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Brusatol synergizes with venetoclax to induce cell death in aggressive lymphomas both in vitro and ex vivo.

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Aggressive lymphomas are the most common lymphoid malignancies in adults and their incidence is constantly increasing. Despite available chemo-immunotherapy, one-third of patients with DLBCL experience treatment failure. Therefore, the development of new therapies is urgently needed.

The aim of this study was to investigate the potential of Brusatol, which has been shown to have anti-tumour properties, in the treatment of aggressive lymphoma cells as a single agent or in combination with venetoclax.

Ten cell lines representing different types of lymphoma were treated with increasing concentrations of Brusatol for 24h to determine the IC50 values. Apoptosis induction and cell cycle distribution were assessed over 48h of brusatol treatment, together with collecting samples for Western blot. Nascent protein synthesis was evaluated by click-chemistry after brusatol treatment (4h). Co-treatment of brusatol with seven different inhibitors, including venetoclax, was performed and apoptosis induction was measured (24h). Finally, the potential of brusatol was investigated in lymphoma patient samples using an AI-driven functional precision medicine platform.

In ten cell lines representing different types of lymphomas, Brusatol induced cell growth inhibition in a concentration-dependent manner. Based on the results of apoptosis assays, they can be grouped into cell lines more and less sensitive to Brusatol. Cell cycle analysis showed an increased cell number in the subG1 phase in more sensitive cell lines. Western blot results of the more sensitive cell lines showed reduced levels of Bcl-2, Bcl-XL, Mcl-1, p53 and Myc. Interestingly, the protein expression profile of untreated cells indicated that cell lines with higher Myc levels were more sensitive to Brusatol. mRNA expression analysis showed that the reduction of affected proteins occurred mainly at the protein level. Thus, we examined the

effect of Brusatol on protein biosynthesis using click chemistry and observed inhibition of protein translation. Finally, the combination of Brusatol and Venetoclax synergistically increased lymphoma cell killing, either in an in vitro or ex vivo model.

Our data indicate that brusatol induces cell death in aggressive lymphomas in vitro and ex vivo. Additionally, the combination of brusatol with venetoclax results in enhanced induction of apoptosis. Thus, our study suggests that brusatol represents an interesting agent for the development of novel anti-lymphoma therapies.