The novel WEE1i, APR-1051, is a potentially well tolerated and effective treatment for Cyclin E-overexpressing cancers

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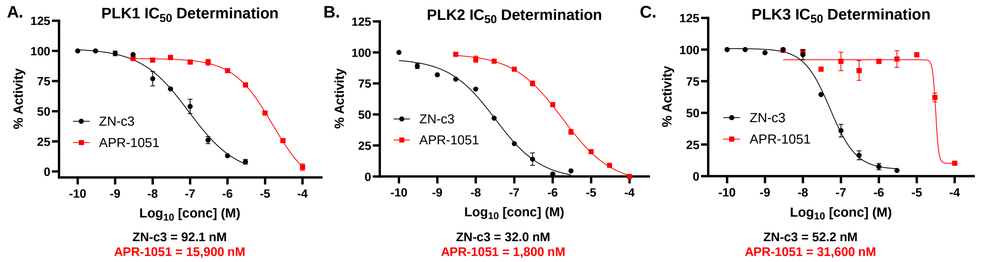
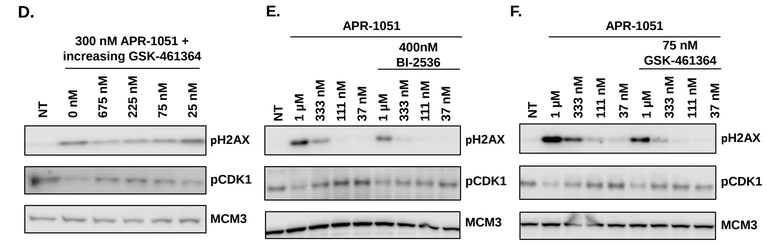
Abstract

Previous studies have demonstrated that WEE1 inhibitors (WEE1i) are a promising cancer therapeutic through synthetic lethality with Cyclin E overexpression. One challenge for two of the leading WEE1i, AZD1775 and ZN-c3, has been the observed off-targeting of PLK family members, namely, PLK1, PLK2 and PLK3. In addition, the therapeutic application of these inhibitors in clinical trials has been limited by adverse hematological effects, including anemia. Herein, we describe the testing of a novel WEE1i (APR-1051) that, based on preclinical studies: 1) demonstrates reduced off-targeting of PLK1, PLK2 and PLK3, and 2) has the ability to suppress the growth of CCNE1-overexpressing xenografted tumors while causing little impact on red blood cell and reticulocyte levels. APR-1051 has an IC50 of 2.2 nM for WEE1 in vitro and has shown the ability to limit the proliferation of various cancer cell lines in culture. Interestingly, the genotoxicity of APR-1051 is suppressed by increasing concentrations of PLK1 inhibitors, suggesting that off-targeting of PLK1 by other WEE1i may compromise efficacy of this targeted cancer treatment in addition to the risk of producing PLK1i-associated adverse effects. APR-1051 demonstrated effectiveness in suppressing the growth of Cyclin E-overexpressing breast and ovarian cancer cells lines, thus pinpointing Cyclin E as a potential biomarker for APR-1051 treatment. Importantly, dose and scheduling of APR-1051 that causes significant suppression of CCNE1-amplified high-grade serious ovarian cancer tumors in mice is well tolerated, with red blood cell and platelet counts remaining within non-pathogenic ranges after a 28-day treatment period. In addition, inhibition WEE1 by APR-1051 occurs at an IC50 that is 200-fold lower on average than the IC50 of hERG potassium channel inhibition, implying that APR-1051 will potentially exhibit low cardiotoxicity. APR-1051 is now progressing through IND-enabling studies. Together, these findings underscore the potential of APR-1051 as a novel WEE1i for the treatment of Cyclin E-overexpressing cancers.

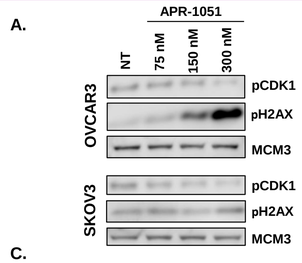
2. APR-1051 does not substantially off-target PLK1, PLK2 or PLK3

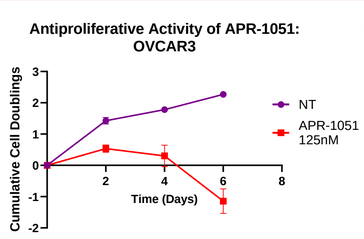


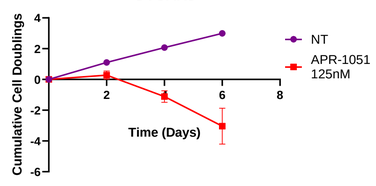


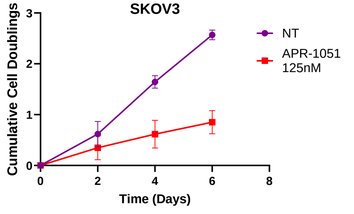
(**A.-C.**) In vitro kinase IC50 determination of APR-1051 vs. ZN-c3: APR-1051 IC50 values show >150-fold, >50-fold, and >600-fold IC50 values compared to ZN-c3 for PLK1, PLK2, and PLK3, respectively, potentially showing higher selectivity. (**D.**) Dose range of PLK inhibitor GSK-461364 in combination with a single dose of APR-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 APR-1051's ability to increase pH2AX.

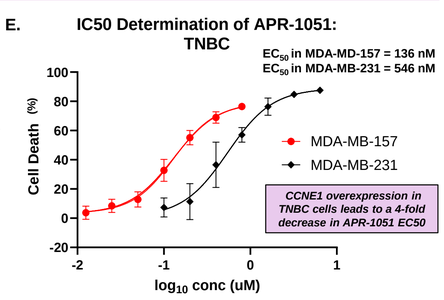
3. APR-1051 suppresses the growth of CCNE1-o/e cells





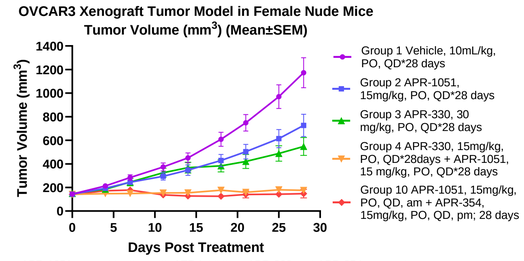






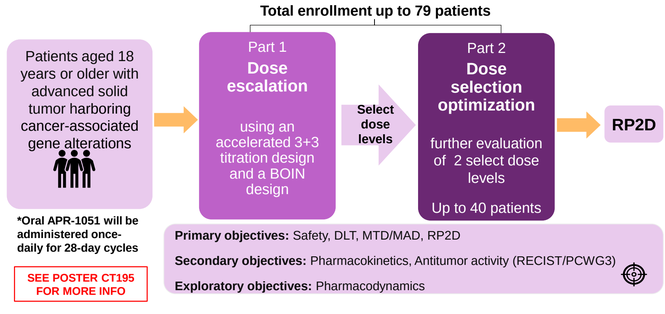
(**A.**) Western blot in OVCAR3 (CCNE1-AMP) and SKOV3 (CCNE1-WT) cells shows that Cyclin E overexpression causes more APR-1051-driven genotoxic stress (**B.,C.**) Cumulative cell doublings exhibits antiproliferative effect of APR-1051 in Cyclin E1-overexpressing ovarian cancer cell lines (OVCAR3, CCNE1-AMP and OVCAR8, CCNE1-GAIN) (**D.**) Anti-proliferative effect of APR-1051 is less significant in ovarian cancer cells with normal levels of CCNE1 (SKOV3). (**E.**) Cell proliferation assay of TNBC cells with APR-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. APR-1051 + ATRi activity



APR-1051 in combination with ATR Inhibitors APR-330 and APR-354 appear to reduce volume of OVCAR3 xenograft tumors in female nude mice.

6. APR-1051 Clinical Trial Plan



***Oral APR-1051 will be administered once-daily for 28-day cycles**

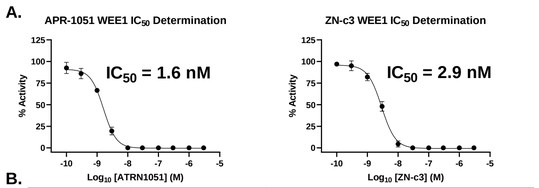
SEE POSTER CT195 FOR MORE INFO

Primary objectives: Safety, DLT, MTD/MAD, RP2D

Secondary objectives: Pharmacokinetics, Antitumor activity (RECIST/PCWG)

Exploratory objectives: Pharmacodynamics

1. APR-1051 vs. WEE1 and PLK



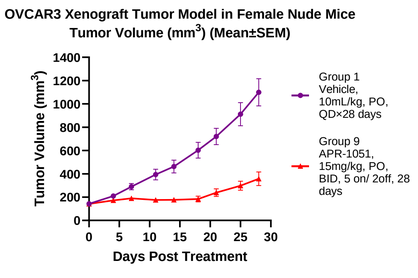
B.

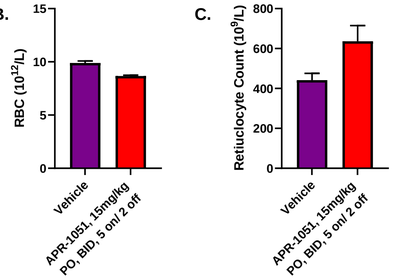
	On-Target IC_{50} (nM)	Off-Target Inhibition at 1 µM (%)		
	WEE1	PLK1	PLK2	PLK3
APR-1051	1.9*	17	33	12
ZN-c3 (1)	3.4*	79	96	92
AZD-1775 (1)	3.9	70	101	91

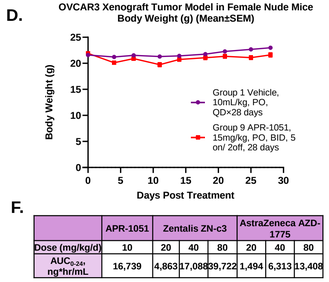
(1) Head-to-head studies have not been conducted.

Cell-free IC50 determination of WEE1 (**A.**) shows that the IC50 values of APR-1051 and ZN-c3 for WEE1 inhibition are 1.6 nM and 2.9 nM, respectively. (**B.**) Comparison of APR-1051 to known WEE1 inhibitors in regard to on-target WEE1 inhibition and off-target PLK inhibition implies increased APR-1051 potency and selectivity. * Values represent average of two kinase assay.

4. APR-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)

(**A.**) Preclinical studies show that APR-1051 suppresses the growth of CCNE1-amplified HGSOC xenografted tumors (**B.,C.**) Preclinical studies suggest that APR-1051 admin. at the dose and schedule shown has a low impact on red blood cell (RBC) counts. (**D.**) Effect of APR-1051 on body weight. (**E.**) The IC50 of APR-1051 for WEE1 appears to be substantially lower than the IC50 for hERG channels. (**F.**) Comparisons of APR-1051 AUC to those of ZN-c3 and AZD-1775 a single administration in mice.

F.

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

Conclusions

- APR-1051 exhibits high potency for WEE1 inhibition in vitro
- APR-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 APR-1051 appear to be enhanced in multiple CCNE1 overexpressing cell lines
- APR-1051 suppresses the growth of CCNE1-amplified HGSOC xenografted tumors and is relatively well-tolerated in mice
- Combination treatment of APR-1051 and Aprea's second-generation ATR inhibitors is efficacious in xenografted tumors
- In March 2024, APR-1051 received U.S. FDA clearance for a clinical trial, now with plans to dose the first patient in June 2024

Acknowledgements

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