PB2168 Brusatol Induces Apoptosis in Aggressive Lymphoma Cells In Vitro and Synergizes with Venetoclax

Topic: 20. Lymphoma Biology & Translational Research

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Background: Aggressive lymphomas include Burkitt lymphoma, follicular lymphoma (grade 3), mantel cell lymphoma and the most frequent type - diffuse large B-cell lymphoma (DLBCL). These types of lymphoma represent the most common lymphoid malignancies in adults and their incidence rate is still increasing. Despite chemoimmunotherapy (R-CHOP: Rituximab, Cyclophosphamide, Doxorubicin Hydrochloride, Vincristine Sulfate and Prednisone) more than one-third of patients experiences treatment failure, due to primary refractory disease or relapse, thus indicating the great need for the development of novel therapeutic strategies. One of the investigated drugs for these disease entities is Venetoclax (FDA approved Bcl-2 inhibitor), which shows an overall response rate of 44% in a phase 1 trial. In order to improve the effectiveness of the available treatment, we tested a flavonoid named Brusatol, that has anti-cancer activity in human cancer cells including leukemic cells as previously shown.

Aims: Here, we investigated the potential of Brusatol to treat aggressive lymphomas as a single agent or in combination with Venetoclax.

Methods: Seven cell lines (BL2, Jurkat, Karpas422, Raji, RI-1, SuDHL4 and U2932) representing different types of aggressive lymphomas (Burkitt lymphoma, germinal-center B-cell-like DLBCL, activated B-cell DLBCL) and T-cell leukemia, were treated with increasing concentrations of Brusatol (up to 10µM) and IC50 values were determined. After 24 and 48 hours of treatment with Brusatol (50nM and 250nM), induction of apoptosis (Annexin V staining, Caspase-3 cleavage, PARP cleavage) and changes in cell cycle were assessed. Furthermore, samples from untreated and Brusatol-treated cells were collected for Western blot analysis. Moreover, co-treatment of Brusatol and Venetoclax for 24 hours was performed in four cell lines (BL2, SuDHL4, RI-1 and U2932) and induction of apoptosis was analyzed by Annexin V staining.

Results: In all seven cell lines, Brusatol caused cell growth inhibition in a concentration-dependent manner. However, they could be grouped into more (BL2, Jurkat, Raji, SuDHL4) and less (Karpas422, RI-1, U2932) Brusatol-sensitive cell lines in the apoptosis assays. Moreover, cell cycle analysis revealed an increase of cells in sub-G1 phase – indicative of cell death induction – in the more sensitive cell lines. Interestingly, Western blot analysis of Brusatol-sensitive cell lines showed decreased protein levels of pro-survival apoptosis-regulating proteins Bcl-2, Bcl-XL, and Mcl-1. Furthermore, we detected reduced p53 and Myc protein expression in Brusatol-sensitive cell lines upon treatment. Notably, the protein expression profile of untreated cells indicates that cell lines with higher Myc-levels are more sensitive to Brusatol treatment. Interestingly, the combination of Brusatol with the Bcl-2 inhibitor Venetoclax synergistically enhanced the cell killing, both in more (SuDHL4, BL2) and less sensitive (RI-1, U2932) cell lines to Brusatol.

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Summary/Conclusion: Our data indicate that Brusatol is able to efficiently induce cell death in aggressive lymphoma cells by reducing the expression of pro-survival proteins. Interestingly, cells with higher Myc-levels were especially sensitive to this flavonoid. Additionally, the combination of Brusatol with Venetoclax results in enhanced induction of apoptosis. Thus, our study suggests that Brusatol, alone or in combination with Venetoclax, represents a very interesting agent to further develop regarding novel anti-lymphoma therapies.