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ORAL ABSTRACTS

605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

Depletion of the Mitochondrial E3 Ligase MARCH5 Induces Synthetic Lethality to BCL2 Inhibitor (Venetoclax) Therapy in Cell Lines Representative of Diverse Blood Cancers

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Combination therapies incorporating the BCL2 inhibitor, venetoclax (VEN), are standards of care in chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML), and display promising activity against mantle cell lymphoma (MCL), multiple myeloma (MM) and many other hematologic malignancies. However, primary resistance is observed in some diseases and even across responsive malignancies, enduring cells typically persist, ultimately driving relapse and precluding cure. Genome wide CRISPR/Cas9 screens have the potential to identify novel mediators of venetoclax resistance. However, conventional knock-out/drop out (KO/DO) screens have several limitations including the frequent identification of common essential genes as false positives and the large scale required to detect DO with high statistical confidence.

We devised a novel augmentation on the conventional CRISPR/Cas9 KO/DO screen utilizing two MCL cell lines (Jeko1, Z138) which were both resistant to apoptosis induced by VEN +/- ibrutinib (IBR)(Fig 1). Rather than detecting sensitizing hits through DO, we positively enriched for sensitizing hits by flow-sorting the rare (~10%) population of cells initiating apoptosis as indicated by AnnexinV positivity after brief (6hr) BH3-mimetic exposure. At this early time point, cells whose KO had led to a lowered apoptotic threshold could be identified without sufficient time for DNA degradation. DNA yield from dying cells approached 100% efficiency. sgRNA frequencies among the control cells and those undergoing early apoptosis were analyzed using MAGecKE-MLE. This method was anticipated to reduce common essential gene contamination, increase statistical power to detect sensitizing hits due to enrichment rather than DO of relevant genes, and identify targets likely to exhibit synthetic lethality (i.e. neither VEN nor KO alone are lethal, but combination achieves potent killing).

The screens in both cell lines demonstrated significant depletion of common essential genes defined by DepMap by D14 (prior to treatment). Consistent with the increased power expected from a positive selection screen, both yielded >50 statistically significant VEN-sensitizing hits, after correction for multiple hypothesis testing. These included the expected KOs of *MCL1* and *BCL2L1* (BCL-X_L), confirming the validity of the method. sgRNAs resulting in KO of the E3 ligase *MARCH5* were among the most enriched within dying (VEN-sensitized) cells in both Jeko1 and Z138 screens, with either VEN only or VEN+IBR treatment (Fig 1). The screen method was repeated in Jeko1 cells using the MCL1 inhibitor AMG176 and BCL-X_L inhibitor A1331853, and again yielded *BCL2* as well as novel hits, including *MARCH5* KO as a sensitizing hit to BCL-X_L inhibition.

KO of *MARCH5* markedly sensitized multiple human blood cancer cell lines to venetoclax (Table 1), including sensitive and resistant lines, with and without functional *TP53*. In Jeko1 (MCL) cells, which are highly resistant to BCL2 or BCL-X_L inhibition when wildtype (10-20% apoptosis at 6μM), *MARCH5* KO induced marked sensitization to single-agent VEN (LC50 8nM) or A1331853 (LC50 375nM).By introducing a degron dTAG *MARCH5* construct into *MARCH5* KO Jeko1 cells, we demonstrated dose-dependent sensitisation to VEN with MARCH5 depletion, marked synergy (mean Bliss score: 22.97) and synthetic lethality. Degron dTAG-mediated depletion of MARCH5 demonstrated synergy with venetoclax in MOLM13 (AML) and Z138 (MCL) cell lines, and enhanced *in vitro* killing of MOLM13 by VEN + azacytidine, and killing of Jeko1 and Z138 by VEN + IBR.

CRISPR "death" represents a powerful modification to conventional CRISPR KO screens, with reduced common essential gene false positives, increased statistical power and enrichment for synthetic lethal hits. We demonstrate the pan-hematologic, dose-dependent VEN-sensitization achieved by MARCH5 depletion across multiple blood cancer lines with and without normal *TP53* function. MARCH5 depletion also sensitizes blood cancer cell lines to BCL-X_L inhibition. Addition of MARCH5 depletion to VEN-combination treatment improved MCL and AML killing *in vitro* and this target should be prioritized for accelerated drug development.

Disclosures Lew: *WEHI:* Patents & Royalties: Employee of the Walter and Eliza Hall Institute of Medical Research, which receives milestone and royalty payments related to venetoclax. Recipient of share in royalty payments. ; *AbbVie:* Honoraria. **Van Delft:** *WEHI:* Patents & Royalties: Employee of the Walter and Eliza Hall Institute of Medical Research, which receives milestone and royalty payments related to venetoclax. Recipient of share in royalty payments. . **Riffkin:** *WEHI:* Patents & Royalties: Employee of the Walter and Eliza Hall Institute of Medical Research, which receives milestone and royalty payments related to venetoclax. Recipient of share in royalty payments. . **Angela:** *WEHI:* Patents & Royalties: Employee of the Walter and Eliza Hall Institute of Medical Research, which receives milestone and royalty payments related to venetoclax. Recipient of share in royalty payments. . **White:** *WEHi:* Patents & Royalties: Employee of the Walter and Eliza Hall Institute of Medical Research, which receives milestone and royalty payments related to venetoclax. Recipient of share in royalty payments. . **Yuan:** *WEHI:* Patents & Royalties: Employee of the Walter and Eliza Hall Institute of Medical Research, which receives milestone and royalty payments related to venetoclax. Recipient of share in royalty payments. . **Anderson:** *WEHI:* Patents & Royalties: Employee of the Walter and Eliza Hall Institute of Medical Research, which receives milestone and royalty payments related to venetoclax. Recipient of share in royalty payments.; *Roche, Novartis, Takeda, Kite, Abbvie, Janssen, Beigene, AstraZeneca, Gilead, CSL:* Honoraria. **Huang:** *WEHI:* Patents & Royalties: Employee of the Walter and Eliza Hall Institute of Medical Research, which receives milestone and royalty payments related to venetoclax. Recipient of share in royalty payments. . **Dawson:** *Cambridge Epigenetix / Biomodal:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *GlaxoSmithKline:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Storm Therapeutics:* Consultancy, Membership on an entity's Board of Directors or advisory committees. **Roberts:** *WEHI:* Patents & Royalties: Employee of the Walter and Eliza Hall Institute of Medical Research, which receives milestone and royalty payments related to venetoclax. Recipient of share in royalty payments. ; *AbbVie:* Research Funding.

Figure 1. CRISPR "death" screening identifies *MARCH5* deletion as a sensitizer to VEN +/- IBR in resistant MCL cell lines.

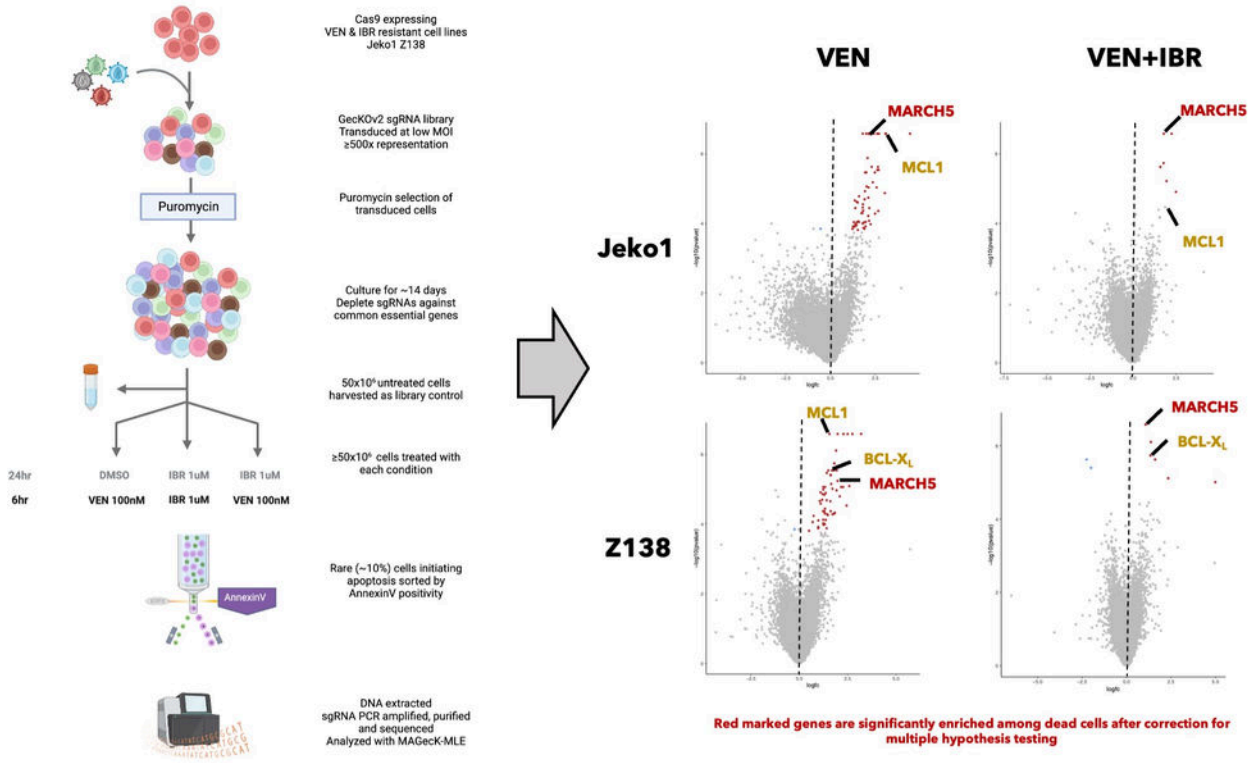

Table 1. *MARCH5* KO sensitizes multiple blood cancer cell lines to VEN, irrespective of *TP53* function.

Human cell line	Disease type	TP53 function	VEN LC50		Fold change
			MARCH5 WT	MARCH5 KO	
Jeko1	MCL	Null	>10μM	8nM	>1250x
Z138	MCL	Normal	1.9μM	11nM	173x
Mino	MCL	Null	300μM	10nM	30x
MOLM13	AML	Normal	12nM	0.9nM	13x
KMS12PE	MM	Null	8nM	1.4nM	6x

Figure 1

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