Novel therapeutics: response and resistance in ovarian cancer

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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 II/I trial in 28 patients with advanced platinum-resistant non-BRCA-mutant ovarian cancer demonstrated a promising response
rate of 33%. 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. 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. 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 chemotherap. 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, which eventually drives adaptive resistance to PARP inhibitors (NT-100). Finally, Dr Annina 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. 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 synergetic (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. 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. 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. 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. PARP 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. These 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 GO arrest. While in vitro 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 chemotherapynonresponse, 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 metabolism21 (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. This generates a strong rationale for evaluation of anal- 

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**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 consistently demonstrates strong synergies in multi-drug combinations. 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 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. Dr Satish Kumar Ramraj and colleagues used the p53 reactivator drug PRIMA-1MET in combination with ShetA2, a small molecule that inhibits mortalin (mitochondrial heat shock protein 70). 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. 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. Dr Junming Yue and colleagues demonstrated that knockdown 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 Ricciardi 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. 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 serious 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 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 identified three compounds—two targeting tyrosine kinases—which inhibited ovarian cancer adhesion, invasion, and growth (NT-97).

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, epithelial to 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.

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