Development and testing of a first-in-class series of macrocyclic ATR inhibitors for cancer treatment

PB336

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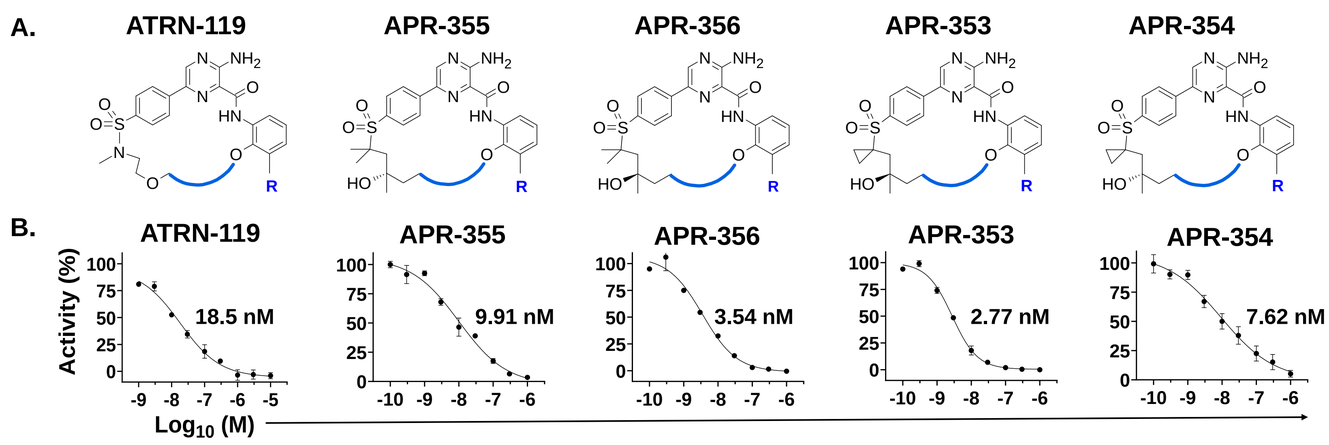
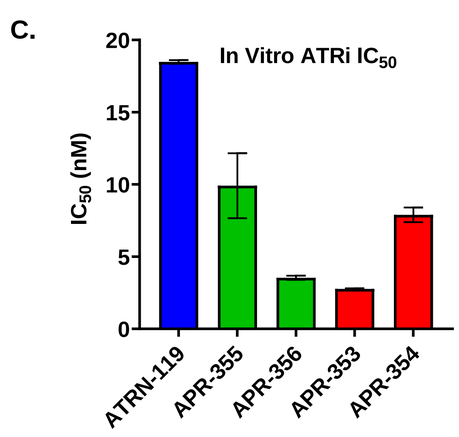
Abstract

The phosphatidyl-inositol kinase-related kinase (PIKK) ATR plays key roles in cellular responses to replication stress. Previous studies have demonstrated the promise of ATR inhibitors (ATRi) as cancer therapeutics. However, toxicity to normal tissues, mainly in the form of myelosuppression, has limited the potential therapeutic value of previously developed ATRi. It is conceivable that the combination of ATR inhibition with yet-to-be-identified off-targets, including lipid kinases, may contribute to the narrow therapeutic value. One means to limit off-targeting and increase drug potency is to utilize macrocyclic small molecules, which typically assume fewer structurally distinct conformations than equally complex non-macrocyclic compounds. Here we describe the first macrocyclic ATRi to have entered clinical trials, ATRN-119, which is one of a series of Aprea's macrocyclic ATRi. Aprea's macrocyclic ATRi are highly potent in vitro biochemical kinase assays for ATR inhibition, with IC50s below 20 nM. Importantly, ATRN-119 demonstrates minimal off-target inhibition of other PIKKs (ATM, DNA-PK, and mTOR). Western blot data confirms the potency of the macrocyclic ATRi series in multiple cancer cell lines in culture, as indicated by decreased phosphorylation of its direct target (CHK1 on S345) and increased phosphorylation of H2AX, which is an indication of double-strand break formation. Cell culture proliferation assays show that ATRN-119 and other macrocyclic ATRi series members significantly limit or completely compromise cellular viability, with EC50s in the low nanomolar range. Furthermore, a substantial increase in potency is observed when ATRN-119 or other macrocyclic Aprea ATRi are combined with cancer treatment agents, such as topoisomerase and PARP inhibitors. Finally, in vivo studies demonstrate that ATRN-119 has broad-spectrum single-agent activity in xenografted tumors from colon and prostate cancer cell lines and suppresses the growth of BRCA2-deficient ovarian cancer PDX tumors both alone and in combination with PARP inhibition. In conclusion, macrocyclic ATRi represent a promising new class of potent and selective ATRi with the potential to treat a wide range of cancers.

1. Macrocyclic ATR inhibitors show high potency and selectivity in *in vitro* kinase assays



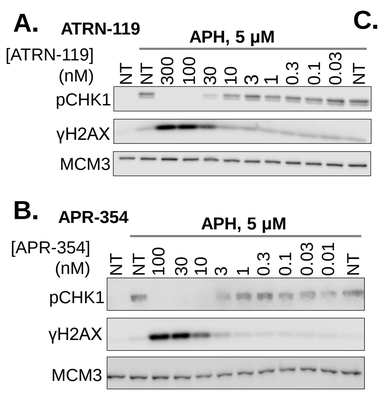
(A) Markush structures for ATRN-119 and 300-series ATR inhibitors. The blue curve represents a linker region. Structures for APR-355 and APR-356 (enantiomers of APR-330), and APR-353 and APR-354 (enantiomers of APR-333), are shown. (B) Assessment of the inhibitory activity of ATRN-119 and APR-series compounds against ATR using *in vitro* kinase assays. ATR inhibition was evaluated using the Eurofins ATR /ATRIP Human PIKK Kinase Enzymatic ELISA / EIA [Km ATP] KinaseProfiler LeadHunter Assay. Varying concentrations of each compound was incubated with the reaction mixture for 40 minutes before measuring the fluorescence signal. N=2±SD. (C) Comparison of IC50 values for ATRN-119 and various APR-series analogs, derived from the *in vitro* kinase assays for ATR performed in (B). (D) Comparison of the inhibitory activity of ATRN-119 and APR-series analogs for ATR compared to ATM, DNA-PK, and mTOR. IC50 values were derived from the *in vitro* kinase assays for ATR performed in (B) as well as from corresponding assays for ATM, DNA-PK, and mTOR. N=2±SD.



D. IC_{50} Data From In Vitro Kinase Assays, (nM)

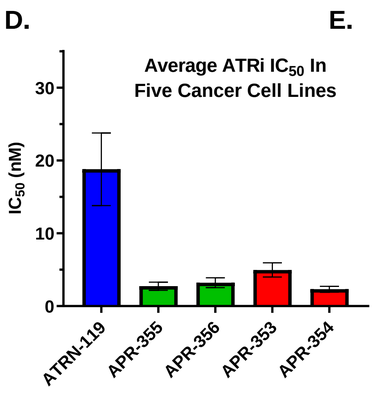
Compound		ATR	ATM	DNA-PK	mTOR
ATRN-119	–	18.5 ± 0.12	>10,000 (>541-fold)	3,974 ± 1,148 (215-fold)	>10,000 (>541-fold)
APR-355	Enantiomers of APR-330	9.91 ± 2.25	108 ± 12 (11-fold)	7,674 ± 125 (774-fold)	3,275 ± 160 (330-fold)
APR-356		3.54 ± 0.14	534 ± 6 (151-fold)	9,031 ± 734 (2,551-fold)	4,370 ± 284 (1,234-fold)
APR-353	Enantiomers of APR-333	2.77 ± 0.04	708 ± 149 (256-fold)	9,678 ± 2,435 (3,494-fold)	5,568 ± 453 (2,010-fold)
APR-354		7.62 ± 0.26	2,114 ± 34 (277-fold)	6,767 ± 759 (888-fold)	1,749 ± 163 (230-fold)

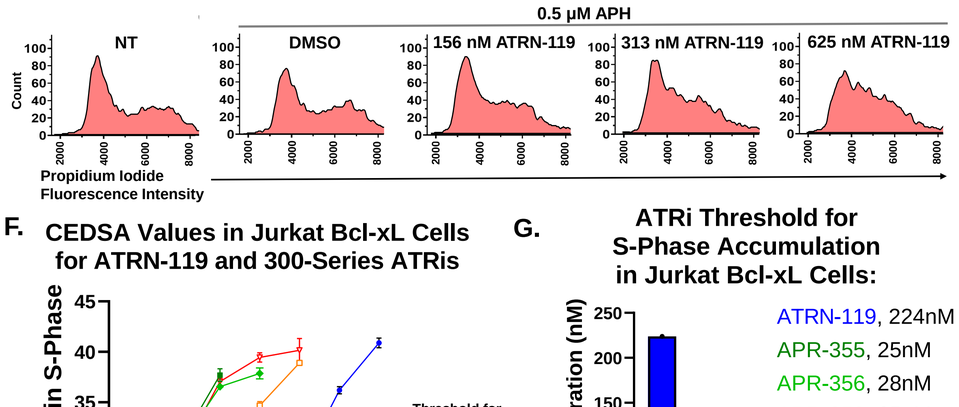
2. Western blot and flow cytometry data confirm high potency across several cancer cell lines

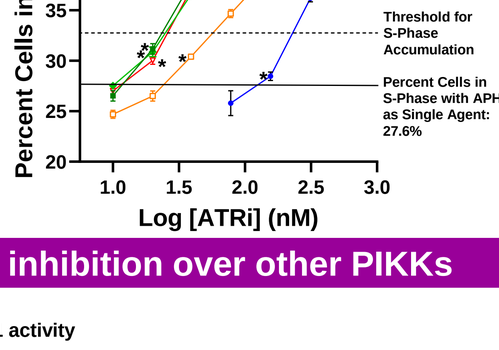


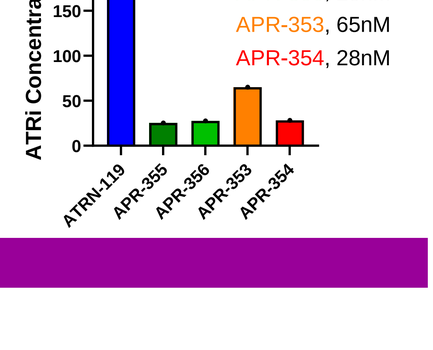
C. IC_{50} Data for CHK1 Phosphorylation

Compound	OVCAR3	OVCAR8	SKOV3	22Rv1	HCT116
ATRN-119	20.3	35.5	18.2	15.4	4.55
APR-355	2.81	2.16	1.34	4.69	2.68
APR-356	1.89	5.76	2.71	3.44	2.29
APR-353	6.64	7.06	1.57	4.95	4.61
APR-354	3.12	2.62	0.97	1.89	3.00



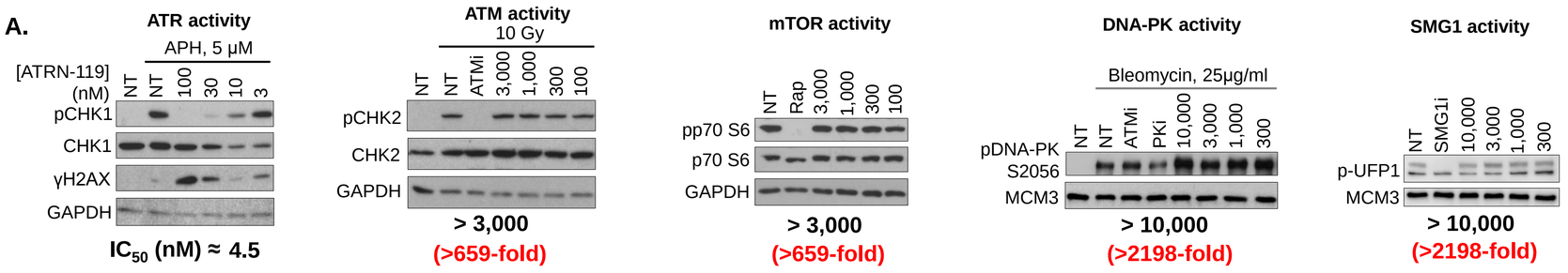






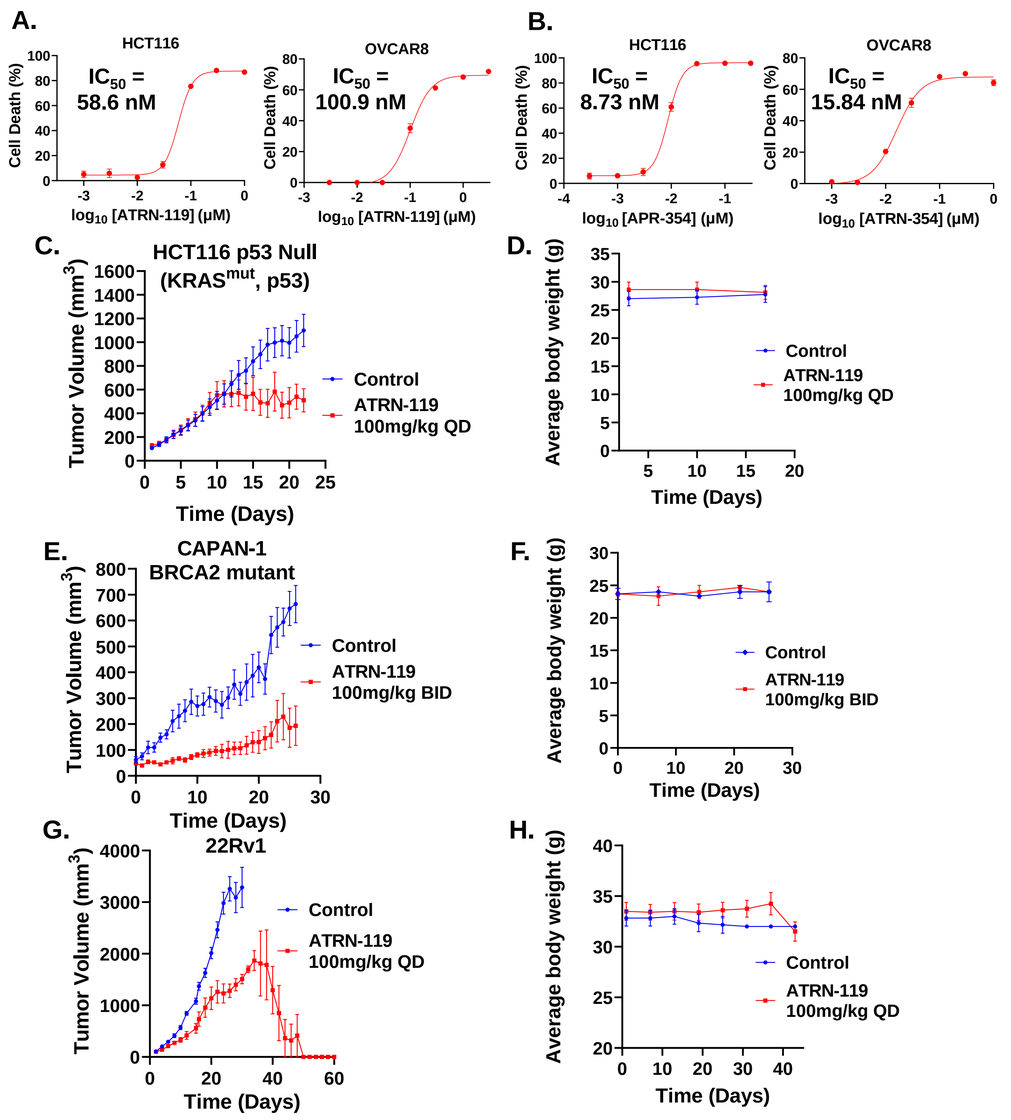
(A) Western blots showing phospho-CHK1 levels as a marker of ATR inhibition in OVCAR3 cells following treatment with ATRN-119. (B) Western blots showing phospho-CHK1 levels as a marker of ATR inhibition in OVCAR3 cells following treatment with APR-354. (C) Summary of IC50 values (nM) for phospho-Chk1 (Ser345) inhibition by macrocyclic ATR inhibitors in ovarian cancer cell lines OVCAR3, OVCAR8, and SKOV3, prostate cancer cell line 22Rv1, and colorectal cancer line HCT116 Bcl-xL. IC50 values were derived by quantifying the phospho-Chk1 (Ser345) signal from western blots using Image Studio and normalization to the loading control. (D) Average IC50 values for phospho-Chk1 (Ser345) inhibition by ATRN-119 and APR-300 series ATR inhibitors across the 5 cancer cell lines shown in (C). (E) Flow cytometry histograms showing S-phase build-up of Jurkat Bcl-xL cells treated with ATRN-119 after pre-treatment with APH (to elicit replicative stress). (F) Average Concentration Eliciting Detectable S-Phase Accumulation (CEDSA) (above treatment with APH alone) values of ATRN-119 in Jurkat Bcl-xL cells shown in (E) and APR-300 series ATR inhibitors in Jurkat Bcl-xL cells. Asterisks label CEDSA concentrations. Dotted line at 33.5% cells in S-phase represents the threshold for S-Phase accumulation for ATRN-119 and the 300-series. N=3±SEM. (G) Threshold values for ATRN-119 and the 300-series derived from 33.5% cells in S-phase in (F).

3. Western blot data confirm high selectivity of ATRN-119 for ATR inhibition over other PIKKs



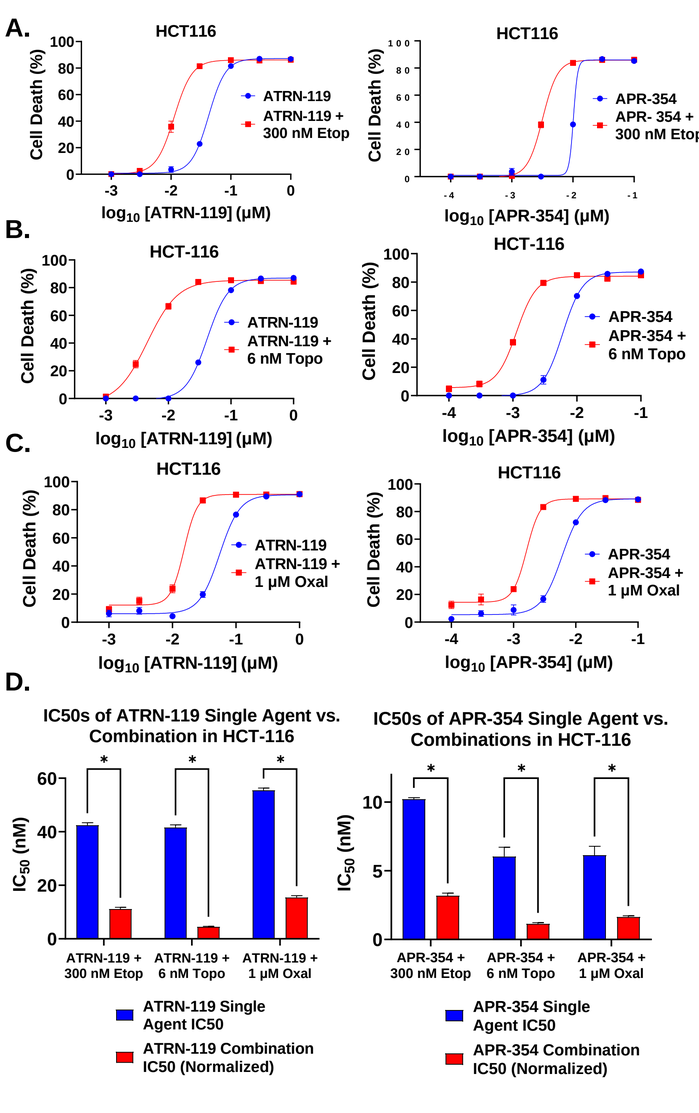
Western blots in HCT116 Bcl-L xcells showing specificity of ATRN-119 for ATR over PIKK kinases ATM (CHK2 Thr368 phosphorylation), mTOR (p70 S6K Thr389 phosphorylation), DNA-PK (autophosphorylation of Ser2056), and SMG1 (phosphorylation of Upf1(Ser1127)). IC50 values for the inhibition of each target's phosphorylation are indicated below the blots, and numbers in brackets indicate IC50 fold-change compared to phospho-Chk1 inhibition. ATMi, KU-60019, 10 μM; Rap, rapamycin, 50 nM; PKi, NU7441, 10 μM; SMG1i, hSMG-1 inhibitor 11e, 3 μM.

4. ATRN-119 suppresses cancer cell and tumor growth as a single agent



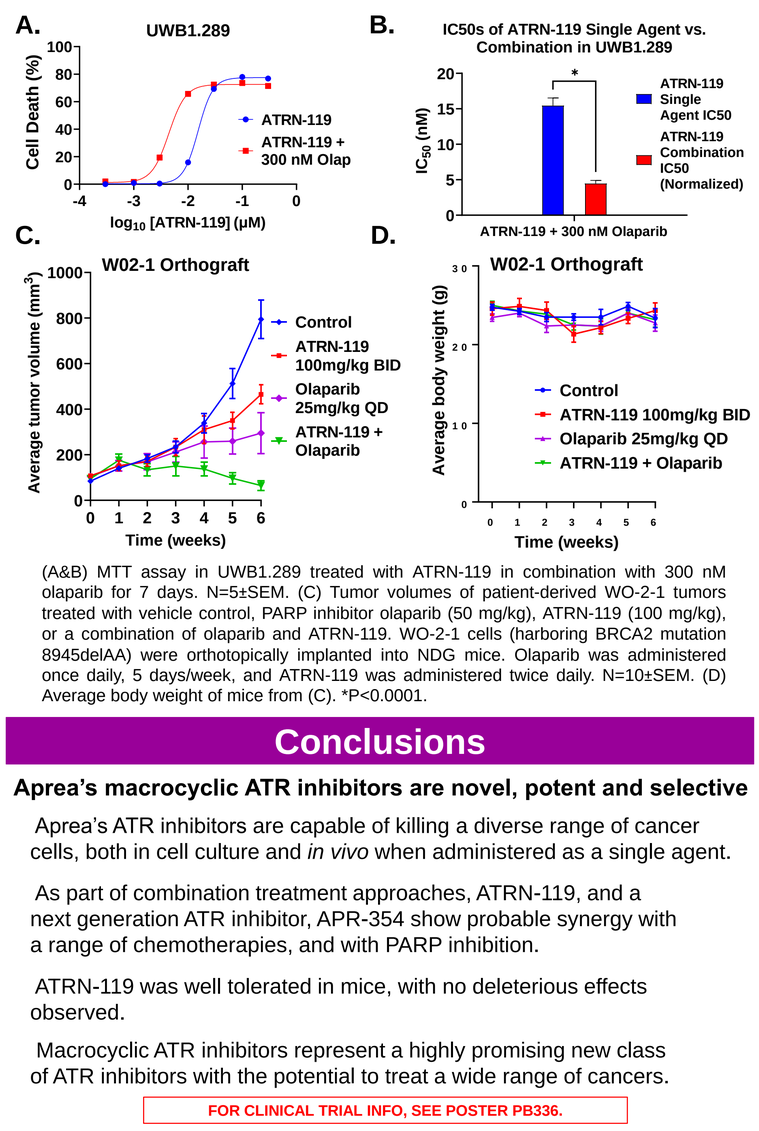
(A&B) MTT assays in HCT116 (WT) and OVCAR8 cells treated with ATRN-119 (A) or APR-354 (B) for 7 days. N=5±SEM. (C) Tumor volumes of implanted HCT116 (KRAS mutant, p53null) flank tumors treated with vehicle control or ATRN-119 (100 mg/kg) administered orally once daily. N=16±SEM. (E) Tumor volumes of implanted CAPAN1 (BRCA2-mutant) flank tumors treated with vehicle control or ATRN-119 (100 mg/kg), administered orally twice daily. N=6±SEM. (G) Tumor volumes of implanted 22Rv1 flank tumors treated with vehicle control or ATRN-119 (100mg/kg) administered orally once daily, 6 days/week for up to 60 days. N=6±SEM. (D, F, H) Average body weight of mice from (C, E, G) respectively.

5. 119/354 sensitize to DNA damaging agents



(A-C) MTT assays in HCT116 (p53null) cells treated with ATRN-119 or APR-354 in combination with etoposide (A), topotecan (B), or oxaliplatin (C) for 7 days. N=5±SEM. (D) Bar graphs displaying IC50s of ATRN-119 or APR-354 from single agent and combination treatments in A-C. Combination IC50s were normalized to the single agent chemotherapy effect. N=5±SEM. *P<0.0001.

6. ATRN-119 synergizes with PARPi in BRCA2^{mut} OvCa



(A&B) MTT assay in UWB1.289 treated with ATRN-119 in combination with 300 nM olaparib for 7 days. N=5±SEM. (C) Tumor volumes of patient-derived WO-2-1 tumors treated with vehicle control, PARP inhibitor olaparib (50 mg/kg), ATRN-119 (100 mg/kg), or a combination of olaparib and ATRN-119. WO-2-1 cells (harboring BRCA2 mutation 8945delAA) were orthotopically implanted into NDG mice. Olaparib was administered once daily, 5 days/week, and ATRN-119 was administered twice daily. N=10±SEM. (D) Average body weight of mice from (C). *P<0.0001.

Conclusions

Aprea's macrocyclic ATR inhibitors are novel, potent and selective

Aprea's ATR inhibitors are capable of killing a diverse range of cancer cells, both in cell culture and *in vivo* when administered as a single agent.

As part of combination treatment approaches, ATRN-119, and a next generation ATR inhibitor, APR-354 show probable synergy with a range of chemotherapies, and with PARP inhibition.

ATRN-119 was well tolerated in mice, with no deleterious effects observed.

Macrocyclic ATR inhibitors represent a highly promising new class of ATR inhibitors with the potential to treat a wide range of cancers.

FOR CLINICAL TRIAL INFO, SEE POSTER PB336.

Acknowledgements

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