The Dual PI3/PIM-kinase Inhibitor, IBL-202, is Highly Synergistic with Venetoclax against CLL Cells, and *TP53* knock-out Cells, and Under Conditions that Mimic the Tumor Microenvironment.

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Introduction

Constitutive activity of the B-cell receptor (BCR) signaling pathway and the dysregulation of the pro-survival Bcl-2-family of proteins play crucial roles in the pathogenesis of chronic lymphocytic leukemia (CLL). Extended follow-up from trials of the fludarabine, cyclophosphamide and rituximab (FCR) regimen show a significant proportion of patients either do not respond or relapse with drug-resistant disease. Drug-resistance and complications have also been reported with novel agents such as ibrutinib and Venetoclax. Treatment options remain limited for patients, in particular those with *TP53* lesions. In a recent study (Crassini et al., 2018), we demonstrated efficacy of the PI3/PIM kinase inhibitor, IBL-202, against CLL cells via a mechanism that involves an increase in the Noxa/Mcl-1 ratio. Given that the sensitivity of CLL cells to venetoclax is influenced by the expression of Mcl-1, we proposed that combining IBL-202 with venetoclax may represent a rational combination which increases the sensitivity of CLL cells to the BH3-mimetic, irrespective of their interaction with stromal cells or *TP53* status.

Aims

1. **To investigate the efficacy of combining the novel dual inhibitor of PIM and PI3-kinase (IBL-202) and Venetoclax against primary CLL cells and a *TP53* knock-out CLL cell line.**
2. **To investigate the mechanism of the synergy between IBL-202 and venetoclax against CLL cells cultured under conditions that mimic the tumour microenvironment.**

Methods

Patient samples

Peripheral blood samples were collected from CLL patient following written consent. Peripheral blood mononuclear cell (PBMC) fractions were isolated by ficoll-density centrifugation and stored in liquid nitrogen.

TP53 knock-out OSU-CLL cell line

OSU-CLL cells were obtained from the human genetics sample bank at Ohio State University. The *TP53* knock-out OSU-CLL (OSU-CLL-*TP53*) line was generated using a lentiviral CRISPR/Cas9 system developed at the Walter and Eliza Hall Institute (WEHI), Melbourne, Australia. The methods employed for virus production, transduction of cell lines and the induction of sgRNA expression by dox hyclate were as described in the paper by Aubrey *et al.,* 2015 (2).

Assessment of cytotoxicity and synergy between IBL-202 and venetoclax

The drugs were combined at ratios based on their IC50 values as single agents. Apoptosis was determined using the mitochondrial membrane potential dye $DiIC_1(5)$/propidium iodide (PI) and flow cytometry. Combination indices (CI) were calculated using the Compusyn software (ComboSyn, Inc., NJ, USA). CI values of < 1 are indicative of synergism.

Cell Migration

The *in vitro* migratory capacity of CLL cells was determined by assessing their ability to migrate down an stroma-derived factor-1α (SDF-1α) gradient with or without prior drug treatment.

Immunoblotting

CLL cells were treated with IBL-202, venetoclax or the drugs in combination while in co-culture with CD40L-fibroblasts. All antibodies to the proteins indicated were obtained from Cell Signaling Technology (Danvers, MA, USA).

Results

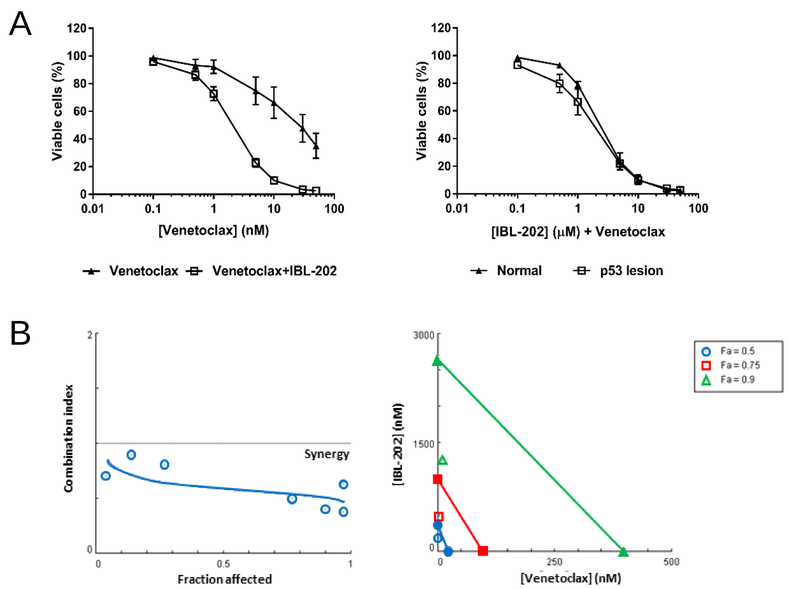

Fig 1. Synergy between IBL-202 and venetoclax against CLL cells.

A. Combining IBL-202 and venetoclax significantly reduced the IC50 for both drugs as shown (n = 10, 5 TP53 normal and 5 with TP53 lesion). No significant difference in the IC50 values for either drug as single agents or in combination was observed against CLL patient samples with TP53 aberrations.

B. The combination index plot (left) demonstrates strong synergy between IBL-202 and venetoclax against CLL cells. Analysis of the data by isobologram (right) illustrates the significant degree to which combining the drugs reduces the dose of each drug necessary to induce apoptosis in 50, 75 and 90% of cells.

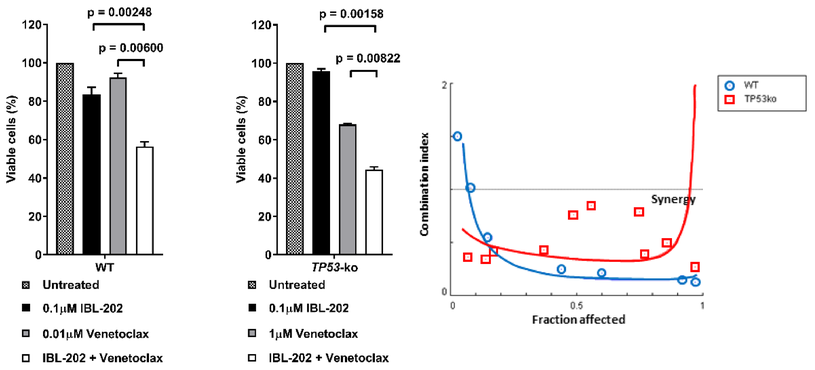

Fig 2. IBL-202 and venetoclax are synergistic against TP53-ko OSU-CLL cells

The combination of IBL-202 and venetoclax had a significantly greater cytotoxic effect than either drug as a single agent ($p < 0.05$) against both the OSU-CLL and OSU-CLL-*TP53* lines (n = 3).

Combination indices of < 1 were observed at fractional effects > 0.1 in both cell lines indicating strong synergy between the drugs.

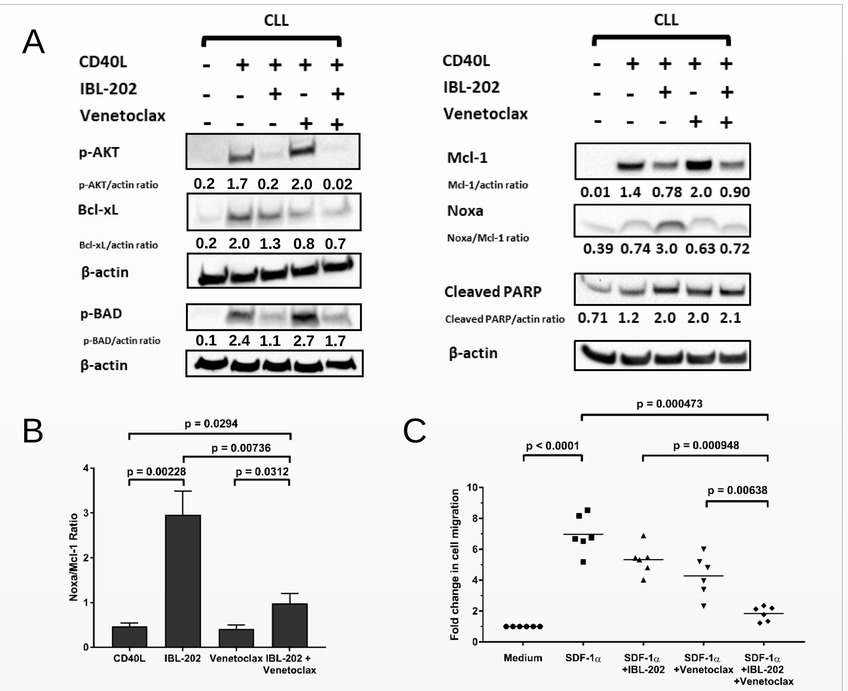

Fig 3. Effects of IBL-202 and venetoclax in combination on signaling and migration of CLL cells.

A. IBL-202, but not venetoclax, decreased phosphorylation of BAD and AKT and expression of Mcl-1.

B. IBL-202 as a single agent and in combination with venetoclax increased expression of Noxa and the Noxa/Mcl-1 ratio (n = 4), despite attenuation of Noxa expression by venetoclax.

C. IBL-202 and venetoclax in combination significantly reduced the migratory capacity of CLL cells towards SDF-1α compared to either drug as a single agent (n = 6).

Summary

1. IBL-202 and venetoclax were highly synergistic against primary CLL cells with *TP53* aberrations, the*TP53* knock-out OSU-CLL cell line and patient samples cultured under conditions that mimic the tumour microenvironment.
2. Synergy between IBL-202 and venetoclax is concomitant with a pro-apoptotic shift in the balance of expression of Bcl-2 family proteins.
3. IBL-202 and venetoclax in combination significantly reduced the migratory capacity of CLL cells.

Conclusions

1. **IBL-202 and venetoclax in combination may represent an effective therapeutic option for CLL, particularly for patients with poor risk disease.**
2. **The effects of IBL-202 in combination with venetoclax on cell survival and migration suggest the drugs may target the proliferative, drug-resistant compartment of CLL disease and reduce CLL infiltration into the lymph nodes and bone marrow.**

References

1. Crassini, K., et al., Br J Haematol, 2018. 182(5): p. 654-669.
2. Aubrey, B.J., et al., Cell Rep, 2015. 10(8): p. 1422-32.

The authors have no conflicts of interest to declare.