Objective: The aim of this study was to summarize developments in novel therapeutics for ovarian cancer presented at the Ovarian Cancer Research Symposium held at the University of Washington.

Methods: A symposium of the leaders in ovarian cancer research was convened to present and discuss current advances and future directions in ovarian cancer research.

Results: The fourth session was held on September 13, 2016, and focused on Novel Therapeutics for Ovarian Cancer. The session featured a keynote presentation on Novel Immunotherapeutics for Ovarian Cancer from Nora Disis and an invited oral presentation from Scott Kaufmann that discussed poly (ADP-ribose) polymerase (PARP) Inhibitor Combinations for the Treatment of Ovarian Cancer. Eight additional oral presentations were selected from abstract submissions. Thirty-eight abstracts were presented as posters highlighting recent advances in tumor immunology, PARP inhibition, chemoresistance, and novel targets for ovarian cancer therapy.

Conclusions: PARP inhibitors, immunotherapies, and targeted therapies are but some of the expanding number of treatment options for ovarian cancer patients. Identification of the subsets of patients who will benefit most from these treatments remains the subject of intense preclinical and clinical research. Evidence presented at this symposium suggests that non-BRCA patients also benefit from PARP inhibitor therapies. Improved understanding of the mechanisms of chemoresistance and encouraging preclinical data presented for combinatorial approaches may soon yield new therapies for ovarian cancers that are resistant and refractory to standard treatments.

Key Words: Ovarian cancer, PARP inhibitors, Immunotherapy

Received June 23, 2017, and in revised form June 25, 2017.
Accepted for publication July 3, 2017.

(Int J Gynecol Cancer 2017;00: 00–00)
activation and lytic activity of CD8 T cells. This phenotype of immune response has been observed in high-grade serous ovarian cancer where markers of activated CD8 T cells are associated with a more favorable prognosis. Foreign antigens have been thought to drive signaling toward a type-1 phenotype, whereas aberrantly expressed self-antigens bias toward a type-2 response. The goal for cancer immunotherapy is then to seek levers to tip the balance of immune responses toward the inflammatory, type-1 responses that lead to tumor regression. Approaches taken to optimizing therapeutic immune include increasing the numbers of effector T cells, enhancing existing immunity, and modulating the tumor microenvironment. Recent applications of agents targeting PD-L1/PD-L1 in ovarian cancer have shown modest response rates. When 124 patients with refractory or recurrent ovarian cancer, unscreened for PD-L1 expression, were treated with avelumab, which is an antibody targeting PD-L1, in a phase-I/II study, 9.7% of patients had a partial response, and 44.4% of patients achieved stable disease with diverse patterns of response. Although expression of PD-L1 in tumor has been reported to correlate with responses to these agents in other tumors, this was not seen with avelumab in ovarian cancer. BRCA1 and BRCA2 mutation status also did not correlate with response. To enhance existing immunity, vaccines are being investigated for their ability to stimulate antigen specific T cells in ovarian cancer; however, these attempts in other malignancies have been sometimes hampered by a phenotype of immune response that proves to be unfavorable to the elimination of tumors. To generate antigen specific T cell responses with the desired type 1 phenotype, epitopes can be screened for those that preferentially induce these responses, as has been demonstrated with a vaccine targeting IGFBP-2. Epigenetic reprogramming through DNA methylation has also been shown to alter T-cell trafficking to tumors. Alternate approaches to prevent immune escape of tumor may rest with strategies such as the selective depletion of macrophages and targeting the unique features of the tumor microenvironment.

Immune therapy approaches may also be applied through epigenetic control of critical antitumor immune functions. Hengrui Zhu from the Wistar Institute in Philadelphia presented a study examining the roles of epigenetic mutations in regulating PD-L1 gene expression and modulating antitumor immunity. A small molecule library of epigenetic inhibitors was screened for their ability to inhibit PD-L1 expression in epithelial ovarian cancer. Positives were confirmed in vivo using the ID8 mouse model. When a bromodomain and extraterminal protein (BET) inhibitor, JQ1, which targets BRD4, is used in ovarian cancer cells, PD-L1 expression in tumor and immune cells are suppressed. ChIP-seq and ChIP assays showed that BRD4 directly binds to the PD-L1 gene promoter and regulates transcription. When tested in mouse models, BET inhibition suppresses tumor growth and improves the survival of mice through activation of antitumor T cells. These findings suggest that BET inhibitors could be used to target the PD-L1/PD-1 interaction in ovarian cancer and generate antitumor immune responses.

PARP Inhibitors

PARP inhibitors (PARPi) have shown promising single-agent activity in ovarian cancer patients with BRCA1 and BRCA2 mutations. Moreover, subsets of patients that do not have germline mutations in BRCA1 or BRCA2 genes have also demonstrated responses to PARPi. The PARPi olaparib was awarded Food and Drug Administration approval for the treatment of ovarian cancer patients with BRCA1 and BRCA2 mutations who have received 3 or more chemotherapies. In Europe, olaparib was approved by the European Medicines Agency for use as a maintenance treatment of patients with platinum-sensitive relapsed BRCA mutated ovarian cancer who had a complete or partial response to platinum therapy. In December 2016, the poly (ADP-ribose) polymerase (PARP) inhibitor rucaparib was granted accelerated approval by the Food and Drug Administration for the treatment of patients with deleterious BRCA mutations. Of significance, rucaparib is approved for the treatment of women with both germline and somatic BRCA mutations associated with advanced ovarian cancer who have been treated with 2 or more chemotherapies.

The most effective strategy for the use of PARPi in different patient subgroups continues to be the subject of clinical research. Saul Rivkin, MD, presented an update on a phase-I/II expansion study of patients who were treated with the maximum tolerated dose of olaparib plus weekly (metronomic) carboplatin and paclitaxel in relapsed ovarian cancer patients. In this trial, a total of 54 patients were evaluated who had all failed first-line platinum containing therapy. Patients received metronomic therapy of paclitaxel 60 mg/m2 IV and carboplatin AUC 2 IV weekly for 3 of 4 weeks, and olaparib tablets at 150 mg twice daily administered orally for 3 consecutive days every week for each cycle. Dr Rivkin reported that 25% patients had a complete response (CR), 31% partial response (PR), 23% stable disease, 21% progressive disease. Of the 13 CRs, 4 were BRCA negative. Progression-free survival for BRCA-positive subjects was 12.6 months versus 4.8 months for BRCA negative. Median overall survival for BRCA-positive subjects was 24 months versus 16 months for BRCA negatives. This study represents a successful combination of olaparib with carboplatin and paclitaxel that has been well tolerated and can be safely administered. A previous similar study also supports these findings. In addition, this work reinforces previous clinical studies showing that olaparib is highly effective in BRCA-positive patients, but there are subsets of patients without BRCA mutations that have demonstrated similar benefit. Many efforts are ongoing to generate methods that accurately identify these patients.

Studies led by Elizabeth Swisher, MD, have sought to identify BRCA-negative patients with alternative genomic defects that result in PARPi sensitivity. It was previously untested whether BRCA1/1 promoter methylation impacts PARPi response. The Cancer Genome Atlas and others failed to show improved survival in ovarian cancers with BRCA1 methylation. ARIEL2 is a phase-2 study of the PARPi rucaparib in patients with recurrent platinum sensitive high-grade ovarian and peritoneal or fallopian tube carcinoma. Swisher and colleagues assessed BRCA1 and RAD51C promoter hypermethylation using methylation-sensitive polymerase chain reaction in paired archival and pretreatment biopsies from ARIEL2 patients. Of 165 cases, 21 (12.7%) had BRCA1 and 4 (2.4%) had RAD51C promoter methylation. Methylation of either was mutually exclusive with mutations in these genes or other homologous recombination...
(HR) genes tested. Confirmed Response Evaluation Criteria In Solid Tumors (RECIST) responses were seen in 52.4% (11/21) of BRCA1 and 75% (3/4) RAD51C-methylated cases. In summary, this study demonstrated that BRCA1 and RAD51C methylation correlated with high response to PARPi. However, Dr Swisher cautioned that loss of methylation also frequently occurs and could account for lack of differences in overall survival despite initial improved therapeutic sensitivity.

Anna Piskorz, PhD, described her group’s work examining circulating tumor DNA (ctDNA) from patients in the ARIEL2 study as a means to examine tumor response to rucaparib treatments. Piskorz and colleagues developed a targeted deep sequencing approach to detect low-frequency mutations throughout the TP53 gene in ctDNA. Clinical response rates were evaluated by RECIST v1.1 and GCIG CA-125 criteria. Median TP53 mutant allele frequency at screening and cycle 1 day 1 was 5.1% and 3.8%, respectively. Dr Piskorz described that 7/9 patients demonstrated a greater than 50% reduction TP53 mutant allele frequency in ctDNA at cycle 2 also achieved a RECIST-confirmed PR. No patients with less than 50% reduction at cycle 2 achieved a RECIST response. This work demonstrates that ctDNA is a promising biomarker for response to PARPi rucaparib and could have the potential to predict response to other therapeutics. In another approach, allelic imbalance (AI)/loss of heterozygosity, referred to as “genomic scarring,” was used as a surrogate measure of HR deficiency and as a predictor of response to PARPi and angiogenesis inhibitor therapy. Here, Dr Wang demonstrated that a high AI score positively correlated with the degree of clinical response to either olaparib alone or in combination with cediranib. These data provide evidence that AI scores may be useful for predicting clinical responses to single-agent or PARPi combination therapy.

In addition to exploring strategies for PARPi deployment, understanding therapy resistance mechanisms is crucial for determining how best to prevent the onset of resistance as well as to generate biomarkers that predict therapeutic outcomes. Moreover, understanding the biology of resistance aids drug development efforts targeting resistance-driving pathways. Neil Johnson, PhD, presented work describing a new mechanism of resistance to PARPi and platinum in BRCA1 mutation carriers. The BRCA1185delAG allele is a common inherited mutation located close to the protein translation start site, which is thought to produce a short peptide devoid of function. Johnson presented work demonstrating PARPi and cisplatin resistant cancers that did not harbor secondary reversion mutations. Rather, increased expression of a really interesting new gene domain-deficient BRCA1 protein (Rdd-BRCA1) was required for resistance. Translation initiation occurred downstream of the frameshift mutation, likely at the BRCA1-Met-297 codon (Fig. 1). Furthermore, Rdd-BRCA1 protein expression was detectable in recurrent carcinomas from germline BRCA1185delAG mutation carriers. Going forward, it will be important to determine the frequency of Rdd-BRCA1 expression in the patient population and if specific mutations predict particular resistance mechanisms and therapeutic outcomes.

Efforts to increase the potency and activity of PARP inhibitors may prevent or delay the onset of resistance. Drs Baldwin and Sridhar presented their work using nanoolaparib for the treatment of ovarian cancer. Olaparib is administered by oral dosage and requires the drug to undergo first-pass metabolism, inactivating a significant fraction of the dose. The presenters developed a nanoparticle delivery system to allow for local delivery to the intraperitoneal cavity. In preclinical studies using mouse models, nanoolaparib provided a more uniform antitumor response to treatment compared with oral olaparib. However, toxicity issues arose with long-term treatments. Further studies are required to determine the therapeutic efficacy of nanoolaparib.

In addition to using PARPi as a monotherapy, another strategy includes combining PARPi with DNA damaging chemotherapy, where there is evidence that PARPi can prevent the repair of DNA damage and sensitize cancers to chemotherapy. Scott Kaufmann, MD, PhD, described preclinical and clinical studies that combined PARPi with cytotoxic chemotherapies. Previous preclinical work showed that PARPi enhances the cytotoxicity of topotecan by trapping PARPi at sites of topotecan-induced DNA damage. Among 52 evaluable patients, objective responses (1 CR, 3 PRs) and stable disease (22 patients, 6 lasting ≥8 cycles) were observed. There was no correlation with germline BRCA1 or BRCA2 status; however, of note a patient with a RAD51D mutation had stable disease for 14 cycles.

In addition, the PARPi enzyme is involved in the base excision repair process, and PARPi inhibitors have been shown to block this DNA repair pathway. In preclinical studies, PARPi enhanced the cytotoxicity of floxuridine, which is an agent that was previously shown to have clinical activity in Figure 1. Novel mechanisms of PARP inhibitor resistance. BRCA1 germline mutations often result in stop codons and an early end to translation. Evidence presented suggests that cancers with BRCA1 mutations located at the 5’ end of the gene may generate therapy resistance-inducing BRCA1 proteins. Here, protein translation initiates at in-frame start sites located downstream of the mutation-inducing stop codon. The resulting BRCA1 proteins lack the RING domain but retain other domains important for HR DNA repair and PARPi inhibitor resistance. RING indicates really interesting new gene.
Floxuridine is metabolized to 5-fluorouracil and induces DNA damage that can be repaired by base excision repair. Building on these preclinical observations, a phase 1 trial of veliparib and floxuridine in patients with recurrent ovarian cancer confined to the peritoneal cavity was conducted (NT01749397). Patients were treated with veliparib PO twice daily on days 1 to 10 and floxuridine on days 3 to 5 every 21 days. The trial is currently ongoing, however, among 20 evaluable patients, treatment duration has ranged from 2 to 33 cycles. These studies build on the different aspects of PARPi biological functions and illustrate two different approaches to enhance the activity of PARPi in relapsed ovarian cancer.

Chemoresistance and Novel Targets for Therapy

Although most of the advanced ovarian cancers are initially responsive to platinum-based chemotherapy, resistance to chemotherapies ultimately develops in recurrent tumors. Lidia Hernandez of the National Cancer Institute presented a study based on the finding that ovarian cancers with low expression of caspase-8 have poorer overall survival. Combination therapy with second mitochondrial-derived activator of caspases (SMAC) mimetics can target ovarian cancer cells with low caspase-8 and induce necroptotic cell death. The SMAC mimetics synergized with carboplatin and paclitaxel chemotherapy in vitro. Testing in mouse xenograft tumors using this combination showed 50% decrease in the growth of low caspase-8 expressing xenografts. These findings suggest that ovarian cancers with low expression of caspase-8 may benefit from the addition of treatments that promote nonapoptotic forms of cell death-like SMAC mimetic agents.

ARID1A is mutated in approximately 50% and 30% of clear cell (OCCC) and endometrioid (OEC) ovarian cancers, respectively. The OCCC carries a worse prognosis and is more chemoresistant compared with other histotypes of ovarian cancer, therefore there is an urgent need for improved therapeutic strategies targeting this disease. Benjamin Bitler, PhD, discussed his group’s work investigating the role of histone deacetylases (HDACs) in ARID1A wild type and mutated OCCC cell lines. Bitler discovered that ARID1A contributes to the repression of HDACs and ARID1A inactivation promotes aberrant transcriptional regulation of HDACs. Furthermore, HDAC inhibitors had preferential cell-killing effects on ARID1A mutated rather than wild-type cancer cell lines. Significantly, HDAC inhibition improved the survival of mice bearing ARID1A mutated tumors. Therefore, HDAC inhibition could represent a novel therapeutic strategy for ARID1A mutated cancers.

Small cell carcinoma of the ovary, hypercalcemic type (SCCOHT) is a rare, poorly differentiated tumor that is seen in younger women characterized by rapid progression and high-mortality rates. Yemin Wang, PhD, from the University of British Columbia presented data studying whether the histone methyltransferase, EZH2, may be a target potential in tumors with a deficiency in the SWI/SNF chromatin remodeling complex. Immunohistochemistry showed expression of EZH2 in all 24 SCCOHT. Compared with other ovarian cancer cell lines, SCCOHT cells had enhanced sensitivity to EZH2 inhibitors and EZH2 shRNA, with evidence of cell cycle arrest, apoptosis, and differentiation. Experiments carried out in xenograft-bearing mice confirmed these findings, with delayed tumor progression and improved survival. They also reported that SCCOHT cells are hypersensitive to other histone deacetylase inhibitors and showed synergy with EZH2 inhibition. Their studies suggest that EZH2 could be a novel target for therapy in SCCOHT.

Additional studies presented using inhibitors of epigenetic modifiers included work presented by Dineo Khabele, MD. Here, the bromodomain inhibitor JQ1 was shown to sensitize HR repair proficient ovarian cancers to PARPi treatment. In another study presented by James Duncan, PhD, JQ1 inhibitors were effective as single agents for the treatment of ovarian cancer cell lines. However, resistance quickly developed and was mediated by dynamic reprogramming of the kinome. Dr. Duncan demonstrated that combining JQ1 with kinase inhibitors provided longer responses and prevented the emergence of resistance.

Concluding Remarks

In summary, PARP inhibitors and immunotherapies represent meaningful life-improving treatment options for ovarian cancer patients. However, the optimal patient groups that will benefit from these agents and the fine-tuning of their administration remain the subject of preclinical and clinical research. In particular, evidence presented at this symposium indicates that non-BRCA patients also benefit from PARPi therapy. The ability to readily identify these patients remains a challenge and multiple methods are currently being explored. Although PARP inhibitors have demonstrated survival improvements, similar to platinum-based therapies, drug resistance invariably emerges and these agents are not curative. An improved understanding of therapy-resistance mechanisms may provide opportunities for the rationale design of approaches to prevent or subdue resistant disease. Furthermore, encouraging data from several preclinical investigations characterizing new combination therapies should be translated into clinical studies and could provide new opportunities to improve outcomes for patients who develop recalcitrant ovarian cancers.

REFERENCES


