Editorial

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Future Medicinal Chemistry

Targeting anti-apoptotic BCL-2 family proteins for cancer treatment

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"A number of strategies, such as BCL-X_L-targeting ADCs and BCL-X_L PROATCs, have been developed to circumvent the on-target thrombocytopenia associated with BCL-X_L inhibition. Similar strategies could also be employed to reduce the potential on-target cardiac toxicity associated with MCL-1 inhibition."

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Apoptosis, also known as programmed cell death, contributes to cellular homeostasis, normal development and clearance of abnormal cells [1]. Dysregulation of apoptotic pathways plays an important role in tumorigenesis and is considered to be one of the hallmarks of cancer [2]. Moreover, resistance to apoptosis is associated with desensitization to conventional cytotoxic and targeted therapies. Therefore, the induction of cell death by targeting apoptotic pathways is an attractive therapeutic strategy. BCL-2 family proteins are the key regulators of the mitochondria-mediated apoptotic pathway and can be divided into three subsets; pro-apoptotic BH3-only proteins (apoptosis initiators, such as BIM, BID and PUMA), pro-apoptotic proteins BAK and BAX (apoptosis effectors) and anti-apoptotic proteins (apoptosis gatekeepers, such as BCL-2, BCL-X_L, MCL-1 and BCL-w) [3]. Among those, several anti-apoptotic members including BCL-2, BCL-X_L, and MCL-1 are well-validated anticancer targets. Displacement of pro-apoptotic proteins from the binding groove of anti-apoptotic members results in homo-oligomerization of BAK and BAX, permeabilization of mitochondrial outer membrane, release of cytochrome *c* and activation of caspases to trigger apoptosis [1].

The development of first FDA approved BCL-2 antagonist

Targeting protein–protein interactions with small molecules has long been considered as a very challenging task for medicinal chemists. However, with fragment-based drug design approach coupled with novel drug discovery techniques such as nuclear magnetic resonance (NMR) spectroscopy and x-ray crystallography, many successful examples have been reported. The discovery of ABT-737 [4], the first potent dual inhibitor of BCL-2 and BCL-X_L, was based on the above-mentioned approaches. Due to its unfavorable pharmacokinetic properties, researchers at Abbvie developed the orally bioavailable successor, navitoclax (ABT-263), which entered clinical trials in 2006. However, navitoclax induces rapid and dose-dependent thrombocytopenia [3]. This clinical observation is in accordance with preclinical evidence that platelets depend on BCL-X_L for survival [5].

To overcome the on-target and dose-limiting toxicity associated with BCL-X_L inhibition, a highly selective BCL-2 inhibitor, venetoclax (ABT-199), was developed by Abbvie. Venetoclax shows robust anti-tumor effects against various hematological malignancies while sparing platelets. It was approved by the US FDA for the treatment of chronic lymphocytic leukemia and small lymphocytic lymphoma as a single agent and for acute myeloid leukemia in combination with azacitidine, decitabine, or low-dose cytarabine [6].

Ongoing strategies in targeting BCL-X_L

Venetoclax has remarkable clinical performance and great potential for further investigation, both as monotherapy and in combination with other drugs to treat hematological malignancies. However, it has limited applications for

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the treatment of solid tumors. In contrast, BCL-X_L is overexpressed in various solid tumors and a subset of leukemia and lymphoma cancerous cells. Bioinformatics analyses also reveal a strong correlation between the levels of BCL-X_L expression and resistance to chemotherapies [7]. Therefore, even though the clinical applications of ABT-263 as a single agent is hampered by its dose-limiting platelet toxicity, several clinical trials to circumvent platelet toxicity of ABT-263 through combination with chemotherapeutic agents are ongoing [8]. In addition, APG-1252, a prodrug derived from a BCL-2/X_L dual inhibitor, is designed to minimize the drug exposure to platelets and is currently in clinical trials to treat patients with small cell lung cancer and other solid tumors [9]. Recently, utilizing a novel drug delivery technology, AstraZeneca's DEP® BCL-2/X_L conjugate, AZD-0466, received FDA authorization for clinical trial [10].

It is worth noting that the exacerbation of neutropenia was observed when ABT-263 was combined with docetaxel clinically, which is likely attributed to the BCL-2 inhibitory effect of navitoclax [11]. By contrast, selective BCL-X_L inhibitor A-1331852 enhanced the efficacy of docetaxel while sparing neutropenia, indicating the potential and benefit to recruit selective BCL-X_L inhibitor for further investigation. By converting a selective BCL-X_L inhibitor into an antibody–drug conjugate (ADC), the selectivity to specific cancer cells over platelets can be improved. A representative ADC, ABBV-155, which targets B7H3-expressing cancer cells, is currently in Phase I clinical trial for the treatment of advanced solid tumors [12]. However, no small-molecule BCL-X_L selective antagonists have been advanced for clinical evaluation.

A novel strategy to reduce on-target toxicity is to utilize proteolysis targeting chimera (PROTAC) technology. The rationale is based on the differentially expressed E3 ligases in different cells/tissues. Thus, cell/tissue specificity could be achieved by converting an inhibitor to a PROTAC. In proof of concept studies, both Von Hippel–Lindau (VHL) cullin-2 and cereblon (CRBN) cullin-4A RING E3 ligases, which were found to be minimally expressed in human platelets, have been recruited to degrade BCL-X_L [13,14]. The preliminary data support the reduction of on-target platelet toxicity for both CRBN-based and VHL-based PROTAC degraders. Further, the lead candidate, DT2216, a VHL-based PROTAC derived from ABT-263, achieved better potency to a variety of BCL-X_L dependent cancer cells with a significantly improved therapeutic window than its parent compound ABT-263 [14].

MCL-1 inhibition for cancer therapy

Targeting MCL-1 for cancer treatment has also been explored for over a decade. Several hematological cancers, including multiple myeloma and acute myeloid leukemia and some solid tumors (e.g., hepatocellular carcinoma and non-small-cell lung cancer) rely on MCL-1 for survival [15], while showing modest response to BCL-2 inhibition. Earlier reported MCL-1 inhibitors in the literature are often with low selectivity over BCL-2 and/or BCL-X_L or work via alternative mechanisms rather than direct MCL-1 inhibition.

Inspired by the increasing biological evidence that a variety of cancer cells depend on MCL-1 for their viability, several highly selective and potent MCL-1 inhibitors have been developed recently [16]. In comparison with BCL-X_L and BCL-2 inhibitors, MCL-1 antagonists are smaller in size but more complicated in structure. To occupy the large and shallow binding groove as much as possible to achieve sufficient on-target cellular efficacy, numerous chiral centers, atropisomerism and macrocyclization have been introduced into the scaffold to maintain a favorable binding conformation. Tremendous efforts in lead optimization yielded several highly selective MCL-1 inhibitors with binding affinity at picomolar levels, which is required for on-target cellular activity [15].

It is important to realize that the preclinical evaluation of MCL-1 inhibitors is challenging since a variety of MCL-1 inhibitors have less binding affinities to mice MCL-1 than to human MCL-1. As a result, the *in vivo* studies of MCL-1 inhibitors using wide-type mice may not be suitable for the prediction of the drug efficacy and toxicity. The introduction of human MCL-1 knock-in mice mostly resolved this issue, and so far, five MCL-1 inhibitors (AMG-176, AMG-397, AZD5991, S64315/MIK665 and ABBV-467) have entered Phase I clinical trials [17].

Late in 2019, the FDA handed out a hold for AMG-397 in clinical trials due to 'safety signal for cardiac toxicity'. Meanwhile, Amgen has voluntarily halted enrollment for another early-stage test for AMG-176 [18]. Given that pro-survival role of MCL-1 may be essential for normal cardiac myocyte mitochondrial activity [17], the observations of clinical cardiac events are likely because of the on-target toxicity. If that is the case, conventional MCL-1 inhibitors may all have limited therapeutic window regardless of their chemotypes. However, the fate of the MCL-1 inhibitors will likely be determined by the outcomes of the currently ongoing clinical trials.

Future perspective

The FDA approval of BCL-2 selective inhibitor, venetoclax, is a milestone in targeting the intrinsic apoptotic pathway for cancer treatment. Further clinical trials of venetoclax hold the potential of additional applications in hematologic cancers. Small molecules that target BCL-X_L or MCL-1 have the potential of broader applications in cancer treatments compared with BCL-2 inhibitors. However, the clinical development of BCL-X_L or MCL-1 inhibitors has been hampered by their on-target, dose-limiting toxicities. A number of strategies, such as BCL-X_L-targeting ADCs and BCL-X_L PROATCs, have been developed to circumvent the on-target thrombocytopenia associated with BCL-X_L inhibition. Similar strategies could also be employed to reduce the potential on-target cardiac toxicity associated with MCL-1 inhibition. MCL-1 PROTACs have been recently developed [19,20]. It will be interesting to evaluate whether these degraders have the benefit of reduced on-target toxicity.

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