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POSTER ABSTRACTS

605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

Enriched Signalling Pathways in Venetoclax-Relapsed Chronic Lymphocytic Leukemia (CLL) Cells and Targeting Using a Protac-Based Bcl-2/Bcl-Xl Degrader

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Venetoclax is a specific inhibitor of Bcl-2, the key protein which protects CLL cells from intrinsic apoptosis, whereas the Bruton's Tyrosine Kinase (BTK) inhibitor ibrutinib kills CLL cells via blockade of B-cell receptor (BCR) signalling. Unfortunately, some patients develop treatment resistance after receiving these agents in combination or sequentially. We aimed to evaluate whether CLL cells collected upon venetoclax and ibrutinib relapse retain survival dependency on alternative targets of the BCR pathway and Bcl-2 family, and to propose new vulnerabilities based on gene expression changes.

Using single cell RNA sequencing (sc-RNA-seq), we analyzed paired samples from a CLL patient collected at baseline and at relapse, which occurred after cessation of fixed-duration treatment with venetoclax and ibrutinib. The proportion of cells in each CLL cluster was vastly altered at relapse. Cluster 3 represented 4% of CLL cells at baseline versus 36% at relapse and had a high BCR score, including high expression of *MAP3K8 FOSB8* and *NFKB1* genes. Moreover, at relapse, it contained more CLL cells expressing transcripts from the Bcl-2 family of anti-apoptotic proteins, including Bcl-2 and Bcl-xL.

We also integrated sc-RNA-seq data from three primary CLL samples collected at relapse after venetoclax and ibrutinib treatment. The four clusters of CLL cells identified were enriched for BCR, interleukin-mediated, ErbB and the Fc epsilon receptor I signalling pathways. Importantly, most of the relapsed CLL cells which expressed high levels of Mcl-1 or Bcl-xL also expressed Bcl-2, suggesting that these resistant CLL cells became dependent on various anti-apoptotic proteins for survival. These observations led us to test Bcl-2/Bcl-xL targeting PROTACs PZ18753b and WH25244 in venetoclax-resistant CLL. These PROTACs were derived from navitoclax (Bcl-2/Bcl-xL dual inhibitor) linked to a VHL E3 ligase ligand to target Bcl-2 and Bcl-xL for degradation, with improved specificity to cancer cells while sparing platelets. We used three models of venetoclax-resistant CLL: (1) OSU-CLL cells expressing mutant Bcl-2 (G101V, F104L, R107-110dup or A113G), (2) treatment-naïve primary CLL cells co-cultured with NK.Tert stromal cells for 24h (CLL+stroma), and (3) primary CLL cells collected at venetoclax relapse. CLL cells were treated with venetoclax, navitoclax, PZ18753b, WH25244, or negative controls (NC) lacking VHL recruitment, PZ18753b-NC or WH25244-NC. Protein degradation was evaluated by Western Blot; viability, by Cell Titer Glo; and apoptosis, by flow cytometric detection of active BAX and BAK, mitochondrial cytochrome C, and AnnexinV binding.

WH25244 was superior to venetoclax at targeting OSU-CLL cells expressing either wildtype or mutant Bcl-2, upon 72h treatment. Cell death by WH25244 was preceded by degradation of Bcl-xL and partial degradation of Bcl-2, despite presence of Bcl-2 mutations. In treatment-naïve primary CLL cells, Bcl-xL degradation by PZ18753b and WH25244 proceeded in a dose-, time- and VHL-dependent manner. The potency of these PROTACs fell in between that of venetoclax and navitoclax, with mitochondrial apoptosis occurring at low nanomolar concentrations known to spare human platelets. Stepwise, activation of BAK and BAX, and cytochrome C release were evident at 14h of treatment, followed by phosphatidyl serine exposure at 18h of treatment.

A rightward shift in the dose-response curves of PZ18753b (16-fold) and WH25244 (8-fold) was noted for CLL+stroma cells relative to paired CLL cells treated in suspension. This reduction in potency was associated with (1) uptake of the PROTACs by

NK.Tert cells, since Bcl-xL degradation was noted in stromal cells yet without evident toxicity, and (2) up to 2.5-fold increased levels of Bcl-xL in CLL+stroma, which shifted the dose-dependent degradation of Bcl-xL. Finally, 2 out of 3 venetoclax-relapsed primary CLL samples showed more active BAK and cytochrome C release upon treatment with WH25244 than venetoclax, evaluated at 10 nM.
In conclusion, WH25244 is a PROTAC-based Bcl-2/Bcl-xL degrader with the potential to overcome venetoclax-resistant CLL dependent on Bcl-xL and mutant Bcl-2. Relative to its precursor, navitoclax, it shows increased potency against CLL cells and decreased toxicity against platelets *in vitro*, due to its VHL-dependent activity and minimal expression of VHL in platelets.

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