Poster # 528P

Discovery of ZN-d5, a potent BCL-2 inhibitor with improved selectivity for BCL-2

Joseph R. Pinchman*, Hooman Izadi, Chad D. Hopkins, Noah Ibrahim, Kevin D. Bunker, Fernando Doñate, Ahmed A. Samatar, Peter Q. Huang.
Zentalis Pharmaceuticals, San Diego, CA.

Background

The B-cell lymphoma-2 (BCL-2) family of proteins are essential components in the intrinsic apoptosis pathway. While a primary mechanism for the removal of damaged or unwanted cells, dysregulation of this pathway is observed in many human cancers. Specifically, BCL-2 overexpression has been associated with pro-survival of multiple hematological and solid tumor malignancies. In the clinic, BCL-2 inhibitors have demonstrated impressive efficacy in hematological diseases, including chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), and non-Hodgkin lymphoma (NHL). However, development of more selective BCL-2 inhibitors is warranted, as thrombocytopenia, resulting from BCL-x_L inhibition, is a common adverse reaction in patients receiving the approved BCL-2 inhibitor, venetoclax. ZN-d5 is a potent, selective, and orally-bioavailable BCL-2 inhibitor with increased selectivity for BCL-2 vs. BCL-x_L compared to venetoclax, and shows significant preclinical anti-tumor activity in multiple cancer models.

Methods

BCL-2 family binding affinity was measured using the BCL2scan™ ligand binding assay, and binding to BCL-2 mutants was measured using homogeneous time-resolved fluorescence (HTRF, BAK displacement). Cell proliferation in tumor cell lines and platelet viability was measured using CellTiter-Glo® (Promega). Anti-tumor efficacy was determined in xenograft models of NHL, AML, and other hematological and solid tumor types.

Disclosure Statement: Joseph R. Pinchman is an employee and shareholder of Zentalis Pharmaceuticals, Inc. and a member of Recurium IP Holdings, LLC, which retains future rights to royalties and milestones associated with ZN-d5.

Correspondence: jpinchman@zentalis.com

Funding for this research was provided by Zentalis Pharmaceuticals, Inc.

Results

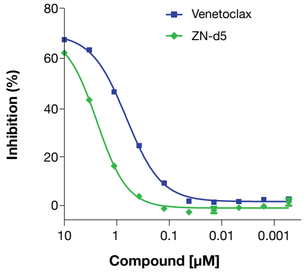
Table 1. ZN-d5 has 10x Improved Selectivity for BCL-2 vs BCL-x_L and Binds With Higher Affinity to BCL-2 Mutants than Venetoclax

Compound ID	Affinity (Kd, nM)			IC_{50} (nM) BCL-2 Type			
	BCL-2	BCL-x_L	MCL-1	WT	G101V	F104L	D103Y
Venetoclax	0.41	28	>30000	1.3	7.3	8.4	18.3
ZN-d5	**0.29**	**190**	**>30000**	**1.4**	**3.7**	**1.4**	**5.0**

Table 2. ZN-d5 Exhibits Potent *In Vitro* Activity Across Multiple Tumor Cell Lines

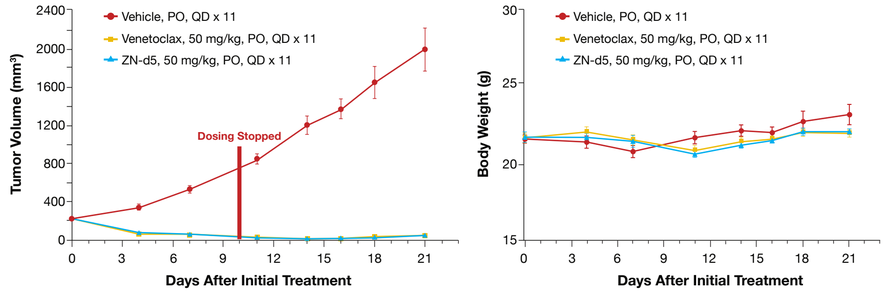
Compound ID	CTG IC_{50} (nM)						
	ALL	MCL	DLBCL		AML		
	RS4;11	Granata-519	DOHH-2	Toledo	HL-60	Molm-13	MV4-11
Venetoclax	2.9	161	43	191	26	18	3.8
ZN-d5	**5.1**	**89**	**50**	**92**	**21**	**39**	**5.1**

Figure 1. ZN-d5: Less Human Platelet Toxicity Compared to Venetoclax in an *In Vitro* Assay



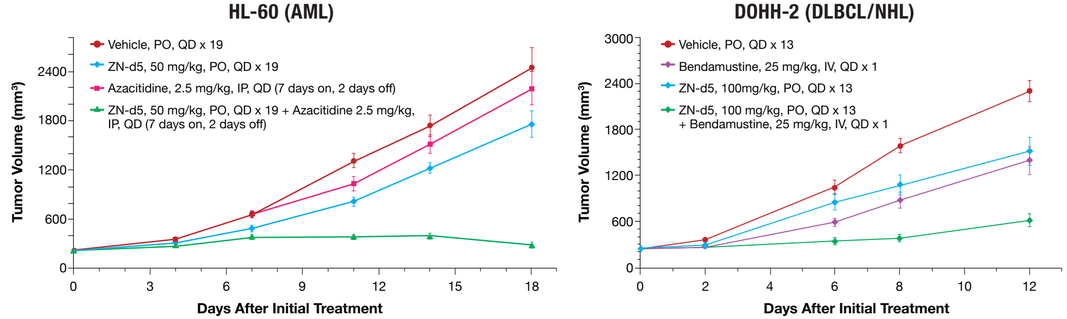
	CTG (24 h) IC_{50} (µM)
Venetoclax	**0.6**
ZN-d5	**2.4**

Figure 2. ZN-d5 Demonstrates Potent *In Vivo* Efficacy in a RS4;11 (ALL) Model



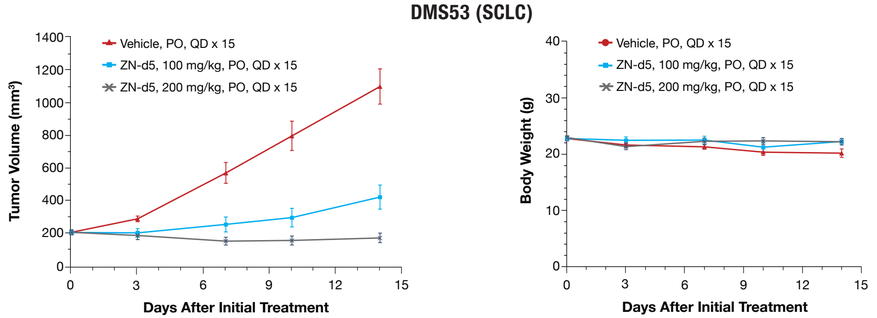
NOD/SCID mice bearing RS4;11 (ALL) tumor cells were orally dosed with ZN-d5 and venetoclax for 11 days, and animal body weights (10 mice per group) were measured twice a week.

Figure 3. ZN-d5 Exhibits Enhanced Efficacy in Combination with Azacitidine or Bendamustine



HL-60: BALB/C nude mice bearing HL-60 (AML) tumor cells were treated with ZN-d5 (PO), Azacitidine (IP) or in combination for 19 days.
DOHH-2: CB17 SCID mice bearing DOHH-2 (DLBCL/NHL) tumor cells treated with ZN-d5 (PO), Bendamustine (IV), or in combination for 13 days.
Combination treatments were tolerated well in both studies.

Figure 4. ZN-d5 Demonstrates Single Agent Efficacy in a Solid Tumor Model



DMS53: BALB/C nude mice bearing DMS53 (SCLC) tumor cells were treated with ZN-d5 (PO) for 15 days, and animal body weights (10 mice per group) were measured twice a week.

Conclusions

ZN-d5 is a promising new BCL-2 inhibitor with improved selectivity for BCL-2 vs. other BCL-2 family members, and demonstrates potent single-agent and combination activity in multiple *in vivo* tumor models. ZN-d5 is currently in Phase 1 clinical trials for the treatment of NHL and AML (NCT04500587).

zentalis