Single-cell transcriptomic analysis of CLL cells at ibrutinib plus venetoclax relapse and targeting using BCL-2/BCL-xL PROTACs PZ18753b and WH25244

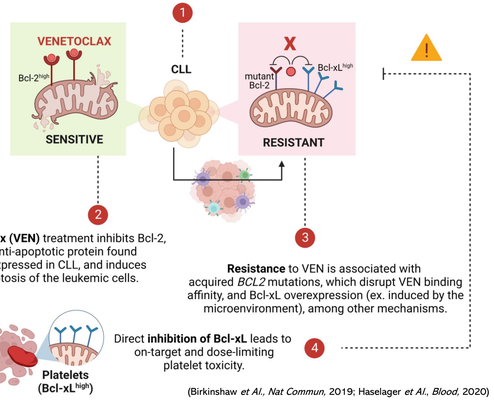
Daisy Díaz Rohena[1], Arnau Peris Cuesta[1], Bailey Slawin[1], Janani Ravikrishnan[2], Chaomei Liu[1], Charmelle Williams[1], Wanyi Hu[3], Peiyi Zhang[3], Phillip Thompson[4], William Wierda[1], Nitin Jain[1], Sachet Shukla[1], Guangrong Zheng[3], Daohong Zhou[5], Jennifer Woyach[2] and Deepa Sampath[1]

[1]The University of Texas MD Anderson Cancer Center, Houston, TX; [2]The Ohio State University Comprehensive Cancer Center, Columbus, OH; [3]University of Florida, Gainesville, FL; [4]Peter MacCallum Cancer Centre, Australia; [5]University of Texas Health Science Center at San Antonio, San Antonio, TX

dydiaz@mdanderson.org

TARGETING VENETOCLAX RESISTANT CLL

In **CLL** patients, abnormal mature B-lymphocytes progressively accumulate in the blood, bone marrow and lymphoid tissues.



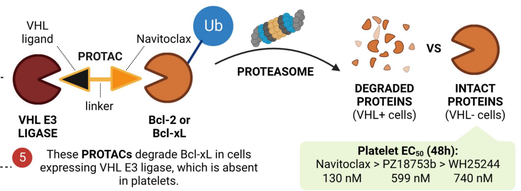
Venetoclax (VEN) treatment inhibits Bcl-2, an anti-apoptotic protein found overexpressed in CLL, and induces apoptosis of the leukemic cells.

Resistance to VEN is associated with acquired *BCL2* mutations, which disrupt VEN binding affinity, and Bcl-xL overexpression (ex. induced by the microenvironment), among other mechanisms.

Direct **inhibition of Bcl-xL** leads to on-target and dose-limiting platelet toxicity.

(Birkinshaw *et Al., Nat Commun,* 2019; Haselager *et Al., Blood,* 2020)

We hypothesized that venetoclax-resistant CLL cells can be targeted by novel PROTAC-based Bcl-2/Bcl-xL protein degraders, PZ18753b and WH25244, while sparring platelets.

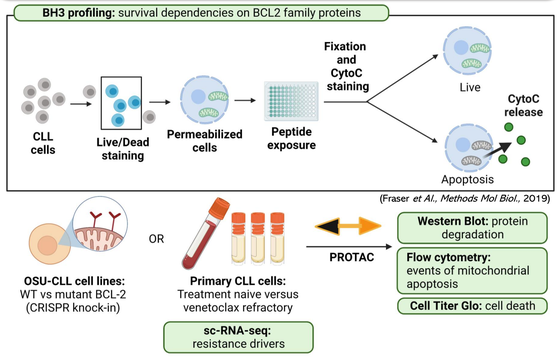


These **PROTACs** degrade Bcl-xL in cells expressing VHL E3 ligase, which is absent in platelets.

Platelet EC_{50} (48h):
Navitoclax > PZ18753b > WH25244
130 nM 599 nM 740 nM

(Dongwen *et Al., Nat Commun,* 2021; Zheng Laboratory, *unpublished*)

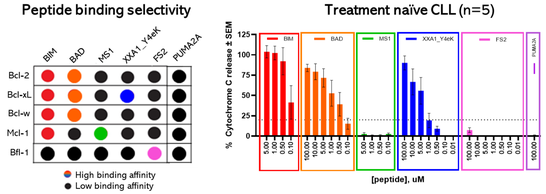
METHODS



(Fraser *et Al., Methods Mol Biol.,* 2019)

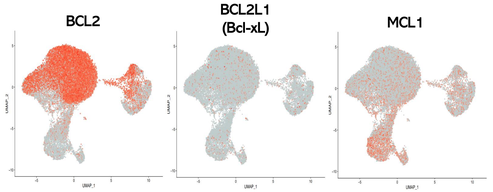
BCL-2/BCL-XL PROTACS IN VENETOCLAX RELAPSED CELL: RATIONALE AND MECHANISM OF ACTION

1 Bcl-2 and Bcl-xL are the main proteins that protect CLL cells from apoptosis.



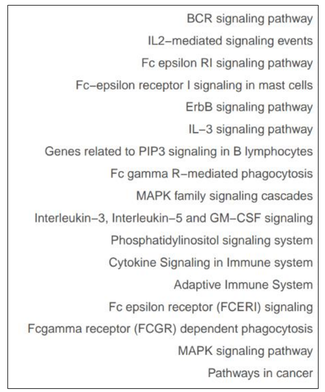
BH3 profiling in treatment naïve CLL cells (n=5) showed sensitivity to BIM, BAD and XXA1_Y4eK peptides, assessed by cytochrome C release.

2 Venetoclax-relapsed CLL retain survival dependency in anti-apoptotic proteins from the BCL2 family.

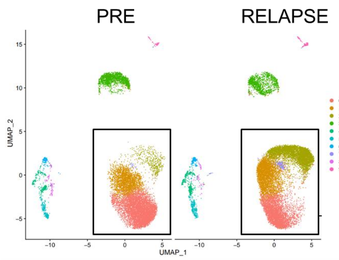


Integrated data from sc-RNA-seq of CLL samples (n=3) collected at venetoclax relapse. (Analysis by Arnau Peris Cuesta)

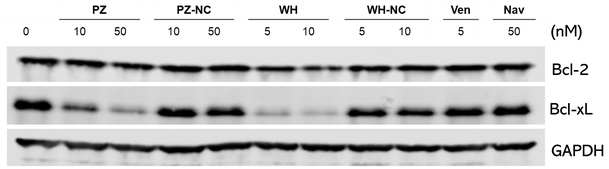
3 Top enriched pathways at venetoclax relapse (n=3 CLL samples).



4 Expansion of CLL cells with abundant BCR and BCL-2 family transcripts at relapse after cessation of ibrutinib plus venetoclax.

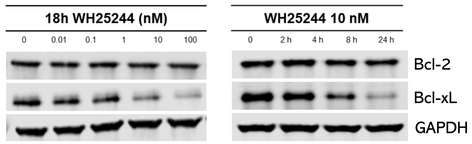


5 PZ18753b and WH25244 recruit the VHL E3 ligase to degrade Bcl-xL within primary CLL cells.



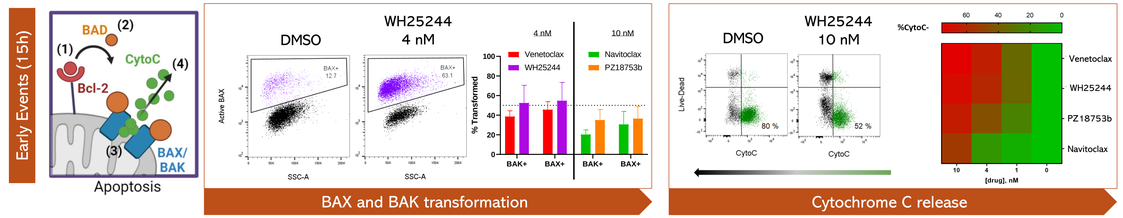
Treatment naïve CLL cells were treated for 14h with PROTACs PZ18753b (PZ), WH25244 (WH) or negative controls (NC) lacking an active VHL ligand (n=5).

6 The extent of Bcl-xL degradation in CLL increases with PROTAC dose and exposure time.

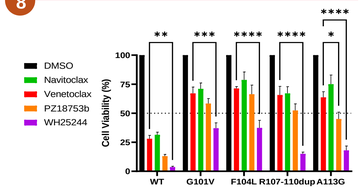


DC_{50} (18h)	PZ18753b	WH25244
Bcl-xL	1.82 nM	0.26 nM

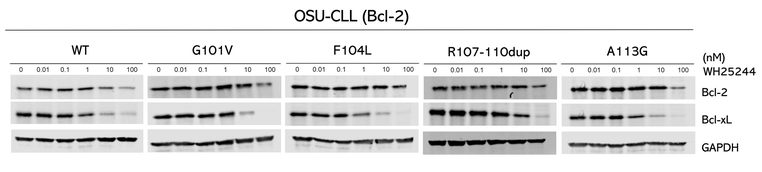
7 Treatment with Bcl-2/Bcl-xL PROTACs kills CLL cells via mitochondrial apoptosis (n=4).



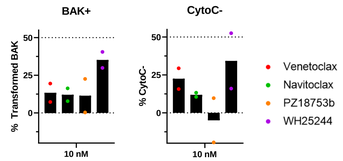
8 WH25244 is superior to venetoclax at killing Bcl-2 mutant OSU-CLL cells (72h, 200 nM).

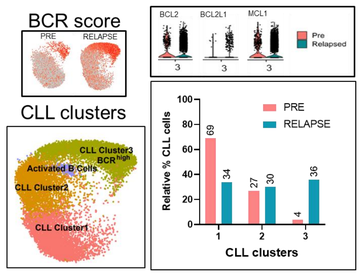


9 WH25244 degrades wildtype and mutant Bcl-2, and Bcl-xL within 24h in OSU-CLL cell lines.



10 Primary CLL cells resistant to venetoclax initiate apoptosis upon treatment with WH25244 (n=2).





KEY FINDINGS

WH25244 is a PROTAC-based degrader that has the potential to re-sensitize venetoclax-resistant CLL cells to apoptosis, via degradation of wildtype and mutant Bcl-2, and Bcl-xL, in a VHL-dependent manner.

Its therapeutic index is improved when compared to its precursor, navitoclax, as observed *in vitro* by:

- Increased potency against CLL cells (on-target effect)
- Decreased potency against platelets (on-target toxicity)

ACKNOWLEDGEMENTS

Dr. Sampath Laboratory
Dr. Woyach Laboratory
Dr. Shulkla Laboratory
Dr. Zheng Laboratory
Dr. Zhou Laboratory
Dr. Bachireddy Laboratory
MDACC CLL Moonshot
Dr. Nitin Jain
MDA UTHealth Houston GSBS Cancer Biology Program
MDACC South Campus Flow Cytometry Core
MDACC Advanced Technology Genomics Core
Department of Leukemia
CLL patients

NIH (TL1TR003169 and UL1TR003167).