(PB3875) CELL CYCLE–FOCUSED CHEMICAL SCREEN REVEALS MITOTIC KINASE INHIBITORS THAT ENHANCE THE EFFICACY OF A CD30-TARGETED ANTIBODY–DRUG CONJUGATE

Topic: 20. Other aggressive lymphomas

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Background

Brentuximab vedotin (BV), an antibody–drug conjugate (ADC) composed of an anti-CD30 monoclonal antibody conjugated to the microtubule-disrupting drug monomethyl auristatin E (MMAE), is widely used as a key therapeutic agent for the treatment of peripheral T-cell lymphoma (PTCL). Nevertheless, most patients fail to achieve durable remission, highlighting the need for improved BV-based strategies. We previously performed a genome-wide loss-of-function CRISPR screen and found that knockout of *MAD2L1BP* and *ANAPC15* genes, which promote mitotic progression, increased BV sensitivity in CD30-positive PTCL cells (Suto, et al. Leukemia. 2025), though inhibitors targeting MAD2L1BP or ANAPC15 have not yet been developed. Given the link between mitotic regulation and BV efficacy, we hypothesized that pharmacological targeting of cell cycle regulators could provide a therapeutically actionable approach to enhance BV activity.

Aims

To identify therapeutically actionable cell cycle regulators that enhance BV efficacy in CD30-positive PTCL.

Methods

We established a focused compound library of 118 cell cycle-related agents. Two CD30-positive PTCL cell lines—the ALK-positive anaplastic large cell lymphoma (ALK+ ALCL) line DEL and the adult T-cell leukemia/lymphoma (ATLL) line ED40515(–)—were treated with BV and the drug panel for 72 hours. Cell viability was assessed, and combinatorial effects were analyzed using the Bliss independence model.

Results

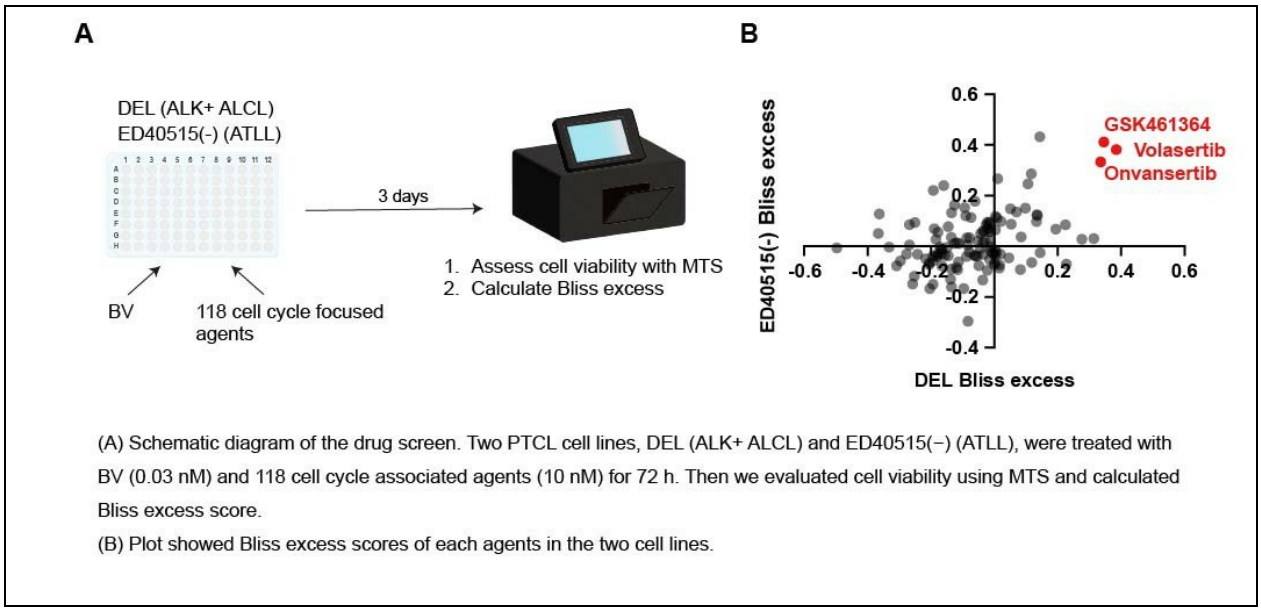
The screen identified three compounds—volasertib, GSK461364, and onvansertib—that exhibited strong synergy with BV in both cell lines. Notably, all three agents were polo-like kinase 1 (PLK1) inhibitors, supporting biological coherence. To validate these findings, we performed cell proliferation assays in four CD30-positive PTCL cell lines. BV combined with each PLK1 inhibitor demonstrated synergistic effects across all four cell lines. Among the three PLK1 inhibitors, volasertib was selected for further experiments given its advanced clinical development and established safety profile.

To elucidate the mechanism underlying the combinatorial effects, we performed cell cycle analyses and found that the proportion of cells in the G2/M phase significantly increased upon treatment with BV and volasertib. Western-blot analysis also revealed elevated expression of M phase markers, cyclinB1 and phospho-Histone H3 (Ser10), in cells treated with the combination, indicating enhanced mitotic arrest. In addition, the DNA damage marker phospho-histone H2A.X (Ser139) and the apoptosis marker cleaved caspase-3 were increased, suggesting that mitotic arrest led to DNA damage and apoptotic cell death. Inhibition of Mps1 abolished the combinatorial induction of mitotic arrest markers, establishing that SAC activation is essential for the synergistic cytotoxicity of BV and volasertib.

In vivo, the combination of BV and volasertib suppressed tumor growth more than either monotherapy in the DEL xenograft model. Similarly, in the ALK-negative ALCL PDX model, the combination resulted in significantly greater tumor reduction compared with individual treatments.

Summary/Conclusion

Our findings highlight the therapeutic potential of combining BV with PLK1 inhibitors—particularly volasertib—as a rational strategy to enhance cytotoxicity in CD30-positive PTCL. This study provides a mechanistic and preclinical foundation for future clinical evaluation of this combination therapy.



(A) Schematic diagram of the drug screen. Two PTCL cell lines, DEL (ALK+ ALCL) and ED40515(−) (ATLL), were treated with BV (0.03 nM) and 118 cell cycle associated agents (10 nM) for 72 h. Then we evaluated cell viability using MTS and calculated Bliss excess score.

(B) Plot showed Bliss excess scores of each agents in the two cell lines.