Apoptosis: Directly Targeted at Last

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Every cell in our bodies is encoded with pathways of programmed cell death so that useless or harmful cells can be eliminated for the benefit of the organism as a whole. Among these, the mitochondrial pathway of apoptosis is prominent for its role in oncogenesis and response to cancer therapy. The selective activation of apoptosis in cancer cells has been an important goal in oncology for decades. Recently, a class of drugs called BH3 mimetics has emerged. In the original report that accompanies this article, Harrison et al[1] present another novel application of BH3 mimetic therapy with navitoclax in the context of myelofibrosis.

The point of commitment to cell death in the mitochondrial apoptotic pathway is mitochondrial outer membrane permeabilization (MOMP). MOMP is directly regulated by the BCL-2 family of proteins, named after the first protein characterized in this family. The BCL-2 family contains both proapoptotic and antiapoptotic proteins. BAX and BAK, and perhaps less certainly BOK, are so-called effectors.[2,3] Following allosteric activation, they can homo-oligomerize and form large pores in the mitochondrial outer membrane, permitting egress of intermembrane space contents. Among these contents are cytochrome c and second mitochondria-derived activator of caspase which facilitate the activation of a family of cysteine proteases called caspases.[4,5] Caspases are required for many of the characteristics of apoptotic cell death, including widespread proteolysis, oligonucleosomal DNA fragmentation, and tagging of the cell with signals that facilitate phagocytosis (Fig 1).[6]

BAX and BAK can be activated by another subset of proapoptotic BCL-2 family proteins, the BH3-only activators. Activators, which include BIM and BID, can interact with BAX and BAK and induce an allosteric change that permits BAX and BAK homo-oligomerization and MOMP.[7,8] Antiapoptotic proteins, which include BCL-2, BCL-XL, MCL-1, BCL-w, and BFL-1, block apoptosis by binding the BH3 domains of monomeric activators or effectors, sequestering and neutralizing them.[9] Another class of proapoptotic proteins, the BH3-only sensitizers, exert their proapoptotic function by competing for the hydrophobic BH3 binding site in the antiapoptotic proteins, displacing effectors bound there, driving progression to MOMP.[10]

BCL-2 homology 3 (BH3) domains are amphipathic alpha-helices possessed by all members of the BCL-2 family. Although there are four such BH domains, BH1-4, the BH3-only proteins contain only the BH3. The BH3 domains of BH3-only proteins act as natural inhibitors of the function of the antiapoptotic proteins. Some are broadly inhibitory, interacting with all the antiapoptotic proteins, whereas others act more narrowly, in some cases inhibiting only a single antiapoptotic protein.[11-14] Thus, the natural oligopeptide BH3 domains served as prototypes of cancer therapeutics that could inhibit BCL-2 or BCL-XL or MCL-1, albeit ones with very poor pharmacologic properties.[10]

A team at AbbVie was responsible for ground-breaking strategies to produce the first pharmacologically useful small molecule mimetics of the sensitizer BH3 domains.[15] They used rapid NMR-based screening of ligands for two different aspects of the hydrophobic BH3 binding site in BCL-XL to create the first effective BH3 mimetic inhibitor, ABT-737. ABT-737 antagonized BCL-2, BCL-XL, and BCL-w, mimicking the selectivity of the BH3 domain of the BAD protein. Building on this program, AbbVie later developed ABT-263 (navitoclax),[16] with specificity similar to ABT-737, and later ABT-199 (venetoclax),[17] a selective BCL-2 antagonist. Venetoclax, the first effective drug directly targeting an apoptotic pathway, has received regulatory approval so far in acute myelomonocytic leukemia (AML) and chronic lymphatic leukemia (CLL) by regulatory bodies worldwide.[18,19] Moreover, the success of venetoclax has spawned an entire new class of BH3 mimetic drugs made by several companies targeting BCL-2, BCL-XL, and MCL-1 with varying degrees of specificity. These are currently being tested in more than 100 clinical trials internationally.

Given the expanding number of BH3 mimetics, one might ask, how might one determine which drug is best for an individual patient or type of cancer? First, we should understand that cellular sensitivity to a BH3 mimetic requires that cell to be dependent on the antiapoptotic protein targeted by that BH3. Thus, cells that are sensitive to the BCL-2 selective venetoclax are those that are dependent on BCL-2. Dependence on an individual antiapoptotic protein requires that protein to be primed with a proapoptotic protein: mere expression of the antiapoptotic protein is not sufficient.[20] For example, in CLL, a disease broadly sensitive to venetoclax, abundant BCL-2 is expressed, but critically a large proportion of that BCL-2 is sequestering the activator protein BIM. When venetoclax

ASSOCIATED CONTENT

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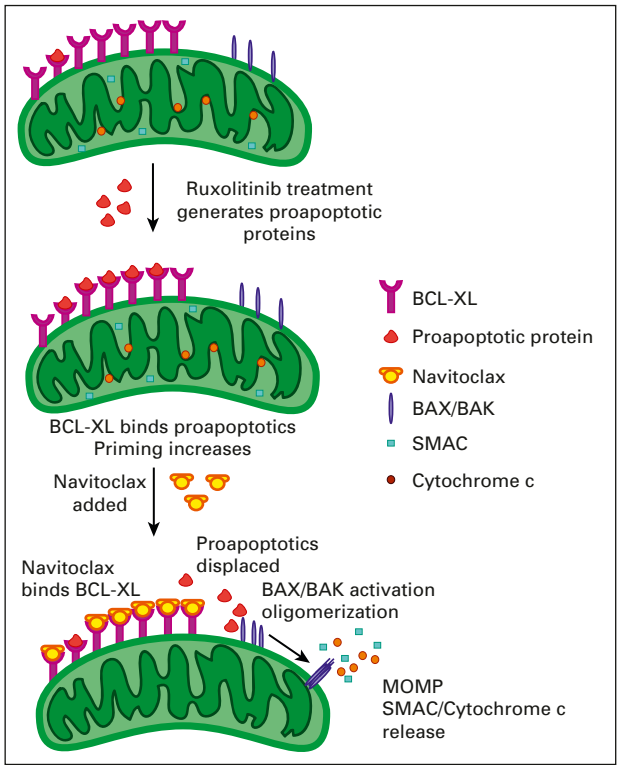

FIG 1. Mitochondrial apoptotic priming drives sensitivity to BH3 mimetics. At top, ruxolitinib treatment generates proapoptotic proteins that are sequestered by antiapoptotic BCL-XL at the mitochondrial outer membrane (middle), priming BCL-XL and rendering the cell more dependent on BCL-XL on survival. Treatment with a BH3 mimetic such as navitoclax, which competes for the BH3 binding site in BCL-XL, displaces proapoptotic proteins from BCL-XL. Some of these displaced proteins, such as BIM, can activate BAX or BAK, initiating their homo-oligomerization and MOMP. MOMP allows egress of cytochrome c and SMAC, which facilitate widespread activation of caspases, which in turn cleave many proteins. These cleavage events lead to DNA endonuclease activation and tagging of the cell with eat-me signals to facilitate phagocytosis, completing the process of apoptosis. Figure credit: Jeremy Ryan. MOMP, mitochondrial outer membrane permeabilization; SMAC, second mitochondria-derived activator of caspase.

competes for the BH3-binding domain of BCL-2, some BIM is displaced, freeing it to activate BAX and induce MOMP and commitment to cell death.[21] This can happen very rapidly in CLL, where a patient with an elevated WBC can see it normalize within hours of starting venetoclax.

However, quantitative measurement of the protein complexes necessary to know the degree of priming of an individual antiapoptotic protein in a patient tumor is technically very challenging. One alternative is to use BH3 profiling, an assay in which mitochondria of patient cancer cells are exposed to a standardized set of BH3 peptides to functionally gauge dependence on individual antiapoptotic proteins.[21,22] Another alternative is to simply expose patient tumor cells ex vivo directly to the BH3 mimetics in question and measure a property indicative of cell death.[15,23] Both approaches were critical to correctly identify the BCL-2 dependence in CLL and AML, which led to successful clinical trials and regulatory approvals in both those diseases for venetoclax.

In the myelofibrosis trial reported in the *Journal of Clinical Oncology*,[1] the choice of navitoclax was made primarily because it can target BCL-XL. Dependence on BCL-XL was identified in myelofibrosis by several lines of evidence, including BH3 profiling.[24,25] The combination with ruxolitinib was chosen because it is a drug with demonstrated activity in myelofibrosis. This is in concordance with a principle that has already been supported clinically, with the activity of venetoclax with anti-CD20 and BTK inhibitors in CLL,[26] or venetoclax with hypomethylating agents in AML[27]: the right BH3 mimetic makes drugs that already work in a particular disease work better.

The future of BH3 mimetics in cancer lies in identifying active combinations. To identify the right BH3 mimetic for a distinct histology or even an individual patient will likely require continued dependence on functional strategies such as direct exposure of tumor to drugs or BH3 profiling. Static precision medicine tools such as next-generation sequencing have not been of much utility in this regard. There are many creative ideas working their way from the laboratory to the clinic in identifying novel, mechanistic synergy–based combinations with BH3 mimetics. In the meantime, however, identifying the right combination partners for BH3 mimetics might be as simple as selecting what is already given in a particular disease.

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