NORTHWEST GYNECOLOGICAL CANCER SYMPOSIUM

PROGRAM & ABSTRACTS

DECEMBER 4, 2017

PELTON AUDITORIUM | FRED HUTCH | SEATTLE, WA

Rivkin Center for Ovarian Cancer

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Fred Hutch
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Rivkin Center for Ovarian Cancer

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Knight Cancer Institute
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British Columbia Cancer Agency
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Rivkin Center for Ovarian Cancer

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POSTERS

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Deborah Bowen, PhD
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Barbara Norquist, MD
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Marc Radke
University of Washington
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Chaoyang Sun, PhD
Knight Cancer Institute
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Engineering immunosuppressive pathways operative within T cells for enhancing adoptive therapy of ovarian cancer

Kristin Anderson
Fred Hutch & University of Washington

Breanna M. Bates[1], Edison Y. Chiu[2], Christopher B. Morse[1,2], Nicolas Garcia[1], Philip D. Greenberg[1,2]
[1]Fred Hutch, [2]University of Washington

In the United States, one of every 75 women will develop advanced ovarian cancer in her lifetime, and one of every hundred will die from the disease. This mortality rate has changed very little over the last 20 years—better treatments are urgently needed. One new therapeutic approach employs immune T cells reprogrammed to selectively target tumor cells, and is achieving provocative results. T cells can potentially control tumor growth without toxicity to healthy tissues when they are engineered to target immunogenic proteins (antigens) that are selectively overexpressed in transformed cells. Mesothelin (Msln) is a promising tumor antigen in ovarian cancer; it contributes to the malignant/invasive phenotype and has limited expression in healthy cells.

Human T cells engineered to target Msln induce death in human ovarian cancer cell lines in vitro. However, this setting does not recapitulate the numerous immunosuppressive obstacles that adoptive T cell immunotherapy faces in solid tumors. In considering a more predictive model, we validated immunosuppressive features shared by human and murine ID8-VEGF tumors. We then tested whether engineered T cell therapy is effective in an immunocompetent mouse model of advanced stage disease.

We demonstrated that Msln-targeting engineered T cells kill ID8-VEGF tumor cells in vitro and safely provide therapeutic benefit in vivo. However, the immunosuppressive features of the tumor microenvironment (TME) limited anti-tumor activity. The ovarian cancer TME is a nutrient- and oxygen-deprived milieu, and adaptive metabolic responses by infiltrating T cells can have protean effects on their function. Thus, strategies that modulate T cell metabolic pathways and influence the TME might enhance T cell function and improve anti-tumor efficacy by overcoming a critical component of immune evasion in many solid tumors. Efforts to characterize and modulate the immunosuppressive pathways in a murine model of advanced stage ovarian cancer will be discussed, focusing on the goals of enhancing and sustaining the anti-tumor activity of therapeutic T cells for clinical translation.

[Oral Presentation]

Advanced Methods for Cancer Detection by Vaginal Screening: feasibility and assessment of deep sequencing based self-sampling methods

Michael Anglesio, University of British Columbia

Tinker A (UBC), Rushton C (SFU), Yang W (BCCA), Yun JP (BCCA), Rufin K (BCCA), Alcaide M (SFU), Morin R (SFU)

Background:
Detection of non-invasive precursors or occult lesions may allow for interventions or cure before progression to advanced, invasive cancer has occurred. Ovarian and endometrial cancer (OC/EC) are associated with familial cancer syndromes, BRCA-related and Lynch Syndrome respectively. Neither OC nor EC have effective screening tools to help "high-risk" women detect early lesions or help in decision making around fertility preservation or invasive prophylactic surgeries. Recent studies suggest DNA from vaginal sampling may contains tumour-specific, somatic mutations and may be useful for monitoring occult/early cancers.

Methods:
Cancer affected (n=90) and healthy women (n=30) were recruited for minimally-invasive self-screening using a vaginal swab and tampon. DNA from swabs, tampons (and tumours in cancer-affected women), were subjected to digital next generation sequencing targeting 10 genes frequently altered in OC/EC.

Results:
4 EC and 2 healthy control specimens have been analyzed: swab sampling was able to corroborate at least one somatic EC mutation in 2/4 EC specimens, while tampon sampling detected alterations in 4/4 EC specimens. Although "cancer hotspot" mutations were not detectable in healthy controls (0/2), a number of alteration were predicted that were not present in saliva-derived germline samples.

Conclusion:
Although our rate of detection is consistent with previous reports, training of prediction algorithms and greater depth of sequencing is needed to improve accuracy. Self-screening was rated as acceptable by participants and has a greater potential for implementation compared to invasive screening methods such as uterine lavage. Additional data is needed to determine utility of this assay for detecting early lesions and understanding of alterations found in healthy women.

[Poster Presentation]

Vaccine targeting antigens associated with epithelial to mesenchymal transition in combination with cisplatin significantly inhibits ovarian cancer growth

Denise Cecil, University of Washington

Ekram Gad and Lauren Corulli, University of Washington

There is a subpopulation of cancer stem cells (CSCs) in ovarian cancer that contributes to tumor growth and treatment resistance. It has been shown that chemotherapy, such as platinum-based therapies, not only fails to eliminate CSCs, the treated tumors often contain an even higher frequency of chemo-resistant CSCs than observed before treatment. On the other hand, platinum-based chemotherapies can exhibit immune potentiating effects. Cisplatin can reduce the expression of T-cell inhibitory molecules on both dendritic and tumor cells as well as inducing the expression of chemokines that recruit T-cells with antitumor activity. Thus, we hypothesized that combining cytotoxic chemotherapy with a CSC-targeted immunotherapy could offer greater clinical utility in eradicating the treatment-resistant subpopulation. CSCs often exhibit properties associated with epithelial to mesenchymal transition (EMT) and it was demonstrated that an EMT signature was a universal finding in the metastases in ovarian cancer. We have identified four biologically relevant antigens in ovarian cancer that target proteins associated with drug resistance, self-renewal, and EMT. We used a combined scoring system from five algorithms for predicting class II binding to determine Th epitopes for IGFBP-2, Survivin, HIF-1α and IGF-IR. Those epitopes that induced a selective Th1 response, determined via IFN-g and IL-10 ELISPOT responses in human PBMC, were included in a plasmid-based multi-epitope vaccine and verified to induce a robust Th1 immune response in mice. The percentage of stem cells in the epithelial ovarian cancer cell line, ID8, were determined to be at low levels as measured via expression of the stem cell marker Sca-1 by flow cytometry. We utilized the implanted ID8 ovarian cancer model and monitored tumor growth via bioluminescent imaging. The ID8 tumor was confirmed to be resistant to cisplatin treatment and significantly more cells in the tumors from 4/6 treated mice expressed Sca-1 as compared to the untreated mice ($p=0.0316$). Cisplatin treatment also increased the percentage of CD8+ cells in the ascites of the treated mice compared to untreated mice ($p=0.01$). There was also no control of tumor growth by vaccination targeting the EMT antigens. However, if vaccination was performed in combination with cisplatin treatment, tumor growth was inhibited by 68% compared to monotherapy ($p=0.0314$). These data suggest that although cisplatin can promote CSCs in the tumor, active immunization against antigens associated with EMT and CSCs can target this resistant population resulting in a more effective and specific treatment.

[Oral Presentation]

MAGENTA: a large randomized study to define an optimal genetic counseling strategy in women undergoing genetic testing at home for ovarian cancer risk

Tara Coffin, University of Washington

Deborah Bowen PhD, Tara Coffin, Barbara Norquist M.D, Jamie Crase, Elizabeth Swisher MD for the Stand up to Cancer Ovarian Cancer Dream Team

Approximately 20% of ovarian carcinomas are caused by inherited mutations ovarian cancer susceptibility genes and are therefore potentially preventable with more widespread identification of genetic risk. Risk-reducing salpingo-oophorectomy is a proven cancer prevention strategy that reduces cancer and overall mortality in women with BRCA1 and BRCA2 mutations and is recommended from women with elevated ovarian cancer risk. Genetic testing for cancer risk has become increasingly sensitive and exponentially cheaper, raising the possibility of population testing for cancer risk in the not too distant future. However, the current paradigm for genetic testing for cancer risk creates many barriers to delivery of genetics services, and is particularly burdensome to underserved populations and those at distance from specialized genetics providers. The Stand Up to Cancer Ovarian Cancer Dream Team initiated a randomized trial called MAGENTA (MAking GENetic Testing Accessible) to assess a novel internet-based delivery of genetic counseling and testing. MAGENTA is a randomized four arm 2 by 2 trial comparing standard pre and post testing genetic counseling (delivered via the phone) versus pre- and post-test delivery of genetic information using internet materials. MAGENTA is enrolling 3000 women nationally who have a personal or family history suggestive of increased ovarian cancer risk or with a known ovarian cancer gene mutation in a family member. The primary outcome is cancer worry at 3 months post receiving test results. Secondary outcome include completion of testing, anxiety, depression, quality of life, family communication of results, and utilization of ovarian cancer screening or risk reduction surgery as well as outcomes at 1 and 2 years post testing. The MAGENTA trial is pushing the boundaries of standard genetics services and clinical trial design in multiple ways including 1. Use of complete internet-based clinical trial access including electronic eligibility screen and consent, 2. Delivery of genetic services entirely at home to patients on their schedule, 3. Subject recruitment using social medial outreach including targeted ads designed to increased enrollment in genetically underserved populations, 4. Testing the hypothesis that not all patients needs traditional in person pre- and post-test genetic counseling. As of October 2017, we have consented and randomized 560 patients. An exponential increase in enrollment followed a social media outreach by advocacy partners. We anticipate competing enrollment for MAGENTA in late 2018.

[Poster Presentation]

The origins of endometriosis associated cancers

Dawn Cochrane, British Columbia Cancer Agency

Basile Tessier-Cloutier (UBC), Katherine M Lawrence (BC Cancer Agency), Tayyebeh Nazeran (UBC), Anthony N. Karnezis (BC Cancer Agency), Jennifer Ji (UBC), Clara Salamanca (BC Cancer Agency), Evan Gibbard (BC Cancer Agency), Angela S Cheng (BC Cancer Agency), Jessica N McAlpine (UBC), Lien N Hoang (UBC), C Blake Gilks (UBC)

Both clear cell ovarian carcinoma (CCOC) and endometrioid ovarian carcinoma (ENOC) are associated with ovarian endometriotic cysts, which is believed to be the precursor lesion of these cancers. Women with endometriotic cysts have up to a 3 fold increased risk of developing CCOC and ENOC. It is perplexing that these two clinically distinct histotypes of ovarian cancer arise from the same precursor lesion. We have performed whole genome sequencing of ovarian cancer histotypes and found that while some genomic features are more common to one histotype than the other, there is not a single feature that is unique to either histotype. Lacking genomic evidence that could explain the differences between these histotypes, we hypothesized that these cancers arise from distinct cells of origin within endometrial tissue, and the cellular context accounts for their differences. We performed global proteomic analysis of ovarian cancer histotypes and identified CTH as a marker for CCOC. Upon examination of normal Müllerian tissues, we found that CTH is highly expressed in the ciliated cells of endometrium (both ectopic endometrium and endometriosis), and of the fallopian tube, with very little expression in the secretory cells of these tissues. We also find that other ciliated cell markers are expressed in CCOC, whereas endometrial secretory cell markers are expressed in ENOC. We propose a new model of CCOC and ENOC histogenesis wherein ENOC is derived from cells of secretory cell lineage whereas CCOC is derived from, or share similarities to, cells of ciliated cell lineage. There remains, however, many unanswered questions. For example, while CCOC and ENOC occur at roughly equal prevalence, ciliated cells of the endometrium are rare compared to secretory cells. Cells in the endometriotic cyst are exposed to factors such as inflammation and reactive oxygen species, which could influence differentiation of endometrial progenitor cells into the secretory or ciliated cell lineage. To test factors that promote ciliated cell differentiation in normal endometrium, we treated organoid cultures of normal endometrium with IL-6 and Notch pathway modulators. We propose that ovarian cancer histotypes arise from different cells of origin and that the biology of the normal cells will be partly responsible for determining the phenotype of the cancers.

[Oral Presentation]

The Central Role of Advocates on the Stand up To Cancer Ovarian Cancer Research Fund Alliance- National Ovarian Cancer Coalition Ovarian Cancer Dream Team

Jaime Crase, University of Washington

Jamie Crase, Kathleen Gavin, Deborah Polinsky, Deborah Bowen, Barbara Norquist, Elizabeth Swisher

Ovarian cancer is the deadliest of the gynecological cancers, resulting in about 14,000 deaths and 22,000 new cases every year. There is no screening test for ovarian cancer and with few symptoms present in the early stages of the disease, early detection lags and with it, treatment and prognosis. The SU2C Ovarian Cancer Dream Team is working on two trials focusing on ovarian cancer prevention in which advocates have played leading roles in trial design and implementation.

WISP (Women ChoosIng Surgical Prevention) is a surgical prevention trial to determine whether interval salpingectomy, followed by delayed oophorectomy (ISDO) can improve sexual functioning and menopausal symptoms compared to standard risk-reducing salpingo-oophorectomy (RRSO). Women at increased risk of ovarian cancer based on a genetic mutation are recommended to undergo removal of the fallopian tubes and ovaries (RRSO) by age 40 for BRCA1 and by age 45 for BRCA2. For other gene mutations associated with elevated risk for ovarian cancer there are recommendations to consider RRSO, although age is not specified.

MAGENTA (MAking GENetic Testing Accessible) will provide genetic testing from your comfort of your home. There are a number of barriers that prevent women from getting the genetic testing they may benefit from. This SU2C initiative seeks to improve the availability of genetic testing for hereditary cancer syndromes to at-risk individuals using an online genetic testing service. Genetic testing has the potential to address the high morbidity and mortality rates associated with ovarian cancer through innovative prevention and treatment efforts.

Advocates are involved in all aspects of the study design including surveys, policy decisions, outcome indicators and graphics. Advocates and advocacy groups have been essential in promotion and implementation through the use of social media outlets like Facebook, Instagram, and Twitter in the hopes of reaching women who are at greater risk, but find the barriers to traditional genetic testing to great to overcome. Advocates vetted all community outreach efforts prior to approval by the IRB. Advocates participate in regular teleconferences to monitor study progress. They will be essential to translate and publicize study findings to the community in order to maximize the impact of study findings and clinical practice.

[Poster Presentation]

Gain-of-function and loss-of-function TP53 mutations in ovarian carcinomas with and without concurrent BRCA1 or BRCA2 mutations

Talayeh Ghezelayagh, University of Washington

Talayeh Ghezelayagh, MD (University of Washington), Kathryn Pennington, MD (University of Washington), Barbara Norquist, MD (University of Washington), Maria I. Harrell, PhD (University of Washington), Kathy Agnew, BS (University of Washington), Marc Radke (University of Washington), Garrett Collett (University of Washington), Ming Lee, PhD (University of Washington), Elizabeth M. Swisher, MD (University of Washington)

Objectives: Most high grade ovarian carcinomas (OC) have mutations in the TP53 tumor suppressor gene. A subset of missense mutations confer oncogenic properties and have been termed gain-of-function (GOF) mutations. We assessed the distribution and impact of GOF versus loss-of-function (LOF) TP53 mutations in OC with and without concurrent BRCA mutations.

Methods: We performed BROCA sequencing on 375 unselected OC prospectively followed for survival. For patients with stage II-IV, high grade, non-clear cell OC, overall survival was compared using Kaplan-Meier curves and logrank testing, with multivariate Cox regression analysis examining the contribution of BRCA and TP53 mutations. Logistic regression was used to analyze the effects of TP53 mutation type on platinum resistance.

Results: Of 375 total OC evaluated, 248 (66.1%) had TP53 mutations, 98 (26.1%) with GOF, and 150 (40%) with LOF. Of 272 high grade serous cancers, GOF and LOF TP53 mutations were present in 82 (30.1%) and 122 (44.9%). TP53 mutations were more common in grade 2-3 OC vs. grade 1 (69.3% vs 11.8% ($p<0.001$), and stage III-IV disease vs stage I-II (72.2% vs 32.7%, $p<0.001$) but the ratio of LOF to GOF mutation did not differ. BRCA mutated OC had a higher total TP53 mutation rate (70/86, 81.4% vs. 178/289, 61.6%, $p=0.001$), which was entirely explained by a higher rate of LOF mutations (55.8% vs. 35.3%, $p=0.001$). Median survival was 43 months (95% CI 27-53) for women without TP53 mutations, 54 months (95% CI 42-60) for LOF, and 43 months (95% CI 33-54) for GOF (logrank $p=0.51$). Survival curves did not statistically diverge when further stratified by both TP53 mutation type and BRCA mutation status (logrank $p=0.28$). After adjusting for clinicopathologic factors and BRCA status, neither GOF (HR 1.17, $p=0.49$) nor LOF (HR 1.23, $p=0.39$) mutations affected overall survival. TP53 LOF and GOF mutations trended toward an association with platinum resistance but the results were not statistically significant (HR 0.51, $p=0.12$ and HR 0.57, $p=0.23$ respectively).

Conclusions: OC with BRCA mutations have a higher frequency of TP53 mutations relative to BRCA wild type cases, secondary to an increased frequency of LOF mutations and a similar fraction of GOF mutations. The presence of a LOF versus a GOF TP53 mutation did not impact overall survival after controlling for BRCA mutation status.

[Oral Presentation]

Development and implementation of a targeted next generation sequencing methylation panel for the assessment of methylation in ovarian and endometrial cancers

Maria Isabel Harrell, University of Washington

Steven J. Salipante, Department of Laboratory Medicine, University of Washington; Angela L. Jacobson, Department of Laboratory Medicine, University of Washington; Sarah S. Bernards, Department of Obstetrics and Gynecology, University of Washington; Tom Walsh, Department of Medical Genetics, University of Washington; Ming K. Lee, Department of Medical Genetics, University of Washington; Eric Q. Konnick, Department of Laboratory Medicine, University of Washington; Colin C. Pritchard, Department of Laboratory Medicine, University of Washington; Elizabeth M. Swisher, Department of Obstetrics and Gynecology, University of Washington

Objectives: To develop a tool for assessing the contribution of methylation of DNA repair genes in ovarian and endometrial carcinoma.

Methods: A methylation sequencing panel targeting the CpG islands and adjacent shores of 57 genes associated with DNA repair was designed based on Agilent's Sureselect MethylSeq technology and was implemented to study methylation in carcinomas. For initial analysis we included 13 ovarian carcinoma cases from a university-based gynecologic oncology tissue bank. Four endometrial cancer cases with normal germline and somatic sequencing at all the Lynch syndrome DNA mismatch repair genes and unexplained IHC loss of MSH2 and MSH6 were referred for methylation analysis from a university clinical laboratory. DNA from frozen and formalin fixed paraffin embedded cancer was prepared for target enrichment, hybridized, and captured to regions of interest. Post hybridization, the captured DNA libraries were bisulfite treated to differentiate methylated and un-methylated DNA segments. Bisulfite modified and captured DNA libraries were then amplified, indexed and pooled for multiplex deep sequence analysis using an Illlumina HiSeq. The bioinformatics pipeline included alignment to a theoretical bisulfite modified genome to obtain annotated, interpretable output. The Integrative Genomics Viewer (IGV) was used to visualize output in each region and assess depth of coverage.

Results: Six ovarian carcinomas were identified as methylated at three genes by methylation sensitive PCR including 2 each at the BRCA1, RAD51C and MLH1 promoters. All were found to be methylated with MethylSeq generating 100% concordance. Methylation sequencing of 7 additional ovarian carcinoma tumors, whose methylation status was unknown showed one case, LS519, with methylation of MLH1. One of 4 unsolved endometrial cancers with unexplained MSH2/MSH6 deficiency in the tumor was found to be positive for methylation at the MSH2 promoter in the region previously linked to down-regulation of MSH2.

Conclusions: The methylation panel for genes associated with DNA repair is a useful tool to assess promoter methylation at many DNA repair genes simultaneously. We are now using this panel to assess methylation in DNA repair genes in ovarian carcinomas and relating to response to treatment and development of resistance.

[Poster Presentation]

Rivkin Center for Ovarian Cancer

Disease course and treatment patterns of unselected patients with ovarian carcinoma and germline BRCA mutations

Soledad Jorge, University of Washington

Heidi Gray, Barbara Norquist, Kathryn Pennington, Rochelle Garcia

Objectives: Patients with hereditary ovarian, peritoneal or fallopian tube carcinoma (OC collectively) associated with germline BRCA1 and BRCA2 (BRCA) mutations have better 5 year survival than patients without mutations, but little has been reported on their long-term disease course. The goal of this study was to describe the cumulative treatments and outcomes of a cohort of OC patients with germline BRCA mutations.

Methods: A retrospective study of women with germline BRCA mutations and OC from a database in which all patients were genotyped was conducted. Women with stage II or greater epithelial high-grade tumors, diagnosed between 2004 and 2014, and for whom complete medical records were available were included. Demographic and treatment data were abstracted, survival analysis completed, and a Swimmer plot used to illustrate disease timelines.

Results: Forty BRCA mutation carriers (26 BRCA1, 14 BRCA2) met inclusion criteria. Median age was 54 years (52 BRCA1, 57 BRCA2), 80% were white, 63% had Stage IIIC disease, and 75% had serous histology. Median follow up time was 49.3 months (interquartile range (IQR) 33.7 - 85.4). In total, 38% of patients received neoadjuvant chemotherapy, and the remainder had upfront cytoreductive surgery (56% no residual, 32% less than 1 cm, 12% suboptimal). All patients initially received adjuvant platinum-based chemotherapy, and 40% received intraperitoneal chemotherapy. Most (90%) were platinum sensitive, with a median first platinum-free interval of 11.8 months (IQR 3.6-21.9). The median number of treatment lines was 3 (IQR 1-6) with a median of 2 (IQR 1-3) platinum lines. Greater than 7 treatment lines were administered to 18% of patients. Half (50%) received bevacizumab and 20% received PARP inhibitors. On average, patients spent 43% (range 6-87%) of the time after diagnosis in active treatment. The median overall survival was 77.8 months from diagnosis, 70.2 months after first-line treatment, and 19.4 months after second-line treatment. A full 15% of women had complete responses to first line treatment lasting longer than 5 years. Half (n=3) of these women received maintenance therapy and the other half did not. Their characteristics did not differ significantly from the larger group.

Conclusions: Beyond standard first-line platinum, there was treatment and outcome heterogeneity for OC patients with BRCA mutations. After diagnosis, these women spent nearly half their life on treatment. Nevertheless, there was an important subset who did not recur, even without maintenance therapy.

[Oral Presentation]

Establishment and characterization of human ovarian cancer xenograft models using bioluminescence imaging

Toni Jun, Crown Biosciences Inc.

Nektaria Papadopoulou, Louise Wainwright, Vicky Lacey, Jane Wrigley, Jamie Wood, Louise Woolley, Jason King, Simon Jiang, Yinfei Yin and Rajendra Kumari (Crown Bioscience UK Ltd1, Loughborough, UK)

BACKGROUND: Ovarian cancer is the most common gynaecological cancer and a major cause of cancer-related death in women. The high lethality of this cancer is mainly due to late diagnosis and treatment failure. Therefore, better understanding of ovarian cancer biology, further improvements in detection technology and advanced models for more efficient pre-clinical testing are much needed. It is widely accepted that orthotopic models are much more clinical relevant because orthotopic tumours establish at organ specific sites, form more patient comparable tumour vasculature/microenvironment and facilitate spontaneous metastatic spread. The use of bioluminescent imaging allows non-invasive longitudinal monitoring of orthotopic tumour burden, assist optimal randomization and also provide the tool to evaluate end stage (metastatic) disease, which produce much more data from each individual animal. Here we describe the set-up of orthotopic human ovarian cancer models using bioluminescence imaging.

MATERIALS & METHODS: Bioluminescent ovarian cell lines were generated by transducing the wild type cell lines using in-house packaged lentiviral particles containing a firefly luciferase expressing plasmid (pLVC-puro-CMVLucSh luciferase). Transduced cells were selected using puromycin to establish stable cell lines and assessed for DNA changes from their respective parental cell lines by STR profiling (IDEXX Labs Inc). Xenograft models were set up by intraperitoneal injection of bioluminescent ovarian cells to mimic late stage ovarian cancer. Tumour burden was monitored by weekly bioluminescent imaging. Ex vivo bioluminescent imaging was used to detect tumour seeding.

RESULTS: The success rate of tumour formation was 100% as confirmed by both in-life and ex-vivo imaging at termination. In-life imaging detected positive bioluminescent signal from day 7 and the models could be taken to around day 30 which provided a dosing window of about 3 weeks. Paclitaxel treatment produced reduced tumour burden quantified by real-time bioluminescent imaging. Ex vivo imaging confirmed tumour mass in the liver, genital tract and diaphragm."

[Poster Presentation]

Deep Sequencing of Pap Smears Reveals Low-Frequency Somatic TP53 Mutations in Women With and Without Serous Ovarian Cancer

Jeffrey Krimmel-Morrison, University of Washington

Shenyi Lian2, Kathryn Baker2, Daniela Nachmanson2, Maria I. Harrell3, Kathy J. Agnew3, Elizabeth M. Swisher3, Rosa Ana Risques2

1. Department of Medicine, University of Washington; 2. Department of Pathology, University of Washington; 3. Department of Obstetrics and Gynecology, University of Washington

Ovarian carcinoma has poor prognosis because it is usually diagnosed at an advanced stage. The development of screening methods to detect early-stage disease has been long hoped to reduce mortality but has remained an elusive goal. TP53 mutations are present in nearly all high-grade serous ovarian carcinomas (HGSOC) and their identification in bodily fluids could facilitate cancer diagnostics. An earlier study demonstrated that ovarian tumor DNA is detectable in 41% of Pap tests from women with ovarian tumors. However, the sensitivity of this approach may have been limited by the threshold of detection of the sequencing technology used, Safe-SeqS. We used Duplex Sequencing, the most accurate sequencing technology known to date, to sequence Pap tests from women with HGSOC and without cancer to improve this limitation in sensitivity and better define the utility of Pap tests to detect ovarian cancer cells. Whereas most conventional sequencing methods cannot distinguish low frequency mutations from errors due to PCR amplification and DNA damage, Duplex Sequencing is a molecular barcoding technique that dramatically decreases sequencing errors by scoring only mutations present in both strands of DNA. This has allowed the detection of single mutant alleles among >107 sequenced nucleotides. Here we used a new version of Duplex Sequencing that employs CRISPR/Cas9 for target enrichment, which increases efficiency and reduces DNA input. We analyzed 5 Pap tests from women with HGSOC and 24 from women without cancer. Consistent with the earlier study, in 40% of women with HGSOC (2/5), the tumor TP53 mutation was detected in the Pap test at low mutant-allele fractions (0.67% and 0.029% respectively). We also detected extremely low-frequency TP53 coding mutations in Pap tests from all (29/29) patients with and without cancer. The mean number of low-frequency mutations per Pap test was 6.9, and the median mutant allele frequency was 0.036%. These mutations were primarily deleterious to the protein (86% non-synonymous), clustered in hotspots, and increased with age. These mutations also displayed a spectra that is consistent with known mutational signatures of aging and cancer. Our results demonstrate the ability of Duplex Sequencing to detect very rare cancer cells well below thresholds of other sequencing methods. However, these tumor-specific TP53 mutations are difficult to distinguish from the other widespread, low frequency, age-associated TP53 mutations in Pap tests from women without malignant disease. This is the first time that widespread somatic driver mutations have been demonstrated in Pap test DNA from women without cancer across a wide range of ages, and this contributes to the mounting evidence that somatic cancer-like mutations are common in gynecologic and non-gynecologic non-cancerous tissues.

[Oral Presentation]

Adaptive response of ovarian cancer cells to PARP and DNA damage checkpoint inhibitors

Marilyne Labrie, MD Anderson Cancer Center (Knight Cancer Institute)

Marilyne Labrie, UT MD Anderson Cancer Center; Daniel J McGrail, UT MD Anderson Cancer Center; Gordon B Mills, UT MD Anderson Cancer Center"

High grade serous ovarian carcinoma (HGSOC) is the most common form of OC and is characterized by chromosomal instability, universal TP53 mutation and it is frequently associated with defects in the homologous recombination (HR) DNA damage repair (DDR) pathway. These particularities identify poly (ADP-ribose) polymerase (PARP) as a potential target for HGSOC patients' treatment. PARP is a key player in several DDR pathways, including base excision repair (BER) of DNA single strand breaks and non-homologous end joining (NHEJ) repair of double strand breaks. Thus, PARP is considered to be essential for cells with defective HR and becomes an interesting target for the treatment of OC patients harboring HR deficiency. Because OC cells are known to rapidly develop PARP inhibitor (PARPi) resistance, OC patients would benefit from a PARPi-based combination therapy, preventing the development of drug resistance. Studies from several groups, including ours, indicate a synergistic effect of PARPi in combination with G2/M cell cycle checkpoint inhibitors in several cancer cell lines as well as xenograft and PDX models. Because of TP53 mutations, HGSOC harbor defective cell cycle G1 DNA damage checkpoint. Blocking the G2/M checkpoint prevent the cells from efficiently repairing the DNA damage induced by PARPi, leading to mitotic catastrophe and cancer cell death. Although the results obtained with this drug combination are promising, not all OC patients are expected to respond to PARP and DNA damage checkpoint inhibitors and an adaptive response of the cancer cells to those inhibitors is expected to occur, reinforcing the need for informed and effective combination therapies. The goal of this project is to study the adaptive response of OC cells to PARP and WEE1 or ATR inhibitors combination. To investigate the mechanisms of resistance, we produced several resistant OC cell lines and found one CaOV3 WEE1 resistant cell line that displayed a cross resistance to PARP and ATR inhibitors when compared to the CaOV3 parental cell line. In order to identify the functional and molecular events leading to this cross resistance, we analyzed genomic alterations of this cell line and performed proliferation, cell cycle and DNA fiber assays. We also used reverse-phase protein array to monitor the signaling pathways that are altered by PARP, WEE1 and ATR inhibitors used either alone or in combination. This study will lead to a better understanding of the mechanisms of resistance of OC cells to PARP and DNA damage checkpoint inhibitors. It will also help identify patients that are most likely to respond to this combination therapy and find new targets for patients who don't respond optimally.

[Poster Presentation]

Proteomic Biomarkers of MEK inhibitor resistance in low-grade serous ovarian cancer cell lines

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BACKGROUND: Low-grade serous ovarian carcinoma (LGSC) occurs at low frequency in premenopausal women. It harbours few mutations, but common aberrations in RAS-MAPK genes have led to several clinical trials of MEK inhibitors (MEKi). Few experimental systems exist, and there has been a lack of preclinical drug testing. Because recent results from a clinical trial of the MEKi ARRY-142886 show a 15% response in LGSC patients, it is essential to identify biomarkers of patient response and alternate targets for combination therapy to improve treatment outcomes.

Methods: Nine cell lines were derived from advanced/recurrent LGSC. Responses to MEKi (1μM ARRY-142886, ARRY-438162, RDEA-119; or 0.1μM JTP-74057) by IC50, proliferation, viability, and apoptosis assays were evaluated. On-target MEKi effects were verified by Western blot (WB). Reverse-phase protein array (RPPA) was used to investigate proteomic differences between LGSC cells. 2 MEKi-sensitive and 7 MEKi-resistant LGSC lines were treated with control or MEKi for 24 hours. Protein lysates were analyzed by RPPA, to study expression levels of 90 proteins involved in cancer-signaling pathways. SPSS software was used to identify significant changes in protein expression before and after treatment (Mann-Whitney U Test, $p < 0.05$). Two potential biomarkers of MEKi response (EGFR and PKCa) were validated by WB. MEKi-resistant LGSC lines were treated with MEKi and erlotinib (an EGFR inhibitor; EGFRi). Effects on cell proliferation and viability were evaluated. On-target drug effects and pathway interactions were analyzed by WB. Compusyn software was used to measure drug synergism.

Results: Two of nine LGSC cell lines showed exquisite sensitivity to MEK inhibition. Analysis of RPPA data showed 12 differentially-expressed proteins between MEKi-sensitive and MEKi-resistant LGSC cell lines. Two of these proteins, EGFR and PKCa, were overexpressed in the MEKi resistant cells (at basal level), and poorly expressed in MEKi-sensitive cells. These results were validated by WB in an expanded cohort of LGSC cell lines. The combination of MEKi and EGFRi treatment in MEKi-resistant LGSC cells show a synergistic reduction in LGSC cell proliferation and viability. Compensatory effects to drug treatments in EGFR and MAPK pathways were seen by WB.

Conclusions: Using RPPA and WB we have identified differentially-expressed proteins between MEKi sensitive/resistant LGSC cell lines. Validation of these findings in LGSC tumor tissues from patients treated with a MEKi is needed. If these results are validated, measurement of these proteins could help to select LGSC patients who might benefit from MEKi therapy. EGFR was overexpressed in MEKi-resistant LGSC cells. The synergistic drug effects of MEKi and EGFRi will need to be validated in mice models. If synergism is confirmed, dual treatment could be considered as a treatment option for LGSC patients who may be resistant to MEKi therapy.

[Oral Presentation]

Adoptive T cell therapy for ovarian cancer: Development of a surgically relevant model

Christopher Morse, University of Washington & Fred Hutch

Kristin G. Anderson, Breanna M. Bates, Edison Y. Chiu, Nicolas M. Garcia, Philip D. Greenberg (Fred Hutchinson Cancer Research Center)

Objectives: We are developing immunotherapy for ovarian cancer targeting Mesothelin (MSLN), a protein selectively overexpressed in ovarian malignancies. CD8 T cells engineered with a MSLN-specific T cell receptor (TCR-MSLN) kill ovarian cancer cells in vitro. In a model with intraperitoneal (IP) tumor injection, transferred TCR-MSLN T cells reduce tumor burden and prolong survival in mice with advanced disease. To assess T cell function in a more clinically relevant setting, we have developed an orthotopic tumor model, with surgical cytoreduction prior to T cell therapy.

Methods: Murine ID8 ovarian cancer cells were transduced with an enhanced luciferase (eLuc) construct to permit imaging of microscopic tumors, and 1x106 tumor cells were injected beneath the ovarian bursa of C57/Bl6 mice. Upon detection of metastases, mice underwent hysterectomy and bilateral salpingo-oophorectomy to remove all macroscopic disease. Mice were then treated with either MSLN-specific T cells, tumor-irrelevant T cells, or no further therapy. Peripheral blood was collected serially after T cell therapy, and tumors collected at necropsy.

Results: eLuc-transduced ID8 cells were 27x brighter than cells transduced with standard luciferase, enabling in vivo visualization of microscopic intra-abdominal metastases in mice that developed large primary ovarian tumors six weeks after orthotopic tumor injection. Primary surgical cytoreduction at ~6 weeks produced minimal residual disease in all mice. In previous studies in our IP model, MSLN-specific T cells isolated from tumors expressed activation markers (CD69 and 41BB) at both day 8 and 21 post-transfer, but expressed high levels of T cell-inhibitory proteins (PD-1, Tim-3, Lag-3, TIGIT) by day 21. This expression correlated with reduced cytokine production, suggesting diminution of function. We anticipate that surgical debulking prior to therapy will favor T cell function in the orthotopic model and new findings will be presented.

Conclusions: Adoptive transfer of high-affinity TCR-MSLN-engineered T cells improves survival in the IP mouse ovarian cancer model. Ongoing studies are characterizing the function and persistence of such MSLN-specific T cells in a clinically relevant surgical model, with the goal of clarifying properties associated with tumor eradication versus progression for eventual design of effective clinical trials.

[Oral Presentation]

"Look" before you LEEP? An economic evaluation of the Pap see-and-treat strategy for the management of cervical pre-cancer

Van Nghiem, Fred Hutch

J. Robert Beck (Fox Chase Cancer Center), Michele Follen (Brookdale University Hospital Medical Center), Scott B. Cantor (MD Anderson Cancer Center)

Introduction:
In developed countries, women with high-grade or cancer result on a Pap test could be immediately treated with loop electrosurgical excision procedure (LEEP) and skip the "look" step of colposcopy and cervical biopsy, hereafter called "Pap see-and-treat". Although U.S. guidelines suggest this management strategy for women of 25 years or older, little is known about the strategy's effectiveness and cost-effectiveness. This study evaluated the cost-effectiveness of the Pap see-and-treat strategy compared with usual care (which included colposcopy and potential biopsy after abnormal Pap).

Methods:
We modelled a hypothetical cohort of 12-year-old females and followed them through their lifetimes using a Markov model. From a U.S. health system perspective, we conducted the cost analysis in 2012 dollars and estimated health benefit in quality-adjusted-life-years (QALYs). Cost data were derived from national costing databases and existing literature. We estimated short-term disutility of LEEP procedure and patient's adherence to follow-up abnormal Pap test results from previous studies. The incremental cost-effectiveness ratio (ICER) comparing the Pap see-and-treat strategy with usual care was evaluated at the willingness-to-pay threshold of $50,000/QALY. Sensitivity analyses varying key parameters investigated changes of the ICERs. We ran 10,000 microsimulations to estimate the LEEP usage.

Results:
In the base case, the Pap see-and-treat strategy gained an extra 0.004 QALY at the cost of $83, yielding an ICER of $18,477/QALY. The ICERs were sensitive to several costs (Pap test, LEEP, and colposcopy), operating characteristics of Pap test, LEEP disutility, and adherence to follow-up an atypical squamous cells of undetermined significance result from Pap test. At our given willingness-to-pay threshold, the Pap see-and-treat strategy had a 94.3% chance to be cost-effective compared to usual care. The strategy yielded the same overtreatment rate as usual care. Approximately 15.5% of the women in the Pap see-and-treat strategy had at least one LEEP procedure, compared to 9.3% in usual care. The proportion of patients having multiple LEEP procedures during their lifetime was 1.2% and 0.5%, respectively for Pap see-and-treat and usual care.

Conclusion:
Our study provided support to implement Pap see-and-treat strategy in the US because of its cost-effectiveness. The strategy may help overcome low adherence to follow-up abnormal Pap results."

[Oral Presentation]

WISP: a surgical prevention study evaluating bilateral salpingo-oophorectomy versus interval salpingectomy and delayed oophorectomy in women with inherited mutations in ovarian cancer susceptibility genes

Barbara Norquist, University of Washington

Barbara Norquist M.D, Deborah Bowen PhD, Jamie Crase, Kathy Agnew, Elizabeth Swisher MD for the Stand Up To Cancer Ovarian Cancer Dream Team

Approximately 20% of ovarian carcinomas are caused by inherited mutations in BRCA1, BRCA2 and at least 8 other DNA repair genes including BRIP1, PALB2, RAD51C, RAD51D and the DNA mismatch repair genes MSH2, MSH6, MLH1, and PMS2. Women with mutations in these genes have an elevated risk of ovarian carcinoma. Early detection strategies have only minimal efficacy without a clear impact on mortality. Therefore, current clinical recommendation are for women with inherited ovarian cancer risk to undergo risk-reducing salpingo-oophorectomy (RRSO) after completion of child-bearing, an intervention with proven efficacy. However, many high-risk women are reluctant to undergo RRSO because of its profound hormonal impact on quality of life and long-term health and a perceived impact on sexual function. We and others have shown that many so-called "ovarian" carcinomas in women with inherited risk actually arise in the fallopian tube. These data support the hypothesis that salpingectomy and delayed oophorectomy could provide cancer risk reduction with improved hormonal function and quality of life relative to standard RRSO. To address this question, the Stand Up To Cancer Ovarian Cancer Dream Team initiated the clinical trial WISP (Women choosIng Surgical Prevention) which allows pre-menopausal women (age 30-50) with a damaging mutation in an ovarian cancer susceptibility gene to self-select standard RRSO versus interval salpingectomy and delayed oophorectomy (ISDO). Providers recommend RRSO for BRCA1 mutation carriers by age 40 and for BRCA2 carriers by age 45, but women are allowed to self-select either arm regardless of age. The primary outcome is change in sexual function from baseline to 6 months after surgery as measured by the Female Sexual Function Index (FSFI). Secondary outcomes include quality of life, menopausal symptoms, cancer worry, age and completion of delayed oophorectomy, identification of occult cancers and follow-up cancers. The trial is open at 7 centers nationally (University of Washington, University of Chicago, Mayo Clinic, MD Anderson Cancer Center, Dana Farber Cancer Center, New York University and Memorial Sloan Kettering). To date we have enrolled 86 women of a planned 270 with relatively equal distribution between the two arms. Women in the ISDO arm are younger than in the RRSO arm. One woman with a BRCA1 mutation at age 41 was diagnosed with breast cancer within 2 month of bilateral salpingectomy. Another 39 year old subject who chose RRSO with a PALB2 mutation had high grade intraepithelial neoplasia in the fallopian tube (also called serous intra-epithelial carcinoma (STIC)). We anticipate completing enrollment by July of 2019.

[Poster Presentation]

Somatic reversion mutations in hereditary ovarian carcinomas predict platinum sensitivity

Marc Radke, University of Washington

Maria I. Harrell PhD, Kathy J. Agnew, and Sarah S. Bernards, University of Washington Dept of OB/GYN, Rochelle Garcia MD, University of Washington Dept. of Pathology, Beth Y. Karlan MD and Jenny Lester, Cedars-Sinai Women's Cancer Program, and Barbara M. Norquist MD, University of Washington Dept of OB/GYN

Objectives: Secondary somatic reversion mutations restoring BRCA1, BRCA2, RAD51C, and RAD51D function in hereditary ovarian carcinomas can be a mechanism of chemotherapy resistance. We tested for somatic reversion mutations in BRCA1 and BRCA2 as well as other homologous recombination repair (HRR) genes in a large series of primary and recurrent ovarian, fallopian tube, or primary peritoneal cancer (collectively termed OC), assessing their relationship with chemotherapy resistance.

Methods: OC from patients with known damaging germline mutations in key HRR genes were tested. In OC with low neoplastic cellularity, malignant cells were purified with laser-capture microdissection and haplotyping was performed by Sanger sequencing the germline mutation and 2-3 nearby intragenic heterozygous single-nucleotide polymorphisms (SNPs). OC had a reversion event if a novel frameshift mutation was identified that restored the open reading frame or if wildtype sequence was identified in association with the mutant haplotype.

Results: 76 paired primary and recurrent OC from 35 patients as well as 71 unpaired primaries and 10 recurrences were analyzed. Patients had deleterious germline mutations in BRCA1 (74, 63.2%), BRCA2 (30, 25.6%), RAD51C (3, 2.6%), RAD51D (3), BRIP1 (3), PALB2 (2, 1.7%), and BARD1 (2). Three (2.8%) reversion mutations were identified in 106 primary OC, 2 from patients with previous chemotherapy exposure for breast cancer. In 51 recurrent OC, 14 (27.5%) had reversion mutations. Of 27 patients with platinum-resistant or refractory recurrences, ten (37%) had reversions, compared with 1 (5.3%) in 19 patients with platinum sensitive recurrences (p=.016). Three secondary somatic mutations (17.6%) were novel frameshift mutations that restored the open reading frame, while 14 (82.4%) were reversion-to-wildtype sequence. All reversion mutations were identified in BRCA1 or BRCA2.

Conclusions: Reversion mutations were, with one exception, seen only in women previously treated with chemotherapy, and were associated with resistance to platinum chemotherapy. 80% of the reversion mutations were reversion-to-wildtype sequence, suggesting that current next-generation sequencing methods and cell free DNA tests will fail to identify the vast majority of reversion events.

[Poster Presentation]

Risk-reducing salpingo-oophorectomy: a single institutional experience in over 500 women at increased risk for ovarian carcinoma

Shannon Rush, University of Washington

Elizabeth Swisher MD, Rochelle Garcia MD, Kathy Agnew, Mark Kilgore MD, Barbara Norquist MD: all UW

Background: Risk-reducing salpingo-oophorectomy (RRSO) is an effective strategy to decrease cancer incidence and mortality in women at increased risk of ovarian carcinoma. We aimed to summarize our findings from a large prospective series of risk-reducing surgeries in high risk women.

Methods: Patients undergoing RRSO at the University of Washington between 1992 and 2017 who participated in a prospective registry were reviewed. All patients had a personal history of breast cancer, a family history of breast or ovarian cancer, or a known mutation in an ovarian cancer susceptibility gene. Genetic testing results were confirmed from original test reports or were obtained after surgery using the BROCA sequencing assay. All patients provided informed written consent.

Results. Risk-reducing surgery was performed on 511 women; 503 women underwent RRS0 and 7 women had a bilateral salpingectomy with plan for delayed oophorectomy in the future. 403 (78.9%) women had a pathogenic mutation in a gene increasing their risk for ovarian carcinoma including 193 in BRCA1, 174 in BRCA2, 7 in BRIP1, 6 in PALB2, 4 in BARD1, 3 in RAD51C, 2 in RAD51D, 5 in MSH2, 4 in MSH6, 2 in MLH1, 2 in PMS2, AND 1 in ATM. 108 (21.1%) women had negative genetic testing. 42 (8.2%) surgeries were performed prior to 2001 when a standardized protocol was adopted to serially section the entire fallopian tube for detailed pathological evaluation. 17 occult carcinomas or in situ high grade neoplasia were identified, all in patients with BRCA1 or BRCA2 mutations. Occult neoplasia was significantly more common in women with BRCA1 versus BRCA2 mutations occurring in 15 BRCA1 carriers (8.3%, median age 47 years, range 37-63 years) versus 2 BRCA2 carriers (1.1%, p=0.002, ages 46, 65 years). One occult carcinoma was a stage 1A endometrioid endometrial cancer in a thin 43 year old patient with a BRCA1 mutation and one was a stage 1A high grade carcinoma of the ovary in a 46 year old BRCA2 carrier. The remaining 15 (88.2%) occult neoplasia were all identified in the fallopian tube in cases with complete serial sectioning. One case of primary peritoneal carcinoma occurred 3 years after RRSO in a BRCA1 carrier being treated with chemotherapy for breast cancer at the time of RRSO. 36 (7.0%) RRSOs were performed for mutations in ovarian cancer susceptibility genes other than BRCA1 and BRCA2 and most took place in the last five years.

Conclusions: Women with BRCA1 mutations are at highest risk for occult carcinoma at the time of RRSO with the vast majority located in the fallopian tube. Primary peritoneal carcinoma after RRSO in patients with negative pathology and complete serial sectioning occurs only rarely and appears to be lower than the incidence identified in large registry studies with variable pathology. RRSO for women with mutations in ovarian cancer susceptibility genes beyond BRCA1 and BRCA2 is likely to become increasingly common.

[Oral Presentation]

Personalized Medicine: A CLIA-Certified High-Throughput Drug Screening Platform for Ovarian Cancer

Franz Schaub, SEngine Precision Medicine, Inc

Franz X Schaub, Rachele Rosati, Hallie Swan, Michael Churchill, Reid Shaw, Roland Watt, Robert Diaz, Stephanie Tatem Murphy, Shalini Pereira, VK Gadi and Carla Grandori

Background: Metastatic disease in ovarian cancer is difficult to treat and patients often exhaust standard-of-care regimens. To get a better understanding of potential treatments, genomic data is used in some cases. However, this only points to therapeutic options in a minority of cases highlighting an urgent need to develop assays to identify potential therapies. Here we present a CLIA certified high-throughput functional assay employing organoid cultures derived from primary patient material to directly benefit patients for personalized treatment selection (PARIS test).

Experimental Procedures: Organoids are treated for 6 days with a library of 123 clinically relevant drugs and viability is assessed by measuring ATP levels. The normalized dose-response curves are used to calculate the area under the curve (AUC) which is compared to an internal database of drug responses including more than 15 different tumor types. Unique responders are selected based on z-score calculation and lowest AUC. The results are further integrated with genomic data and reported to the clinician to highlight treatment options. SEngine has performed >100 drug screens and established high reproducibility including multiple ovarian cancer cases from either ascites, surgical or core biopsies.

Results: Here we present two n-of-one patient studies. Patient 1 is a 48 year old woman with positive family history who was diagnosed with late stage serous ovarian cancer. The tumor was found to be in-operable and positive for BRCA1 and TP53 mutations. SEngine generated cancer organoids and performed high-throughput screening with a panel of 123 drugs. Sensitivities to PARP inhibitors prompted SEngine to advise for BROCA testing which revealed a germline BRCA1 mutation. The cells were also uniquely sensitive to paclitaxel and the combination with carboplatin resulted in remission indicating concordance with clinical data. In addition, highlighting the importance of functional screening, a unique response to a sub-group of EGFR inhibitors was identified, of potential consideration in case of recurrence. Patient 2 also has a family history of ovarian cancer, but no BRCA mutation was detected. Genomic data indicated FGF6 and FGF23 amplification which directly corresponded to a unique sensitivity to one FGFR inhibitor (AZD-4547). The organoids were also resistant to PARP inhibitors, consistent with the lack of BRCA mutations.

Impact: We developed a robust ex vivo screening platform to objectively quantify patient specific sensitivity to a panel of 123 oncology drugs. SEngine is compiling a registry capturing clinical data, outcome following the PARIS test as well as genomic data. The power of high throughput technology and organoid isolation will enable the rapid selection of optimal individualized therapies as single agents or in combination.

[Oral Presentation]

BRD4 inhibition is synthetic lethal with PARP inhibitors through the induction of homologous recombination deficiency

Chaoyang Sun, MD Anderson Cancer Center (Knight Cancer Institute)

Chaoyang Sun, Jun Yin, Yong Fang, Gordon B. Mills

Poly(ADP-ribose) polymerase inhibitors (PARPi) are selectively active in cells with homologous recombination (HR) deficiency (HRD) caused by mutations in BRCA1/2 and other pathway members. We thus sought small molecule inhibitors that would induce HRD in HR competent cells. We demonstrate that depletion or inhibition of bromodomain containing 4 (BRD4) induced HRD, which was associated with increased PARPi sensitivity in vitro and in vivo. BRD4 inhibition (BRD4i) sensitizes cells to PARPi regardless of BRCA1/2, TP53, RAS, or BRAF status. Furthermore, BRD4 inhibition could reverse multiple mechanisms of resistance to PARPi. The combination of PARPi and BRD4i warrants clinical assessment in both PARPi sensitive and resistant cancers across multiple tumor lineages.

[Poster Presentation]

Validation of ProMisE molecular classifier in a large population based series: a new era in endometrial carcinoma diagnosis and treatment

Aline Talhouk, University of British Columbia

Stephan Kommoss, Melissa McConechy, Frieder Kommoss, Samuel Leung, David Huntsman, Blake Gilks, Jessica McAlpine

Background: Endometrial carcinoma (EC) is the most common gynecological malignancy in Canada. The current EC classification system and risk stratification tools are inadequate, prompting a push to incorporate molecular features that may provide insight to tumor biology. Based on the Cancer Genome Atlas, we previously developed and confirmed a pragmatic molecular classifier named ProMisE (Proactive Molecular Risk Classifier for Endometrial Cancer), which identifies four prognostically distinct molecular subtypes, and can be applied to diagnostic specimens (e.g., endometrial biopsy), thus enabling earlier informed decision-making. We are now on the final validation step following the Institute of Medicine (IOM) guidelines for genomic biomarker validation.

Methods: We assessed ProMisE molecular subtypes in an independent cohort (n=450) from the Tübingen University Women's Hospital, Germany. The prognostic ability of ProMisE was validated and compared to other risk stratification tools. We also validated the concordance of ProMisE subtype assignment between diagnostic endometrial specimens and final hysterectomy (n=156). Results: ProMisE is a prognostic marker for progression and disease-specific survival even after adjusting for known risk factors. Concordance between diagnostic and surgical specimens was highly favorable with accuracy of 0.91, and kappa0.87.

Conclusion: We have developed, confirmed and now validated a pragmatic molecular classification tool that provides consistent categorization of tumors and identifies four distinct prognostic molecular subtypes. The tool works on biopsy samples and thus could be used for surgical triage decisions. The assay can be considered valid and, based on the Institute of Medicine process, ready for clinical evaluation, likely through a prospective clinical trial.

[Oral Presentation]

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