# Restoring cancer's death sentence

**In this issue of *Cancer Cell*, two groups present data on the function of an antagonist of BCL-2, ABT-737. Both groups find that expression of MCL-1, an antiapoptotic protein related to BCL-2, is a key determinant of resistance to ABT-737. Lowering MCL-1 levels is an effective adjunct to BCL-2 antagonism, and both groups suggest ways that this might be accomplished practically in a clinical setting. The mechanism by which ABT-737 selectively kills cancer cells is discussed below in the context of these and prior reports of ABT-737's function. Antagonism of BCL-2 is an exciting anticancer strategy that may soon become a clinical reality.**

Cancer cells get away with murder. In order to grow, spread, and, all too often, kill their hosts, cancer cells exhibit behaviors that ought to secure their own demise. For instance, genomic instability, oncogene activation, and anoikis, commonly exhibited by cancers, all provoke death signals that would kill ordinary cells, particularly by the mitochondrial, or intrinsic, pathway of programmed cell death (PCD). Yet cancer cells find a way to escape this death sentence. For this reason, it is widely believed that a block in PCD is a requirement for oncogenesis.

The BCL-2 protein blocks PCD. The *bcl-2* gene was discovered as a participant in the t(14;18) that drives BCL-2 overexpression in follicular lymphoma cells, but BCL-2 expression is found in many tumor types. ABT-737 is a molecule that tightly binds and antagonizes the antiapoptotic function of BCL-2 and related proteins BCL-$X_L$ and BCL-w (Oltersdorf et al., 2005).

In order to understand how ABT-737 kills cells, we must understand two competing models of how BCL-2 (and related antiapoptotic proteins) oppose PCD. One model holds that prodeath "activator" BH3-only proteins, including BID and BIM, activate BAX or BAK to provoke mitochondrial outer membrane permeabilization (MOMP) (Certo et al., 2006; Kuwana et al., 2005; Letai et al., 2002). Binding and sequestering these activators before they can contact BAX or BAK is the key role for BCL-2 (and related antiapoptotic proteins) in this model (Figures 1Aa and 1Ac). In addition, BCL-2 may also bind BAX or BAK once they are activated. Cells with a significant amount of prodeath proteins held at bay by BCL-2 proteins we refer to as "primed." Other "sensitizer" BH3-only family proteins cannot activate BAX or BAK but rather exert their prodeath function by competing for the hydrophobic cleft on BCL-2 that binds the activators, acting essentially as BCL-2 antagonists. An important criticism of this model, that an activating interaction between activator BH3-only molecules and BAX or BAK is difficult to observe directly, has been largely lifted by recent direct biochemical demonstrations of such an interaction (Oh et al., 2006; Walensky et al., 2006). In the competing model, BAX and BAK do not require activation by BH3-only

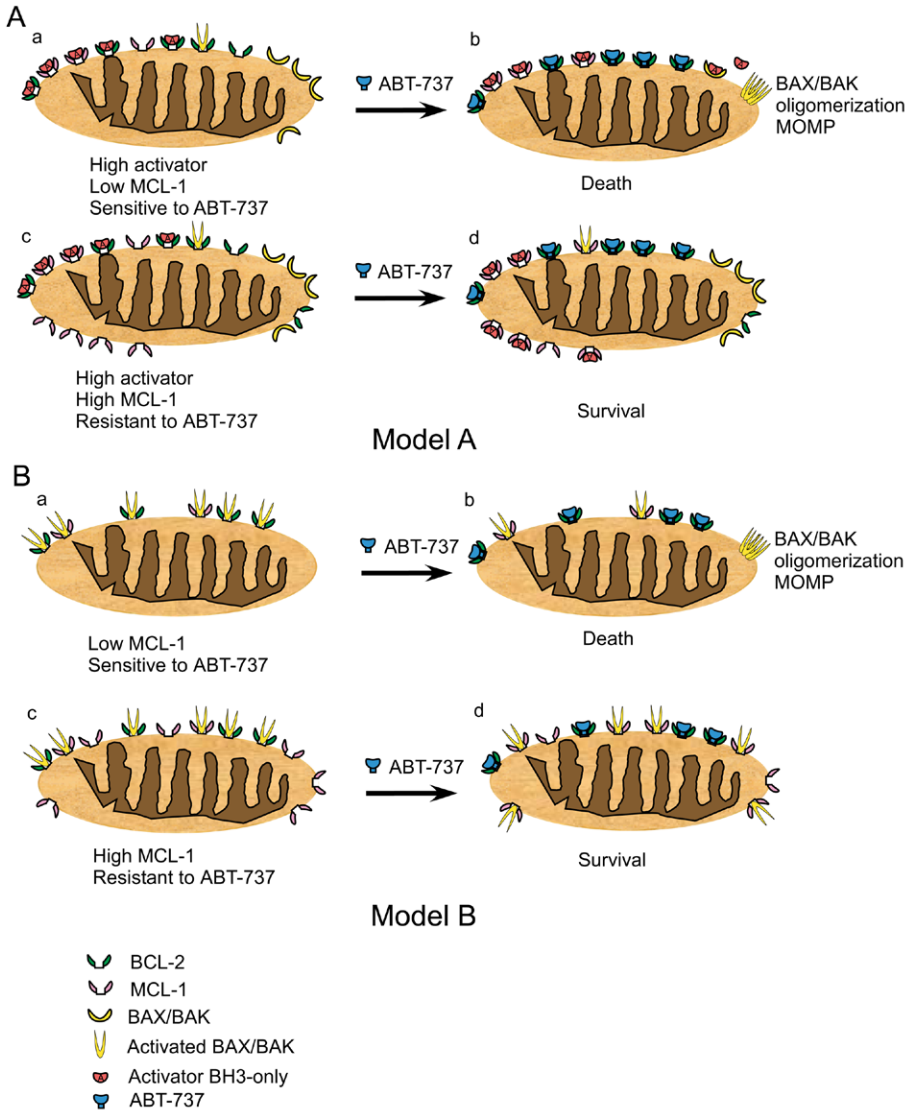


**Figure 1.** Two models of BCL-2 inhibition of PCD

**A:** In Model A, activator BH3-only proteins are sequestered by BCL-2 at the mitochondrion, preserving survival (**Aa**) until treatment with ABT-737 displaces the activators, which then activate BAX or BAK, which oligomerize and induce MOMP (**Ab**). When abundant MCL-1 is present, activator displaced from BCL-2 by ABT-737 is bound by MCL-1, maintaining survival (**Ac** and **Ad**).
**B:** In Model B, BCL-2 and MCL-1 keep activated BAX or BAK at bay (**Ba**). ABT-737 frees BAX or BAK from BCL-2, allowing oligomerization and MOMP (**Bb**). When abundant MCL-1 is present, BAX or BAK displaced from BCL-2 can be bound by MCL-1 to prevent PCD (**Bc** and **Bd**).

molecules like BIM or BID, but are rather always activated, and must thus be always bound and sequestered by BCL-2 (or related antiapoptotic proteins) to maintain survival (Chen et al., 2005; Willis et al., 2005) (Figures 1Ba and 1Bc). While binding of BAX or BAK to BCL-2 and other antiapoptotic proteins can be observed, the quantity of BAX or BAK bound generally represents the minority of the protein available, so that other interactions may also be important in keeping BAX and BAK in check. The BH3-only proteins in this model have no activator function, but rather act only as antagonists of the antiapoptotic proteins like BCL-2.

In an earlier study, an activator protein (BID) was indeed required for ABT-737 to induce mitochondrial apoptosis (Supplemental Figure 3 in Oltersdorf et al., 2005). ABT-737 lacks the ability to directly activate BAX or BAK, defining it as a "sensitizer" BH3 mimetic (Certo et al., 2006; Oltersdorf et al., 2005). In addition, we found that priming of BCL-2 with activators correlated with cellular sensitivity to ABT-737 (Certo et al., 2006). Thus, prior work has supported ABT-737's operation through a mechanism consistent with model A (Figure 1A).

The current reports focus more on the role of MCL-1 in determining resistance to ABT-737 (van Delft et al., 2006; Konopleva et al., 2006). Both groups show in selected cell lines that high expression of MCL-1 correlates with relative resistance to ABT-737. Both groups furthermore demonstrate that reduction of MCL-1 levels significantly enhances sensitivity to ABT-737. Both groups interpret this finding as supporting model B, in that it shows that loss of function of both MCL-1 and BCL-2 is needed to free BAX or BAK to induce apoptosis (Figures 1Bc and 1Bd). However, it must be noted that this finding does not necessarily exclude operation according to the mechanism of model A. If activators are indeed critical to the activation of BAX or BAK, and they are displaced by ABT-737 from BCL-2, excess MCL-1 present would be able to buffer the death signal by sequestering activators (Figures 1Ac and 1Ad). Therefore, loss of MCL-1 would foster ABT-737 toxicity in either model.

Konopleva et al. perform additional experiments to investigate whether activator displacement is critical. They demonstrate that ABT-737 disrupts interactions between BCL-2 and either BAX or BAK or BIM. When they knock down BIM in HL-60 cells, they show that there is little effect on apoptosis induced by ABT-737, suggesting that BIM plays little role in the apoptotic signaling. However, it is important to observe that the knockdown of BIM is subtotal and has little effect on the toxicity of Taxol, which depends in part on BIM for death signaling. In addition, while BID and BIM are the only BCL-2 family members thus far identified as activators, it may be that other proteins with BAX- or BAK-activating function exist either in or out of the BCL-2 family.

An important issue for clinical use is whether ABT-737 selectively kills cancer cells. In an initial report, ABT-737 showed single-agent toxicity against chronic lymphocytic lymphoma cells, follicular lymphoma cells, lymphoma cell lines, and small-cell lung cancer cell lines. When tested in mouse xenograft models, tumor remission was observed, with observed toxicity to normal mouse tissues limited to lymphopenia and thrombocytopenia (Oltersdorf et al., 2005). Konopleva et al. use primary malignant cells obtained from patients with acute myelogenous leukemia to examine this issue. ABT-737 caused a dramatic reduction in colony formation in all five AML samples tested. Importantly, the effect on colony formation of normal bone marrow cells was much more modest. Taken together, these two results demonstrate the presence of a therapeutic window between normal and malignant myeloid bone marrow cells. As an interesting adjunct, the authors also demonstrate that cells identified as leukemia stem cells are generally quite sensitive to ABT-737 as well. This is an important observation, as it must be presumed that leukemia stem cells must also be killed if a therapeutic is expected to cure a malignancy. It will be very interesting to see whether the same observation can be made in vivo when such a drug makes its way into clinical trials of AML.

What determines sensitivity to ABT-737? In these two reports, a key factor seems to be lack of MCL-1. However, determination of sensitivity and resistance may be a more complex matter. For instance, in the very AML experiments described above, the normal $CD34^+$ bone marrow cells express a relatively modest level of MCL-1 protein yet show much less sensitivity to the drug than do the malignant AML cells. In addition, a wide range of expression of BCL-2, MCL-1, and BAX can be observed across murine tissues. Yet normal tissues, with the exception of platelets and lymphocytes, are relatively insensitive to ABT-737 (Oltersdorf et al., 2005). What other factors may be important? A prior report points to the importance of priming BCL-2 with activator BH3-only proteins such as BIM to allow sensitivity to ABT-737 (Certo et al., 2006). Evidence is provided that cancer cells may in general be more primed than nonmalignant cells, explaining the observed therapeutic window. Whether this can be extended to a general predictor for cell toxicity to ABT-737 remains to be seen. It is also possible that explanations for different sensitivities between normal and malignant cells and explanations for different sensitivities among various malignant cells may diverge.

While this preview has focused somewhat on competing ideas regarding the mechanism of ABT-737 function, it is important to summarize the very significant points on which there appears to be general agreement. ABT-737 works through its expected mechanism of competing for the BH3-binding cleft of BCL-2 and displacing prodeath BCL-2 family members. High MCL-1 levels are likely to contribute to resistance to ABT-737. ABT-737 has shown impressive single-agent toxicity in cell lines ex vivo and in vivo, and against primary human malignant cells. These areas of agreement alone are sufficient to prompt considerable excitement at the prospect of adding such an interesting drug to our anticancer armamentarium.

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# Can't kick that oncogene habit

**One of the most exciting developments in recent cancer treatment has been the move away from crude cytotoxic agents toward drugs that inhibit specific targets in specific cellular pathways. One assumption of this strategy is that maintenance of human cancers is dependent upon a limited cadre of therapeutically tractable oncogenic lesions. In this issue of *Cancer Cell*, an intriguing paper from Sharma et al. endorses this approach by showing that evolution appears to be working for us. They show that an innate asymmetry in the dynamics of intracellular signaling biases pathway inhibition in favor of cell death. This bias may significantly potentiate targeted cancer therapies.**

Intrinsic tumor suppression pathways are innate, self-defeating programs that evolution has attached to those engines of cell expansion whose unbridled activities would otherwise constitute severe neoplastic risk (Lowe et al., 2004). Early examples included the unexpected propensity for activated RAS to induce growth arrest and the equally paradoxical proclivity of MYC to drive apoptosis. Such observations are now understood as examples of how the mitogenic actions of individual oncoproteins can be exploited by the cell only when their inherent growth-inhibitory properties are quelled by collateral signals. In the classical paradigm of oncogene cooperation, such obligate collateral signals are provided by the collaborating oncogene: hence, each oncogenic lesion is dependent on the properties of the other for its oncogenic potential to be manifest. Thus, the tumor phenotype is an emergent property of oncogenic lesions acting in concert (Evan and Littlewood, 1998)—something a geneticist might term "synthetic viability" (Figure 1A).

Such observations offered the earliest clue that tumor cells might be preternaturally dependent for their survival upon the aberrant signaling networks that drive them, by suggesting that cutting individual oncogenic cords within the tumor ensemble can expose the latent intrinsic tumor suppression pathways directed by any remaining oncogenic lesions. On the other hand, since oncogenes harbor the seeds of their own destruction, such ideas also raised the disturbing counter-possibility that inactivation of individual oncogenes might actually accentuate tumor growth by staunching the associated intrinsic tumor suppressor pathway. Only with the advent of reversibly switchable transgenic mouse cancer models, in which the activities of a specific oncogene targeted to a specific tissue can be toggled on and off at will, could the net consequences of acute oncogene ablation be directly tested in vivo. Such animals are, in essence, genetic surrogates for targeted drugs, which can be used to establish the extent to which maintenance of experimental tumors remains dependent upon the oncogenic mutations that drove their evolution, and what the nature of that dependency might be. Such in vivo studies indicated that deactivation of pivotal oncogenic mutations typically triggered profound tumor apoptosis that would frequently (Chin et al., 1999; Felsher and Bishop, 1999; Fisher et al., 2001; Pelengaris et al., 1999, 2002), but not always (Boxer et al., 2004), lead to marked tumor regression. Even though such regression was often superseded by the emergence of resistant clones, such studies confirmed, in principle, the idea that tumor cells acquire de novo a dependence upon the lesions that drive and maintain them. With the advent of targeted cancer therapies, it has finally become possible to explore this idea in human cancers in vivo, and the successful treatment of CML with Gleevec is the poster child for the notion that acquired dependency on oncogenic mutations holds for spontaneously occurring human cancers. Naysayers may point to the fact that resistant clones eventually cause relapse of Gleevec-treated patients. However, even here the news is good: the great majority of relapses involve resistant mutations in the ABL kinase rather than wholesale replacement of ABL by a newly evolved oncogenic edifice (Shah and Sawyers, 2003). This suggests that dependence upon ABL is, indeed, profound and that, notwithstanding the genomic instability that characterizes the accelerated phase of CML, room for evolutionary maneuver by surviving tumor cells is extremely constrained.

Why should tumor cells acquire a dependence upon their oncogenic mutations? The answer seems fairly straightforward in situations where an oncogenic mutation confers survival properties on the cell—for example, overexpression of BCL-2/BCL-$x_L$, or constitutive signaling through survival factors receptors and their intracellular transducers. In such cases, removal of the constitutive survival signal exposes the targeted tumor cell to the full onslaught of preexisting proapoptotic flux rife in cancers—hypoxic and nutrient-poor microenvironments, internal havoc wrought of genotoxic injuries and aberrant protein folding, and the continuous pumping of apoptotic pathways by proproliferative mutations like activated MYC and E2F or loss of RB. By contrast, the dependence that tumor cells exhibit for