Improving the efficacy of PARP inhibition with a novel ATR inhibitor, ATRN119, in ovarian high grade serous cancers

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OBJECTIVE

We hypothesize that ATRi, using a new selective small molecule inhibitor (ATRN119), in combination with PARPi, will increase tumor regression compared to PARPi monotherapy with acceptable toxicity.

ABSTRACT

INTRODUCTION:

PARP inhibitors (PARPi) inhibit the repair of single-stranded breaks (SSBs), leading to DSBs at replication forks. ATR responds to replicative stress by stabilizing replication forks leading to cell cycle arrest, allowing DNA to repair. ATR inhibition (ATRi) is synthetically lethal with many genetic alternations found in ovarian HGSCs including cyclin E overexpression as well as *BRCA1/2 and TP53* mutations. While ATRi's are in early clinical trial development, improvement in specificity to minimize off-target effects and toxicity are needed. *We hypothesize that ATRi, using a new selective small molecule inhibitor (ATRN119), in combination with PARPi, will increase tumor regression compared to PARPi monotherapy with acceptable toxicity.*

METHODS:

ATRi (ATRN119), carboplatin and PARPi (AZD2281; olaparib) were evaluated in PEO1 (*BRCA2*-mutant), JHOS4 (*BRCA1*-mutant), PEO4 (*BRCA* wild-type), and WO-24 primary ovarian cells (*BRCA* wild-type, Cyclin E high). Viability and colony formation was assessed. Drug effects on target and off-target pathways were performed. Drugs were tested as monotherapy and in combination using an orthotopic ovarian cancer patient-derived xenograft (PDX) model.

RESULTS:

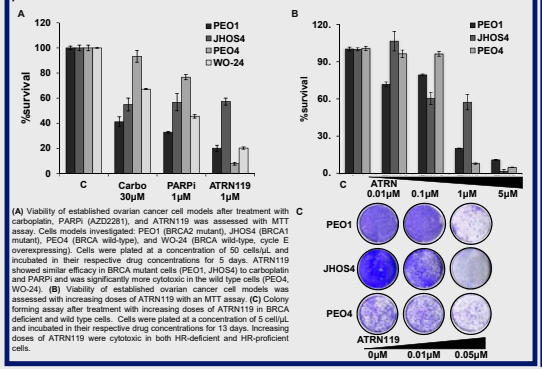
ATRIN119 treatment alone significantly decreased cell viability (<20%) in *BRCA* mutant, CCNE1-overexpressing HGSOC cells which were PARPi-resistant and platinum-resistant. Similarly, ATRN119 was synergistic in decreasing cell survival (<10%) when in combination with AZD2281 in these cell models. ATRN119 targeted ATR by decreasing pCHK1 at nanomolar concentrations with no effect on mTOR/ATM showing selectivity. Increases in pCHK1 with PARPi treatment were overcome with ATRi using ATRN119. ATRN119 alone had similar anti-tumor effects as PARPi alone in a *BRCA2*-deficient PDX model. Combination of ATRN119 with PARPi was tolerable and resulted in complete tumor regression unlike monotherapy in a *BRCA2* mutant PDX model.

CONCLUSION:

ATRN119 is a new ATRi that shows increased selectivity and potency compared to older ATRi. ATRN119 is as effective as PARPi monotherapy resulting in tumor suppression in BRCA mutant PDXs. However, combination ATRN119 with PAPRi is tolerable and results in complete tumor regression suggesting combination therapies may make PARPi more effective in the recurrent setting.

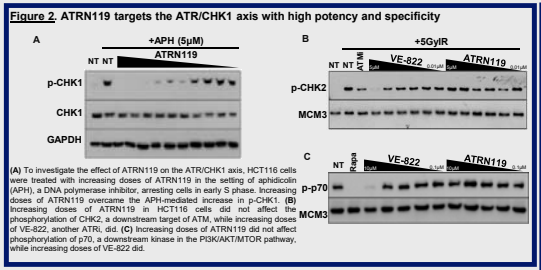
RESULTS

Figure 1. Targeting the ATR/CHK1 pathway with ATRIN119 is effective in both HR deficient and proficient established ovarian cancer cell models.



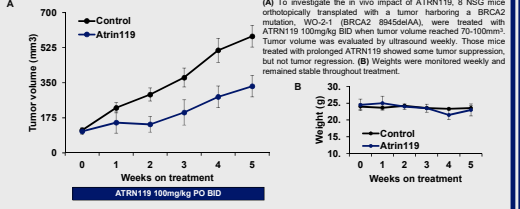
(A) Viability of established ovarian cancer cell models after treatment with carboplatin, PARPi (AZD2281), and ATRN119 was assessed with MTT assay. Cells models investigated: PEO1 (BRCA2 mutant), JHOS4 (BRCA1 mutant), PEO4 (BRCA wild-type), and WO-24 (BRCA wild-type, cycle E overexpressing). Cells were plated at a concentration of 50 cells/μL and incubated in their respective drug concentrations for 5 days. ATRN119 showed similar efficacy in BRCA mutant cells (PEO1, JHOS4) to carboplatin and PARPi and was significantly more cytotoxic in the wild type cells (PEO4, WO-24). **(B)** Viability of established ovarian cancer cell models was assessed with increasing doses of ATRN119 with an MTT assay. **(C)** Colony forming assay after treatment with increasing doses of ATRN119 in BRCA deficient and wild type cells. Cells were plated at a concentration of 5 cell/μL and incubated in their respective drug concentrations for 13 days. Increasing doses of ATRN119 were cytotoxic in both HR-deficient and HR-proficient cells.

Figure 2. ATRN119 targets the ATR/CHK1 axis with high potency and specificity



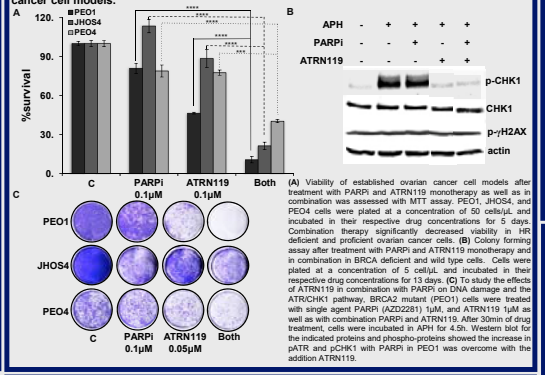
(A) To investigate the effect of ATRN119 on the ATR/CHK1 axis, HCT116 cells were treated with increasing doses of ATRN119 in the setting of aphidicolin (APH), a DNA polymerase inhibitor, arresting cells in early S phase. Increasing doses of ATRN119 overcame the APH-mediated increase in p-CHK1. **(B)** Increasing doses of ATRN119 in HCT116 cells did not affect the phosphorylation of CHK2, a downstream target of ATM, while increasing doses of VE-822, another ATRi, did. **(C)** Increasing doses of ATRN119 did not affect phosphorylation of p70, a downstream kinase in the PI3K/AKT/MTOR pathway, while increasing doses of VE-822 did.

Figure 3. ATRIN119 has single agent activity in a BRCA2 mutant HGSOC PDX model.



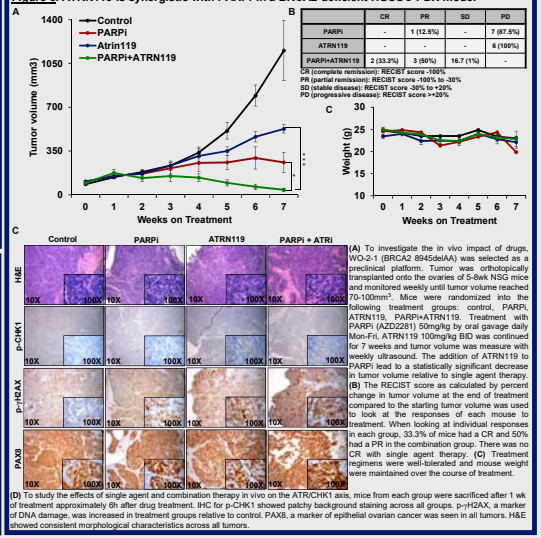
(A) To investigate the in vivo impact of ATRN119, 8 NSG mice orthotopically transplated with a tumor harboring a BRCA2 mutation, WO-2-1 (BRCA2 8945delAA), were treated with ATRN119 100mg/kg BID when tumor volume reached 70-100mm³. Tumor volume was evaluated by ultrasound weekly. Those mice treated with prolonged ATRN119 showed some tumor suppression, but not tumor regression. **(B)** Weights were monitored weekly and remained stable throughout treatment.

Figure 4. ATRN119 is synergistic with PARPi in HR deficient and proficient established ovarian cancer cell models.



(A) Viability of established ovarian cancer cell models after treatment with PARPi and ATRN119 monotherapy as well as in combination was assessed with MTT assay. PEO1, JHOS4, and PEO4 cells were plated at a concentration of 50 cells/μL and incubated in their respective drug concentrations for 5 days. Combination therapy significantly decreased viability in HR deficient and proficient ovarian cancer cells. **(B)** Colony forming assay after treatment with PARPi and ATRN119 monotherapy and in combination in BRCA deficient and wild type cells. Cells were plated at a concentration of 5 cell/μL and incubated in their respective drug concentrations for 13 days. **(C)** To study the effects of ATRN119 in combination with PARPi on DNA damage and the ATR/CHK1 pathway, BRCA2 mutant (PEO1) cells were treated with single agent PARPi (AZD2281) 1μM, and ATRN119 1μM as well as with combination PARPi and ATRN119. After 30min of drug treatment, cells were incubated in APH for 4.5h. Western blot for the indicated proteins and phospho-proteins showed the increase in pATR and pCHK1 with PARPi in PEO1 was overcome with the addition ATRN119.

Figure 5. ATRN119 is synergistic with PARPi in a BRCA2 deficient HGSOC PDX model

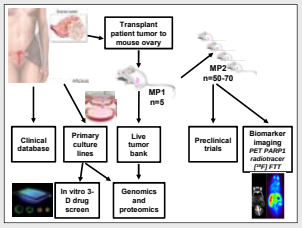


(A) To investigate the in vivo impact of drugs, WO-2-1 (BRCA2 8945delAA) was selected as a preclinical platform. Tumor was orthotopically transplanted onto the ovaries of 5-8wk NSG mice and monitored weekly until tumor volume reached 70-100mm³. Mice were randomized into the following treatment groups: control, PARPi, ATRN119, PARPi+ATRN119. Treatment with PARPi (AZD2281) 50mg/kg by oral gavage daily Mon-Fri, ATRN119 100mg/kg BID was continued for 7 weeks and tumor volume was measure with weekly ultrasound. The addition of ATRN119 to PARPi lead to a statistically significant decrease in tumor volume relative to single agent therapy. **(B)** The RECIST score as calculated by percent change in tumor volume at the end of treatment compared to the starting tumor volume was used to look at the responses of each mouse to treatment. When looking at individual responses in each group, 33.3% of mice had a CR and 50% had a PR in the combination group. There was no CR with single agent therapy. **(C)** Treatment regimens were well-tolerated and mouse weight were maintained over the course of treatment.

(D) To study the effects of single agent and combination therapy in vivo on the ATR/CHK1 axis, mice from each group were sacrificed after 1 wk of treatment approximately 6h after drug treatment. IHC for p-CHK1 showed patchy background staining across all groups. p-γH2AX, a marker of DNA damage, was increased in treatment groups relative to control. PAX8, a marker of epithelial ovarian cancer was seen in all tumors. H&E showed consistent morphological characteristics across all tumors.

METHODS

Schema for the development of a novel OVCA preclinical platform



Tumors are removed during debulking surgery and transplanted onto the ovaries/fallopian tubes of NSG female mice and grown in culture to establish a primary cell line. Mouse tumors are passaged and expanded to establish a live tumor bank and a platform for preclinical trials. Maintenance of mutational status, tumor heterogeneity, and copy number variation through cell culture and mouse passages is confirmed with genomics and proteomics. Primary culture lines may be used for in vitro 3-D drug screening.

CONCLUSION

- **ATRN119 is a potent and specific ATRi that is cytotoxic in both HR-deficient and HR-proficient ovarian cancer cell models.**
- **ATRN119 shows efficacy in a BRCA2 mutant orthotopic ovarian cancer PDX model and is well-tolerated.**
- **Combination therapy with PARPi and ATRN119 is synergistic in both HR-deficient and proficient ovarian cancer cell models and in a BRCA2 mutant orthotopic ovarian cancer PDX model.**
- **ATRN119 is a viable therapeutic option in HR-deficient and HR-proficient ovarian cancer and further preclinical work is ongoing to optimize bioavailability and to identify the maximum tolerated dose.**

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