EDITORIAL

Close encounter of the covalent kind: Inhibiting MCL1’s proapoptotic activity with covalent inhibitors

Cell Death Discovery (2017) 3, 16094; doi:10.1038/cddiscovery.2016.94; published online 23 January 2017

Targeting the BCL-2 family proteins to combat cancer is now a fait accompli. With the recent FDA approval of venetoclax for the treatment of 17p-deleted Chronic Lymphoma Leukemia (CLL), patients suffering from a subtype of the disease associated with very poor prognosis can now access an entirely new class of drugs, which works by re-establishing apoptotic cell death in cancer cells.1 This achievement validates the bold idea that arose from the discovery of BCL-2’s role in cancer in the early 1990’s.2 Since then, much has been discovered about the BCL-2 family and its members,3 which are divided in two main subgroups: one is characterized by its prosurvival activity (that is, preventing cell death) and includes proteins such as BCL-2 itself, BCL-XL, or MCL1. A second subgroup promotes cell death and is itself divided into the executioners BAX/BAK (and BOK), which form pores on the mitochondrial outer membrane to allow apoptogenic factors such as cytochrome c to be released leading to caspase activation and cell demolition. The other group of proapoptotic proteins, the so-called ‘BH3 only proteins’ (for example, BIM, BAD, NOXA), operate upstream of the prosurvival BCL-2 family proteins, resulting in the release of proapoptotic activity of BAX and BAK. This cascade of inhibition between pro- and anti-apoptotic proteins is mediated by protein-protein interactions through helix-in-groove interactions.2 Overexpression of prosurvival proteins, which results in a block of the apoptotic response, is an important trait of most cancers. Reestablishing this response with small molecule drugs, coined BH3-mimetics,4,5 that is, induce apoptosis by directly interacting with one or several pro-survival proteins, which works by re-establishing apoptotic cell death in cancer cells.1 This specter of toxicity was partly relieved through some recent studies using heterozygote animals that suggested that full therapeutic potential could be achieve with the removal of only one mcl1 allele with little impact on the animal health,6-9 suggesting that a potential therapeutic window could be achieved.

Because of the challenges associated with targeting the BCL-2 family of proteins in general and MCL1 in particular, scientists have been forced to devise new strategies to design small molecule BH3-mimetics. In their paper, Akcay et al.,6 a group from Astra Zeneca, describe an interesting approach based on the formation of a covalent but reversible bond between their inhibitor and MCL1 (Figure 1). While the idea of covalent inhibitors (reversible or irreversible) is not novel (largely explored in the field of kinase), it has so far never been used to target the BCL2 family of proteins with small molecules (a close example has been recently described with a reactive stapled peptide10). Akcay et al. use a recently described formyl boronic acid moiety, which acts as the reactive group to specifically and preferentially create a covalent bond with lysine residues.

As a proof of concept for their approach, they took advantage of a known class of MCL1 inhibitors and available X-ray structures.11,12 With this information in hand, they elegantly designed new inhibitors placing the reactive moiety in close proximity of Lysine 234 located in the BH3 binding groove. Beyond the innovative approach, the strength of the paper resides also in the efforts made to characterize the activity of these reactive compounds. The authors first demonstrate that the inhibitor bearing the best reactive moiety have enhanced binding affinity (4.2 nM compared with 383 nM for the parent compound) associated with significant induction of apoptosis (measured through caspase activation) in a cell line relying on MCL1 for survival (MOLP-8). This activity was then confirmed using a panel of myelomas with various MCL1 dependencies.

BAX/BAK dependency is one of the hallmarks of intrinsic, mitochondrial apoptosis.3 Interestingly, Akcay et al. demonstrate that the activity of their reactive compound seems mainly mediated by BAX as SiRNA-mediated knockdown of BAK led to a significant decrease in activity. Through a series of binding experiments using Surface Plasmon Resonance, they provide information regarding the reaction kinetics with the lysine residue. Finally, using MS experiments together with the expression of a variant of MCL1 lacking the key lysine residue Lys324, they prove that the reactive compound forms an adduct with this amino acid located in the groove, as intended. Altogether, this paper demonstrates that reactive inhibitors of BCL-2 family proteins can be developed to improve activity of the parent compound: the single agent activity of the best analogue presented by Akcay et al. is far better than that of an unreactive analogue.13 This is a notable result because designing a successful reactive moiety is not trivial even when structural information is available. Will this type of reactive compound be suitable for clinical development? Only time will tell, especially since no in vivo data are presented in this study. Notably, a recent publication on potent MCL1 inhibitor shows that such a compound need not be reactive to achieve very high binding affinity and potent single agent activity.11 At the
molecular level, the paper also raises questions about the impact of these compounds on MLC1 stability. Indeed, MCL1 levels are tightly regulated via multiple mechanisms, in particular proteasomal degradation, making MCL1 a short-lived protein. Will a reactive inhibitor have the same stabilizing effect as that observed with S63845? How does the formation of a covalent bond with MCL1 play out in vivo considering MCL1’s limited half-life?

Despite these fascinating questions, the paper from Akcay et al. is a clear advance in the field of BH3-mimetics as it provides a new type of weapon in a slowly expanding armamentarium. We can expect more examples and hopefully drugs derived from this concept.

COMPETING INTERESTS
GL is an employee of the Walter and Eliza Hall Institute of Medical Research, which receives research funding and milestone payments in relation to venetoclax (ABT-199). GL also receives research funding from Servier.

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