The novel WEE1i, APR-1051, does not substantially off-target PLK1, PLK2, or PLK3 and exhibits favorable in vivo characteristics for treating CCNE1-overexpressing cancers.

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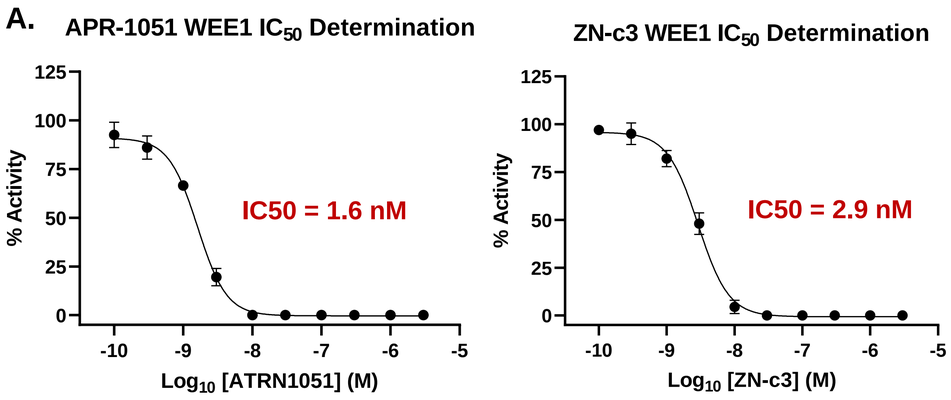
PB323

Abstract

WEE1 inhibitors (WEE1i) are potential cancer therapeutics. Previous studies indicate that WEE1 inhibition is synthetically lethal with various cancer-associated alterations that lead to Cyclin E overexpression. However, previously developed WEE1i have been shown to significantly off-target PLK1, PLK2, and PLK3, which in aggregate could contribute to the myelosuppression and sepsis observed clinically. Here we describe in vitro and in vivo data from a structurally distinct WEE1i (APR-1051) that is a highly potent WEE1i and shows limited off-targeting of PLK1, PLK2, and PLK3. APR-1051 inhibits WEE1 kinase activity in the low nanomolar range in biochemical assays (IC50 = 1.8 nM). Importantly, APR-1051 inhibits PLK1, PLK2, and PLK3 only at much higher concentrations (IC50s = 15,900 nM, 1,800 nM, and 31,600 nM, respectively). These values indicate that APR-1051 is 8,800-fold, 1,000-fold, and 17,500-fold more potent in inhibiting WEE1 than PLK1, PLK2 or PLK3, respectively. For comparison, similar assays were performed with a previously developed WEE1i (ZN-c3). This compound exhibited a 3.2 nM IC50 for inhibiting WEE1, but off-targeted PLK1, PLK2, and PLK3 with IC50s that were only 29-fold, 10-fold, and 16-fold greater than the IC50 for inhibiting WEE1. Notably, ZN-c3 was recently placed on a partial clinical hold because of two recent drug-related deaths due to presumed sepsis on the DENALI study. A Phase 1 clinical trial of APR-1051 has now been initiated (Dr. Timothy Yap, Lead PI). To determine the ability of APR-1051 to suppress the growth of CCNE1-amplified tumors and evaluate potential adverse effects, APR-1051 was applied using various schedules over a 28-day treatment period. APR-1051 strongly suppressed the growth of CCNE1-overexpressing tumors. However, APR-1051 had little impact on body weight, the loss of which is a well-known characteristic of sepsis and intestinal distress. These data imply that APR-1051's potency for WEE1 inhibition and its substantially reduced off-targeting of PLK1, PLK2, and PLK3 may decrease the risk of sepsis-related deaths and other adverse effects associated with other WEE1 inhibitors.

1. APR-1051, ZN-c3 and AZD1775 inhibitory activities against WEE1 and PLK1, PLK2 and PLK3



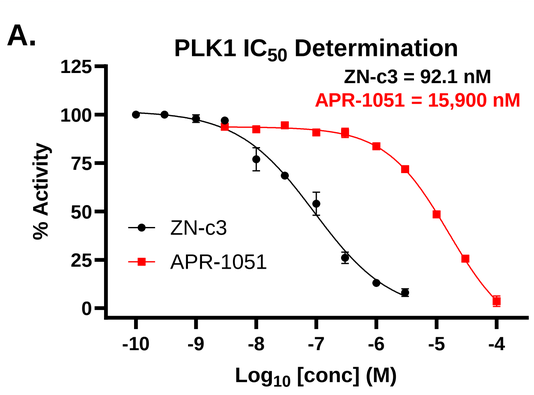
B.

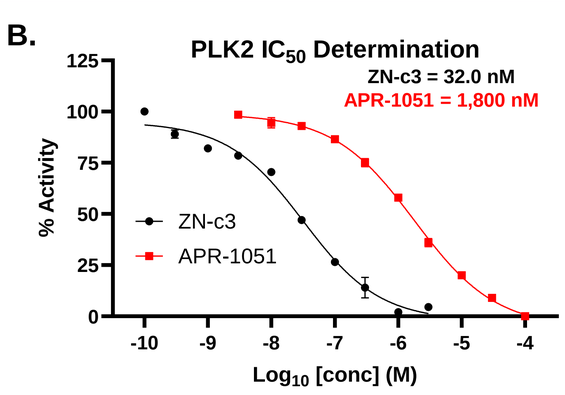
	On-Target IC_{50} (nM)	Off-Target Inhibition at 1 µM (%)		
	WEE1	PLK1	PLK2	PLK3
APR-1051	1.9*	17	33	12
ZN-c3 (1)	3.4*	79	96	92
AZD1775 (1)	3.9	70	101	91

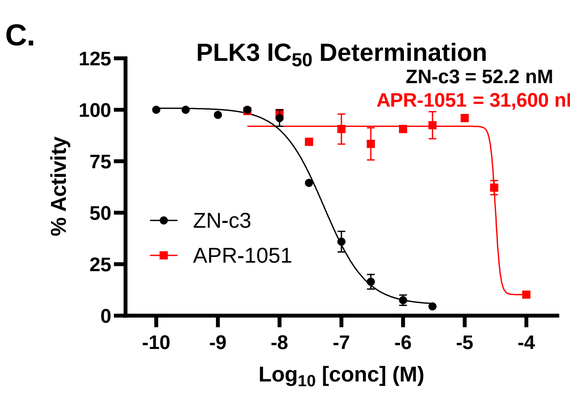
(1) Head-to-head studies have not been conducted.

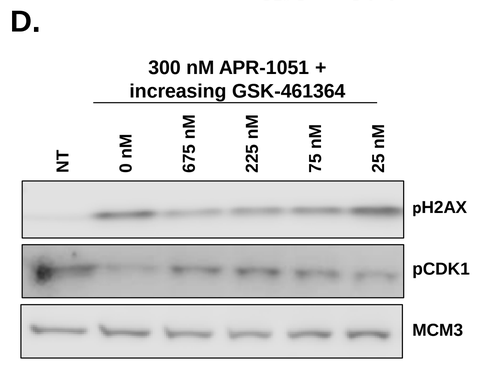
Cell-free IC50 determination of WEE1 (**A.**) shows that the IC50 values of APR-1051 and ZN-c3 for WEE1 inhibition are 1.6 nM and 2.9 nM, respectively. (**B.**) Comparison of APR-1051 to known WEE1 inhibitors in regard to on-target WEE1 inhibition and off-target PLK inhibition implies increased APR-1051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

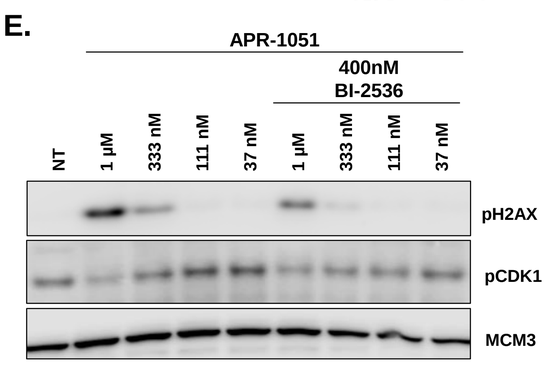
2. APR-1051 does not substantially off-target PLK1, PLK2 or PLK3 and comparison to ZN-c3

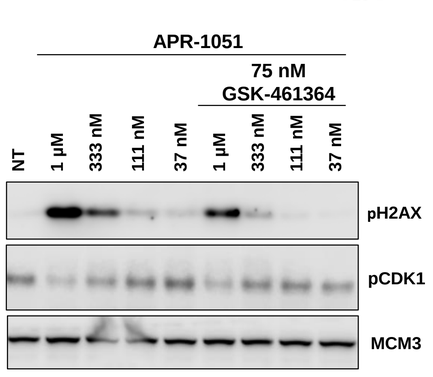






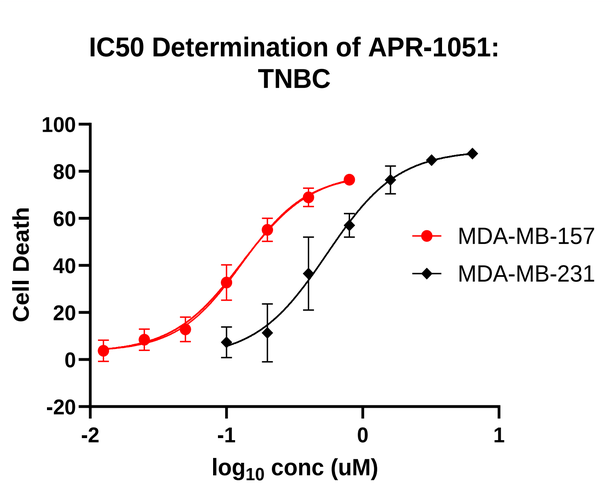






(**A.-C.**) In vitro kinase IC50 determination of APR-1051 vs. ZN-c3: APR-1051 IC50 values show >150-fold, >50-fold, and >600-fold IC50 values compared to ZN-c3 for PLK1, PLK2, and PLK3, respectively, potentially showing higher selectivity. (**D.**) Dose range of PLK inhibitor GSK-461364 in combination 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. CCNE1-o/e is a biomarker for APR-1051

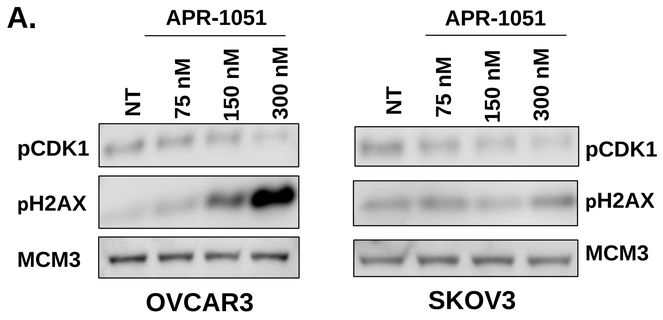


EC_{50} in MDA-MD-157 = 136 nM
EC_{50} in MDA-MB-231 = 546 nM

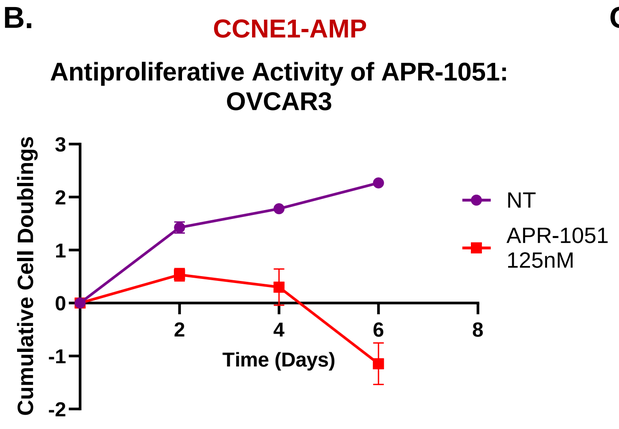
CCNE1 overexpression in TNBC cells leads to a 4-fold decrease in APR-1051 EC50

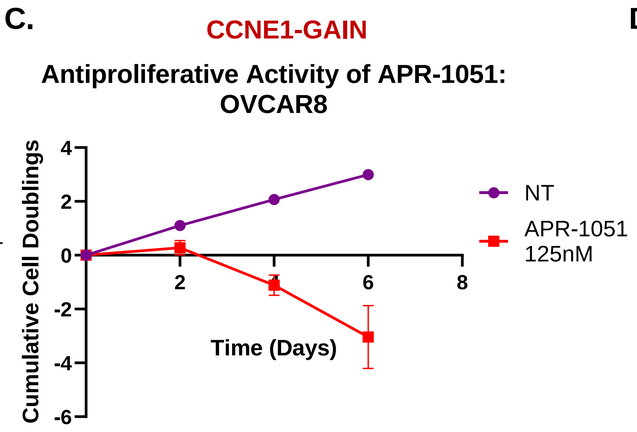
Cell proliferation assay of TNBC cells with APR-1051 shows CCNE1-overexpressing MDA-MB-157 cells exhibit a 4-fold lower EC50 than MDA-MB-231 cells, which express CCNE1 at normal levels.

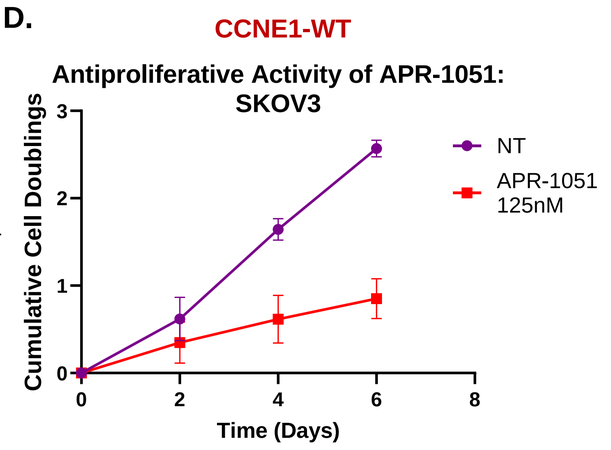
3. CCNE1-o/e is a potential biomarker for APR-1051 (continued)



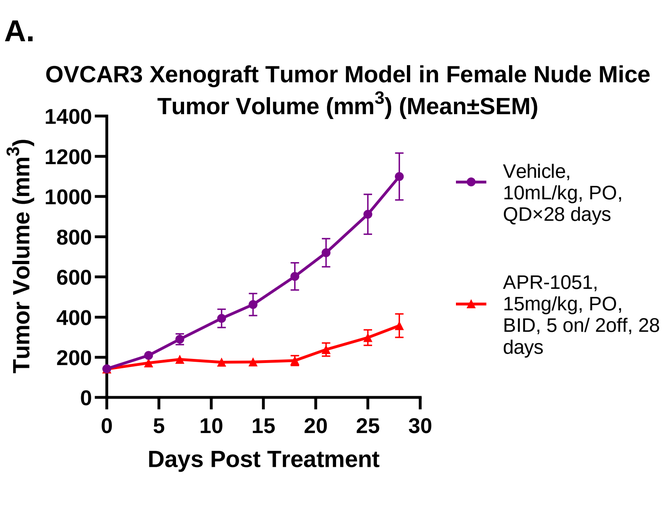
(**A.**) Western blot in OVCAR3 (CCNE1-AMP) and SKOV3 (CCNE1-WT) cells shows that Cyclin E overexpression causes more APR-1051-driven genotoxic stress (**B.,C.**) Cumulative cell doublings exhibits antiproliferative effect of APR-1051 in Cyclin E1-overexpressing ovarian cancer cell lines (OVCAR3, CCNE1-AMP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

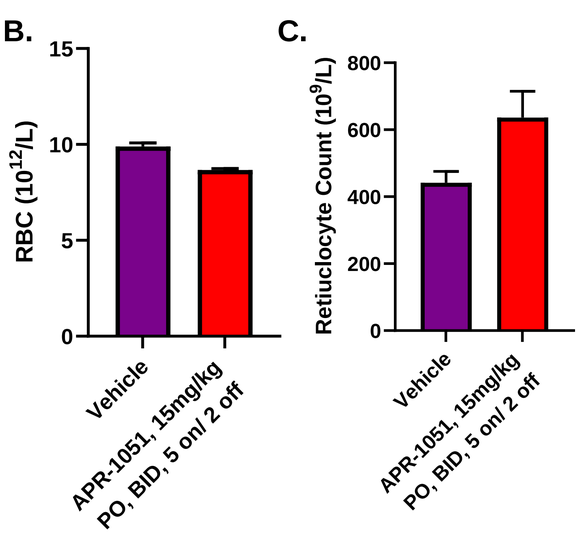


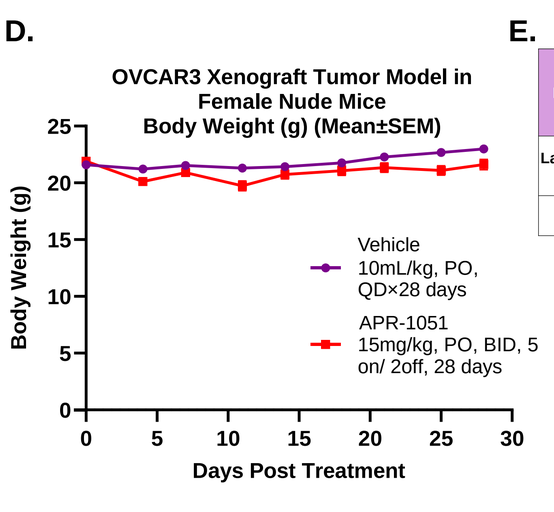




4. APR-1051 suppresses the growth of CCNE1-o/e tumors in mice with limited toxicity with respect to RBC counts, body weight and QT prolongation







E.

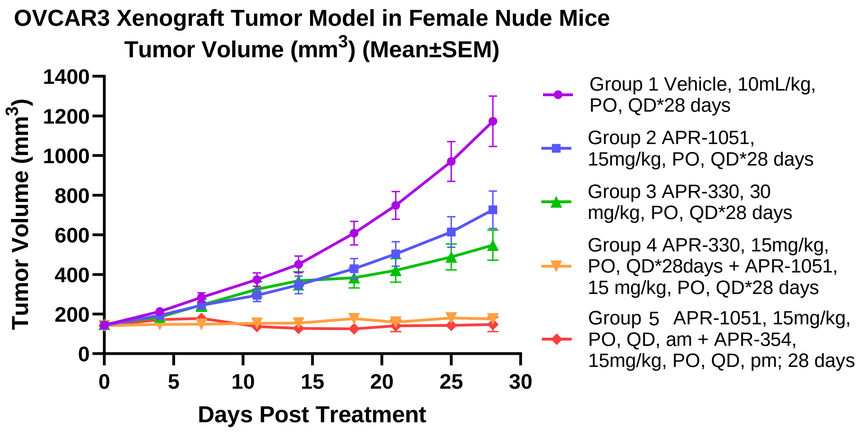
In vitro kinase assays		Average WEE1 kinase IC50	hERG inhibition		Average hERG IC50	Fold difference between kinase and hERG inhibition
LanthaScreen (Thermo)	Hotspot (Reaction Biology)	21.8 nM	HEK293 cells (Medicilon)	CHO cells (WuXi)	4,750 nM	hERG inhibition over WEE1 kinase inhibition
2.2 nM	41.4 nM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

F.

	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

(**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.

5. APR-1051 + ATRi suppresses tumor growth



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 Phase I clinical trial plan

Total enrollment up to 79 patients

Patients aged 18 years or older with advanced solid tumor harboring cancer-associated gene alterations

Part 1
Dose escalation
using an accelerated 3+3 titration design and a BOIN design
Up to 39 patients

Select dose levels

Part 2
Dose selection optimization
further evaluation of 2 select dose levels
Up to 40 patients

RP2D

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

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

Secondary objectives: Pharmacokinetics, Antitumor activity (RECIST/PCWG3)

Exploratory objectives: Pharmacodynamics

SEE POSTER PB065 FOR MORE INFO

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, and initiated Phase 1 in June of 2024

Acknowledgements

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