Poster #1965

Discovery of ZN-c3, a Potent Wee1 Inhibitor with a Differentiated Pharmacologic and Kinase Selectivity Profile

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Introduction

Genome instability initiated by DNA damage or DNA replication stress is a driver of tumor development. To ensure the accuracy of DNA replication, DNA damage response (DDR) mediated by various cell cycle checkpoints activates the DNA repair system. Activation of the DNA damage response pathway leads to Wee1 kinase activation to prevent entry into mitosis by suppressing cyclin-dependent kinase 1 (CDK1) activity. The activation of Wee1 induces cell cycle arrest, which prevents damaged cells from entering mitosis. Inhibition of Wee1 leads to unscheduled mitotic entry and mitotic catastrophe. Small-molecule inhibitors of Wee1 are currently being tested in several tumor types. Preliminary results show promising clinical activity of AZD1775, a potent inhibitor of Wee1; however, achieving an optimal dose and a schedule that is well tolerated has been a challenge.

Here, we describe the characterization of ZN-c3, a potent, selective and orally-bioavailable small-molecule inhibitor of Wee1.

ZN-c3, with its key differentiated pharmacologic and kinase selectivity profile, is a promising new generation of Wee1 inhibitor that is currently in clinical development for the treatment of advanced solid cancers.

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Results

As displayed in Table 1A, ZN-c3 has similar potency as AZD1775, but demonstrates better ADME properties.

Table 1B displays the potent anti-proliferative activity of ZN-c3 compared to AZD1775 in a panel of cell lines representing several tumor types.

Table 1A. ZN-c3 has Similar Potency as AZD1775 but Demonstrates Better ADME Properties

Compound ID	Biochem. assay IC_{50} (nM) Wee1	Log D	Solubility (μM)	hERG (μM)
ZN-c3	3.8	2.4	2131	>30
AZD1775	2.8	2.2	60	15.1

ZN-c3 is a potent Wee1 inhibitor with an IC_{50} of 3.8 nM in *in vitro* kinase assay, and has a better profile regarding solubility and hERG inhibition than AZD1775.

ADME = absorption, distribution, metabolism, excretion; hERG = human-Ether-à-go-go-Related Gene

Table 1B. ZN-c3 Anti-Proliferative Activity in Panel of Cell Lines Representing Several Tumor Types

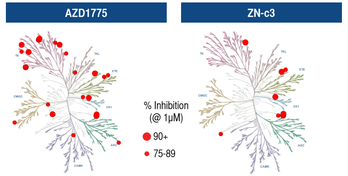
Compound ID	CTG IC_{50} (nM)							
	NSCLC		SCLC		TNBC		Ovarian cancer cells	
	NCI-H23	A427	DMS-53	NCI-H1048	MDA-MB-231	HCC1806	OVCAR3	UWB1.289
ZN-c3	124	88	118	92	190	95	69	54
AZD1775	108	94	130	97	233	94	124	57

Proliferation Assay. ZN-c3 anti-proliferative activity was evaluated in a panel of tumor cell lines representing several tumor types. Cell viability was measured using CellTiter-Glo assay.

NSCLC = non-small cell lung cancer; SCLC = small cell lung cancer; TNBC = triple-negative breast cancer

Selectivity profiles of ZN-c3 and AZD1775 were performed *in vitro* across a large human kinase panel. Figure 1 shows that ZN-c3 is highly selective for Wee1. Compared to AZD1775, ZN-c3 has a superior selectivity profile against this panel of kinases.

Figure 1. ZN-c3 is Highly Selective for Wee1 Against a Large Kinase Panel



Selectivity profiles of ZN-c3 and AZD1775 were performed *in vitro* across a panel of 480 human kinases at a concentration of 1μM.

Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com)

In a proliferation assay, cells grown in media were treated with either ZN-c3 or the PARP inhibitor, talazoparib. As shown in Table 2, ZN-c3 has activity in BRCA1 null or wild-type BRCA1 UWB1.289 ovarian cancer cells.

Table 2. ZN-c3 has Activity in BRCA1 Null or Wild-Type BRCA1 UWB1.289 Ovarian Cancer Cells

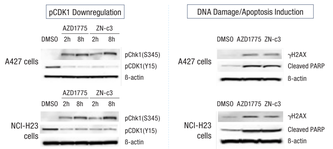
Compound ID	IC_{50} (nM)	
	UWB1.289 (BRCA1 null)	UWB1.289+BRCA1
ZN-c3	53.7	141
Talazoparib	29	>10000

Proliferation Assay. UWB1.289 and UWB1.289+BRCA1 (in which wild-type BRCA1 was restored) cells grown in media were treated with ZN-c3 or talazoparib (PARP inhibitor). Cell viability was measured using CellTiter-Glo assay.

BRCA1 = breast cancer gene 1; PARP = poly (ADP ribose) polymerase; UWB = ultra-wideband

A427 and H23 NSCLC cell lines were treated with either ZN-c3 or AZD1775. Immunoblotting determined the phosphorylation status of CDK1 and Chk1 kinases, the induction of apoptosis, and the extent of DNA damage. Figure 2 shows the effects of ZN-c3 on this downstream signaling.

Figure 2. ZN-c3 Effects on Downstream Signaling



A427 and H23 NSCLC cell lines were treated with 300 nM of ZN-c3 or AZD1775. Immunoblotting was performed to determine phosphorylation status of CDK1 and Chk1 as well as the induction of apoptosis (cleaved PARP) and DNA damage (γH2AX).

CDK = cyclin-dependent kinase; DMSO = dimethyl sulfoxide; NSCLC = non-small cell lung cancer; PARP = poly (ADP ribose) polymerase

The pharmacokinetic and efficacy profiles of ZN-c3 and AZD1775 were compared in tumor xenograft models. ZN-c3 showed higher plasma and tumor exposure than AZD1775, which correlated with more potent tumor growth inhibition. Table 3 displays the high plasma and tumor exposure of ZN-c3 in the A427 NSCLC efficacy model.

Table 3. ZN-c3 Demonstrates High Plasma and Tumor Exposure in A427 NSCLC Efficacy Model

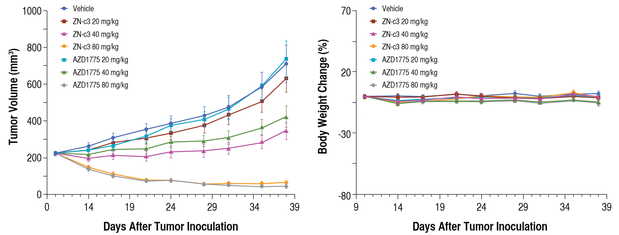
Study (A427 NSCLC)	ZN-c3			AZD1775		
Dose (mg/kg/day)	20	40	80	20	40	80
C_{max} (ng/mL)	1,167	1,997	5,100	635	2,460	4,703
T_{max} (hr)	1	1	1	1	1	1
AUC_{0-24hr} (ng·hr/mL)	4,863	17,088	39,722	1,494	6,313	13,408
Tumor Conc. (ng/mL)	10.5	48.0	811	BQL	BQL	6.95

Steady state pharmacokinetics (PK) of ZN-c3 and AZD1775 in plasma and tumor tissues in A427 NSCLC Model.

BQL = below quantification limit; NSCLC = non-small cell lung cancer

As presented in Figure 3, ZN-c3 causes tumor regressions as a single agent in the NSCLC (A427) xenograft model. Both ZN-c3 and AZD1775 induced dose-dependent antitumoral activity and were well tolerated at the tested doses.

Figure 3. ZN-c3 Causes Tumor Regression in A427 Human NSCLC Tumor Xenograft

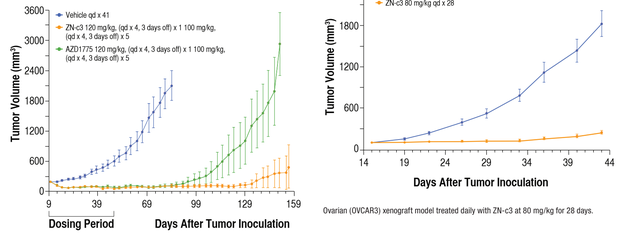


NSCLC (A427) xenograft model treated with ZN-c3 or AZD1775 at various doses orally once daily for 29 consecutive days. Both compounds induced dose-dependent antitumoral activity and were well tolerated at the tested doses.

NSCLC = non-small cell lung cancer

In the A427 NSCLC model, ZN-c3 or AZD1775 dosed intermittently for 42 days induced similar tumor regressions (Figure 4). Tumor regrowth was monitored in the treatment arms after treatment cessation until Day 159. ZN-c3 induced prolonged tumor growth delay compared to AZD1775 (Figure 4).

Figure 4. ZN-c3 Induces Prolonged Tumor Growth Delay in A427 Human NSCLC Tumor Xenograft Model



ZN-c3 or AZD1775 were dosed intermittently (4 days on / 3 days off) at 120 mg/kg for one week followed by 100 mg/kg for 5 weeks. Treatment with ZN-c3 or AZD1775 was stopped on Day 42, and the tumor regrowth in the treatment arms was monitored until Day 159. These doses were well tolerated.

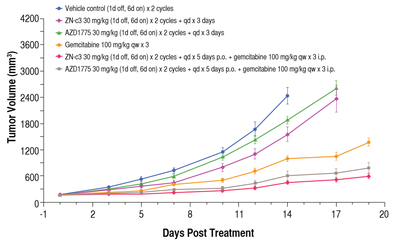
Figure 5 displays the antitumor activity of ZN-c3, tracked for 28 days, in the OVCAR3 ovarian tumor xenograft model.

Figure 5. ZN-c3 Antitumor Activity in OVCAR3 Ovarian Tumor Model

Ovarian (OVCAR3) xenograft model treated daily with ZN-c3 at 80 mg/kg for 28 days.

A SJSA-1 osteosarcoma tumor xenograft model was treated with ZN-c3, AZD1775, or gemcitabine, as single agents and combinations. ZN-c3 was shown to have enhanced antitumor activity in combination with gemcitabine in this tumor model (Figure 6).

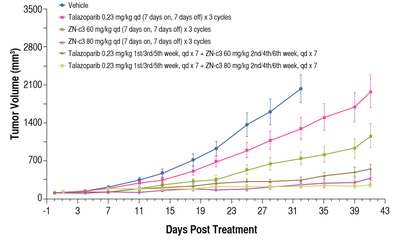
Figure 6. Enhanced Antitumor Activity of ZN-c3 Combined with Gemcitabine in SJSA-1 Osteosarcoma Tumor Xenograft Model



SJSA-1 osteosarcoma xenograft model treated with single agents (ZN-c3 30 mg/kg, AZD1775 30 mg/kg) or gemcitabine 100 mg/kg and combinations (ZN-3 plus gemcitabine; AZD1775 plus gemcitabine) for 20 days.

The OVCAR3 ovarian tumor xenograft model was treated with ZN-c3, talazoparib, and their combinations over 42 days. ZN-c3 was shown to be effective in combination with talazoparib in this ovarian cancer model (Figure 7).

Figure 7. ZN-c3 Efficacy in Combination with Talazoparib in OVCAR3 Ovarian Cancer Model



Ovarian (OVCAR3) xenograft model treated with single agents (ZN-c3 60 mg/kg and 80 mg/kg) or talazoparib 0.23 mg/kg and combinations (ZN-c3 plus talazoparib) for 42 days.

Conclusions

- ZN-c3 is a potent and highly-selective Wee1 inhibitor that demonstrates anti-proliferative activity across several tumor types
- ZN-c3 induced significant antitumor efficacy as a single agent or in combinations
- ZN-c3 induced a prolonged tumor growth delay after treatment was stopped
- ZN-c3 showed improved pharmaceutical properties, including high solubility compared to AZD1775
- The higher plasma and tumor exposure of ZN-c3 was associated with a prolonged tumor growth delay in the efficacy model
- ZN-c3 exhibits the potential for an improved therapeutic window due to higher selectivity, lower doses needed, and superior efficacy (vs. AZD1775)
- ZN-c3 is currently undergoing Phase 1/2 clinical trials as a single agent and in combinations

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