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POSTER ABSTRACTS

618.ACUTE LYMPHOBLASTIC LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Identification of vulnerabilities for targeting BCL-2 family members in T-Cell Acute Lymphoblastic Leukemia

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T-Cell acute lymphoblastic leukemia (T-ALL) is a disease caused by the malignant transformation of T-cell lineage progenitors. With the use of intensive chemotherapies survival rates have improved, but outcomes particularly of relapsed patients remain poor. In addition, currently used intensive chemotherapies are associated with high rates of treatment-related morbidity and mortality, emphasizing the need to develop new and improved therapies.

The intrinsic apoptosis pathway, one of the key pathways controlling cell death, is dysregulated in many cancers and contributes to leukemogenesis and treatment failure. The main steps of this pathway are controlled by proteins of the B-cell lymphoma (BCL-2) family at the outer mitochondrial membrane. Hence, targeting this pathway by BH3-mimetics has emerged as an effective new treatment strategy in different cancers. Venetoclax is a selective BCL-2 inhibitor and is successfully used in CLL and AML, but heterogeneous sensitivity to venetoclax has been described in ALL and inhibitors of other BCL-2 family members including BCL-XL-selective A-1331852 and MCL-1 selective AZD5991 have been developed. Moreover, a dual inhibitor of the anti-apoptotic BCL-2 family members BCL-2 and BCL-XL (AZD4320) with its dendrimer conjugate (AZD0466) has recently shown anti-tumor activity in hematologic cancer models with manageable toxicity.

The aims of this study are to evaluate susceptibilities of T-ALL to inhibitors of BCL-2 family proteins, to identify markers of response, to elucidate mechanisms of action and to assess combination effects.

First, we analyzed the anti-leukemia activities of inhibitors targeting BCL-2 (venetoclax), BCL-XL (A-1331852) and MCL-1 (AZD5991) and of the dual BCL-2/BCL-XL inhibitor AZD4320 in T-ALL cell lines (N=6) and a series of patient-derived xenograft (PDX) samples (N=8). Inhibition of MCL-1 alone was not effective in the cell lines tested (EC50 >1 μM), but heterogeneous sensitivity was found in PDX samples (EC50 <1μM in 3/8 samples). In cell lines, sensitivity for BCL-2 inhibition was exclusively found in a cell line with an early T-cell precursor ALL phenotype (Loucy). Similar to cell lines, we found insensitivity for BCL-2 inhibition in all PDX samples tested (N=8). For inhibition of BCL-XL, we found heterogeneous sensitivities with 4/6 cell lines and 5/8 PDX samples showing an EC_{50} <1 μM. Interestingly, BH3-profiling confirmed BCL-2 dependence in Loucy and BCL-XL dependence in the other cell lines. Importantly, dual inhibition of BCL-2 and BCL-XL showed clear responses in cell lines and PDX samples (median EC_{50}s 796.1 nM and 721.6 nM). Notably, sensitivity was associated with priming of the BH3-peptide BAD (r_s=0.89, p=0.03), providing a marker of response.

We next sought to elucidate the molecular mechanisms by which different BH3-mimetics induce cell death in T-ALL. Analyzing protein interactions of BCL-2 family members by co-immunoprecipitation studies, we found that AZD4230 acts on-target by releasing BIM from BCL-2 and BCL-XL, but with compensatory increased protein complexes of BIM and MCL-1. Accordingly, combined inhibition of BCL-2 and BCL-XL with inhibition of MCL-1 leads to release of BIM from all three anti-apoptotic proteins enabling downstream apoptosis signaling. These findings suggest that combined inhibition of BCL-2 and BCL-XL together with MCL-1 may provide synergistic benefits. To further validate these data, we applied dynamic BH3-profiling and determined dependencies of T-ALL samples on BCL-2 family members in response to BCL-2/BCL-XL inhibition. Here, we found that the MCL-1 dependence strongly increases upon BCL-2/BCL-XL inhibition, suggesting MCL-1 as a target for combined inhibition.

Based on these results, we performed dose-response matrix analyses to test the effects of combined BCL-2/BCL-XL and MCL-1 inhibition. Most importantly, combined inhibition showed synergism in all cell lines and PDX samples tested (Bliss scores >10), including leukemias being insensitive to single compounds.

Taken together, we found vulnerabilities of T-ALL to BCL-2 family inhibition, particularly to dual BCL-2/BCL-XL inhibition by AZD4320, and we demonstrated on-target activities. Using BH3-profiling, we identified BAD-priming as a marker of response

for AZD4320 and MCL-1 dependence as a resistance mechanism that can be targeted by combination treatment, suggesting further clinical evaluation.

Disclosures No relevant conflicts of interest to declare.

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