCC – lung squamous cell carcinoma; OCCC – ovarian clear cell carcinoma, OSC – ovarian serous carcinoma

Conclusions: These data confirm that 1) endometrial and colorectal carcinomas are the most commonly seen MMR-deficient solid tumors, followed by ovarian carcinomas; and 2) MMR-deficient tumors of other organs are uncommon.

1224 Whole Exome Analysis of Incidental Tubal Precursors Without Concurrent Carcinomas

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Background: Serous tubal intraepithelial carcinoma (STIC) of the fallopian tube has been proposed as the immediate precursor for most pelvic high-grade serous carcinomas (HGSCs). Molecular studies that support this paradigm mainly come from the analysis of STICs in the presence of HGSCs. The rapidly expanding carcinoma may destroy the tubal tissues, resulting in the extinction of extant precursor species in the habitat, and moreover these cancer cells may disseminate onto the tubal surface, mimicking an intraepithelial lesion, obscuring the reconstruction of early genetic landscape during tumor evolution.

Design: To overcome the above limitations, we used laser capture micro-dissection to harvest cells of interest, extracted DNA and analyzed the whole exomes in 14 incidental tubal lesions including 12 STICs and two p53 signatures (p53 positive normal-appearing epithelium) collected from 8 fallopian tubes in the absence of carcinoma. In addition, 4 cases containing synchronous HGSC and STIC/p53 signatures were also analyzed.

Results: Our results showed that as compared to STICs associated with HGSC, incidental STICs had a lower number of somatic mutations ($p< 0.05$) and there was no significant difference among incidental STICs and p53 signatures or between HGSC and STICs in synchronous cases. All incidental tubal lesions, like those associated with HGSCs, harbored *TP53* mutations and, interestingly, in one case with multiple geographically distinct lesions (4 STICs and two p53 signatures), all had distinct *TP53* sequence variations. Likewise, another tube contained two incidental STICs, both harboring different *TP53* mutations.

Conclusions: We demonstrated, for the first time, the mutation landscape of tubal precursor lesions of HGSC without synchronous carcinomas. Our data suggest a parallel polyphyletic evolution of multiple precursor clones arising from fallopian tubes but only one of them may progress to HGSC. The universal *TP53* mutation in all tubal lesions without concurrent HGSC ensures that detecting *TP53* sequence variations should be sensitive for detecting ovarian cancer precursors using cervical-vaginal fluid or endometrial lavage.

1225 p53 is a Useful Marker to Distinguish dVIN from Inflammatory Dermatoses and Other Squamous Lesions in the Vulva

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Background: Vulvar squamous cell carcinoma (SCC) constitutes over 90% of vulvar malignancies. dVIN (differentiated vulvar intraepithelial neoplasia) is the precursor lesion to HPV (human papillomavirus)-independent vulvar SCC. Unlike usual-type VIN, which is driven by HPV infection and exhibits conspicious histologic changes, the histologic features of dVIN can be very subtle and very difficult to distinguish from benign inflammatory dermatoses in the vulva. It has been reported that p53 shows increased staining in dVIN, but the percentage, intensity and patterns of staining have not been well characterized. In addition, the p53 staining characteristics in the broad entities that reside in the differential diagnosis of dVIN have not yet been described in detail.

Design: We studied the p53 and Ki67 immunohistochemical staining patterns in 12 cases of dVIN and 27 cases of vulvar squamous lesions commonly included in the differential for dVIN (5 lichen sclerosus (LS); 5 lichen simplex chronicus (LSC); 3 lichen planus (LP); 6 psoriasis (PSO); 4 spongiotic dermatoses (SPD); 4 candidiasis (CAN)). Due to the diagnostic challenges of diagnosing dVIN on small biopsies, all dVIN cases were based on resection specimens where the dVIN was identified adjacent to a p16-negative invasive SCC.

Results: We observed that dVIN demonstrated moderate-strong uniform p53 staining in over 70% of basal cells, with moderate to strong parabasal staining extending to two-thirds of the epidermis in the majority of cases. This was compared to weak or weak-moderate patchy p53 staining in the majority of other lesions. Moderate-strong and increased basal p53 staining was also observed in lichen sclerosus and lichen planus, however in both categories, this was limited to the basal layer and any staining in the parabasal layers was patchy (unlike the uniform intense staining seen in dVIN). Null-type p53 staining was unique to dVIN. Ki67 staining was variable in dVIN, ranging from 10-90% of basal cell positivity.

FOR TABLE DATA, SEE PAGE 469, FIG. 1225

Conclusions: p53 is a useful marker in distinguishing dVIN from other inflammatory/benign mimics in the vulva. dVIN exhibited strong and uniform staining, extending uniformly into the parabasal layers, or a complete absence of staining (null-type). Although lichen sclerosus and lichen planus also exhibited increased basal staining (intensity and percentage), the parabasal staining was patchy in quality. Ki67 staining was variable, and was not helpful in distinguishing dVIN from the other vulvar squamous lesions.

1226 Neoadjuvant Chemotherapy for Tubo-ovarian High-grade Serous Carcinoma: Correlation of Clinical Response with Tumor Morphology and Immunophenotype

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Background: Increasingly, select patients with advanced stage tubo-ovarian high-grade serous carcinoma (HGSC) are treated with neoadjuvant chemotherapy (NACT) prior to debulking surgery. NACT alters both tumor morphology and protein expression and can significantly decrease tumor volume, albeit with variable response. Comparing patients with optimal (<1cm residual disease) and suboptimal (≥1cm residual disease) response to NACT, we sought to characterize NACT-induced tumor morphology and immunophenotype alternations in correlation with clinical response.

Design: All patients with newly diagnosed tubo-ovarian HGSC treated with NACT followed by interval debulking surgery at a single academic institution from 2009 to 2017 were included. Pre- and post-NACT tumor specimens were reviewed. Tumor morphology and P53, PAX8, and WT1 protein expression were assessed by immunohistochemistry. Clinical data was abstracted from medical charts.

Results: 29 patients met study criteria with a median age of 62 years (range 45-81). 89% (n=26) had stage III disease. Patients received an average of 5 cycles (range 3-9) of platinum-based combination NACT. Median time between diagnostic biopsy and interval debulking surgery was 5 months (range 2.5-9). Post-NACT, 88% of patients showed significant decrease of serum CA-125 values (>90%). Debulking specimens revealed optimal response in 13 patients (45%), whereas suboptimal response was seen in 16 (55%). Compared to NACT tumors with optimal response, those with suboptimal response showed higher mitotic activity (5-20/HPF), less cytological alternation (i.e. multinucleation, chromatin smudging, cytoplasmic eosinophilia), and less stromal alternation (i.e. fibrosis, inflammation, foamy histiocytes). P53 and PAX8 showed similar positivity pre- and post-NACT in all patients. WT1 showed complete absence or decreased expression in 71% of NACT tumors with suboptimal response but not in tumors with optimal response to NACT.

Conclusions: We demonstrated that HGSC tumors exhibit significantly different cytological and stromal alternations in response to NACT. While P53 and PAX8 protein expression remain constant, the key immunophenotype alternation is WT1 whose complete loss or decreased expression correlates with less favorable clinical response.

1227 High mRNA Levels of Endometrial Stem/progenitor Cells (ES/PCs) Identify a Sub-group of Endometrial Carcinoma with Particularly Poor Overall Survival

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Background: The endometrium is a rich source of endometrial stem/progenitor cells (ES/PCs), and a number of different markers have been used to isolate a heterogeneous group of ES/PCs that vary in their proliferative and pluripotent capacities. When malignancies arise in the endometrium, they form a heterogeneous group of carcinomas, and four different, well-established molecular subtypes have been described. Among them, the serous-like endometrial carcinoma (SLEC) molecular subtype shares a number of molecular features with triple-negative breast carcinomas, which encompasses the claudin-low subtype. Claudin-low subtype is characterized by low expression of adhesion molecules and high expression of mammary stem cell markers.

Design: A set of 33 genes was generated through literature review. The mRNA levels for these genes from the cancer genome atlas (TCGA) cohort were downloaded from cBioPortal. Survival analyses were performed after dichotomizing the TCGA cohort based on the median Z-score values for each marker.

Results: Four genes, CD9, LGR5, MSI1 and ST3GAL2, were found to be expressed at higher levels in SLECs, compared to normal tissue and other subtypes. Their expression levels did not show strong correlations with each other. In general, higher expression of each marker corresponded to worse overall survival (OS), with the worst OS seen in the cases with high expression of all four markers (median survival 46.8 months *vs.* not reached for others, log-rank *p*-value < 0.0001). High expression remained prognostic in a sub-group analyses restricted grade 3 (long-rank *p*-value = 0.0384), serous histology (*p* = 0.0308), or SLEC molecular subtype (median survival 46.8 months *vs.* not reached, log-rank *p*-value 0.0077). In a multivariate model that included stage (> II), grade (> 2), age (> 60 years), molecular subtype (SLEC *vs.* other) and ES/PC marker levels, high expression of all four markers remained strongly prognostic (risk ratio 6.5, 95% confidence interval range 2.3-18.2).

Conclusions: Taken together, our analyses, based on mRNA levels, identify a subgroup of tumours with high expression of a specific set of ES/PC markers enriched in the SLEC molecular subtype. The prognostic value of this phenotype was significant and independent of tumour grade, stage and molecular subtype. Current efforts are underway to confirm the prognostic significance in an independent cohort, examining protein levels.

1228 Molecular Profiles of Mixed Endometrial Carcinomas

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Background: Mixed endometrial carcinomas are defined as a combination of two or more distinct histologic subtypes, one component of which must be a Type II tumor comprising at least 5% of the tumor volume. Recent estimates show that mixed endometrial carcinomas account for anywhere between 3 to 10% of all endometrial carcinomas, and have historically been a source of both great interest and diagnostic difficulty. In light of the increasingly appreciated morphologic overlap between different subtypes of endometrial carcinoma, the oncogenesis of these tumors remains unclear. The goal of the current study was to evaluate these tumors at the molecular level in the hopes of better understanding the oncogenic mechanisms at play.

Design: 8 cases of mixed endometrial carcinoma previously evaluated with immunohistochemical markers were selected. These included 5 mixed endometrioid (EC)/serous (SC), one SC/clear cell (CC) , and two mixed EC/CC cases. Each component was interrogated with the NGS Oncomine Comprehensive Assay v3 that covers 161 genes including 87 hotspot mutations, 91 copy number variants (CNV), and 51 fusion drivers. HDx positive controls were used and ≥ 90% of expected variants were detected.

Results: All tumors shared mutations in both components. In 6 cases containing SC, shared mutations involved ARID1A, PIK3CA, TP53 and PTEN. 5/6 of these also showed additional mutations in the SC component only: POLE (2 cases), DDR2 and MAP2K1 (1 case), CCNE1

(1 case) and a second TP53 mutation (1 case). Further, in EC/SC mixed tumors, ERBB2 amplification was found in both components of 1 case, and ERBB2 activating mutation in the SC component in another case. The two EC/CC cases showed FGFR2 activating mutations in the EC component only. The median coverage for all cases was 1120x. In 12/16 components where RNA sequencing was successful, no fusion drivers were identified.

Conclusions: Our data support that the majority of these tumors begin as a single clone and diverge along two pathways: 1) tumor progression, in which one component displays additional mutations, and 2) tumor divergence, in which tumors have both shared mutations and mutations unique to their classified histology. Our findings are clinically relevant since targetable mutations may only be present in one component of mixed tumors.

1229 Molecular Evaluation of Low-Grade Low-Stage Endometrial Cancer With and Without Recurrence

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Background: Low-grade, low-stage endometrioid carcinomas (LGLS EC) demonstrate 5-year survival rates up to 95%. However, a small subset of patients recur and there are neither prognostic markers nor established mutation profiles associated with recurrence. Using whole-exome sequencing (WES), we previously described increased rates of PIK3CA mutation and broad copy number gains in the primary tumors from a cohort of LGLS EC with recurrence. The goal of the current study was to identify genomic differences between primary tumors and subsequent recurrence.

Design: Three of four previously described cases of LGLS EC with recurrence and 8 cases without recurrence were evaluated with the Oncomine Comprehensive Assay v3, which covers 161 genes including 87 hotspot mutations, 91 copy number variants (CNV), and 51 fusion drivers. HDx positive controls were used and ≥ 90% of expected variants were detected. Exclusion of the fourth case was based upon insufficient material. The resulting molecular profiles of the primary tumor were also compared to prior WES results.

Results: Two of the three recurrent cases showed additional mutations in the recurrence. One case included an additional loss-of-function TP53 mutation and the other case showed loss-of-function POLE and activating DDR2 mutations. The third case did not accrue additional mutations. PIK3CA mutations were detected in 4 of 4 LGLS EC with recurrence and in 3 of 8 disease-free cases. The median coverage for all cases was 1362x. No fusion drivers were identified.

Conclusions: The underlying cause as to why certain LGLS ECs recur despite a lack of histologic or clinical evidence portending aggressive behavior has not yet been elucidated. This pilot study showed two of three recurrent cases gained a mutation associated with genetic instability (TP53 and POLE) and one of them also acquired a mutation in the DDR2 kinase, a potential therapeutic target. We also corroborated the previously noted higher frequency of PIK3CA mutations in the primary tumors that later recurred.

1230 A Case Series of Incidental Maternal Endometrial Polyp Within the Placenta (MEPP)

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Background: Endometrial polyps are common lesions that are found within the uterine cavity of women, and can be asymptomatic, associated with abnormal uterine bleeding or infertility. As common as endometrial polyps are, their presence as an incidental finding with in the placenta has not been previously described. Our institution has accumulated three cases of term placentas with an incidental finding of a retained endometrial polyp, also known as the maternal endometrial polyp within the placenta (MEPP). MEPPs should not to be confused with placental polyps, a radiologic term used to describe retained placental tissue. We report three cases of maternal endometrial polyps incorporated into each placenta over the course of gestation and identified during the gross placental evaluation.

Design: We searched our institutional archives for the terms "placenta" and "endometrial polyp" from 1997 to 2016 and yielded 3 cases. All H&E slides and gross photos were re-reviewed with special attention to the histopathology and relevant clinical history.

Results: All three placental cases were submitted for histologic review under the 1996 CAP guidelines. The maternal ages ranged from 29-37, and all three cases delivered a healthy term infant. Grossly, all cases showed a well-circumscribed white nodule associated within the placental specimen. In each case, the nodules were located in different locations including the disk parenchyma, amnionic membranes, and retroplacental maternal-fetal interface. Based on the gross examination, the differential diagnosis included a placental infarct, an organizing intervillous thrombus, or a chorangioma. Histologically, the nodules were endometrial polyps with prominent decidualized stroma, thick vascular bundles, and endometrial glands with gestational changes, consistent with MEPPs. No pregnancy complications occurred in association with the MEPPs.

Conclusions: We describe three cases of incidental MEPP (maternal endometrial polyps within a placenta). Incidental MEPPs should be distinguished from the previously described "placental polyp" due to the clinical implications of this term. Placental polyps are chorionic remnants that are retained within the uterine cavity and can cause post-delivery uterine bleeding and elevations in beta-HCG, and thus require removal. MEPPs are histologically maternal in origin becoming associated with the placenta over the course of gestation. In our case series, these lesions were benign and incidental with no associated clinical outcomes.

1231 Application of an Ovarian Cancer Histology-Based Testing Strategy for BRCA1/2 Mutation Detection

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Background: Background: Personalized therapies for ovarian cancer include PARP inhibitors, which are active against tumors with BRCA1/2 mutations, either germline or somatic. Additionally, there is interest in identifying germline carriers of BRCA1/2 mutations to enroll them and their family members in cancer control and prevention programs. Thus, there is currently a growing need to introduce BRCA1/2 mutation testing into the routine laboratory diagnosis of ovarian cancer.

Design: Design: During a 16-month period ovarian tumor tissue from 111 patients was reflexively tested based on the tumors histologic subtype, of which 93 gave consent for inclusion. High-grade tumors, such as HGSC, endometrioid FIGO 3, undifferentiated and carcinosarcoma were tested.

Results: Results: The mean age of the 93 patients was 66 years. Testing was performed on 60 FFPE and 33 fresh frozen tumor samples. In 22/93 (24%) of patients a BRCA1/2 mutation was detected in their resected tumor material, including 14 BRCA1 and 8 BRCA2 mutations. The average age of patients whose tumors had a BRCA1/2 mutation compared to those without was 61 yrs., compared to 67 yrs. (p=0.02). The average age of patients whose tumors had a BRCA1 vs a BRCA2 mutation was also different (58 yrs. vs 67 yrs., p=0.02). A nonsignificant difference in detection of BRCA1/2 mutations was observed between testing on fresh frozen vs. FFPE material (Fresh: 10/33= 30%; FFPE: 12/60=20%).

After tumor testing, 2/22 (9%) patients had not yet been referred for genetic counselling, and 4/22 (18%) were found to harbour nonspecific BRCA-mutations. Germline mutational analysis was performed on 16 patients and 9/16 (56%) were identified as harbouring a germline BRCA1/2 mutation, including 5 BRCA1 and 4 BRCA2 carriers.

Conclusions: Conclusion: This data indicates that reflex testing of tumor tissue is an effective method for detecting patients that might benefit from PARPi therapy. Additionally, tumor testing allowed a number of previously unidentified BRCA1/2 germline mutation carriers to be identified. Of note, one BRCA1/2 mutation carrier had a carcinosarcoma, indicating that reflex testing should not only be restricted to HGSC. There was a significantly lower age to patients whose tumors had a BRCA1/2 mutation. These results indicate that in a consecutive series a significant number of tumors with BRCA1/2 mutations, that might potentially respond to PARPi therapy, are not found in germline carriers but are the result of somatic mutations.

1232 Characterization of the Immune Microenvironment of High Grade Serous Ovarian Carcinomas in African American Women: A Study of 112 Cases from the African American Cancer Epidemiology Study (AACES)

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Background: African American (AA) women with high grade ovarian serous carcinoma (HGOSC) show worse outcomes when compared to women of European descent. This can be partially attributed to differences in access to and quality of care, but these factors cannot fully account for the observed discrepancy. Immune context is increasingly understood to influence tumor behavior, and prior work has shown racial differences in systemic and intratumoral inflammation. The African American Cancer Epidemiology Study (AACES) is a multi-center population-based case-control study of ovarian cancer in AA women and represents the largest available cohort of this population. We herein characterized the immune milieu in 112 HGOSC from AACES with attention to lymphocyte infiltration and the targetable immune regulatory molecules PD-L1 and IDO.

Design: IHC for PD-L1, IDO, CD8, and FOX3p was performed on whole section slides from 112 HCOSC. CD68 IHC was performed to facilitate distinction between tumor cells and associated macrophages. The extent of PD-L1 and IDO immunostaining was scored separately in tumor and immune cells. CD8 and FOX3p-positive lymphocytes were enumerated over 10 high-power fields (HPF) and mean/HPF was calculated. Statistics were performed using Fisher's exact and Kruskal-Wallis tests.

Results: Tumor cells were positive for PD-L1 and IDO in 29% and 58% of cases, respectively. The majority (including all PD-L1-positive cases) showed <10% staining; 6% expressed IDO in 10-25% of cells, and no cases had >25% positivity. Immune PD-L1 and IDO staining was seen in 73% and 54% of cases, respectively. Immune staining was most often focal, however ≥10% staining was seen for PD-L1 and IDO in 24% and 2%, respectively. Median CD8/HPF count was 13 (range 0-204), median FOX3/HPF was 3 (range: 0-39). Higher CD8 and FOX3 numbers were significantly associated with increased PD-L1 and IDO expression (p=0.05).

Conclusions: PD-L1 and IDO expression is seen in a subset of HGSOC from AA women and is associated with elevated cytotoxic and regulatory T lymphocyte infiltration. Tumoral PD-L1 expression rates are higher than has been reported in racially unselected cohorts. However, diffuse expression for both PD-L1 and IDO remains rare, invoking caution regarding the potential for immunotherapeutic response. Clinical trials are needed to determine thresholds predictive of response to immune modulatory molecule antagonism in HGOSC, as are comparison studies with European American control groups.

1233 ALK is a Specific Diagnostic Marker for Inflammatory Myofibroblastic Tumor of The Uterus

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Disclosures:
Josh Haimes: *Employee*, ArcherDx
Brian Kudlow: *Employee*, ArcherDX

Background: Inflammatory myofibroblastic tumor (IMT) is a myofibroblastic/fibroblastic tumor of intermediate malignant potential. It is frequently characterized by genetic fusion of *ALK* with a variety of partner genes, which results in activated ALK signaling pathway that can be targeted with kinase inhibitors. IMTs can occur in the gynecologic tract, with uterus being the most frequent site. Recent studies have shown that IMTs in the gynecologic tract are under-recognized and a low-threshold for performing ALK immunohistochemistry is proposed. The aim of this study is to evaluate the specificity of ALK immunohistochemistry for IMTs among uterine mesenchymal and mixed epithelial/mesenchymal tumors.

Design: We performed ALK immunohistochemistry on 15 molecularly confirmed uterine IMTs and 245 uterine pure mesenchymal and mixed epithelial/mesenchymal tumors (35 leiomyosarcomas, 63 leiomyomas, 41 low-grade endometrial stromal sarcomas, 5 high-grade endometrial stromal sarcomas, 6 undifferentiated uterine sarcomas, 75 carcinosarcomas and 20 adenosarcomas). Cases showing any positive cytoplasmic and/or membranous staining of the tumor cells were considered to be ALK-positive.

Results: All 15 uterine IMTs were previously confirmed to harbor *ALK* genetic fusion/rearrangement by RNA-sequencing and/or genetic rearrangement by fluorescence *in situ* hybridization analysis. Diffuse ALK immunostaining in the form of granular cytoplasmic positivity and paranuclear accentuation was observed in all 15 IMTs, although the intensity was variable within the same tumor in some cases. ALK was negative (with complete absence of staining) in all other pure uterine mesenchymal tumors and in all uterine mixed epithelial/mesenchymal tumors.

Conclusions: Our findings show that ALK is a highly specific diagnostic immunohistochemical marker for *ALK* fusion in the uterus. ALK immunopositivity in the appropriate histologic context strongly suggests the diagnosis of uterine IMT and should prompt confirmatory molecular analysis.

1234 L1 CAM – A Potential Biomarker for Recurrent and Aggressive Endometrial Carcinoma

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Background: Endometrial cancer (EC) is the most common gynecologic malignancy in the United States and the second most common gynecologic cancer worldwide. Type I EC with endometrioid histology has an excellent prognosis, with 10-year survival rate over 80%. However, some patients show recurrences and poor survival. L1CAM (CD171) is a cell adhesion molecule from the immunoglobulin family expressed in neuronal cells and in several tumors (ovarian, endometrial, colo-rectal, pancreatic cancer). It enhances proliferation, migration, invasion and metastasis. Lately, L1CAM was shown to be present in patients with stage I EC, predicting a worse prognosis with lower progression-free and overall survival. The purpose of this study is to evaluate L1CAM as a potential biomarker for aggressive EC.

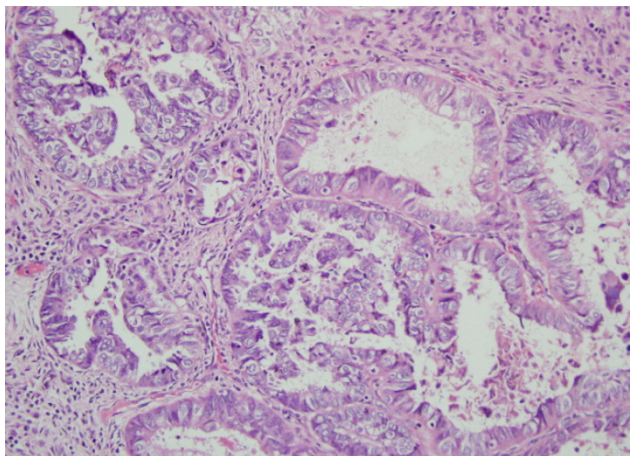
Design: A retrospective study was performed. 135 random cases of endometrioid EC were identified from UMMC pathology database (1996-2004). The cases were reviewed for confirmation of the diagnosis of EC and FIGO grade and a representative tumor block was chosen. Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded sections using a commercially available monoclonal antibody for L1CAM. Each slide was and assigned a numeric "H score" (range 0-300) based on the percentage and intensity of staining. An H-score of ≥ 10 was considered positive. Tumor recurrence, defined as evidence of clinical recurrence >6 months following therapy, was assessed from the electronic health record and pathology records.

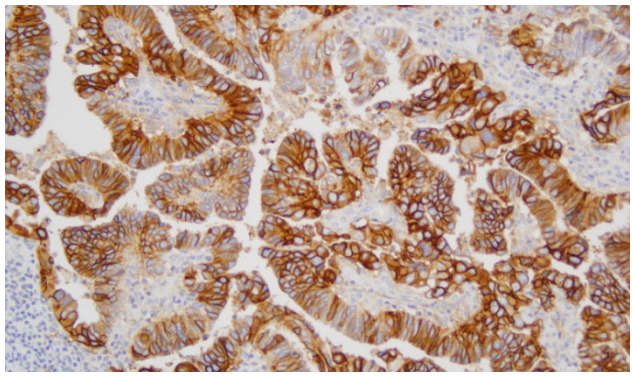
Results: We analyzed 135 cases of EC from our institution, evaluating L1CAM expression as an independent factor for tumor recurrence. We used a chi-squared test with a significance level of .05 for our calculations. Of the 135 cases, 30 were L1CAM positive (+) and 105 were L1CAM negative (-). Of the 30 positive cases, 6 had recurrence (recurrence rate =.20), and of the 105 negative cases, only 5 had recurrence (recurrence rate = .04) (see table).

L1CAM expression by immunohistochemistry and recurrent endometroid carcinoma

L1CAM IHC	Recurrent EC	Non-recurrent EC	Total
L1CAM +	6	24	30
L1CAM-	5	100	105
Total	11	124	135

P value = 0.007133





Conclusions: L1CAM+ tumors have a higher rate of recurrence than L1CAM- ones and this difference is statistically significant (p-value =.0007133). Our overall recurrence rate of 8.1% is similar to the rates found in other studies, which include all grades and stages of type 1 EC. Our results further support the importance of L1CAM expression in EC. Ultimately, testing EC for L1CAM expression in clinical setting may help identify women at higher risk for tumor recurrence and guide adjuvant therapy, including future L1CAM-targeted therapies.

1235 Gastric Differentiation in Vaginal Adenosis and Primary Vaginal Mucinous Adenocarcinomas

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Background: Primary adenocarcinomas of the vagina are rare and the WHO Classification includes endometrioid, clear cell, mucinous and mesonephric types. Gastric differentiation is uncommon but well described in a range of benign, premalignant and malignant cervical glandular lesions; the malignant lesions are termed gastric-type adenocarcinoma. We report the clinicopathological features of 5 cases of primary vaginal mucinous adenocarcinoma exhibiting gastric differentiation (gastric-type adenocarcinomas). We describe gastric differentiation in vaginal adenosis and suggest a neoplastic pathway to the development of vaginal gastric-type adenocarcinoma.

Design: The clinicopathological features of the 5 cases were studied and a panel of immunohistochemical markers performed.

Results: The cases occurred in women aged 41-69 years with no known history of in-utero exposure to diethylstilbestrol. Symptoms included a vaginal mass, vaginal discharge, urinary symptoms and abnormal glandular cells on cervical smear. Follow-up was available in 2 cases and both patients died of tumour after 3 years. The tumours ranged in size from 3 to 4 cm. Histology showed moderately or poorly differentiated mucinous adenocarcinomas with abundant clear or eosinophilic cytoplasm, morphological appearances identical to gastric-type cervical adenocarcinomas. In one case, there were focal goblet cells and in another basaloid and sarcomatous components; the latter two features have not been described in gastric-type cervical adenocarcinomas. Immunohistochemistry for the gastric marker MUC6 showed positivity in all 3 cases tested. p16 and ER were negative in all cases tested (5 and 4 respectively). p53 exhibited mutation-type staining in 3 of 5 cases. PR, CK7, CK20, CEA and CDX2 were positive in 1/4, 4/4, 1/4, 2/3 and 4/4 cases respectively. Areas of benign adenosis with mucinous features were identified in all cases which sometimes exhibited nuclear atypia (atypical adenosis) with MUC6 positivity. We also studied several cases of benign vaginal adenosis and have observed gastric differentiation with MUC6 positivity and goblet cell formation.

Conclusions: Gastric-type adenocarcinomas, identical to those occurring in the cervix, may occur as primary vaginal neoplasms. They arise from adenosis/atypical adenosis which may exhibit gastric differentiation and the latter may also occur in benign vaginal adenosis. The prognosis appears poor, although follow-up of larger numbers of cases is required to confirm this.

1236 PD-L1 and Mismatch Repair Protein Immunohistochemical Expression in Small Cell Carcinoma of the Uterine Cervix

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Background: Small cell carcinoma of the uterine cervix (SmCC-Cx) is a rare tumor related to HPV infection. Therapeutic options are limited for this aggressive neoplasm. Merkel cell carcinoma, another virus-related neuroendocrine malignancy, has significant rates of PD-L1 expression, and targeted treatment with immune checkpoint inhibitors is a consideration in this disease. PD-L1 expression has also been reported in other malignancies of the cervix. Thus, we aimed to determine the prevalence of PD-L1 and mismatch repair protein (MMR) expression in SmCC-Cx.

Design: We identified patients with a diagnosis of SmCC-Cx seen in our institution in the last 16 years. Cases with material available for review and immunohistochemistry (IHC) testing were included. Diagnosis was confirmed by two gynecologic pathologists. IHC panel included PD-L1 IHC (clone SP263, Ventana), MLH1, MSH2, MSH6 and PMS2. PD-L1 expression in tumor cells was scored quantitatively (H-score). MMR IHC was interpreted as lost (absence of nuclear staining) or retained (any staining). Clinico-pathologic variables were recorded, including patient outcome.

Results: Ten patients with material available and confirmed diagnosis were included. PD-L1 positivity (any staining in tumor cells), was seen in 7 cases, mostly focal (H-score ranging from 2-170, median 8). 3/9 cases showed MMR deficiency (MLH1/PMS2 loss). PD-L1 expression levels correlated with MMR loss status: all three MLH1/PMS2-deficient cases had a ≥5% PD-L1 staining and an H-score ≥15 (p=0.01, two-tail Fisher's exact test). All patients received standard chemotherapy +/- radiation. With a median follow-up of 3 months (range 1-35), 2 patients had persistent tumor and 8 showed quick progression of the disease with multiorgan metastases.

Conclusions: In our cohort, MLH1/PMS2 loss was observed in a third of SmCC-Cx tumors, all of which showed 5% or more PD-L1 expression. Our findings suggest that a subset of patients with SmCC-Cx may be amenable to immune checkpoint inhibitor therapy, a potential alternative to standard treatments for this aggressive and often refractory disease. MMR and PD-L1 IHC can be used to identify such patients. Validation of our findings and proposed cut-off is necessary.

1237 The Expression of Neuroendocrine Markers in Endometrial Tumors Without Classic Histologic Features of Neuroendocrine Carcinoma

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Background: Neuroendocrine (NE) tumors are relatively uncommon in the gynecologic tract. In addition to their characteristic histopathologic features, what defines a NE carcinoma is the expression of markers such as chromogranin, synaptophysin and neural cell adhesion molecule (CD56) by immunohistochemistry (IHC). Although limited data have demonstrated that some high-grade uterine tumors may focally express these markers, the incidence of such labeling in endometrial carcinomas (EC) in general is not well known. The goal of this study was to characterize the expression of NE markers in a cohort of EC.

Design: We searched our institutional surgical pathology database for hysterectomy specimens containing EC. Cases demonstrating classic morphologic features of NE carcinomas were excluded. IHC for synaptophysin, chromogranin and CD56 was performed in whole-tissue sections of FFPE tumors. Thyroid transcription factor 1 (TTF-1) was also included, given its positivity in a subset of small cell carcinomas. Marker expression was graded based on percentage of positive tumor cells (0= not detected, 1= 1-25%; 2= 25-50%; 3= >50%). Statistics were done using chi-square; significance set at $p<0.05$.

Results: 71 carcinomas of endometrioid (EMC; 26 cases), serous carcinoma (SC; 20), clear cell carcinoma CCC; 12), undifferentiated (2) and dedifferentiated (1) histologies were obtained, as well as 10 carcinosarcomas (CS). The majority expressed one or more NE markers (51/71; 71%), with most positive cases showing focal (1+) staining of a single marker. Significantly more tumors stained positive for CD56 than synaptophysin (58% vs. 7%, $p<0.01$). CCC were the least likely to express any NE marker (4/12; 33%), whereas SC (80%) and CS (100%) were the most likely. CD56 labeling was seen in 9/10 CS, in both epithelial (7/9) and mesenchymal (5/9) elements. A slightly greater proportion of non-endometrioid histologic types stained positive for TTF-1 compared with endometrioid type (31% vs. 12%, p = 0.06).

Endometrial Carcinomas Immunohistochemically Positive For One or More NE Markers	
Histology	Positive/Total
Endometrioid FIGO 1	7/12
Endometrioid FIGO 2	4/6
Endometrioid FIGO 3	6/8
Serous (SC)	16/20
Clear Cell (CCC)	4/12
Carcinosarcoma (CS)	10/10
Dedifferentiated	1/1
Undifferentiated	2/2
Total	51/71

Incidence of at Least Focal NE Marker Expression in Endometrial Carcinomas Lacking Conventional NE Features	
Marker	Positive/Total
Synaptophysin	5/71
TTF-1	17/71
Chromogranin	21/71
CD56	41/71

Conclusions: Immunohistochemical expression of NE markers is relatively common in EC that lack classic NE histology. The most frequent pattern encountered in our study was focal (1-25%) labeling of a single marker. Synaptophysin appeared reliably negative, while CD56 was commonly present in non-NE histology. CCC tend to be consistently negative, whereas CS and SC frequently express at least one marker. Awareness of these data may help to avoid misdiagnosis of a neuroendocrine carcinoma in limited samples.

1238 Pathologic Examination of Placenta: A Study On 500 Live Births to Assess Conformity to CAP Guidelines and Clinicopathologic Correlation

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Background: Placental examination is a valuable tool in understanding the pathophysiology of adverse outcome including the identification of potentially treatable conditions, improving management of future pregnancies and in medicolegal risk assessment. College of American Pathologists (CAP) published guidelines for placental pathologic examination which include underlying maternal diseases, pregnancy complications, fetal/neonatal and placental indications. However, conformity to these guidelines is highly variable from one institution to other. We aim to examine the appropriateness of placental pathologic examinations at our institution, their conformity with CAP guidelines and evaluate clinico-pathologic correlation (CPC) between the indications and pathologic findings.

Design: Obstetrical records(history and delivery reports) were reviewed for a total of 500 consecutive live births noting whether placenta was sent for pathologic evaluation according to CAP guidelines. The cases were divided into two main categories: A) compliant with CAP guidelines whether 1) sent (true positive/TP) or 2) not sent for pathologic examination (true negative/TN) and B) non-compliant with CAP guidelines whether 3) sent and should not have been sent (false positive/FP) or 4) not sent but should have been sent (false negative/FN). CPC was also assessed on TP cases.

Results: Of the 500 live births, 213 placentas should have been sent, out of which 135 placentas were sent for pathologic examination (63.4%). Of the 287 placentas not indicated to be sent, 263 were appropriately not sent (91.6%). We found concordant CPC in 87/135 placentas submitted (TP cases). The most common unsuspected diagnosis was chorioamnionitis. A total of 91/135 placentas were submitted for maternal, fetal (33/135) and placental (11/135) indications. Interestingly, some FP cases had significant pathologic findings. Non-reassuring fetal heart tones turned out to be a common indication for placental submssion in our study which is not an established criterion in the CAP guidelines.

Sent	Should have been sent	
	Yes	No
Yes	135	24
No	78	263
	Sensitivity: 63.4 %	Specificity: 91.6%

Conclusions: Placental examination is an important element in maternal and fetal medicine. The CAP guidelines suggest establishing customized lists of indications by inter-department consultation. Certain indications (gestational hypertension, diabetes, oligohydramnios) need more characterization of severity to warrant pathologic examination. More awareness and understanding of these guidelines is needed for appropriate submission of placentas for pathologic examination.

1239 Gastric-type Cervical Adenocarcinoma in Small Biopsy and Cytology Specimens

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Background: Gastric-type cervical adenocarcinomas (GCAs) are human papillomavirus (HPV)-negative, aggressive, chemo-refractory tumors. They include so-called "minimal deviation adenocarcinomas (MDAs)" and may exhibit bland appearances that belie their aggressive behavior. This could potentially lead to under-recognition or misdiagnosis, particularly in limited (biopsy or cytology) specimens. We sought to document the morphologic features of GCA in biopsy and cytology specimens.

Design: We identified patients with histologic diagnoses of GCA or MDA diagnosed between 2004-2017 with available slides from cervical biopsy (CB), vaginal biopsy (VB), endocervical curettage (ECC), endometrial biopsy/curettage (EMB/EMC) and cytology specimens, which were reviewed.

Results: There were 27 patients aged 29-83 (median 52) years, with slides from biopsy, cytology and both biopsy and cytology specimens in 11, 9 and 7 patients, respectively; there was >1 biopsy in 10 patients. Biopsies included: VB (n=4), CB (n=7), ECC (8), EMB (n=5) and EMC (n=6), pelvis (n=1) and umbilicus (n=1). Cytologic specimens included: cervicovaginal smears (n=9), pelvic fluid (n=4), imprint of pelvic lymph node biopsy (n=1), and fine-needle biopsies of neck lymph node (n=1) and pelvic mass (n=1).

Biopsies showed well or moderately differentiated adenocarcinomas. Neoplastic glands were composed of polygonal or columnar cells with pale or foamy cytoplasm, with well defined cytoplasmic borders. Nuclei exhibited mild to moderate pleomorphism and nucleoli were identified in many tumor cells. The diagnosis was readily apparent in most biopsies, and was only challenging in a minority in which the neoplastic glandular epithelium was scant, fragmented and/or very well differentiated.

Cytologic slides showed crowded and disorganized groups of tumor cells, as well as single cells. Cells were columnar or polygonal in shape, with pale, foamy &/or vacuolated cytoplasm with well-defined cytoplasmic borders. Nuclei were round to oval, moderately pleomorphic, and mostly contained finely granular/vesicular chromatin, with one or more nucleoli.

Mitoses and apoptotic bodies were rare in both biopsy and cytology specimens.

Conclusions: Diagnosis of GCA, particularly well-differentiated tumors, in small-volume biopsy and cytology specimens can be challenging. Awareness of the morphologic features with confirmatory immunochemistry (eg markers of gastric differentiation and HPV), will allow recognition and accurate diagnosis of these aggressive tumors.

1240 BCOR Expression in Mullerian Adenosarcoma: A Potential Diagnostic Pitfall

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Background: BCOR expression has emerged as a robust tool in the evaluation of high-grade endometrial stromal sarcoma with *YWHAE* rearrangement and *BCOR* genetic abnormalities. Mullerian adenosarcoma with stromal overgrowth can rarely mimic high-grade endometrial stromal sarcoma with *ZC3H7B-BCOR* fusion that may show entrapped glands and often exhibits diffuse BCOR expression. We encountered diffuse BCOR expression in rare adenosarcomas and sought to define its frequency among a larger cohort of these tumors.

Design: All available slides and pathology reports of adenosarcomas diagnosed between 2013 and 2017 were reviewed for confirmation of diagnosis. BCOR immunohistochemistry was performed on archival formalin-fixed paraffin-embedded tumor tissue in all cases. Staining intensity (negative, weak, moderate, strong) and percentage of positive tumor nuclei in the mesenchymal component were evaluated.

Results: The diagnosis of adenosarcoma was confirmed in 11 tumors, including 9 primary and 2 metastatic, with available material. These included 2 low-grade, 1 high-grade and 8 with stromal overgrowth. BCOR staining was seen in the mesenchymal component of 8 of 11 (73%) tumors, including 5 with stromal overgrowth, 1 high- and 2 low-grade tumors without stromal overgrowth. Moderate to strong staining in >70% of cells was seen throughout 4 of 6 high-grade tumors with stromal overgrowth. BCOR expression was seen in 20-100% of cells in the periglandular stroma of 4 tumors, including 2 low- and 2 high-grade, 1 of which showed stromal overgrowth. No staining was seen in 2 low- and 1 high-grade tumor with stromal overgrowth.

Conclusions: BCOR expression may be seen in adenosarcomas with higher frequency among high-grade tumors with and without stromal overgrowth. Strong and diffuse staining is seen in a subset of tumors, suggesting a potential diagnostic pitfall in the interpretation of BCOR expression in uterine mesenchymal neoplasms, particularly with limited tissue sampling.

1241 Molecular Landscape of Gastric-Type Endocervical Adenocarcinomas (GAS)- Next Generation Sequencing of 14 Cases

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Background: GAS are unusual, aggressive endocervical tumors that are not associated with human papilloma virus (HPV), usually diagnosed at a higher stage and can be challenging in regards to diagnosis and treatment. The molecular characteristics of these tumors have not been explored yet. In this study, we sought to understand the molecular profile of GAS using Next Generation Sequencing (NGS) with a panel focused on detecting relevant SNVs (single nucleotide variant), CNVs (copy number variations), gene fusions, and indels from 161 unique cancer driver genes.

Design: DNA and RNA were extracted from formalin-fixed paraffin-embedded tumor of 14 GAS cases (PCR confirmed negative HPV) and 2 controls. Genomic DNA and cDNA (from RNA) was profiled using the Oncomine Comprehensive V3 NGS panel (ThermoFisher Scientific). Variant data was assessed by Ion Reporter software (ThermoFisher). HPV status by PCR and p53 IHC (immunohistochemistry) staining were performed using standard

procedures. Clinico-pathological features were reviewed and documented.

Results: The mean age at diagnosis was 57 years. A total of 92 variants (7 variants on an average) were observed across 14 tumor samples tested. *TP53* was the most recurrently mutated gene followed by *MSH6, SLX4, POLE, CDKN2A/B, FANCA, ARID1A, STK11, BRCA2* and *MSH2.* Abnormal p53 expression was observed in 9 cases by immunohistochemistry (3 diffuse overexpression, 6 null expression). *TP53* variants were present in 4/6 cases that were p53 null by IHC but no correlation was observed between overexpressed p53 staining and presence of *TP53* mutations. 2 cases showed *MDM2* gene amplification in 12q15 (69202190-69233452) locus. There were 4 cases with *STK11* null (frameshift/nonsense) variants, three of which were previously reported in Peutz-Jeghers Syndrome.

Conclusions: This study is the first to explore the molecular features of this aggressive variant of cervical cancer. In general, genes involved in the DNA damage, cell cycle, DNA recombination and repair, PI3K-AKT, Fanconi anemia and HTLV1 infection pathways were found to be mutated. Many of the genes known to be tumorigenic as acquired or inherited variants in colorectal or gastric cancers, such as *TP53, STK11, KRAS, MSH6, MSH2, PMS2* were enriched in our dataset. Interestingly, finding *MDM2* 12q15 amplification (seen in well-differentiated liposarcoma) opens the possibility of use of MDM2 antagonists in treating these tumors. Overall, our study unravels pathways that may enhance therapeutic options for these tumors.

1242 Squamous Morules with Central Necrosis in Endometrial Biopsies: A Clinicopathologic Follow-Up Study

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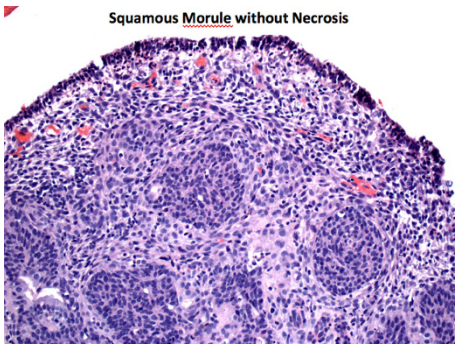
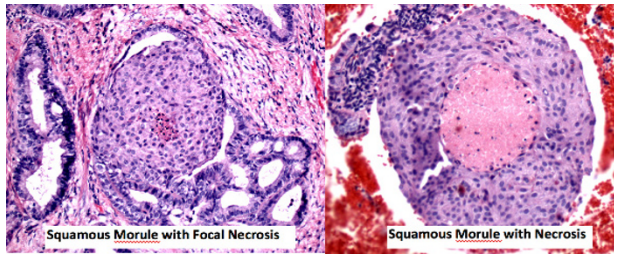
Background: Squamous morules may be encountered in endometrial biopsies often in association with endometrial hyperplasia or endometrioid adenocarcinoma (EAC). On occasion, necrosis may be identified within squamous morules. The significance of necrosis in this setting is unknown. In this study, we describe the morphology, assess the prevalence, and evaluate the potential significance of necrosis in squamous morules through review of clinicopathologic follow-up in patients showing this feature.

Design: Endometrial biopsies showing squamous morules from 2007 to 2010 were reviewed. Clinicopathologic data and subsequent specimens were examined. Morphologic features of each case were noted. Cases were classified as containing necrosis and lacking necrosis. Statistical comparison was performed using Fisher's exact test.

Results: 879 endometrial biopsies were identified with 202 meeting inclusion criteria. Of these, 60 endometrial biopsies demonstrated squamous morules including 30% (n=18) with necrosis and 70% (n=42) without necrosis. Morphologically, the necrosis was typically centrally located in the morules with either single-cell to focal involvement or a more extensive comedo-like pattern. Squamous morules with necrosis were most frequently seen in EIN (39%, 7/18) and complex hyperplasia (33%, 6/18). Morules without necrosis were most frequently seen EIN (52%, 22/42) and EAC (19%, 8/42). Taken together, no significant differences in endometrial pathology between the two groups were identified (p=0.219). Thirty-eight patients (63%, 38/60) underwent hysterectomy. Among these, 9 (24% 9/38) showed squamous morules with necrosis and 12 (32%, 12/38) morules without necrosis. In the follow-up specimens, the endometrial pathology did not significantly differ between the two groups (p=0.557). Five patients (13%, 5/38) demonstrated higher grade lesions associated with squamous morules on hysterectomy compared to their respective biopsies. Among these, one had squamous morules with necrosis on initial biopsy.





Conclusions: Necrosis is a rare finding in squamous morules within the endometrium with either focal or extensive comedo-like morphology. To our knowledge, we are first to describe and examine the significance of this finding. While necrosis is regarded as a histologic feature of poor outcome in some tumors, significant prognostic effects of this feature in squamous morules of the endometrium were not identified.

1243 Gestational Trophoblastic Disease in the Largest National Teaching Hospital. A retrospective Review And Analysis

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Background: Gestational trophoblastic disease (GTD) has varying prevalence across geographic regions of the globe. Prevalence of GTD is said to be lower in Europe and North America, and higher in Africa, Middle East, and Latin America. This study aimed to determine; the institutional prevalence of GTD, and histologic and demographic characteristics of GTDs among patients seen in Korle-Bu Teaching Hospital (KBTH) in Accra, Ghana between 1999 and 2013.

Design: A retrospective study which included all histologically confirmed GTDs in the Pathology Department during the study period. Surgical request forms, Haematoxylin & Eosin (H&E) stained tissue slides, and paraffin embedded tissue blocks were retrieved. Demographic characteristics, baseline β-hCG levels, and ultrasound findings were extracted from the surgical pathology request forms. The H&E-stained slides of the GTD cases were reviewed microscopically and classified using WHO/FIGO system of classification. The total number of deliveries registered at the Department of Obstetrics and Gynaecology (O&G) of KBTH for the study period was obtained. Institutional prevalence, and age distribution of the different histological types of GTDs were determined. Clinical parameters were compared using paired t-test for significance at 95% confidence interval. For all comparisons, a p-value of 0.05 or less was considered statistically significant.

Results: A total of 157,875 deliveries were registered in the Department of Obstetrics and Gynaecology (O & G).317 cases of GTD were seen during the period. No cases of PTT or ETT was seen. Out of the 317 GTD cases, 170 cases were from the O&G Department of KBTH, giving an institutional prevalence of 0.94 in 1000 deliveries. Forty cases did not meet the inclusion criteria. 77.6% of GTDs were seen in patients between the ages of 20 to 39 years. GTDs were less common in patients who were 50 years and above. 85% of GTDs seen were hidatidiform mole (HM). 49.45% of HM cases were diagnosed in the first trimester. Bleeding per vaginam was the commonest presenting complaint. Invasive mole was the least common entity of GTD seen. 92% of GTDs seen were in pre-menopausal women.

Conclusions: Institutional prevalence of GTD in KBTH is 0.94 per 1000 deliveries. This appears to differ from that of other centers in Nigeria, Netherlands, United Kingdom, Dubai, and Brazil, but closer to that of a center in Tunisia. All entities of GTD were more common in women between the ages of 20 to 39 years.

1244 Cancer Associated Mutations in Eutopic Endometrium

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Disclosures: David Huntsman: *Employee*, Contextual Genomics

Background: Recent evidence illustrates that endometriosis may carry cancer driver mutations with virtually no risk of malignant transformation.So far, the role of cancer associated mutations in endometriosis is unclear. To better understand the potential origin of the mutations in endometriosis we examine normal eutopic

endometrium for cancer hotspot mutations.

Design: We reviewed and macrodissected endometrium from histologically normal archival endometrial samples from 21 women who underwent hysterectomy for benign diagnoses. All samples were analyzed using a cancer hotspot sequencing panel. Mutations were validated using digital droplet polymerase-chain-reaction (ddPCR). Immunohistochemistry for ARID1A and PTEN was used as a surrogate for somatic alterations and immunohistochemistry for ER was done to validate functional, normal endometrium. All markers were reviewed and scored by two pathologists.

Results: Mean patient age was 35.5 years (ranged from 29 to 48 years). Initial data analysis revealed 7 of 21 cases harbored one or two known cancer driver mutations in KRAS, PIK3CA and FGFR2. PTEN was inactivated in individual glands consistent with heterogeneous pattern in 4 cases. All cases expressed normal ER and ARID1A.

Conclusions: We found well-known cancer driver mutations in 33% of histomorphologicaly normal utopic endometrium obtained from women in reproductive age group. Cancer associated mutations occur at a moderate frequency in normal endometrium as part of the normal biology. Further analysis is warranted to examine whether these cell mark an early event in endometriosis, may be more prone to colonizing endometriosis lesions, and/or may be at higher risk for later malignant progression. A larger cohort of normal endometrial tissue is currently being tested for validation.

1245 HER2 and p95HER2 Protein Expression Varies in Primary Versus Metastatic Serous Endometrial Carcinomas

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Background: Previous studies have shown overexpression of HER2 in many cancers including endometrial. Unfortunately, use of anti-HER2 therapies in HER2 over expressing endometrial cancers has not been very successful. Recent studies have shown that this resistance may be related to overexpression of p95HER2, a truncated version of the normal protein which lacks the extracellular domain, therefore rendering targeted therapies ineffective. To date, there have been studies that have shown overexpression of both HER2 and p95HER2 in uterine serous carcinoma (USC), but none investigating whether this expression varies between primary and metastatic tumors.The purpose of this study was to determine whether HER2 and p95HER2 expression differ in primary and metastatic USC.

Design: A database was queried for eligible cases which included patients having undergone surgery for uterine serous carcinoma between 2004 and 2014 and for whom there were both primary and metastatic tumor samples available. Thirteen cases (26 tumor samples including 13 primary tumor samples and 13 paired metastatic tumor samples) were identified. HER2 and p95HER2 protein expression was quantified using the VeraTag assay from Monogram Biosciences. Nonparametric statistical methods were used to analyze these data. The Spearman correlation coefficient was used to assess the association between metastatic and primary samples with respect to HER2, p95, and the p95:HER2 ratio. Wilcoxon signed-rank test was used to determine whether the distributions of HER2, p95, and the p95:HER2 ratio differed between the paired primary and metastatic samples.

Results: Serous endometrial cancers express higher levels of p95HER2 than those previously reported in breast cancer. For paired tumor samples, there was a direct positive correlation between primary and metastatic samples for both p95HER2 protein expression (P-value 0.03) and the p95HER2:HER2 protein expression ratio (P-value 0.02). p95HER2:HER2 protein expression ratio is significantly increased in metastatic tumor samples compared to primary tumor samples (P-value 0.01).

Conclusions: Our results corroborate that p95HER2 protein expression in USC is higher than that seen breast cancer. This provides some rationale for the trastuzumab resistance observed in USC. The decreased HER2 protein expression and the increased ratio of p95HER2:HER2 protein expression observed in this study when comparing metastatic tumor samples to primary tumor samples gives additional insight into this observation.

1246 PREVIOUSLY PUBLISHED

1247 PD-L1 Expression in Adult Granulosa Cell Tumors

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Background: Adult granulosa cell tumors (AGCT) are typically indolent tumors; however, recurrences can result in significant patient morbidity. In the setting of recurrence, patients often fail to respond to treatment. More effective therapeutic options for patients with recurrent AGCTs, as well for as patients who present with high stage disease are needed. The development of drugs targeting the PD-1/PD-L1 checkpoint has resulted in responses in several cancers. A clinical trial that includes the use of a PD-L1 checkpoint inhibitor in patients with rare gynecologic tumors, including AGCT is underway. PD-L1 immunohistochemical expression may identify patients that are most likely to benefit from such therapeutic agents. An initial study has shown increased PD-L1 expression in AGCTs. We sought to further evaluate PD-L1 expression in AGCT, to assess if drugs targeting the PD-1/PD-L1 checkpoint may be of therapeutic utility.

Design: We searched an 11 year period (2006-2017), in our institutional pathology database, for all cases of AGCT. Immunohistochemistry for PD-L1 (clone SP142,Spring Biosciences) was evaluated. PD-L1 staining was scored in both the tumor, and in the peritumoral immune cells. The percentage of membranous staining was assessed in the tumor, and the degree of staining in the peritumoral immune cells was classified as minimal, moderate or brisk.

Results: 54 AGCTs with slides available for review were retrieved. 46 cases were of primary tumors at initial diagnosis, and 8 cases represented recurrent tumors. Of the 46 initial primary tumors, 43 were stage I (IA-35; IB-1; IC1-1; 1C2-6), 1 was stage IIB, and 2 were stage III (IIIA-1; IIIC-1). 9/54 (16.7%) showed staining for PD-L1 in tumor cells (7/9 with 1%-5% staining; 1/9 with 6%-10% staining, and 1/9 with 10% staining). 12/54 (22.2%) showed staining for PD-L1 in immune cells (10/12 with minimal staining, 2/12 with moderate staining and 0/12 with brisk staining). Tumor leutinization, correlated with PD-L1 expression in both tumor cells, and immune cells (p=0.049 & p = 0.032, respectively). PD-L1 expression was not significantly associated with any other clinicopathologic parameter.

Conclusions: PD-L1 is not commonly expressed in AGCT; however, the presence of tumor leutinization does increase the likelihood of weak PD-L1 expression. Overall, our findings suggest that PD-1/ PD-L1 inhibitor therapies may be of utility in only a very small subset of AGCTs, and not in the majority of AGCTs.

1248 The Role of Methylation Silencing of Tumor Suppressor Genes in Cervical HSIL: A Cytologic-Histologic Correlation Study

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Background: Epigenetic changes of precancerous lesions are poorly understood and rarely studied. Methylation silencing of tumor suppressor genes has been suggested as one of the epigenetic changes promoting carcinogenesis. Based on cell-line and liquid based cytology (LBC) studies it was established that methylation status of CADM 1, MAL, hsa-miR-124 genes plays an important role in cervical HSIL lesions. The aim of this study was to evaluate the role of methylation status of selected genes in cervical HSIL using a cytologic-histologic correlation study.

Design: A prospective study was carried out in 2013-2014 to identify histologically confirmed cases of HSIL with prior screening LBC diagnosis of HSIL within 3 months interval. 70 cases were selected and their clinical and demographic data were obtained. Histologic and cytologic diagnoses were confirmed. Pertinent histologic information including 1) number of blocks harbouring dysplastic squamous epithelium, 2) number of blocks containing glandular extension of dysplastic epithelium and 3) the depth of glandular extension (which was assessed semiquantitatively as grade 1-3). From residual LBC materials HPV subtyping was performed using LINEAR ARRAY HPV Genotyping Test and in house PCR targeting E1 gene. Detection of methylation silencing of tumor suppressor genes was performed by multiplex methylation specific rtPCR.

Results: The number of dysplastic cells present on slide varied. Positive methylation status was detected in 41 cases (58.6%). Number of blocks with dysplastic epithelium varied from 1 to 13. Glandular extension was seen in 44 cases with number of blocks involved ranging from 1 to 10. The depth of glandular extension of dysplastic epithelium varied. Statistically, number of blocks presenting glandular extension of dysplastic epithelium or the depth of glandular extension did not correlated with methylation status. There was no correlation between HSIL methylation status and the number of blocks harbouring dysplastic squamous epithelium (P > 0.05). Only a trend was noted showing that the number of blocks with HSIL (more than 7) may increase the probability of positive methylation status (P = 0,13).

Conclusions: Our findings show that methylation status of CADM 1, MAL, hsa-miR-124 genes in cervical HSIL does not correlate with the

size of the lesion (measured by the number of blocks involved) or with HSIL propensity for endocervical glandular extension.

1249 Paradoxical Intraepithelial Lesions in HPV-associated Squamous Cell Carcinomas of the Vulva

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Background: Most human papillomavirus (HPV)-associated vulvar squamous cell carcinomas (VSCC) originate from high-grade intraepithelial lesions, also named vulvar intraepithelial neoplasia of usual type (HSIL/uVIN), which have a basaloid or warty histology. However, growing evidence suggests that the morphology has limitations to predict the HPV status in vulvar lesions. In this study we aim to explore the adjacent intraepithelial lesions in a large series of DNA HPV-positive VSCCs, focusing on paradoxical histological patterns mimicking differentiated vulvar intraepithelial neoplasia (dVIN) or lichen sclerosus (LS).

Design: Three hundred and twenty-six VSCC positive for HPV DNA, having at least 1 cm of skin adjacent to the invasive tumor were studied. HPV typing, HPV E6*I mRNA and p16 immunohistochemistry were analyzed in all cases, and a thorough histological evaluation was conducted. A conclusive association with HPV was based on a positive result for p16, HPV E6*I mRNA or both, in addition to the HPV DNA. Cases positive for HPV DNA but negative for p16, HPV and E6*I mRNA were classified as non-conclusively associated with HPV.

Results: 121 tumors (37.1%) had a normal adjacent skin, 191 (58.6%) had only HSIL/UVIN. Paradoxical intraepithelial lesions were identified in 14 (4.3%) tumors. Seven cases showed features of dVIN, 5 showed adjacent LS and in 2 cases, dVIN and LS lesions were identified simultaneously. Eight cases were considered as non-conclusively associated with HPV. All were positive for HPV DNA but were negative for p16 both in the tumor and in the dVIN, and the HPV mRNA was either negative or not tested. Six out of the 14 cases had a conclusive association with HPV (three dVIN, two LS, one with combined dVIN and LS features). All of these six cases were associated with HPV16 and were positive for both p16 and HPV mRNA. p16 was also positive in the dVIN and LS.

Conclusions: A small proportion of VSCC conclusively associated with HPV may arise in intraepithelial lesions that closely simulate dVIN and LS, the intraepithelial precursors of HPV-independent VSCC. Awareness of this paradoxical feature may help to correctly classify these lesions as HPV-associated.

Funded in part by the Instituto de Salud Carlos III (ICSIII)-Fondo de Investigacion Sanitaria, and ERDF 'One Way to Europe' (PI15/00546)

1250 Molecular and Immunohistochemical Characterization of Intravenous Leiomyomatosis: A Study of 28 Cases

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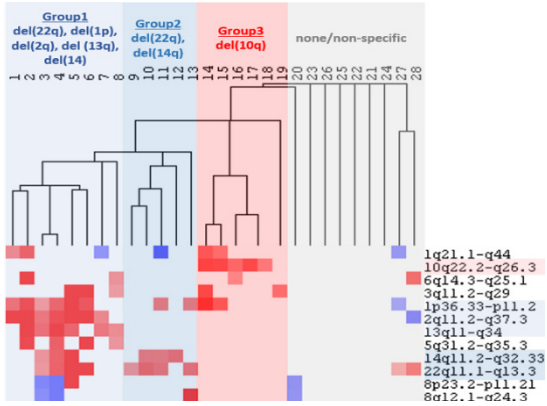
Background: IVL is a benign intravascular smooth muscle proliferation outside the confines of a leiomyoma. Despite the well delineated molecular genetic alterations of uterine leiomyomas and leiomyosarcomas, there is only a single comprehensive molecular genetic study of IVL showing several recurrent copy number alterations. Studying correlation of protein expression patterns with their genetic background may provide insight into its pathophysiology.

Design: 28 IVL were evaluated by aCGH. Hierarchical clustering analysis was performed looking for the presence of recurrent aberration(s) detected in at least 4 different cases. 5 immunohistochemical markers were analyzed based on involved chromosome positions: p16, CyclinD1, Carbonic Anhydrase IX, INI1 and FH. Staining was evaluated semiquantitatively in tumor (tumor cells and vessels) and myometrium: 0 (absent), 1 (minimal, <5%), 2 (focal, 5-24%), 3 (multifocal, 25-50%) and 4 (diffuse, >50%). Data were analyzed by student t-Test.

Results: Hierarchical clustering analysis revealed 3 distinct molecular groups: Group 1 and 2 with del(22q) and group 3 with del(10q) (Figure 1, n=8, 5 and 5; respectively). The presence of 22q and 10q deletion were mutually exclusive. All IVLs showed differential expression of either CyclinD1 or p16 (mean scores 1.07 and 1.78) in comparison to myometrium (mean scores 0.00 and 0.28) (p<0,001). Differential staining scores with increased tumor vessel staining compared to tumor cells was seen only in IVLs with chromosome aberrations (12 of 20 copy number alteration positive cases) except for one (out of 8) with no genetic alterations, with <1% expression only in vessels; showing epithelioid morphology. Multifocal/diffuse CyclinD1 positivity was seen in distinct morphologic groups (2/2 epithelioid, 2/11 focally hydropic/hydropic, 1/3 cellular), however it did not correlate with presence of chromosome aberrations. INI1 and FH were retained and Carbonic Anhydrase IX was negative in all cases.



Conclusions: This study represents the largest series of IVL characterized by molecular and immunohistochemical studies. The detected recurrent chromosome alterations have a signature distinct from those described in leiomyomas. The vascular staining of p16 in IVL with chromosome abnormalities, as well as the increased Cyclin D1 expression particularly in the different morphologic variants may provide further insight into our understanding of IVL tumorigenesis.

1251 A Machine Learning Approach to Classifying Benign, Premalignant, and Malignant Endometrial Biopsies

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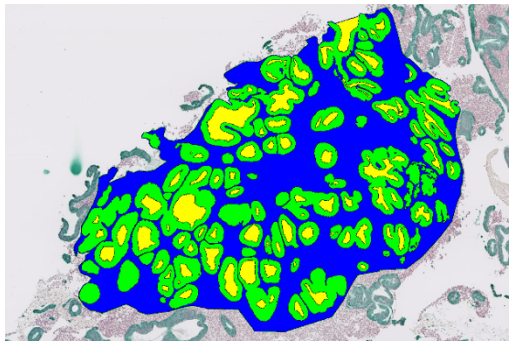
Background: Benign normal (NL), premalignant (endometrial intraepithelial neoplasia, EIN) and malignant (cancer, EMCA) endometrium must be precisely distinguished for optimal patient management. EIN was first defined in part by a logistic regression classification algorithm trained using variables from manually traced, segmented histologic structures. The algorithm classified cases based on volume percentage stroma, gland outer surface density, and standard deviation of shortest nuclear axis, features used to define diagnostic criteria.

Design: Here, we develop an automated approach to segmentation, and we use advanced machine learning tools to objectively classify endometrial biopsy fragments. Endometrial tissue from 148 patients was randomly separated into independent 72-patient training (40 NL, 16 EIN, and 16 EMCA) and 76-patient validation (43 NL, 17 EIN, 16 EMCA) cohorts. Each patient had an average of 1.8 (261/148) fragments analyzed. We used Visiopharm Integrator System (Horsholm, Denmark) image analysis software to automatically segment whole-slide digital images into epithelium, cells, and nuclei (Figure 1). 1413 variables per image were extracted and used to train and test random-forest classification algorithms.

Results: Weakly contributory variables were culled reducing the dimensionality of input data from 1413 to 75 variables in a 3-group (NL, EIN, EMCA) classification algorithm. This algorithm correctly classifies cases with 3-class error rates of 0.04 (training set) and 0.058 (validation set). Our algorithm classifies cases across the benign-neoplastic boundary (NL vs. EIN+EMCA) with errors of 0.016 (training set) and 0 (validation set). The 4 most important variables are surrogates of those previously identified, including stromal and epithelial area percentages, and normalized epithelial surface lengths. Additional, previously unrecognized predictors include gland and lumen axis lengths and ratios, and individual cell measures.



Conclusions: Automated image analysis and random forest classification algorithms can classify normal, premalignant, and malignant endometrial tissues with excellent accuracy. The most important variables are architectural, but individual cell features are also contributory. Incorporation of variables across a hierarchy of scale into an automated analysis platform provides an option for assisted diagnosis in a digital pathology workflow.

1252 Morphologic Patterns of Secondary Involvement of the Uterine Cervix by Non-gynecologic Neoplasms

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Background: Secondary involvement of the uterine cervix by non-gynecologic neoplasms is exceedingly uncommon accounting for <2% of metastases to the gynecologic tract. The aim of this study was to describe the morphologic patterns of cervical involvement by non-gynecologic tumors.

Design: The institutional databases were searched for patients diagnosed with non-gynecologic neoplasms of the uterine cervix 2004-2017. Pathology reports and all available slides were reviewed. The following clinicopathologic features were recorded: clinically visible or microscopic lesion, tumor size, patterns of spread (pagetoid or infiltrating), depth of invasion and lymphovascular invasion (LVI). Pertinent electronic medical records were reviewed.

Results: A total of 20 cases were identified: 13 carcinomas - 3 breast (BC), 4 urothelial (UC), 4 colorectal (CRC), 1 appendiceal signet ring cell (ASRCC), 1 cholangiocarcinoma (CC); 6 lymphomas - 4 diffuse large B-cell (DLBCL), 1 centroblastic lymphoma (CL), 1 chronic lymphocytic lymphoma (CLL); and 1 malignant melanoma (MM). Patient median age was 58.5 (range 25-86). The cervix was the first site of gynecologic presentation in 3 cases (1 UC, 2 DLBCLs), while synchronous cervical involvement was present in 5 cases (1 MM, 2 DLBCLs, 1 CC, 1 CL). In a subset of 11 patients who had more detailed clinical information available, vaginal bleeding was the most common presenting symptom (45.5%). Only 3 patients (1 UC, 1 ASRCC, 1 DLBCL) had clinically and/or radiologically detected cervical lesions. Assessment of morphologic patterns in these 11 cases revealed pagetoid growth pattern in 2 UCs (high grade squamous intraepithelial lesion like pattern) and 1 CRC (adenocarcinoma in situ like pattern). Infiltrating growth pattern from the outer cervical wall spanning 1.2 cm was seen in 1 CRC. Both pagetoid and infiltrating patterns were identified in 2 UCs, with ≤3 mm depth of invasion. Pattern of spread could not be evaluated in 2 cases (1 CRC, 1 ASRCC) due to specimen fragmentation. Lymphocytic infiltrate was diffuse in DLBCLs and perivascular in CLL. LVI was present in 2 CRCs and 1 UC. Two cases (1 UC, 1 DLBCL) were initially misdiagnosed as gynecologic primary. Clinical history and/or ancillary studies were essential in diagnosing all tumors.

Conclusions: Involvement of the uterine cervix by non-gynecologic tumors can mimic primary in situ or invasive carcinomas in both ecto- and endocervix. Clinical history and a high level of suspicion are crucial to ensure an accurate diagnosis.

1253 Uterine Myxoid Smooth Muscle Tumors with Malignant Potential: Clinico-Pathologic Characterization of a Case Series with Follow-Up

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Background: Uterine myxoid leiomyosarcoma (mLMS) is a rare and aggressive tumor. Myxoid tumors of unknown malignant potential (mSTUMP) are even more infrequent, and criteria for their distinction from mLMS are lacking. Our aim was to compare the pathologic characteristics of these tumors in the context of patient outcome.

Design: Cases diagnosed as mSTUMP or mLMS in our institution were identified. those with diagnostic material available for review and follow-up of at least 12 months were further selected. Pathologic features of malignancy were recorded (% myxoid matrix, tumor border, mitoses/10HPFs, necrosis, nuclear atypia). Additional clinical and pathologic characteristics were assessed including outcome.

Results: Seven patients were identified, 2 diagnosed as mSTUMP and 5 diagnosed as mLMS. Median age at presentation was 40 years (range 35-71). Median tumor size was 8.5 cm (range 6-35). All contained an abundant myxoid stroma representing ≥50% of the tumor volume. 6/7 tumors had an infiltrative border; 4/7 had moderate to severe nuclear atypia. Median follow-up was 34 months (range 12-77): widespread recurrence was observed in two mLMS patients at 13 and 27 months, respectively. Recurrent tumors had high mitotic counts (14 and 20 mitoses in 10 HPFs, respectively); in contrast, all cases with indolent course had 0-2 mitoses / 10 HPFs. Necrosis was observed in 2/2 recurrent tumors, versus 1/5 without recurrence.

Conclusions: In this institutional series of myxoid smooth muscle tumors with clinical follow-up, those with 0-1 mitoses and no necrosis were associated with favorable follow-up. Although mitotic activity in mLMS can be low, high mitotic counts and presence of necrosis may be stronger predictors of tumor recurrence, as previously observed in the literature. Whole exome sequencing is being performed to better understand this clinical heterogeneity and to identify potential biomarkers.

1254 Identification of Prognostically Significant Subsets of Ovarian Clear Cell Carcinoma Using an Endometrial Carcinoma Molecular-Based Classifier

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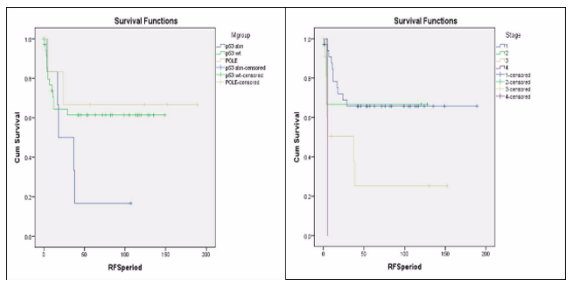
Background: The PROMISE algorithm is a reliable surrogate of the molecular profile in endometrial carcinoma, and testing for p53, mismatch repair proteins (MMR) and polymerase ? *(POLE)* exonuclease domain mutations have been shown to predict clinical outcome in endometrial carcinoma as well as ovarian endometrioid carcinoma. Moreover, similar prognostic grouping has been recently demonstrated in endometrial clear cell carcinoma. Thus, we aim to explore the role of these markers in ovarian clear cell carcinoma (OCCC), another endometriosis-associated malignancy.

Design: OCCCs resected in a 14-year period were retrieved, and those with confirmed OCCC diagnosis were included. *POLE* mutational analysis and immunohistochemistry for p53, MLH1, MSH2, MSH6 and PMS2 was performed in formalin-fixed, paraffin-embedded tissue. Following the molecular classifier for endometrial carcinoma (*B J Cancer 2015;113:299-310*), cases were classified as: *POLE*-mutated, MMR-abnormal, p53-abnormal (abn) and p53-wild type (wt). Features recorded included age, tumor size, laterality, surface involvement, LVI, presence of endometriosis and patient outcome [disease-free survival (DFS) and disease-specific survival (DSS)].

Results: 50 tumors were included; 42(84%) were endometriosis-associated. Six (12%) OCCC were p53-abn and 44 (88%) were p53-wt. Among the latter, 7 (14%) had *POLE* mutations. All tumors in the cohort had intact MMR expression. Mean follow-up period was 53 months (median 45, range 8-107). Tumor recurrence was documented in 5/6(83%) p53-abn, versus 15/44(31%) p53-wt (p=0.03, Fisher's exact test). DFS based on abnormal p53 expression approached statistical significance (p=0.07 log rank test, HR=2.2, 95% CI 0.8–6.2). Stage at presentation was the only significant variable for DFS (p=0.007, HR=1.8, 95% CI 1.2-2.8) and DSS (p=0.001, HR=2, 95% CI 1.4-3) in univariate analysis (Figure 1). Among the 7 *POLE*-mutated OCCC, 2 tumors developed recurrence; both harbored the same mutation (exon 13, c.1337G>A). Remaining 5 *POLE*-mutated cases had an indolent outcome.



Conclusions: A molecular-based classifier identified subsets of OCCC with potential prognostic differences. Abnormal p53 expression appears to correlate with worse disease-free survival. *POLE* exonuclease mutations tend to be associated with good prognosis; however, we identified a mutation in two OCCCs manifesting with recurrence, which requires further investigation. Based on our current data, the use of these markers in OCCC has potential prognostic value.

1255 Smooth Muscle Tumors of the Adnexal Ligaments: A Clinicopathologic Study of 60 Cases Emphasizing Prognostic Criteria

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Background: The adnexal ligaments that anchor the female reproductive organs to the pelvis are embryologically derived from the gubernaculum or paramesonephric ducts. Smooth muscle tumors (SMTs) arising from these ligaments are essentially limited to case reports, and criteria for malignant potential have yet to be established. To determine whether morphologic criteria validated for SMTs of the uterus, ovary, vagina and vulva are applicable to SMTs of the adnexal ligaments, we studied a large cohort of cases.

Design: Cases from 2 institutions were centrally reviewed for site of origin (correlated with imaging, intraoperative reports and gross descriptions), clinical presentation, gross tumor size, degree of cytologic atypia, mitotic index, morphology (spindled and/or epithelioid and/or myxoid) and tumor cell necrosis. Consultation cases, cases associated with endometriosis and cases that had physical connection to the abdominal or pelvic organs were excluded. Clinical data were obtained from internal medical records.

Results: A total of 60 tumors consisting of 52 leiomyomas (86%), 1 STUMP (2%) and 7 leiomyosarcomas (12%) from 60 patients were identified. Follow up was available for 52 patients (86%) ranging from 1 to 296 months (mean 101, median 80). Leiomyomas ranged 0.5 to 20 cm, had predominantly spindled morphology, mild or mild to moderate cytologic atypia, no TCN and a mitotic index of 0 to 4 per 10 HPFs. In the 46 cases of leiomyoma that had clinical follow up (88%), none recurred. The 1 STUMP was a 7.5 cm tumor with purely spindled morphology, diffusely moderate cytologic atypia, 3 mitotic figures per 10 HPFs and no tumor cell necrosis. This patient had no evidence of disease at last follow up (178 months). The 7 leiomyosarcomas ranged 3.2 to 21 cm in size, had predominantly spindled morphology, moderate to severe cytologic atypia and tumor cell necrosis. Six of 7 cases of leiomyosarcoma had ≥ 10 figures per 10 HPFs. Clinical status was available for all 7 leiomyosarcomas and 4 patients were dead of disease at last follow up.

Conclusions: Morphologic criteria derived from uterine SMTs and subsequently validated in the ovary, vagina and vulva are equally effective at classifying SMTs of the adnexal ligaments. Our findings allow for a universal method to evaluate malignant potential of SMTs of the gynecologic tract.

1256 Immune Microenvironment in Endometrial Adenocarcinoma with Mucinous Differentiation

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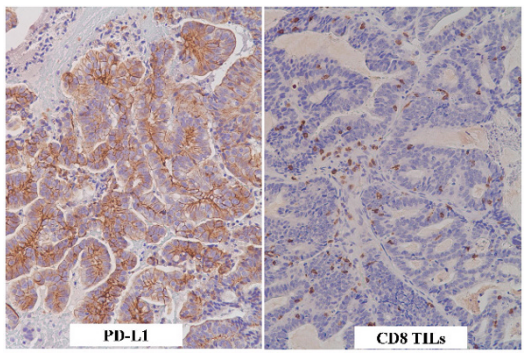
Background: Immune microenvironment is gaining increasing importance in a number of malignancies. We intended to study the immune microenvironment in recurrent and non-recurrent endometrial adenocarcinomas with mucinous differentiation to determine possible therapeutic importance.

Design: We queried our records for 10 cases each of recurrent (primary and secondary) and non-recurrent endometrial adenocarcinoma with mucinous differentiation. Tissue microarrays were constructed. Immunohistochemistry for programmed death ligand 1 (PD-L1), and for tumor infiltrating lymphocytes (TILs) expressing programmed cell death protein 1 (PD1), CD3, CD8, and FoxP3 was performed. PD-L1 membranous expression in >1% tumor cells was considered positive and scored as >1 to 25% score 1, 25 to 50% score 2 and >50% score 3. TILs were counted per 100 epithelial cells and in the stroma per high power field (hpf). Statistical analysis was performed by Fisher's test and paired t-tests.

Results: The clinicopathological features of tumors are shown in table 1 [Table 1]. Membranous expression of PD-L1 was noted in all tumors, shown in figure 1 left, and representative image of CD8 TILs on right [Figure 1]. Comparison of immune microenvironment markers between primary non-recurrent and primary recurrent tumors is shown in table 1. There was no difference between primary recurrent and secondary recurrent tumors.

Table 1: Comparison of clinicopathologic and immune microenvironment parameters between primary non-recurrent and primary recurrent endometrial adenocarcinomas with mucinous differentiation.

Parameter	Primary non-recurrent (n=10)	Primary recurrent (n=10)	P value
Age Median (range)	59 (52-90)	74 (47-93)	0.09
Size Median (range)	4.5 (1.5-7)	4 (3.2-6.5)	0.69
FIGO grade n(%) I II III	10 (100%)	7 (70%) 2 (20%) 1 (10%)	0.21 0.47 1.00
Stage n(%) 1a 1b 2 3a	10 (100%)	6 (60%) 2 (20%) 1 (10%) 1 (10%	0.08 0.47 1.0 1.0
Myometrial invasion Median (range)	36 (6.5-40)	30 (7.6-100)	0.20
Lower uterine segment Involved n(%)	3 (30%)	5 (50%)	0.65
Cervix involved n(%)	0 (0%)	1 (10%)	1.0
Ovary involved n(%)	0 (0%)	0 (0%)	1.00
Fallopian tube involved n(%)	0 (0%)	1 (10%)	1.0
Lymphovascular invasion Present n(%)	1 (10%)	4 (40%)	0.30
Margin positive n(%)	0 (0%)	0 (0%)	1.00
Lymph node n(%) Positive Negative Not Sampled	0 4 (40%) 6 (60%)	2 (20%) 4 (40%) 4 (40%)	0.47 1.00 0.65
Squamous features n(%)	1 (10%)	5 (50%)	0.14
Time to recurrence Median (range)	Not applicable	13 (4-206)	
PD-L1 positive n(%)	10 (100%)	10 (100%)	1.0
PD1 positive n(%)	6 (60%)	7 (70%)	1.0
CD3 per 100 epithelial cells	6.5 (3-8)	4 (2-10)	0.07
CD3 stroma/hpf	120 (50-300)	77 (10-240)	0.15
CD8 per 100 epithelial cells	2 (1-8)	2 (1-15)	0.59
CD8 stroma/hpf	47 (10-130)	35 (2-320)	0.76
FoxP3 per 100 epithelial cells	2 (1-3)	2 (1-4)	0.80
FoxP3 stroma/hpf	32 (1-55)	12 (1-33)	0.03



Conclusions: The primary recurrent tumors had low stromal FoxP3 regulatory TILs compared to non-recurrent tumors. This finding emphasizes role of immune microenvironment in tumor progression of endometrial adenocarcinomas with mucinous differentiation. All tumors including primary and secondary expressed PD-L1, which indicates possible therapeutic role of PD1/PD-L1 checkpoint blockade.

1257 Lymphoepithelioma-Like Carcinoma of the Uterine Cervix: A Pathologic Study of Seven Cases

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Background: Lymphoepithelioma-like carcinoma (LELC) of the uterine cervix is an extremely uncommon malignant tumor, considered by the World Health Organization as a variant of non-keratinizing squamous cell carcinoma. Given its rarity, data in regards to the pathogenesis of this neoplasm is still a topic of debate, as most studies are based on isolated reports. While evidence points to human papilloma virus (HPV) to play a role in the etiology of LELC, limited data predominantly from Asian populations hypothesize that, similarly to LELC occurring in other anatomic sites, it is an Epstein-Barr virus (EBV)-driven process.

The goal of this study was to evaluate a series of cases of cervical LELC and describe the most common histopathologic findings, as well as to investigate possible presence of HPV and/or EBV genome by in situ hybridization studies.

Design: We retrospectively searched our surgical pathology database for cases of LELC of the uterine cervix. Immunohistochemistry (IHC) for p63, p16, HLA-DR, and BCL-2 was done on formalin-fixed paraffin-embedded sections. In situ hybridization (ISH) for both high-risk HPV and EBV (Epstein-Barr encoding region; EBER) was performed. Slides were then reviewed by two pathologists.

Results: Seven (7) cases of LELC of the cervix were found. They corresponded to tumors occurring in patients from 26 to 81 years of age (mean: 55; median 58). The cases consisted of three biopsy and four resection specimens. The tumors showed poorly differentiated morphology, with sheets of cells containing vesicular nuclei, prominent nucleoli and eosinophilic or amphiphilic cytoplasm. All lesions had a dense lymphocytic infiltrate within and around the tumor islands. Lymph vascular invasion was present in 4 of 7 cases. By IHC, all tumors (7/7) were positive for p63, p16 and HLA-DR. Two of seven cases (2/7) were also positive for BCL-2. By ISH, six of seven tumors (6/7) were positive for high risk HPV, and were all negative for EBER.

Conclusions: Lymphoepithelioma-like carcinoma (LELC) of the uterine cervix is a rare neoplasm with unique morphologic and immunohistochemical profiles. This study shows that in contrast to LELC occurring in other anatomic sites (e.g. nasopharyngeal), cervical LELCs are associated with HPV, and do not appear to be related to EBV.

1258 Distribution of p53 and p16 Immunohistochemical Staining Identifies Different Pathways of Vulvar Carcinogenesis

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Background: Vulvar squamous cell carcinoma (VSCC) comprises 5% of female genital tract cancers. Despite therapeutic advances, the prognosis of advanced-stage and recurrent VSCC remains poor. Two distinct pathways of carcinogenesis of VSCC are known; one related to hrHPV infection & the other through p53 gene mutation. The aim of this study is to evaluate the distribution of p53 and p16 immunohistochemical (IHC) staining in VSCC.

Design: One hundred eighty six cases of VSCC were retrieved. All cases were stained with p53 & p16 antibodies on representative whole tissue sections. P53 nuclear and p16 nuclear & cytoplasmic expression were scored using the H-score method [sum of intensity (0-3) x % positive cells]. An H-score of 200 or higher (equivalent to diffuse strong expression) was considered a positive result. Based on the coordinate expression of p53 & p16, 4 different "tumor pathways" were assigned: p53 pathway (p53 positive, p16 negative), p16/HPV pathway (p53 negative, p16 positive), dual negative (p53 negative and p16 negative), dual positive (p53 positive, p16 positive). Although, complete absence of staining for p53 is considered indicative of p53 mutation, no definitive data exist. Therefore, the p16 negative cases with p53 H-score of 0 were included in the "dual negative" category.

Results: The mean and median age of the patients was 69.4 and 74 years, respectively (26-95 years). The average size of the tumor was 3.3 cm. Seventy one tumors (41.5%) were stage I, 41 (24.0%) were stage II, 49 (28.7%) were stage III & 10 (5.8%) were stage IV. Based on the distribution of p53/p16 staining, 46 tumors were dual negative, 6 were dual positive, 67 were p53 positive only and 67 were p16 positive only. Patients with HPV pathway tumors were significantly younger than the patients with p53 pathway tumors (62.8 versus 74.4 years, $p<0.0001$). p53 pathway tumors were significantly larger than HPV pathway tumors (3.5 cm vs 2.5 cm, $p=0.0053$). However, there was no significant difference regarding tumor stage at presentation between different pathways.

Pattern (Pathway)	Cases (%)	p53 H-Score mean	p53 H-score range	p16 H-score mean	p16 H-score range	Mean Age	Mean tumor size
Dual Negative	46 (25%)	42	0-130	17	0-190	71.2	3.9 cm
Dual Positive	6 (3%)	263	200-300	247	200-300	74.2	4.5 cm
p16/HPV	67 (36%)	23	0-130	295	200-300	62.8	2.5 cm
p53	67 (36%)	285	210-300	7	0-120	74.4	3.5 cm

Conclusions: The p53 and p16 IHC staining of VSCC confidently identifies the two well-known pathways of vulvar carcinogenesis. Each of these pathways accounts for 36% of the cases. One quarter of VSCC are negative for both p53 and p16 suggesting a yet another unidentified pathway of vulvar carcinogenesis. Further studies with a larger cohort of patients with treatment and outcome data are warranted to evaluate the prognostic significance of these pathways.

1259 Frequency of Rare and Multi Viral High-Risk HPV Subtype Infection in Low-grade Squamous Intraepithelial Lesion (LSIL) with Cervical Cytology Correlation: A Follow-Up Study

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Background: Cervical cancer is among the top 3 cancers in the United Arab Emirates (UAE). Multi-viral and rare subtype high-risk HPV (hrHPV) is common in high grade squamous intraepithelial lesions (HSIL) in UAE as we reported previously (Modern Pathol 2017;30 (Suppl 2), 305A). Frequency of multi-viral and rare subtype hrHPV in low grade squamous intraepithelial lesions (LSIL) is not known and this information is required to planning mass vaccination program. Persistence of such infection may enhance the risk of disease progression even though the initial histologic diagnosis was LSIL.

Design: Fourteen cervical cone biopsies with LSIL were selected from a larger study performed on UAE population to assess hrHPV subtypes in a tertiary care hospital in UAE. DNA extracted from five, 10-µ-thick, tissue sections using the QIAamp DNA FFPE Tissue Kit (QIAGEN Inc, Valencia, CA 91355, Cat No. 56404). GenomeMe™'s GeneNav™ HPV One qPCR Kit (GenomeMe, Richmond, BC, Canada) was used for specific detection of hrHPV 16&18; non-16/18 samples were further typed by GenomeMe™'s GeneNav™ hrHPV Genotyping qPCR Kit.

Results: The mean age was 35 years (range 25-59 years) and the majority, (78.6%) were UAE nationals. hrHPV subtypes identified were 16, 18, 31, 33, 39, 52, 56, 66 & 68. Four cases had co-infection (28.6%); two cases had two, one had three, and one had 5 hrHPV subtypes. The patient with 5 hrHPV subtypes was 59 years old. hrHPV 16, 31, 33, 52, 56 and 68 were identified in co-infection. Most frequent types were hrHPV 31 (35.7 %), followed by hrHPV 16 (28.57%), hrHPV 33 (21.4%), and hrHPV 68 (21.4%). hrHPV 16/18 were identified in 5/14 (35.7%) cases. Two patients were lost to follow-up. The duration of follow up in 12 cases ranged from 4-60 months. Seven cases had two follow-up Pap smears, three had three Pap smears, one had four and one case had five follow up visits. All cases were negative for cervical neoplasia. Two cases demonstrated ASCUS with subsequent negative cytology. One had hrHPV and the other was negative. In 7/14 cases LSIL persisted beyond 6 months after cone biopsy.

Conclusions: Multi-viral hrHPV infection is common even in LSILs and viral infection appears to persist beyond 6 months in some cases. This is the first report also showing rare subtype infection by hrHPV is the dominant cause of LSIL in this Middle Eastern country, as hrHPV 31 is the most frequent subtype, followed by hrHPV 33 and hrHPV 68, a finding to be remembered during mass vaccination program planning.

1260 Topically-Treated Vulvar High Grade Squamous Intraepithelial Lesion: Post-Treatment Morphology and p16 Immunoexpression, Correlated with In Situ Hybridization for High Risk Human Papilloma Virus RNA

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Background: Treatment-induced alteration of tumor morphology is well-described in various organs and may create difficulty in diagnostic evaluation of post-treatment (post-TX) specimens. The effect of topical treatment of vulvar high grade squamous intraepithelial lesion (HSIL) of the usual type (i.e. associated with high risk human papilloma virus (hrHPV) is not well described in residual lesions. This study evaluated the morphology and p16 immunoexpression pattern in post-TX biopsies from women with vulvar HSIL treated with topical immune

response modifier and/or topical chemotherapy. The findings were correlated with in situ hybridization (ISH) for hrHPV RNA performed on the post-TX biopsies.

Design: Post-TX biopsy specimens were evaluated from 20 women with vulvar HSIL (usual type) treated with topical immune response modifier (n=13), topical chemotherapy (n=6) or both (n=1). The degree of nuclear atypia and mitotic activity was compared to the pre-TX biopsy in 15 women for whom a pre-TX biopsy was available for review. p16 staining was performed on all post-TX biopsies. Diffuse, strong "block" staining of at least the lower third of the epithelium was defined as a positive p16 result. ISH for hrHPV RNA was performed on all post-TX biopsies and interpreted blinded to the morphologic diagnosis and to the p16 stain results.

Results: Post-TX residual dysplasia showed morphologic features of HSIL in 13/20 cases and features of condyloma/LSIL in 7/20 cases. Mitotic activity was significantly reduced in 3/13 post-TX HSIL and the degree of nuclear atypia appeared less severe in 2/13 cases compared to the pre-TX biopsy. p16 was negative in 2/13 post-TX HSIL but hrHPV was present in both cases. hrHPV RNA was present in 1/7 post-TX biopsies classified as condyloma/LSIL/ p16 negative; no further HSIL developed in follow up of this patient

Post-TX HSIL N=13				Post-TX condyloma/LSIL N=7		
P16 positive		P16 negative		P16 positive	P16 negative	
11		2		0	7	
hrHPV RNA present	hrHPV RNA absent	hrH-PV RNA present	hrH-PV RNA absent		hrHPV RNA present	hrHPV RNA absent
11	0	2	0		1	6

Conclusions: Residual vulvar HSIL following topical treatment may occasionally exhibit a lesser degree of atypia and mitotic activity than expected, and/or negative p16 staining. In these settings, ISH for hrHPV may be of value to confirm the diagnosis of vulvar HSIL. The clinical significance of residual post-treatment vulvar HSIL that exhibits LSIL morphology, negative p16 staining, and positive hrHPV RNA merits additional long term studies.

1261 Validation of a DNA Methylation Panel for the Triage of HPV Positive Women in a HPV Primary Screening Population

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Background: The aim of this study is to evaluate triage options to manage HPV positive women from a primary HPV cervical screening programme. Host methylation factors have repeatedly shown to be hypermethylated in cervical cancer and pre-cancer and have the potential to triage women at high risk of progression or who have CIN2+ precancerous lesions. This is part of a larger study within CERVIVA.

Design: In partnership with CervicalCheck, The National Cervical Screening programme, in Ireland, CERVIVA are undertaking a longitudinal HPV primary screening pilot study which will evaluate several triage strategies for management of a HPV-positive primary screening test. We are recruiting 13,000 women attending for routine smear tests. HPV testing is performed using the Cobas HPV DNA test and all HPV positive samples are tested for a panel of methylation specific biomarkers [CAD M1 M18, MAL M1, hsa-mir-124-2] this is done via bisulfite conversion followed qPCR. A validation panel of 200 samples with confirmed histology is being created to provide clinically relevant cut off points.

Results: To date 10,800 women have been recruited, (median age 38 years) the abnormal cytology rate is 7.23% and HPV DNA positivity rate is 15.39%. A subset of 157 samples (HPV Positive n=107; HPV negative n=50) with histology confirmed CIN1-3 (n=107), and no abnormality detected (NAD) (n=50) have been run as a validation panel to define clinically relevant cut off points for the detection of CIN2+. The panel shows statistically different methylation scores between CIN1 and NAD samples for MAL M1 and hsa-mir-124-2(unpaired t-test p=0.036,0.019) and statistically different methylation scores for all markers between CIN3 and NAD samples (CAD M1, MAL M1, hsa-mir-124-2 (p=0.015,0.016,<0.001)). There is also a statistical difference in methylation patterns between CIN1 and CIN3 sample (p= 0.018,0.018,0.001).

Conclusions: Currently the combination of CAD M1 M18, MAL M1 and hsa-mir-124-2 shows promise in differentiating CIN3 pre-cancers from a negative population as well as CIN1 from CIN3. Inclusion of CIN2 samples is to follow to determine the panels ability to differentiate relevant from non-relevant lesions. Once complete all HPV positive women will have their methylation scores accessed. These women have consented to a 10 year follow up allowing the panel to be assessed in both a first round screening situation and longitudinally.

1262 Maternal Red Blood Cell Distribution Width Differences in Preterm versus Term Births and Spontaneous versus Indicated Births

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Background: Preterm birth (PTB) is defined as birth under 37 weeks of gestational age (GA). PTB is the largest contributor to perinatal morbidity and mortality. Spontaneous PTB occurs without indication for preterm delivery and is difficult to predict. Of all preterm deliveries in the USA, an average of 58% are spontaneous and around half of spontaneous PTB have unknown causes. PTB is often due to maternal vascular malperfusion or pathologic inflammation, such as from chorioamnionitis. Elevated RDW is a prognostic predictor in different inflammatory and vascular conditions. In this study, we seek to determine whether there are significant differences in median pre-birth maternal RDW among the following comparator groups: spontaneous PTB vs. indicated PTB, preterm vs. term births, very preterm vs. term births, and overall spontaneous vs. overall indicated births.

Design: Maternal chart review was performed for births with banked placental tissue from 2013 to 2016. Birth was defined as term, preterm, or very preterm if GA was 37 weeks or above, under 37 weeks, or under 32 weeks at time of delivery. Cases were enriched for PTB. Indications for delivery were identified by chart review. Each birth was coded as either spontaneous or indicated. The most recent maternal RDW before birth was recorded.

Results: In total 257 births were studied. Of these, 76 were excluded on the basis of multiple gestation, insufficient clinical information, missing RDW data, and intrauterine fetal death. Of the remaining 181 births, 142 (78%) were preterm and 39 (22%) were term. Of the preterm births, 87/142 (61%) were spontaneous and 55/142 (39%) were indicated. Of the term births, 24/39 (62%) were spontaneous and 15/39 (38%) were indicated. The Wilcoxon rank-sum test of medians was used. Indicated PTB RDW was not significantly different from spontaneous PTB RDW (13.7 vs. 13.5, p=0.1349). All PTB RDW was not significantly different from all term birth RDW (13.5 vs. 13.9, p=0.0684). Overall spontaneous birth RDW was not significantly different from overall indicated birth RDW (13.5 vs. 13.7, p=0.2511). Very PTB RDW was significantly different from term birth RDW (13.35 vs. 13.9, p=0.0489).

Conclusions: The study finds a statistically significant difference in pre-delivery maternal RDW in a cohort of very PTB vs. term birth. Further investigation includes validation in an independent set of cases and the possible development of a risk stratification system using clinical information and RDW values to predict PTB.

1263 Beta-catenin Immunohistochemistry as a Surrogate for CTNNB1 Mutational analysis in Endometrial Carcinoma Biopsies

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Background: *CTNNB1* mutations are commonly detected in endometrial carcinomas (TCGA 30%). They are prevalent in the copy-low molecular subtype and its prognostic value seems context dependent.

Design: The purpose was to test whether beta-catenin IHC reliably predicts *CTNNB1* mutations identified by next generation sequencing (NGS) in EC biopsy samples. Cases of serous, non-serous high-grade and grade 1-2 endometrioid EC were selected in a 2:1:1 ratio. Beta-catenin IHC carried out centrally on full sections from EC biopsy/curetting specimens from 5 centres (24-49 cases/centre) were compared to tagged-amplicon NGS *CTNNB1* sequencing results in a total of 182 cases. Molecular subtype was assessed by immunohistochemical staining for p53 and mismatch repair (MMR)

proteins as well as mutational analysis of DNA polymerase epsilon (POLE) exonuclease domain.

Results: Beta-catenin staining pattern was nuclear in 11.6% of cases, occurring mutually exclusive from abnormal p53 expression pattern. Nine different *CTNNB1* hotspot mutations affecting three codons were identified in 10 cases (5.5%). Beta-catenin IHC had a sensitivity of 80.0% (95% CI 44.4-97.5%) and specificity of 93.0% (95% CI 88.1 – 96.4%) to detect a *CTNNB1* mutation with an overall accuracy of 92%.

Beta-catenin IHC	**CTNNB1** hotspot mutation		TOTAL
	Present	Absent	
Nuclear	8	12	20
Membranous	2	160	162
TOTAL	10	172	182

Conclusions: Our preliminary analysis suggests that beta-catenin immunohistochemistry is a reasonable surrogate for *CTNNB1* mutation analysis. A particular issue identified was common nuclear expression without a detectable mutation. This could indicate a higher sensitivity of beta-catenin IHC to detect subclonal *CTNNB1* mutation compared to sequencing.

1264 Cervical Glandular Neoplasia in the United States: Increasing Prevalence and Inadequate Screening Practices

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Background: Since the 1970s cervical glandular neoplasia (CGN) (encompassing adenocarcinoma in situ (AIS) and adenocarcinoma) has increased in prevalence in absolute, not just relative, terms when compared to squamous cell carcinoma. CGN can present a diagnostic challenge because current screening methods for cervical neoplasia are most appropriate for squamous lesions. With increasing prevalence of CGN, appropriate screening protocols need to be determined.

There is an abundance of literature on HPV-related squamous neoplasia but very limited regarding glandular lesions. A recently published collaborative study by 12 European groups found variations in HPV prevalence and ADC type-distribution by country. However, we were unable to find publications of large CGN cohorts from the United States.

Design: We retrospectively reviewed available cytology, human papilloma virus (HPV), and histologic, and clinical data in patients diagnosed with adenocarcinoma in situ (AIS) and adenocarcinoma at the University of Michigan between 1990 through 2015. We then performed sensitive HPV PCR testing (Multiplex PCR) on all cases to determine HPV-type.

Results: The prevalence of CGN at our institution has increased 5-fold since 1990 (Figure 1).

Of the 147 cases of glandular neoplasia, 116 were found to have HPV infection in the distribution seen in Table 1. Seventeen cases were negative due to low volume of DNA, 11 cases were presumed false negatives based on histologic review, 9 cases were negative due to a histology not associated with HPV infection.

Fifty cases had concurrent molecular and cytology preceding tissue diagnosis of glandular neoplasia (Table 2). 41/50 cases detected cytologic atypia, either squamous and/or glandular (sensitivity of cytology alone 82%). Of the nine cases with negative cytology, on biopsy five had only glandular disease and four had both glandular and squamous disease. 1/50 cases was negative for HPV DNA (sensitivity of molecular alone - 98%). The one case with a negative molecular study was read on cytology as ASCUS and on biopsy this case had both glandular and squamous disease. The sensitivity of combined molecular and cytology was 100%.

Please see attached "Figure 2" for Tables 1 and 2.

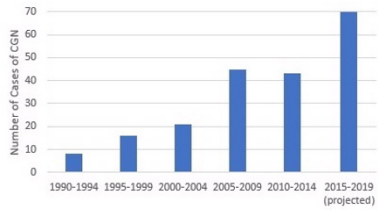

Figure 1. Increasing Prevalence of CGN from 1990 to 2015

Table 1. HPV Type-specific prevalence in HPV-positive cases

	All Infections (Single or Multiple)		
	Total (n=116)	AIS (n=47)	Invasive (n=69)
HPV 16	72 (62.1%)	23 (48.9%)	49 (71.0%)
HPV 18	31 (26.7%)	17 (36.2%)	14 (20.3%)
HPV 33	1 (0.9%)	-	1 (1.4%)
HPV 45	6 (5.2%)	3 (6.4%)	3 (4.3 %)
HPV 52	1 (0.9%)	1 (2.1%)	-
HPV 56	2 (1.7%)	2 (4.3%)	-
HPV 59	1 (0.9%)	1 (2.1%)	-
HPV 68	2 (1.7%)	-	2 (2.9%)

Table 2. Molecular and cytology results at the time of screening prior to tissue diagnosis of glandular neoplasia

	Molecular screening results				
Cytology	HPV16 (n=5)	HPV18 (n=7)	HR HPV (n=34)	Equivocal (n=2)	Negative (n=1)
AGUS, NOS		1	9		
AGUS, FN	1	1	3	1	
AdenoCA			3		
ASCUS	1	2	11		1
LSIL			1		
HSIL			5	1	
Negative	4	3	2		

Conclusions: The prevalence of CGN has increased significantly since 1990. Pap smear alone is an insensitive method of screening for glandular neoplasia and should be accompanied by molecular testing. HPV infection accounts for at least 80% of glandular neoplasia and should be incorporated into first line cervical screening.

1265 Reproducibility of Pattern-Based Classification for Endocervical Adenocarcinoma - An Institutional Experience

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Background: A pattern-based classification system for invasive endocervical adenocarcinoma has been shown to be predictive of nodal metastases and recurrence and can spare patients with no risk of recurrence after complete resection, the morbidities of further treatment. However, the reproducibility of this pattern classification system has had mixed results. We sought to determine if the pattern classification system could be applied to our patient population and pathology group.

Design: We retrospectively reviewed available histologic and clinical data in patients diagnosed with invasive endocervical adenocarcinoma at the University of Michigan between 1990 through 2015. We then assigned pattern of invasion based on the recently proposed classification system by Diz De Vivar et al. and performed sensitive HPV PCR testing (Multiplex PCR) on all cases to determine HPV-type.

Results: We assigned pattern of invasion to 54 of 69 invasive adenocarcinomas. We had 7 cases of pattern A (13%), 28 cases of pattern B (52%), and 18 cases of pattern C (33%), and 1 case of mixed pattern B and C (2%). None of the patients with pattern A invasion recurred (average follow up time 8 years). Two patients with pattern B invasion recurred both at 3 years. Four patients with pattern C recurred at 1, 2, 3 and 6 years.

Conclusions: Our data support the prognostic value of pattern classification. Based on our cohort, there is no risk of recurrence in pattern A invasive endocervical adenocarcinoma. There is a trend for higher risk of invasion with increasing pattern classification from A to C. The pattern classification system is reproducible within our pathology group.

1266 Positive p16 Expression by Immunohistochemistry in Morphologic Subsets of Endometrioid Carcinoma

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Background: Uterine endometrioid carcinoma typically are p16 positive with a interrupted 'striped' pattern, or they are negative. However, occasionally, strong p16 expression akin to serous carcinoma can be seen. As a result, endometrioid carcinomas that are p16-positive are at risk for being misdiagnosed as serous carcinoma in routine practice, particularly in biopsy material with scant tissue. We hypothesized that variability in p16 immunostaining may be related to morphologic subtypes in endometroid carcinoma. We evaluated p16 staining patterns in endometrioid carcinomas with a focus on tumors with eosinophilic or mucinous morphology.

Design: We reviewed tumor-containing slides from 150 consecutive cases of endometrial carcinoma who underwent hysterectomy at our institution in one year (2012). From these, we identified tumors with abundant cytoplasm or mucinous differentiation (n=30). These included tumors containing oncocytic and admixed mucinous areas, and carcinoma with solid areas with abundant cytoplasm closely resembling the former. Lesional tissue was independently assigned a final designation as eosinophilic/mucinous or conventional based on presence or absence of distinctive areas within tumor exhibiting these features. p16 immunostaining in tumor was evaluated as either confluent or striped based on expression patterns in the areas of interest.

Results: Of the selected cases, 18 were classified as 'eosinophilic/mucinous' and 12 as conventional. Two modes of p16 positivity were seen: one, with a characteristic striped pattern with strongly positive cells alternating with weak or negative tumor cells, and a

second, with nuclear and cytoplasmic confluent positivity (2+ to 3+) in glands. Of the 18 cases with distinctive eosinophilic/mucinous areas, 15 showed confluent strong p16-positivity, whereas this pattern was absent in all conventional endometrioid carcinoma. The correlation between morphologic subtype and p16 expression patern was highly statistically significant with the chi-square test ($\chi 2 = 20.0$, p=0.0001)

Conclusions: We hereby demonstrate that strong p16 positivity can be seen in a subset of endometrioid carcinoma other than serous carcinoma. Additionally these observations suggest that areas containing variant morphology, particularly those with mucinous features, are highly likely to be p16-positive. Confirmation and further characterization of p16 expression in endometroid carcinoma may be useful in avoiding diagnostic pitfalls.

1267 HER2 Expression in Gynecologic Carcinosarcoma: A Seven-Year Single-Institution Experience

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Background: HER2 overexpression/amplification has been reported in a third of serous endometrial carcinomas (EC) and a randomized phase II clinical trial was recently completed to assess the role of trastuzumab therapy in these tumors. Serous EC has been found to have unique HER2 immunostaining patterns, including significant intratumoral heterogeneity and lack of apical membrane staining. We aimed to systematically evaluate the characteristics of HER2 expression in CS of the female genital tract using standardized staining methods and scoring criteria.

Design: Text search for CS cases with available HER2 status was performed in our departmental archives between 2010 and 2017. All H&E and immunohistochemistry (IHC) slides were reviewed along with available FISH results. HER2 scores were assigned to both the carcinomatous and sarcomatous components – when present on the HER2 IHC slide - per the 2007 and current (2013) ASCO/CAP breast scoring criteria.

Results: CSs from 62 patients (53 uterine, 9 ovarian or fallopian tube) were included. The carcinoma component was serous (61%), endometrioid (10%), clear cell (3%), undifferentiated (3%), neuroendocrine (1%), or mixed (24%); the sarcoma component was either homologous (57%) or heterologous (43%). Using the 2013 criteria, 10 cases (9 uterine, 1 ovarian, 16%) were HER2 positive (either by IHC or FISH) in the carcinoma component, while only 8 cases were HER2 positive per the 2007 criteria. Six cases showed discrepancy in their HER2 status between the two scoring criteria: 4 cases changed from 1+ (2007) to 0 (2013) and 2 cases from 2+ (2007) to 3+ (2013). Nine cases with 2+ staining on review had no FISH results available. Twelve cases showed significant intratumoral heterogeneity of HER2 expression. The sarcoma component was stained in 29 cases, 4 of which (14%) showed 2+, equivocal HER2 expression. No 3+ staining was observed in the sarcoma component in any of the cases.

HER2 Score (2013)	Carcinoma (n)	Sarcoma (n)	FISH amplified (n)	FISH not amplified (n)	FISH not done (n)
0	20	15	0	0	20
1+	15	8	0	1	14
2+	20	4	3	7	10
3+	7	0	1	3	3

HER2 Score (2007)	Carcinoma (n)	Sarcoma (n)	FISH amplified (n)	FISH not amplified (n)	FISH not done (n)
0	16	15	0	0	16
1+	19	8	0	1	18
2+	22	4	3	9	10
3+	5	0	1	1	3

Table. HER-2 score of the carcinomatous and sarcomatous components using the ASCO/CAP 2007 and 2013 breast criteria.

Conclusions: Using the current breast HER2 scoring criteria, 16% of gynecologic CS showed HER2 overexpression and/or amplification. HER2 expression by IHC was typically stronger in the carcinoma component, although 14% of sarcoma components showed 2+ HER2 staining. Heterogeneity of HER2 expression was observed in 44% of 2+ and 3+ HER2 IHC cases combined, and lack of apical membrane staining was seen in a significant number of cases. This pilot study provides informative data for future possible therapeutic options for patients with gynecological CS.

1268 Malignant Peritoneal Mesothelioma in Patients with Endometriosis

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Background: Florid mesothelial hyperplasia is known to result from endometriosis. Well-differentiated papillary mesothelioma and multiloculated peritoneal inclusion cysts have also been described in women with endometriosis. To our knowledge, peritoneal diffuse malignant mesothelioma (MM) arising in the setting of endometriosis has not been reported.

Design: The surgical pathology files of a tertiary academic medical center and the consultation files of one of the study authors were reviewed for cases with MM and endometriosis.

Results: Six women with MM and endometriosis ranging in age from 29-55 years (median=45 years) were identified. All had peritoneal MM and endometriosis involving the peritoneum and/or adnexa. Five had epithelial MM and 1 had biphasic MM. Two had a history of paraoccupational exposure to asbestos. The clinicopathologic features are summarized in the table.

Case	Age	Endometriosis duration	MM type	Distribution of MM	Time since MM diagnosis	Died of disease	Asbestos exposure (duration)
1	29	Diagnosed synchronously with MM	Epithelial	Pelvic perito-neum, uterine serosa	9 years		Father - plumber (10 years)
2	55	Diagnosed synchronously with MM	Epithelial	Pelvic peritoneum, appendiceal and bowel serosa, me-soappendix, uterine serosa, subserosal myometrium	12 years		Uncle - ironworker (8 years)
3	41	18 years	Epithelial	Ovarian sero-sa, omentum, peritoneum	10 years		Not iden-tified
4	48	5 years	Biphasic	Bowel serosa	1 year	Y	Not identified
5	53	30 years	Epithelial	Pelvic perito-neum	3 years		Not identified
6	37	Diagnosed synchronously with MM	Epithelial	Uterine serosa	4 months		Not identified

Conclusions: MM occurs rarely in women with endometriosis and interestingly has only been seen in the peritoneum, but not other serosal cavities. Chronic inflammatory processes involving the serosa have been associated with the development of MM in rare instances. The findings in the present study suggest that endometriosis may also be a precursor of MM. While the role of endometriosis in the carcinogenesis of peritoneal MM remains to be established, emerging evidence suggests that inflammasome activation is both caused by endometriosis and also plays a part in the induction of various malignancies, including MM.

1269 MYC Gene Amplification in High-Grade Serous Ovarian Carcinoma and its Association with BRCA1/2 Mutation Status

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Background: The Cancer Genome Atlas (TCGA) identified significant molecular alterations in high-grade serous ovarian carcinomas (HGSOC), including *MYC* gene amplification in a subset of cases. Aberrations in *MYC* have been described in other tissues as activators of malignant transformation and cancer progression. Since prior studies have found that *MYC* is amplified in a group of *BRCA1*-associated breast cancer, we intended to evaluate the potential association of *MYC* amplification with *BRCA1/2* status in HGSOC.

Design: Data from the TCGA website was retrieved and evaluated. Diagnostic odds ratio for cases with alterations in *MYC, BRCA1* and *BRCA2* genes was calculated. Statistical significance was set at p<0.05. Concurrently, a cohort of HGSOC cases from patients who had tumors analyzed via different commercial next-generation sequencing (NGS) panels was obtained, and the results were compared with TCGA findings. Additionally, immunohistochemistry (IHC) for MYC protein was performed in paraffin embedded formalin-fixed tissue in a subset of the cases, and scored as positive (≥40% of cells) or negative (<40% of cells).

Results: The cohort from TCGA comprised of 316 HGSOC cases. *MYC* amplification was positive in 102 (32%). Genomic alterations, including germline and somatic mutations, copy number alterations, and mRNA up- and down-regulations in *BRCA1*/2 occurred in 56 (18%)

samples each. Of the 102 cases with *MYC* amplification, 33 had *BRCA1* (15 germline, 18 somatic) and 26 *BRCA2* (9 germline, 17 somatic) alterations. Significant tendency towards co-occurrence between *MYC* and *BRCA1* (p<0.001) and *MYC* and *BRCA2* (p=0.019) was noted. We found that a tumor with *MYC* amplification has 3.9 times the odds of having genomic alterations in *BRCA1* and/or *BRCA2* genes compared to tumors without it (95% CI: 2.35 to 6.42, p<0.0001). IHC for MYC was done on 18 cases in our cohort. All cases showed varying degree of expression (1-80%). However, only 2 of 6 patients with germline *BRCA1/2* mutations had intense (>40%) MYC expression, in contrast to 6 of 12 *BRCA1/2* negative cases.

Conclusions: Our results indicate that HGSOC with *MYC* amplification have increased odds of harboring *BRCA1/2* genomic alterations based TCGA data analysis. MYC protein expression by IHC, however, did not support this finding probably due to a limited number of tested cases. Nevertheless, as limited data is available regarding *MYC* aberrations in the setting of *BRCA1/2* mutations and ovarian carcinogenesis, additional studies are needed for further investigation.

1270 The Molecular Landscape of Benign Metastasizing Leiomyomas

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Background: Benign metastasizing leiomyoma (BML) is a controversial entity which arises in women with extreme rarity. BMLs have been previously described in the lungs, bone, soft tissue and lymph nodes and invariably arise in the setting of a previously or synchronously diagnosed uterine leiomyoma which led to the hypothesis that they represent metastases from a uterine primary. However, the histologic features of BMLs are those of bland smooth muscle neoplasms which is at odds with the notion that they represent distant metastases. As such, competing alternative hypotheses emerged which postulated that BMLs could represent metastatic low-grade leiomyosarcomas or could be primary leiomyomatoses unrelated to the concomitant uterine leiomyoma. To help understand the pathogenesis, we devised this study to determine the molecular alterations present in BMLs.

Design: We retrospectively identified 6 patients who had surgical resections for BMLs at our U.S. tertiary care hospital. Four of the patients had paired BMLs and uterine leiomyomas available for analysis. Next generation sequencing (NGS) was performed on all of the lesions to determine underlying mutations.

Results: In our patient cohort, BMLs were identified in the lungs, liver, lymph nodes and soft tissue. Four specimens were sequenced with the remaining six specimens in process. We found novel mutations in ASTML (variant allele frequency = 37.84%), ATM (9.43%), BCR (6.56%), BMPR1A (11.85%), BRCA1 (36.36%), BTK (5.68%), CTNNB1 (6.25%), CYP2D6 (28.57%), ECT2L (5.1%), ERG (39.76%), FANCA (39.55%), FZR1 (81.72%), MAGEA1 (99.25%), MEN1 (73.11%), NOTCH2 (24.3%), PCLO (7.29%), PDE4DIP (17.09%), PIK3CG (11.96%), PMS1 (33.75%), RELN (6.59%), RPN1 (8.05%), SYNE1 (36.54%), TBX22 (7.5%) and TP53 (6.06%). While prior reports indicate MED12 hotspot mutations are found in up to 70% of uterine leiomyomas, we found no evidence of MED12 mutation in the BML or uterine leiomyoma. Gene ontology enrichment analysis revealed the identified mutations were most commonly part of the DNA repair pathway.

Conclusions: NGS analysis revealed numerous novel mutations in the tumors with the mutations most commonly involving genes involved in DNA repair. All tumors were wild-type for MED12 (which is mutated in the majority of uterine leiomyomas) and no recurring mutations were present. More investigation is necessary to uncover the pathogenesis of BMLs.

1271 Histologic Features of Hysterectomy Specimens from female-male Transgender Individuals

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Background: Histologic changes in the female genital tract after prolonged androgen stimulation have been described in the past. However, these changes have not been systematically addressed in hysterectomy specimens from subjects undergoing surgical gender-reassignment, typically after treatment with exogenous androgens. The current study aims to provide practicing pathologists with a list of expected histologic features in hysterectomy specimens from female-male transgender individuals.

Design: Twenty four hysterectomy with bilateral salpingo-oophorectomy specimens (2003 -2017) were identified from our Laboratory Information System. Slides were retrieved and reviewed for features associated with androgen exposure, without knowledge of the clinical history. Clinical information from the 24 patients was then obtained from the electronic medical records.

Results: Twenty three subjects ranged from 21-46 years of age (mean = 31). The twenty fourth patient was a 78-year-old with untreated congenital adrenal hyperplasia with androgen excess. Ectocervical transitional cell metaplasia (TCM) was present in 15/24 (63%) subjects. Inactive endometrium with focal stromal decidualization (SD) was present in 16/24 (67%) subjects. Bilateral cystic follicles (CFs) were present in 19/24 (79%) subjects, with observed primordial follicles density higher than that expected for age. Additionally, 3/24 (13%) subjects had benign endometrial polyps and 3/24 (13%) had uterine leiomyomas. Incidentally, unilateral mature cystic teratomas were found in 3/24 (13%) subjects. Prolonged preoperative androgen therapy was documented in 18/23 while the hormone therapy profile was unknown in one subject. All specimens with TCM, SD, and CFs were from subjects with preoperative androgen exposure.

Conclusions: Ectocervical transitional cell metaplasia, inactive endometrium with focal stromal decidualization and bilateral cystic follicles are present in the majority of specimens from female-male transgender individuals (>60%). These histologic changes correlate with prolonged preoperative androgen administration. The significance of these findings relies on recognizing the spectrum of androgen-related histologic alterations and not confusing TCM with dysplasia. We are exploring a morphometric model of assessing the age-related decline of primordial follicle density in a control group, to objectively compare to the observed high density in this series.

1272 Interobserver Agreement for Mismatch Repair Protein Immunohistochemistry in Endometrial and Ovarian Carcinomas

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Background: Immunohistochemistry (IHC) for mismatch repair proteins (MMR) is an established tool to identify Lynch Syndrome (LS) among patients with colorectal cancer (CRC) and is being increasingly used to identify LS in women with endometrial and/or ovarian cancer. Reported kappas for interobserver agreement among pathologists for MMR interpretation in colorectal specimens have ranged from moderate to substantial. Interobserver reliability in gynecologic specimens has not been reported to our knowledge. The aim of this study was to assess the interobserver variability in the interpretation of MMR-IHC on endometrial and ovarian carcinomas

Design: The study sample consisted of 73 consecutive endometrial cancers (n=48) and non-serous, non-mucinous epithelial ovarian (n=25) carcinomas from a single cancer center. Tissue sections were stained with MMR-IHC for MLH1, MSH2, MSH6, and PMS2. Five pathologists and a pathology fellow from two cancer centers evaluated the MMR stains: 1 pathologist with >10 years experience interpreting MMR-IHC in colorectal and gynecologic cancers, and the others with no previous experience. Prior to the study, a review of 10 teaching cases was led by the experienced pathologist. A decision tool was developed for use as a guide in MMR staining interpretation. Staining was interpreted as intact, deficient or equivocal for each protein, and a final interpretation of the patient MMR status was also reported.

Results: Interobserver agreement for the patient MMR status was categorized as "almost perfect" with kappa= 0.919 (95%CI: 0.863-0.976). Consensus interpretation identified 51 cases as MMR-intact and 22 as deficient. All observers were in agreement in 66 (92%) tumors. Except for the most experienced pathologist, each observer had at least 1 discrepant interpretation. There were 7 discordant cases: 3 tumors considered MMR-deficient and 2 as MMR-intact were called equivocal by at least one of the observers, and in 2 discordant cases, the disagreement interpretation was MMR- intact while the consensus was MMR-deficient. Two of 73 (3%) patients would have been misclassified without opportunity for further testing.

Conclusions: The reliability of MMR-IHC on endometrial and ovarian cancer specimens appears to be comparable to that of MMR interpretation in CRCs. While the interobserver agreement was high, pathologists less experienced with MMR-IHC may be more inclined to call cases MMR- equivocal and may miss a proportion of cases which are MMR deficient.

1273 Napsin-A as an Immunohistochemical Marker for Clear Cell Carcinoma of the Uterine Cervix

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Background: Clear cell carcinoma (CCC) of the uterine cervix is a rare tumor, accounting for approximately 4% of cervical adenocarcinomas. Histomorphology is generally used to distinguish CCC from other endocervical adenocarcinoma variants. However, morphologic

overlap exists, necessitating a sensitive and specific marker for CCC. Napsin-A is an aspartic proteinase used as a diagnostic marker, in addition to TTF-1, for adenocarcinoma of the lung. It has also been shown to be a sensitive and specific marker for CCC arising in the ovary and endometrium. However, this marker has not been studied in CCC of the uterine cervix. The goal of this study was to determine the Napsin-A immunohistochemical staining pattern of CCC arising in the cervix compared to other variants of cervical adenocarcinomas.

Design: This study consisted of 19 cases of primary cervical adenocarcinomas, including clear cell carcinoma (CCC, n=5), mixed serous/clear cell carcinoma (MSER/CCC, n=1), gastric-type adenocarcinoma (GAS, n=5) and usual-type endocervical adenocarcinoma (UEA, n=8). Four cases of ovarian CCC were also included as controls. All cases were immunostained with anti-Napsin-A and semiquantitatively scored as follows: 0, no staining, focal (+), <25%, patchy (++), 25-75%, and diffuse (+++), > 75%.

Results: Napsin-A staining was detected in all cervical clear cell carcinomas with 3 showing diffuse (+++) staining, one showing patchy (++) staining and one showing focal (+) staining. All four ovarian CCC stained positive for Napsin-A with 2 showing diffuse (+++) staining and 2 showing patchy (++) staining. Napsin-A staining was also detected in 4 of 5 gastric-type adenocarcinomas with 1 showing diffuse (+++) staining, 2 showing patchy staining (++) and 1 showing focal (+) staining. The MSER/CCC did not stain with Napsin-A. Only 1/8 usual-type endocervical adenocarcinoma was Napsin-A positive, and it was focal (+); the remaining 7 cases were negative.

Conclusions: A majority of clear cell carcinomas arising in the uterine cervix were positive with Napsin-A and showed a similar staining pattern to ovarian CCC. Napsin-A was only focally positive in 1/8 cases of usual-type endocervical adenocarcinomas. Therefore, Napsin-A may be a useful marker for distinguishing cervical CCC from the most common type of cervical adenocarcinoma. Unexpectedly, a majority of gastric-type adenocarcinomas were positive, at least focally, for Napsin-A. This finding suggests that Napsin-A may not be optimal to differentiate CCC from GAS.

1274 Lipoblastoma-like Tumor of the Vulva: A Clinicopathologic, Immunohistochemical, Fluorescence In Situ Hybridization and Genomic Copy Number Profiling Study of 6 Cases

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Background: Lipoblastoma-like tumor of the vulva (LLTV) is a rare mesenchymal neoplasm of adipocytic differentiation with morphologic features that overlap those of lipoblastoma, myxoid liposarcoma, myxoid well-differentiated liposarcoma and spindle cell lipoma. Only 12 cases have been reported to date. Recently, LLTV has been shown to frequently lack RB expression by immunohistochemistry (IHC), suggesting a link to other RB-deficient tumors such as spindle cell lipoma, mammary-type myofibroblastoma and cellular angiofibroma. The mechanism underlying RB protein loss in LLTV is unknown.

Design: Six cases of LLTV were retrieved from our archives. All cases were known to be negative for *PLAG1* or *DDIT3* rearrangement and *MDM2* amplification. RB IHC, *RB* fluorescence in situ hybridization (FISH) using an enumeration probe set, and genomic copy number profiling by OncoScan microarray were performed.

Results: The referring diagnosis in consultation cases was myxoid liposarcoma (5 cases) and myxoid spindle cell lipoma (1 case). The patients ranged from 21 to 39 years of age (mean 31 years). All cases showed typical morphological features of LLTV, with a lobulated, myxoid and highly vascular proliferation of bland spindled cells with intermixed mature adipocytes and lipoblasts. RB protein expression was absent in 3 cases tested to date. *RB* deletion was not identified by FISH in 5 cases. One case demonstrated an equivocal result for monosomy 13 by FISH. No evidence of 13q or other regional deletions were detected by genomic copy number profiling in 3 cases tested to date (FISH-equivocal case not yet tested).

Conclusions: Although LLTV frequently lacks RB expression by IHC, the mechanism for this is unclear. Our data suggest that most LLTV do not show *RB* deletions by FISH, or loss of chromosome 13q by genomic copy number profiling. Ongoing immunohistochemical, FISH and genomic copy number profiling of additional cases should clarify LLTV's relationship to other RB-deficient neoplasms, in particular spindle cell lipoma.

1275 Hotspot TERT Promoter Mutations in Adult-Type Granulosa Cell Tumors of the Ovary as Potential Drivers of Progression

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Background: Adult-type granulosa cell tumors (GCTs) of the ovary have a low malignant potential, however these tumors have a propensity for local spread and late recurrences. We sought to define the repertoire of somatic mutations, in addition to the pathognomonic *FOXL2* hotspot mutation, and to identify potential markers of recurrence in this rare type of sex cord-stromal tumor of the ovary.

Design: Primary GCTs, which did not develop recurrences after 4 years of follow-up (n=10), primary and matched recurrent/metastatic GCTs (n=9) and recurrences only (n=3) were subjected to DNA extraction and massively parallel sequencing targeting all exons and selected introns and regulatory regions of 410 cancer-related genes and/or to Sanger sequencing analysis of *TERT* and *FOXL2*. Bioinformatics analyses were performed using validated algorithms.

Results: All GCTs of the ovary included in this study harbored the somatic p.C134W *FOXL2* mutation, confirming their diagnosis. Sequencing analyses revealed hotspot mutations in the *TERT* promoter (c.-124C>T or c.-146C>T) in 67% (8/14) of primary GCTs that recurred and/or recurrences/metastases, while only 10% (1/10) of primary GCTs that did not recur harbored *TERT* promoter hotspot mutations (p=0.01, Fisher's exact test). No *TERT* amplifications were found in the GCTs analyzed. In a subset of recurrent/metastatic GCTs lacking *TERT* promoter mutations, other pathogenic genetic alterations, including *TP53* hotspot mutations and/or *CDKN2A* homozygous deletions, were found.

Conclusions: The presence of *TERT* promoter hotspot mutations may identify subsets of ovarian GCT patients who are at risk for recurrence and who may require prolonged surveillance and/or adjuvant therapy.

1276 CADM1, MAL and miR-124 Promoter Methylation as Biomarkers of Progression in Cervical Cancer.

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Background: Virtually all cervical cancers (CC) result from persistent infection by high-risk human papillomaviruses (hrHPV) and are preceded by intraepithelial lesions. Molecular tests for hrHPV detection have shown a sensitivity of 90-95% for the detection of high-grade intraepithelial lesions/cervical intraepithelial neoplasia grade 2-3 and CC (HSIL/CIN2+), but have a relatively low specificity, as many hrHPV infections will never progress to CC. This prospective study analyzes whether the CADM1, MAL and miR124 promoter methylation analysis could detect those patients with HSIL/CIN2+, and thus, have a role in the triage of hrHPV-positive women.

Design: Prospective study that included cervical biopsies form 131 women referred to the Colposcopy Clinic from January 2013 to December 2015. Eight patients had a negative biopsy and were negative for hrHPV, 19 had a low-grade squamous intraepithelial lesion/cervical intraepithelial neoplasia grade 1 (LSIL/CIN1), 30 had HSIL/CIN2, 60 a HSIL/CIN3, and 14 a CC. DNA extraction was performed from formalin-fixed paraffin-embedded tissue after bisulfite treatment. A standardized, multiplex quantitative methylation specific PCR (prototype PreCursor-M test, Self-screen B.V., Amsterdam, The Netherlands) was used to identify the methylation status of the promoter regions. The proportions of methylation-positive samples per histological diagnosis were compared using the Mann-Whitney U test.

Results: Table 1 shows the positivity rates of MAL, CADM1 and miR-124 promoter methylation in the different diagnostic groups. The sensitivity and specificity of the methylation of at least one biomarker for HSIL/CIN2+ was 84.6% and 74.1%, respectively. The sensitivity and specificity of the methylation of two biomarkers for HSIL/CIN2+ was 57.7% and 96.3%, respectively.

Table 1.

Biomarker	Negative (n=8)		LSIL/CIN1 (n=19)		HSIL/CIN2 (n=30)		HSIL/CIN3 (n=60)		CC (n=14)		p
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	
CADM1	0	(0.0)	2	(10.5)	2	(6.6)	31	(51.6)	8	(57.4)	<0.001
MAL	0	(0.0)	2	(10.5)	17	(56.6)	36	(60.0)	13	(92.8)	<0.001
miR124	1	(12.5)	3	(15.7)	20	(66.6)	41	(68.3)	11	(78.5)	<0.001
At least 1	1	(12.5)	6	(31.5)	25	(83.3)	49	(81.6)	14	(100)	<0.001
At least 2	0	(0.0)	1	(5.2)	13	(43.3)	36	(60.0)	12	(85.7)	<0.001
All 3	0	(0.0)	0	(0.0)	1	(3.3)	23	(38.3)	6	(42.8)	<0.001

Conclusions: CADM1, MAL and miR-124 promoter methylation could be a useful biomarker of progression in women with hrHPV associated cervical lesions.

Funded in part by the Instituto de Salud Carlos III (ICSIII)-Fondo de Investigacion Sanitaria, and ERDF 'One Way to Europe' (PI15/00546).

1277 Molecular Differences Between Mucinous Ovarian Tumors Associated with Brenner Tumors and Teratomas and Their Precursors

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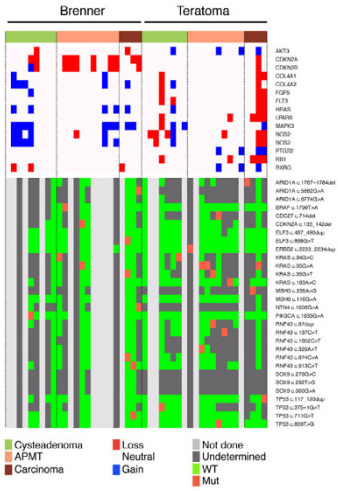
Background: The cell of origin of primary mucinous ovarian tumors (MOT) is unknown. The aim of this study was to examine clonal relationship between MOT and associated Brenner tumors or teratomas based on molecular changes.

Design: A national Pathology Registry search was carried out to identify unilateral Brenner tumor associated mucinous tumors (BAMT) and unilateral teratoma associated mucinous tumors (TAMT). Metastatic ovarian tumors were excluded based on pathology history. Seventeen cystadenomas, 21 atypical proliferative mucinous tumors (APMT) and eight carcinomas were included. DNA was isolated from all 46 tumor pairs and shallow genome-wide sequencing for copy number analysis (CNA) was performed. For 14 BAMT pairs and 17 TAMT pairs single molecule molecular inversion probe (smMIP) based Next Generation Sequencing was carried out for 37 cancer related genes.

Results: CNA showed significant differences between BAMT and TAMT in 49 genomic regions coding for 542 genes. Of these, 14 are cancer related genes according to the KEGG database. BAMT showed more gains of *MAPK3* and *HRAS* and loss of *CDKN2A* and TAMT showed more gains of *AKT3* and loss of *RB1*, mainly in APMTs and carcinomas. Mutation analysis did not show differences between BAMT and TAMT, with most frequent mutations (*KRAS, RNF43* and *TP53)* present in cystadenomas, APMTs as well as carcinomas, suggesting early events in carcinogenesis. *ARID1A, ELF3, MSH3* and *NTN4* mutations occurred solely in mucinous carcinomas. Brenner tumors showed mutations in *ARID1A, ELF3, RNF43* and teratomas in *CDC27* and *MSH6*. Mutations in *SOX9* occurred only in Brenner tumors. No identical mutations between tumor pairs were observed. Hierarchical clustering based on CNA showed four tumor pairs cluster together (one BAMT and three TAMT). One Brenner tumor and associated cystadenoma both showed a loss of *AKT3*, one Brenner tumor and associated carcinoma gains of *COL4A2, MAPK3* and *HRAS*.



Conclusions: CNA showed indications for clonality between MOT and associated Brenner tumors and teratomas, based on hierarchical clustering en identical CNA. No clonality was found using mutation analysis, due to low mutational load in the Brenner tumors and the teratomas. *KRAS, TP53* and *RNF43* mutations seem to be early events in MOT carcinogenesis, but could not be detected in Brenner tumors and teratomas. *SOX9* mutations seem to be specific for the transitional cell phenotype.

1278 Technical and Interpretive Performance of p53 Immunostaining in Ovarian Carcinoma Across Laboratories: Results of an International Collaborative Project

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Background: Optimized p53 Immunohistochemistry is a surrogate for underlying *TP53* mutation. Interpretation is dependent on technical factors as well as accurate recognition of mutation-associated patterns.

Design: Members of the British Association of Gynaecological Pathologists were invited to participate in this project. Sections from a tissue microarray of 42 ovarian carcinomas with known *TP53* mutation status were immunohistochemically stained for p53 by individual laboratories. Pathologists and biomedical scientists (range 1-7/laboratory) interpreted these staining results independently as normal (wild-type, WT), abnormal (over expression (OE), complete absence (CA) or cytoplasmic (CY)) or not assessable (NA).

Results: Thirty-one laboratories returned slides for central review accounting for 87 participants. Four cores were excluded from analysis due to absence of an internal control. There were thus 38x87=3306 core results for analysis (Table 1). The number of uninterpretable cores per laboratory ranged from 0-25, with 10/31 labs having 5 or more uninterpretable cores. Excluding uninterpretable results there was 91.7% (2671/2913) absolute concordance between participant and central review results. The discrepant results included 11% of all abnormal results interpreted as normal (105/948), and 6% of all normal results interpreted as abnormal (130/2030). No significant difference was observed in technical protocols in labs with and without high numbers of uninterpretable cores and/or discrepant results, which were largely due to due to weak staining.

Participant Results	Central Review Results				TOTAL
	OE	CA	WT	NA	
oe	383	1	9	1	394
ca	3	435	93	162	693
cy	3	0	28	4	35
wt	35	70	1853	106	2064
na	7	11	47	55	120
TOTAL	431	517	2030	328	3306

Conclusions: It is vital to monitor performance characteristics of p53 technical staining quality as variations can lead to significant differences in interpretation with clinical impact. The major single reason for both uninterpretable staining due to lack of an internal control and incorrect interpretation was weak staining. These shortfalls can be addressed through a combination of improvement in protocols, including the use of an appropriate on-slide control, and awareness of the range of mutation-related staining patterns.

1279 p53 Immunohistochemistry as a Surrogate for TP53 Mutational Analysis in Endometrial Carcinoma Biopsies

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Background: *TP53* mutations are considered a surrogate biomarker of the serous-like "copy-number high" molecular subtype of endometrial carcinoma (EC). While p53 immunohistochemistry (IHC) is widely considered a surrogate for *TP53* mutation, as it accurately reflects mutational status in ovarian carcinoma with close to 100% specificity, its accuracy in EC has not been established.

Design: The purpose was to test whether p53 IHC reliably predicts *TP53* mutations identified by next generation sequencing (NGS) in EC biopsy samples. Cases of serous, non-serous high-grade and grade 1-2 endometrioid EC were selected in a 2:1:1 ratio. p53 IHC carried out locally on EC biopsy/curetting specimens from 5 centres (24-49 cases/centre) was compared to reference p53 IHC as well as tagged-amplicon NGS *TP53* sequencing results in a total of 183 cases. Molecular subtype was assessed by immunohistochemical staining for mismatch repair (MMR) proteins and mutational analysis of DNA polymerase epsilon (POLE) exonuclease domain.

Results: Local and central p53 IHC results were concordant in 97% cases. The p53 staining pattern was heterogeneous (i.e. abnormal in a subclone or two abnormal patterns) in 7/183 cases, frequently occurring in a background of MMR deficiency (n=2) and POLE mutation (n=3).The IHC staining pattern was abnormal in 84/93 cases with a detectable *TP53* mutation and was "wild type" in 69/83 cases where no mutation was detected (Table 1). Performance characteristics for p53 IHC were: sensitivity 90.3% and specificity 83.1% with overall accuracy of 85%.

Reference p53 IHC	Deleterious **TP53** Mutation		TOTAL
	Present	Absent	
Abnormal (overexpression)	68	7	75
Abnormal (complete loss)	11	6	17
Abnormal (cytoplasmic)	5	1	6
Normal	9	69	78
TOTAL Normal/abnormal	93	83	176
Heterogeneous (subclonal/mixed abnormal)	2	5	7

Conclusions: On preliminary analysis the performance of optimised p53 IHC as a surrogate for underlying *TP53* mutation in EC is good though inferior to what has been observed in ovarian carcinoma. A particular issue identified was the relatively poor agreement between the complete loss abnormal staining pattern and mutational assessment. Further analysis of discordant cases may improve concordance.

1280 JAZF1 Fusion Detection by FISH: Valuable Marker for Diagnosis of Endometrial Stromal Tumor in Curettage Specimens

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Background: When the overall histological structure is lost due to severe fragmentation in curettage specimens, entertaining the diagnosis of endometrial stromal tumor and its distinction from the much more common adenomyosis - particularly the gland-poor variant – may be problematic. Immunohistochemistry offers no help in this setting. Since the *JAZF1*/*SUZ12*(*JJAZ1*) fusion resulting from t(7;17)(p21;q15) is the signature molecular event in the majority of endometrial stromal tumors, we investigated the utility of *JAZF1* FISH testing in endomyometrial curettage specimens containing an endometrial stromal component suspicious for endometrial stromal tumor.

Design: Fourteen cases of small, fragmented endometrial specimens were retrieved, including 5 endometrial biopsies, 6 endometrial curettings, and 3 fragmented myomectomy specimens. Additionally, two leiomyomas from hysterectomy specimens were included as negative controls. Cases were histologically reviewed and appropriate target tissue sections were subjected to *JAZF1* fusion using a break-apart FISH probe.

Results: *JAZF1* FISH analysis was informative in 8 recent cases (tissue < 8 years old) and unsuccessful in the remaining 6 older specimens. Of the 8 samples analyzed, 3 were positive and 5 were negative. Table 1 summarizes these informative cases. Three cases had the initial diagnosis of endometrial stromal tumor; two of them showed positive *JAZF1* gene fusion by FISH. Three cases had suspicion for endometrial stromal tumor and none were positive for *JAZF1* fusion, although one was confirmed to be an endometrial stromal tumor by hysterectomy. Two cases had no concern for endometrial stromal tumor at the initial myomectomy, one of which (case 3) was positive for the *JAZF1* rearrangement. This case was initially diagnosed as consistent with a fragmented leiomyoma admixed with endometrium, adenomyosis and solid stromal fragments. Low grade endometrial stromal sarcoma was eventually confirmed by hysterectomy in this case.

Table 1. JAZF1 FISH Analysis Results in 8 Successful Cases

Case	Age	Biopsy Procedure	Histological Diagnosis	FISH Results	Follow-up Hysterectomy
1	65 yo	EMC	Endometrial Stromal Tumor	Positive	-
2	70 yo	EMC	Endometrial Stromal Tumor	Positive	Endometrial Stromal Sarcoma
3	35 yo	Myomectomy	Consistent with Leiomyoma	Positive	Endometrial Stromal Sarcoma
4	49 yo	EMBx	Weakly Proliferative Endometrium with Marked Breakdown Changes	Negative	Endometrial Stromal Tumor*
5	74 yo	EMBx	Inactive Endometrium with Prominent Endometrial Stroma and Features of Endometrial Polyp	Negative	N/A
6	50 yo	EMC	Endometrial Stromal Sarcoma	Negative	No residual endometrial stromal sarcoma
7	40 yo	EMC	Spindle Cell Proliferation with Endometrial Stromal Differentiation	Negative	N/A
8	50 yo	Myomectomy	Consistent with Leiomyoma	Negative	N/A

EMC=Endometrial Curettage, EMBx=Endometrial Biopsy

*Specimen is received fragmented; differential includes endometrial stromal nodule and low grade endometrial stromal sarcoma.

Conclusions: Fragmentation of curettage specimens may result in a significant challenge in the diagnosis of endometrial stromal tumors. *JAZF1* fusion gene detection by FISH is a valuable molecular marker in the separation of endometrial stromal tumors from non-neoplastic endometrial stroma and adenomyosis. A positive result can turn an otherwise equivocal case into diagnostic certainty.

1281 Lack of Genomic Homozygosity in Pediatric Teratomas: Divergent Genetic Pathogenesis from that of Ovarian Teratomas in Adults

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Background: In adults, both immature and mature ovarian teratomas show frequent genetic homozygosity consistent with tumorigenesis arising from germ cells after meiosis I. The genetic zygosity of various teratomas in children has not been explored.

Design: Slides from thirteen sacrococcygeal, twelve ovarian, and three testicular teratomas in children were retrieved from our departmental archives and histologically reviewed. Tumor and paired normal tissues were microdissected and subjected to short tandem repeat (STR) genotyping.

Results: DNA genotyping was informative in twelve sacrococcygeal teratomas, eight ovarian teratomas, and three testicular teratomas. Table 1 shows the overall results in informative cases. Sacrococcygeal teratomas included 7 mature teratomas, 4 immature teratomas, and 1 mixed germ cell tumor, and the patient age ranged from 0 days to 3 years. All but two patients were female. Ovarian teratomas included 5 mature and 3 immature teratomas, and the patient age ranged from 2 to 18 years. Testicular teratomas included 2 mature teratomas and 1 immature teratoma; the patients ranged in age from 3 months to 3 years. All sacrococcygeal, testicular and ovarian teratomas in patients younger than 4 years showed no evidence of genetic homozygosity. In contrast, all ovarian teratomas in patients older than 9 years showed either partial (>30% homozygosity in tested alleles) or complete homozygosity.

Location	Age	Gender	Diagnosis	# of Heterozygous Alleles
Sacrococcygeal	0 d	F	IT	8/9
Sacrococcygeal	1 d	F	IT	5/5
Sacrococcygeal	1 d	F	IT	6/6
Sacrococcygeal	1 d	F	MT	5/5
Sacrococcygeal	3 d	F	IT	11/12*
Sacrococcygeal	3 d	F	MT	7/7
Sacrococcygeal	6 d	F	MT	10/13*
Sacrococcygeal	2 wk	F	MT	12/12
Sacrococcygeal	2 wk	F	MT	8/8
Sacrococcygeal	2 yo	F	MT	9/9
Sacrococcygeal	2 yo	M	MGCT	10/10
Sacrococcygeal	3 yo	M	MT	9/9
Ovarian	2 yo	F	MT	9/10
Ovarian	3 yo	F	MT	9/11
Ovarian	4 yo	F	MT	5/6
Ovarian	4 yo	F	IT	7/9
Ovarian	9 yo	F	IT	6/14
Ovarian	11 yo	F	IT	5/9
Ovarian	16 yo	F	MT	0/12
Ovarian	18 yo	F	MT	5/8
Testicular	3 mo	M	IT	12/12
Testicular	1 yo	M	MT	7/9
Testicular	3 yo	M	MT	9/9

M=Male, F=Female, MT=Mature Teratoma, IT=Immature Teratoma, MGCT=Mixed Germ Cell Tumor

*No molecular data available for normal tissue

Conclusions: Unlike adult and adolescent ovarian teratomas, pediatric sacrococcygeal and gonadal teratomas lack genetic homozygosity, suggesting that pediatric teratomas of sacrococcygeal or gonadal origin may develop at a stage of germ cell development different from that of adult ovarian teratomas.

1282 Potential Diagnostic Pitfalls in Evaluating Immunohistochemistry for Cervical Myofibroblastomas

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Background: Cervico-vaginal myofibroblastoma (CVM) is a rare benign mesenchymal tumor of the lower female genital tract that typically presents as an expansile, polypoid and unencapsulated, subepithelial mass composed of spindled to stellate shaped cells within a collagenous, focally myxoid and edematous stroma. CVMs are thought to be genetically related to mammary myofibroblastomas (MM) with both showing chromosomal loss of 13q14 (RB1 gene located in this region). However, whereas MMs have demonstrated loss of Rb expression, no study to date has examined this association in CVM. The aim of this study was to investigate the utility of immunohistochemistry (IHC) for desmin, CD34 and Rb in diagnosing CVM.

Design: All cervical polyps diagnosed from 7/2016 to 7/2017 (n=175) were retrospectively reviewed by 2 pathologists. Representative blocks from cases showing morphologic myofibroblastic differentiation were evaluated by IHC for desmin, CD34 and Rb. Staining for desmin and CD34 was recorded as positive or negative. Rb nuclear staining was graded as follows with grade 0 considered deficient: 0 (<10%), 1 (10-25%), 2 (>25-50%), 3 (>50-75%), or 4 (>75%). Intact nuclear expression of Rb in endothelial cells served as an internal positive control. Exclusion criteria included cases with non-expansile stroma, increased inflammation or glands within the lesion, and an exuberant epithelial proliferation.

Results: IHC was performed on 76 cases showing myofibroblastic differentiation. 14 were excluded from the final case cohort due to poor Rb internal control. Of the remaining 62 cases, 61 (98.4%) were positive for desmin and CD34. The distribution of Rb staining for these 61 cases was as follows: grade 0 (n=53, 86.9%), grade 1 (n=5, 8.2%), grade 2 (n=2, 3.3%), and grade 3 (n=1, 1.6%). One case negative for desmin and CD34 (1.6%) showed grade 3 Rb staining. Upon re-review of the histology, only 7 of the original 175 cases (4%) were morphologically and immunohistochemically compatible with CVM (strong, diffuse positivity for desmin and CD34; grade 0 Rb staining). The average size of the lesions was 0.8 cm (range: 0.4-1.7 cm).

Conclusions: CVM is a rare and under-recognized entity (4% of cervical polyps) for which morphology remains the mainstay of diagnosis. Reliance on IHC serves as a potential diagnostic pitfall as 86.9% of cases showing myofibroblastic differentiation demonstrated the staining pattern of desmin and CD34 positivity and Rb deficiency.

1283 PD-L1, p16, and PTEN Expression Profiles in Uterine Smooth Muscle Tumors and Their Diagnostic and Therapeutic Implications

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Background: Smooth muscle tumors of the uterus continue to represent diagnostic and therapeutic dilemmas. Recently, it has been indicated that the status of tumor suppressor genes can influence patient response to immunotherapy. We examined expression of PD-L1 as well as tumor suppressor genes PTEN and p16 in uterine leiomyosarcomas and compared it with atypical leiomyomas, STUMPS, and usual leiomyomas.

Design: Immunohistochemical staining was performed with PD-L1, PTEN, and p16 antibodies on representative sections from 107 uterine smooth muscle tumors, including 54 leiomyosarcomas (LMS), 19 smooth muscle tumors of uncertain malignant potential (STUMP), 12 atypical leiomyomas (ALM), and 22 leiomyomas (LM). Results for PD-L1 were scored in tumor and in tumor infiltrating immune cells, with ≥ 5% staining considered positive. For PTEN and p16, H-scores were calculated and ranged from 0 (no immunoreactivity) to 300 (highest immunoreactivity). The Kruskal-Wallis Test was used to compare results between categories.

Results: PD-L1 was positive in 10 LMS, ranging from 5 to 100%, with an average of 27.7 %. PTEN was positive in 52 LMS, with H-scores ranging from 20 to 300, average of 218; p16 was positive in 53 LMS, ranging from 5 to 300, average of 252. All 10 LMS showing PD-L1 positivity were positive for PTEN and p16. Only 1 STUMP and no ALM or LM were positive for PD-L1. For PTEN and p16, average H scores in STUMP were 216 and 221, in ALM 279 and 143, and in LM 104 and 30. All markers showed statistically significant differences between LMS and LM. In distinction between LMS and ALM only p16 showed significant differences, and in distinction between LMS and STUMP, no statistically significant differences were found in any of the 3 markers.

Conclusions: Almost all LMS show strong immunopositivity for PTEN and p16 tumor suppressor genes and only 18.5 % were positive for PD-L1. Few tumors show strong PD-L1 expression, while no cases showed concurrent lack of expression of PTEN or p16. In view of recent reports, the 10 LMS positive for PD-L1 with concurrent strong expression of PTEN should respond well to immunotherapy, and therefore PD-L1 and PTEN panel may be used to identify good immunotherapy candidates. These findings may have important therapeutic implications and warrant further clinico-pathologic studies with long-term follow-up.

1284 Silva-type Patterns of Invasion and Correlates (Tumor Size, Lymph-vascular Invasion and Lymph Node Metastasis) in HPV Unassociated Endocervical Adenocarcinomas

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Background: International Endocervical Adenocarcinoma Criteria and Classification (IECC criteria) has been recently proposed to classify endocervical adenocarcinomas (ECAs) based on morphological features assessed on H&E slides that are linked to etiology (i.e. HPV infection). The Silva invasion pattern-based classification system has been proposed for ECAs of HPVA type only, separating cases with low risk (pattern A) from cases with higher risk (pattern B and C) of lymph-vascular invasion and lymph node metastasis. The main aim of this study was to compare the Silva system in ECAs unassociated with HPV (NHPVA) and the ones associated with HPV (HPVA).

Design: Complete slide sets from 338 surgical specimens were collected from 7 institutions worldwide. 289 (85.5%) of ECAs were HPVA (86.5% usual, 3.8% mucinous NOS, 3.8% intestinal, 0.4% signet-ring, 3.1% iSMILE, 1.0% villoglandular, 1.4% adenocarcinoma NOS-type), while 49 (14.5%) were NHPVA (69.4% gastric, 22.5% clear cell, 4.1% endometrioid, 2.0% serous, 2.0% mesonephric).

Results: The median size of HPVAs was 21 mm, compared to 38 mm in NHPVAs (p<0.001). 100% of the HPV-unassociated cases (NHPVAs) were of C pattern, while 78.4% of the HPVA cases were similarly categorized. 49.3% of HPVA cases were associated with lymph-vascular invasion (LVI) in contrast to 72.9% of NHPVAs (p: 0.002). Including both HPVAs and NHPVAs, LVI was detected in 0% of pattern A, 28.0% of pattern B and 63.1%% of pattern C cases (p: <0.001). 11.4% of HPVA cases and 32.7% of NHPVAs were associated with lymph node metastases (LNM) (p: <0.001). 0% of the pattern A and B cases were associated with LNM, in contrast to pattern C cases (17.0%).

Conclusions: Compared to HPVAs, NHPVA tumors are larger and more frequently associated with LVI and LN metastases. Also, it is feasible to assign the Silva pattern to all the HPVA and NHPVA cases, but clinical outcomes in the latter group must be driven by other factors such as histotype and stage.

1285 Clinical Outcomes of HPV-associated and Unassociated Endocervical Adenocarcinomas (ECAs) Support the IECC Classification

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Background: The IECC classifies ECAs based on morphological features linked to etiology (i.e. HPV infection), resulting in separation of ECAs into HPV-associated (HPVA) and unassociated (NHPVA) types. Our aim was to determine if clinical outcomes of the newly described categories supports the IECC classification.

Design: Full slide sets of 409 endocervical adenocarcinomas were collected from 7 different institutions worldwide. Data concerning age, tumor size, stage, follow-up were collected. Statistical analysis of relapse free survival (RFS) and disease specific survival (DSS) was conducted using the Cox proportional hazard approach stratifying on institution. The results are presented as hazard ratios (HRs).

Results: The most frequent HPVA was usual-type, while the most frequent NPHVA type was gastric type (100% HPV-negative). Less frequent than usual-type HPVAs were mucinous HPVAs of various

subtypes and NHPVAs (i.e. gastric-type, clear cell, etc). NHPVA tumors were larger and occurred in significantly older patients, compared to HPVA tumors (p<0.001).

HPVAs had a better RFS than NHPVAs (HR 0.36; 95%CI 0.20-0.64; p 0.001), but the effect was reduced when adjusted for stage and no longer statistically significant (HR 0.64; 95%CI 0.33-1.23; p 0.18). HPVAs also had a better DSS than NHPVAs (HR 0.36; 95%CI 0.18-0.73), but the effect was again much reduced when adjusted for stage and no longer statistically significant (HR 0.85; 95%CI 0.38-1.90; p 0.70). Mucinous HPVAs had a worse RFS and DDS than other HPVAs - HR 2.74 and 2.15 after adjustment for stage – but the numbers of events was too small to achieve statistical significance (p 0.058 and 0.31), although the result for RFS was almost significant.

Conclusions: The IECC segregates endocervical adenocarcinomas into different pathogenetic categories, all of which can be recognized on H&E slides. Clinical outcomes studies support these findings. HPVAs should be rigorously separated from NHPVAs. Current data also suggest that mucinous HPVAs continue to be separated from non-mucinous HPVAs.

1286 PI3K Pathway Effectors as Novel Markers of Endometrial Intraepithelial Neoplasia

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Background: The diagnosis of endometrial intraepithelial neoplasia (EIN) can be challenging owing to limited sampling, fluctuations in endometrial histology due to hormonal status/normal cycling, and other confounding histologic variables. New markers such as Pax2 can delineate EIN, but have not proven wholly reliable. Clearly, new markers of EIN are needed. *PTEN*, a potent inhibitor of PI3K signalling, is the most frequently mutated gene in endometrial cancer, and other common mutations (e.g. in *FGFR2*, *PIK3R1*, *PIK3CA*, *AKT1/2/3*) also promote PI3K pathway hyperactivation. PTEN has proven to be of limited utility as an EIN marker, likely because 1) some of these factors function biochemically "downstream" of PTEN; 2) mutations in "upstream" components do not alter PTEN expression; and 3) most PTEN inactivating mutations do not result in loss of protein. Thus, downstream PI3K signalling factors, particularly phosphorylated AKT (pAKT) and FOXO1, stand out as conceptually attractive—and potentially useful—EIN markers.

Design: In preliminary studies, benign (n=14) and neoplastic (n=20) endometrial samples were analyzed by immunohistochemistry against PTEN, PAX2, pAKT, and FOXO1. Staining patterns in endometrial epithelium were interpreted, tabulated, and scored with attention to "clonal distinctiveness" in neoplastic lesions; i.e. pattern alterations relative to background normal glands. Alterations of expression were compared in a pairwise fashion to determine how frequently two or more markers delineated the same neoplastic lesion.

Results: Interestingly, in normal endometria, FOXO1 showed a phase-dependent alteration in subcellular localization. In the proliferative phase, FOXO1 is cytoplasmic, but in the secretory phase is imported to the nucleus; interval endometrium has both nuclear and cytoplasmic FOXO1. These results implicate PI3K/FOXO1 signalling in normal physiology and demonstrate that FOXO1 can serve as a readout of PI3K status. FOXO1 or pAKT expression was altered in the majority of neoplastic lesions (14/17, 82%), with FOXO1 and pAKT being co-altered only in some cases (5/17, 29%).

Conclusions: This is the first study to demonstrate the potential of pAKT and FOXO1 as biomarkers in the histopathologic evaluation of EIN. However, unexplained variability in the expression of these markers still poses challenges in interpretation. We propose that a panel of biomarkers including FOXO1 and pAKT may prove superior to the use of a single marker in the evaluation of EIN.

1287 Detection of Tumor-Specific TP53 Signatures from Archival Endometrial Biopsies Obtained Prior to the Diagnosis of High-Grade Serous Carcinoma

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Disclosures:
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Background: Recent studies have detected high-grade serous cancer (HGSC) specific gene mutations in concurrent lower genital tract secretions. The purpose of this study was to determine the feasibility of detecting HGSC-associated *TP53* mutations in archival endometrial samples obtained prior to the HGSC diagnosis.

Design: The pathology database at our institution was queried for HGSC samples that had an endometrial biopsy (EMB) performed prior to or concurrent with the HGSC diagnosis. DNA was isolated from formalin-fixed, paraffin embedded (FFPE) tissue blocks of patient-matched EMB and tumor samples. A multiplexed PCR approach using barcoded primers (PlexSeq Diagnostics, Cleveland, OH) was employed to amplify all coding regions of *TP53*. Amplicon libraries were sequenced on a MiSeq instrument. If identical mutations that met criteria were found in both tumor and paired EMB, tumor samples were submitted for Oncopanel Next-Generation Sequencing (NGS) as orthogonal confirmation of mutation status. As a control, endometrial biopsies from 20 non-high risk patients were assessed in a similar manner.

Results: A total of 19 patients with both primary HGSC tumor and EMB samples (obtained from 0 to 19 months before diagnosis) were evaluated by multiplex PCR. Seven (7) cases contained matching mutations of convincing type and frequency to prompt orthogonal testing (i.e., NGS). Of the 7 cases, 3 (43%) were confirmed by NGS, which had endometrial sampling 0, 12, and 74 days prior to diagnosis. Of the 3 mutation-positive endometrial biopsies, 1 of the 3 contained visible tumor on original histologic evaluation.

Case	Nucleotide Mutation (NGS)	Amino Acid Alteration	Mutation Type	Tumor DNA Yield (ng/ul)	Average Tumor Reads/ Coverage (multiplex)	EMB DNA Yield (ng/ul)	Average EMB Reads/ Coverage (multiplex)	Time from EMB to Diagnosis (days)
1	c.713G>T	p.C238F	Missense	26.07	64%	0.53	58%	0
2	c.452C>A	p.P151H	Missense	6.20	10%	0.71	31%	12
3	c.538G>T	p.E180*	Nonsense	5.43	not found	2.94	not found	16
4	c.536A>C	p.H179P	Missense	46.41	90%	0.33	not found	36
5	c.267delC	p.S90Pfs*33	Frameshift	14.74	63%	0.25	28%	74
6	c.526T>G	p.C176G	Missense	2.30	90%	11.74	not found	338
7	c.747G>T	p.R249S	Missense	2.18	not found	4.15	not found	577

Conclusions: This study demonstrates that HGSC-associated *TP53* signatures can be detected in EMBs obtained prior to histologic diagnosis using a multiplexed PCR approach. This study proves the feasibility of testing existing archival endometrial tissue for research purposes. More importantly, this study highlights the potential for using lower genital tract tissues and fluids to diagnose asymptomatic or low-stage extrauterine HGSC.

1288 Molecular Characterization of Ovarian Carcinosarcoma Reveals Heterogeneous Oncogenic Mechanisms

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Background: Carcinosarcoma can occur anywhere along the gynecologic tract, most frequently in the uterus followed by the ovary. Like its uterine counterpart, ovarian carcinosarcoma is a biphasic tumor composed of a mix a high-grade carcinoma and sarcoma, and is generally regarded as a type of metaplastic carcinoma. There is currently little known about the oncogenesis of ovarian carcinosarcoma.

Design: We examined 30 ovarian carcinosarcomas by immunohistochemistry (IHC) and by targeted sequencing using a custom Illumina TruSeq amplicon panel that interrogated 26 gynecologic cancer genes.

Results: The average age of patients in this study cohort was 65 years. Nearly all patients (97%) presented with stage 2 or 3 disease. 26 of 30 tumors exhibited mutated pattern of p53 staining (16 diffuse strong nuclear staining and 10 "Null" pattern) with the patterns being concordant between the carcinoma and sarcoma components, while 4 tumors showed a wild-type pattern of p53 staining. Targeted sequencing identified *TP53* mutations in 24 tumors (61% missense, 39% non-sense/frame-shift). All 24 *TP53*-mutated tumors exhibited mutated p53 immunostaining, including 8 with "Null" p53 pattern harboring either frameshift or non-sense mutations. The results of TP53 IHC and sequencing analysis were concordant except for 2 tumors showing "Null" staining pattern and no *TP53* mutations. Among the 30 tumors, 4 showed wild-type TP53 by IHC and sequencing, and 3 of these 4 harbored mutations in *KRAS, CTNNB1, RPL22* and/or *ARID1A* (genes more frequently mutated in non-serous carcinomas). 5 tumors harbored exon 9 or 20 activating *PIK3CA* mutations. All tumors showed intact expression of mismatch repair protein with 86%, 67% and 66% showing PAX8, WT1 and ER nuclear expression respectively in the carcinoma components. All 4 tumors with wild-type TP53 by IHC and sequencing lacked WT1 nuclear expression. Among 20

patients with available clinical follow-up, 18 died from their disease (median survival of 15.5 months); there was no difference in survival based on *TP53* mutation status.

Conclusions: Our results show that while TP53 mutation appears to be an oncogenic driver in the majority of ovarian carcinosarcoma (akin to that seen in high-grade serous carcinoma), a minor subset (13%) develop without TP53 molecular alterations. The mutation profile and the consistent lack of WT1 immunoreactivity suggests that this subset likely arise through alternative oncogenic pathway(s).

1289 p16 Upregulation in Uterine Adenomatoid Tumor

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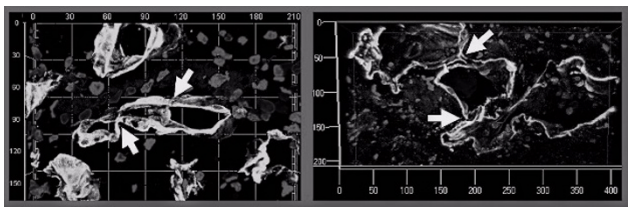
Background: Uterine adenomatoid tumor (AT) is a benign tumor presumed to be of mesothelial origin. The incidence of uterine AT is strongly associated with iatrogenic immunosuppression. However, the pathogenesis of AT, including the genes and proteins that regulate its growth, remains unknown. In this study, we first examined factors reportedly involved in immunosuppression-associated tumors, including p16 expression, integration of known oncogenic viruses, and abnormality in mismatch repair (MMR) proteins, in uterine ATs. p16 expression in other mesothelial lesions was also assessed. Because we believe that the peculiarity of AT lies in the invagination of mesothelial cells and infiltrating pattern of growth in the myometrium, we investigated the three-dimensional (3D) architecture of AT by confocal image analysis.

Design: We retrieved 14 cases of uterine ATs from the archives of the Department of Pathology, Akita University Hospital. Immunohistochemistry for p16, human herpesvirus type 8 (HHV-8), Merkel cell polyomavirus (MCPyV), SV40 large T antigen (SV40), and MMR proteins (MLH1, MSH2, MSH6, and PMS2), and EBER and HPV *in situ* hybridization (ISH), were performed. p16 expression was also evaluated in two cases of peritoneal malignant mesothelioma, one case of well-differentiated papillary mesothelioma and nine cases of mesothelioma of the uterine serosa. Lastly, we attempted electrophoretic tissue clearing of a thick section of a uterine AT, followed by immunofluorescence double staining for cytokeratin and histone H3, to capture the 3D structure of AT by confocal microscopy.

Results: p16 was upregulated in the uterine ATs (Table 1). Of note, a majority of ATs (10/14, 71%) revealed characteristic block-type (> 90%) positivity for p16, whereas p16 expression in most other mesothelial lesions was focal and patchy. No AT was positive for HHV 8, MCPyV, SV40, EBER-ISH, or HPV-ISH. The MMR proteins were retained in all of the ATs. The 3D immunofluorescence analysis demonstrated anastomoses between the luminal structures of ATs (Figure 1).

p16 positivity rate	Adenomatoid tumor (n=14)	Malignant mesothelioma (n=2)	Well-differentiated papillary mesothelioma (n=1)	Mesothelium of the uterine serosa (n=9)
90% >	10	0	0	0
71-90%	2	0	0	0
51-70%	2	0	0	0
31-50%	0	0	1	0
11-30%	0	1	0	4
1-10%	0	1	0	4
<1%	0	0	0	1



Conclusions: Uterine AT is a distinct mesothelial lesion characterized by p16 upregulation. Our data suggest that p16 plays an important role in the pathogenesis of uterine AT. This is the first 3D architectural study of AT to demonstrate a connection between the luminal structures, and the findings are compatible with the mesothelial invagination hypothesis.

1290 Variations in DNA Mismatch Repair Protein MSH6 Expression in Endometrial Carcinoma Independent of Germline or Somatic Gene Mutation

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Background: Endometrial cancers are examined by immunohistochemistry (IHC) for DNA mismatch repair (MMR) proteins to evaluate for Lynch Syndrome. Germline mutation of a MMR gene is associated with complete IHC loss of the corresponding MMR protein. It is known that hypoxia down-regulates expression of MMR proteins, especially MSH6, independent of gene mutation. In patients with glioblastoma multiforme treated with temozolomide, the post-treatment tumor can lose MSH6 IHC expression, and this is associated with tumor progression. We therefore examined MSH6 protein expression, compared to expression of its binding partner MSH2, in different endometrial cancer groups to determine if MSH6 protein expression can be variable.

Design: MSH6 and MSH2 IHC expression were assessed in 153 endometrial carcinomas (62 endometrioid grade 2 MMR intact, 56 endometrioid grade 2 MLH1 negative, 35 serous/mixed serous MMR intact). For each tumor, per cent positive MSH2 and MSH6 nuclear staining and nuclear staining intensity were quantitated using Aperio ImageScope image analysis software in a 1 mm^2 hotspot and in 4 random 1 mm^2 tumor areas, each with more than 90% tumor cells. The four random foci were identical in the MSH2 and MSH6 slildes. A total of 3,825 data points were analyzed.

Results: Compared to MSH6, there was minimal difference in mean MSH2 scores between hotspot and the random foci, suggesting that MSH6 expression is more variable, especially for the endometrioid groups. For all 3 tumor groups, % positive staining in the hotspot was over 90% for MSH2, but only 66-71% for MSH6. In the endometrioid MMR intact group, % tumor cells with positive MSH6 expression was significantly lower than MSH2 expression in both the hotspot and the random 4 foci. The percentage of tumor cell nuclei with 3+ intensity staining was significantly lower for MSH6, especially for the random 4 loci. The endometrioid group with MLH1 IHC loss showed similar findings, suggesting that MMR deficiency from another locus does not impact MSH6 expression.

	Endometrioid MMR Intact	Endometrioid MLH1 lost	Serous/mixed serous MMR Intact
Mean MSH2 % positive hotspot	91.6%	92.5%	93.2%
Mean MSH6 % positive hotspot	66.5%	68.7%	70.5%
Mean MSH2 % positive random	87.9%	91.6%	91.5%
Mean MSH6 % positive random	40.6%	47.5%	57.6%
Mean MSH2 %3+ random	74.1%	71.8%	76.1%
Mean MSH6 %3+ random	47.8%	48.6%	58.3%

Conclusions: MSH6 IHC expression is significantly lower than MSH2 in all tumor groups, even when overall the tumor is considered "MSH6 positive." Per cent MSH6 positive cells is higher in serous compared to endometrioid, suggesting that higher tumor grade influences protein expression. Variable MSH6 expression needs to be accounted for when assessing for Lynch Syndrome. Future studies will examine MSH6 IHC expression in relation to important clinical/pathological variables.

1291 PD-L1 Expression in Ovarian Endometrioid and Clear Cell Carcinoma Correlates with Mismatch Repair Protein Expression and Lynch Syndrome

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Background: Mismatch repair (MMR) protein deficiency is an indicator of programmed cell death receptor ligand 1 (PD-L1) protein expression in certain solid organ tumors, a subset of which exhibit a response to targeted immunotherapies. The current study evaluated the incidence of PD-L1 and MMR protein expression in endometrioid and clear cell carcinomas of the ovary.

Design: A retrospective review of ovarian carcinomas diagnosed at our institution over 18 years revealed 53 endometrioid, 29 clear cell, and 4 mixed endometrioid/clear cell carcinomas. Immunohistochemical staining for MMR and PD-L1/22C3 expression was performed on tissue microarrays. Cases with MLH1 loss of expression were assessed for promoter hypermethylation. PD-L1 expression ≥1% was considered positive. Clinicopathologic parameters, including genetic testing for Lynch syndrome, were collected.

Results: The average age at diagnosis was 57.6 years (range 38-84). PD-L1 was positive in 7/53 (13%) endometrioid carcinomas, 6/29 (21%) clear cell carcinomas, and none of the mixed carcinomas. MMR loss of expression was identified in 8/53 (15%) endometrioid carcinomas and none of the clear cell or mixed carcinomas. PD-L1 positivity was identified in 4/8 (50%) cases with MMR loss of expression compared to 9/78 (12%) cases with retained MMR (p =

0.0163). Further subclassification showed PD-L1 expression in 4/8 (50%) MMR deficient endometrioid carcinomas as compared to 3/45 (7%) MMR proficient endometrioid carcinomas (p = 0.0068). Of the 8 MMR deficient cases, 3 patients carried a deleterious mutation diagnostic of Lynch syndrome and 2 cases demonstrated mutations indicative of Lynch syndrome. PD-L1 positivity was identified in 4/5 (80%) Lynch syndrome cases compared to 9/81 (11%) non-Lynch cases (p = 0.0015).

Conclusions: Overall, PD-L1 expression was observed in 15% of ovarian endometrioid and clear cell carcinomas. PD-L1 expression appears to be significantly correlated with both MMR protein deficiency and Lynch syndrome. These findings suggest clinical utility of PD-L1 testing and potential efficacy of PD-L1 checkpoint inhibitors in ovarian endometrioid carcinomas with aberrant expression of MMR proteins as well as patients with Lynch syndrome.

1292 Molecular Characterization of Differentiated Vulvar Intraepithelial Neoplasia (dVIN) and HPV-independent Vulvar Squamous Cell Carcinoma (SCC)

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Disclosures:
Leah Prentice: *Employee*, Contextual Genomics
David Huntsman: *Employee*, Contextual Genomics

Background: The recognition and understanding of human papillomavirus (HPV)-independent vulvar SCC is of growing importance. The adoption of widespread HPV-vaccination in many countries will lead to a relative increase in HPV-independent vulvar neoplasms in the near future. In addition, HPV-independent vulvar SCC exhibit more aggressive clinical behavior compared to their HPV-driven counterpart. In the vulva, dVIN is the precursor lesion to HPV-independent SCC. To date however, dVIN remains a diagnostic challenge and very little is known about the molecular profile and progression of dVIN to HPV-independent vulvar SCC.

Design: We selected 11 cases of vulvar SCC with adjacent dVIN and lichen sclerosus. In 4 cases, dVIN and SCC could be separately dissected and both components were analyzed individually. Mutations were investigated using next generation sequencing technology, which targeted 123 hotspot mutations and 17 exons in 33 known cancer-related genes. Immunohistochemistry (IHC) for p53 and p16 (surrogate marker for high-risk HPV) were also performed.

Results: Results are summarized in Table 1. All cases were negative for p16.

Mutations in *TP53* were by far the most common, occurring in 4/4 (100%) and 10/11 (91%) of dVIN and vulvar SCC respectively. Approximately half of cases also harbored mutations in genes involved in either the PI3K pathway (2 cases, *PTEN/PIK3CA*), RAS family (3 cases, *NRAS/HRAS*) or ErbB family (1 case, *EGFR*). A *MET* mutation was identified in one case. In the 4 cases of matched dVIN/SCC, 2 cases (50%) demonstrated evidence of molecular progression, with the acquisition of additional mutations in the SCC component (1 acquired *TP53* mutation, 1 acquired *HRAS* mutation). One case was found to have multiple *TP53* mutations in the dVIN and SCC components.

p53 IHC patterns correlated with *TP53* mutation status in 9/11 (82%) cases. Case 5 had a 18bp in-frame deletion and strong p53 staining. Case 1 had 3 missense mutations and showed very weak (almost null) p53 staining.

Mutation Findings and p53 IHC Results in dVIN and Adjacent Vulvar SCC

Case No	Mutations in dVIN	Mutations in VSCC	p53 IHC in dVIN and VSCC
1	TP53 (E287D, E271Q), *HRAS*, PTEN, PIK3CA	TP53 (E287D, E271Q, R248Q), PTEN, PIK3CA	Very weak
2	TP53	TP53, *HRAS*	Strong
3	TP53, PIK3CA	TP53, PIK3CA	Strong
4	TP53 (nonsense)	TP53 (nonsense)	Absent/Null
5	n/a	TP53 (deletion)	Strong
6	n/a	TP53, NRAS	Strong
7	n/a	TP53 (frameshift), MET	Absent/Null
8	n/a	TP53	Strong
9	n/a	TP53 (frameshift)	Absent/Null
10	n/a	TP53	Strong
11	n/a	EGFR	Weak/Patchy

Conclusions: *TP53* mutation is an early event in HPV-independent vulvar SCC, occuring in all dVIN tested. p53 IHC correlated with *TP53* mutation status in the majority of cases, serving as a good marker for ancillary testing. Additional mutations in the PI3K, RAS and ErbB family were seen in half of cases. Multiple *TP53* mutations in one tumor raises the possibility of a "field effect" phenomenon. A larger cohort of dVIN and vulvar SCC is currently being tested to expand on our findings.

1293 SWI/SNF-Deficient Undifferentiated Endometrial Carcinomas Are Highly Aggressive and Resistant to Conventional Chemotherapy

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Background: Undifferentiated/dedifferentiated endometrial carcinoma (UEC/DDEC) is an aggressive type of endometrial cancer. We recently identified genomic inactivation of key components of switch/sucrose non-fermentable (SWI/SNF) complex proteins in 50% of UEC and 70% of DDEC, and showed that SWI/SNF-inactivated tumors are associated with worse outcome than SWI/SNF-proficient tumors. The primary aim of this study is to characterize the clinical behavior of SWI/SNF-deficient UEC/DDEC.

Design: We included 41 SWI/SNF-deficient UEC/DDEC showing BRG1 loss, INI1 loss or ARID1A/1B co-loss in the undifferentiated tumor by immunohistochemistry and wild-type *POLE* by sequencing. Clinical follow-up including treatment response was available in 37 patients.

Results: The average age of patients with SWI/SNF-deficient UEC/DDEC was 60 years. The diagnosis of UEC/DDEC was made in 26% of the cases on preoperative biopsy. Mismatch repair (MMR) protein deficiency was seen in 63% of tumors. The tumor was confined to the uterus in 39% of cases (14 stage 1 and 2 stage 2) while 61% had extrauterine disease spread (14 stage 3 and 11 stage 4) at presentation. In patients with clinical follow-up, 4 (29%) with stage 1/2 tumor died of disease, while all 23 (100%) with stage 3/4 tumor died of disease (typically within a year of diagnosis). Among patients with advanced stage disease and/or postoperative residual disease (21 patients), 14 received adjuvant or neoadjuvant chemotherapy (platin/taxol-based) and all experienced disease progression either during chemotherapy (11 patients) or within 6 months after its completion. Chemotherapy was not given because of rapid disease progression in 4 patients. Disease progression was usually characterized by increasing extent of lymphadenopathy with omentum, lung and liver being the next most frequent metastatic sites, while metastases to adrenal gland, bone and brain were seen in a subset of cases.

Conclusions: SWI/SNF-deficient UEC/DDEC is highly aggressive with frequent extrauterine disease spread that is inevitably associated with rapid disease progression and resistance to platin/taxol-based chemotherapy. These findings underscore the need for more effective systemic therapy in these tumors and novel approaches might include immune check point inhibitors given frequent MMR deficiency and epigenetic modulators given underlying SWI/SNF-deficiency.

1294 Assessment of ALK Gene Rearrangements by Immunohistochemistry and Next Generation Sequencing Fusion Analysis in Uterine Mesenchymal Tumors Diagnosed as Leiomyosarcomas

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Background: Inflammatory myofibroblastic tumor (IMT) of the uterus is rare and under-recognized because of its variable morphology that can overlap with uterine smooth muscle tumors. However, gene rearrangement of the anaplastic lymphoma kinase (ALK) gene, which can be detected by (1) FISH, (2) next generation sequencing (NGS) fusion analysis, and (3) ALK expression by IHC, appears to be both sensitive and specific for uterine IMT. Recent literature suggests that tumors with ALK gene rearrangements are targetable with tyrosine kinase inhibitors, which have been shown to be a reasonable treatment option for aggressive ALK-positive IMTs. Therefore, distinction between uterine leiomyosarcoma (LMS) and IMT carries important clinical and therapeutic implications.

Design: Our database was searched and 31 cases diagnosed as uterine high grade LMS with available material were identified. ALK IHC was

performed on all of these cases and a subset (n=17) also underwent molecular analysis. DNA was extracted from formalin-fixed paraffin embedded tissue. Capture-based NGS was performed using an assay that targets the coding regions of over 500 cancer genes as well as select introns, covering a total of 2.8 megabases.

Results: Two of 31 cases were positive for ALK by IHC and the rest were completely negative. The two cases that were positive by IHC also showed ALK gene fusion by NGS (ALK1-IGFBP5 and ALK1-FN1 fusions). One of these cases also showed a Tp53 mutation while the other showed NF2 and RAD50 mutations. Of the two cases, one patient was alive with widely metastatic disease at the time of this study and the second died of metastatic disease within two years of presentation. Fifteen additional cases that were ALK negative by IHC were also negative for ALK fusion by NGS. On histologic review, one of the ALK positive cases demonstrated myxoid features which could raise the possibility of an IMT, while the other showed features of a conventional high grade LMS.

Conclusions: In our study, 2 of 31 (6%) uterine mesenchymal tumors originally diagnosed as LMS were ALK positive by IHC with ALK fusion confirmed by NGS fusion analysis, raising the question of whether these tumors might be more appropriately classified as uterine IMTs. One of the two cases lacked any histologic features typically associated with IMT. This raises the question of whether it may be worthwhile to perform ALK IHC in all uterine sarcomas, particularly in the metastatic setting, from the point of view of providing options for targeted therapy.

1295 Does the size, number, and location of regional lymph node metastases from endometrial carcinomas have prognostic significance?

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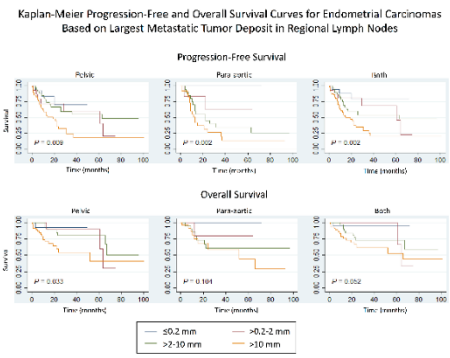
Background: The 7th edition of the AJCC TNM staging system for endometrial carcinoma categorized pelvic lymph node (LN) involvement as pN1, Stage IIIC1, and para-aortic LN involvement as pN2, Stage IIIC2. The 8th AJCC TNM staging manual has now incorporated the size of the LN metastasis into the "pN" category, further subcategorizing into isolated tumor cells (≤0.2 mm), micrometastasis (>0.2-2 mm), or macrometastasis (>2 mm). The purpose of this study is to determine whether the size, number, and location of LN involvement have prognostic implications in endometrial carcinoma.

Design: 168 patients with FIGO stage IIIC1 or IIIC2 endometrial carcinoma (including all histologies and grades) were identified from 2 participating institutions. 31 patients were excluded due to absence of follow-up information, resulting in 137 included patients. LN metastases were grouped according to size of the tumor deposit (≤0.2 mm, >0.2-2 mm, >2 mm-10 mm, or >10 mm), location of nodal involvement (pelvic vs para-aortic), and number of lymph nodes involved (1 vs >1). Multivariate analysis was performed to evaluate the prognostic significance of the number of lymph nodes involved, as well as their location and size of the largest tumor deposit.

Results: The median follow-up period was 23 months. 50 (37%) patients showed evidence of disease recurrence, and 30 (22%) patients died of disease. A statistically significant decrease in progression-free survival was shown with increasing size of the LN metastasis (Figure 1). While not statistically significant in this cohort, a similar trend was identified with regards to overall survival. Cox regression analysis for overall survival demonstrated that >1 LN involvement was statistically significant (HR=2.38, 95% CI: 1.02-5.56, p=0.045) when compared to only 1 LN involved.



Conclusions: Size of the metastatic LN tumor deposit is associated with prognosis in endometrial carcinoma; therefore measurement of the largest LN tumor deposit is of clinical relevance. Similarly, involvement of >1 lymph node by metastatic carcinoma portends a worse overall survival than single lymph node involvement.

1296 Whole Genome DNA Methylation Profiling Distinguishes High Grade Endometrial Carcinomas

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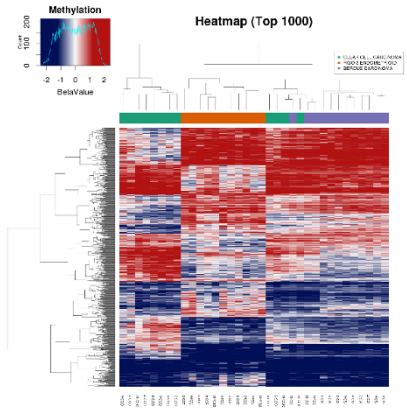
Background: FIGO grade 3 endometrioid (EC3), serous (SC), clear cell (CCC) and ambiguous (AC) or mixed high grade endometrial carcinomas are often diagnostically challenging due to extensive morphologic overlap. Immunohistochemistry and DNA sequencing are not always conclusive. Genome-wide DNA methylation profiling has proven robust in classifying primary brain tumors and bone sarcomas. We sought to establish methylation-based molecular classes to distinguish between different subtypes of high grade endometrial carcinoma.

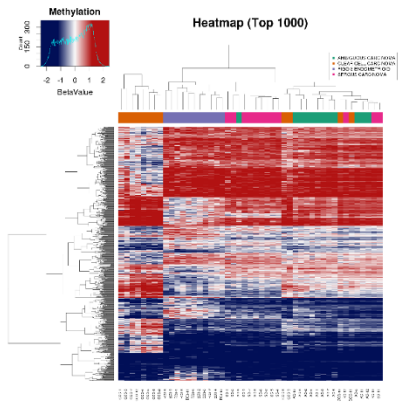
Design: DNA methylation status of 792,388 methylation sites in 11 EC3, 12 SC and 12 CCC of classic morphology were analyzed to establish a reference set. We also profiled 12 AC with highly heterogenous morphology (mixed EC, SC and/or CCC appearance). DNA was extracted from microdissected formalin-fixed paraffin-embedded tumor tissue and profiled using the Illumina MethylationEPIC array. Methylation data were analyzed with the R Bioconductor package minfi, including quality control, data normalization and differentially methylated CpG site analysis. Subsequent filtering was performed using a p-value cutoff of 0.01 and a minimal mean difference of the Beta-value of 0.1.

Results: Hierarchical clustering separated all tumors of classic morphology into 3 major groups (Fig. 1). While all EC3 (11/11, 100%) and a subset of CCC (8/12, 66.7%) formed distinct molecular groups, all SC and a smaller subset of CCC (4/12, 33.3%) formed a separate cluster. This cluster was further divided into 2 subgroups: a subset of SC and misclassified CCC; and SC only. However, AC clustered with the supergroup containing all SC and misclassified CCC; even those with EC histologic features had methylation profiles distinct from the EC3 group (Fig. 2).

Fig. 1. Methylation profiling of endometrial carcinomas of classic morphology.

Fig. 2. Methylation profiling of endometrial carcinoma





Conclusions: EC3 and the majority of CCC and SC form distinct molecular subgroups by methylation. A subset of histologically defined CCC clusters with SC. High grade carcinomas with ambiguous morphology share epigenetic signatures with SC and CCC and are

unlikely to represent EC3 epigenetically. Our study is the first to show that methylation profiling can be employed in distinguishing high grade endometrial carcinomas with ambiguous morphology and/or immunophenotype.

1297 Prognostic Value of Clinicopathologic Variables in Synchronous Endometrial and Ovarian Carcinomas: Metastases or Independent Primary Tumors?

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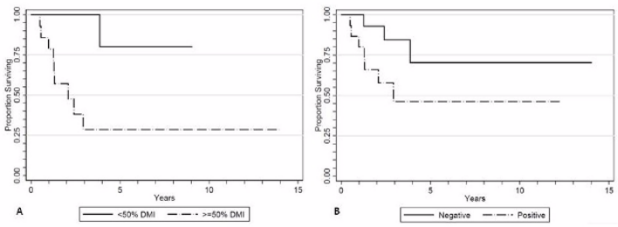
Background: Synchronous carcinomas occurring in the endometrium and ovary are uncommon and pose a differential diagnostic challenge. Distinguishing between independent primary tumors (IPTs) and endometrial cancers with ovarian metastases (at least stage IIIA endometrial cancers, IIIA-EC) using clinicopathologic criteria proposed by Scully *et al* is not always possible in practice. Although these tumors have been suggested to represent IPTs due to their good prognosis, sporadic synchronous endometrioid adenocarcinomas (EA) have recently been reported to be clonally related. We sought to investigate the prognostic value of clinicopathologic features in synchronous endometrial and ovarian carcinomas.

Design: Our cohort included patients who underwent primary surgery for endometrial cancer and had ovarian involvement at our institution in 1993-2014. Pathology reports were reviewed to determine the pathologists' diagnosis as IPTs or IIIA-EC. Patients with stage IV disease were excluded. Overall survival (OS) and recurrence free survival (RFS) analyses were performed using the log-rank test.

Results: Of 74 cases, 19 (25.7%) were considered IPTs per original report. Only 1 (5.3%) of the 19 IPTs had died compared to 16 (29.1%) of the 55 IIIA-ECs (hazard ratio (HR)=5.6, p=0.093). There were 41 (55.4%) cases of EA and 33 (44.6%) non-endometrioid adenocarcinomas (NEA) of the endometrium. OS and RFS analyses of endometrial EA did not identify any risk factors that separated favorable and unfavorable prognostic groups. In contrast, depth of myometrial invasion (DMI) and lymph node status (LNS) were significantly associated with prognosis in NEA: mortality in patients with >50% DMI was significantly higher compared to those with <50% DMI (hazard ratio (HR) =14.5, 95% CI 1.8-114.9, p=0.01), and remained significantly higher after adjusting for LNS (HR=12.6, 95% CI 1.6-101.7, p=0.02). The recurrence rate was also higher in NEA with >50% DMI compared to those with <50% DMI (HR=4.1, 95% CI 1.5-11.1, p=0.005).

Figure 1. Analysis of OS by depth of myometrial invasion (A) and lymph node status (B) in endometrial NEA



Conclusions: Our study suggests that OS and RFS rates are similar in synchronous endometrial and ovarian EA, irrespective of classification as IPTs or IIIA-EC. As clinical outcomes of synchronous NEA, however, are associated with DMI and LNS, they should be considered IIIA-EC, with these two variables further distinguishing cases with relatively favorable and unfavorable clinical outcomes.

1298 BRAF Mutations and Expression of Anti-BRAF-V600E (VE1) in Low-grade Serous Tumors of the Ovary

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Background: Ovarian low-grade serous tumors often show activating mutations in *KRAS* and *BRAF*. *BRAF*-mutated serous borderline tumors (SBTs) have been reported to show typical morphologic features, including cytoplasmic eosinophilia and cell budding. The most common *BRAF* mutation involves substitution of valine by glutamic acid at position 600 (V600E). Immunohistochemistry (IHC) with anti-BRAF-V600E monoclonal antibody VE1 has been shown to be specific for detection of V600E *BRAF* mutation in small studies. We sought to investigate expression of VE1 protein in ovarian low-grade serous tumors and its association with histologic features and *BRAF* mutation status.

Design: We identified patients diagnosed with SBTs and low-grade serous carcinomas (LGSCs) of the ovary between 2000-2012. We reviewed pathology reports and available slides for histologic features suggestive of *BRAF* mutation. VE1 IHC was performed on archival formalin-fixed, paraffin-embedded tissue sections. VE1 expression was scored based on the percentage of positive cells as follows: 0-10%, 25%, 50%, 75% and 90%. Tumors with ≥50% positive tumor cells were considered positive for VE1 expression. Statistical analyses were performed using SPSS 24.0.

Results: Of 121 low-grade serous tumors, there were 69 SBTs, 9 SBTs with micropapillary features (SBTmp); 3 SBTs with microinvasion (SBTi); 22 LGSCs; and 18 metastatic LGSCs (mLGSC). VE1 was positive in 54% (37/69) of SBT, 11% (1/9) of SBTmp and 9% (2/22) of LGSC, and in none of the SBTi and mLGSC (p<0.0001). The distribution of the percentage of positive cells was not significantly different between the groups (p>0.05). *BRAF* mutation status was available in 76 (63%) tumors, of which 42 (55%) were *BRAF*-mutated. *BRAF* mutations were seen in 75% (36/48) of SBT, 33% (1/3) of SBTmp, 67% of SBTi (2/3) and 14% (3/22) of LGSC and mLGSC (p<0.0001). Identified *BRAF* mutations were: V600E (n=26, 34%); G12D (n=8, 11%); and G12V (n=8, 11%). V600E *BRAF* mutations were present in 50% (24/48) of SBT, 33% (1/3) of SBTmp and 5% (1/22) of LGSC and mLGSC (p=0.004). Positive VE1 IHC was associated with the presence of histologic features of *BRAF* mutation (38/38, 100%, p<0.0001), and tumors with V600E *BRAF* mutation were VE1-positive in 25/26 (96%) cases (p<0.0001).

Conclusions: VE1 expression is strongly associated with V600E *BRAF* mutation status and histologic features. VE1 IHC is a robust method for the detection of V600E *BRAF* mutations which are significantly more common in SBT than in LGSC.

1299 Biomarker Analysis in Uterine Leiomyomas with MED12, HMGA2 and FH Mutations

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Background: Uterine leiomyomas (ULM) consist of a group of histologically and genetically heterogeneous neoplasms with many benign morphologic subtypes, yet little is known about the molecular features of leiomyomas. Previous studies have identified gene mutations including MED12, HMGA2, and fumarate hydratase (FH) that are present in ULM. Although each of these driver mutations affect different functional pathways that have the potential to influence tumor growth, their differences are largely unknown. In this study, we examined ULM with specific driver gene mutations to determine how they affect tumor development and focused on the expression of sex steroid hormones (ER, PR), cell cycle markers (p16 and p63), and AKT to determine if any of these features may allow for novel therapeutic strategies in treating specific molecular subtypes of ULM.

Design: We previously ran sequencing and mutational analysis on 178 ULM and randomly selected 25 MED12 cases, 15 HMGA2 cases, and 27 FH cases (by IHC) as well as 40 myometrial controls. We then reviewed ER, PR, p16 and Ki-67 IHC staining patterns for these cases as well as the pAKT expression for each of the three subtypes.

Results: In general, MED12 tumors were the smallest (6.7 cm) while HMGA2 tumors were the largest (11.2 cm). We found that MED12 and HMGA2 ULM showed similar ER and PR expression while FH tumors had significantly lower ER expression. In contrast, MED12 and FH ULM showed low p16 and low Ki-67 expression while HMGA2 tumors showed significantly higher expression of both, correlating with the clinical findings of larger and faster-growing tumors. (Table 1) HMGA2 also showed much stronger staining intensity for pAKT, correlating with the finding that AKT is a downstream marker in the HMGA2 molecular signaling pathway.

	MED12 ULM	HMGA2 ULM	FH ULM	Controls	p value
No. Cases	25	15	27	40	
Size (cm)	6.7 ±0.8	11.2 ±2.4	8.5 ±0.8	N/A	
ER %/Intensity (Median)	60/2	80/3	45/1	70/2	0.0007/0.0001
PR %/Intensity (Median)	70/2	80/2	90/3	75/3	0.0056/0.0044
p16 %	5	1	5	1	0.0060
Ki-67 %	2	10	1	1	0.0001
pAKT Intensity	1	3	1	2	0.0001

Conclusions: We found that the molecular subtypes of ULM show different cellular responses and growth behavior that correlate with their expression of sex steroid hormones, cell cycle markers, and AKT expression. In particular, HMGA2 ULM were more locally aggressive when compared with MED12 and FH ULM. These findings may be valuable for future clinical applications utilizing new therapeutic strategies since these specific driver gene mutations lead to completely different growth behavior.

1300 Thickened Endometrium in Postmenopausal Women with an Initial Biopsy of Limited, Benign, Surface Endometrium: Clinical Outcome and Subsequent Pathologic Diagnosis

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Background: Endometrial biopsy is indicated for post-menopausal bleeding (PMB) or when transvaginal ultrasound (TVUS) shows an abnormally thickened endometrium (> 4 mm). A substantial portion of these biopsies yield limited benign surface endometrium, which may indicate insufficient sampling. This study addresses the clinical significance of this pathologic diagnosis.

Design: Reports of endometrial biopsies from 2012-2015 in women aged ≥ 55 years with a history of thickened endometrium were reviewed. For the subset with an initial biopsy diagnosed as limited benign surface endometrial epithelium, parameters including body mass index (BMI), endometrial thickness, subsequent follow-up diagnoses and treatment were documented.

Results: Among a total of 369 endometrial biopsies, 194 (52%) were diagnosed as limited benign surface endometrial epithelium, 22 (6%) as hyperplasia without atypia, and 33 (9%) as hyperplasia with atypia, endometroid adenocarcinoma, or other malignancies. The clinical presentation of the 194 women with limited sampling included PMB (44%), pelvic pain (26%), enlarged uterus (10%), and other (20%). One hundred and ten (57%) had no subsequent follow-up endometrial sampling, primarily because the thickened endometrium was of low level of concern, the biopsy was interpreted as "benign", or bleeding ceased. Among the 84 women who underwent endometrial sampling, 6 (7%) had hyperplasia with atypia or malignancy, 21 (25%) had a repeat diagnosis of limited surface sample, and 49 (58%) had other benign findings. The average BMI was significantly higher among women with a subsequent diagnosis of hyperplasia with atypia or malignancy compared to the remaining women (37.5 vs 30.7 kg/m^2, p=0.0105). The average endometrial thickness of 8.5 mm among those without subsequent follow-up was significantly lower than those with subsequent follow-up (8.5 vs 11.93 mm, p=0.0066), and those with a subsequent diagnosis of hyperplasia with atypia or malignancy (13.15 mm, p=0.0041).

Conclusions: A slight majority of post-menopausal women with PMB or thickened endometrium on TVUS had an initial limited surface endometrial sample. Most had no subsequent biopsy follow-up. Even though women with subsequent sampling had a higher average endometrial thickness, the majority of them had benign findings, with only 7% having hyperplasia with atypia or malignancies. The study highlights the inconsistencies in adequacy criteria for endometrial sampling and the lack of standardization of subsequent management.

1301 Positive CK7 expression associated with progression of cervical squamous low-grade intraepithelial lesions

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Background: The CK7 positive cells at cervical squamocolumnar junction (SCJ) have been proposed as the progenitor cells, from which the majority of high-grade squamous intraepithelial lesions (HSIL) arise. Low-grade squamous intraepithelial lesions (LSIL) or CIN1 is a common disease. However, the low concordance rate of LSIL diagnosis among pathologists has been a consistent issue. It remains an unanswered question why about 30% LSIL lesion progress to high-grade lesions, while remaining cases regress within 2 years. Some studies have suggested that CK7 positive LSILs have a higher risk to develop into HSIL than CK7 negative LSILs. However, this remains controversial. This study is to further address whether the CK7 positive LSILs carry higher risk for developing HSIL.

Design: Immunohistochemical (IHC) staining using antibody against CK7 was performed on a total of 120 cervical biopsy specimens with confirmed diagnosis of LSIL. LSILs were categorized into 2 group: CK7 positive LSIL and CK7 negative LSIL. CK7 positive lesion is defined as diffuse cytoplasmic staining of > 5 contiguous cells (Figure1). Follow-up period ranged from 6 to 48 months, with an average of 32 months. Follow-up methods can be either Pap smear or cervical biopsy. Follow-up ended when HSILs were identified by either histology or cytology. Follow-up lesions were classified into 2 groups: non-HSILs (including LSIL or less conditions) and HSILs (CIN2 or higher) confirmed by p16 IHC staining. Ectocervical squamous epithelia and benign SCJ endocervical glands were used as negative and positive control for CK7 IHC staining. The results were analyzed using Chi-square test.

Results: All control sections worked properly with negative stains in ectocervical squamous cells and positive in either normal SCJ cells and/or endocervical glands. Among 120 LSILs, 36 cases (30%) were CK7+, while 84 cases (70%) were CK7-. Among CK7+ LSILs, 56% developed into HSILs during follow-up period. This was significantly higher than the CK7- lesions, of which only about 6% cases developed into CIN2+ disease (p < 0.0001, Chi-square test) in a 4-year follow-up period. The detailed results are summarized in Table 1.

Table 1. 48-month outcome of LSIL cases with CK7 status

	# Cases (%)	Follow-up with LSIL or less (%)	Follow-up with CIN2+ (%)	P value
CK7 positive	36 (30)	16 (44)	20 (56)	--
CK7 negative	84 (70)	76 (94)	5 (6)	--
Total	120	92	28	<0.0001

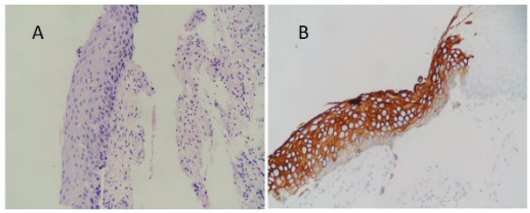

Figure 1.Example of CK7 staining of LSIL. A. H&E staining of LSIL; B.CK7 staining of LSIL.

Conclusions: CK7 positive LSILs carry significantly higher risk for developing HSIL than CK7 negative LSILs. CK7 can be a good prognostic biomarker for identifying high risk LSIL, hence guiding the clinical management of patient with LSILs.

1302 CCND2 Is a Useful Biomarker for Discriminating the Uterine Endometrial Stromal Tumors from the Endometrial Polyps with Hypercellular Stroma In the Curretage Specimens

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Background: The uterine endometrial stromal tumors may mimic endometrial polyps with hypercellular stroma in the curretage specimens in which the fragmental tissues present sparse endometrial glands and cellular stroma. Here, we found a new biomarker, CCND2, is expressed specifically in the endometrial stromal tumor parts, which helps with the differential diagnosis of endometrial stromal tumo rs in the curretage specimens.

Design: A total of 102 cases in the Obstetrics and Gynecology Hospital of Fudan University were recruited in our study from 2016-2017. 12 cases diagnosed as endometrial polyps with hypercellular stroma in the curretage specimens were included. All these cases underwent hysterectomy after the curretage. Besides, 30 cases of low-grade endometrial stromal sarcoma (LGESS), 30 cases of cellular leiomyoma diagnosed in the hysterectomy specimens and 30 cases of classic endometrial polyps diagnosed in the curretage specimens were collected. The histochemistry was performed on the parraffin-embedded tissues. CCND2 antibody (1:200, Sigma Alderich, USA) was used. A staining in the nucleus was considered as postive for CCND2.The staining scores were as follows: 3 × percentage of strongly staining cells + 2 × percentage of moderately staining cells + percentage of weakly staining cells, which ranged from 0-300. Scores 0-100 were considered as negative (0), 101 - 200 as mildly positive (low), and 200-300 as moderate to strongly positive (high). SPSS 20 was used for statistical analysis and a P value under 0.05 was considered significant.

Results: The CCND2 expression in LGESS (18/30, 60.00%) was significantly higher than that either in cellular leiomyoma (3/30, 10.00%, P<0.01) or in classic endometrial polyp (0/30, P<0.01). In the 12 cases diagnosed as endometrial polyps with hypercellular stroma, 1 case was endometrial stromal nodule in the hysterectomy specimen and 7 cases were diagnosed as LGESS. In 5 out 8 cases, CCND2 was postive in the tumor parts but negative in the adjacent endometrial stromal cells in the curretage and the hysterectomy specimens (5/8, 62.50%). The rest 4 cases maintained the original diagnosis and only 1 cases showed low CCND2 expression (1/4, 25.00%, P<0.05).

Conclusions: The specific expression of CCND2 in the uterine endometrial stromal tumors helps to differentiate the true tumors from the polyps with hypercellular stroma in the curretage specimens. Patients with such mimicry histological features may benefit from the use of CCND2 in the clinical practice.

1303 DICER1 Hotspot Mutations in Ovarian Gynandroblastoma

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Background: Gynandroblastoma is a rare sex-cord stromal tumor of the ovary with malignant potential. It is characterized by the appearance of both male (Sertoli cells, Leydig cells) and female (granulosa cells) differentiation. However, the molecular changes driving the oncogenesis of gynandroblastoma are unclear.

Design: We have collected 16 ovarian gynandroblastoma samples and confirmed the diagnosis. The mutational status of FOXL2 at C134, where C134W mutation is a definitive feature of almost all adult granulosa cell tumor (aGCT), was determined by Taqman discrimination qPCR. The mutational status of DICER1 RNase IIIb domain, mutation of which occurs in more than half of ovarian Sertoli-Leydig cell tumor (SLCT), was analyzed by Sanger sequencing and Mi-Seq methods. Protein expression of FOXL2 was assessed by immunohistochemistry.

Results: Sertoli cell tumor or SLCT were identified in all the sixteen cases of gynandroblastoma analyzed. Among them, six cases contained aGCT, nine had juvenile granulosa cell tumor (jGCT), and one contained components of both aGCT and jGCT. None of the sixteen cases harbored the C134W (402 C-->G) mutation of *FOXL2*, while three cases contained mutations at the hotspot regions of RNase IIIb domain of *DICER1*, all of which were mixed tumors of SLCT and jGCT. Interestingly, both jGCT and SLCT components displayed DICER1 hotspot mutations in all three cases. FOXL2 immunostaining was observed in both components of all sixteen tumors.

Conclusions: Our data indicate that *DICER1* hotspot mutation is the key-driving event in a subset of gynandroblastomas with jGCT and the molecular basis of gynandroblastoma containing aGCT is different from pure aGCT.

1304 Prognostic Significance of CDX2 and Beta-Catenin Expression in Ovarian Endometrioid Carcinoma

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Background: Ovarian endometrioid carcinoma (EC) generally has good prognosis with an over-all 5-year survival of 70% and more than 95% in low stage disease. Thus, the standard treatment for low stage disease includes optional chemotherapy after surgical resection of the tumor. Currently histological grade and stage are the only prognostic factors in deciding adjuvant chemotherapy. The most commonly mutated gene among EC is CTNNB1/Beta-catenin, while CDX2 has been implicated as a cooperative partner of the Wnt/Beta-catenin signaling in the pathogenesis of gynecological cancers. Here, we assessed whether CDX2 expression and beta-catenin nuclear localization have prognostic significance in ovarian EC.

Design: Retrospective study was conducted on tissue microarray composed of ovarian EC from 357 patients from two cohorts: discovery set from Alberta Ovarian Tumor Study (N=183) and validation set from British Columbia (N=174). Centralized immunohistochemistry for CDX2 and beta-catenin was performed. The primary endpoint was set to be ovarian cancer specific survival across all stages of the disease.

Results: CDX2 expression was present in 48.2% of all cases, with higher frequency in the discovery set (56.4%) compared to the validation set (38.4%). Nuclear beta-catenin staining was observed in 42.4% of all cases, with similarly higher frequency in the discovery set (46.4%) compared to the validation set (37.7%). Frequent co-expression of both markers was seen: 75% and 83% of CDX2-positive cases also showed nuclear beta-catenin in the discovery and validation sets respectively. In addition, CDX2 expression was significantly associated with better prognosis with a hazard ratio of 0.23 in the discovery set (95% CI: 0.11-0.45, p<0.0001) and 0.34 in the validation set (95% CI: 0.11-0.85, p=0.022). Nuclear beta-catenin localization was also significantly associated with better prognosis with a hazard ratio of 0.38 in the discovery set (95% CI: 0.18-0.73, p=0.0035) and 0.41 in the validation set (95% CI: 0.14-0.97, p=0.042). Within the low stage (I/II) disease, CDX2 expression and nuclear beta-catenin staining were also significantly associated with better prognosis in the discovery set. Similar trend was seen in the validation set, however statistical significance was not reached.

Conclusions: CDX2 and beta-catenin nuclear staining are significantly associated with a lower risk of ovarian cancer related death among all stages of ovarian EC, but their prognostic value in low stage ovarian EC remain elusive.

1305 POLE and Mismatch Repair Complex Abnormalities in Endometriosis of Women with Ovarian Endometrioid Carcinoma

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Background: Endometriosis (EMS) is a prevalent gynecologic disease and a precursor of certain types of ovarian cancer including endometrioid carcinoma (OEC). Even in patients without cancer, EMS has been found to harbor oncogenic abnormalities seen in EMS-associated ovarian cancers, such as mutations in *PTEN*, *ARID1A*, *KRAS*, and *PIK3CA*. Following evidence for a genomic-based classification of endometrial cancers, subsets of OECs have been shown to harbor polymerase ε (*POLE*) exonuclease domain or mismatch repair complex (MMR) alterations, which similarly carries prognostic value. Our aim was to determine whether EMS also harbors *POLE* or MMR deficiencies in the context of concurrent OEC.

Design: From a total of 72 confirmed OEC cases, 6 EMS-associated OECs harboring pathogenic *POLE* mutations and 5 with MMR deficiency were identified. EMS areas were micro-dissected and tested for MMR immunohistochemistry and *POLE* sequencing analysis. Findings in EMS were compared to *POLE* / MMR status in the corresponding OEC.

Results: In none of the 6 OECs with pathogenic *POLE* mutations was a matching mutation in EMS identified. In three of these patients, EMS harbored a different *POLE* mutation. Among the 5 patients with MMR-deficient OECs, all had intact MMR expression in EMS (Table 1).

POLE mutated OEC	CASE NO.	POLE mutation OEC	POLE mutation EMS
	1	Exon 13a p.P436PS	
	2	Exon 14a p.A456AP	EXON 13 c.1359+5G>A
	3	Exon 9 p.P286L	EXON 13 c.1355C>A, c.1337G>A
	4	Exon 13a p.W410WX	EXON 13 c.1304C>A
	5	Exon 13a p.V411VL	
	6	Exon 9 p.V270VM	
MMR deficient OEC	CASE NO.	MMR status OEC	MMR status EMS
	1	MSH6 loss	Retained
	2	MLH1/PMS2 loss	Retained
	3	MLH1/PMS2 loss	Retained
	4	MSH2/MSH6 loss	Retained
	5	MLH1/PMS2 loss	Retained

Conclusions: Matching MMR or *POLE* alterations were not identified in EMS of patients with *POLE*-mutant or MMR-deficient OEC. Nonetheless, certain *POLE* mutations were identified in EMS, which merits further investigation. *POLE* mutations and (theoretically) MMR deficiency can occur in EMS; however, based on our findings, if it does it is likely restricted to cell populations that evolve to OEC, and not as a "field" defect in EMS per se. The use of these markers in predicting the risk of developing OEC in patients with EMS is uncertain.

1306 PTEN Immunohistochemistry and PTEN Mutational Analysis in Endometrial Carcinoma Biopsies

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Background: *PTEN* plays a central role in the pathogenesis of endometrial carcinoma (EC) preferentially in the endometrioid histotype, and thus it may be of diagnostic value in differentiating serous EC from endometrioid EC particularly the copy-number high group. Previous studies showed high interobserver reproducibility for the interpretation of PTEN immunohistochemistry (IHC), and IHC has been shown to detect more PTEN abnormalities compared to exon-based sequencing.

Design: The purpose of this study was to assess the concordance between IHC and next generation sequencing (NGS) in identifying *PTEN* mutations among serous EC (63 cases), non-serous high-grade

EC (58 cases) and low-grade endometrioid EC (62 case). IHC was performed centrally on full biopsy/curetting samples from 5 centres (24-49 cases/centre) and tagged-amplicon NGS *PTEN* sequencing was performed on a total of 183 selected cases. The molecular subtypes of endometrioid EC was assessed by mutational analysis of DNA polymerase epsilon (POLE) exonuclease domain, IHC staining of mismatch repair (MMR) proteins and p53.

Results: NGS detected *PTEN* mutations in 158 cases with a total of 85 unique mutations (64 loss of function and 21 nonsynonymous mutations). PTEN IHC staining was retained in 93 cases (50.8%), heterogeneous/subclonal loss of expression in 11 cases (6.0%), reduced in 13 cases (7.1%), and absent in 66 cases (36.1%). IHC detected loss of PTEN protein expression 71.8% (46/64) of the time when loss of function *PTEN* mutation was confirmed by NGS. In cases with heterogeneous or reduced PTEN expression, mutations were infrequently detected (27% and 38% respectively). Furthermore, *PTEN* genetic alterations were significantly enriched in MMR deficient and p53 wild-type endometrioid EC, while POLE molecular subtype had the highest frequencies of nonsynonymous *PTEN* mutations and more frequently showed heterogeneous (subclonal loss of expression) PTEN IHC staining.

PTEN IHC	**PTEN** mutation			TOTAL
	Loss of function mutation	Nonsynonymous mutation	No mutation detected	
Absent	46	5	15	66
Reduced	4	1	8	13
Heterogeneous	2	1	8	11
Retained	12	14	67	93
TOTAL	64	21	98	183

Conclusions: Our preliminary analysis shows moderate concordance between PTEN IHC and NGS. However, neither assay alone is sufficient for the comprehensive assessment of the molecular PTEN status.

1307 Next Generation Sequencing of Uterine PEComas as Compared to Uterine Leiomyosarcomas

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Background: A subset of uterine mesenchymal tumors (UMTs) with epithelioid morphology show overlapping morphologic and immunophenotypic characteristics of PEComa and leiomyosarcoma (LMS). This distinction has important clinical and therapeutic implications. Many PEComas show TSC1 or TSC2 gene alterations, making them potentially targetable with mTOR inhibitors. This study employed next generation sequencing (NGS) to compare uterine PEComa to uterine LMS and correlate with morphology and immunophenotype.

Design: NGS was performed on 16 uterine LMS (8 with epithelioid features) and 10 uterine PEComas. (Three additional PEComas yielded insufficient DNA.) The capture-based NGS assay targeted the coding regions of over 500 cancer genes as well as select introns, covering a total of 2.8 megabases (MB). H&E slides were reviewed and, if not done previously, IHC for desmin, caldesmon, SMA, SMM, HMB45, Melan-A, MITF, and TFE3 was retrospectively performed. At least focal staining was considered positive.

Results: In PEComas, NGS detected mutations in TSC1 (2/10), TSC2 (5/10), TP53 (1/10), and LZTR1 (1/10) genes. LMS were characterized by multiple mutations including ATRX (9/16), TP53 (7/16) and MED12 (3/16). One case of PEComa, and one case originally diagnosed as epithelioid LMS, each had mutations in both TSC2 and TP53 genes. IHC for smooth muscle markers showed no significant differences except SMM which was more prevalent in LMS ($p<0.05$). HMB45 showed at least focal expression in both PEComas and LMS, but was more common in PEComas ($p<0.05$). While half of PEComas ($p<0.05$) expressed Melan A, all LMS were negative. Regarding morphology, TSC-mutated PEComas showed predominantly epithelioid features (n=5) while 2 were spindled and all had abundant clear to eosinophilic granular cytoplasm. Epithelioid LMS showed overlapping morphology with PEComa, although in general they had less abundant, non-granular cytoplasm.

Conclusions: TSC mutations were found in 70% of uterine PEComas, while LMS most frequently harbored mutations in ATRX, TP53 and MED12. One case each of PEComa and epithelioid LMS showed both TSC2 and TP53 mutations, supporting reclassification of the epithelioid LMS as PEComa. This study confirms that PEComas and epithelioid LMS can show overlapping morphology and immunoprofiles. The findings support testing all epithelioid UMTs for TSC mutation to maximize eligibility for mTOR inhibitor therapy.

1308 Cytotoxic T Lymphocyte Associated Molecule-4 is Expressed in a Significant Number of Endometrial Carcinomas

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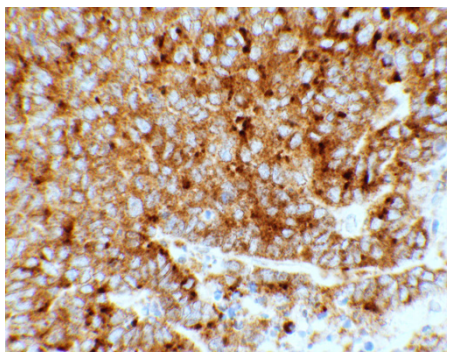
Background: Cytotoxic T lymphocyte-associated molecule-4 (CTLA-4) is an immune checkpoint molecule and a member of the immunoglobulin superfamily which encodes a protein that transmits an inhibitory signal to T-lymphocytes. Blockage of CTLA-4 enhances T-cell activation and therefore antibody-based immunotherapies may enhance anti-tumor effects of the immune system. Relatively new therapies such as ipilimumab activate the immune system by targeting CTLA-4 and enhancing its function. Ipilimumab has been FDA approved for melanoma and is undergoing clinical trials for other cancers such as non-small cell lung carcinoma, small cell lung cancer, bladder cancer, etc. However, little is known about the localization and expression of CTLA-4 in the endometrium. In this study, we investigated the expression of CTLA-4 in endometrial tissue and carcinomas and the possibility of extending the role of CTLA-4 to endometrial malignancies.

Design: CTLA-4 expression was evaluated by immunohistochemistry performed on a tissue microarray of 98 normal, hyperplastic, and neoplastic endometrial tissues. Hematoxylin & eosin and CTLA-4 immunohistochemical stainings (clone F8, mouse monoclonal antibody) were performed. CTLA-4 expression was scored as positive when strongly present in the cytoplasm in a granular pattern (Figure 1).

Results: Table 1 summarizes the frequency of CTLA-4 positivity in 98 endometrial tissues. Overall, CTLA-4 was positive in 36% (33/91) of endometrial malignancies. Sub-categorically, 37% (31/82) of endometrioid adenocarcomas, 33% (1/3) of clear cell carcinomas, and 17% (1/6) of serous carcinomas were positive for CTLA-4. Of the endometrioid adenocarcinomas, 28% (2/7) of tumors with squamous differentiation showed CTLA-4 expression. CTLA-4 was negative in five normal endometrial samples and two samples of endometrial hyperplasia.

Table 1	PD-L1 Expression in Endometrium and its Neoplasms		
n = 98	n	Expression (n)	Expression (%)
Malignant Tumors	91	33	36%
Endometrioid	82	31	37%
Endometrioid - Sq	75	29	38%
Endometrioid + Sq	7	2	28%
Serous	6	1	17%
Clear Cell	3	1	33%
Hyperplasia	2	0	0%
Normal	5	0	0%

+ Sq, squamous differentiation; **-**, without; **+**, with



Conclusions: This study shows significant expression of CTLA-4 in 36% of the endometrial malignancies, suggesting a role for future investigation of anti-CTLA-4 immunotherapies in the treatment of CTLA-4 positive endometrial tumors.

1309 Microsatellite Instability High Endometrial Cancer: Combined Loss of MLH1/PMS2 Expression Coincides with High Frequency of Minimal Microsatellite Shifting

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Background: Microsatellite instability (MSI) testing and analysis of mismatch-repair (MMR) protein expression of colorectal and endometrial cancers play a critical role in screening for Lynch Syndrome. Compared with colorectal cancer, strategies for cost-effective screening of endometrial cancer patients are still evolving. MSI-high endometrial cancers have been found to have significant differences, e.g. rare BRAF mutation in the presence of MLH-1 promoter hypermethylation. The aim of this study was to compare MMR protein expression in correlation with microsatellite shifting patterns by MSI PCR between MSI-high endometrial and colorectal cancers.

Design: The study cohorts included 50 MSI-high endometrial and 19 MSI-high colorectal cancers. Immunohistochemistry for four MMR proteins and MSI PCR testing at 5 NCI recommended loci were performed. Loss of MMR protein expression and microsatellite shifting patterns were compared between the two study cohorts. Minimal microsatellite shifting was defined by one to three nucleotide repeat shifting at the involved locus and major shifting was defined by more than 3 repeat shifting.

Results: Table 1 summarizes the MSI PCR and MMR IHC results. The endometrial cancer cohort included 44 endometrioid, 2 serous, and 4 clear cell carcinomas. Overall, 52% (26/50) of MSI-high endometrial cancers showed minimal microsatellite shifting involving 3 loci in 5 cases (6%) and 2 loci in 21 cases (46%). Among MSI-high endometrial cancers with minimal microsatellite shifting, 65% (17/26) showed combined MLH1 and PMS2 loss, 8% (2/26) had MSH2 and MSH6 loss, 12.5% (3/26) and 14.6% (4/26) showed loss of MSH6 or PMS2, respectively. In contrast, in MSI-high colorectal cancer, only 15.8% (3/19) had minimal microsatellite shifting with corresponding loss of MLH1/PMS2, or MSH2/MSH6 or MSH6. In addition, 15% (7/50) of endometrial cancers showed loss of MSH6, in contrast to 6.7% (1/15) of colorectal cancers.

Table 1: Summary of MSI PCR Testing and MMR Immunohistochemistry

			Endometrial Cancers (N=50)	Colorectal Cancers (N=19)
MSI	No. Loci Involved	2 to 3 Unstable Loci	33 (66.0%)	15 (78.9%)
		4 to 5 Unstable Loci	17 (34.0%)	4 (21.1%)
	Shifting Pattern	Minimal Shift	26 (52.0%)	3 (15.8%)
		Major Shift	24 (48.0%)	16 (84.2%)
MMR	MLH1/PMS2 Loss	Overall	30 (62.5%)	8 (53.3%)
		With minimal shift	17 (65%)**	1 (33%)**
	MSH2/MSH6 Loss	Overall	5 (10.4%)	4 (26.7%)
		With minimal shift	2 (8%)**	1 (33%)**
	PMS2 Loss	Overall	6 (12.5%)	2 (13.3%)
		With minimal shift	4 (15%)**	0**
	MSH6 loss	Overall	7 (14.6%)	1 (6.7%)
		With minimal shift	3 (12%)**	1 (33%)**
	Not available		2	4

**: Percentages are calculated based on the total number of cases with minimal microsatellite shifting, i.e. 26 endometrial and 3 colorectal carcinomas.

Conclusions: Compared with colorectal cancers, MSI-high endometrial carcinomas demonstrate high frequency of minimal microsatellite shifting that coincides with high percentage of combined loss of MLH1/PMS2. MSI-high endometrial cancers also have more frequent loss of MSH6, than colorectal carcinomas. These findings further suggest, that there are fundamental pathogenetic differences between MSI-high endometrial and colorectal cancers. Diagnostically, recognition of minimal microsatellite shifting is important for accurate interpretation of MSI PCR data for endometrial cancer.

1310 Recurrent Genetic Alterations in Small Cell Neuroendocrine Carcinoma of the Uterine Cervix

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Background: Small cell neuroendocrine carcinoma (SCNEC) of the uterine cervix is a rare but extremely aggressive tumor. While high-risk HPV is involved at an early stage of oncogenesis, additional driving events have been postulated to facilitate the progression of SCNECs. Using next generation sequencing, we performed mutational analysis of SCNECs of the cervix to identify oncogenic drivers amenable to targeted therapy.

Design: Clinicopathologic features of 10 cases are reported. Immunohistochemical analysis of p16, synaptophysin, chromogranin, and p53 expression and in situ hybridizations for high-risk HPV and/or HPV 18 were performed. Genomic DNA was extracted from paraffin-embedded tumor tissues and next generation sequencing based on a 640-gene panel was performed.

Results: The patients ranged in age from 28 to 68 years (mean, 45.6; median, 40.5). All tumors had diffuse p16 and synaptophysin expression. All but one tumor was positive for chromogranin (extent of staining ranged from focal to diffuse). p53 expression was aberrant in 3 tumors (2 diffuse, 1 null) and wild-type in 7. HPV 18 was detected in 5 of 10 tested tumors. The 5 tumors in which HPV 18 was not detected also lacked detectable HPV with a high-risk probe. . At least one driver mutation was detected in 8 of 10 cases. Three cases harbored *TP53* somatic mutations which correlated with an aberrant p53 staining pattern. Four *PIK3CA* mutations, p.G106A, p.N345T, p.E545K and p.E545D, were detected in 3 cases, of which 2 cases also harbored *TP53* mutation. Oncogenic driver mutations involving *KRAS*, *Erbb2*, and *TGFBR2* were also detected in individual cases.

Conclusions: Targeted next-generation gene sequencing identified genetic alterations involving the MAPK, PI3K/AKT/mTOR, and TP53 pathways in SCNECs. The presence of genetic alterations that are amenable to targeted therapy in SCNECs offers the potential for individualized management strategies for treatment of this aggressive tumor.

*D.X. and G.Z contribute equally

1311 Immunohistochemical Expression of Anaplastic Lymphoma Kinase (ALK) Protein in Gynecologic Clear Cell Carcinomas

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Background: Clear cell carcinomas of the ovary, endometrium, and cervix are uncommon tumors with an adverse prognosis. Currently, there are limited therapeutic options for non-surgical candidates who present with high stage diseases and currently no targeted therapy is available for these tumors. Anaplastic lymphoma kinase (ALK) inhibitors have been shown to be effective for pulmonary adenocarcinomas with *ALK* translocation. However, there has been no study on ALK protein expression and *ALK* status in gynecologic clear cell carcinomas. In this study we evaluated immunohistochemistry expression of ALK in a large series of gynecologic clear cell carcinomas. This might provide insights in potential therapeutic options like ALK inhibitors for these tumors.

Design: Ninety-six gynecologic clear cell carcinomas were included for this study (ovary 63, endometrium 26, cervix 7). Tissue microarrays of 3.0 mm cores were constructed in triplet for each case. Immunohistochemistry staining with antibody to ALK (Ventana, Clone D5F3, prediluted) was performed on a Ventana Benchmark automated immunostainer (Ventana Medical Systems, Inc., Tucson, AZ) according to standard protocols with appropriate positive and negative controls. Immunostain for ALK shows a cytoplasmic and membranous granular pattern and was visually scored in consensus by 3 pathologists as 0 (negative), 1+ (weak cytoplasmic staining or intense membrane staining), 2+ (moderate cytoplasmic staining), and 3+ (intense cytoplasmic staining). In cases with a heterogenous staining pattern, the highest score is assiged for that case.

Results: Positive immunohistochemical staining for ALK was seen in 6/7 cervical (86%, 1+ in 4, 2+ in 2), 25/26 endometrial (96%, 1+ in 20, 2+ in 4, 3+ in 1), and 61/63 ovarian (97%, 1+ in 40, 2+ in 20, 3+ in 1) clear cell carcinomas, respectively. There is no association between ALK expression and tumor site ($p > 0.05$).

Conclusions: Gynecological clear cell carcinomas often demonstrate immunohistochemical overexpression of ALK but most of these tumors show weak staining (1+). However, nearly one thirds (28/96 or 29%) of these tumors show moderate to intensity staining (2-3+). In

the lung moderate to strong ALK expression by immunohistochemical staining is correlated with *ALK* translocation. FISH study to assess *ALK* status in these gynecologic clear cell carcinomas is in process.

1312 Immunohistochemical Expression of PTEN and PAX2 and Genomic Status of Endometrial Glands in the Endometrioid Intraepithelial Neoplasia and Endometrioid Adenocarcinoma After Progestin treatment

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Background: Progestin therapy can help to preserve fertility in young patients with endometrial intraepithelial neoplasia (EIN) or well-differentiated endometrioid adenocarcinoma (WDEC). Intercurrent endometrial biopsy is commonly used to monitor treatment response and exclude tumor progression, however, it is often difficult to determine the clinical significance of isolated glands that are architecturally simple, but lined by partially atrophic (columnar) epithelium or small nests of complex glands lined by partially atrophic epithelium, both of which do not fulfill the diagnostic criteria of EIN or WDEC.

Design: To determine the histological threshold of residual neoplasm in the follow-up curettage specimens, immunohistochemical expression/loss of PTEN and PAX2 expression was compared between the partially atrophic glands (132 foci) and atrophic glands (132 foci) in 84 follow-up curettage samples from 43 patients. In addition, glandular epithelia of the partially atrophic and atrophic glands in follow up samples as well as initial neoplastic glands were microdissected in four patients using laser captured microdissection on paraffin embedded tissue, and genomic alterations by targeted next-generation sequencing technique were compared in each patient.

Results: In partially atrophic glands, complete loss of PTEN or PAX2 expression was identified in 61% and 56%, respectively, while the loss was identified only in 3.8% and 0.8% of atrophic glands, respectively (p<.0001). In three of four patients, histologically atrophic as well as partially atrophic glands shared the same mutations of *CTNNB1*, *PTEN*, *KRAS*, *TSC2* and *ATRX* genes, with those of initial neoplastic glands, and those three patients had a persistent disease during the follow-up. In contrast, one patient in which partially atrophic and atrophic glands did not show any mutation showed a complete remission during follow up period for 34 months,

Conclusions: Partially atrophic glands may retain abnormalities at the genomic and proteomic levels. Thus, partially atrophic glands during progestin therapy are better considered as a residual disease, and histological threshold for complete remission on the follow up curettage samples should be further defined in a larger cohort.

1313 Molecular Features of Uterine Adenosarcoma and Rhabdomyosarcoma: From Dicer1 Mutational Status to Phenotype

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Background: Embryonal rhabdomyosarcoma (RMS) and adenosarcoma of the uterine cervix/corpus are two cancers with high degree of morphological resemblance, and the pathological distinction between the two entities is fraught with challenges. Embryonal RMS has been implicated in the DICER1 syndrome, a syndrome with increased risks for an eclectic set of tumours, including pleuro-pulmonary blastoma and a subset of ovarian sex cord stromal tumours and loss-of-function mutations in the *Dicer1* gene. The *Dicer1* gene encodes for a RNase III family, with a crucial role in micro-RNA (miRNA) processing. Adding to the diagnostic challenge, *Dicer1* mutations have been also found in recent studies of adenosarcomas.

Design: Cases of adenosarcoma and rhabdomyosarcoma were collected and reviewed by experts in gynaecological pathology. Both tumour and normal sections were used to sequence the complete *Dicer1* gene for somatic and germline mutations, respectively, by Fluidigm sequencing, followed by Sanger sequencing validation.

Results: We have thus far accrued a total of twenty-three cases, comprised of fourteen cases of tumours with the referring diagnosis of adenosarcoma and 9 cases diagnosed as RMS. Loss-of-function Dicer1 mutations were found in 8/9 of RMS cases. 5 of the Dicer1-mutated cases were tested for germline mutations, and 4 of the 5 cases harboured germline DIcer1 mutations. In the fourteen adenosarcomas, loss-of-function *Dicer1* mutations were found in two cases (2/14, 14%). Analyses for germline mutations in cases of adenosarcoma are currently underway.

Conclusions: We confirm that loss-of-function Dicer1 mutations are not exclusive to RMS and confirm that a subset of uterine adenosarcomas to harbour *Dicer1* mutations. However, not all cases of RMS harbour *Dicer1* mutations, raising the possibility that both adenosarcoma and RMS may be heterogeneous groups of tumours. Efforts are currently underway to better understand the differences between the Dicer1-mutated vs. –wildtype tumours with regards to their histo-morphological and miRNA expression profile features.

1314 Indoleamine 2,3-Dioxygenase in Endometrial Cancer: A Targetable Mechanism of Immune Resistance in Mismatch Repair-Deficient and Intact Endometrial Carcinomas

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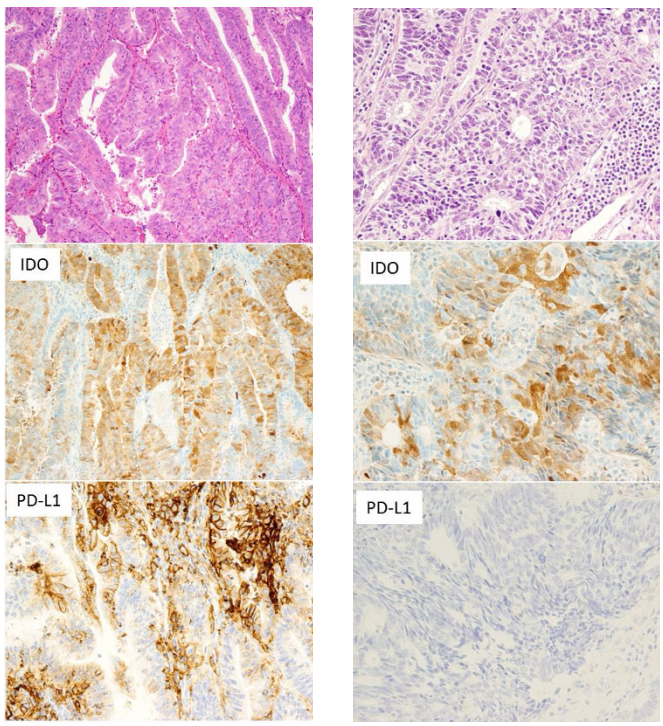
Background: Mismatch repair (MMR)-deficient endometrial carcinomas (EC) are optimal candidates for immunotherapy given their high neoantigen loads, robust cytotoxic T cell infiltrates, and frequent PD-L1 expression. However, the PD-1/PD-L1 pathway is just one of many mechanisms that tumors can utilize to evade host immunity. Another immune modulatory molecule that has been demonstrated in ECs is the enzyme indoleamine 2,3-dioxygenase (IDO). IDO is of interest because it may dampen the effectiveness of anti-PD-1/PD-L1 therapies and anti-IDO therapies are clinically available. We herein evaluate IDO expression in 60 ECs in relation to PD-L1 and MMR status.

Design: IDO immunohistochemistry (IHC) was performed on whole sections from 60 ECs [20 Lynch Syndrome (LS)-associated, 20 MLH1-hypermethylated, and 20 MMR-intact] that had previously been assessed with PD-L1 IHC. Tumor staining extent was categorized as negative, 1-5%, 6-10%, 11-25%, 26-50%, or >50%.

Results: The majority (85%) of ECs showed IDO tumor staining. 25% were positive in >25% of tumor cells and only 7% exceeded 50% staining. While there were no significant differences in IDO expression by MMR status at the 1% cutoff, at the 25% threshold MMR-deficient cancers were more likely than MMR-intact cancers to be IDO-positive [35% vs. 5%, p=0.024]. Differences were amplified when LS-associated cases were evaluated in isolation [50% LS-associated vs. 10% MMR-intact/MLH1-hypermethylated, p=0.001]. Of the 4 cases showing >50% staining, 3 were LS-associated and 1 was MLH1-hypermethylated. IDO expression was more common than PD-L1 expression (85% vs. 40%). 43% of IDO-positive tumors were also positive for PD-L1, whereas 2 cases showed PD-L1 expression in the absence of IDO. (Figure 1: LS-associated tumor with IDO and PD-L1 co-expression; Figure 2: MMR-intact tumor with positive IDO and negative PD-L1).



Conclusions: Overall, IDO expression is prevalent in EC and diffuse staining is significantly more common in MMR-deficient cancers, particularly LS-associated EC. This suggests MMR status alone may not be fully predictive of immunotherapeutic response and specific

molecular alterations may be of interest. Given that the majority of PD-L1 positive cancers are also positive for IDO, synergistic combination therapy with anti-IDO and anti-PD-1/PD-L1 may be relevant in these tumors. Furthermore, anti-IDO therapy may be an option for a subset of MMR-intact cancers which are less likely to benefit from drugs targeting the PD-1/PD-L1 axis.

1315 Molecular Features of Serous Borderline Ovarian Tumor with Micropapillary Pattern: A More Aggressive Premalignant Lesion

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Background: Despite the poor prognosis of serous borderline ovarian tumors with micropapillary patterns (SBMT), it is not completely known the role of this precursor in the pathogenesis of low grade serous carcinoma (LGSC) due to the low literature data about the genetic alterations.

Design: We analyzed the KRAS, NRAS and BRAF mutational status in 30 LGSCs, 15 SBMTs, 30 serous borderline ovarian tumors (SBT), 30 serous cysts (SC) and 30 serous high grade carcinomas (HGSC). In this cohort, we analyzed the expression of miRNA PCR Array (miScript miRNA, Qiagen). Altered miRNAs were confirmed by real-time PCR. Then we analyzed the immunohistochemical expression of several genes regulated by the altered miRNAs. Results were correlated with the clinical and pathological features.

Results: We found a significant higher rate of BRAF mutation in SBT (61%) than in LGSC (10%) and SBMT (20%). Conversely, the rate of RAS mutation significantly increased from SBT (6%) to SBMT (20%) to LGSC (40%). The miRNAs analysis showed a significant altered pattern in the different subgroups, with a progressive upregulation of miRNA155 from SC to LGSC whilst a significant down-regulation of miRNA99, miRNA143 and 144 in the same categories. Interestingly, SBMT and LGSC had a very similar miRNAs pattern except for miRNA155 that was significantly upregulated in LGSC (p=0.002). As miRNA99, miRNA143 and miRNA155 are involved in the regulation of RNF2 (the first two miRNAs) and ARID2 genes, respectively; we analyzed the expression of these proteins in the different diseases. We found significant and similar upregulation of RNF2 in LGSC and SBMT respect to SBT (p=0.007). Furthermore a significant lower expression of ARID2 in LGSC than that in SBMT (p=0.004).

Conclusions: Our data reinforces the idea that SBMT represents a different and more aggressive malignant precursor in comparison with SBT in the pathogenesis of LGSC. MiRNAs pattern and the expression of RNF2 and ARID2 could be useful in the diagnosis of SBMT, and primarily in a new clinical management and treatment approach.

1316 Expression of Novel Neuroendocrine Marker Insulinoma-associated Protein 1 (INSM1) in Gynecologic High Grade Neuroendocrine Carcinomas: An Immunohistochemical Study of 30 Cases With Comparison to Chromogranin and Synaptophysin

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Background: Insulinoma-associated protein 1 (INSM1) is a transcription factor involved in the development of neuroendocrine cells and is directly responsible for the transcription of synaptophysin and chromogranin. INSM1 has been shown to be a more sensitive marker than chromogranin and synaptophysin for pulmonary neuroendocrine tumors. However, no study has investigated the expression of INSM1 in gynecologic high grade neuroendocrine carcinomas. In this study, using immunohistochemical staining, we evaluated INSM1 expression in 30 such tumors with comparison to chromogranin and synaptophysin for their sensitivity.

Design: Thirty high grade neuroendocrine carcinomas of the female genital tract were included for this study (cervix 13, endometrium 15, fallopian tube 1, ovary 1; 21 pure, 9 mixed with another non-neuroendocrine tumor component). For each case, one formalin fixed paraffin block containing tumor was used to generate unstained slides for immunohistochemical stainings with antibodies to INSM1 (Santa Cruz Biotechnology, Clone A8, 1:200 dilution), chromogranin (Ventana, clone LK2H10, prediluted) and synaptophysin (Cell Marque, Clone MRQ-40, prediluted). The immunohistochemical stainings were semi-quantitatively scored as 0 (no tumor staining), 1+ (1-25% cells staining), 2+ (26-50%), 3+ (51-75%), and 4+ (76-100%). The percentage of tumor cells stained for each marker was visually estimated. The staining for INSM1 is nuclear and it is cytoplasmic for chromogranin and synaptophysin.

Results: Positive immunohistochemical staining for INSM1, chromogranin, synaptophysin was seen in 28/30 (93%, 1+ in 6, 2+ in 4, 3+ in 11, 4+ in 7), 25/30 (83%, 1+ in 6, 2+ in 4, 3+ in 3, 4+ in 12) and 30/30 (100%, 2+ in 1, 3+ in 4, 4+ in 25) cases, respectively (INSM1 vs chromogranin, p = 0.1270; INSM1 vs synaptophysin, p = 2.0E-05; chromogranin vs synaptophysin, p = 0.002). The mean number of tumor cells stained for INSM1, chromogranin, and synaptophysin was 53% (median 60%, range 0-95%), 51% (median 50%, range 0-100%), and 91% (median 98%, range 40-100%), respectively (INSM1 vs chromogranin, p = 0.7359; INSM1 vs synaptophysin, p < 0.00001; chromogranin vs synaptophysin, p < 0.00001).

Conclusions: INSM1 is a slightly more sensitive marker than chromogranin for gynecologic high grade neuroendocrine carcinomas (93% vs 83%). However, INSM1 is not as sensitive as synaptophysin for these tumors.

FIG. 1147

Case	Specimen type	Age	Multiple leiomyomas (weight in grams)	Morphology			FH IHC result	FH germline mutation type
				Conventional LM	Atypical LM	FH-morph?		
1	Myomectomy	31	Yes (433)	Yes	None	Some	N/A	c.278+2T>C
2	Myomectomy	30	No (1896)	Yes	None	All	Retained staining in all LM	c.934T>C(p. Phe312Leu)
3	Myomectomy/ Hysterectomy	28/31	Yes (NA/1958)	Yes	Yes	Some	Retained staining in all LM	c.1020T>A(p. Asn340Lys)
4	Myomectomy/ Hysterectomy	34/40	Yes (850/2290)	Yes	Yes	Some	Loss of staining in some LM, retained in other LM	c.204T>A(p. Tyr68*)
5	Myomectomy	30	Yes (279)	Yes	None	All	Loss of staining in all LM	c.204T>A(p. Tyr68*)

Table 1: Summary of Positivity Rates, Strength and Extent For All Stains and Entities

	Ovary				Endometrium				Kidney			
Positive Mesothelin	18/21 (85.7%)				16/17 (94.1%)				3/25 (12.0%)			
Positive CK7	21/21 (100.0%)				17/17 (100.0%)				8/25 (32.0%)			
Positive CD10	5/21 (23.8%)				5/17 (29.4%)				24/25 (96.0%)			
Positive RCC	7/21 (33.3%)				8/17 (47.1%)				22/25 (88.0%)			
Positive AMACR	20/21 (95.2%)				12/17 (70.6%)				17/25 (68.0%)			
Positive catenin	21/21 (100%)				17/17 (100%)				24/25 (96.0%)			
Positive CAIX	11/21 (52.4%)				8/17 (47.1%)				21/25(84.0%)			
	Rare cells	<10%	10-50%	>50%	Rare cells	<10%	10-50%	>50%	Rare cells	<10%	10-50%	>50%
Mesothelin Weak	3/18 (16.7%)	1/18 (5.6%)	1/18 (5.6%)	3/18 (16.7%)	0	1/16 (6.3%)	1/16 (6.3%)	3/16 (18.8%)	1/3 (33.3%)	0	0	1/3 (33.3%)
Mesothelin Strong	0	0	0	5/18 (27.8%)	0	0	2/16 (12.5%)	2/16 (12.5%)	0	0	0	0
Mesothelin Patchy	0	3/18 (16.7%)	2/18 (11.1%)	0	0	2/16 (12.5%)	3/16 (18.8%)	2/16 (12.5%)	0	0	0	1/3 (33.3%)
CK7 Weak	0	0	0	0	0	0	1/17 (5.9%)	0	1/8 (12.5%)	0	1/8 (12.5%)	0
CK7 Strong	0	0	1/21 (4.8%)	20/21 (95.2%)	0	0	1/17 (5.9%)	15/17 (88.2%)	2/8 (25.0%)	0	1/8 (12.5%)	3/8 (37.5%)
CK7 Patchy	0	0	0	0	0	0	0	0	0	0	0	0
CD10 Weak	0	0	0	0	0	0	0	0	1/24 (4.2%)	0	2/24 (8.3%)	0
CD10 Strong	3/5 (60.0%)	1/5 (20.0%)	0	1/5 (20.0%)	5/5 (100.0%)	0	0	0	1/24 (4.2%)	0	3/24 (12.5%)	7/24 (29.2%)
CD10 Patchy	0	0	0	0	0	0	0	0	0	1/24 (4.2%)	4/24 (16.7%)	5/24 (20.8%)
RCC Weak	1/7 (14.3%)	0	0	0	1/8 (12.5%)	1/8 (12.5%)	0	0	0	0	1/22 (4.6%)	0
RCC Strong	1/7 (14.3%)	1/7 (14.3%)	1/7 (14.3%)	3/7 (42.9%)	1/8 (12.5%)	0	1/8 (12.5%)	3/8 (37.5%)	1/22 (4.6%)	1/22 (4.6%)	5/22 (22.7%)	7/22 (31.8%)
RCC Patchy	0	0	0	0	0	1/8 (12.5%)	0	0	0	5/22 (22.7%)	1/22 (4.6%)	1/22 (4.6%)
AMACR Weak	1/20 (5.0%)	2/20 (10.0%)	2/20 (10.0%)	1/20 (5.0%)	0	1/12 (8.3%)	0	1/12 (8.3%)	1/17 (5.9%)	1/17 (5.9%)	7/17 (41.2%)	1/17 (5.9%)
AMACR Strong	0	0	4/20 (20.0%)	7/20 (35.0%)	0	0	3/12 (25.0%)	3/12 (25.0%)	0	0	0	4/17 (23.5%)
AMACR Patchy	0	0	2/20 (10.0%)	1/20 (5.0%)	0	2/12 (16.7%)	1/12 (8.3%)	1/12 (8.3%)	0	3/17 (17.6%)	0	0
catenin Weak	0	0	0	1/21 (4.8%)	0	0	0	1/17 (5.9%)	1/24 (4.2%)	0	1/24 (4.2%)	12/24 (50.0%)
catenin Strong	0	0	0	20/21 (95.2%)	0	0	0	14/17 (82.4%)	0	0	0	2/24 (8.3%)
catenin Patchy	0	0	0	0	0	0	0	2/17 (11.8%)	0	0	4/24 (16.7%)	4/24 (16.7%)
CAIX Weak	3/11 (27.3%)	1/11 (9.1%)	0	3/11 (27.3%)	1/8 (12.5%)	0	3/8 (37.5%)	2/8 (25.0%)	1/21 (4.8%)	0	4/21 (19.1%)	13/21 (61.9%)
CAIX Strong	0	0	0	0	0	0	0	0	0	0	0	1/21 (4.8%)
CAIX Patchy	0	3/11 (27.3%)	1/11 (9.1%)	0	0	0	1/8 (12.5%)	1/8 (12.5%)	0	1/21 (4.8%)	0	1/21 (4.8%)

Case #	Tumor type	TMB/Mb	Molecular classification	Relevant genes altered	FIGO Stage	Followup status	Time to last followup
1	mixed LC-NEC+EA	487.95	POLE (n=1)	TP53 RB1 ARID1A PTEN PIK3CA PIK3R1 CTNNB1	1A	ND	ND
2A	LCNEC	10.65	MSI (n=7)	TP53 (n=4; 3 in EA only) RB1 (n=6) ARID1A (n=7) PTEN (n=6) PIK3CA (n=4) PIK3R1(n=5) CTNNB1 (n=3)	IIIB	ND	ND
2B	EA	13.69					
3A	LCNEC	20.53			IA	DOD	10 months
3B	EA	31.18					
4A	SCNEC	39.52			IIIC1	recent case	N/A
4B	EA	45.60					
5A	LCNEC	51.68			IA	ND	ND
5B	EA	67.64					
6A	SCNEC	19.00			IIIC1	ANED	20 months
6B	EA	24.32					
7A	SCNEC	33.44			IIIC1	ANED	39 months
7B	EA	34.96					
8	Mixed SC-NEC+CS	105.65			ND	recent case	N/A
9A	LCNEC	9.13	CN-L (n=4)	ARID1A (n=4) PTEN (n=2) CTNNB1 (n=2) PIK3CA (n=2)	IB	ANED	6.5 months
9B	EA	9.13					
10	LCNEC	6.08			IIIB	DOD	4.7 months
11	LCNEC	6.08			II	ANED	12 months
12A	LCNEC	4.56			IIIC1	DOD	7 months
12B	EA	3.80					
13	SCNEC	8.36	CN-H (n=2)	TP53(n=2) RB1 DEL (n=2) NKX2-1 HA (n=1) MYC HA (n=1) PIK3CA (n=1)	IV	DOD	2 months
14	SCNEC	4.56			IB	ND	ND

NEC=neuroendocrine carcinoma, EA=endometrioid adenocarcinoma, CS=carcinosarcoma, TMB=tumor mutational burden, Mb=megabase, SC=small cell, LC= large cell, DEL=deletion, DOD=dead of disease, ANED=alive with no evidence of disease, ND= no data, N/A= not applicable

Table 1: p53 and Ki67 staining patterns in dVIN and vulvar squamous lesions.
***Parabasal staining is divided into 1/3 (lower one-third of the epithelium), 2/3 (lower two-thirds of the epithelium) and 3/3 (upper two-thirds to full thickness of the epithelium)**

No	Dx	p53			Ki67	
		Basal %	Pattern	Parabasal	Basal %	Parabasal
1	LS	80	Weak-Moderate, Patchy	1/3	<10	1/3
2	LS	90	Moderate-Strong, Uniform	1/3	60	1/3
3	LS	60	Weak-Moderate, Patchy	1/3	20	1/3
4	LS	90	Moderate-Strong, Uniform	1/3	60	1/3
5	LS	60	Weak-Moderate, Patchy	1/3	40	1/3
6	LSC	10	Weak-Moderate, Patchy	1/3	<10	1/3
7	LSC	10	Weak-Moderate, Patchy	1/3	<10	1/3
8	LSC	10	Weak-Moderate, Patchy	1/3	<10	1/3
9	LSC	10	Weak-Moderate, Patchy	1/3	<10	1/3
10	LSC	50	Moderate-Strong, Patchy	1/3	<10	1/3
11	LP	10	Weak-Moderate, Patchy	2/3	30	1/3
12	LP	80	Moderate-Strong, Patchy	1/3	30	1/3
13	LP	50	Moderate-Strong, Patchy	1/3	<10	1/3
14	PSO	<10	Weak-Moderate, Patchy	1/3	<10	1/3
15	PSO	50	Moderate-Strong, Patchy	1/3	40	1/3
16	PSO	50	Weak-Moderate, Patchy	1/3	20	1/3
17	PSO	10	Weak-Moderate, Patchy	1/3	<10	1/3
18	PSO	40	Weak-Moderate, Patchy	1/3	<10	1/3
19	PSO	20	Weak-Moderate, Patchy	1/3	30	1/3
20	SPD	20	Weak-Moderate, Patchy	2/3	<10	1/3
21	SPD	40	Weak-Moderate, Patchy	1/3	20	1/3
22	SPD	<10	Weak-Moderate, Patchy	1/3	<10	1/3
23	SPD	<10	Weak-Moderate, Patchy	1/3	<10	1/3
24	CAN	10	Weak-Moderate, Patchy	1/3	60	1/3
25	CAN	10	Weak-Moderate, Patchy	1/3	40	1/3
26	CAN	10	Weak-Moderate, Patchy	1/3	20	1/3
27	CAN	<10	Weak-Moderate, Patchy	1/3	<10	1/3
28	dVIN	100	Strong	2/3	90	1/3
29	dVIN	100	Strong	2/3	70	1/3
30	dVIN	90	Moderate-Strong	2/3	40	3/3
31	dVIN	100	Strong	1/3	<10	1/3
32	dVIN	0	None/Null	None	<10	1/3
33	dVIN	100	Strong	2/3	40	1/3
34	dVIN	0	None/Null	None	20	3/3
35	dVIN	0	None/Null	None	20	1/3
36	dVIN	90	Strong	2/3	80	3/3
37	dVIN	100	Strong	2/3	70	2/3
38	dVIN	<10	Weak/Null	1/3	<10	2/3
39	dVIN	70	Moderate-Strong	1/3	60	3/3