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POSTER ABSTRACTS

605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

Discovery of a Novel, First-in-Class Bfl-1 BH3 Mimetic with Pro-Apoptotic Activity

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Background: Intrinsic apoptosis is regulated by the B Cell lymphoma 2 (BCL-2) protein family. Anti-apoptotic family members, such as BCL-2, MCL-1 and BFL-1, sequester their pro-apoptotic counterparts through a highly conserved BH3 binding groove. Disruption of this balance leads to mitochondrial pore formation and subsequent cell death. To evade apoptosis, tumors often upregulate the expression of one or more anti-apoptotic family members and as such they have become attractive targets for anti-cancer drug development. BFL-1 ('Bcl-2 related gene expressed in fetal liver', gene name *BCL2A1*) is a lesser-known anti-apoptotic family member, physiologically mainly expressed in the hematopoietic system.
BCL2A1 overexpression has been reported in many types of B cell lymphoma. Diffuse Large B Cell Lymphoma (DLBCL) patient sample analysis showed that *BCL2A1* expression levels are second only to *MCL1* within the anti-apoptotic family. Expression of BFL-1 was shown to be upregulated in *MYC+/BCL2+* double hit lymphoma cell lines treated with the BCL-2 inhibitor, Venetoclax, *in vivo*. Interestingly, BFL-1 positive lymphomas are less sensitive to inhibition of BCL-2 and MCL-1. Hence, targeting BFL-1 is a rational and attractive strategy in DLBCL to improve clinical outcome.
Methods: Using hit identification screens followed by iterative structure-activity relationship (SAR) analyses and structure-based design, compound A, a first-in-class, selective BFL-1 BH3 mimetic, was identified. Compound A was evaluated using biochemical, cellular *in vitro* and *in vivo* tumor efficacy models.
Results: Compound A is a highly potent and selective compound in biochemical assays. In BFL-1 sensitive lymphoma cell lines characterized by high BFL-1 protein expression, compound A was shown to displace BAK and BIM from BFL-1, leading to downstream cleavage of caspase 3 and cell death. Interestingly, these cellular models were resistant to Venetoclax. Compound A exhibited tumor growth inhibition in an engineered BFL-1 overexpressing, MCL-1 KO human multiple myeloma xenograft model. However, in the high BFL-1 expressing SU-DHL-2 DLBCL human xenograft model, compound A did not induce significant PD (cleaved caspase 3) and efficacy *in vivo*.
Conclusion: First-in-class selective BFL-1 BH3 mimetics are shown to specifically inhibit cell growth of lymphoma cell lines *in vitro* and can induce efficacy *in vivo* in a BFL-1 overexpressing model. Further studies may be useful to determine whether compound A elicits anti-tumor efficacy in lymphoma alone or in combination with other anti-apoptotic agents.

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