ATRN-W1051, a potent WEE1 inhibitor for the treatment of CCNE1-overexpressing ovarian cancer

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Abstract

Previous studies have highlighted the potential for WEE1 inhibitors in the treatment of high grade serous ovarian cancer (HGSOC), particularly those that overexpress CCNE1. However, a key limitation to this approach in clinical trials has been the occurrence of adverse hematological effects, including anemia, neutropenia and thrombocytopenia. Although it has been suggested that these effects are the result of on-target inhibition of WEE1, it remains possible that off-target inhibition of other kinases may contribute to these hematological toxicities. Notably, most clinical reports involve the use of inhibitors that exhibit significant off targeting of the PLK family of kinases, specifically PLK1, PLK2 and PLK3. Given the important role of these kinases in the cell cycle, it is conceivable that their off-target inhibition may contribute to the adverse event profiles observed in clinical trials.

Herein, we report the development of a potent and selective WEE1 inhibitor (ATRN-W1051) that exhibits low off-target inhibition of the PLK family of kinases, has potentially favorable pharmacokinetic properties, and suppresses the growth of CCNE1-overexpressing HGSOC tumors in xenografted mice. In vitro, ATRN-W1051 inhibits WEE1 with an IC50 of 2.2 nM and limits the proliferation of ovarian cancer cell lines in the 100 nM to 200 nM range. Importantly, at concentrations of ATRN-W1051 that are more than 450-fold higher than the IC50 of WEE1 inhibition (1 µM), ATRN-W1051 suppresses the activity of PLK1, PLK2 and PLK3 by 17%, 33% and 12%, respectively. Reported inhibition of PLK1, PLK2 and PLK3 by AZD1775 and ZN-c3, two WEE1 inhibitors that are in clinical trials, is 70%, 101% and 90% (AZD1775) and 79%, 96% and 92% (ZN-c3) at 1 uM, respectively, despite IC50 for inhibition of WEE1 by AZD1775 and ZN-c3 that is similar to that of ATRN-W1051 (AZD1775: 3.9 nM; ZN-c3: 3.8 nM)[1,2]. ATRN-W1051 also has potentially favorable pharmacokinetic properties. Mice administered ATRN-W1051 at 10 mg/kg/day had an AUC_{0-24} of >14,000 ng*hr/ml, and this observed AUC_{0-24} was approximately equivalent to data previously reported for AZD1775 at 80 mg/kg/day and ZN-c3 at 20-40 mg/kg/day[3]. Notably, daily oral dosing of ATRN-W1051 at 30 mg/kg/day for 28 days suppressed the growth of CCNE1-amplified OVCAR3 HGSOC xenografted tumors and was well tolerated. These encouraging preclinical data suggest that ATRN-W1051 may be a promising therapeutic candidate for the unmet medical need of patients with CCNE1-overexpressing HGSOC.

(1) Head-to-head studies have not been conducted
(2) Huang et al, (2021) J Med Chem
(3) Data from study in A-427 NSCLC xenograft model as reported in Zentalis Corporate Overview, March 2022

Disclosures

Joseph Vacca: Serves as a Scientific Consultant for Aprea Therapeutic.

Oren Gilad: Serves as the Chief Executive Officer of Aprea Therapeutics.

Eric J. Brown: Serves as a Scientific Advisory Board Member and Scientific Consultant for Aprea Therapeutics. He formally served on the Scientific Advisory Board of Sierra Oncology.

Forward-Looking Statements

Certain information contained in this presentation includes “forward-looking statements”, within the meaning of Section 27A of the Securities Act of 1933, as amended, and Section 21E of the Securities Exchange Act of 1934, as amended, related to our clinical trials, regulatory submissions and strategic plans. We may, in some cases use terms such as “predicts,” “believes,” “potential,” “continue,” “anticipates,” “estimates,” “expects,” “plans,” “intends,” “may,” “could,” “might,” “likely,” “will,” “should” or other words that convey uncertainty of the future events or outcomes to identify these forward-looking statements. The forward-looking statements are based on current beliefs and expectations of our management team that involve risks, potential changes in circumstances, assumptions, and uncertainties. Any or all of the forward-looking statements may turn out to be wrong or be affected by inaccurate assumptions our management team might make or by known or unknown risks and uncertainties. These forward-looking statements are subject to risks and uncertainties including, without limitation, risks related to the success and timing of our clinical trials or other studies and the other risks set forth in our filings with the U.S. Securities and Exchange Commission, including our Annual Reports on Form 10-K and Quarterly Reports on Form 10-Q. Forward-looking statements regarding our product candidates are also subject to additional risks and uncertainties, including without limitation, with respect to: our dependence on additional financing to fund our operations and complete the development and commercialization of our product candidates, and the risks that raising such additional capital may restrict our operations or require us to relinquish rights to our technologies or product candidates; our limited history and preclinical status of the assets we acquired from Atrin Pharmaceuticals Inc.; our business plan or the likelihood of the successful implementation of such business plan; the timing of initiation of planned clinical trials for our product candidates; the future success of such trials; the successful implementation of our research and development programs and collaborations and the interpretation of the results and findings of such programs and collaborations and whether such results are sufficient to support the future success of our product candidates; the success, timing and cost of our anticipated clinical trials for our current product candidates; the timing of initiation, futility analyses, data presentation, reporting and publication and receipt of interim results (including, without limitation, any preclinical results or data); any statements about our understanding of product candidates mechanisms of action and interpretation of preclinical and early clinical results from its clinical development programs and any collaboration studies; and other factors, including legislative, regulatory, political and economic developments not within our control. For all these reasons, actual results and developments could be materially different from those expressed in or implied by our forward-looking statements. You are cautioned not to place undue reliance on these forward-looking statements, which are made only as of the date of this presentation. We undertake no obligation to update such forward-looking statements to reflect subsequent events or circumstances, except to the extent required by law or regulation.

WEE1i: A means to target CCNE1-overexpressing cancers

More than 20% of HGSOCs overexpress Cyclin E1 (CCNE1) through amplification, copy number gain or other means[(1)].

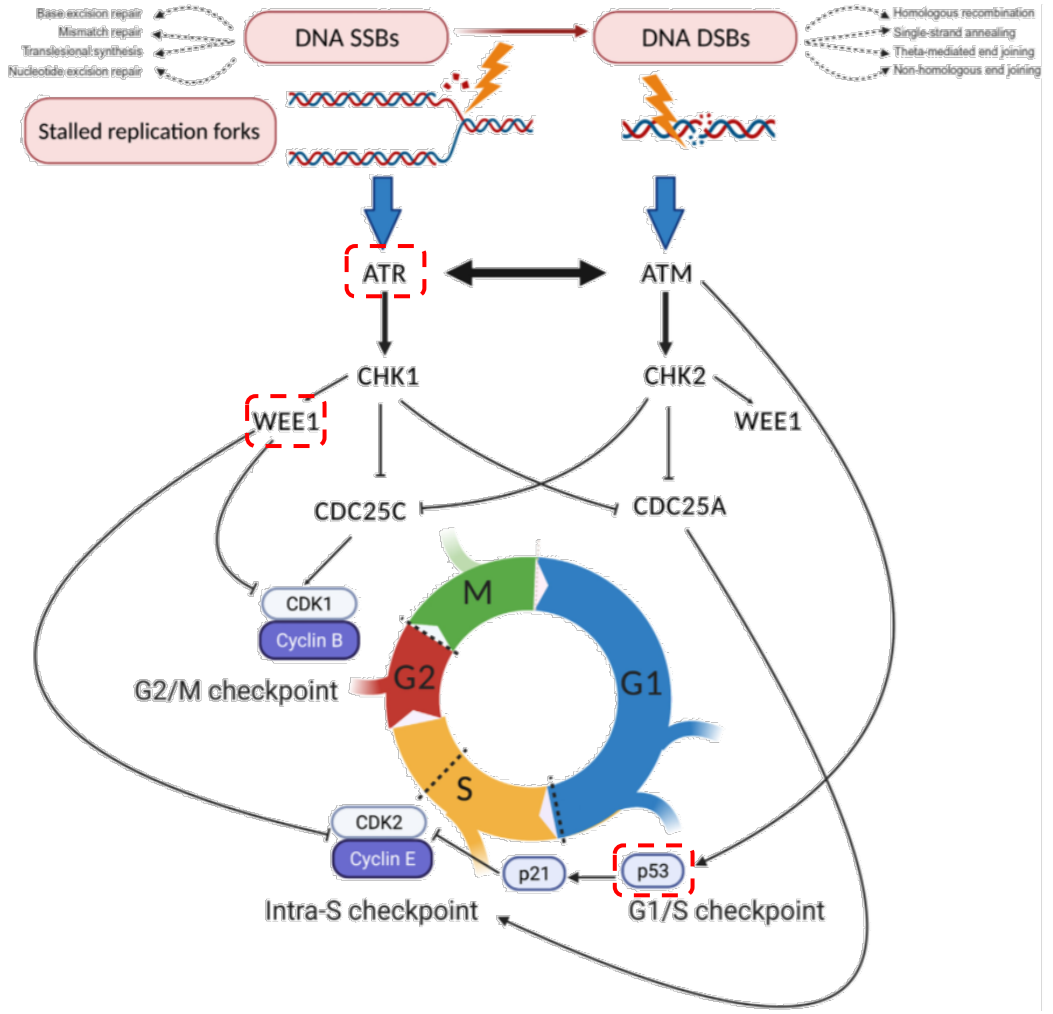
While PARP inhibitors are effective treatments for homologous recombination-deficient cancers, they are ineffective in CCNE1-overexpressing HGSOC.

WEE1 regulates the G1-S and G2-M cell cycle checkpoints through inhibition of CDK2-CCNE and CDK1-CCNB complexes, respectively

WEE1 inhibition (WEE1i) causes excessive activation of CDK2-CCNE1 in CCNE1-amplified HGSOC cells and synergizes with ATR inhibition (ATRi) in cancer cell killing[(2)].

WEE1i is a potential therapy for CCNE1-overexpressing cancers

Overview of Key Proteins in DDR



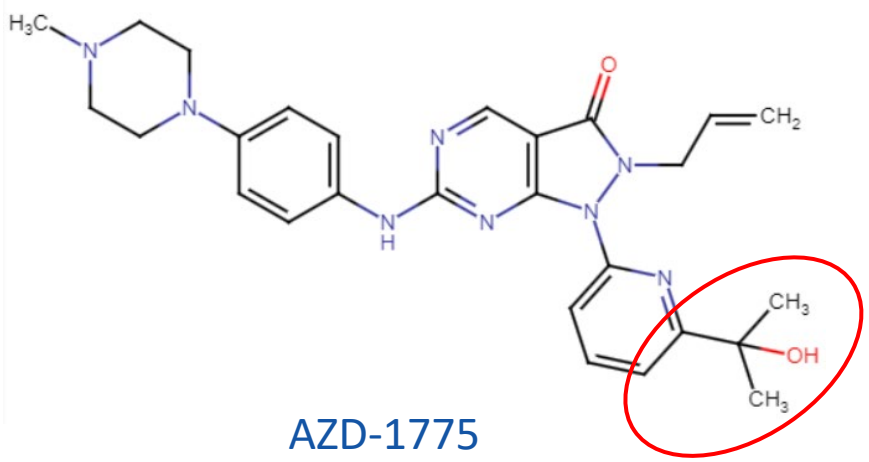
Ngoi et al., *Trends in Cancer*, 2021

(1) Nakayama et al., *Cancer*, 2020
(2) Xu et al., *Cell Rep Med*, 2021

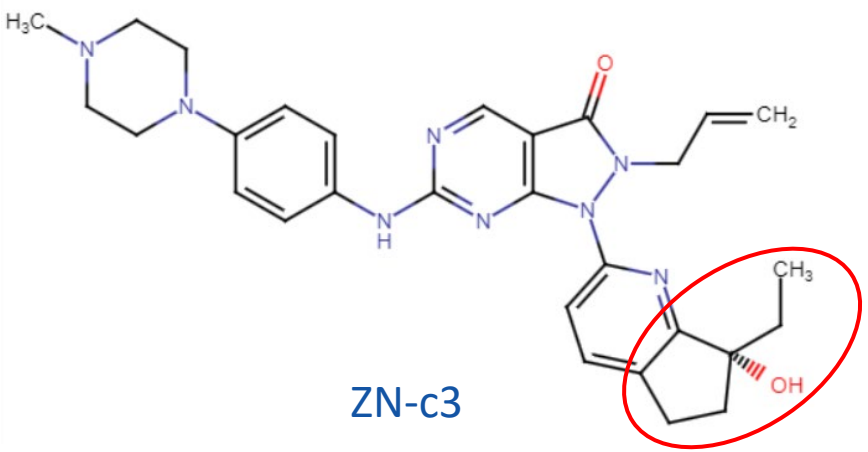
Leading WEE1i are potent but off-target PLK1, PLK2 and PLK3

	On-Target IC_{50} (nM)	Off-Target Inhibition at 1 μM (%)		
	WEE1	PLK1	PLK2	PLK3
ZN-c3 (1)	3.8	79	96	92
AZD-1775 (1,2)	3.9	70	101	91

AZD-1775 and ZN-c3 are structurally similar to one another



AZD-1775



ZN-c3

(1) Huang et al, *J Med Chem*, 2021

(2) AstraZeneca announced discontinuation of AZD-1775 development on June 29, 2022

Red ovals include structurally distinct regions of the molecules

PLK inhibition may be associated with hematologic toxicity

PLK family members have important roles in normal cellular proliferation, including the proliferation of hematopoietic cells, and they protect against unchecked proliferation through their tumor suppressor functions.

MOLECULAR AND CELLULAR BIOLOGY, Nov. 2008, p. 6870–6876 Vol. 28, No. 22
0270-7306/08/$08.00+0 doi:10.1128/MCB.00392-08

Polo-Like Kinase 1 Is Essential for Early Embryonic Development and Tumor Suppression▽‡

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MOLECULAR AND CELLULAR BIOLOGY, Oct. 2003, p. 6936–6943 Vol. 23, No. 19
0270-7306/03/$08.00+0 DOI: 10.1128/MCB.23.19.6936–6943.2003

Role of *Plk2* (*Snk*) in Mouse Development and Cell Proliferation

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Research Article

Polo-like Kinase 3 Functions as a Tumor Suppressor and Is a Negative Regulator of Hypoxia-Inducible Factor-1α under Hypoxic Conditions

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Cancer Res 2008

Hematologic toxicity is frequently associated with PLK1 inhibition in clinical trials

AE	Volasertib (n = 54)		Chemotherapy (n = 55)	
	All Grade	Grade ≥ 3	All Grade	Grade ≥ 3
Any drug-related AE	46 (85.2)	33 (61.1)	41 (75.4)	17 (30.9)
Neutropenia	31 (57.4)	24 (44.4)	10 (18.2)	3 (5.5)
Anemia	24 (44.4)	8 (14.8)	15 (27.3)	1 (1.8)
Thrombocytopenia	22 (40.7)	9 (16.7)	2 (3.6)	2 (3.6)
Leukopenia	15 (27.8)	9 (16.7)	7 (12.7)	0 (0.0)
Asthenia	14 (25.9)	1 (1.9)	16 (29.1)	2 (3.6)
Alopecia	14 (25.9)	0 (0.0)	10 (18.2)	0 (0.0)
Nausea	11 (20.4)	0 (0.0)	15 (27.3)	1 (1.8)
Lymphopenia	7 (13.0)	2 (3.7)	7 (12.7)	1 (1.8)
Vomiting	7 (13.0)	0 (0.0)	7 (12.7)	0 (0.0)
Diarrhea	7 (13.0)	0 (0.0)	8 (14.5)	0 (0.0)
Decreased appetite	6 (11.1)	1 (1.9)	6 (10.9)	0 (0.0)
Platelet count decreased	5 (9.3)	3 (5.6)	0 (0.0)	0 (0.0)
Fatigue	4 (7.4)	1 (1.9)	10 (18.2)	2 (3.6)
Febrile neutropenia	3 (5.6)	3 (5.6)	0 (0.0)	0 (0.0)
Peripheral neuropathy	2 (3.7)	0 (0.0)	7 (12.7)	1 (1.8)

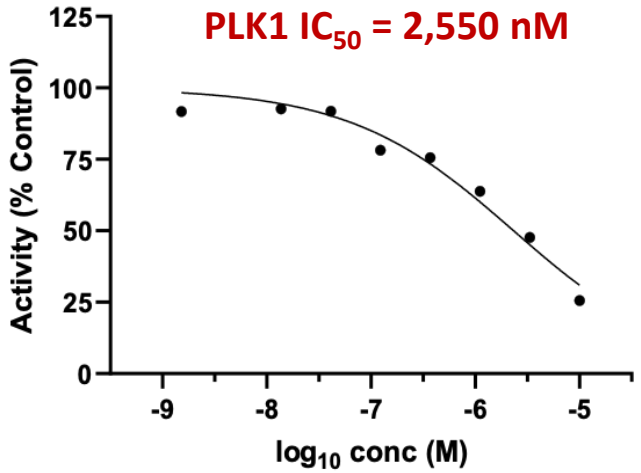
Pujade-Lauraine et al., *J Clin Onc*, 2016

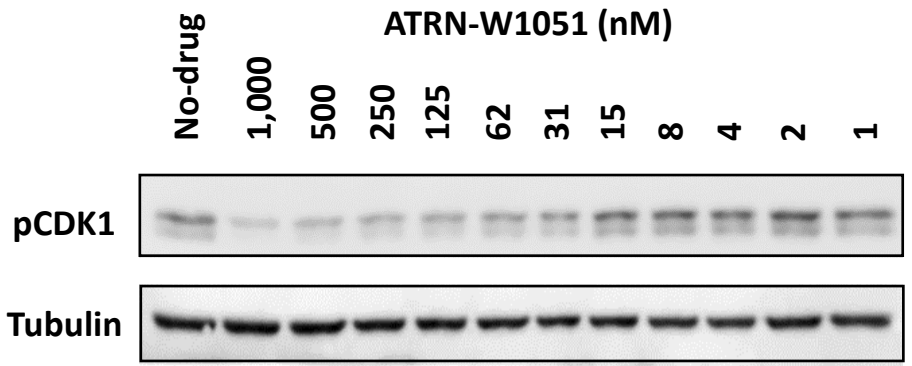
ATRN-W1051 is potentially differentiated from other WEE1 inhibitors

ATRN-'W1051 is a potent WEE1i with low off-target inhibition of PLK1, PLK2 and PLK3

	On-Target IC_{50} (nM)	Off-Target Inhibition at 1 μM (%)		
	WEE1	PLK1	PLK2	PLK3
ATRN-W1051 (1)	2.2	17	33	12
ZN-c3 (1,2)	3.8	79	96	92
AZD-1775 (1,2)	3.9	70	101	91

ATRN-W1051 IC50 for PLK1 inhibition is >1000-fold higher than for WEE1 inhibition

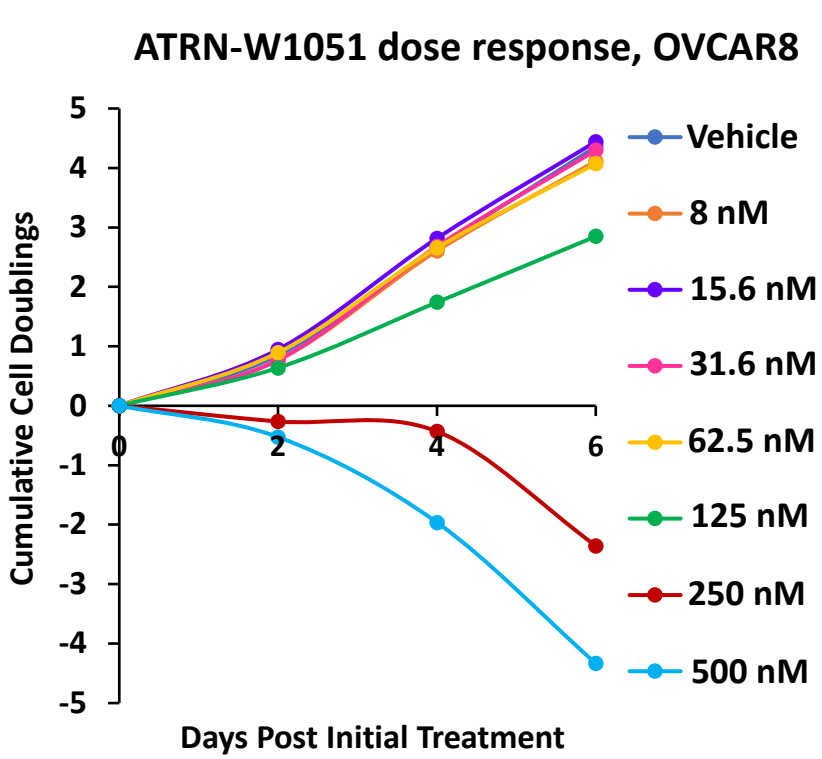


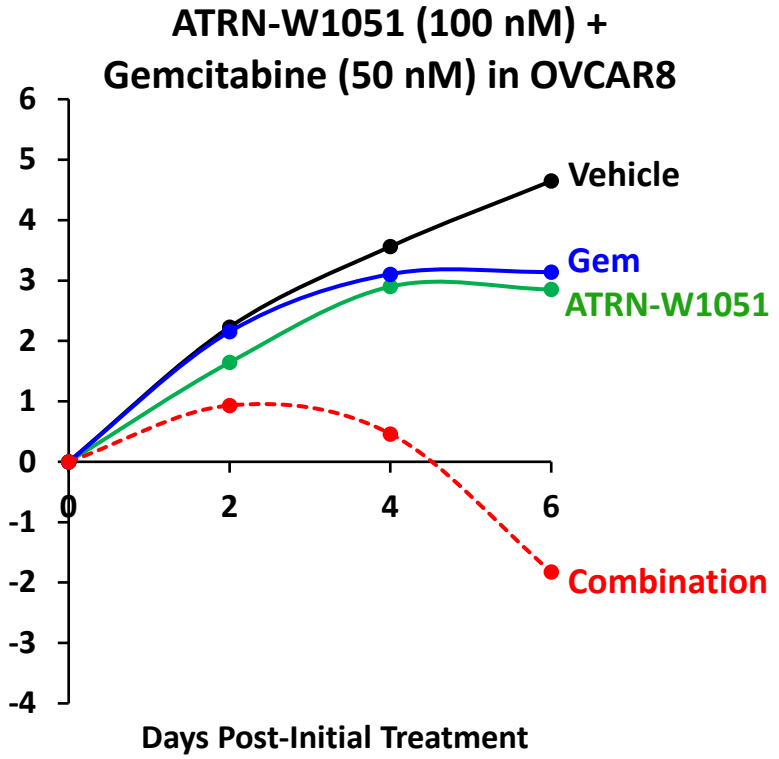


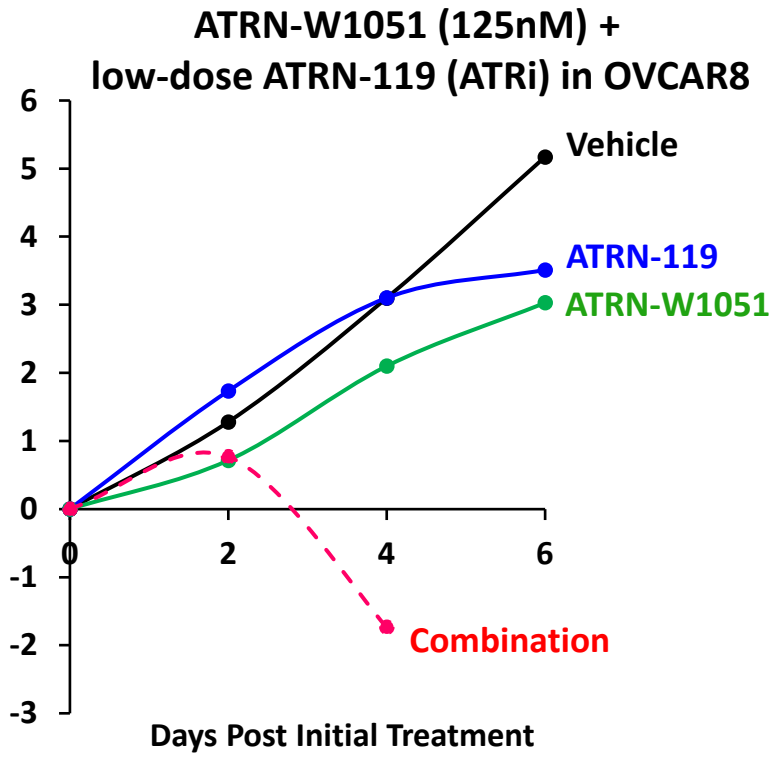
Cellular activity

(1) Head-to-head studies have not been conducted.
(2) Huang et al, *J Med Chem*, 2021

ATRN-W1051 potently inhibits OvCa cell proliferation both alone and in combination







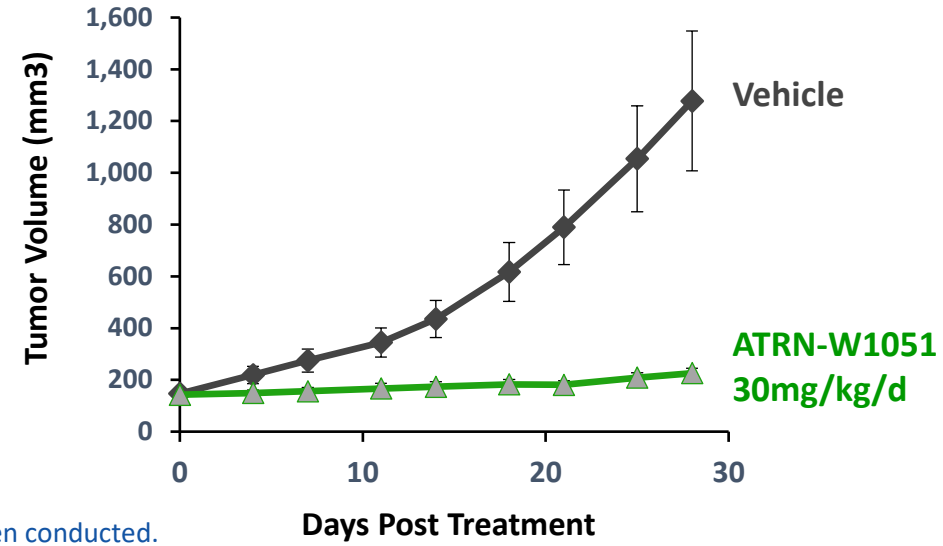
ATRN-W1051 has potentially compelling PK and anti-tumor activity

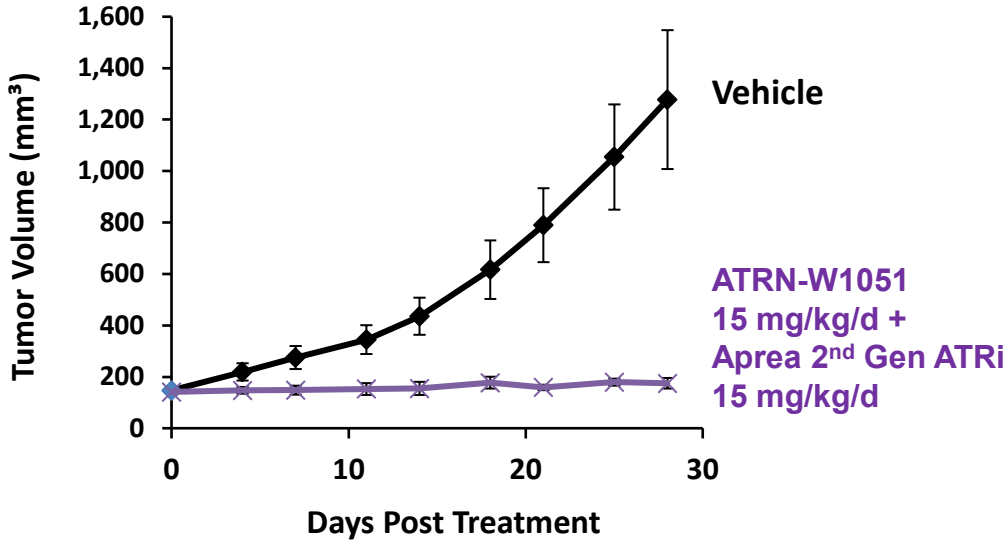
Preclinical data highlight potentially favorable PK properties of ATRN-W1051

	ATRN-W1051 (1,2)	ZN-c3 (1,3)			AZD-1775 (1,3)		
Dose (mg/kg/d)	10	20	40	80	20	40	80
C_{max}, ng/mL	1219	1167	1997	5100	635	2460	4703
T_{max}, hr	2	1	1	1	1	1	1
AUC_{0-24}, ng*hr/mL	14,211	4863	17,088	39,722	1494	6,313	13,408
Tumor concentration, ng/mL	9000 ng/gr (@ 15 mg/kg/d)	10.5	48	811	BQL	BQL	6.95

Anti-tumor activity of ATRN-W1051 – Oral administration

OVCAR3 CCNE1-amplified xenograft model





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

(2) Data from study in normal mice

(3) Data from study in A-427 NSCLC xenograft model as reported in Zentalis Corporate Overview, March 2022

Summary of results

- **ATRN-W1051 is a potent WEE1i (2.2 nM IC_{50}) with low off-target inhibition of PLK1, PLK2 and PLK3.**
- **ATRN-W1051 has the potential to limit the proliferation of ovarian cancer cells in culture.**
- **ATRN-W1051 has potentially favorable pharmacokinetic properties in mice.**
- **Based on data to date, ATRN-W1051 dosed daily for 28 days suppresses the growth of CCNE1-amplified ovarian cancer tumors in vivo.**
- **These encouraging preclinical data suggest that ATRN-W1051 may be a promising therapeutic candidate for patients with CCNE1-overexpressing HGSOC.**

If you have any questions about this presentation, please feel free to email me at brownej@upenn.edu.