August 2019 Volume 29 Issue Supplement 2

INTERNATIONAL JOURNAL OF GYNECOLOGICAL CANCER

jgc.bmj.com

ESGO European Society of Gynaecological Oncology

IGCS INTERNATIONAL GYNECOLOGIC CANCER SOCIETY

BMJ

INTERNATIONAL JOURNAL OF
GYNECOLOGICAL CANCER

Editorial Office
International Journal of Gynecological Cancer, BMJ Journals,
Two Hudson Square, 3rd Floor,
Hoboken, NJ 07030 USA
E: **ijgc@jjeditorial.com**

Senior Production Editor
Eleanor Naylor
E: **production.ijgc@bmj.com**

Customer support
For general queries and support with existing and new subscriptions:
W: **support.bmj.com**
T: **+44 (0)20 7111 1105**
E: **support@bmj.com**

Self-archiving and permissions
W: **bmj.com/company/products-services/rights-and-licensing/**
E: **bmj.permissions@bmj.com**

Advertising
W: **bmj.com/company/for-advertisers-and-sponsor/**

Display Advertising–Rest of world
Sophie Fitzsimmons
T: **+44 (0)20 3655 5612**
E: **sfitzsimmons@bmj.com**

Online Advertising–Rest of world
Marc Clifford
T: **+44 (0)20 3655 5610**
E: **mclifford@bmj.com**

Display & Online Advertising Americas
American Medical Communications (AMC)
T: **+1 973 214 4374**
E: **rgordon@americanmedicalcomm.com**

Reprints
Author Reprints
BMJ Reprints Team
E: **admin.reprints@bmj.com**

Commercial Reprints–Rest of world
Nadia Gurney-Randall
M: **+44 (0)7866 262 344**
E: **ngurneyrandall@bmj.com**

Commercial Reprints Americas
Ray Thibodeau
T: **+1 267 895 1758**
M: **+1 215 933 8484**
E: **ray.thibodeau@contentednet.com**

The International Journal of Gynecological Cancer (*IJGC*) is published by BMJ Publishing Group Ltd and typeset by Exeter Premedia Services Private Limited, Chennai, India.

Aims and Scope: The *International Journal of Gynecological Cancer* (*IJGC*) is the official scientific journal of the International Gynecologic Cancer Society (IGCS) and the European Society of Gynaecological Oncology (ESGO). Published 9 times per year, *IJGC* features articles relevant to detection, prevention, diagnosis, and treatment of gynecologic malignancies. *IJGC*'s audience is gynecologists, medical oncologists, radiation oncologists, radiologists, pathologists, and research scientists with a special interest in gynecologic oncology.

Guidelines for Authors and Reviewers

Full instructions are available online at https://ijgc.bmj.com/pages/authors/. Articles must be submitted electronically https://mc.manuscriptcentral.com/ijgcancer. Authors retain copyright but are required to grant the *International Journal of Gynecological Cancer* an exclusive licence to publish https://ijgc.bmj.com/pages/authors/.

Subscription Information

International Journal of Gynecological Cancer is published nine times a year; subscribers receive all supplements

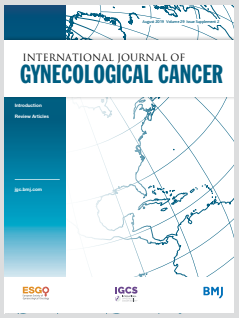
Personal rates 2019

ISSN 2056-6697 (online)
Online only £275/€372/$575

Site licences are priced on FTE basis and allow access by a whole institution.

For details on how to subscribe, institutional pricing and payment methods contact the Subscription Manager in London: tel +44 (0)20 7111 1105; support@bmj.com

Residents of some EC countries and Canada must pay VAT for online subscriptions: for details please visit support@bmj.com or contact the Subscription Manager in London: tel +44 (0)20 7111 1105; support@bmj.com



Editorial Office
International Journal of Gynecological Cancer, BMJ Journals
Two Hudson Square, 3rd Floor,
Hoboken, NJ 07030 USA
E: ijgc@jjeditorial.com

Senior Production Editor
Eleanor Naylor
E: production.ijgc@bmj.com

Customer support
For general queries and support with existing and new subscriptions:
W: support.bmj.com
T: +44 (0)20 7111 1105
E: support@bmj.com

Self-archiving and permissions
W: bmj.com/company/productsservices/rights-and-licensing/
E: bmj.permissions@bmj.com

The International Journal of Gynecological Cancer (*IJGC*) is published by BMJ Publishing Group Ltd and typeset by Exeter Premedia Services Private Limited, Chennai, India.

Introduction

Review Articles

INTERNATIONAL JOURNAL OF GYNECOLOGICAL CANCER

OPEN ACCESS

Introduction: the 12th Biennial Rivkin Center Ovarian Cancer Research Symposium

Nita J Maihle,[1] Douglas A Levine,[2] Kiran Dhillon,[3] Deborah Kay Armstrong[4]

[1]University of Mississippi Medical Center, Jackson, Mississippi, USA
[2]NYU Langone Medical Center, New York City, New York, USA
[3]Rivkin Center for Ovarian Cancer, Seattle, Washington, USA
[4]Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, Maryland, USA

Correspondence to
Dr Kiran Dhillon, Rivkin Center for Ovarian Cancer, Seattle, WA 98122, USA; kiran.dhillon@swedish.org

Received 5 April 2019
Accepted 10 April 2019

To cite: Maihle NJ, Levine DA, Dhillon K, *et al. Int J Gynecol Cancer* 2019;**29**:s1.

ABSTRACT

In September 2018, the 12th Biennial Ovarian Cancer Research Symposium was presented by the Rivkin Center for Ovarian Cancer and the American Association for Cancer Research, in Seattle, WA, USA. The 2018 Symposium focused on four broad areas of research: Detection and Prevention of Ovarian Cancer, Genomics and Molecular Mechanisms of Ovarian Cancer, Tumor Microenvironment and Immunology of Ovarian Cancer, and Novel Therapeutics: Response and Resistance of Ovarian Cancer. In addition, a special panel on the 'Role of Advocates in Ovarian Cancer Research' was featured.

In September 2018, the 12th Biennial Ovarian Cancer Research Symposium was presented by the Rivkin Center for Ovarian Cancer and the American Association for Cancer Research (AACR). The goal of this Symposium was to bring together clinicians and researchers from across disciplines and institutions worldwide to share new knowledge to advance the field of ovarian cancer research. The conference sought to enhance our understanding of this disease, with the intent of using this information to improve the prevention, early detection, and treatment of ovarian cancer.

A particular focus of this Symposium was the inclusion of early-career investigators during the planning process. During this year's Symposium, established scientists partnered with early-career investigators to facilitate each session. The 2018 Symposium focused on four broad areas of research: Detection and Prevention of Ovarian Cancer, Genomics and Molecular Mechanisms of Ovarian Cancer, Tumor Microenvironment and Immunology of Ovarian Cancer, and Novel Therapeutics: Response and Resistance of Ovarian Cancer.

The abstracts[1] from each of these research areas will be published in *Clinical Cancer Research*[2] and are reviewed in this supplement to the *International Journal of Gynecological Cancer*. Reflecting a priority of the Rivkin Center, to encourage and support young investigators in the field of ovarian cancer research, these reviews are authored by early-career investigators who are scholars in the Department of Defense-sponsored Ovarian Cancer Academy (OCA) (http://www.ovariancanceracademy.org). OCA members compete for these mentored awards through the Department of Defense's Congressionally Directed Medical Research Programme (https://cdmrp.army.mil/ocrp/).

The Rivkin Center (www.rivkin.org) was founded in 1996 by Swedish Cancer Institute medical oncologist Saul E Rivkin, MD (now retired), in memory of his wife, Marsha, who lost her life to ovarian cancer. The organization provides seed funding for researchers exploring promising new areas of investigation in the field of ovarian cancer. Areas of priority include innovative research pilot studies, scientific scholar awards for new investigators, and bridge funding awards. To date, the Rivkin Center has awarded more than $13 million in scientific research awards and has a goal to continue to increase its research funding annually. A major focus of the Rivkin Center has been the biennial meetings, held now for more than 20 years, to promote educational and intellectual interactions and collaborations across the spectrum of ovarian cancer clinical and basic science research.

In 2014, the Rivkin Center partnered with the AACR to broaden the scope of research opportunities and information sharing for investigators focused on the study of ovarian cancer. The AACR now organizes biennial fall research symposia alternating with the Rivkin meetings. This fruitful partnership has served to expand the range of clinical, translational, and basic science collaborations in the field of ovarian cancer research, as reflected in the spectrum of abstracts reviewed in the research summaries presented in this supplement. In addition to these summaries, a representative of the patient advocacy community has highlighted perspectives shared during the panel discussion on emerging roles for consumer advocates as medicine and biomedical research become more patient-centric.

Contributors This introduction was written by NJM and DKA, with contributions from DAL and KD.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; internally peer reviewed.

REFERENCES

1. Symposium abstract book pdf. Available: https://www.rivkin.org/wp-content/uploads/2018/09/2018-Ovarian-Cancer-Research-Symposium-Program.pdf
2. Available: http://aacrjournals.org/site/Meetings/meeting_abs.xhtml

Review Article

INTERNATIONAL JOURNAL OF GYNECOLOGICAL CANCER

OPEN ACCESS

Meeting report from the 2018 12th Biennial Ovarian Cancer Research Symposium detection and prevention of ovarian cancer

Wa Xian,[1] Sophia George[2]

[1]IMM, University of Texas Health Science Center at Houston, Houston, Texas, USA
[2]Sylvester Comprehensive Cancer Center, Miami, Florida, USA

Correspondence to
Dr Wa Xian, IMM, University of Texas Health Science Center, Houston, TX 77030, USA; wa.xian@uth.tmc.edu

Received 11 April 2019
Accepted 3 May 2019

To cite: Xian W, George S. *Int J Gynecol Cancer* 2019;**29**:s2–s6.

ABSTRACT

The objective of this review is to summarize recent research advances in the detection and prevention of ovarian cancer and discuss the experts' opinions of future directions. The 12th Biennial Ovarian Cancer Research Symposium was held in Seattle, Washington, in September 2018. At this meeting, experts in ovarian cancer research gathered to present and discuss recent breakthroughs and their visions of future ovarian cancer research. Session 1 of the symposium focused on the detection and prevention of ovarian cancer. It included two invited oral presentations from Ranjit Manchanda, MD, PhD (Barts Cancer Institute) and Rosana Risques, PhD (University of Washington). Another eight oral presentations were selected from abstract submissions. Fifteen abstracts were presented in poster format. These presentations covered topics including cellular origin of high-grade serous cancer, risk factors for ovarian cancer, new methods for early detection of ovarian cancer, mechanisms underlying ovarian cancer development, and new therapeutic approaches for preventing ovarian cancer from forming or progressing. In conclusion, a clear understanding of the cellular origin and molecular mechanisms underlying the initiation of high-grade serous cancer is essential for developing effective means for early detection and prevention of this most devastating type of ovarian cancer. Recognizing the complexity of ovarian cancer and appreciating that ovarian cancer is not a single disease will help us to generate proper models, design rational experiments, and collect and analyze patient data in a meaningful way. A concerted effort in the field will help to bridge the basic science and clinical applications and lead to more precise and effective detection and treatment.

Epithelial ovarian cancer is a disease with poor prognosis. It is the fifth most common cause of death from cancer in women, and is the most lethal of all gynecological cancers. The lifetime risk of a woman developing ovarian cancer is 1 in 71, and 1 in 200 women will develop ovarian cancer between their 50th and 70th birthday. Worldwide, 224 747 new cases of ovarian cancer are diagnosed annually and there are an estimated 140 163 disease-related deaths.[1] Ninety percent of all deaths from ovarian cancer are due to high-grade serous cancer, and this cancer sub-type accounts for 75% of all cases.[2] Despite recognition of the importance of early detection and rapid progress in our understanding of the cellular origin of high-grade serous cancer, only 2% of cases can be identified at stage I.[3] As a consequence, up to 80% of women present with stages III/IV disease, and the 5-year survival rate is just 30%. This severe mortality and poor survival rates have not changed much since the 1930s.[4] Therefore, there is an urgent and unmet medical need for precise diagnosis and effective treatment for this disease at earlier stages, where the survival rate is >90%, as we have achieved in cervical cancer and breast cancer[5] after the biology/origin of these two women's cancers were unraveled.

At the 12th Ovarian Cancer Research Symposium at the Rivkin Center for Ovarian Cancer in Seattle, Washington, one session focused on the detection and prevention of ovarian cancer. Diverse topics were covered in this session, including basic biological questions such as the cellular origin of high-grade serous cancer and clinical applications such as diagnostic markers for early detection. Dr Ranjit Manchanda (Barts Cancer Institute) presented a population-based test for ovarian cancer gene mutations. He discussed the limitations associated with the current system of genetic testing, based on clinical criteria/family history, and presented data supporting the promising outcome of population-based *BRCA* testing in the Jewish population. Dr Manchanda further extended the testing for established cancer genes in non-Jewish women and suggested surgical prevention is a cost-effective approach in women at high risk identified by this test.

Dr Rosana Risques (University of Washington) also presented her recent research on using ultra-sensitive sequencing tools for detection of ovarian cancer. The presentation by Dr Risque centered on deep sequencing of genital tract fluids for *TP53* mutations using techniques to identify very small numbers of affected cells. They demonstrated the presence of *TP53* mutations in these fluids in women with ovarian cancer and also in 100% of controls. These findings underline the fact that cells containing *TP53* mutations are present in the peritoneal or other genital tract fluids of virtually all women, increasing with age and in keeping with a 'pre-malignant mutation background' discussed in their previous paper.[6] Interestingly, it complements the model proposed by Soong

et al in a subsequent presentation, in which genetically altered but non-malignant precursor cells escape from the fallopian tubes and, in some instances, culminate in high-grade serous cancer in the peritoneal cavity.[7] The two studies superimposed on prior work attest to both the frequency of *TP53* mutations in the fallopian tube and the likelihood that such cells commonly exist in the peritoneal fluid. The mechanism(s) by which malignancy occurs and who is at risk for a rare occurrence of malignant transformation remains to be determined.

In addition, Ms Heydrich and colleagues (Color Genomics) presented their studies in Texas supporting the benefit of providing broad access to genetic testing in the obstetrics and gynecology care setting. Using a next-generation, sequencing-based assessment of 30 genes associated with hereditary cancer risk, they identified 90 patients with increased risk for cancer among 1113 people included in this study. Significantly, 70 of the 90 identified as at increased risk had no significant family history of ovarian cancer and would not have been obvious candidates for screening. This finding suggests that broader genetic testing would be beneficial in identifying a larger set of patients at risk.

Developing rational and effective screening and cancer preventive strategies for ovarian cancer, particularly high-grade serous cancer, requires a better understanding of the origin of this disease. During the past decade, clinical and molecular evidence has increasingly suggested that many, if not all, cases of high-grade serous cancer arise from the fallopian tube.[8] In the original serous carcinogenesis model, high-grade serous cancer was thought to arise through dissemination of tumor cells from serous tubal intra-epithelial carcinomas.[9] However, many cases are found not to be associated with serous tubal intra-epithelial carcinomas. Drs Thing Rinda Soong and Christopher Crum (University of Washington and Harvard Medical School, respectively) presented data to support a new alternative model termed 'precursor escape'. In this model, a pre-cancerous lesion termed 'early serous proliferation'" bearing p53 mutations is found in the distal fallopian tube of patients with high-grade serous cancer.[10] Their study indicates for the first time a lineage continuity between early serous proliferation in the distal tube and some metastatic high-grade serous cancer via shared, site-specific TP53 mutations, explaining the apparent sudden onset of cancers without co-existing serous tubal intra-epithelial carcinomas. This new pre-cancer–cancer paradigm for high-grade serous cancer suggests that precursor initiation and progression to malignancy are separated spatially and temporally.

The study presented by Dr Burdette (University of Illinois, Chicago) explained why the ovary is the primary metastasis site for tumors derived from the fallopian tube. Using imaging mass spectrometry, Dr Burdette and colleagues found that norepinephrine and a series of new molecules may facilitate the colonization of fallopian tube-derived tumor cells on the ovarian surface. Therapeutic intervention blocking ovarian metastasis can be developed targeting pathways activated by these molecules.

Using genetic mouse models, Drs Shuang Zhang and Benjamin Neel (New York University-Langone Medical Center) showed that PAX8 + fallopian tube cells can be transformed in the presence of combined retinoblastoma inactivation and *TP53* mutations and give rise to serous cancer. Dr Zhang and colleagues also claimed that under the same conditions, ovarian epithelial cells can be transformed to form serous-like tumors. It is important to notice that the serous-like tumors generated from transformed ovarian surface epithelial cells do not express PAX8, a hallmark of high-grade serous cancer, whereas the tumors generated from transformed fallopian tube epithelial cells express all the hallmarks of this cancer as shown in publications from various laboratories.[11 12] A presentation given by Dr Sophia George (University of Miami) further investigated the fallopian tube as the site of origin of high-grade serous cancer by performing RNAseq analysis of fallopian tube from patients with and without *BRCA* mutations. The women carrying a deleterious mutation in the *BRCA1/2* genes have an increased risk of this cancer of up to 40%. Dr George and colleagues found that the fallopian tube epithelium of a *BRAC1/2* carrier shows distinct unique pre-neoplastic processes, such as increased metabolic activity and aberrant regulation of DNA repair pathways. Their study using a *BRCA* model may provide new insights into the early development of ovarian cancer.

Kendall Greening from Dr David Huntsman's laboratory (University of British Columbia) presented preliminary data suggesting that 42% of post-menopausal women had p53 lesions in the fallopian tube. They plan to study the effect of the use of oral contraceptive pills and its association with >40% reduction in the risk of high-grade serous cancer by studying p53 lesions in the fallopian tubes. Their study may disclose the impact of oral contraceptive pills on the earliest known precursors of serous cancer. Dr Angela Russo and colleagues (University of Illinois at Chicago) presented their work on the effect of loss of PAX2 and PTEN in the fallopian tube epithelium.[13] Their data suggested that loss of PTEN or PAX2 mediated a cancer stem cell phenotype that initiates formation of high-grade serous cancer. These findings may help to define early events of carcinogenesis and help to refine the strategies of targeted therapeutics or marker discovery for early detection of serous cancer. Dr Kara Bernstein and colleagues (University of Pittsburg) presented their work on characterization of cancer-associated mutations in the RAD51 paralogs on homologous recombination proficiency. This may help us to develop more effective predictive models of targeted therapies, such as poly-ADP-ribose polymerase inhibitors. Using yeast 2/3-hybrid assays, they found that mutations in RAD51 disrupted the interaction between RAD51 and XRCC2, a protein required for the early response of RAD51 to DNA damage,[14] and subsequently affect homologous recombination proficiency.

Several presentations mentioned recent progress with tools to deal with the unmet clinical need for early detection of ovarian cancer. The recent discovery of fallopian tube as the site of origin of high-grade serous cancer has re-shaped strategies of early detection.[15] For instance, Dr Jennifer Barton (University of Arizona) presented her work on a second-generation falloposcope for minimally invasive imaging of the fallopian tube. They propose to use this imaging test as an adjunct confirmatory test after an initial positive or suspicious blood test with known or recently uncovered markers for serous tubal intra-epithelial carcinoma lesions in their laboratory. Their continuous work on identifying sensitive and specific serum protein markers and optimizing the falloposcope may create a reliable and efficient detection method for general screening of the general population for early epithelial ovarian cancer.

Dr Amy Skubitz (University of Minnesota) and colleagues presented their work using Proseek Multiplex Oncology II plates to simultaneously measure the expression of 92 cancer-related

proteins in serum in order to bypass the inability of CA125 and HE4 screening of the general population to identify early-stage disease. Their analysis of women with advanced serous cancer compared with age-matched healthy women showed that CA125 alone achieved a sensitivity of 93.4%, but by adding five proteins to CA125, they increased sensitivity to 98.4%. They hope that this Proseek technology will help to identify biomarkers to improve the sensitivity and specificity of detection methods for early stages of high-grade serous cancer.

Dr Karen Belkic (Karolinska Institution) summarized the key achievement of the fast Pade transform in magnetic resonance spectroscopy for diagnosis of early ovarian cancer. Their meta-analysis showed that cancerous and benign ovarian lesions are inadequately distinguished via fast Fourier transform-based magnetic resonance spectroscopy. In contrast, the high-order, non-parametric fast Pade transform has clear display with identification and exact quantification of key metabolic transformation, including the ovarian cancer biomarker phosphocholine. Their next step is pursuing this strategy in vivo for diagnosis of ovarian cancer. Dr Kristin Boylan and colleagues from the University of Minnesota, also used mass spectrometry-based proteomics methods for early detection. They hypothesized that ovarian cancer cells can be detected during a routine Pap test performed for cervical cancer prevention. Given the convincing data supporting the fallopian tube as the site of origin of ovarian cancer, they think it is likely that ovarian cancer protein can be found in the lower genital tract, perhaps even in the early stages. Using the extract from patient's tumor tissue run on 2D-liquid chromatography tandem mass spectrometry, followed by bioinformatics integration, they identified thousands of protein markers shared by several patients, including well-known markers of ovarian cancer such as CA125. Their further analysis using patient-matched normal tissues will help to uncover cancer-specific markers that could be used for the quantification of proteins from Pap test fixatives and cervical swabs for ovarian cancer protection.

Dr Naoko Sasamoto (Brigham and Women's Hospital) and colleagues presented their efforts on improving the efficiency and accuracy of using CA125 as a prediction method. They hypothesized that the distinct personal characteristics among individuals contributed to the low specificity of CA125 as an ovarian cancer screening biomarker. Thus they proposed to identify personal characteristics that influence CA125 levels in order to create personalized thresholds for CA125, thereby improving its performance as an ovarian cancer screening biomarker. They conducted internal and external validation of two prediction models (linear and dichotomous) of circulating CA125 among post-menopausal women using 28 842 controls without ovarian cancer in four large population-based studies. Although both models appeared to provide some improvement to the CA125 method, a further fine-tuning of these models is required to increase the predictive ability of these models.

Estrogen-induced DNA damage may contribute to the early development of ovarian cancer.[16] To overcome the technical challenges in detecting and analyzing the variety of different DNA lesions that are formed by estrogen compounds, Dr Kaushlendra Tripathi (University of Alabama) and colleagues have developed a new method. They used biotinylated estrogens to allow immunodetection of estrogen-induced DNA adducts by slot-blot and single-cell molecular cloning and proximity ligation assays. Using this method, they quantitatively detected these adducts on DNA and showed that estrogen activates replication-associated DNA damage response and induces chromosomal instability. Thus, the biotin-labeled estrogens could be used as a tool to detect the early stage of ovarian carcinogenesis.

Several research groups presented their work on identifying risk factors for the development of ovarian cancer that might reduce its burden. Dr Kara Michels (National Cancer Institute) presented a study examining the association between metabolic dysregulation and development of ovarian cancer by a case–control study within the Surveillance, Epidemiology and End Results Medicare linked database. Their results suggest that individual components of metabolic syndrome, rather than the syndrome itself, are associated with ovarian cancer. Thus high levels of triglycerides are associated with an increased risk of high-grade serous cancer, whereas a high level of fasting glucose is linked with a reduced risks for this cancer.

Dr Holly Harris (Fred Hutchinson Cancer Research Center) and colleagues presented their work on the use of Mendelian randomization to examine the association between polycystic ovary syndrome and ovarian cancer. They evaluated the single nucleotide polymorphisms associated with polycystic ovary syndrome using publicly available data from genome-wide association studies. Based on seven associated single nucleotide polymorphisms, they found an inverse association between genetically predicted polycystic ovary syndrome and high-grade serous cancer and endometrioid tumors.

As full-term births have been known to be protective for ovarian cancer,[17] Dr Alice Lee (California State University) and colleagues presented their work on incomplete pregnancies and risk of ovarian cancer based on the pooled epidemiologic data from 16 population-based, case–control studies from the Ovarian Cancer Association Consortium. They found that both incomplete and complete pregnancies are protective against ovarian carcinogenesis, although full births are more protective. The protective association is strongest for clear cell ovarian cancer and less apparent for high-grade serous cancer and mucinous ovarian cancers. Randomized trials and recent meta-analysis have shown better survival for women who took hormone therapy after their diagnosis in comparison with women who did not.[18] Dr Celeste Pearce from the same research group (California State University) presented their study on the relationship between use of hormone therapy before ovarian cancer diagnosis. They analyzed 4700 patients with ovarian cancer recorded in the Ovarian Cancer Association Consortium and found that women who used hormone therapy before diagnosis had an 11% decreased risk of death compared with those who did not. It appears that the longer duration confers better survival in both serous and mucinous ovarian cancer.

Other factors linked with reduced ovarian cancer risks are higher parity and oral contraceptive use.[19] In order to understand the association between the lifetime number of ovulatory cycles and ovarian cancer risk, Dr Britton Trabert and colleagues (National Cancer Institute) analyzed 3866 cases of ovarian cancer collected by the Ovarian Cancer Cohort Consortium. In this large prospective analysis of pooled cohort study data, they observed a positive association between increased risk of ovarian cancer of several histotypes, including serous, endometrioid, and clear cell tumors, but not mucinous tumors. Their data suggest that a DNA damage-rich

environment created by ovulation at the ovary surface and within the fallopian tube increases the risk of ovarian carcinogenesis.

Dr Faina Linkov (University of Pittsburgh) presented their study of 1840 patients with ovarian cancer at the University of Pittsburgh Medical Center facilities and concluded that intra-peritoneal chemotherapy showed enhanced long-term survival of patients. Since intra-peritoneal chemotherapy has not been widely used outside specialty hospitals, increasing its use in clinical practice for the treatment of patients with ovarian cancer may improve outcomes.

Christina Clarke (Kaiser Permanente Colorado) and colleagues presented a retrospective observational study of high-grade serous cancer to explore predictors of long-term survival, using data from five participating health plans in the Cancer Research Network. They confirmed that younger age, lower stage, and receipt of chemotherapy were statistically significantly associated with long-term survival.

In this session, a few researchers also presented their findings for the development of new approaches for early treatment. Tiffany Lam and colleagues (University of Minnesota) hypothesized that some tumor cells can evade initial chemotherapy treatment by entering a dormant state. They used a silica gel encapsulation platform to capture the subset of cells capable of dormancy. They observed a connection between cells' ability to enter dormancy and chemoresistance in ovarian cancer. Therefore they suggest that silica gel technology might be used as a predictive clinical tool to identify patients at risk of early recurrence or serve as a research tool to study the mechanisms underlying dormancy, chemoresistance, and recurrence.

Dr Chang Li (University of Washington) and colleagues presented their attempts to develop preventive approaches for patients with ovarian cancer at high-risk of disease recurrence or patients with cancer-predisposing inherited mutations. Their approach is based on in vivo genetic modification of hematopoietic stem cells.[20] By microRNAseq and microRNA arrays, these researchers identified microRNAs that were absent in tumor-associated leukocytes, allowing for tumor-restricted therapeutic transgene expressions by inserting these microRNAs in the 3' untranslated region of the transgenes. They hypothesized that these genetically engineered tumor-associated neutrophils and macrophages can overcome the immunosuppressive tumor environment allowing effector T-lymphocyte cells to stop tumor growth at an early stage. They are using oncogene-transgenic mice that develop spontaneous tumors to test this hypothesis.

CONCLUSION

The American Cancer Society describes the 'signs' of ovarian cancer as 'bloating, abdominal pain, a feeling of fullness'—clearly all indications of late-stage disease. Indeed markers such as CA125 and imaging by transvaginal ultrasound also report frank cancer which, if not cured by surgery, represents a largely protracted medical struggle with limited odds of success.[21] It would seem that the future should include early detection afforded by the large period of time presented by most pre-cancerous lesions and non-invasive cancer form of other organs. A molecular analysis of an early lesion that could be tied to high-grade serous cancer would provide an array of targets that are either secreted by these cells or presented on the cell surface, with potential screening and therapeutic value, respectively. Monoclonal antibodies to secreted proteins have the potential to form the basis of population-wide screening methods from blood or cervical fluid for those at risk who might benefit from salpingectomy. Monoclonal antibodies to surface proteins of the cells in these lesions might assist in alternative detection via imaging technologies through the fallopian tube isthmus, similar to the confocal endoscopy performed today with Barrett's esophagus. They might also provide potentially non-invasive means of eradicating these early lesions though cytotoxic effects. While it seems that imaging technologies akin to the endoscopy and colonoscopy employed are not yet available for monitoring the fallopian tube, it would seem with the rapid pace of development that the fallopian tube will soon be within range of these modalities.

One goal of all cancer therapy is to provide early screening and pre-emptive intervention to avoid the challenges presented by highly metastatic cancers. Thus, the stages of cancer have a huge influence on the outcome. Early diagnosis of cancer will fundamentally affect the management of these tumors. The Ovarian Cancer Research Society has started to explore the current hypothesis that the fallopian tube is the origin of high-grade serous cancer and aims to develop new, sophisticated and yet simple strategies to detect the cancer in its earliest stage: the pre-cancerous lesion. If successful, we would have filled an important unmet medical need that has been troubling the medical profession for decades. Continuously developing new knowledge in this field is essential for us to develop medical diagnostic technologies that might save lives and improve the quality of global healthcare.

Acknowledgements We thank Dr Christopher P Crum for advice and support.

Contributors WX and SG wrote the paper together.

Funding This work was supported by grants from the U.S. Department of Defense (W81XWH-17-1-0123 to WX and W81XWH1810072 to SG), the Cancer Prevention Research Institute of Texas (CPRIT; RR150104 to WX). The work was supported by the DOD OCRP Early Career Investigator Award W81XWH-17-1-0123 (WX) and DOD OCRP Early Career Investigator Award W81XWH1810072 (SG).

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Commissioned; internally peer reviewed.

REFERENCES

1. Ferlay J, Shin H-R, Bray F, *et al*. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010;127:2893–917.
2. Cho KR, Shih I-M, IeM S. Ovarian cancer. *Annu. Rev. Pathol. Mech. Dis.* 2009;4:287–313.
3. Seidman JD, Zhao P, Yemelyanova A. "Primary peritoneal" high-grade serous carcinoma is very likely metastatic from serous tubal intraepithelial carcinoma: Assessing the new paradigm of ovarian and pelvic serous carcinogenesis and its implications for screening for ovarian cancer. *Gynecol Oncol* 2011;120:470–3.
4. Jemal A, Siegel R, Ward E, *et al*. Cancer statistics, 2008. *CA Cancer J Clin* 2008;58:71–96.
5. Surveillance epidemiology and end results fact sheet (Cancer of the breast) from. Available: www.cancer.gov

6. Krimmel JD, Schmitt MW, Harrell MI, *et al*. Ultra-deep sequencing detects ovarian cancer cells in peritoneal fluid and reveals somatic *TP53* mutations in noncancerous tissues. *Proc Natl Acad Sci U S A* 2016;113:6005–10.
7. Soong TR, Dinulescu DM, Xian W, *et al*. Frontiers in the pathology and pathogenesis of ovarian cancer: cancer precursors and "Precursor Escape". *Hematol Oncol Clin North Am* 2018;32:915–28.
8. Lee Y, Miron A, Drapkin R, *et al*. A candidate precursor to serous carcinoma that originates in the distal fallopian tube. *J Pathol* 2007;211:26–35.
9. Carlson JW, Miron A, Jarboe EA, *et al*. Serous tubal intraepithelial carcinoma: its potential role in primary peritoneal serous carcinoma and serous cancer prevention. *J Clin Oncol* 2008;26:4160–5.
10. Soong TR, Howitt BE, Miron A, *et al*. Evidence for lineage continuity between early serous proliferations (ESPs) in the fallopian tube and disseminated high-grade serous carcinomas. *J Pathol* 2018;246:344–51.
11. Yamamoto Y, Ning G, Howitt BE, *et al*. *In vitro* and *in vivo* correlates of physiological and neoplastic human Fallopian tube stem cells. *J Pathol* 2016;238:519–30.
12. Perets R, Wyant GA, Muto KW, *et al*. Transformation of the fallopian tube secretory epithelium leads to high-grade serous ovarian cancer in Brca;Tp53;Pten models. *Cancer Cell* 2013;24:751–65.
13. Russo A, Czarnecki AA, Dean M, *et al*. PTEN loss in the fallopian tube induces hyperplasia and ovarian tumor formation. *Oncogene* 2018;37:1976–90.
14. Tambini CE, Spink KG, Ross CJ, *et al*. The importance of XRCC2 in RAD51-related DNA damage repair. *DNA Repair* 2010;9:517–25.
15. Crum CP, McKeon FD, Xian W. The oviduct and ovarian cancer: causality, clinical implications, and "targeted prevention". *Clin Obstet Gynecol* 2012;55:24–35.
16. Cavalieri EL, Rogan EG. A unified mechanism in the initiation of cancer. *Ann N Y Acad Sci* 2002;959:341–54.
17. Fortner RT, Ose J, Merritt MA, *et al*. Reproductive and hormone-related risk factors for epithelial ovarian cancer by histologic pathways, invasiveness and histologic subtypes: results from the EPIC cohort. *Int J Cancer* 2015;137:1196–208.
18. Temkin SM, Mallen A, Bellavance E, *et al*. The role of menopausal hormone therapy in women with or at risk of ovarian and breast cancers: misconceptions and current directions. *Cancer* 2019;125:499–514.
19. Doherty JA, Jensen A, Kelemen LE, *et al*. Current gaps in ovarian cancer epidemiology: the need for new population-based research. *J Natl Cancer Inst* 2017;109. doi:10.1093/jnci/djx144
20. Wang H, Georgakopoulou A, Psatha N, *et al*. In vivo hematopoietic stem cell gene therapy ameliorates murine thalassemia intermedia. *J Clin Invest* 2019;129:598–615.
21. Reade CJ, Riva JJ, Busse JW, *et al*. Risks and benefits of screening asymptomatic women for ovarian cancer: a systematic review and meta-analysis. *Gynecol Oncol* 2013;130:674–81.

INTERNATIONAL JOURNAL OF GYNECOLOGICAL CANCER

OPEN ACCESS

Genomics and molecular mechanisms of high grade serous ovarian cancer: the 12th Biennial Rivkin Center Ovarian Cancer Research Symposium

Erinn B Rankin

Radiation Oncology and Obstetrics and Gynecology, Stanford University, Stanford, California, USA

Correspondence to
Dr Erinn B Rankin, Radiation Oncology and Obstetrics and Gynecology, Stanford University, Stanford, CA 94305, USA; erankin@stanford.edu

Received 4 April 2019
Revised 19 June 2019
Accepted 21 June 2019

To cite: Rankin EB. *Int J Gynecol Cancer* 2019;**29**:s7–s11.

ABSTRACT

Objective The aim of this study was to review current research efforts in genomics and molecular mechanisms of high grade serous ovarian cancer, presented at the 12th Biennial Rivkin Center Ovarian Cancer Research Symposium, held at the University of Washington.

Methods The 12th Biennial Rivkin Center Ovarian Cancer Research Symposium brought together leaders in the field to discuss recent advances in ovarian cancer research and therapy.

Results The genomics and molecular mechanisms of ovarian cancer session featured invited speaker presentations by Dr Alan D' Andrea on 'Deoxyribonucleic acid (DNA) repair in ovarian cancer' and Dr Kathleen Cho on 'Modeling the genomics of high grade serous carcinoma in the mouse'. Eight additional oral presentations and 46 poster presentations were selected from the submitted abstracts that highlighted current research efforts in p53, DNA repair, genomic instability and modeling disease in mice, and organoids in high grade serous ovarian cancer.

Conclusions New technologies utilizing clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (CAS9) approaches in mice, organoids, and cell based screens continue to advance our knowledge of key molecular drivers of ovarian cancer initiation, progression, and drug resistance. Improved understanding of the mechanisms of poly ADP ribose polymerase inhibitor resistance may lead to new therapeutic strategies to enhance outcomes in women with high grade serous ovarian cancer.

INTRODUCTION

Ovarian cancer is a heterogeneous disease with multiple subtypes that are classified based on distinct histological and genetic features. The most common and lethal subtype of ovarian cancer is high grade serous ovarian cancer. Approximately 70% of women diagnosed with high grade serous ovarian cancer present with advanced disease where the tumor has disseminated beyond the ovaries and pelvic organs to the peritoneum and abdominal organs, including the diaphragm, stomach, omentum, liver, and intestines.[1 2] Common mutations associated with the development of high grade serous ovarian cancer include *TP53* mutations and *BRCA1/2* mutations.[3] At the genomic level, high grade serous ovarian cancer is also characterized by recurrent deoxyribonucleic acid (DNA) copy number alterations, making this cancer genomically unstable.[4] These clinical findings suggest an important role for p53, DNA repair, and genomic instability in the pathogenesis of high grade serous ovarian cancer, and were a focus of the research presented at the 12th Biennial Rivkin Center Ovarian Cancer Research Symposium during the session on genomics and molecular mechanisms of ovarian cancer.

Here we will summarize the key topics discussed in this session that was held on September 13–15, 2018, at the University of Washington.

P53 AND HIGH GRADE SEROUS OVARIAN CANCER

The tumor suppressor p53 is known as the guardian of the genome due to its central role in regulating DNA damage responses. A variety of stimuli, including DNA damage, nutrient starvation, and oncogenic signaling, activate p53 signaling to modulate cell cycle arrest, apoptosis, ferroptosis, senescence, oncogenic signaling, metabolic reprogramming, differentiation, invasion, and signaling within the tumor microenvironment (for a recent review see Mello and Attardi[5]). Mutations in *TP53* are found in 96% of cases of high grade serous ovarian cancer, making p53 a critical tumor suppressor for ovarian cancer.[3] *TP53* mutations are found within early serous tubal intraepithelial lesions found in the fallopian tube, suggesting that p53 loss is an early event in the pathogenesis of high grade serous ovarian cancer (for an recent review see Soong et al[6]). In the genomic and molecular mechanisms of ovarian cancer session, Dr Kathy Cho presented data from murine models of ovarian cancer demonstrating that recombination of *Trp53* inactivation along with *Brca1* and *Rb1* inactivation in fallopian tube epithelium utilizing Cre-lox technology requires a latency period of more than a year for early serous tubal intraepithelial carcinoma lesions to progress to high grade serous ovarian cancer, suggesting that tumor initiation and progression require additional events.[7 8]

Dr Rong Wu presented on new technologies utilizing clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (CAS9) mediated somatic gene editing in fallopian tube epithelium to model gynecologic cancers in mice. Importantly, he reported that the Cre-CRISPR/Cas9 sgRNA system could recapitulate morphology and immunophenotypic characteristics of *Apc-Pten* or *Brca1-Trp53-Rb1-Nf1* endometrioid carcinomas and high grade serous ovarian cancer produced by Cre-lox technology. Within the Rivkin Symposium, we also learnt of additional research efforts utilizing CRISPR-Cas9 mediated gene targeting in organoid cultures derived from mouse oviduct and ovarian surface epithelium to identify gene combinations that result in tumor formation in transplantation studies.[9] Future work utilizing these technologies and model systems will expedite the identification of genetic events that work in concert with p53 mutations to promote high grade serous ovarian cancer formation and metastasis within murine models.

Similar to most cancers, the majority of *TP53* mutations in high grade serous ovarian cancer are missense mutations that reside within the DNA binding domain. Mutant p53 proteins result in loss of wild type p53 function through multiple mechanisms, including loss of DNA binding and protein structural mutations. *TP53* mutations can facilitate p53 protein aggregation leading to loss of p53 function, dominant negative effects, and gain of function activities. Previous studies have shown that R248 and R280 mutant p53 proteins stimulate p53 protein aggregation *in vitro* and enhance p53 protein aggregates within the nucleus of cancer cells to inactivate p53 function.[10] Dr Nicole Heinzl discussed the development of an enzyme linked immunosorbent assay recently published by Maritschnegg et al[11] that may be utilized to detect p53 protein aggregates in high grade serous ovarian cancer patient specimens. Ongoing studies are focused on determining whether p53 aggregates correlate with patient survival and/or response to platinum based chemotherapy in high grade serous ovarian cancer.

There are likely multiple mechanisms by which p53 mediates its tumor suppressor functions in ovarian cancer. One target that may contribute to the pathogenesis of high grade serous ovarian cancer is forkhead box protein M1 (FOXM1). FOXM1 is a forkhead box transcription factor that controls cell cycle progression and cell proliferation in normal cells through activation of G2 specific genes.[12 13] In cancer cells, FOXM1 has additional functions in controlling apoptosis, angiogenesis, invasion, genomic instability, inflammation, and metabolism.[12] In ovarian cancer, aberrant activation of FOXM1 has been shown to promote tumor migration, invasion, chemoresistance, and poly ADP ribose polymerase inhibitor resistance.[14–16] The FOXM1 transcription factor network is overexpressed at the messenger ribonucleic acid (RNA) level in 87% of high grade serous ovarian cancer.[3] FOXM1 is a repressed p53 target, indicating that p53 loss may contribute to enhanced FOXM1 signaling in high grade serous ovarian cancer.[17] In support of this notion, FOXM1 expression is increased in p53 and Rb deficient murine ovarian surface epithelial cells and tumors compared with p53 and Rb wild type cells.[18] Dr Carter Barger recently demonstrated that FOXM1 is highly expressed in human cancers with p53 inactivation and Rb-E2F deregulation. Moreover, FOXM1 expression was associated with genomic instability.[18] Overall, these studies suggest that FOXM1 may be an important therapeutic target for the treatment of high grade serous ovarian cancer .

DNA REPAIR IN HIGH GRADE SEROUS OVARIAN CANCER

Defective homologous recombination plays an important role in the pathogenesis and therapeutic response of high grade serous ovarian cancer. It has been estimated that approximately 50% of high grade serous ovarian cancers have defects in DNA repair and homologous recombination. Most notably, 20% of high grade serous ovarian cancers exhibit germline or somatic mutations in the homologous recombination proteins BRCA1/2 and an additional 11% of high grade serous ovarian cancers loose BRCA1 expression through promoter methylation. Additional genomic changes within genes such as EMSY, PTEN, RAD51, ATM, recombination plays, and Fanconi anemia also result in defective homologous recombination in 25% of high grade serous ovarian cancer tumors.[3]

The homologous recombination pathway plays an important role in repairing DNA double strand breaks that occur during DNA replication. Defects in homologous recombination result in the accumulation of chromatid breaks.[19] If chromatid breaks are not repaired, the cells become dependent on alternative end joining double strand break repair for survival. Alternative end joining will repair the breaks by joining the double strand breaks, resulting in chromosomal rearrangements and genomic instability.[20] Homologous recombination deficient tumors are particularly sensitive to intrastrand and interstrand crosslinks induced by platinum based chemotherapeutic agents.[21]

In addition to chemotherapy, homologous recombination deficient high grade serous ovarian cancers are also particularly sensitive to poly ADP ribose polymerase inhibitors. In 2014, olaparib was first approved by the Food and Drug Administration for the treatment of BRCA1/2 mutant epithelial ovarian cancer for those who have received three or more chemotherapy regimens.[22] Subsequently, rucaparib was approved for women with advanced ovarian cancer who have been treated with two or more chemotherapies and have germline or somatic BRCA mutations.[23] Niraparib was the first approved poly ADP ribose polymerase inhibitor for maintenance therapy in recurrent ovarian cancer patients who are in complete or partial response to platinum based chemotherapy, regardless of BRCA mutation status.[24] Currently, olaparib, niraparib, and rucaparib are approved by the Food and Drug Administration for maintenance therapy in patients with recurrent epithelial ovarian cancer who are in complete or partial response to platinum based chemotherapy.[25] There are a number of ongoing combination studies evaluating the safety and efficacy of poly ADP ribose polymerase inhibitors with chemotherapy, radiation therapy, immunotherapy, antiangiogenic agents, PI3K pathway inhibitors, and inhibitors of DNA damage repair.[26] Phase II clinical trials combining olaparib with paclitaxel in BRCA mutated cancers have reported improved clinical responses compared with single agent platinum or topoisomerase inhibitors.[27] However, significant myelosuppression limits the combination of poly ADP ribose polymerase inhibitors with standard doses of platinum and topoisomerase inhibitors.[28 29]

A recent study by Drs Sarah Hill and Alan D'Andrea demonstrated that independent of DNA repair gene mutational status, high grade serous ovarian cancer patient organoids with a functional defect in homologous recombination, as determined by defective RAD51 foci assembly following irradiation, correlates with response to poly ADP ribose polymerase inhibition.[30] These studies highlight the importance of homologous recombination deficiency in mediating

poly ADP ribose polymerase inhibitor response in high grade serous ovarian cancer and suggest that functional testing of homologous recombination activity may be most effective to predict which patients may respond to poly ADP ribose polymerase inhibitors. There are multiple mechanisms by which homologous recombination defective tumors are sensitive to poly ADP ribose polymerase inhibition. First, poly ADP ribose polymerase is a single strand DNA repair protein. If single strand breaks are not repaired by poly ADP ribose polymerase, they are converted into double strand breaks during replication that are repaired by RAD51 and homologous recombination.[20 31] Additional mechanisms for poly ADP ribose polymerase inhibitor mediated sensitivity of homologous recombination deficient high grade serous ovarian cancer cells may also include activation of classic non-homologous end joining and inhibition of DNA repair mediated by the accumulation of poly ADP ribose polymerase1-DNA complexes.[32]

Despite the enthusiasm for the addition of poly ADP ribose polymerase inhibitors to the clinical landscape of high grade serous ovarian cancer, many patients who initially respond to poly ADP ribose polymerase inhibitors develop resistance. There are multiple mechanisms by which high grade serous ovarian cancers develop resistance to poly ADP ribose polymerase inhibitors. Dr Alan D'Andrea, an invited speaker at the Rivkin Symposium, spoke about the current research efforts investigating mechanisms that drive sensitivity and resistance to poly ADP ribose polymerase inhibitors. In BRCA1/2 deficient tumors, a common mechanism driving poly ADP ribose polymerase inhibitor resistance is the restoration of BRCA1 or BRCA2 protein activity through genetic or epigenetic events.[32] Loss of poly ADP ribose polymerase1 expression, the target of poly ADP ribose polymerase inhibitors, has also been linked to poly ADP ribose polymerase inhibitor resistance in human cancer cell lines.[33] In addition to loss of poly ADP ribose polymerase1 expression, mutations within poly ADP ribose polymerase1 that prevent poly ADP ribose polymerase trapping by poly ADP ribose polymerase inhibitors at sites of DNA damage can also contribute to drug resistance.[34] Recent studies have utilized unbiased screening methods to identify novel mediators of poly ADP ribose polymerase inhibitor resistance.

Screening for factors that mediate resistance of BRCA1 deficient tumors to poly ADP ribose polymerase inhibitors identified mitotic arrest deficient 2 like 2 (REV7) and tumor protein p53 binding protein 1 (53BP1).[35] 53BP1 is a chromatin binding protein that is rapidly recruited to double strand breaks where it regulates DNA repair choices by inhibiting DNA end resection and homologous recombination and promotes non-homologous end joining.[36] During homologous recombination, BRCA1 promotes the displacement of 53BP1 from chromatin near double strand breaks to activate DNA end resection.[37 38] Loss of 53BP1 in a *Brca1* deficient setting is sufficient to promote homologous recombination and confer poly ADP ribose polymerase inhibitor resistance.[36 39 40] REV7 has recently been identified as a component of the shieldin complex, a downstream effector of 53BP1 in DNA double strand break repair.[32 35 41] Dr Yizhou He presented data on another CRISPR mediated screen for genes that mediate poly ADP ribose polymerase inhibitor response where the multifunctional homodimeric protein hub dynein light chain LC8-type I (DYNLL1) was identified as an another important factor mediating poly ADP ribose polymerase inhibitor sensitivity. Studies revealed DYNL11 loss resulted in poly ADP ribose polymerase inhibitor resistance. In these studies, He et al found that DYNLL1 is a negative regulator of DNA end resection.[42] These findings are consistent with a recent report demonstrating that DYNLL1 acts to regulate 53BP1 non-homologous end joining by promoting 53BP1 oligomerization and chromatin interactions.[43] Overall, these findings highlight the importance of the p53BP1 pathway in poly ADP ribose polymerase inhibitor resistance and have revealed novel mechanisms governing the DNA damage response.

In addition to the p53BP1 pathway, Dr Jeremy Chien presented work ongoing in his laboratory that has identified the TP53 induced glycolysis regulatory phosphatase TIGAR as an important mediator of poly ADP ribose polymerase inhibitor response. Knockdown of TIGAR enhanced responses to olaparib in ovarian cancer cells *in vitro*. Moreover, TIGAR expression is amplified and correlates with poor overall survival in high grade serous ovarian cancer, suggesting that TIGAR may be an important therapeutic target for ovarian cancer. Overall, these data demonstrate that there are multiple mechanisms that may drive poly ADP ribose polymerase1 resistance, suggesting that analysis of each individual patient may be necessary to inform treatment strategies for these patients. Dr Elizabeth Stover performed a near genome CRISPR/Cas9 screen in BRCA2 mutant high grade serous ovarian cancer cell lines to identify genes that mediate survival to platinum based chemotherapy. In this screen, overexpression of the proapoptotic genes BCL-like 1 (BCL-XL), BCL2 apoptosis regulator (BCL-2), and MCL1 apoptosis regulator mediated resistance to platinum based chemotherapy. In preliminary studies, antiapoptotic inhibitors against BCL-XL, MCL1, or BCL2/BCL-XL synergized with cisplatin or paclitaxel, suggesting that antiapoptotic targets such as BCL-XL and MCL1 may be additional therapeutic targets driving chemotherapy resistance in high grade serous ovarian cancer. Moreover, BCL-XL, MCL1, or BCL2/BCL-XL inhibitors also synergized with the poly ADP ribose polymerase inhibitor olaparib, suggesting that BCL-XL and MCL1 may be therapeutic targets to use in combination with DNA damaging agents.

GENOMIC INSTABILITY OF HIGH GRADE SEROUS OVARIAN CANCER

High grade serous ovarian cancer ranks among the top cancers with chromosome structural variants.[1 4] In addition to recurrent mutations in *TP53* and the homologous recombination pathway, high grade serous ovarian cancer exhibits a high degree of somatic copy number alterations. Dr James Brenton presented work from his laboratory where they utilized copy number signatures derived from whole genome sequencing of core biopsies as a novel approach to identify mutational processes in high grade serous ovarian cancer. Their study identified seven distinct copy number signatures that are present in high grade serous ovarian cancer patient specimens at the time of diagnosis. Importantly, a copy number signature associated with oncogenic RAS signaling (neurofibromin 1, NF1; KRAS proto-oncogene GTPase, KRAS; and NRAS proto-oncogene GTPase, NRAS) predicts platinum resistant relapse and poor survival in high grade serous ovarian cancer patients. Moreover, they identified a copy number signature associated with BRCA1/2 related homologous recombination defects

that is associated with improved patient survival.[44] Their study also reveals the majority of high grade serous ovarian cancer patients have a mixture of copy number signatures, suggesting multiple mutational processes may coevolve during the pathogenesis of high grade serous ovarian cancer.[44] These studies reveal new information regarding mutational processes that occur during the evolution of high grade serous ovarian cancer and have important therapeutic implications in the use of copy number signatures for patient stratification to targeted therapies in high grade serous ovarian cancer.

EMERGING AREAS WITHIN HIGH GRADE SEROUS OVARIAN CANCER

The analysis and identification of driver non-coding somatic mutations in epithelial ovarian cancer is an emerging area of research in the ovarian cancer field. Whole genome sequencing studies within epithelial ovarian cancer have identified thousands of non-coding somatic mutations.[45] Dr Rosario Corona presented ongoing work in the Lawrenson laboratory analyzing non-coding somatic mutations in epithelial ovarian cancer to distinguish between driver and passenger non-coding mutations. They hypothesized that driver non-coding mutations may localize to regulatory elements (promoters, enhancers) of genes known to be involved in the pathogenesis of ovarian cancer. They utilized genome wide histone 3 methylates Lys27 (H3K27) acetylation ChIP-sequencing of fresh primary ovarian cancer tissue samples from each major ovarian cancer histotype combined with RNA sequencing to identify common active regulatory elements across all histotypes. They then integrated these data with whole genome sequencing data to identify common non-coding mutations within active regulatory elements in ovarian cancer. Preliminary data identify several commonly mutated regulatory elements within each ovarian cancer histotype, including RNA polymerase III subunit E (POLR3E) and coiled-coil-helix-coiled-coil-helix (CHCHD6) for high grade serous ovarian cancer. Future studies are needed to further explore the identification and validation of these novel non-coding somatic mutation in epithelial ovarian cancer.

SUMMARY

In summary, new technologies utilizing CRISPR-CAS9 approaches in mice, organoids, and cell based screens continue to advance our knowledge of key molecular drivers of ovarian cancer initiation, progression, and drug resistance. Poly ADP ribose polymerase inhibitors, targeting homologous recombination defects in high grade serous ovarian cancer, have significantly altered the clinical management of ovarian cancer. However, resistance to these agents has emerged as an important clinical challenge. The development of predictive biomarkers for single agent poly ADP ribose polymerase inhibitors are needed for patient stratification. Additionally, improved understanding of the mechanisms of poly ADP ribose polymerase inhibitor resistance may lead to new therapeutic strategies to enhance outcomes in women with high grade serous ovarian cancer.

Contributors EBR wrote the research summary.

Funding This study was supported by US Department of Defense (W81XWH-15-1-0097).

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Commissioned; internally peer reviewed.

REFERENCES

1. Bowtell DD, Böhm S, Ahmed AA, *et al*. Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. *Nat Rev Cancer* 2015;15:668–79.
2. Tan DSP, Agarwal R, Kaye SB. Mechanisms of transcoelomic metastasis in ovarian cancer. *Lancet Oncol* 2006;7:925–34.
3. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. *Nature* 2011;474:609–15.
4. Ciriello G, Miller ML, Aksoy BA, *et al*. Emerging landscape of oncogenic signatures across human cancers. *Nat Genet* 2013;45:1127–33.
5. Mello SS, Attardi LD. Deciphering p53 signaling in tumor suppression. *Curr Opin Cell Biol* 2018;51:65–72.
6. Soong T, Kolin D, Teschan N, *et al*. Back to the future? The fallopian tube, Precursor escape and a dualistic model of high-grade serous carcinogenesis. *Cancers* 2018;10:468.
7. Zhai Y, Wu R, Kuick R, *et al*. High-grade serous carcinomas arise in the mouse oviduct via defects linked to the human disease. *J Pathol* 2017;243:16–25.
8. Cho KR. Is "ovarian" cancer a misnomer? Exploring ovarian cancer origins in the mouse. *Trans Am Clin Climatol Assoc* 2018;129:40–7.
9. Kopper O, de Witte CJ, Lõhmussaar K, *et al*. An organoid platform for ovarian cancer captures intra- and interpatient heterogeneity. *Nat Med* 2019;25:838–49.
10. Ano Bom APD, Rangel LP, Costa DCF, *et al*. Mutant p53 aggregates into prion-like amyloid oligomers and fibrils: implications for cancer. *J Biol Chem* 2012;287:28152–62.
11. Maritschnegg E, Heinzl N, Wilson S, *et al*. Polymer-ligand-based ELISA for robust, high-throughput, quantitative detection of p53 aggregates. *Anal Chem* 2018;90:13273–9.
12. Halasi M, Gartel AL. FOX(M1) news--it is cancer. *Mol Cancer Ther* 2013;12:245–54.
13. Laoukili J, Kooistra MRH, Brás A, *et al*. FoxM1 is required for execution of the mitotic programme and chromosome stability. *Nat Cell Biol* 2005;7:126–36.
14. Lok GTM, Chan DW, Liu VWS, *et al*. Aberrant activation of ERK/FOXM1 signaling cascade triggers the cell migration/invasion in ovarian cancer cells. *PLoS One* 2011;6:e23790.
15. Chiu W-T, Huang Y-F, Tsai H-Y, *et al*. FOXM1 confers to epithelial-mesenchymal transition, stemness and chemoresistance in epithelial ovarian carcinoma cells. *Oncotarget* 2015;6:2349–65.
16. Fang P, Madden JA, Neums L, *et al*. Olaparib-induced adaptive response is disrupted by FOXM1 targeting that enhances sensitivity to PARP inhibition. *Mol Cancer Res* 2018;16:961–73.
17. Barsotti AM, Prives C. Pro-proliferative FoxM1 is a target of p53-mediated repression. *Oncogene* 2009;28:4295–305.
18. Barger CJ, Zhang W, Hillman J, *et al*. Genetic determinants of FOXM1 overexpression in epithelial ovarian cancer and functional contribution to cell cycle progression. *Oncotarget* 2015;6:27613–27.
19. Sonoda E, Sasaki MS, Buerstedde JM, *et al*. Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death. *Embo J* 1998;17:598–608.
20. Farmer H, McCabe N, Lord CJ, *et al*. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 2005;434:917–21.
21. Yang D, Khan S, Sun Y, *et al*. Association of BRCA1 and BRCA2 mutations with survival, chemotherapy sensitivity, and gene mutator phenotype in patients with ovarian cancer. *JAMA* 2011;306:1557–65.
22. Kim G, Ison G, McKee AE, *et al*. FDA approval summary: olaparib monotherapy in patients with deleterious germline BRCA-mutated advanced ovarian cancer treated with three or more lines of chemotherapy. *Clin Cancer Res* 2015;21:4257–61.
23. Balasubramaniam S, Beaver JA, Horton S, *et al*. FDA approval summary: rucaparib for the treatment of patients with deleterious

BRCA mutation-associated advanced ovarian cancer. *Clin Cancer Res* 2017;23:7165–70.
24. Ison G, Howie LJ, Amiri-Kordestani L, *et al*. FDA approval summary: niraparib for the maintenance treatment of patients with recurrent ovarian cancer in response to platinum-based chemotherapy. *Clin Cancer Res* 2018;24:4066–71.
25. LaFargue CJ, Dal Molin GZ, Sood AK, *et al*. Exploring and comparing adverse events between PARP inhibitors. *Lancet Oncol* 2019;20:e15–28.
26. Beaver JA, Coleman RL, Arend RC, *et al*. Advancing drug development in gynecologic malignancies. *Clin Cancer Res* 2019.
27. Oza AM, Cibula D, Benzaquen AO, *et al*. Olaparib combined with chemotherapy for recurrent platinum-sensitive ovarian cancer: a randomised phase 2 trial. *Lancet Oncol* 2015;16:87–97.
28. Kummar S, Chen A, Ji J, *et al*. Phase I study of PARP inhibitor ABT-888 in combination with topotecan in adults with refractory solid tumors and lymphomas. *Cancer Res* 2011;71:5626–34.
29. Rajan A, Carter CA, Kelly RJ, *et al*. A phase I combination study of olaparib with cisplatin and gemcitabine in adults with solid tumors. *Clin Cancer Res* 2012;18:2344–51.
30. Hill SJ, Decker B, Roberts EA, *et al*. Prediction of DNA repair inhibitor response in short-term patient-derived ovarian cancer organoids. *Cancer Discov* 2018;8:1404–21.
31. Bryant HE, Schultz N, Thomas HD, *et al*. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 2005;434:913–7.
32. Konstantinopoulos PA, Ceccaldi R, Shapiro GI, *et al*. Homologous recombination deficiency: exploiting the fundamental vulnerability of ovarian cancer. *Cancer Discov* 2015;5:1137–54.
33. Liu X, Han EK, Anderson M, *et al*. Acquired resistance to combination treatment with temozolomide and ABT-888 is mediated by both base excision repair and homologous recombination DNA repair pathways. *Mol Cancer Res* 2009;7:1686–92.
34. Pettitt SJ, Krastev DB, Brandsma I, *et al*. Genome-wide and high-density CRISPR-Cas9 screens identify point mutations in PARP1 causing PARP inhibitor resistance. *Nat Commun* 2018;9.
35. Xu G, Chapman JR, Brandsma I, *et al*. REV7 counteracts DNA double-strand break resection and affects PARP inhibition. *Nature* 2015;521:541–4.
36. Bunting SF, Callén E, Wong N, *et al*. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell* 2010;141:243–54.
37. Chapman JR, Sossick AJ, Boulton SJ, *et al*. BRCA1-associated exclusion of 53BP1 from DNA damage sites underlies temporal control of DNA repair. *J Cell Sci* 2012;125:3529–34.
38. Densham RM, Garvin AJ, Stone HR, *et al*. Human BRCA1-BARD1 ubiquitin ligase activity counteracts chromatin barriers to DNA resection. *Nat Struct Mol Biol* 2016;23:647–55.
39. Bouwman P, Aly A, Escandell JM, *et al*. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. *Nat Struct Mol Biol* 2010;17:688–95.
40. Jaspers JE, Kersbergen A, Boon U, *et al*. Loss of 53BP1 causes PARP inhibitor resistance in *Brca1*-mutated mouse mammary tumors. *Cancer Discov* 2013;3:68–81.
41. Gupta R, Somyajit K, Narita T, *et al*. DNA repair network analysis reveals shieldin as a key regulator of NHEJ and PARP inhibitor sensitivity. *Cell* 2018;173:972–88.
42. He YJ, Meghani K, Caron M-C, *et al*. DYNLL1 binds to MRE11 to limit DNA end resection in BRCA1-deficient cells. *Nature* 2018;563:522–6.
43. Becker JR, Cuella-Martin R, Barazas M, *et al*. The ASCIZ-DYNLL1 axis promotes 53BP1-dependent non-homologous end joining and PARP inhibitor sensitivity. *Nat Commun* 2018;9:5406.
44. Macintyre G, Goranova TE, De Silva D, *et al*. Copy number signatures and mutational processes in ovarian carcinoma. *Nat Genet* 2018;50:1262–70.
45. Phelan CM, Kuchenbaecker KB, Tyrer JP, *et al*. Identification of 12 new susceptibility loci for different histotypes of epithelial ovarian cancer. *Nat Genet* 2017;49:680–91.

Review Article

INTERNATIONAL JOURNAL OF GYNECOLOGICAL CANCER

OPEN ACCESS

Tumor microenvironment and immunology of ovarian cancer: 12th Biennial Rivkin Center Ovarian Cancer Research Symposium

Anirban K Mitra,[1] Yang Yang-Hartwich[2]

[1]Medical Sciences Program, Indiana University School of Medicine, Bloomington, Indiana, USA
[2]Obstetrics, Gynecology & Reproductive Sciences, Yale School of Medicine, New Haven, Connecticut, USA

Correspondence to
Dr Anirban K Mitra, Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN 46202, USA; anmitra@indiana.edu

Received 31 May 2019
Revised 5 July 2019
Accepted 15 July 2019

To cite: Mitra AK, Yang-Hartwich Y. *Int J Gynecol Cancer* 2019;**29**:s12–s15.

ABSTRACT

The 12th Biennial Ovarian Cancer Research Symposium organized by the Rivkin Center for Ovarian Cancer and the American Association for Cancer Research held on September 13–15, 2018 covered cutting edge and relevant research topics in ovarian cancer biology and therapy. Sessions included detection and prevention, genomics and molecular mechanisms, tumor microenvironment and immunology, novel therapeutics, and an education session. In this article we provide an overview of the key findings presented in the tumor microenvironment and immunology session.

INTRODUCTION

Ovarian cancer is the most lethal gynecologic malignancy and the fifth leading cause of cancer-related deaths among women in the USA.[1 2] Recent developments in the field of ovarian cancer biology have uncovered several key findings that help us understand the disease better and provide us with new directions for improving patient outcome.[3] Tumor microenvironment (TME) has emerged as an important area of interest. The TME includes the blood vessels, fibroblasts, immune cells, extracellular matrix (ECM), and all the signaling molecules surrounding the tumor.[4] TME closely interacts with the tumor and mediates its initiation, progression, and metastasis.[4–6] The vital TME components have become new therapeutic targets since they can profoundly affect patients' responses to treatments.[7] Accumulating evidence suggests that the efficacy of chemotherapy and the promising technique of immunotherapy can be improved through the modulation of TME.[8] Therefore, research interest in understanding the complexity and diversity of TME has exponentially increased in recent years. This aspect of ovarian cancer research was well represented in the 'Tumor Microenvironment and Immunology of Ovarian Cancer' session of the 12th Biennial Ovarian Cancer Research Symposium.

TUMOR MICROENVIRONMENT AND IMMUNOLOGY SESSION

The session included two invited speaker presentations followed by eight talks from selected abstracts. The first invited talk by **Dr Frances Balkwill** from Barts Cancer Institute presented her laboratory's efforts to better understand ovarian cancer metastasis by first deconstructing metastasis in patient tumors and then developing experimental models based on the knowledge gained to accurately study the mechanism of regulation of metastatic colonization of the omentum. Using biopsies of metastasis from high-grade serous ovarian cancer (HGSOC) patients representing a spectrum of disease progression from marginal to aggressive, they profiled the dynamic interactions and changes in the tumor and stroma as the metastasis progressed. Extensive studies were performed using the same biopsy samples including gene expression, matrisome, ECM organization, biomechanical properties, cytokine/chemokine levels, and cellular profiles. The matrisome in humans consists of about 300 proteins that form the ECM, growth factors associated with the ECM, proteases and other ECM-modifying enzymes, and other ECM-associated proteins.[9] Changes in the matrisome was a key feature identified, which could be correlated with prognosis and immune cell signatures that can themselves affect patient outcome. Through the reorganization of fibrillar collagens and the expression of glycoproteins and proteoglycans, the matrisome signature also determined the stiffness of the tumors. Moreover, there was a strong association between the number of α-smooth muscle actin and α-fibroblast-activated protein (FAP)-positive cancer associated fibroblasts (CAFs) and metastasis progression. Combining multiple different types of analysis on the same biopsies, and utilizing biopsies from patients exhibiting different extents of metastatic tumor progression, Dr Balkwill's group provided a comprehensive picture of the process. This compliments the data from primary tumors provided by The Cancer Genome Atlas Program and can effectively form a platform for launching detailed studies deciphering the mechanism of metastatic progression in HGSOC as well as provide novel therapeutic targets to treat metastasis.

Dr Balkwill proceeded to describe her group's efforts to reconstruct the omentum in a petri dish to provide effective models to further study the mechanisms of the key factors identified by their multi-parameter analysis of ovarian cancer metastasis. Using

HGSOC cells, fibroblasts, and adipocytes embedded in a collagen I gel, her group has developed tri-cultures representing the omental metastasis. Similarly, they have generated a quadri-culture model by including omental mesothelial cells. These models have been characterized for the expression of the matrix proteins identified in the deconstruction experiments, and transforming growth factor beta (TGF-β) was found to be a regulator of five of the six matrix molecules identified in the matrisome signature. Dr Balkwill also presented their characterization of mouse HGSOC models and presented data pointing towards the similarities in the ECM and immune subsets in the mouse and human tumors. Taken together, the models presented provide unique opportunities to study detailed mechanisms of metastatic colonization as well as drug discovery.

In the next invited talk, **Dr Ernst Lengyel** from the University of Chicago reported his group's recent findings on the role of cancer/testis antigen 45 (CT45) in increasing chemosensitivity of ovarian tumors. To study the role of the metastatic tumor proteome on the HGSOC patient outcome following chemotherapy, his group collaborated with Matthias Mann from the Max Planck Institute of Biochemistry. They developed a high-sensitivity, label-free proteomic mass spectrometry-based work-flow to analyze the proteome from formalin-fixed paraffin-embedded (FFPE) tumors. Using this approach they quantified more than 9000 proteins in chemosensitive and chemoresistant tumors. CT45 was the most upregulated in the chemosensitive tumors. It was found to affect DNA damage repair pathways by regulating protein phosphatase 4. The expression of CT45 is suppressed in the normal ovary by DNA methylation and is significantly upregulated in ovarian tumors through the loss of methylation. By combining immunopeptidomics and mass spectrometry, Dr Lengyel identified the role of CT45 as a cancer antigen presented on human leucocyte antigen class I receptors. These CT45 peptides were found to be potent in activating patient-derived cytotoxic T cells and inducing cancer cell killing. This novel comprehensive proteomics strategy incorporating proteome quantification, phosphoproteomics, interactome studies, and immunopeptidomics was effective in analyzing achieved FFPE tumor samples and identifying a unique biomarker, which is particularly relevant to long-term patient survival and immunotherapy.

Dr Lengyel also described the reciprocal signaling between CAFs and ovarian cancer cells identified by quantitative label-free mass spectrometry-based phosphoproteomics in co-cultures of CAFs and ovarian cancer cells. His group had previously shown that the metastasizing ovarian cancer cells utilized lipids released from the adipocytes in the omentum to drive their growth. His present findings demonstrate that the reciprocal signaling between CAFs and the cancer cells help the cancer cells switch their metabolism towards utilizing glycogen once the fat reserves are depleted. The activation of p38 mitogen-activated protein kinase signaling in the CAFs by the cancer cells resulted in the increased secretion of the cytokines interleukin 6 (IL-6), C-X-C motif chemokine ligand 10 (CXCL10), and C-C motif chemokine ligand 5 (CCL5). The secreted cytokines activated glycogenolysis in the cancer cells in a paracrine manner by activating phosphoglucomutase 1. Glycogen phosphorylase inhibition reduced metastasis in mice indicating that blocking glycogen mobilization could be a potentially effective therapeutic strategy to treat metastasis.

Dr Laurie Ailles from the University of Toronto presented her group's recent findings on the differential gene expression profiles of ovarian cancer CAFs and cancer cells. CAFs could be subdivided into two groups based on the expression levels of FAP. FAP high and FAP low CAFs had distinct transcriptional programs, and analysis of The Cancer Genome Atlas data demonstrated a shorter progression-free and overall survival in FAP high patients. FAP high CAFs were functionally distinct from FAP low CAFs, with the FAP high CAFs having a greater ability to promote cancer cell invasion in vitro and tumor growth in mice. It would be interesting to analyze the matrisome signature described by Dr Balkwill in these FAP high and FAP low CAFs. The heterogeneity and potential functions of the subsets of CAFs would be relevant in future strategies involving targeting of the tumor stroma. **Dr Katherine Fuh** from Washington University presented her group's findings on the role of discoidin domain receptor 2 (DDR2) expression in fibroblasts in promoting ovarian cancer metastasis. Inhibition or silencing DDR2 in fibroblasts decreased cancer invasion and mesothelial cell clearance as well as decreased collagen staining (trichrome) intensity and quantity in tumors. This suggests the possibility of targeting the stromal DDR2 as a potential therapeutic option, and again calls for the analysis of the secretome of the fibroblasts and the cancer cells regulated by DDR2. **Dr Sara Zanivan** from the University of Glasgow reported the role of oxidoreductase chloride intracellular channel protein 3 (CLIC3) as a secreted factor and key contributor of tumor-stromal interaction. Through the mass spectrometry-proteomic comparative analysis of CAFs and their normal counterparts, CLIC3 was identified as the most upregulated and the most deposited in ECM. Further analysis revealed that the abundant CLIC3 in tumors was secreted by both stromal and cancer cells and could activate tissue transgutaminase-2 to promote blood vessel growth and increase tumor invasiveness. Their findings suggest a new mechanism of TME factor-mediated tumor invasion.

Understanding the complexity and diversity of immune cells in the TME has become critical for maximizing the clinical benefits from immunotherapy. **Dr Alan D'Andrea's** group at Harvard Medical School applied a novel high-multiplex tissue cyclic immunofluorescence (t-CycIF) platform to understanding the dynamics between DNA damage in cancer cells and the immune context in HGSOC TME. This platform quantifies the expression of 60 antigens at single cell resolution. Data from over 10^6 cells showed distinct cell compositions in the TME of BRCA1/2 mutant and homologous recombination wild-type HGSOCs. On one hand, tumors with high programmed cell death protein 1 (PD-1) and its ligand (PD-L1) expression have high infiltration of CD1c+dendritic cells, which indicates the suppression of antigen presenting pathway and that these tumors are likely to respond to immune checkpoint blockade. Conversely, a subset of tumors with high levels of DNA damage show active interferon signaling and high CD8+ cytotoxic T-cell infiltration suggesting an immunogenic phenotype in this subset of HGSOCs. The application of new technologies like t-CycIF will contribute to the development of rational combination therapies and predictive biomarkers for DNA damaging agents and immune checkpoint blockade. **Dr Pamela Kreeger's** group at the University of Wisconsin-Madison analyzed the secretome of macrophages and identified fms-related tyrosine kinase 3 ligand, heparin-binding epidermal growth factor, IL-6, IL-8, and leptin to be associated with tumor spheroid spreading in a macrophage-HGSOC spheroid co-culture using a 35-cytokine-multiplex assay. Although each ovarian cancer cell line (eg, OVCAR3, OVCA433, and OV90) responded to a different

set of cytokines secreted by macrophages, they utilized a common signaling pathway to regulate spheroid spreading, which is the Janus kinase 2/signal transducers and activators of transcription 3 (JAK2/STAT3) activation leading to matrix metallopeptidase-9 (MMP-9)-promoted tumor spreading. These findings suggest that multiple macrophage-secreted factors drive the tumor metastasis in HGSOC patients. However, they may share the same downstream signaling pathways, such as JAK2/STAT3/MMP-9. The identification of this molecular mechanism indicates the possibility of controlling tumor metastasis in a broad group of patients by targeting the main common signaling pathways for macrophage-tumor interaction. **Dr Ronny Drapkin's** group at the University of Pennsylvania revealed the role of ring finger protein 20 (RNF20)/histone H2B monoubiquitylation (H2Bub1) loss as an early event in HGSOC that modulate the immune signaling pathways during tumor initiation. H2Bub1 is an epigenetic regulator and tumor suppressor that is lost in serous tubal intraepithelial carcinomas (STICs) and HGSOCs. Ubiquitin ligase RNF20 catalyzes H2Bub1. Their data demonstrated that the inhibition of RNF20 altered immune signaling pathways and led to increased cell migration and clonogenic growth. The loss of RNF20/H2Bub1 functions is possibly responsible for the early oncogenic phenotype in STICs.

Angiogenesis is another important therapeutic target in the TME. **Dr Anil Sood's** group in the University of Texas MD Anderson Cancer Center identified a new target for overcoming the resistance to anti-angiogenic therapy. p130cas (Crk-associated substrate) is a central regulator of focal adhesion kinase (FAK)/Src-mediated angiogenesis. Their data showed that p130cas was highly expressed in the tumor-associated vascular endothelium. Ablation of p130cas gene or inhibition of its expression in mouse models of ovarian cancer increased the sensitivity to anti-vascular endothelial growth factor antibody treatment and inhibited tumor growth through autophagy-regulated cell death in endothelial cells. They have generated nanoparticle-delivered peptide antagonist to p130cas as a targeted therapeutic agent. The antagonist's clinical efficacy and mechanism of action are under evaluation.

It is also very exciting that new imaging technologies have been applied to the quantitative assessment of the architectural features in the TME of ovarian cancer. **Dr Paul Campagnola's** group at the University of Wisconsin-Madison used collagen-specific sensitive second harmonic generation imaging microscopy, 3D texture analysis, and machine learning to extract textural features and build models of the ECM in the ovarian cancer TME. They generated models for normal stroma, high-risk stroma, benign tumor, high-grade serous, low-grade serous, and endometroid carcinoma. By examining the collagen alterations, they developed quantitative biomarkers for assessing the increased collagen concentration and the changes of alignment of collagen molecules within fibrils and fibers. Their data indicate that combining macro/supramolecular probes and the fiber morphology classification improves our understanding of TME evolution in ovarian cancer and the role of ECM alteration in disease etiology. This novel approach has the potential to be developed into new prognostic and diagnostic methods.

The poster session included very interesting presentations covering epigenetic modulators, non-coding RNAs, tumor immunology, in vitro models, cancer stem cells, novel therapeutics, and so on. Coffman reported that ovarian cancer cells mediate EZH2 induction and epigenetic reprogramming to convert mesenchymal stem cells into carcinoma-associated mesenchymal stem cells. Zhang demonstrated that a combination of histone deacetylase 6 inhibition with PD-L1 checkpoint blockade could be a potential strategy to treat ARID1A-mutated clear cell ovarian cancer. Inhibition of nuclear factor-κB activity in macrophages and potentially other cells in the ovarian TME was shown to inhibit tumor progression by Yull. Other studies included association of decreased let-7 with stemness, the role of long non-coding RNAs in metabolism, autophagy, or immune response, and a glycosylation-dependent mechanism involving Sox2 that drives a cancer stem cell phenotype. Posters covering tumor immunology demonstrated that the inositol-requiring enzyme 1/X box binding protein 1 arm of the endoplasmic reticulum stress response pathway in dendritic cells was necessary for accelerated ovarian cancer progression, that neuropilin-1 promotes survival and suppressive function of T_{reg}, and included a study providing new insights into the metabolic pathways that regulate T cell anti-tumor responses in ovarian cancer. Another study demonstrated that ovarian cancer patient monocytes are more tumoricidal when cultured with interferons than monocytes from matched controls, supporting a novel, innate, immune-based approach to immunotherapy of ovarian cancer.

In vitro models were represented by a 3D cell culture model for predicting the response to standard carbo-taxol chemotherapy, a 3D perfused bioreactor that allows the study of tumor biology and anti-tumor drug testing under physiological conditions, and models mimicking shear forces to improve our fundamental understanding of peritoneal metastasis and mechanotransduction. Therapeutics targeting ovarian cancer included the use of myxoma virus (MYXV) as a poxvirus that synergized with chemotherapy, aldehyde dehydrogenase 1A inhibitors increased LKB1 phosphorylation leading to AMP-activated protein kinase a phosphorylation. McLean showed that the combination of inhibiting IL-6/LIF signaling with ruxolitinib with anti-estrogen therapy resulted in a synergistic decrease in ovarian cancer tumor cell viability. A new two-step targeting approach was presented that introduces non-natural targets (azide functional groups) in the tumors followed by the delivery of drug-loaded polymeric nanoparticles that are surface modified to have high affinity for these synthetic targets. Other pathways covered in the abstract session included apelin/APJ pathway in omental metastasis, role of TGFβ1/protein kinase Cα/Twist1 signaling pathway in ovarian cancer metastasis, and the induction of insulin-like growth factor signaling by follicular fluid in fimbrial cells causing stemness, clonal expansion, and transformation. Yang-Hartwich presented data supporting the role of mutant p53 in promoting the initiation of HGSOC from fallopian tube precursors, while Rankin demonstrated that tumor-associated mesothelial cells promoted tumor invasion by increasing collagen deposition and remodeling.

CONCLUSIONS

The TME contributes to the 'hallmarks' of cancer and also shapes therapeutic responses and chemoresistance.[4 6 10] It provides potential prognostic markers and therapeutic opportunities. Significant advances have been made in our understanding of the various components of the TME, including the cellular and acellular constituents. The 12th Biennial Ovarian Cancer Research Symposium reflected the increasing interest in the

TME of ovarian cancer and provided an excellent overview of the cutting-edge research going on in the ovarian cancer TME field, which provides renewed hope for our collective efforts to understand and eventually cure ovarian cancer. The identification of targetable molecular and cellular components in the TME will lead to the development of combination therapies that can simultaneously modulate TME and eliminate cancer cells to treat ovarian cancer more efficiently and effectively.[11] The new breakthroughs in the field of ovarian cancer TME that were presented at this meeting are leading the way in our fight against this deadly disease.

Acknowledgements AKM and YYH were supported by their respective DoD OCRP Ovarian Cancer Academy awards (W81XWH-15-0253 and W81XWH-15-1-0221). The authors thank the Ovarian Cancer Academy Dean and Assistant Dean for their helpful insights.

Contributors AKM and YYH prepared the manuscript.

Funding This study was supported by Congressionally Directed Medical Research Programs (W81XWH-15-0253, W81XWH-15-1-0221).

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Commissioned; internally peer reviewed.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin* 2019;69:7–34.
2. Torre LA, Trabert B, DeSantis CE, *et al*. Ovarian cancer statistics, 2018. *CA Cancer J Clin* 2018;68:284–96.
3. Bowtell DD, Böhm S, Ahmed AA, *et al*. Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. *Nat Rev Cancer* 2015;15:668–79.
4. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell* 2012;21:309–22.
5. Mitra AK. Ovarian cancer metastasis: a unique mechanism of dissemination. In: *Tumor metastasis*. InTech, 2016: 43–58.
6. Dasari S, Fang Y, Mitra AK. Cancer associated fibroblasts: naughty neighbors that drive ovarian cancer progression. *Cancers* 2018;10:406.
7. Hansen JM, Coleman RL, Sood AK. Targeting the tumour microenvironment in ovarian cancer. *Eur J Cancer* 2016;56:131–43.
8. Odunsi K. Immunotherapy in ovarian cancer. *Ann Oncol* 2017;28(Suppl 8):viii1–7.
9. Hynes RO, Naba A. Overview of the matrisome - an inventory of extracellular matrix constituents and functions. *Cold Spring Harb Perspect Biol* 2012;4:a004903.
10. Nieman KM, Romero IL, Van Houten B, *et al*. Adipose tissue and adipocytes support tumorigenesis and metastasis. *Biochim Biophys Acta* 2013;1831:1533–41.
11. Pearce OMT, Delaine-Smith RM, Maniati E, *et al*. Deconstruction of a metastatic tumor microenvironment reveals a common matrix response in human cancers. *Cancer Discov* 2018;8:304–19.

Review Article

INTERNATIONAL JOURNAL OF GYNECOLOGICAL CANCER

OPEN ACCESS

Novel therapeutics: response and resistance in ovarian cancer

Dmitriy Zamarin

Correspondence to
Dr Dmitriy Zamarin, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA; zamarind@mskcc.org

Received 5 April 2019
Accepted 16 May 2019

ABSTRACT

Here we review the latest pre-clinical and clinical developments for treatment of ovarian cancer, presented at the American Association of Cancer Research/Rivkin Center Ovarian Cancer Research Symposium held at the University of Washington in September 2018. Abstracts and presentations pertaining to the 'Novel Therapeutics' session were reviewed and are summarized here. The session featured a keynote presentation from Dr Ursula Matulonis, who summarized the current state of the art of treatment of ovarian cancer, including recent clinical trials incorporating the use of novel agents, including poly-ADP-ribose polymerase (PARP) inhibitors, other DNA-damaging agents, vascular endothelial growth factor receptor inhibitors, mirvetuximab soravtansine, and immune checkpoint blockade. Dr Jung-Min Lee then summarized the rationale and the results of early studies for targeting cell cycle checkpoint kinases for anti-cancer therapy. Eight submissions were selected for oral presentations, and 36 abstracts were presented as posters. The topics covered a range of clinical and pre-clinical strategies and biomarkers, including immunotherapy, mechanisms of chemotherapy, and PARP inhibitor resistance, DNA-damaging agents, and other novel therapeutic strategies. Key studies have highlighted that resistance to chemotherapy and PARP inhibitors remain a major challenge in therapy of ovarian cancer. Cancer stem cells represent an important mechanism of chemoresistance and strategies to target these cells may be a pathway to prevention of ovarian cancer relapse. Advancement of novel therapeutics targeting DNA damage, cell metabolism, and endoplasmic reticulum present some of the novel strategies in the pipeline. Emerging compelling pre-clinical data with novel antibody-drug conjugates targeting various surface receptors in ovarian cancer alone and in combination with immune checkpoint blockade generate a strong enthusiasm for rapid translation of these strategies to clinic.

INTRODUCTION

Since the last symposium in 2016, we have seen a series of developments in ovarian cancer, with approval of several poly-ADP-ribose polymerase (PARP) inhibitors in different treatment settings as well new clinical trials using agents targeting DNA repair, oncogenic pathways, and the immune system. The Novel Therapeutics session focused on the studies that aim to delineate the mechanisms of resistance to existing agents, identify novel targets, and develop new treatment combinations.

CURRENT STATE OF THERAPY OF OVARIAN CANCER AND NOVEL COMBINATIONS IN CLINICAL TRIALS

Dr Ursula Matulonis gave a keynote lecture in this session, summarizing the state of the art of treatment of ovarian cancer and the results of recent clinical trials. Given the advances with PARP inhibitor development in ovarian cancer, she highlighted the multiple ongoing trials using PARP inhibitors in combination with other agents. These include PARP inhibitors in combination with agents targeting other mechanisms of homologous DNA repair, vascular endothelial growth factor and vascular endothelial growth factor receptor inhibitors, such as cediranib and bevacizumab, phosphatidylinositol-3-kinase inhibitors, and programmed cell death protein 1 (PD-1) inhibitors, some of which have demonstrated signals of activity in early clinical trials.[1–3] She further summarized clinical data with folate receptor-targeting antibody drug conjugate mirvetuximab soravtansine, with a single agent response rate of 39% in non-heavily pre-treated patients.[4] Combinations of mirvetuximab soravtansine with other agents, such as pembrolizumab, chemotherapy, and bevacizumab are ongoing. The responses appear to be enriched in the patients with medium/high folate receptor α expression, which will serve as a biomarker for future patient selection.

TARGETING CELL CYCLE CHECKPOINTS AND DNA REPAIR MECHANISMS

Cell cycle checkpoint kinases represent another target for therapy in ovarian cancer, particularly those cancers not carrying inactivating BRCA mutations. Dr Jung-Min Lee summarized the pre-clinical data and early clinical data behind prexasertib, an inhibitor of checkpoint kinase 1/2 in non-BRCA-mutant ovarian cancer. In pre-clinical models, the drug demonstrates synergistic activity when used in combination with olaparib in non-BRCA-mutant cell lines, probably through inhibition of RAD51-mediated homologous DNA repair.[5] A phase I/II trial in 28 patients with advanced platinum-resistant non-BRCA-mutant ovarian cancer demonstrated a promising response

To cite: Zamarin D. *Int J Gynecol Cancer* 2019;**29**:s16–s21.

rate of 33%.[6] Grade 3/4 neutropenia was the most common adverse event, which interestingly was transient. Dr Lee further discussed other ongoing studies targeting DNA checkpoints alone or in combination, including combinations of checkpoint kinase 1 inhibitor and mammalian target of rapamycin inhibitors, and checkpoint kinase 1 inhibitors with PARP inhibitors, which are ongoing. Finally, she presented early clinical data with combinations of carboplatin with Wee1 inhibitors, demonstrating a promising response rate of 43% with 5.3 months' median progression-free survival.

PARP INHIBITORS: RECENT FINDINGS, MECHANISMS OF RESISTANCE, AND NOVEL COMBINATIONS

Dr Kathleen Moore and colleagues presented results of the QUADRA study, which evaluated single agent niraparib at 300 mg daily in patients with high-grade serous ovarian cancer and three or more prior lines of therapy. A total of 463 patients were treated in the study and the results were assessed according to platinum sensitivity, BRCA status, and homologous recombination deficiency status. In 456 patients with measurable disease, the disease control rate was 49%. In the primary efficacy population, defined as homologous recombination deficiency positive, the overall response rate was 29%, with a disease control rate of 71%. The most commonly observed grade 3 and higher adverse events were anemia (26.3%) and thrombocytopenia (20.5%). Niraparib thus demonstrated promising activity in this heavily pre-treated population, including platinum-resistant patients and some non-BRCA-mutant patients (NT-101).

Despite the promising response rate, this trial highlights the sad reality that PARP inhibition is not an option for the majority of patients, particularly those with platinum-resistant disease and BRCA wild-type status. Identification of biomarkers of primary and acquired resistance and strategies to minimize toxicity thus remain a high priority for further development of this class of agents.

Nanoparticles present an innovative and attractive strategy for drug delivery to tumors as a means of minimizing systemic toxicity.[7] Dr Paige Baldwin and colleagues explored nanoparticle encapsulation of talazoparib, followed by intraperitoneal delivery, to minimize systemic toxicities of PARP inhibition. The resultant drug formulation was more efficient in suppressing tumor growth than the parental drug, with no obvious signs of toxicity (NT-87).

A number of studies uncovered several new mechanisms of PARP inhibitor resistance.[8] Dr Neil Johnson presented an oral abstract describing a novel mechanism responsible for acquired PARP inhibitor resistance. BRCA1 mutations in the BRCA1 C-terminal domain normally lead to protein misfolding and degradation, promoting sensitivity to PARP inhibitors. Dr Johnson and colleagues have uncovered a mechanism, whereby retention of intron in a BRCA1 gene containing mutation in the BRCT domain results in intron translation and loss of BRCA1 C-terminal domain. This led to robust BRCA1 C-terminal expression and resistance to chemotherapy. Dr Lu Liu and colleagues demonstrated that short-term and chronic PARP inhibition can drive aldehyde dehydrogenase 1A1 expression, thus conferring stemness to the cancer cells,[9] which eventually drives adaptive resistance to PARP inhibitors (NT-100). Finally, Dr Anniina Färkkilä used *in vitro* exposure to PARP inhibitors to characterize the mechanisms of PARP inhibitor resistance in BRCA1 mutant cells. The resultant resistance to PARP inhibitors was mediated by several mechanisms in different clones, highlighting the potential for heterogeneity in resistance on PARPi treatment (NT-92).

With identification of PARP inhibitor resistance mechanisms comes the rationale for combination therapies to overcome the resistance. Dr Marilyne Labrie and colleagues implemented a "window of opportunity" trial to study adaptive resistance to PARP inhibitors. Using reverse- phase protein array analysis, measuring the expression of over 300 proteins in pre- and post-treatment samples, the authors were able to develop an algorithm that could suggest potential combination partners for PARP inhibition in specific patients (NT-098).

Targeting DNA damage through several targets is a rational strategy to overcome PARP inhibitor resistance.[10] Dr Erin George presented an oral abstract on the potential utility of ataxia telangiectasia and Rad3-related protein (ATR) inhibitors in combination with PARP inhibitors. They identified constitutive activation of the ATR/checkpoint kinase 1 pathway in PARP inhibitor and carboplatin-resistant cell lines. Treatment of such cell lines with an ATR inhibitor in combination with an ataxia-telangiectasia mutated inhibitor was synergistic both *in vitro* and *in vivo*. Interestingly, this approach was also effective in CCNE-amplified tumors, which are known to be highly resistant to therapy. Dr Anne Steino and colleagues have explored VAL-083 as a strategy to overcome platinum and PARP resistance. VAL-083 is a first-in-class DNA-damaging agent, which induces inter-strand DNA crosslinks leading to double-stranded DNA breaks. The authors examined VAL-083 in combination with PARP inhibitors both in homologous recombination-proficient and homologous recombination-deficient cell lines and demonstrated synergistic activity in both settings (NT-109). Dr Ludmila Szabova and colleagues evaluated another example of a DNA-damaging agent—non-camptothecin topoisomerase I inhibitors—in ovarian cancer. BRCA1/2- and PALB2-deficient cell lines were highly sensitive to the new compounds. Moreover, combination between the new compounds and olaparib was synergistic (NT-110). Dr Amber Yasmeen and colleagues explored poly-ADP-ribose glycohydrolase (PARG) as a potential strategy to sensitize ovarian cancer cells to DNA-damaging agents. PARG is responsible for poly-ADP-ribose catabolism which is synthesized by PARP.[11] Faulty poly-ADP-ribose formation or disintegration inhibits single-strand break repair. The authors demonstrate that PARG is expressed in 30% of ovarian cancers in The Cancer Genome Atlas. Inhibition of PARG in ovarian cancer cell lines resulted in reduced cellular proliferation and migration and sensitized the cells to PARP inhibitors and cisplatin (NT-117). Dr Rashid Gabbasov and colleagues focused on targeting heat shock protein 90, which plays a role in mediating maturation and stability of several key proteins required for DNA damage response.[12] They demonstrate that targeted inhibition of heat shock protein 90 with ganetespib sensitizes BRCA1-null cell lines to the effects of talazoparib (NT-094).

A number of studies looked at other signaling pathways that might be targeted in combination with PARP inhibition. Dr Alicia Beeghly-Fadiel and colleagues have demonstrated that nuclear orphan receptor NR4A1/TR3 plays an important pro-growth and pro-survival role in ovarian cancer.[13 14] Inhibition of NR4A1 using a chemical antagonist or small interfering RNA knockdown resulted in tumor growth inhibition, and was synergistic with PARP inhibitor therapy (NT-88). Dr Takeshi Fukumoto and colleagues have shown that up-regulation

of Wnt/β-catenin pathway in BRCA-mutant cancer cells results in PARP resistance.[15] Interestingly, activation of the Wnt pathway was secondary to N6-methyladenosine modification of FZD10 mRNA. PAPR inhibition and Wnt/β-catenin inhibitor showed synergistic suppression of growth of PARP inhibitor-resistant cancer both *in vitro* and *in vivo* in a xenograft ovarian cancer mouse model (NT-93).

OVERCOMING CHEMOTHERAPY RESISTANCE

A number of studies have focused on the mechanisms of resistance to chemotherapy and potential strategies to overcome it. Dr Wa Xian and colleagues used ovarian cancer resection specimens to generate libraries of ovarian cancer stem cells. The authors showed that while the majority of these cells are killed by chemotherapy, a number of clones are resistant to treatment. The resistant clones were characterized by a gene expression profile that was distinct from that of the sensitive clones. A broad screen of small molecules against the resistant clones proved that these cells are also resistant to other chemotherapy drugs. However, a number of compounds were either directly cytotoxic or cytotoxic in combination with paclitaxel, presenting a potential strategy to eliminate the resistant clones with upfront therapy and prevent cancer recurrence (NT-115). Dr Allison Sharrow and colleagues explored ovarian cancer stem cells as a potential mechanism of chemotherapy resistance.[9] Using aldehyde dehydrogenase 1 (ALDH1) as a marker of stemness in ovarian cancer cell lines, they demonstrated the increased resistance of populations with high levels of ALDH1 to chemotherapy. The authors further used gene expression analyses in these populations and identified several up-regulated pathways, including mTOR, FGF18, and CD47, which could be explored therapeutically (NT-107). In support of these findings, Dr Nuzhat Ahmed presented an oral abstract summarizing a proteomic analysis of chemo-naive and chemo-experienced ovarian cancer cells isolated from patients, demonstrating that chemotherapy results in enrichment of markers of cancer stem cells as well as alterations in pathways involved in DNA repair, immune recognition, cell cycle, and metabolism. This study provided important findings about potential mechanisms of chemotherapy resistance that may lead to a relapse in ovarian cancer and generated a rationale for potential novel combinations.

Dr Alex Cole and colleagues presented an oral abstract summarizing data on a new mechanism of chemotherapy resistance mediated by nuclear factor of activated T cells 3 (NFAT3), which is over-expressed in cancer stem cells.[16] They showed that over-expression of NFAT3 promotes quiescent phenotype with G0 arrest. While *in vivo* this results in tumor growth arrest, it also leads to chemoresistance. These data thus highlight the possibility that NFAT3 might be a potential mechanism of chemotherapy targeting and presents an attractive therapeutic target to be used in combination with chemotherapy.

Tumor hypoxia results in a number of biologic changes in the tumor cells and microenvironment.[17] Dr Andrea Nieto-Veloza demonstrated that chemical induction of hypoxia in ovarian cancer cell line results in paclitaxel resistance, although no effect is seen on sensitivity to cisplatin (NT-102). These findings highlight that hypoxia-mediated chemotherapy resistance does not occur with all agents.

Several groups employed new compounds in combination with chemotherapy as potential chemotherapy sensitizers. Dr Amber Yasmeen and colleagues explored whether differential sequencing of PARP inhibitors and chemotherapy might improve efficacy. Regardless of BRCA mutational status, exposure to PARP inhibition before chemotherapy resulted in efficient induction of apoptosis *in vitro* (NT-118). Dr Vermont Dia presented a potential strategy to overcome chemoresistance induced by transforming growth factor β1 using BG-4. BG-4 is a bioactive peptide isolated from the seeds of *Momordica charantia* and exhibits anti-cancer properties.[18] While addition of transforming growth factor β1 to ovarian cancer cell lines in the presence of paclitaxel and cisplatin resulted in chemotherapy resistance, the resistance was ameliorated by addition of BG-4. This was accompanied by reversal of transforming growth factor β1-induced epithelial to mesenchymal transition, suggesting a possible mechanism of BG-4 action (NT-91). Dr John Giannios presented a strategy of targeting chemoresistant ovarian cancer cells through the use of paired guide RNAs targeting microRNA-221/222, which are known to inhibit apoptosis.[19] By encapsulating paired guide RNAs into pegylated nanosomes tagged with anti-EphA2 antibodies, the authors were able to deliver the RNAs into EphA2-expressing ovarian cancer cells. This strategy led to inhibition of microRNA-221/222 biogenesis and downstream pathways, resulting in reversal of chemoresistance (NT-95).

TARGETING ENDOPLASMIC RETICULUM STRESS PATHWAY

Cancer cells are characterized by endoplasmic reticulum stress and unfolded protein response, which contributes to cancer cell survival and resistance to stress caused by chemotherapy, hypoxia, and nutrient deprivation.[20] Dr Carlos Telleria and colleagues explored endoplasmic reticulum stress as a potential mechanism to target ovarian cancer cells. Treatment with anti-progestin mifepristone or HIV protease inhibitor induced endoplasmic reticulum stress in ovarian cancer cell lines. Combination of these drugs with the proteasome inhibitor bortezomib resulted in enhanced ovarian cancer cell death (NT-111). Similarly, Dr Yang Yang-Hartwich and colleagues explored the unfolded protein response pathway as a potential target for overcoming chemoresistance in ovarian cancer. Using a novel sulfonamide SF-Y3, the authors demonstrated inhibition of proliferation and induction of apoptosis in ovarian cancer cells. This effect was primarily seen in cancer cells with high levels of Bip1, a key chaperone protein in the endoplasmic reticulum, implicating its role in SF-Y3-induced unfolded protein response (NT-116).

TARGETING METABOLIC PATHWAYS

Advances in understanding of metabolism and biosynthetic pathway alterations in cancer cells are the rationale for selective targeting of these pathways as a means to improve the efficacy of standard therapies. Dr Adegbite Emmanuel and colleagues revealed preliminary results of in silico screening of inhibitors of lactate dehydrogenase, which plays a role in ovarian cancer metabolism[21] (NT-86). Dr Manish Patankar and colleagues explored the use of oxidative phosphorylation inhibitors in ovarian cancer, given the mounting evidence that the mitochondrial pathway can also contribute to cancer cell metabolism. They showed that atovaquone is an efficient inhibitor of electron transport in ovarian cancer cells and leads to tumor growth inhibition in an ID8 tumor model. The authors highlighted several mechanisms of action of atovaquone, including production of free radicals and

inhibition of ion transport resulting in loss of mitochondrial membrane potential (NT-103).

ANTIBODY DRUG CONJUGATES

Targeting of ovarian cancer surface molecules using antibody drug conjugates is a viable therapeutic strategy, best supported by the initial data from the phase I trial of mirvetuximab soravtansine, a folate receptor-targeting antibody drug conjugate, which demonstrated a 26% response rate in a heavily pre-treated patient population and a 39% response rate in patients with three or fewer lines of therapy.[4] This generates a strong rationale for evaluation of analogous strategies targeting other surface molecules and for further optimization of folate receptor α targeting. Dr Venita De Almeida presented pre-clinical data with STRO-002, a novel antibody drug conjugate targeting folate receptor α, which was optimized by selection of the antibody, drug linker, conjugation site, and drug–antibody ratio that conferred the best pharmacological properties. The resultant drug exhibited high potency *in vitro* and *in vivo*, while exhibiting a high safety profile in toxicology studies (NT-90). Dr Chunsheng Li and colleagues presented an antibody drug conjugate strategy targeting CD248, which is expressed by over 90% of ovarian cancers. In pre-clinical models, the antibody was cytotoxic to cancer cells both *in vitro* and *in vivo* and induced infiltration of lymphocytes into tumors, highlighting a potential dual mechanism of action of this strategy with rationale for combination with immune checkpoint blockade (NT-99). Finally, Dr Wolf Wiedemeyer and colleagues explored SC-003, another antibody drug conjugate targeting dipeptidase 3. By screening a patient-derived xenograft bank of ovarian cancers, the authors identified dipeptidase 3 as a common target in tumor-initiating cells. Treatment of dipeptidase 3 + patient-derived xenograft models with SC-003 resulted in efficient tumor regression. By using dipeptidase 3-expressing syngeneic mouse cells, the authors further showed that such a strategy can synergize with systemic programmed cell death protein 1 (PD-1) blockade (NT-113).

IMMUNOTHERAPY

Treatment with immune checkpoint blockade has been evaluated in epithelial ovarian cancer, but with disappointing response rates to date.[22–25] These findings necessitate the development of rational combinations and identification of biomarkers that might predict response to PD-1/PD-L1 blockade. Dr Dmitriy Zamarin presented an oral abstract discussing the results of a phase II clinical trial evaluating the use of the PD-L1 inhibitor durvalumab in combination with folate receptor α vaccine TPIV200 in 27 patients with heavily pre-treated platinum resistant/refractory ovarian cancer. While the overall response rate was 4%, similar to previously observed results with single agent PD-1/PD-L1 inhibitors, the study demonstrated a median overall survival of 21 months, which is better than the expected overall survival of <1 year in this patient population.[26] There was evidence of potentially enhanced clinical benefit from subsequent chemotherapy in the patients after completion of immunotherapy, highlighting the rationale for the use of chemotherapy in combination with immune checkpoint blockade in this patient population. Dr Denise Cecile presented pre-clinical findings of insulin growth factor binding protein 2 vaccine in ID8-luciferase tumor models. The authors also employed a new method of anti-tumor assessment using multi-view multi-spectral imaging, allowing for more accurate tumor volume assessment. The study was able to demonstrate heterogeneity in metastatic tumor distribution and response, suggesting that this strategy may be a useful tool for evaluation of inter-tumoral heterogeneity in animal models.

OTHER THERAPEUTIC STRATEGIES

A number of abstracts discussed other novel targets and therapeutic modalities with potential application to ovarian cancer. Dr Karen Levy presented an oral abstract discussing pre-clinical data behind the use of a new radiation approach termed FLASH, which consists of short pulses of ultra-high dose radiation given in a single fraction.[27] In a peritoneal model of ID8 ovarian cancer, this approach was effective in controlling tumors, while having no significant toxicity in comparison with conventional radiation. Dr Varatharasa Thiviyanathan and colleagues developed single-stranded nucleic acid aptamers with ability to recognize and bind ovarian cancer endothelial cells through annexin A2. Using this technology, the authors made an RNA/DNA nanoparticle capable of delivering doxorubicin to cancer cells in animal models. This strategy presents a potential mechanism for delivering drugs directly to the tumors while avoiding systemic toxicity (NT-112).

Several studies focused on the alterations common to high-grade serous ovarian cancer and other histologies. Missense mutations in p53 are the most common genetic alterations in ovarian cancer.[28] Dr Satish Kumar Ramraj and colleagues used the p53 reactivator drug PRIMA-1^{MET} in combination with SHetA2, a small molecule that inhibits mortalin (mitochondrial heat shock protein 70).[29] The combination was synergistic in ovarian cancer cell lines with mutant and wild-type p53 and showed additive activity in p53-null cell lines (NT-104).

Clear cell carcinoma of the ovary represents a highly chemoresistant sub-type of ovarian carcinoma and frequently harbors mutations in ARID1A.[30] Dr Shogo Shigeta and colleagues performed small interfering RNA screens against two ovarian clear cell carcinoma cell lines. A number of genes were identified as potential targets, including bromodomain BET family proteins BRD2 and BRD3. Knockdown of these proteins using RNA interference resulted in tumor growth inhibition (NT-108). Dr Shuai Wu and colleagues explored the mechanisms of acquired resistance to enhancer of zeste homolog 2 (EZH2) inhibition. In ARID1A-mutated cancer cells, the switch of the SWI/SNF catalytic sub-unit from SMARCA4 to SMARCA2 was the primary mechanism of resistance, leading to induction of anti-apoptotic genes such as BCL2. Use of BCL2 inhibitor ABT263 was able to overcome EZH2 inhibitor resistance and was synergistic with EZH2 inhibition *in vivo* (NT-114).

Epithelial to mesenchymal transition contributes to ovarian tumor metastasis and chemoresistance.[31] Dr Junming Yue and colleagues demonstrated that knockout of the metal regulatory transcription factor 1 results in inhibition of this transition, leading to reduced cell proliferation, migration, and invasion (NT-120). Dr Carmela Ricciardelli and colleagues explored all-trans retinoic acid as a means of inhibiting the annexin A2-S100A10 signaling pathway, which plays a role in ovarian cancer invasion and metastasis.[32] Treatment of ovarian cancer cells with all-trans retinoic acid led to reduced cell

survival, proliferation, and invasion, although the mechanism was not always S100A10-dependent (NT-105). Dr Flavio Rizzolio and colleagues demonstrated that targeting of peptidyl prolyl cis-trans isomerase (Pin1), which controls different oncogenes and tumor suppressors, can inhibit tumor growth in mouse ovarian cancer model.[33] Chemical or short hairpin RNA inhibition of Pin1 in ovarian cancer cell lines sensitized the cells to the effect of carboplatin (NT-106).

Several studies have characterized new compounds whose mechanisms of action are not yet not fully understood. Dr Alexandria Young and colleagues explored synthetic analogs of phyllanthusmin for cytotoxic activity in ovarian cancer cell lines.[34] The most potent analog, PHY34, has nanomolar potency in high-grade serous ovarian carcinoma cell lines *in vitro* and displayed cytotoxic activity through late-stage autophagy inhibition and activation of apoptosis. The analog was also effective with intra-peritoneal administration in xenograft models (NT-119). Dr Arvinder Kapur and colleagues used fabclavine, a metabolite of *Xenorhabdus budapestensis*, and demonstrated that the compound inhibited ovarian cancer cell line proliferation and induced apoptotic cell killing at nanomolar concentrations (NT-096). Dr Powel Crosley and colleagues generated a TRAIL-expressing recombinant vaccinia virus and demonstrated its activity against granulosa cell tumor cell lines. Combination of the recombinant virus with procaspase-activating compound 1 resulted in further potentiation of lytic activity (NT-89). Dr David Pepin presented the results of the effects of Mullerian inhibiting substance on ovarian cancer cells isolated from ascites.[35] Using single cell RNA sequencing, their study uncovered a high degree of heterogeneity of expression of known and novel markers related to epithelial–mesenchymal states and stemness, both across patients and within patient samples. In addition, they uncovered some unexpected effects of Mullerian inhibiting substance on the immune cells in ascites. These findings will be important in understanding responses to this substance as it progresses through clinical development.

Finally, several groups presented early data on identification of novel compounds and pathways for targeting in ovarian cancer. Dr Hilary Kenny and colleagues used high throughput screening of small molecules against an ovarian cancer organotypic model that recapitulates features of ovarian cancer stroma. They identified three compounds—two targeting tyrosine kinases—which inhibited ovarian cancer adhesion, invasion, and growth (NT-97). Dr Einav Zmora and colleagues analyzed endothelial growth factor-like ligands from ascites of 43 patients with ovarian cancer and found that 86% of them expressed high levels of amphiregulin, which is a cytokine playing a role in tissue repair and inflammation.[36] The authors generated an antibody against amphiregulin and evaluated it in animal tumor models. Treatment with anti-amphiregulin resulted in significant prolongation of survival of mice bearing cancer xenografts (NT-121).

CONCLUDING REMARKS

In summary, the symposium presented a rich influx of information, highlighting novel mechanisms of primary and acquired resistance to chemotherapy and PARP inhibition. The revolving theme of combination therapy to overcome or prevent resistance resonates throughout all studies. Potential combination partners include other agents inhibiting DNA repair, agents targeting cellular checkpoints, and drugs effective against cancer stem cells. Immune checkpoint inhibitors, while effective in a small subset of patients, unfortunately demonstrate limited single-agent activity in ovarian cancer and rational combinations with other immunotherapies, PARP inhibitors, and standard chemotherapy are currently underway. Other novel therapeutic strategies focusing on endoplasmic reticulum stress response, epithelialto mesenchymal transition, and targeting of surface molecules using novel antibody drug conjugates demonstrate compelling evidence of anti-tumor activity in pre-clinical models and generate a strong rationale for evaluation in clinic.

Acknowledgements We thank Dr Deborah Armstrong for the critical review of the manuscript.

Contributors DZ was responsible for the review of the conference data and manuscript composition.

Funding DZ is a member of the Parker Institute for Cancer Immunotherapy at MSKCC. DZ is supported by the Ovarian Cancer Research Foundation Liz Tilberis Award, and the Department of Defense Ovarian Cancer Research Academy (OC150111). This work was supported in part by the MSK Cancer Center Support Grant P30 CA008748.

Competing interests DZ indicated financial activities outside of the submitted work, which include personal fees, grants, and intellectual property.

Patient consent for publication Not required.

Provenance and peer review Commissioned; internally peer reviewed.

REFERENCES

1. Konstantinopoulos P, Munster P, Forero-Torres A, *et al*. *TOPACIO: preliminary activity and safety in patients (PTS) with platinum-resistant ovarian cancer in a phase 1/2 study of niraparib in combination with pembrolizumab*. New Orleans, LA: SGO Annual Meeting, 2018.
2. Liu JF, Barry WT, Birrer M, *et al*. Combination cediranib and olaparib versus olaparib alone for women with recurrent platinum-sensitive ovarian cancer: a randomised phase 2 study. *Lancet Oncol* 2014;15:1207–14.
3. Konstantinopoulos PA, Barry W, Birrer M, *et al*. Phase I study of the alpha specific PI3-kinase inhibitor BYL719 and the poly (ADP-ribose) polymerase (PARP) inhibitor olaparib in recurrent ovarian and breast cancer: analysis of the dose escalation and ovarian cancer expansion cohort. *Cancer Res* 2017;77(13 Suppl). Abstract nr CT008.
4. Moore KN, Martin LP, O'Malley DM, *et al*. Safety and activity of mirvetuximab soravtansine (IMGN853), a folate receptor alpha-targeting antibody-drug conjugate, in platinum-resistant ovarian, fallopian tube, or primary peritoneal cancer: a phase I expansion study. *J Clin Oncol* 2017;35:1112–8.
5. Brill E, Yokoyama T, Nair J, *et al*. Prexasertib, a cell cycle checkpoint kinases 1 and 2 inhibitor, increases *in vitro* toxicity of PARP inhibition by preventing Rad51 foci formation in *BRCA* wild type high-grade serous ovarian cancer. *Oncotarget* 2017;8:111026–40.
6. Lee J-M, Nair J, Zimmer A, *et al*. Prexasertib, a cell cycle checkpoint kinase 1 and 2 inhibitor, in BRCA wild-type recurrent high-grade serous ovarian cancer: a first-in-class proof-of-concept phase 2 study. *Lancet Oncol* 2018;19:207–15.
7. Calzoni E, Cesaretti A, Polchi A, *et al*. Biocompatible polymer nanoparticles for drug delivery applications in cancer and neurodegenerative disorder therapies. *J Funct Biomater* 2019;10.10.3390/jfb10010004
8. Lim J, Tan DSP. Understanding resistance mechanisms and expanding the therapeutic utility of PARP inhibitors. *Cancers* 2017;9.

9. Shah MM, Landen CN. Ovarian cancer stem cells: are they real and why are they important? *Gynecol Oncol* 2014;132:483–9.
10. Pilie PG, Tang C, Mills GB, *et al*. State-of-the-art strategies for targeting the DNA damage response in cancer. *Nat Rev Clin Oncol* 2018;16:81–104.
11. Bu X, Kato J, Moss J. Emerging roles of ADP-ribosyl-acceptor hydrolases (ARHs) in tumorigenesis and cell death pathways. *Biochem Pharmacol* 2018.10.1016/j.bcp.2018.09.028
12. Pennisi R, Ascenzi P, di Masi A. Hsp90: a new player in DNA repair? *Biomolecules* 2015;5:2589–618.
13. Beard JA, Tenga A, Chen T. The interplay of NR4A receptors and the oncogene-tumor suppressor networks in cancer. *Cell Signal* 2015;27:257–66.
14. Wilson AJ, Liu AY, Roland J, *et al*. TR3 modulates platinum resistance in ovarian cancer. *Cancer Res* 2013;73:4758–69.
15. Yin P, Wang W, Zhang Z, *et al*. Wnt signaling in human and mouse breast cancer: focusing on Wnt ligands, receptors and antagonists. *Cancer Sci* 2018;109:3368–75.
16. Lu H, Huan C. Transcription factor NFAT, its role in cancer development, and as a potential target for chemoprevention. *Curr Cancer Drug Targets* 2007;7:343–53.
17. Kim JY, Lee JY. Targeting tumor adaption to chronic hypoxia: implications for drug resistance, and how it can be overcome. *Int J Mol Sci* 2017;18.
18. Dia VP, Krishnan HB. BG-4, a novel anticancer peptide from bitter gourd (Momordica charantia), promotes apoptosis in human colon cancer cells. *Sci Rep* 2016;6.
19. Fu L-lei, Wen X, Bao J-ku, *et al*. MicroRNA-modulated autophagic signaling networks in cancer. *Int J Biochem Cell Biol* 2012;44:733–6.
20. Madden E, Logue SE, Healy SJ, *et al*. The role of the unfolded protein response in cancer progression: from oncogenesis to chemoresistance. *Biol Cell* 2019;111:1–17.
21. Xiang J, Zhou L, Zhuang Y, *et al*. Lactate dehydrogenase is correlated with clinical stage and grade and is downregulated by si-SATB1 in ovarian cancer. *Oncol Rep* 2018;40:2788–97.
22. Matulonis UA, Shapira-Frommer R, Santin A, *et al*. Antitumor activity and safety of pembrolizumab in patients with advanced recurrent ovarian cancer: interim results from the phase 2 KEYNOTE-100 study. *J Clin Oncol* 2018;36(15_suppl).
23. Hamanishi J, Mandai M, Ikeda T, *et al*. Safety and antitumor activity of anti-PD-1 antibody, nivolumab, in patients with platinum-resistant ovarian cancer. *J Clin Oncol* 2015;33:4015–22.
24. Disis ML, Patel MR, Pant S, *et al*. Avelumab (MSB0010718C), an anti-PD-L1 antibody, in patients with previously treated, recurrent or refractory ovarian cancer: a phase Ib, open-label expansion trial. *J Clin Oncol* 2015;33(15_suppl). abstr 5509.
25. Liu YL, Zamarin D. Combination immune checkpoint blockade strategies to maximize immune response in gynecological cancers. *Curr Oncol Rep* 2018;20. 94.Pmc6244932.
26. Pujade-Lauraine E, Hilpert F, Weber B, *et al*. Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: the AURELIA open-label randomized phase III trial. *J Clin Oncol* 2014;32:1302–8.
27. Favaudon V, Caplier L, Monceau V, *et al*. Ultrahigh dose-rate flash irradiation increases the differential response between normal and tumor tissue in mice. *Sci Transl Med* 2014;6.
28. Atlas TCG. Integrated genomic analyses of ovarian carcinoma. *Nature* 2011;474:609–15.
29. Mayer MP, Gierasch LM. Recent advances in the structural and mechanistic aspects of hsp70 molecular chaperones. *J Biol Chem* 2018;294:2085–97.
30. Caumanns JJ, Wisman GBA, Berns K, *et al*. ARID1A mutant ovarian clear cell carcinoma: a clear target for synthetic lethal strategies. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer* 2018;1870:176–84.
31. Deng J, Wang L, Chen H, *et al*. Targeting epithelial-mesenchymal transition and cancer stem cells for chemoresistant ovarian cancer. *Oncotarget* 2016;7:55771–88.
32. Liu Y, Myrvang HK, Dekker LV. Annexin A2 complexes with S100 proteins: structure, function and pharmacological manipulation. *Br J Pharmacol* 2015;172:1664–76.
33. Cheng C-W, Tse E. Pin1 in cell cycle control and cancer. *Front Pharmacol* 2018;9:1367.
34. Young AN, Herrera D, Huntsman AC, *et al*. Phyllanthusmin derivatives induce apoptosis and reduce tumor burden in high-grade serous ovarian cancer by late-stage autophagy inhibition. *Mol Cancer Ther* 2018;17:2123–35.
35. Kushnir VA, Seifer DB, Barad DH, *et al*. Potential therapeutic applications of human anti-müllerian hormone (AMH) analogues in reproductive medicine. *J Assist Reprod Genet* 2017;34:1105–13.
36. Singh B, Carpenter G, Coffey RJ. EGF receptor ligands: recent advances. *F1000Res* 2016;5.

Review Article

INTERNATIONAL JOURNAL OF GYNECOLOGICAL CANCER

OPEN ACCESS

Survivor advocacy: I speak for those who can't

Sachia Stonefeld Powell

Correspondence to
Ms. Sachia Stonefeld Powell, Rivkin Center for Ovarian Cancer, Seattle, WA 98122, USA; sachiaspowell@gmail.com

Received 18 March 2019
Accepted 5 April 2019

ABSTRACT

The 12th Biennial Ovarian Cancer Research Symposium held on September 13–15, 2018, brought together ovarian cancer scientists, clinicians, survivors, and advocates in Seattle, Washington. The Symposium featured a panel on 'The role of advocates in ovarian cancer research' aimed at facilitating discussion between scientists and patient advocates to enable a more patient-centric approach to ovarian cancer research. Here we describe learnings from panelists that included seasoned research scientists and patient advocates.

I attended the panel discussion on 'The role of advocates in ovarian cancer research' during the 12th Biennial Ovarian Cancer Research Symposium and I learnt something new that I have actually known all along: *there aren't many ovarian cancer survivors; therefore, there aren't many ovarian cancer survivor advocates*. Ovarian cancer accounts for only 2.5% of cancer diagnoses in women, and the survival rate is much lower than that of other cancers. Of those who survive, some will not be well enough to advocate or have the mental energy to act. Therefore, I—an ovarian cancer survivor—am one of a relatively small number of people with the experience, ability, and interest to be a survivor advocate.

Survivor advocates are people who have navigated a diagnosis of cancer and its subsequent treatment, and who share the knowledge gained through their personal experience with a broader audience. The goal in disseminating the panel's collective wisdom on the topic of advocates in research is to bridge the gap between the advocate who wants to make an impact on a disease that affected them personally, and the scientist who spends their career trying to affect the same disease but without the perspective of one who has lived through it. Hopefully, by explaining some of the possible ways that advocates and scientists can interact, ovarian cancer scientists will be inspired to incorporate patients' perspectives into all aspects of their studies.

The panel emphasized that survivor advocates can and should be involved in every step along the cancer research path. Advocates bring the patient perspective to researchers for use in grant proposals to get research support. Advocates can play a critical role by posing questions to the scientists to help them better frame the topics to be examined, and to question their assumptions. Advocates can provide feedback about the layperson's summary in grant abstracts and in clinical protocols. Advocates can also help to publicize clinical trials or spread other information through their close-knit network of survivors/friends. And, of course, they can advocate for legislative action and additional research funding. Through the process, advocates, by their very presence, emphasize the needs of the patient and the related need for urgency, paving the way to patient-centric healthcare.

There are many ways to get started in advocacy. To gain a better understanding, advocates can attend cancer research conferences to learn about the current state of research and meet those involved in it. The conferences also provide an excellent opportunity to meet other survivor advocates and scientists; that network can lead to advocacy opportunities. Additionally, several organizations, such as SHARE Cancer Support, the Research Advocacy Network, and the Ovarian Cancer Research Alliance, provide training, including online courses, and conferences specifically for those interested in advocacy. The training in basic medical research terminology and scientific concepts provides advocates with the necessary information to advocate knowledgeably. Some groups also provide advocate mentorship, pairing new advocates with those with experience. Even organizations that are not cancer-specific can provide relevant advocacy information and training.

Advocates can also contact local healthcare institutions and research centers in their area to determine whether there are existing advocacy programmes they can join. These entities may have advisory boards, advocacy networks, or survivor support groups with openings for advocates, or may welcome advocates to share their stories in educational settings. Regional and national organizations such as the National Institutes of Health and the National Cancer Institute (NCI) also have advocacy opportunities through their advisory boards or other programmes. For example, NCI's Office of Advocacy Relations specifically works to connect survivors with NCI to improve cancer research. And finally, organizations that allocate research funds, such as the Department of Defense Ovarian Cancer Research Program, use survivor advocates to review grant applications and clinical trial approvals.

To cite: Powell SS. *Int J Gynecol Cancer* 2019;**29**:s22–s23.

Several simple steps can be taken by advocates at the grassroots level. Those interested in policy adjustments or funding enhancement can write to their congressional representatives to encourage change, especially by emphasizing the amount of money that comes into their districts as a result of the funds they allocate to cancer research. Advocates can themselves raise funds for research through numerous fundraising activities. They can post their survivor status on social media; spreading the word about survivorship status can help lead to advocacy opportunities. Advocates can also use social media to disseminate information about medical research, such as clinical trials, to their networks of survivors/friends. Finally, advocates can simply ask relevant organizations: 'What can I do to help?"

From their experience, survivor advocates gain the opportunity to give back to the scientists who pioneered their life-saving treatment, and to give their survival a purpose. In return for their service, advocates ask only that scientists use their collective voice to inform Congress by contacting their representatives at key times and exercising their right to vote. Advocates also appreciate it when the scientists can cover their out-of-pocket expenses by including those expenses in the grant proposals.

Like the survivor advocates on the panel, *I keep losing my friends*. Once I disclosed my ovarian cancer diagnosis I became a magnet for anyone who knew someone with a similar diagnosis. Suddenly, I knew many women with ovarian cancer, who I now count among my friends. But as ovarian cancer continues its insidious attack I also have more friends who have succumbed to its assault. As a result, I have become even more passionate about advocating. *I am the voice of those who can no longer speak for themselves.*

Contributors This article was written by SSP. SSP is an ovarian cancer survivor, and an instructor and facilitator in the Education Program of the Rivkin Center for Ovarian Cancer.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Commissioned; internally peer reviewed.