C147

The DNA replication checkpoint inhibitors, ATRN-1051 (WEE1i) and ATRN-119 (ATRi), are potentially well-tolerated and effective cancer treatments

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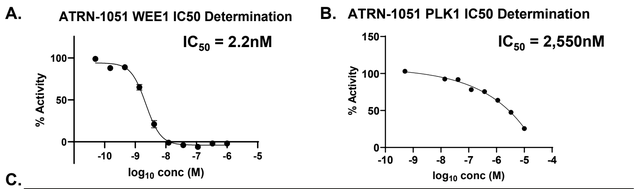
Abstract

Previous studies have demonstrated WEE1i and ATR inhibitors (ATRi and WEE1i) to be promising cancer therapeutics through synthetic lethality with various cancer associated mutations. However, a key limitation to the use of these inhibitors as cancer therapies in prior clinical trials has been the occurrence of adverse hematological effects, including anemia and thrombocytopenia. Herein, we describe two novel inhibitors, ATRN-1051 (WEE1i) and ATRN-119 (ATRi) that are potentially both effective in tumor suppression and well tolerated in animal models. ATRN-1051 was developed to be both a potent WEE1i and selective for WEE1 over other kinases (PLK1, PLK2 and PLK3). ATRN-1051 has an IC50 of 2.2 nM for WEE1 and limits the proliferation of various cancer cell lines in culture in the 100 nM to 200 nM range. Notably, ATRN-1051 suppressed the growth of Cyclin E overexpressing cells lines, pinpointing Cyclin E as a potential biomarker for ATRN-1051. In addition, ATRN-1051 has potentially favorable pharmacokinetic properties that permits 3-8 times lower dosing than other clinical WEE1 inhibitors to achieve similar exposure (AUC, 0-24) levels (1). Consistent with the increase in selectivity of ATRN-1051 fostering increased tolerability, dose-range finding studies indicate that doses potentially expected to cause significant tumor suppression are hematological well tolerated in mice, with red blood cell and platelet counts remaining in a non-pathogenic range. Importantly, daily oral dosing of ATRN-1051 suppresses the growth of CCNE1-amplified high-grade serous ovarian xenografted tumors over the course of 28 days, providing further evidence of the role of CCNE1-overexpression as a biomarker for ATRN-1051 treatment. Based on these data, ATRN-1051 has entered and is now progressing through IND-enabling studies. As a distinct upstream DNA replication checkpoint inhibitor, ATRN-119 is a macrocyclic ATRi that is highly specific for inhibition of ATR over other phosphatidylinositol kinase-related kinases (PIKKs), such as ATM, DNA-PK, and MTOR, implying the potential for increased tolerability. Supporting this implication, daily dosing of ATRN-119 suppresses tumor growth in xenograft mouse models of colon, pancreatic, and prostate cancers and causes no appreciable loss of body weight or hematologic toxicity. Daily dosing of ATRN-119 in combination with PARP inhibition causes significant tumor reduction in a BRCA2-deficient PDX model of high-grade serous ovarian cancer, again with no appreciable loss of body weight. These findings have led to a biomarker driven Phase 1/2a clinical trial of ATRN-119 with daily dosing (Simpkins, PI). We believe these findings underscore the promise of ATRN-1051 and ATRN-119 as DNA replication checkpoint inhibitors for the treatment of a variety of cancers.

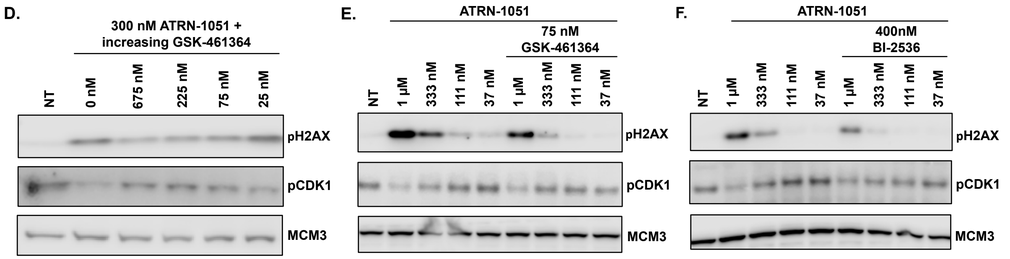
2. ATRN-1051 does not substantially off-target PLK1, PLK2 or PLK3



C.

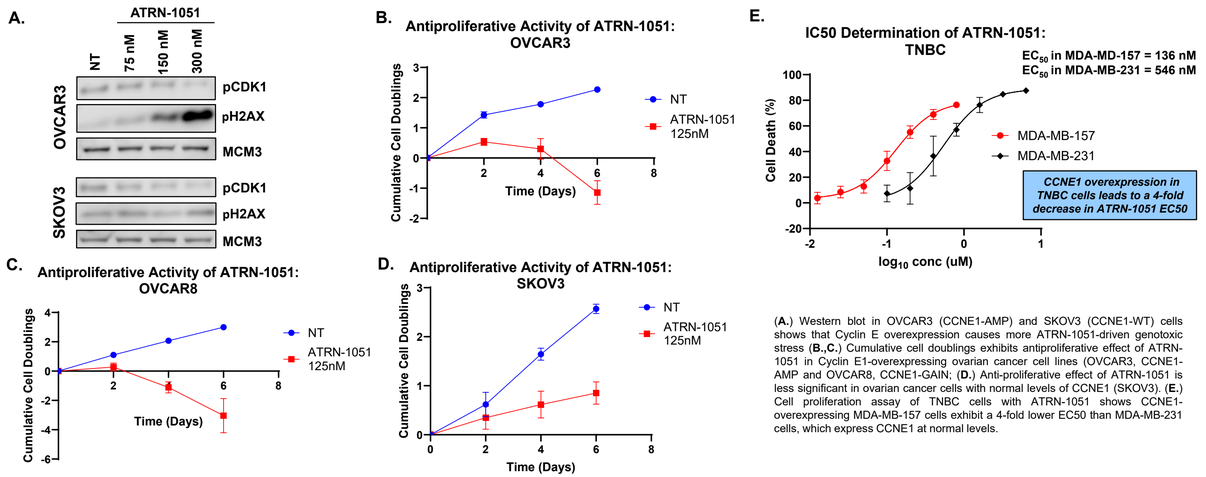
	On-Target IC_{50} (nM)	Off-Target Inhibition at 1 µM (%)		
	WEE1	PLK1	PLK2	PLK3
ATRN-W1051	2.2	17	33	12
ZN-c3 (1)	3.8	79	96	92
AZD-1775 (1)	3.9	70	101	91

(1) Head-to-head studies have not been conducted.



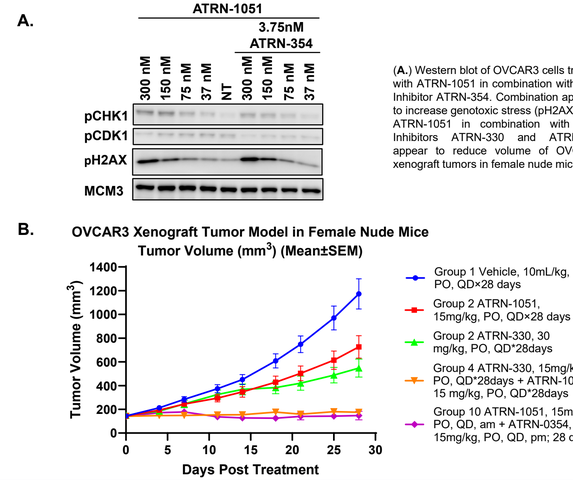
Cell-free WEE1 IC50 determination of WEE1 (**A.**) and PLK1 (**B.**) show that the IC50 of ATRN-1051 for PLK inhibition is 1,000 times higher than WEE1 inhibition. (**C.**) Comparison of ATRN-1051 to known WEE1 inhibitors in regard to on-target WEE1 inhibition and off-target PLK inhibition implies increased ATRN-1051 potency and selectivity. (**D.**) Dose range of PLK inhibitor GSK-461364 in combination with a single dose of ATRN-1051 in OVCAR3 cells suggests interference the genotoxic effects of WEE1 inhibition (pH2AX). Western blot suggests that PLK inhibitor, GSK-461364 (**E.**) and BI-2536 (**F.**), interferes with ATRN-1051's ability to increase pH2AX..

3. ATRN-1051 suppresses the growth of CCNE1-o/e cells



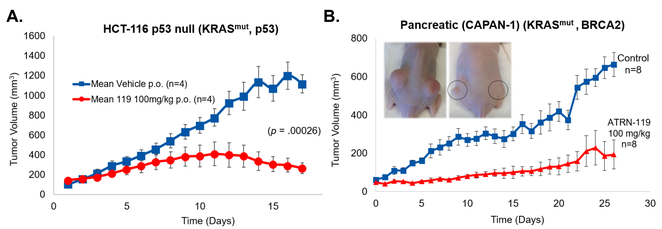
(**A.**) Western blot in OVCAR3 (CCNE1-AMP) and SKOV3 (CCNE1-WT) cells shows that Cyclin E overexpression causes more ATRN-1051-driven genotoxic stress (**B.**,**C.**) Cumulative cell doublings exhibits antiproliferative effect of ATRN-1051 in Cyclin E1-overexpressing ovarian cancer cell lines (OVCAR3, CCNE1-AMP and OVCAR8, CCNE1-GAIN; (**D.**) Anti-proliferative effect of ATRN-1051 is less significant in ovarian cancer cells with normal levels of CCNE1 (SKOV3). (**E.**) Cell proliferation assay of TNBC cells with ATRN-1051 shows CCNE1-overexpressing MDA-MB-157 cells exhibit a 4-fold lower EC50 than MDA-MB-231 cells, which express CCNE1 at normal levels.

5. ATRN-1051 + ATRi activity



(**A.**) Western blot of OVCAR3 cells treated with ATRN-1051 in combination with ATR Inhibitor ATRN-354. Combination appears to increase genotoxic stress (pH2AX). (**B.**) ATRN-1051 in combination with ATR Inhibitors ATRN-330 and ATRN-354 appear to reduce volume of OVCAR3 xenograft tumors in female nude mice.

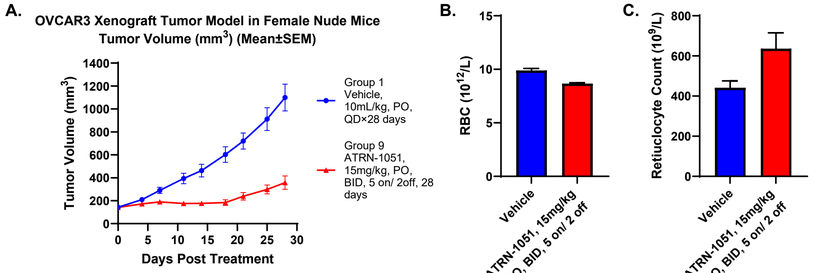
1. ATRN-119 efficacy in vivo



Based on pre-clinical studies, ATRN-119 displays strong tumor control in vivo, including in those with challenging genetic backgrounds like colon (**A.**) and pancreatic (**B.**) cancer.

For more information on the ATRN-119 program, please see preliminary clinical trial findings presented on Poster C034.

4. ATRN-1051 suppresses the growth of CCNE1-o/e tumors



E.

In vitro kinase assays		Average WEE1 kinase IC50	hERG inhibition		Average hERG IC50	Fold difference between kinase and hERG inhibition
LanthaScreen (Thermo)	Hotspot (Reaction Biology)	21.8 nM	HEK293 cells (Medicilon)	CHO cells (WuXi)	4,750 nM	hERG inhibition over WEE1 kinase inhibition
2.2 nM	41.4 nM		8,840 nM	660 nM		218-fold (range 16- to 3,946-fold)

F.

	ATRN 1051 (1)	Zentalis ZN-c3 (2)			AstraZeneca AZD-1775 (2)		
Dose (mg/kg/d)	10	20	40	80	20	40	80
$AUC_{0\text{-}24h}$ ng*hr/ml	16,739	4,863	17,088	39,722	1,494	6,313	13,408

(**A.**) Preclinical studies show that ATRN-1051 suppresses the growth of CCNE1-amplified HGSOC xenografted tumors (**B.**,**C.**) Preclinical studies suggest that ATRN-1051 admin. at the dose and schedule shown has a low impact on red blood cell (RBC) counts. (**D.**) Effect of ATRN-1051 on body weight. (**E.**) The IC50 of ATRN-1051 for WEE1 appears to be substantially lower than the IC50 for hERG channels. (**F.**) Comparisons of ATRN-1051 AUC to those of ZN-c3 and AZD-6738 a single aadmin.in mice.

Conclusions

- ATRN-1051 exhibits high potency for WEE1 inhibition in vitro.
- ATRN-1051 shows low off-target inhibition of the PLK family of kinases
- Inhibition of PLK1 limits the genotoxic effects of WEE1i
- Anti-proliferative effects of ATRN-1051 appear to be enhanced in multiple CCNE1 overexpressing cell lines.
- ATRN-1051 suppresses the growth of CCNE1-amplified HGSOC xenografted tumors and is relatively well-tolerated in mice.
- Combination treatment of ATRN-1051 and Aprea's second-generation ATR inhibitors is efficacious in xenografted tumors.

References

(1) Head-to-head studies have not been conducted. Comparative data from Huang et al, (2021) and J Med Chem and Zentalis Corporate Overview, March 2022
(2) J Med Chem and Zentalis Corporate Overview, March 2022
(3) Xu H, George E, Kinose Y, et al. *CCNE1* copy number is a biomarker for response to combination WEE1-ATR inhibition in ovarian and endometrial cancer models. *Cell Rep Med*. 2021;2(9):100394.

Acknowledgements

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