genotoxic factors. In addition, the strong correlation of ERFSs with CNVs in B cell lymphomas strengthens the argument that common mechanisms likely underlie what might initially appear as distinct phenomena and suggest that replication arrest at ERFSs can trigger CNV formation. This is indeed likely, because similar CNVs that mimic constitutional and cancer-related CNVs are induced in human and mouse cells following replication stress, including at some CFSs (Arlt et al., 2011). It would be interesting to similarly examine de novo CNVs in B-lymphocytes treated to express ERFSs. The identity of the replication and repair factors that create the rearrangements and the endogenous conditions or environmental agents that lead to replication stress are unclear. These are important questions that are being ad-dressed with regard to human germline CNVs and translocations, and they are equally important to understanding rear-rangements in the cancer genome and the risk factors involved.

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ABT-199: Taking Dead Aim at BCL-2

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ABT-199 is a new selective small molecule inhibitor of BCL-2 that appears to spare platelets while achieving potent antitumor activity. Assays that can predict the efficacy of ABT-199 in individual tumors will be critical in determining how best to incorporate this promising agent into the armamentarium of cancer therapies.

The B cell lymphoma/leukemia 2 (BCL-2) family regulates critical life or death decisions of cells via the mitochondrial pathway of apoptosis (Davids and Letai, 2012). BCL-2 inhibits death by binding the BH3 domains of pro-death BCL-2 family proteins, thus preventing mito-chondrial outer membrane permeabiliza-tion, which can be considered the point of commitment to apoptosis. BCL-2 has several anti-apoptotic cousins, including BCL-XL, BCL-w, and MCL-1, each of which possesses a distinct, hydrophobic BH3-binding pocket. Lymphoid malig-nancies are frequently addicted to BCL-2 for their survival. Because most of these cancers, including chronic lymphocytic leukemia (CLL), remain incurable with conventional therapies, agents that specifically target BCL-2 are under urgent investigation.

Early efforts to target the BCL-2 family were met with disappointment in the clinic. Agents such as the antisense oligonucleotide oblimersen sodium and the small molecule obatoclax showed promise as BCL-2 antagonists in pre-clinical testing but had little clinical activity. A potential mechanistic short-coming of these agents is that they were never conclusively shown to specifically engage their purported BCL-2 family targets in patients.

Abbott Laboratories (now AbbVie) has developed a series of BH3-mimetic small molecules that bind to the BH3 bind-ing sites of anti-apoptotic proteins like BCL-2. ABT-737, which binds BCL-2, BCL-XL, and BCL-w, was the first molecule studied extensively preclinically (Oltersdorf et al., 2005). Many subsequent experiments support its killing in an on-target fashion in cell lines, primary human cancer cells, and animal models. ABT-263 (navitoclax) was the first of this series to enter the clinic. Like ABT-737, it binds BCL-2, BCL-XL, and BCL-w, but it has the perceived advantage of being orally bioavailable. Clinical activity was observed, particularly in lymphoid cancers (Roberts et al., 2012); however, because navitoclax binds not only to BCL-2 but also to BCL-XL, the drug causes predictable,

dose-dependent thrombocy-topenia (Figure 1A). This is an on-target effect due to the reliance of platelets on BCL-XL for survival, and it provides pharmacodynamic evidence of the mechanism of action of the drug. Indeed, drugs claim-ing to inhibit BCL-XL that do not cause significant throm-bocytopenia in vivo should be viewed with caution. The modest clinical activity of navitoclax is likely related to the inability to optimize its dose due to this dose-limiting thrombocytopenia.

Souers et al. (2013) recently reported the re-engineering of navitoclax to create ABT-199, a highly potent and selective inhibitor of BCL-2. Through an elegant structure-based reverse engineering process, ABT-199 maintains a sub-nanomolar affinity for BCL-2 but binds over three orders of magnitude less avidly to BCL-XL, suggesting the drug may not cause clinically significant thrombocytopenia (Figure 1B). In an in vitro cell culture model, the authors provide convincing evidence that ABT-199 selectively kills BCL-2 dependent, but not BCL-XL dependent, cells and kills through the mitochondrial pathway of apoptosis. ABT-199 also potently kills a diverse array of non-Hodgkin's lym-phoma (NHL) and acute myelogenous leukemia cell lines, suggesting that the drug has the potential to be efficacious in a wide variety of hematologic malignan-cies. In vivo, ABT-199 suppresses tumor growth in several human hematologic tumor xenograft models and shows at least additive efficacy in combination with chemotherapy. Importantly, the authors also show compelling data from both in vitro and in vivo experiments that, as predicted, ABT-199 causes markedly less thrombocytopenia than navitoclax.

The true measure of a drug's efficacy must come from clinical trials, and the authors provide some very preliminary but promising data in three CLL patients. The rapid reduction of absolute lympho-cyte count in just 8 hr along with a concomitant reduction in palpable lym-phadenopathy in all three patients sug-
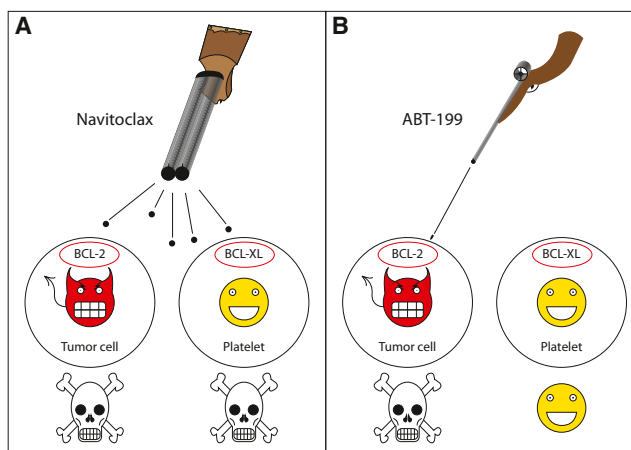
Figure 1. ABT-199 Selectively Kills BCL-2-Dependent Tumor Cells While Sparing Platelets
(A) Navitoclax (ABT-263) binds with high affinity to both BCL-2 and BCL-XL. Because many tumors, particularly lymphoid malignancies, are addicted to BCL-2 for survival, this potently induces tumor cell apoptosis. Platelets depend primarily on BCL-XL for survival and, therefore, are also destroyed by navitoclax.
(B) ABT-199 is specific for BCL-2 and induces selective death of BCL-2 dependent tumor cells while sparing platelets. Figure created by Richard Oakley.

gests that the drug is more potent in CLL patients than navitoclax. The lack of thrombocytopenia in this limited data set is also a promising sign that the preclinical studies predicting less thrombocytopenia will be validated in the clinic. A similar lack of thrombocyto-penia accompanied by significant anti-tumor activity was reported in an early interim analysis of a phase I study of ABT-199 in NHL patients at the 2012 American Society of Hematology Annual Meeting. Also notable in the Souers et al. (2013) article is that markedly elevated LDH and phosphate levels were seen during the same early time frame when the anti-tumor response was observed, reflecting significant tumor lysis, a clinical syndrome with potentially serious consequences if not managed aggressively. Whether modi-fications to the study design for future patients are adequate to reduce the risk of tumor lysis will be critical to the clinical development of ABT-199.

The use of rational biomarkers has become essential to optimizing the devel-opment of targeted cancer therapies, and ABT-199 is no exception. A challenge in developing a predictive biomarker for ABT-199 is understanding the molecular biology underlying BCL-2 addiction. The levels of over a dozen BCL-2 family pro-

teins, both pro- and anti-death, fluctuate rapidly, and protein-protein interactions or posttranslational modifi-cations can have a major in-fluence on the behavior of the system. Functional biomarkers are one way to probe the physiology of this dynamic system in real time. One such assay is BH3 profiling (Certo et al., 2006), which measures the mito-chondrial response to dif-ferent BH3-only peptides, each with its own selectivity of interaction with anti-apoptotic members. Results can be used to determine the relative dependence of a cell on a particular anti-apoptotic protein such as BCL-2, BCL-XL, or MCL-1. In many examples of primary cancer cells and cancer cell lines, BH3 profiling has cor-rectly predicted sensitivity to ABT-737 (Del Gaizo Moore et al., 2007; Deng et al., 2007; Vo et al., 2012).

Central to the fate of a cancer cell exposed to chemotherapy is its proximity to the threshold of apoptosis, a cellular state also detected through BH3 profiling that is known as "mitochondrial priming". Mitochondrial priming helps explain why CLL cells are generally quite sensitive to chemotherapy despite having high levels of BCL-2 protein. Most BCL-2 in primed cells is bound by pro-death BCL-2 family members such as BIM, thereby perching these cells precariously near the precipice of apoptosis. Mitochondrial priming also provides insight into the reason why chemotherapy may be effective against primed malignant cells while sparing rela-tively less primed hematopoietic stem cells. Moreover, pretreatment priming levels in patient samples correlate with clinical response to cytotoxic chemo-therapy (Davids et al., 2012; Ni Chong-haile et al., 2011; Vo et al., 2012). Agents like ABT-199 afford the exciting possi-bility of selectively increasing the mito-chondrial priming of certain cancer cells, identified by the proper predictive biomarker, which might then be more effectively treated with conventional chemotherapy.

Clinical response to navitoclax was strongly associated with high BIM-MCL-1 or BIM-BCL-2 ratios, providing evidence of the selectivity of the drug for targeting the BCL-2 family in patients (Roberts et al., 2012). Additional correlative studies built into the ongoing clinical trials of ABT-199 include analysis of *BCL2* gains, *BCL2* amplification, as well as BH3 profiling. These assays have the potential to help predict the toxicities and efficacy of ABT-199, perhaps even one day to guide personalized therapy of cancer patients. Whether ABT-199 will be most effective as a single agent or in combination with other agents will be important to determine. Moreover, the biological insights gained through correlative studies will help inform the rational design of combination trials to determine the optimal role for ABT-199 in the treatment of cancer.

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