For example, the BH3-only sensitizer protein HRK binds resident proteins that activate cytoplasmic caspase proteases. Of displacing their pore-forming ligands, thus effectuating on the antiapoptotic proteins. Each antiapoptotic protein the proapoptotic proteins interact with hydrophobic grooves targets, BAX and BAK (Fig. 1). The BH3 helical domains of formation in the outer mitochondrial membrane—BH3-only MOMP by binding proteins essential for apoptotic pore proteins (BCL-XL, MCL-1, and BCL-W) provide a barrier against mimetics have a role in this disease.

Although killing cancer cells is a high-priority goal in cancer therapy, predicting responses to therapy is an imprecise art. Even in the era of targeted therapies, the connections between inhibiting a target and cellular demise are still poorly understood. Detailed knowledge of the regulation of mitochondrial outer membrane permeabilization (MOMP) during apoptotic cell death has led to much-improved forecasting of the efficacy of a class of targeted agents, BH3 mimetics, in several types of cancer. As demonstrated by Pan and colleagues in this issue (1), BH3 profiling assays couple a mechanism-based readout of mitochondrial disintegration, tightly linked to apoptotic death, with validation of on-target effects. Application to acute myelogenous leukemia (AML) provides strong evidence that BCL-2–targeted BH3 peptidomimetics have a role in this disease.

BCL-2 and the related mitochondrial antiapoptotic proteins (BCL-XL, MCL-1, and BCL-W) provide a barrier against MOMP by binding proteins essential for apoptotic pore formation in the outer mitochondrial membrane—BH3-only activators (BIM, BID, and PUMA) and their multidomain targets, BAX and BAK (Fig. 1). The BH3 helical domains of the proapoptotic proteins interact with hydrophobic grooves on the antiapoptotic proteins. Each antiapoptotic protein has a profile of different BH3-only sensitizer proteins capable of displacing their pore-forming ligands, thus effectuating MOMP and releasing cytochrome c and other mitochondria-resident proteins that activate cytoplasmic caspase protases. For example, the BH3-only sensitizer protein HRK binds with high affinity only to BCL-XL whereas NOXA selectively binds to MCL-1. Healthy cells may have low to absent levels of proapoptotic proteins bound to the BCL-2 survival protein family, but various exogenous or endogenous stresses lead to mitochondrial loading of proapoptotic proteins. This is referred to as a “primed” state, as the reserve of empty BH3-binding sites is depleted, and additional insults frequently lead to apoptosis. Primed cells are also susceptible to drugs designed to displace the bound proapoptotic factors from their sequestration at BCL-2–related antiapoptotic proteins.

BH3 mimetic drugs, such as navitoclax, resemble BH3 domains from sensitizer proteins (BAD in the case of navitoclax) and preserve their binding specificities to the antiapoptotic proteins. The first BH3 mimetic compounds, ABT-737 and ABT-263 (navitoclax), have subnanomolar binding affinities for BCL-2, BCL-XL, and BCL-W. Initial reports demonstrated preclinical single-agent activity in cancer cell lines derived from B-cell lymphomas and small cell lung carcinomas (two tumor types notable for BCL-2 expression), but not other types of solid tumors (2). In clinical trials, this lack of target selectivity proved to be a disadvantage, as on-target BCL-XL inhibition resulted in severe thrombocytopenia. Structure-based redesign of navitoclax led to the second-generation agent ABT-199, which, despite a higher binding affinity for BCL-2 ($K_i < 0.01$ nmol/L), has >800-fold lower affinity for BCL-XL and minimal effects on platelet counts (3). In several studies, resistance to these BH3 mimetics, observed in many solid tumors, was mediated by expression of MCL-1, a related antiapoptotic protein that is poorly bound by ABT-737, ABT-263, and ABT-199 (4, 5).

By adding BH3 peptides from specific sensitizer proteins to permeabilized tumor cells or purified mitochondria, the identity of any antiapoptotic proteins bound to the pore-forming protein machinery can be inferred from the profile of BH3 peptides that elicit MOMP (measured as cytochrome c release or mitochondrial membrane depolarization). In its current format, digitonin-permeabilized cells in microtiter plates are loaded with a fluorescent probe of mitochondrial membrane potential, $\Delta \Psi_{m}$, and fluorescence followed over time after addition of BH3 peptide. This provides a remarkably simple functional readout to discern whether cancer cells are primed for apoptosis (i.e., BCL-2 and related antiapoptotic proteins are engaged in binding pore-forming components), and which proteins are involved. As MOMP and cytochrome c release are often viewed as points of no return in apoptosis, there is a high correlation between these responses and the efficacy of targeting specific antiapoptotic proteins. With this information in hand, the response to a BH3 mimetic drug is predictable.

Expression of BCL-2, BCL-XL, and MCL-1 in AML is highly variable, and not consistently linked to chemotherapy response (6). In contrast, mitochondrial priming, as measured using the BH3 profiling assay, correlates with clinical response in AML (7). This result is not entirely surprising, as mitochondrial priming integrates expression, subcellular localization, and protein–protein interactions of pro- and antiapoptotic proteins, details that are critical for apoptosis but require
The authors were able to show the expected correlation to ABT-199 efficacy. The difference between cell lines and primary AML blasts may indicate that BCL-XL priming in primary AML blasts is more uniformly distributed between ABT-199-sensitive and -resistant cells than was the case for the smaller series of AML cell lines.

Although an outwardly attractive idea, in vitro chemosensitivity testing has yet to be established in clinical practice (9). Among several potential hindrances to in vitro chemosensitivity and resistance assays (absence in most cases of stroma, vascularization, and systemic drug metabolism), the lack of insight into how DNA damage, oxidative stress, or targeted therapies result in cell death is high on the list. At present, either these molecular events seem to be cell-specific or do not provide usable information on the tipping points that convert damage responses or broken signal transduction relays into apoptosis. BH3 profiling seems to succeed by consolidating a large number of parameters into a measurable quantity that remains directly linked to the mechanism of BH3 mimetics.

In the future, similar strategies may need to be developed in order for more personalized therapies against individual tumors to become a reality.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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Forecast: Rough Seas for Leukemia

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