A novel Selective BCL2 inhibitor with limited immune suppression and improved safety compared to venetoclax.

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BACKGROUND

- BCL2 is a key pro-survival protein that is overexpressed in many cancers
- The first generation BCL2 inhibitor venetoclax has proved highly effective in multiple hematological malignancies including CLL and AML.
- Venetoclax has several limitations:
  - A long *in vivo* half-life
  - metabolism is influenced by Cyp3A/4 inhibition making use in diseases where anti-fungal prophylaxis is required challenging
  - Treatment can also result in significant immunosuppression including dose-limiting neutropenia and thrombocytopenia, and the loss of multiple lymphocyte populations due in part to its effect on BCL-xL

AIMS

- Develop a novel highly selective BCL2 inhibitor
- Assess *in vitro* and *in vivo* efficacy compared to venetoclax
- Assess in immunological impact of our novel compound compared to venetoclax using high parameter flow cytometry

ZE50-0134 PROPERTIES

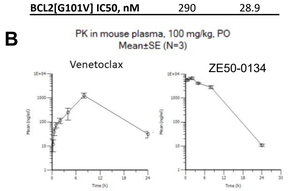
- ZE50-0134 binds the P2 pocket of BCL2, thereby increasing selectivity toward BCL-2
- ZE50-0134 showed a 4600-fold greater selectivity for BCL2 over BCL-xL compared to venetoclax with only an 84-fold greater selectivity (Figure 1A)
- *In vivo* murine and canine pharmacokinetics showed that ZE50-0134 has a substantially shorter half-life than venetoclax suggesting feasibility for periodic pulse dosing to limit the on-target adverse effects of BCL2 inhibition (Figure 1B)
- An *in vivo* murine study examining the pharmacology of ZE50-0134 or venetoclax given with ketoconazole, a strong CYP3A inhibitor suggesting less Cyp3A influence on ZE50-0134 (Figure 1C&D)
- Toxicology in rats and dogs suggest a 19-fold margin between therapeutically effective dose and toxicity with ZE50-0134

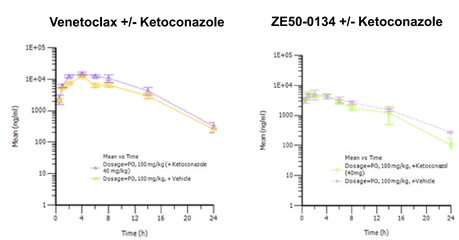
RESULTS

ZE50-0134 is more selective for BCL2, has greater bioavailability, and a shorter half-life than venetoclax

A

Parameter	Venetoclax	ZE50-0134
BCL2[WT] IC50, nM	5.7	1
BCL xL [WT] IC50, nM	480	4600
BCL2[G101V] IC50, nM	290	28.9





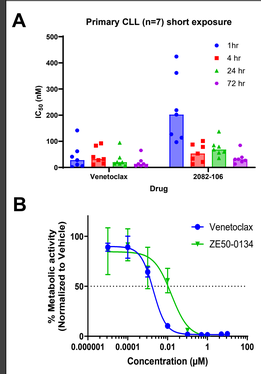
D

Venetoclax		100 mg/kg		Fold increase
Parameter	Units	-ketoconazole	+ketoconazole	
kel	1/h	0.21	0.22	
T1/2	h	3.34	3.09	
Tmax	h	4	4	
Cmax	**uM**	**13.9**	**17.2**	**1.2**
AUClast	**h*uM**	**119.0**	**180.8**	**1.52**
AUCinf	**h*uM**	**120.4**	**182.4**	**1.52**
MRTlast	h	7.41	7.41	

ZE50-0134		100 mg/kg		Fold increase
Parameter	Units	-ketoconazole	+ketoconazole	
kel	1/h	0.15	0.19	
T1/2	h	4.77	3.74	
Tmax	h	2.0	1.0	
Cmax	**uM**	**6.8**	**6.8**	**1.0**
AUClast	**h*uM**	**70.7**	**59.7**	**0.84**
AUCinf	**h*uM**	**73.3**	**60.4**	**0.82**
MRTlast	h	7.37	6.67	

Figure 1: **A.** ZE50-0134 has increased selectivity for BCL2 over BCL-xL compared to venetoclax. **B** Pharmacokinetics of single dose venetoclax and ZE50-0134 in RS4;11 tumor bearing mice **C.** Pharmacokinetics of single dose venetoclax and ZE50-0134 with and without a strong CYP3a inhibitor, ketoconazole. **D.** Tables summarizing data in C.

ZE50-0134 is equally efficacious to venetoclax in Primary CLL cells and the RS4;11 cell line model



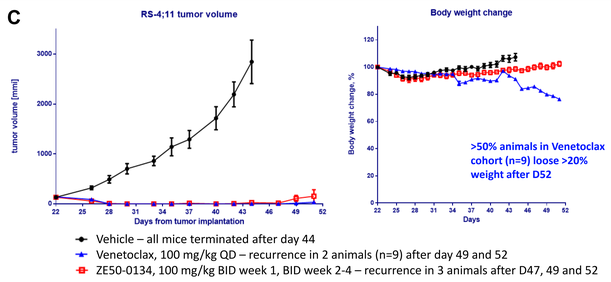


Figure 2 A. ZE50-0134 and Venetoclax have equivalent efficacy after 4 hours exposure in primary CLL patient samples **B.** *in vitro* sensitivity of RS4;11 cells **C.** *in vivo* efficacy and toxicity of venetoclax and ZE50-0134 in subcutaneous RS4;11 mouse model

ZE50-0134 and venetoclax have equivalent efficacy in the MOLM13 AML xenograft mouse model

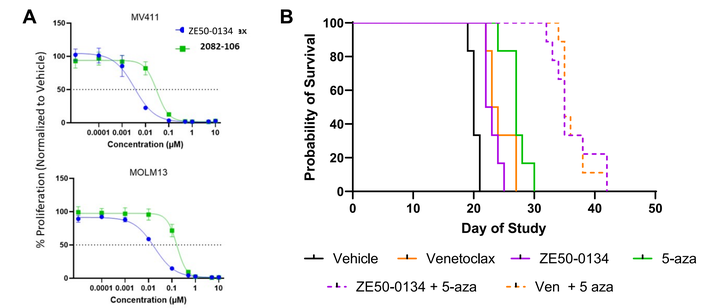


Figure 3. **A.** *in vitro* IC_{50} of venetoclax and ZE50-0134 in AML cell lines **B.** *in vivo* overall survival in of 28 days 100 mg/kg QD venetoclax and ZE50-0134 alone or in combination with 5x 2.5 mg/kg QW Azacytidine (Aza) **C.** Absolute counts of human CD45+ cells in the bone marrow (BM) at endpoint

ZE50-0134 results in significantly less *in vivo* immunosuppression than venetoclax

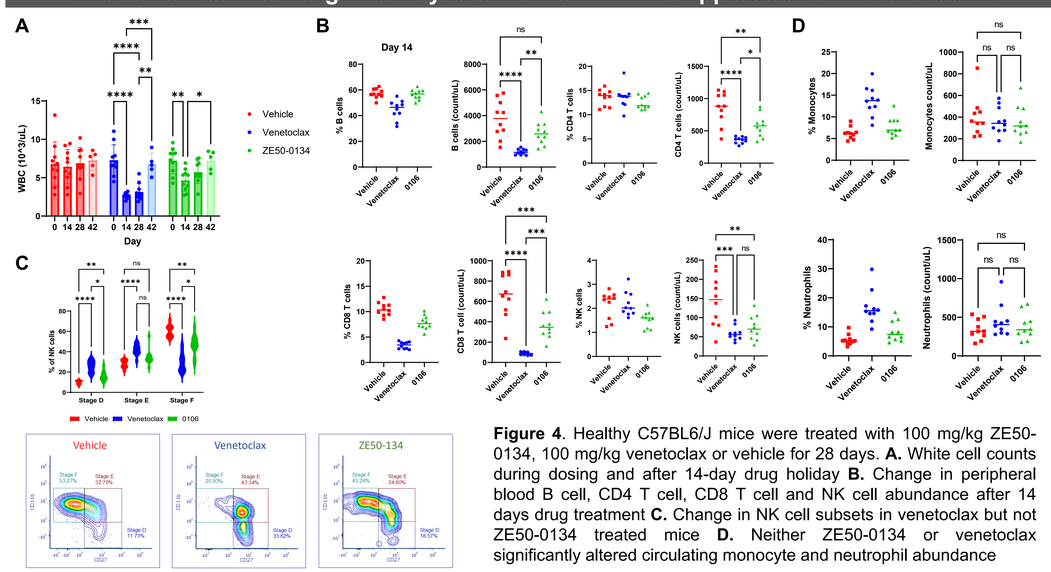


Figure 4. Healthy C57BL6/J mice were treated with 100 mg/kg ZE50-0134, 100 mg/kg venetoclax or vehicle for 28 days. **A.** White cell counts during dosing and after 14-day drug holiday **B.** Change in peripheral blood B cell, CD4 T cell, CD8 T cell and NK cell abundance after 14 days drug treatment **C.** Change in NK cell subsets in venetoclax but not ZE50-0134 treated mice **D.** Neither ZE50-0134 or venetoclax significantly altered circulating monocyte and neutrophil abundance

CONCLUSIONS

- ZE50-0134 is novel highly selective BCL2 inhibitor
- ZE50-0134 is a highly bioavailable molecule with a short half-life and little interaction with CYP3A
- ZE50-0134 has equivalent *in vivo* anti-tumor efficacy to venetoclax in both B cell and myeloid malignancy cell line models
- ZE50-0134 has limited impact on non-malignant immune populations leading to significantly less immunosuppression *in vivo*

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