Trends in Cancer

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Review

Endogenous and imposed determinants of apoptotic vulnerabilities in cancer

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The intrinsic apoptosis pathway is controlled by the BCL-2 family of proteins. Although the prosurvival members of this family can help cancer cells evade apoptosis, they may also produce apoptotic vulnerabilities that can potentially be exploited therapeutically. Apoptotic vulnerabilities can be driven by endogenous factors, including altered genetics, signaling, metabolism, structure, and lineage or differentiation state, as well as imposed factors, the most prominent being exposure to anticancer agents. The recent development of BH3 mimetics that inhibit prosurvival BCL-2 family proteins has allowed these apoptotic vulnerabilities to be targeted with demonstrable clinical success. Here, we review the key concepts that are vital for understanding, uncovering, and exploiting apoptotic vulnerabilities in cancer for the potential improvement of patient outcomes.

Highlights

During cancer development, cells with pre-existing buffers against apoptosis, such as increased expression of prosurvival BCL-2 proteins, can evade transformation-associated apoptosis and form tumors.

The requirement for prosurvival BCL-2 family proteins in cancer cells endows them with vulnerabilities that are exploitable using BH3 mimetic drugs.

Diverse endogenous features of cancer cells modulate BCL-2 family protein dependencies, as do imposed features such as the cellular response to drug treatments.

The parallel development of selective, potent BH3 mimetic drugs, combined with our evolving understanding of the landscape of BCL-2 family vulnerabilities in cancer, has set the stage for the large-scale integration of direct apoptosis-activating therapies into the clinic.

Understanding mitochondrial apoptosis

Intrinsic (mitochondrial) apoptosis is an evolutionarily conserved cell death pathway that is critical for normal development, maintenance of tissue homeostasis, and cancer prevention [1]. This pathway is controlled by the **BCL-2 family of proteins** (see Glossary), which contains both proapoptotic and prosurvival members. To trigger apoptosis, proapoptotic, BH3-only 'activator' proteins (BIM or BID) activate the proapoptotic pore-forming proteins (BAX or BAK), which oligomerize to cause **mitochondrial outer membrane permeabilization (MOMP)** (Figure 1). MOMP releases apoptogenic factors into the cytosol, such as cytochrome c, which binds APAF-1 and activates caspases to dismantle the cell and prepare it for phagocytosis [2,3]. To balance this process, prosurvival proteins (BCL-2, BCL-X_L, MCL-1, etc.) can bind and sequester both classes of proapoptotic proteins to maintain cellular survival. However, when these prosurvival proteins are inhibited, they release the bound proapoptotic proteins, which can then go on to cause MOMP – this establishes the therapeutic index for **BH3 mimetics**, as described in more detail in the following text. Curiously, MOMP has also been shown to be triggered in cells lacking known proapoptotic BH3-only activator proteins when all prosurvival proteins are inhibited [4].

The abundance of proapoptotic and prosurvival proteins varies between cells, which alters their baseline apoptotic sensitivity and consequently their sensitivity to cellular damage and stress signaling. To illustrate, a cell that maintains its survival by expressing just enough prosurvival proteins to barely buffer endogenous prodeath signals is 'primed' for apoptosis (Figure 2). In contrast, a cell that expresses a surplus of prosurvival proteins that can adequately buffer against existing and even potentially additional prodeath molecules is 'unprimed.' Finally, cells that do not express sufficient levels of BAX or BAK to initiate apoptosis or express an overwhelming abundance of prosurvival proteins are considered 'apoptosis refractory.' Apoptotic priming can be assessed using a functional assay, **BH3 profiling**, which measures cytochrome c release in response to titrated doses of proapoptotic BH3 peptides [5–8]. Apoptotic priming is a critical determinant of cellular responses to chemotherapy or radiation in cancer cells [7,8] as well as

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healthy tissues [9]. Furthermore, as described below, cells that are primed for apoptosis may be dependent on prosurvival proteins that are actively sequestering prodeath signals.

Cells experience a myriad of changes while undergoing malignant transformation, including derangement of cellular growth signals, oncogenic activation, loss of microenvironmental support signaling, cell cycle checkpoint violation, ongoing DNA damage, nutrient deprivation, reactive oxygen species signaling, hypoxia, and many others [10,11]. Many of these have previously been linked directly to the increased production of proapoptotic signaling. For example, growth factor deficiency can cause upregulation of proapoptotic BIM, PUMA, and BMF [12,13], whereas nutrient deprivation can upregulate proapoptotic Noxa [14]. This production of proapoptotic signaling can induce apoptosis in cells during the process of transformation and act as an anticancer defense mechanism. Cells that have pre-existing buffers against apoptosis, such as increased expression of prosurvival proteins BCL-2, BCL-w, BCL-X_L, BFL-1 (A1), and MCL-1, can evade transformation-associated apoptosis and form tumors. However, this may come at the cost of subsequently requiring those prosurvival proteins to actively sequester their proapoptotic counterparts – a perilous balance that equates to an apoptotic vulnerability that can be therapeutically exploited by BH3 mimetics, a class of small molecule drugs that potently and selectively inhibit prosurvival BCL-2 family proteins by blocking their interactions with proapoptotic BCL-2 family members. Given that the transformation-associated cell stress presumably does not dissipate, the apoptotic vulnerability continues to be present in the growing tumor. The twin concepts of heightened apoptotic priming and increased dependence on prosurvival BCL-2 family proteins in cancer cells likely underlies the striking clinical success of venetoclax, a BH3 mimetic that selectively inhibits BCL-2, in the context of multiple hematological malignancies [15–17]. There are currently many agents targeting prosurvival BCL-2 family proteins in development, and their specificity, selectivity, and clinical progress have recently been reviewed elsewhere [18,19].

The recent development and deployment of BH3 mimetics targeting diverse prosurvival BCL-2 family proteins with variable selectivity profiles has created major opportunities for targeting survival proteins in cancer. However, the full and timely clinical exploitation of these powerful agents requires a complete understanding of which mimetics should be used in which cancer contexts. Here, we summarize what is known about both the endogenous determinants of apoptotic dependencies, which include tumor genetic, signaling, metabolic, and structural features and lineage or differentiation states, as well as how these dependencies can be further shaped by drug therapies. By defining the determinants of apoptotic vulnerabilities, it will be possible to maximize the clinical impact of BH3 mimetics.

Endogenous determinants of apoptotic vulnerabilities

The endogenous apoptotic vulnerabilities that exist within a cancer cell at baseline (without further perturbations) are known to be driven by numerous factors, including the cell's lineage, differentiation state, oncogene and tumor suppressor signaling pathways, mitochondrial structure, and metabolism, which in turn regulate BCL-2 family protein expression, localization, and protein–protein interactions (Figure 3). The net integration of these and likely other less known factors produces the vulnerability states that dictate responses to BH3 mimetics.

Cell lineage

The most consistent apoptotic vulnerability that has been detected and exploited thus far is the BCL-2 dependence in several hematologic malignancies, including chronic lymphocytic leukemia (CLL) and **acute myeloid leukemia (AML)** [16,17]. These dependencies evidently can be quite strong, given that patients with CLL can remain on treatment with the BCL-2 inhibitor venetoclax

Glossary

Anoikis: a form of cell death triggered by detachment from a surrounding extracellular matrix.
BCL-2 family of proteins: a family of prosurvival and proapoptotic proteins whose interactions dictate a cell's apoptotic equipoise. Prosurvival members include BCL-2, BCL-X_L, MCL-1, BCL-w, and BFL-1, whereas proapoptotic members include the pore-forming proteins BAX and BAK as well as BH3-only activators and sensitizers, including BIM, BID, BAD, PUMA, NOXA, HRK, and BMF.
BH3 mimetics: a class of drugs targeting the prosurvival BCL-2 family proteins, most notably BCL-2, BCL-X_L, and MCL-1.
BH3 profiling: a live cell–based functional assay to measure apoptotic priming and dependencies.
BRD4 inhibitors: inhibitors of BRD4, a bromodomain-containing protein that binds to acetylated histones to regulate gene expression and is important for the viability of certain cancers, including AML.
Epithelial–mesenchymal transition (EMT): a process whereby cancer cells acquire characteristics associated with increased aggressiveness and drug resistance.
Mitochondrial outer membrane permeabilization (MOMP): the BAX/BAK-dependent process that leads to cytoplasmic cytochrome c release and downstream caspase activation during apoptosis.
PERK: an endoplasmic reticulum (ER)-resident protein that signals ER stress.
Sanctuary site: a physiological site where cells are protected, such as from drug-induced cell death.
SMAC: a protein that facilitates activation of caspases downstream of MOMP.

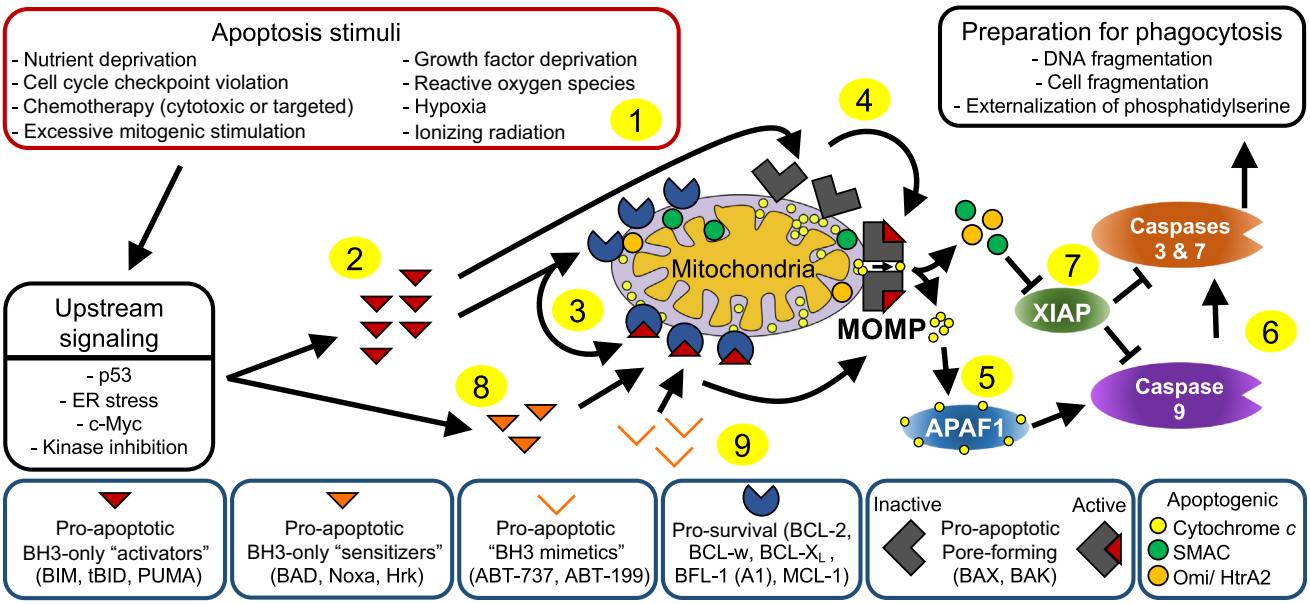

Figure 1. The mitochondrial pathway of apoptosis. To trigger apoptosis, cellular stress or damage signals (1) produce proapoptotic proteins (BH3-only 'activators' of apoptosis) via their upregulation (e.g., BIM or PUMA) or cleavage (BID cleaved to form active, truncated tBID) (2), which can either be sequestered by prosurvival proteins such as BCL-2, BCL-X_L, or MCL1 (3) or, when these prosurvival proteins are saturated or not expressed, can activate the pore-forming proteins BAX and/or BAK (4). Activated BAX or BAK oligomerize and form pores to cause mitochondrial outer membrane permeabilization (MOMP), triggering the release of apoptogenic molecules, including cytochrome c, SMAC, and OMI/HtrA2 from the mitochondrial intermembrane space. Cytochrome c complexes with APAF1 in the cytosol to form the apoptosome (5), which activates initiator caspase 9, which then goes on to activate the effector caspases 3 and 7 (6) to proteolytically dismantle cellular structures and prepare the cell for phagocytosis. Caspase activation can be blocked by XIAP (7), which in turn is inhibited by the released SMAC and OMI/HtrA2 proteins from mitochondria (7). Upstream damage or stress signaling can also activate BH3-only 'sensitizer' proteins that cannot efficiently activate BAX and BAK but inhibit the activity of prosurvival BCL-2 family proteins to release any bound and sequestered BH3-only activators, which can then activate BAX or BAK to trigger MOMP (8). BH3 mimetics are small molecule drugs that mimic the activity of BH3-only sensitizers and can trigger apoptosis outright in apoptotically dependent cells or enhance sensitivity to other anticancer agents in unprimed cells by blocking the activity of prosurvival BCL-2 family proteins (9). Abbreviation: ER, endoplasmic reticulum.

for many years without developing resistance. Cells from the hematopoietic system seem to have the highest expression of BCL-2, and B cells, from which CLL arises, seem to express consistently high levels of this protein [20]. However, the positive expression of a prosurvival protein does not necessarily constitute actionable dependence, because cells must be primed for apoptosis in order to have a significant degree of dependence on prosurvival proteins (Figure 2). Furthermore, all other things being equal, higher expression of BCL-2 may decrease venetoclax sensitivity, because more drug is required to neutralize BCL-2 activity. Another key factor in the success of venetoclax in the treatment of CLL is the consistent lack of expression of other prosurvival proteins that may buffer against BCL-2 inhibition [21]. Similar to CLL, follicular lymphomas also strongly overexpress BCL-2, which is due to the t(14;18) translocation of the BCL-2 gene and the immunoglobulin heavy chain (IGH) promoter [22]; yet, patients with this disease have only modest sensitivity to venetoclax [23]. This is likely due to the presence and buffering capacity of prosurvival proteins BCL-X_L and MCL-1, which have been shown to be upregulated in this disease [24].

Although there is variation in expression levels of BCL-2 family members in cells of the hematopoietic system, these cells seem to express higher levels of BCL-2 specifically and are thus predisposed to being dependent on this protein. This led to hematologic cancers being explored first for therapy with BCL-2 inhibitors with demonstrated success. BCL-2 inhibitor trials in solid cancers have reported mixed results, often with only modest responses evident in subsets of

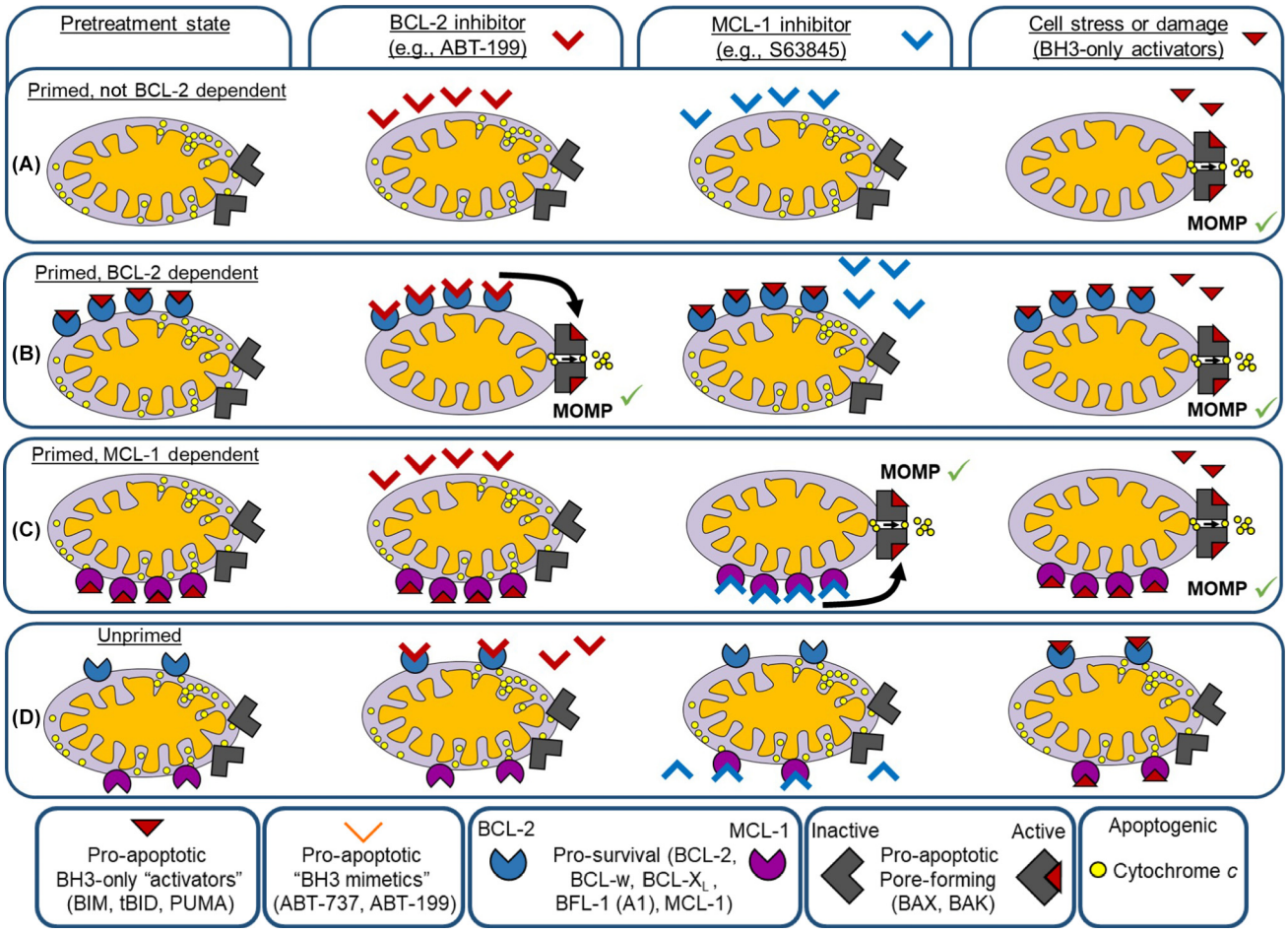

Figure 2. A model of how mitochondrial apoptosis can be targeted directly or indirectly to induce apoptosis. (A) Cells that contain a low reserve of unbound antiapoptotic proteins in their pretreatment state (prior to administration of any agents) but also lack proapoptotic protein expression are considered to be primed for apoptosis. These cells are typically sensitive to cytotoxic chemotherapies that cause cell stress or damage and upregulation of proapoptotic BH3-only proteins but are resistant to BH3 mimetics. (B, C) Cells that contain a low reserve of unbound antiapoptotic proteins (primed for apoptosis) and (B) BCL-2 or (C) MCL-1 molecules that are actively binding and sequestering activator BH3-only proteins such as BIM, BID, or PUMA are sensitive to specific BCL-2 or MCL-1 inhibition, respectively. These cells are typically also sensitive to cytotoxic chemotherapies that cause cell stress or damage. (D) Cells that contain a high reserve of unbound antiapoptotic proteins (unprimed) can buffer stress-induced proapoptotic signals and are therefore resistant to cytotoxic chemotherapies and BH3 mimetics. If additional pan-inhibitor or cytotoxic chemotherapy is administered, however, mitochondrial outer membrane permeabilization (MOMP) could ultimately be triggered. Note that the cells in D that are experiencing cell stress or damage, perhaps due to chemotherapy treatment, would now be sensitive to additional proapoptotic factors as well as BH3 mimetics targeting BCL-2 or MCL-1.

patients. This is consistent with cancer cell line studies demonstrating that only subsets of cell lines from solid malignancies such as breast [25] and lung [26] carcinomas are sensitive to BCL-2 inhibition. The modest activity of BCL-2 inhibitors in solid tumors is believed to be principally due to the higher expression of other prosurvival proteins in these cancers, especially BCL-X_L [27–29]. However, BCL-X_L inhibitors and even dual-targeting BCL-2 and BCL-X_L inhibitors have not been as successful in solid tumors [30–32], perhaps suggesting these tumors may also experience lower overall apoptotic pressure. Consistent with this concept, studies have shown that solid cancers are less primed for apoptosis than hematologic malignancies [8] and that overcoming this apoptotic resistance in solid tumors requires alternative strategies, such as cotreatment with BCL-X_L inhibitors along with direct activators of proapoptotic BAX [33].

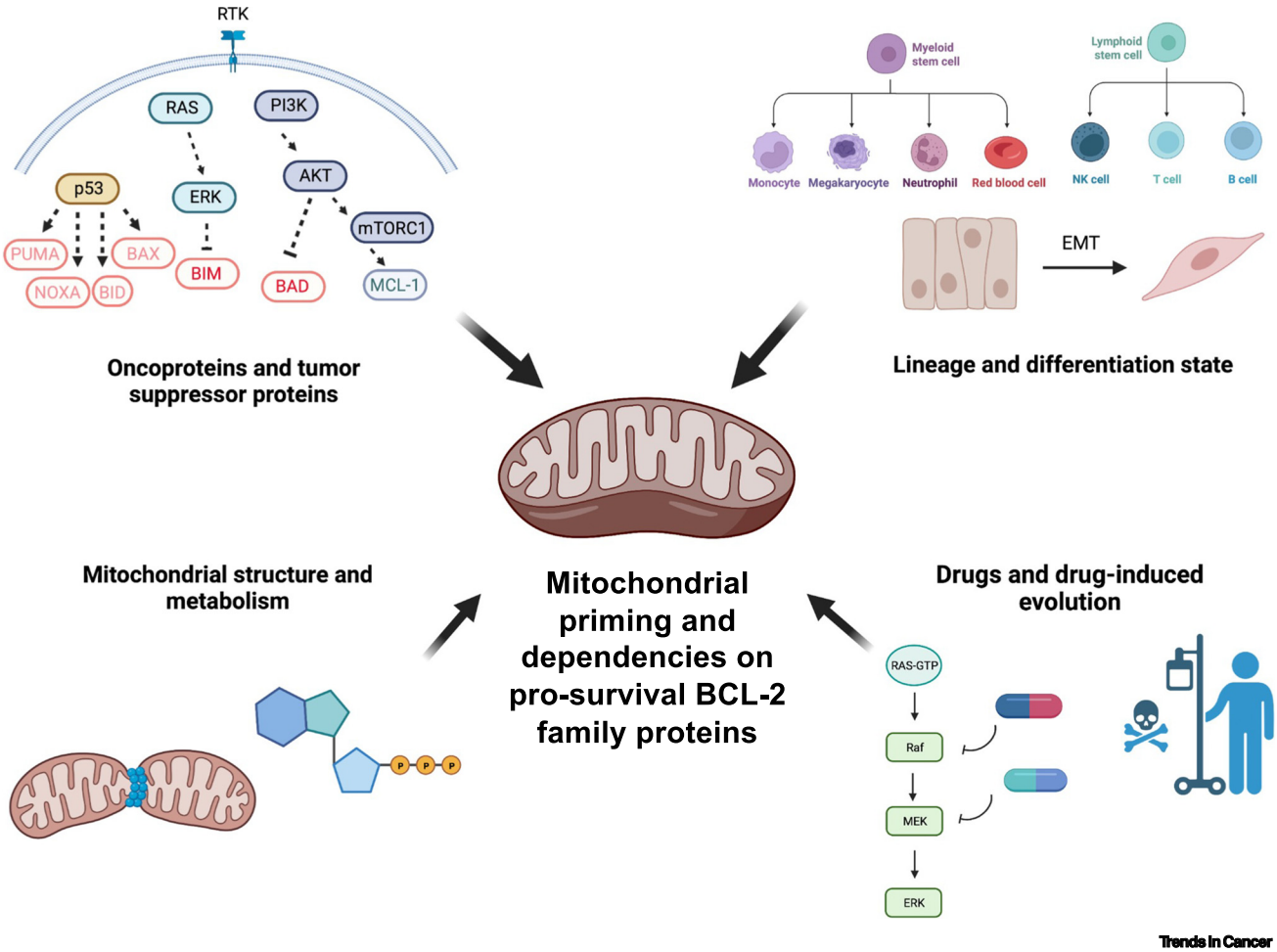

Figure 3. Major endogenous and imposed determinants of apoptotic priming. Key endogenous and imposed factors can affect mitochondrial priming and vulnerabilities in normal and cancer cells. These include endogenous factors such as the presence of activated oncoproteins or inactivated tumor suppressor proteins, the altered structure and metabolic functions of tumor mitochondria, and cellular differentiation states. In addition, drugs can act as exogenous factors shaping apoptotic priming, either by altering signal transduction directly or by selecting for resistant cells with altered apoptotic dependencies. Abbreviations: EMT, epithelial–mesenchymal transition; NK cell, natural killer cell; RTK, receptor tyrosine kinase. Image created with BioRender.com.

Beyond the hematopoietic system, BCL-2 is also expressed in certain cells within the female reproductive system as well as endocrine and thyroid glands [34]. The cancers that arise from these tissues may potentially be sensitive to BCL-2 inhibition. BCL-X_L is expressed at higher levels in neural and epithelial cells [35,36], whereas MCL-1 is enriched in certain hematopoietic cells, including plasma cells [37], certain types of epithelial cells, enterocytes, and microglia [38,39]. The heightened expression of MCL-1 in plasma cells is likely linked to the reported MCL-1 dependence in the plasma cell malignancy multiple myeloma [40,41]. The model coming into focus is that cell lineage is a critical factor driving expression of prosurvival BCL-2 family proteins and may predispose cancers arising from varying precursors to develop lineage-driven apoptotic vulnerabilities. Surprisingly, our understanding of apoptosis regulation in healthy tissues is more limited than that in cancers. Thus, the degree to which BH3 mimetics beyond venetoclax will be tolerated clinically in pediatric and adult patients is not yet clear, a fact that underscores the importance of both a deep understanding of the determinants of apoptotic vulnerabilities and the development of therapeutic strategies that selectively sensitize tumor cells to BH3 mimetics, topics further elaborated later.

Differentiation and maturation stage

Although lineage-associated regulation of apoptosis helps establish initial set points for expression of BCL-2 family proteins, the differentiation or maturation state of normal or cancer cells can modulate their expression. For example, apoptotic priming is dynamically regulated in normal cells during postnatal development, with young tissues that are growing or remodeling being more primed for apoptosis than adult tissues [9,42–44], making young tissues consistently more sensitive to ionizing radiation or chemotherapy than their mature counterparts [9,45–47]. This is consistent with differentiation state being a powerful arbiter of apoptotic sensitivity in both healthy and cancerous cells. It is likely that cancers deriving from more mature and apoptosis-resistant progenitors may exhibit reduced levels of prosurvival protein dependence. Indeed, pediatric malignancies seem to be more sensitive to anticancer therapies than their counterparts in adults, which may be linked to differences in apoptotic sensitivity of the cancers that arise from differently primed cells of varying maturation stages. It has also been shown that the differentiation state of AML cells affects venetoclax sensitivity, with phenotypically primitive AML being sensitive to BCL-2 inhibition, whereas monocytic AML exhibits reduced expression and dependence on BCL-2 [48]. Similarly, T-cell acute lymphoblastic leukemias of the early T cell progenitor subgroup, which are at high risk for relapse, are dependent on BCL-2, whereas those exhibiting features of more mature T cells are instead dependent on BCL-X_L [49].

The plasticity of differentiation states can also modulate apoptotic vulnerabilities. For example, the **epithelial–mesenchymal transition (EMT)**, a cellular program through which cell–cell and cell–substrate interactions are remodeled, leading to epithelial cell detachment and adoption of a mesenchymal cell fate, is critical for the progression of certain solid tumors [50]. Among other properties, the EMT confers on cancer cells a broad ability to resist cell death following treatment with cytotoxic and targeted chemotherapies [50]. Thus, it is reasonable to assume that EMT induction impacts the regulation of the apoptotic signaling network in cancer cells. Indeed, recent studies have begun to demonstrate how this may occur. For example, the EMT transcription factor ZEB1 can directly bind to the BIM promoter to suppress its transcription, inhibiting the apoptotic response to epidermal growth factor receptor (EGFR) inhibitors in mesenchymal *EGFR* mutant lung cancer models [51]. Additional studies have highlighted a particularly critical role for the antiapoptotic BCL-2 family protein, BCL-X_L. Cells that have undergone EMT appear not only to exploit BCL-X_L for chemoresistance but also to rely upon it for their survival. For example, one recent study demonstrated that EMT renders cells more dependent upon BCL-X_L through a **PERK**–NOXA-dependent mechanism [52]. Another recent study corroborated this concept by demonstrating that mesenchymal kidney cancers are dependent upon BCL-X_L, speculating that this dependence may derive from the fact that epithelial shedding triggers cell death via **anoikis** and that cancer cells that undergo EMT may be positively selected due to enhanced BCL-X_L activity in order to avoid anoikis [53], a concept further supported by the observation that transforming growth factor-β1 treatment of epithelial cells can promote both apoptosis and EMT [54]. Thus, changes in differentiation state that promote tumor progression may sensitize cancer cells to BCL-X_L inhibition.

Oncogenes and tumor suppressors

BCL-2 family prosurvival proteins are frequently altered in cancer genomes in a manner analogous to classical oncogenes, a fact which provided early support for the idea that evasion of apoptosis is a hallmark property of cancers that is required in order for neoplastic cells to survive in the face of hostile intracellular and microenvironmental stresses characteristic of the disease [55]. For example, the classic discovery that follicular lymphoma cells frequently harbor the aforementioned t(14;18) translocation established that resistance to apoptosis through increased expression of a prosurvival BCL-2 family protein can promote cancer development [2]. More

recently, cancer genome characterization projects have extended this concept, such as through the discovery that MCL-1 amplifications are recurrent in diverse malignancies, including breast, lung, bladder, ovarian, and prostate cancers [56].

It is now also clear that oncogenes and tumor suppressor genes (TSGs) promote malignant transformation at least in part through their regulation of apoptosis. For example, *TP53*, the most commonly altered TSG in human cancers, encodes a positive regulator of the proapoptotic proteins PUMA, NOXA, BID, and BAX [57–60]. Indeed, p53-mediated, transcriptional *PUMA* induction has been demonstrated to be the major driver of apoptosis in response to certain instances of DNA damage [61]. Likewise, oncogenic pathways, when hyperactivated, can suppress proapoptotic proteins and/or upregulate prosurvival proteins. For example, extracellular signal-regulated kinase (ERK) phosphorylates BIM, leading to its βTRCP-dependent proteasomal degradation; thus, hyperactive ERK pathway activity leads to suppression of BIM levels [62,63]. BAD is phosphorylated at S112 and S136 by ERK and AKT, respectively, leading to 14-3-3 binding and inactivation [64,65]. Finally, phosphatidylinositol-3-kinase pathway hyperactivation can both suppress *PUMA* expression via FOXO3A and promote MCL-1 translation through mTORC1 [66,67].

Complicating the situation is the fact that, contrary to the themes earlier, oncogenes can also promote apoptosis. This concept, most thoroughly established for the *MYC* oncogene, is based on the fact that MYC positively regulates the expression of proapoptotic genes, including *BAX*, *BID*, and *BIM* [9]. Thus, both cancers and actively proliferating, MYC-high nonmalignant tissues exhibit increased apoptotic priming, a fact that may both explain some of the toxicities associated with chemo- and radiotherapy treatments observed in children [9] and offer a novel treatment strategy for aggressive, MYC-high, drug-resistant disease [68,69], topics elaborated elsewhere in this review.

Cytokine, growth factor, and hormone signaling

Signaling pathways regulated by cytokines, extracellular ligands, growth factors, and hormones have been shown to modulate BCL-2 family protein expression and activity and can therefore modulate apoptotic vulnerabilities in cancer cells. For example, estrogen receptor (ER) signaling is known to induce levels of BCL-2 [25] in breast cancer cells, a finding that motivated ongoing trials examining venetoclax in ER^+ breast cancer. Signaling pathways activated by cytokines, including interleukin 6, interleukin 7, and others, have been shown to modulate levels of BCL-2 family proteins and either promote survival, promote apoptosis, or modulate sensitivity to BH3 mimetics. Similarly, CD40 ligation from follicular helper T cells in the lymph node microenvironment can shift CLL cells toward survival and provoke resistance to BCL-2 inhibition. By extension, blocking CD40 signaling may potentially restore sensitivity, even in this so-called **sanctuary site**.

Mitochondrial structure and shape

Mitochondria contain inner and outer mitochondrial membranes, abbreviated as IMM and OMM, respectively. The smooth OMM serves as a physical interface with the cytosol and other cellular compartments, whereas the IMM delineates the mitochondrial matrix and is composed of two subcompartments: an inner boundary membrane that runs parallel to the OMM and the cristae-convoluted invaginations that contain the core proteins required for cellular respiration. The mitochondrial network in living cells is constantly being reshaped through a series of processes known as 'mitochondrial membrane dynamics,' which involve both organelle fusion and fission and ultrastructural remodeling [70]. These dynamic changes, used to meet the changing signaling and metabolic needs of the cell, in turn regulate apoptotic priming. For example, during cell death, cristae undergo significant structural changes that are dependent

upon the mitochondrial fusion regulator OPA1 [71,72]. These changes support the redistribution of cytochrome c from the cristae to the intermembrane space, followed by its complete release into the cytosol through apoptotic pores in the OMM. Similarly, during apoptosis, BAX and BAK initiate mitochondrial fragmentation through stabilization of the fission regulator DRP1 [70]. Even mitochondrial shape has been shown to affect the ability of the pore-forming proteins to induce MOMP: Hyperfragmented mitochondria that have a more spherical shape are less permissive to stabilizing interactions between BAXα9 and the mitochondrial outer membrane [73]. This may protect cells from apoptosis and invalidate any apoptotic vulnerabilities that may exist.

Given the relationship between mitochondrial membrane dynamics and apoptotic cell death, it is intuitive that tumor cells may alter mitochondrial dynamics homeostasis as a means of promoting their growth and survival [74]. Indeed, recent evidence suggests that tumors exhibit recurrent amplification of mitochondrial dynamics–regulating genes [75]. Furthermore, oncogenic signaling pathways exert direct control over mitochondrial membrane dynamics, as exemplified by studies demonstrating that the ERK/mitogen-activated protein kinase pathway, frequently hyperactivated in cancer, directly phosphorylates DRP1 to promote mitochondrial fission, which is essential for tumor growth [76,77]. Evidence suggests that mitochondrial membrane dynamics also powerfully influence tumor responses to apoptosis-inducing therapies. For example, small molecule inhibitors of driver oncogenes sensitize *EGFR*, *BRAF*, *KRAS*, and *PIK3CA* mutant tumors to second mitochondrial activator of caspases (**SMAC**) mimetic therapies via their effects on mitochondrial membrane dynamics [75]. Furthermore, tumor cells with altered mitochondrial membrane dynamics can be positively selected for by apoptosis-inducing therapy because of their increased resistance to apoptosis. This has been observed in leukemia cells, in which the mitochondrial chaperonin CLPB is upregulated upon the acquisition of resistance to venetoclax. CLPB's resistance-conferring function is explained by its interaction with OPA1, which promotes the stabilization of mitochondrial cristae to prevent cytochrome c release following venetoclax treatment, highlighting the possibility that in certain cases, cells may recover from BAX/BAK-mediated MOMP, provided that cytoplasmic cytochrome c release is prevented [78]. Together, these findings provide critical evidence that mitochondrial structure and dynamics modulate tumor sensitivity to apoptosis-inducing therapy, suggesting that mitochondrial structural regulation may be a point of convergence for diverse apoptosis resistance mechanisms.

Cellular metabolism

The intrinsic apoptosis pathway is largely regulated at the mitochondria, an organelle that is also the principal metabolic site of the cell. It is thus understandable that bidirectional crosstalk exists between metabolic and apoptotic regulation. For example, changes in mitochondrial membrane dynamics can simultaneously affect both cellular metabolism and apoptotic commitment [70,74]. Furthermore, discrete metabolic pathways regulate apoptotic potential. For example, changes in pentose phosphate pathway flux can alter the caspase activation state through NADPH-regulated redox inactivation of cytochrome c [79], and BAX activation and consequent MOMP are regulated by ceramide and sphingosine metabolism [80,81]. Reciprocally, phosphorylation of NOXA and BAD can drive glucose consumption through the pentose phosphate pathway and glycolysis, respectively, whereas BCL-X_L has been linked to oxidative phosphorylation via ATP synthase [82–85].

Excitement surrounding venetoclax and other direct activators of cancer cell apoptosis inspired a series of recent studies to more fully articulate the mechanisms of metabolic–apoptotic crosstalk. This included one recent study that used CRISPR-based loss-of-function screens to map cellular metabolic pathways that influence the sensitivity of AML cells to venetoclax-induced apoptosis. Among other findings, this work revealed that heme biosynthesis is a powerful modulator of

apoptotic priming via its effects on the stability and function of the electron transport chain and that the altered regulation of heme biosynthesis may explain, in part, both the therapeutic window that exists for venetoclax between AML cells and normal hematopoietic stem cells as well as the refractoriness of certain leukemias with upregulated heme biosynthesis to induction chemotherapy [86]. In a similar manner, other studies have used unbiased functional genomic or metabolomic approaches to define metabolic pathways that influence apoptosis regulation and clinical sensitivity to venetoclax. Examples include studies identifying metabolic pathways leading to increased oxidative phosphorylation [87], fatty acid oxidation [88], and nicotinamide metabolism [89] as important modulators of apoptotic priming and consequent venetoclax sensitivity in patients with leukemias. These and other studies also suggest that therapeutic targeting of metabolic pathways whose activation status is altered in cancer and that function to suppress apoptotic commitment may be a particularly effective strategy to reverse venetoclax and chemotherapy resistance [90–93].

Imposed determinants of apoptotic vulnerabilities

Although endogenous features of tumors described earlier clearly template their apoptotic priming states, these states are not immutable. Instead, a significant amount of work in recent years has focused on rational strategies to shape tumor apoptotic priming using pharmacological approaches. Most notably, researchers have leveraged the idea that just as driver oncogenes often suppress apoptotic priming through biochemical modulation of the BCL-2 network, therapies targeting oncogenic drivers can reverse these effects, driving apoptosis or sensitizing tumor cells to BH3 mimetics. Many examples of this concept exist, including studies demonstrating that BIM induction is central to the apoptotic response of *EGFR* mutant [94–97] and *ALK* rearranged [98] lung cancers, *HER2* amplified and *PIK3CA* mutant breast cancers [99], and *BRAF* mutant melanoma [100] and colorectal cancer [101]. Furthermore, additional studies have revealed that even when oncopathway targeted therapies fail to induce significant levels of apoptosis on their own, they can modulate the regulation of the BCL-2 network in a manner that primes its sensitivity to blockade of one or more antiapoptotic BCL-2 family members. This concept is demonstrated, for example, in studies identifying strong synergy between mitogen-activated protein kinase kinase (MEK) and either BCL-X_L [28] or MCL-1 [102] inhibitors in *KRAS* mutant tumors, between mTORC1 and BCL-X_L inhibitors in *PIK3CA* mutant breast cancers [67], and between BRAF and MCL-1 inhibitors in *BRAF* mutant tumors [103], among many other examples.

The concept of therapy-induced apoptotic priming extends well beyond oncopathway targeted therapies. In fact, the therapeutic indices of diverse anticancer agents, including those targeting cell cycle checkpoints (e.g., CDK4/6 inhibitors, Aurora kinase inhibitors), metabolism (e.g., glycolysis inhibitors), and DNA integrity (e.g., DNA damaging agents, ionizing radiation, topoisomerase inhibitors), are linked to the fact that already-stressed cancer cells exhibit increased apoptotic sensitivity [104,105]. A wealth of information is available on the specific BCL-2 family protein changes induced by these anticancer agents, changes that can ultimately sensitize cells to BH3 mimetics. For example, topoisomerase inhibitors lead to cleavage and activation of the activator BID [106,107], an effect that, like BIM induction following targeted therapy treatments [7,95,96,108], would be expected to cause an overall increase in the number and strength of apoptotic vulnerabilities, depending on which prosurvival proteins are expressed. Still other agents can cause changes in BCL-2 family protein interactions that very specifically alter apoptotic vulnerabilities. For example, proteasome inhibitors such as bortezomib cause ER stress, an unfolded protein response, and subsequent upregulation of Noxa [109], which binds and inhibits only MCL-1. This essentially mimics the activity of an MCL-1 inhibitor and potently triggers apoptosis in MCL-1–dependent cells. In cells that express both MCL-1 and BCL-2, this Noxa upregulation results in sensitivity to BCL-2 inhibitors [110].

The timing of therapeutic strategies that alter apoptotic vulnerabilities is an important and emerging consideration. For example, administering a DNA damaging agent may increase BCL-X_L dependence only after the damage is first sensed by DNA damage response elements such as p53 to activate downstream transcriptional programs, a process that may not manifest as increases in apoptotic vulnerabilities until 48 to 72 h later in some cases [111]. Thus, the timing of administration of BH3 mimetics designed to synergize with these agents should be dictated by this mechanistic consideration, particularly for those mimetics that are expected to cause stronger on-target toxicities than the well-tolerated venetoclax. More recently, studies have also suggested that drug treatments not only can lead to the modulation of apoptotic priming on short timescales (hours to days) but also can select on longer timescales (weeks to months) for resistant cancer cells that exhibit hypersensitivity to BH3 mimetics. A recently reported example includes the oncogene MYC, which can prime cancer cells for apoptosis through the mechanisms described earlier. MYC is commonly upregulated in AML cells with acquired resistance to **BRD4 inhibitors** and in *BRAF* mutant melanoma cells with acquired resistance to BRAF/MEK inhibitors, causing these resistant cells to exhibit hypersensitivity to BH3 mimetics and laying the groundwork for serial treatment strategies that act as anticancer 'evolutionary traps' [68,69].

Key challenges

Before targetable apoptotic vulnerabilities can be fully exploited for cancer therapy, a series of challenges must be addressed, as outlined below.

- What cancers have endogenous or induced apoptotic vulnerabilities that can be targeted therapeutically? A growing body of mechanistic studies, combined with cell line profiling efforts, have begun to clarify how apoptotic priming states and BCL-2 family dependencies vary across human cancers [52]. Furthermore, the deployment of clinical grade BH3 profiling assays is allowing the more highly resolved mapping of both the overall apoptotic priming state and specific BCL-2 family dependencies in clinical samples in real time, work that is being complemented by emerging *ex vivo* BH3 mimetic profiling efforts [112,113]. These methods can be further complemented by cytogenetic or expression biomarkers of BH3 mimetic sensitivity. For example, the t(11;14) translocation in multiple myeloma, which puts control of cell cycle-promoting cyclin D1 (*CCND1*) under the IGH promoter, is associated with heightened dependence on BCL-2 for reasons that are unclear. Likewise, increased BCL-2 dependence is also observed in ER-positive metastatic breast cancers with confirmed BCL-2 expression, which is upregulated in a subset of these tumors. Finally, the baseline expression of a BCL-2 family member targeted by a BH3 mimetic, universally across the tumor cells present within a patient, should be a minimal criterion for enrollment of patients.
- What are the most effective strategies for combining BH3 mimetics with other anticancer agents? Although in many cases the most effective chemotherapeutic agents for a given cancer will pair well with an appropriate BH3 mimetic, this will not always be the case. For example, chemotherapeutics that drive nonapoptotic forms of cell death, such as senescence or ferroptosis, may fail to synergize with BH3 mimetics. More broadly, the most synergistic, and potentially best tolerated, BH3 mimetic-containing combination therapies are expected to sometimes comprise agents that, on their own, are not particularly effective because one agent creates a tumor-specific apoptotic vulnerability that is exploited by the second agent. For example, *KRAS* mutant tumor models respond impressively to combinations of MEK inhibitors with BCL-X_L or MCL-1 inhibitors, and *PIK3CA* mutant tumor models similarly respond well to combinations of mTORC1 and BCL-X_L inhibitors, despite the fact that, in each case, the constituent drugs are relatively ineffective on their own [28,67,102]. Indeed, BCL-X_L and MCL-1 appear to cooperatively regulate survival in a wide swath of tumor models, where in many cases selective inhibition of either target alone is ineffective [52].

- What regional variation or temporal dynamics of apoptotic vulnerabilities exist in tumors? Apoptotic vulnerabilities exploitable with BH3 mimetics may oscillate in a stochastic or nonstochastic manner over time and space, potentially impacting clinical sensitivity to these agents. The basis for these effects likely includes changing apoptotic competence during the cell cycle and fluctuating or spatially varying nutrient and growth factor availability, though much work is needed to understand these factors and their clinical impact more fully.
- What are the short- and long-term toxicities associated with therapies that include BH3 mimetics, and how do they vary across heterogeneous patient populations? Defining the toxicities associated with BH3 mimetic agents, particularly when they are combined with targeted or cytotoxic chemotherapies, has to date been a largely empirical exercise. Toxicities observed with single-agent BH3 mimetics to date, including the thrombocytopenia that results from the exquisite sensitivity of platelets to direct BCL-X_L inhibition [114] and the cardiovascular toxicity observed with MCL-1 inhibitors, are consistent with the predictions of models systems [115,116]. Similarly, for the case of BH3 mimetic-based combination therapies, the increased rate of infections seen in patients with multiple myeloma treated with bortezomib plus venetoclax is easily understood [117], whereas the mechanistic basis for arrhythmias associated with combined BTK inhibition and venetoclax is less clear [112]. Finally, toxicities experienced in pediatric patients treated with BH3 mimetics may vary from those evident in adults due to the increased apoptotic sensitivity of cells within growing tissues [9].
- How can BH3 mimetics be refined to improve efficacy while reducing toxicity? The development of the first BH3 mimetic, ABT-737, was a stunning achievement of fragment-based drug discovery that opened the door to what is now a family of BH3 mimetic drugs with impressive potency, selectivity, and bioavailability [26]. However, it is clear that drugs targeting additional players in the mitochondrial apoptotic network, including inhibitors of less-studied BCL-2 family prosurvival proteins such as BFL-1 and BCL-w and direct activators of BAX and BAK, could find utility in circumstances where existing BH3 mimetic drugs fail [52,118]. Furthermore, drugs that circumvent the on-target toxicities of existing BH3 mimetics, such as recently described BCL-X_L degraders that spare platelets, could dramatically improve the depth and breadth of activity of these agents [119]. Finally, methods to achieve tumor-selective MCL-1 modulation, such as by targeting of the protein's upstream regulators, may be a useful strategy for overcoming the on-target toxicities observed with direct MCL-1 inhibitors to date [67].
- Can BH3 mimetics drive genomic instability and mutagenesis? A key consideration when targeting endogenous or imposed apoptotic vulnerabilities with BH3 mimetics is the strength of the dependence that is present in a cancer cell. Although strong endogenous or imposed apoptotic vulnerability can support strong sensitivity to BH3 mimetics, mild dependence due to expression of multiple prosurvival proteins can buffer against inhibition of a single protein in a manner that may ultimately make BH3 mimetics in these settings counterproductive. This is based on the recent discoveries showing that inducing MOMP with BH3 mimetics in only a subset of mitochondria within a cell – a phenomenon referred to as 'minority MOMP' – can lead to submaximal caspase and DNase activation, which can be insufficient to carry out all the execution stages of apoptosis and instead cause DNA mutations and other cellular adaptations that promote tumor progression [120,121].
- How do BH3 mimetics modulate antitumor immune responses? Recent preclinical studies have suggested that BH3 mimetics, including venetoclax, can improve the activity of both immune checkpoint inhibitors and natural killer cell therapies [122,123], exciting developments that nevertheless should be considered with care, given the well-established vulnerability of certain immune populations to these drugs. Furthermore, it is also now clear that therapies that induce MOMP generally represent a 'point of no return' after which cell death is inevitable. However, combining MOMP-inducing agents such as BH3

mimetics with deletion of downstream executioner caspases leads to the profound activation of type I interferon (IFN) signaling in dying cells [124,125]. This is important because tumor-selective type I IFN signaling can augment multiple steps in the tumor-immunity cycle, amplifying the therapeutic activity of diverse immunotherapies [126,127]. Thus, decoupling mitochondrial cell death from caspase-driven immune suppression may be a promising strategy for unlocking the immune stimulatory potential of BH3 mimetic therapies.

Concluding remarks and future perspectives

The convergence of three recent events – the development of selective, potent, and *in vivo* bioavailable BH3 mimetic drugs; our increased understanding of the determinants of apoptotic priming and vulnerabilities; and evidence of striking clinical successes with venetoclax – have together set the stage for the large-scale integration of direct apoptosis-activating therapies into the cancer clinic. In fact, it is not implausible to suggest that patients with a very broad range of cancers could potentially benefit from the simple combination of the most effective chemotherapy for their cancer with an appropriate BH3 mimetic agent for their tumor. Nevertheless, fully achieving the therapeutic potential of BH3 mimetic drugs in the clinic requires that we develop a more complete understanding of the determinants of sensitivity and toxicity along with an improved set of pharmacological tools (see Outstanding questions).

Outstanding questions

What is the complete landscape of apoptotic priming states across defined tumor types and subtypes?

What are the most effective strategies for building BH3 mimetic-based combination therapies containing cytotoxic or targeted chemotherapies?

What are the key toxicity challenges associated with BH3 mimetic-based therapies?

Can improved pharmacological strategies make BH3 mimetics safer and more effective?

How do apoptotic vulnerabilities in tumors vary in space and time?

What is the nature and risk of tumor-promoting effects of BH3 mimetics?

How can BH3 mimetics modulate the immune system, and how does this impact therapeutic outcomes?

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Declaration of interests

K.C.W. is a founder, consultant, and equity holder at Tavros Therapeutics and Celldom and has performed consulting work for Guidepoint Global, Bantam Pharmaceuticals, and Apple Tree Partners. K.A.S. declares no potential conflicts of interest.

References

1. Fuchs, Y. and Steller, H. (2011) Programmed cell death in animal development and disease. *Cell* 147, 742–758
2. Danial, N.N. and Korsmeyer, S.J. (2004) Cell death: Critical control points. *Cell* 116, 205–219
3. Wei, M.C. *et al.* (2001) Proapoptotic BAX and BAK: A requisite gateway to mitochondrial dysfunction and death. *Science* 292, 727–730
4. O'Neill, K.L. *et al.* (2016) Inactivation of prosurvival Bcl-2 proteins activates Bax/Bak through the outer mitochondrial membrane. *Genes Dev.* 30, 973–988 Epub 2016 Apr 7. PMID: 27056669; PMCID: PMC4840302
5. Fraser, C. *et al.* (2019) BH3 profiling: A functional assay to measure apoptotic priming and dependencies. *Methods Mol. Biol.* 1877, 61–76
6. Montero, J. *et al.* (2015) Drug-induced death signaling strategy rapidly predicts cancer response to chemotherapy. *Cell* 160, 977–989
7. Sarosiek, K.A. *et al.* (2013) BID preferentially activates BAK while BIM preferentially activates BAX, affecting chemotherapy response. *Mol. Cell* 51, 751–765
8. Ni Chonghaile, T. *et al.* (2011) Pretreatment mitochondrial priming correlates with clinical response to cytotoxic chemotherapy. *Science* 334, 1129–1133
9. Sarosiek, K.A. *et al.* (2017) Developmental regulation of mitochondrial apoptosis by c-Myc governs age- and tissue-specific sensitivity to cancer therapeutics. *Cancer Cell* 31, 142–156
10. Green, D.R. and Walczak, H. (2013) Apoptosis therapy: Driving cancers down the road to ruin. *Nat. Med.* 19, 131–133
11. Lopez, J. and Tait, S.W. (2015) Mitochondrial apoptosis: Killing cancer using the enemy within. *Br. J. Cancer* 112, 957–962
12. Farlie, P.G. *et al.* (1995) bcl-2 transgene expression can protect neurons against developmental and induced cell death. *Proc. Natl. Acad. Sci. U. S. A.* 92, 4397–4401
13. Kristiansen, M. and Ham, J. (2014) Programmed cell death during neuronal development: The sympathetic neuron model. *Cell Death Differ.* 21, 1025–1035
14. Wensveen, F.M. *et al.* (2010) Apoptosis threshold set by Noxa and Mcl-1 after T cell activation regulates competitive selection of high-affinity clones. *Immunity* 32, 754–765
15. Castillo, J.J. *et al.* (2022) Venetoclax in previously treated Waldenstrom macroglobulinemia. *J. Clin. Oncol.* 40, 63–71
16. DiNardo, C.D. *et al.* (2020) Azacitidine and venetoclax in previously untreated acute myeloid leukemia. *N. Engl. J. Med.* 383, 617–629

17. Roberts, A.W. *et al.* (2016) Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia. *N. Engl. J. Med.* 374, 311–322
18. Villalobos-Ortiz, M. *et al.* (2020) BH3 profiling discriminates on-target small molecule BH3 mimetics from putative mimetics. *Cell Death Differ.* 27, 999–1007
19. Osterlund, E.J. *et al.* (2022) Efficacy and specificity of inhibitors of BCL-2 family protein interactions assessed by affinity measurements in live cells. *Sci. Adv.* 8, eabm7375
20. Adams, C.M. *et al.* (2018) Targeting the Bcl-2 family in B cell lymphoma. *Front. Oncol.* 8, 636
21. Del Gaizo Moore, V. *et al.* (2007) Chronic lymphocytic leukemia requires BCL2 to sequester prodeath BIM, explaining sensitivity to BCL2 antagonist ABT-737. *J. Clin. Invest.* 117, 112–121
22. Ott, G. and Rosenwald, A. (2014) Molecular pathogenesis of follicular lymphoma. *J. Clin. Exp. Hematol.* 54, 23–30
23. Anonymous (2014) Phase I study of ABT-199 (GDC-0199) in patients with relapsed/refractory non-Hodgkin lymphoma: Responses observed in diffuse large B-cell (DLBCL) and follicular lymphoma (FL) at higher cohort doses. *Clin. Adv. Hematol. Oncol.* 12, 18–19
24. Zhao, W.L. *et al.* (2004) Prognostic significance of bcl-xL gene expression and apoptotic cell counts in follicular lymphoma. *Blood* 103, 695–697
25. Vaillant, F. *et al.* (2013) Targeting BCL-2 with the BH3 mimetic ABT-199 in estrogen receptor-positive breast cancer. *Cancer Cell* 24, 120–129
26. Oltersdorf, T. *et al.* (2005) An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* 435, 677–681
27. Abed, M.N. *et al.* (2016) Antagonism of Bcl-XL is necessary for synergy between carboplatin and BH3 mimetics in ovarian cancer cells. *J. Ovarian Res.* 9, 25
28. Corcoran, R.B. *et al.* (2013) Synthetic lethal interaction of combined BCL-XL and MEK inhibition promotes tumor regressions in KRAS mutant cancer models. *Cancer Cell* 23, 121–128
29. Stover, E.H. *et al.* (2019) Pooled genomic screens identify anti-apoptotic genes as targetable mediators of chemotherapy resistance in ovarian cancer. *Mol. Cancer Res.* 17, 2281–2293
30. Joly, F. *et al.* (2022) A phase II study of navitoclax (ABT-263) as single agent in women heavily pretreated for recurrent epithelial ovarian cancer: The MONAVI - GINECO study. *Gynecol. Oncol.* 165, 30–39
31. Gandhi, L. *et al.* (2011) Phase I study of navitoclax (ABT-263), a novel Bcl-2 family inhibitor, in patients with small-cell lung cancer and other solid tumors. *J. Clin. Oncol.* 29, 909–916
32. Rudin, C.M. *et al.* (2012) Phase II study of single-agent navitoclax (ABT-263) and biomarker correlates in patients with relapsed small cell lung cancer. *Clin. Cancer Res.* 18, 3163–3169
33. Lopez, A. *et al.* (2022) Co-targeting of BAX and BCL-XL proteins broadly overcomes resistance to apoptosis in cancer. *Nat. Commun.* 13, 1199
34. Lu, Q.L. *et al.* (1993) Bcl-2 expression in adult and embryonic non-haematopoietic tissues. *J. Pathol.* 169, 431–437
35. Gonzalez-Garcia, M. *et al.* (1995) bcl-x is expressed in embryonic and postnatal neural tissues and functions to prevent neuronal cell death. *Proc. Natl. Acad. Sci. U. S. A.* 92, 4304–4308
36. Taylor, J.K. *et al.* (1999) Inhibition of Bcl-xL expression sensitizes normal human keratinocytes and epithelial cells to apoptotic stimuli. *Oncogene* 18, 4495–4504
37. Peperzak, V. *et al.* (2013) Mcl-1 is essential for the survival of plasma cells. *Nat. Immunol.* 14, 290–297
38. Krajewski, S. *et al.* (1995) Immunohistochemical analysis of Mcl-1 protein in human tissues. Differential regulation of Mcl-1 and Bcl-2 protein production suggests a unique role for Mcl-1 in control of programmed cell death in vivo. *Am. J. Pathol.* 146, 1309–1319
39. Krajewski, S. *et al.* (1994) Immunohistochemical analysis of Mcl-1 and Bcl-2 proteins in normal and neoplastic lymph nodes. *Am. J. Pathol.* 145, 515–525
40. Kotschy, A. *et al.* (2016) The MCL1 inhibitor S63845 is tolerable and effective in diverse cancer models. *Nature* 538, 477–482
41. Morales, A.A. *et al.* (2011) Distribution of Bim determines Mcl-1 dependence or codependence with Bcl-xL/Bcl-2 in Mcl-1-expressing myeloma cells. *Blood* 118, 1329–1339
42. Krajewska, M. *et al.* (2002) Dynamics of expression of apoptosis-regulatory proteins Bid, Bcl-2, Bcl-X, Bax and Bak during development of murine nervous system. *Cell Death Differ.* 9, 145–157
43. Polster, B.M. *et al.* (2003) Postnatal brain development and neural cell differentiation modulate mitochondrial Bax and BH3 peptide-induced cytochrome c release. *Cell Death Differ.* 10, 365–370
44. Shi, J. *et al.* (2012) Downregulation of doxorubicin-induced myocardial apoptosis accompanies postnatal heart maturation. *Am. J. Physiol. Heart Circ. Physiol.* 302, H1603–H1613
45. Kahalley, L.S. *et al.* (2016) Comparing intelligence quotient change after treatment with proton versus photon radiation therapy for pediatric brain tumors. *J. Clin. Oncol.* 34, 1043–1049
46. Pulsifer, M.B. *et al.* (2018) Cognitive and adaptive outcomes after proton radiation for pediatric patients with brain tumors. *Int. J. Radiat. Oncol. Biol. Phys.* 102, 391–398
47. Steen, R.G. *et al.* (2001) Effect of ionizing radiation on the human brain: white matter and gray matter T1 in pediatric brain tumor patients treated with conformal radiation therapy. *Int. J. Radiat. Oncol. Biol. Phys.* 49, 79–91
48. Pei, S. *et al.* (2020) Monocytic subclones confer resistance to venetoclax-based therapy in patients with acute myeloid leukemia. *Cancer Discov.* 10, 536–551
49. Chonghaile, T.N. *et al.* (2014) Maturation stage of T-cell acute lymphoblastic leukemia determines BCL-2 versus BCL-XL dependence and sensitivity to ABT-199. *Cancer Discov.* 4, 1074–1087
50. Dongre, A. and Weinberg, R.A. (2019) New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. *Nat. Rev. Mol. Cell Biol.* 20, 69–84
51. Song, K.A. *et al.* (2018) Epithelial-to-mesenchymal transition antagonizes response to targeted therapies in lung cancer by suppressing BIM. *Clin. Cancer Res.* 24, 197–208
52. Soderquist, R.S. *et al.* (2018) Systematic mapping of BCL-2 gene dependencies in cancer reveals molecular determinants of BH3 mimetic sensitivity. *Nat. Commun.* 9, 3513
53. Grubb, T. *et al.* (2022) A mesenchymal tumor cell state confers increased dependency on the BCL-XL anti-apoptotic protein in kidney cancer. *Clin. Cancer Res.* Published online July 1, 2022. https://doi.org/10.1158/1078-0432.CCR-22-0669
54. Song, J. (2007) EMT or apoptosis: A decision for TGF-beta. *Cell Res.* 17, 289–290
55. Hanahan, D. and Weinberg, R.A. (2011) Hallmarks of cancer: The next generation. *Cell* 144, 646–674
56. Beroukhim, R. *et al.* (2010) The landscape of somatic copy-number alteration across human cancers. *Nature* 463, 899–905
57. Villunger, A. *et al.* (2003) p53- and drug-induced apoptotic responses mediated by BH3-only proteins puma and noxa. *Science* 302, 1036–1038
58. Nakano, K. and Vousden, K.H. (2001) PUMA, a novel proapoptotic gene, is induced by p53. *Mol. Cell* 7, 683–694
59. Yu, J. *et al.* (2001) PUMA induces the rapid apoptosis of colorectal cancer cells. *Mol. Cell* 7, 673–682
60. Sax, J.K. *et al.* (2002) BID regulation by p53 contributes to chemosensitivity. *Nat. Cell Biol.* 4, 842–849
61. Yu, J. *et al.* (2003) PUMA mediates the apoptotic response to p53 in colorectal cancer cells. *Proc. Natl. Acad. Sci. U. S. A.* 100, 1931–1936
62. Dehan, E. *et al.* (2009) betaTrCP- and Rsk1/2-mediated degradation of BimEL inhibits apoptosis. *Mol. Cell* 33, 109–116
63. Luciano, F. *et al.* (2003) Phosphorylation of Bim-EL by Erk1/2 on serine 69 promotes its degradation via the proteasome pathway and regulates its proapoptotic function. *Oncogene* 22, 6785–6793
64. Zha, J. *et al.* (1996) Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X(L). *Cell* 87, 619–628
65. Datta, S.R. *et al.* (1997) Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell* 91, 231–241
66. You, H. *et al.* (2006) FOXO3a-dependent regulation of Puma in response to cytokine/growth factor withdrawal. *J. Exp. Med.* 203, 1657–1663

67. Anderson, G.R. *et al.* (2016) PIK3CA mutations enable targeting of a breast tumor dependency through mTOR-mediated MCL-1 translation. *Sci. Transl. Med.* 8, 369ra175
68. Singleton, K.R. *et al.* (2017) Melanoma therapeutic strategies that select against resistance by exploiting MYC-driven evolutionary convergence. *Cell Rep.* 21, 2796–2812
69. Lin, K.H. *et al.* (2020) Using antagonistic pleiotropy to design a chemotherapy-induced evolutionary trap to target drug resistance in cancer. *Nat. Genet.* 52, 408–417
70. Giacomello, M. *et al.* (2020) The cell biology of mitochondrial membrane dynamics. *Nat. Rev. Mol. Cell Biol.* 21, 204–224
71. Frezza, C. *et al.* (2006) OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. *Cell* 126, 177–189
72. Yamaguchi, R. *et al.* (2008) Opa1-mediated cristae opening is Bax/Bak and BH3 dependent, required for apoptosis, and independent of Bak oligomerization. *Mol. Cell* 31, 557–569
73. Renault, T.T. *et al.* (2015) Mitochondrial shape governs BAX-induced membrane permeabilization and apoptosis. *Mol. Cell* 57, 69–82
74. Chen, H. and Chan, D.C. (2017) Mitochondrial dynamics in regulating the unique phenotypes of cancer and stem cells. *Cell Metab.* 26, 39–48
75. Anderson, G.R. *et al.* (2018) Dysregulation of mitochondrial dynamics proteins are a targetable feature of human tumors. *Nat. Commun.* 9, 1677
76. Kashatus, J.A. *et al.* (2015) Erk2 phosphorylation of Drp1 promotes mitochondrial fission and MAPK-driven tumor growth. *Mol. Cell* 57, 537–551
77. Serasinghe, M.N. *et al.* (2015) Mitochondrial division is requisite to RAS-induced transformation and targeted by oncogenic MAPK pathway inhibitors. *Mol. Cell* 57, 521–536
78. Chen, X. *et al.* (2019) Targeting mitochondrial structure sensitizes acute myeloid leukemia to venetoclax treatment. *Cancer Discov.* 9, 890–909
79. Vaughn, A.E. and Deshmukh, M. (2008) Glucose metabolism inhibits apoptosis in neurons and cancer cells by redox inactivation of cytochrome c. *Nat. Cell Biol.* 10, 1477–1483
80. von Haefen, C. *et al.* (2002) Ceramide induces mitochondrial activation and apoptosis via a Bax-dependent pathway in human carcinoma cells. *Oncogene* 21, 4009–4019
81. Chipuk, J.E. *et al.* (2012) Sphingolipid metabolism cooperates with BAK and BAX to promote the mitochondrial pathway of apoptosis. *Cell* 148, 988–1000
82. Lowman, X.H. *et al.* (2010) The proapoptotic function of Noxa in human leukemia cells is regulated by the kinase Cdk5 and by glucose. *Mol. Cell* 40, 823–833
83. Danial, N.N. *et al.* (2008) Dual role of proapoptotic BAD in insulin secretion and beta cell survival. *Nat. Med.* 14, 144–153
84. Alavian, K.N. *et al.* (2011) Bcl-xL regulates metabolic efficiency of neurons through interaction with the mitochondrial F1FO ATP synthase. *Nat. Cell Biol.* 13, 1224–1233
85. Chen, Y.B. *et al.* (2011) Bcl-xL regulates mitochondrial energetics by stabilizing the inner membrane potential. *J. Cell Biol.* 195, 263–276
86. Lin, K.H. *et al.* (2019) Systematic dissection of the metabolic-apoptotic interface in AML reveals heme biosynthesis to be a regulator of drug sensitivity. *Cell Metab.* 29, 1217–1231.e7
87. Guieze, R. *et al.* (2019) Mitochondrial reprogramming underlies resistance to BCL-2 inhibition in lymphoid malignancies. *Cancer Cell* 36, 369–384 e13
88. Stevens, B.M. *et al.* (2020) Fatty acid metabolism underlies venetoclax resistance in acute myeloid leukemia stem cells. *Nat. Cancer* 1, 1176–1187
89. Jones, C.L. *et al.* (2020) Nicotinamide metabolism mediates resistance to venetoclax in relapsed acute myeloid leukemia stem cells. *Cell Stem Cell* 27, 748–764 e4
90. Daniels, V.W. *et al.* (2021) Metabolic perturbations sensitize triple-negative breast cancers to apoptosis induced by BH3 mimetics. *Sci. Signal.* 14
91. Stahl, M. *et al.* (2021) Oncogenic mechanisms and therapeutic targeting of metabolism in leukemia and lymphoma. *Cold Spring Harb. Perspect. Med.* 11, a035477
92. Kuntz, E.M. *et al.* (2017) Targeting mitochondrial oxidative phosphorylation eradicates therapy-resistant chronic myeloid leukemia stem cells. *Nat. Med.* 23, 1234–1240
93. Molina, J.R. *et al.* (2018) An inhibitor of oxidative phosphorylation exploits cancer vulnerability. *Nat. Med.* 24, 1036–1046
94. Cragg, M.S. *et al.* (2007) Gefitinib-induced killing of NSCLC cell lines expressing mutant EGFR requires BIM and can be enhanced by BH3 mimetics. *PLoS Med.* 4, 1681–1690
95. Deng, J. *et al.* (2007) Proapoptotic BH3-only BCL-2 family protein BIM connects death signaling from epidermal growth factor receptor inhibition to the mitochondrion. *Cancer Res.* 67, 11867–11875
96. Costa, D.B. *et al.* (2007) BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. *PLoS Med.* 4, 1669–1680
97. Gong, Y. *et al.* (2007) Induction of BIM is essential for apoptosis triggered by EGFR kinase inhibitors in mutant EGFR-dependent lung adenocarcinomas. *PLoS Med.* 4, e294
98. Takezawa, K. *et al.* (2011) Role of ERK-BIM and STAT3-survivin signaling pathways in ALK inhibitor-induced apoptosis in EML4-ALK-positive lung cancer. *Clin. Cancer Res.* 17, 2140–2148
99. Faber, A.C. *et al.* (2011) BIM expression in treatment-naive cancers predicts responsiveness to kinase inhibitors. *Cancer Discov.* 1, 352–365
100. Wang, Y.F. *et al.* (2007) Apoptosis induction in human melanoma cells by inhibition of MEK is caspase-independent and mediated by the Bcl-2 family members PUMA, Bim, and Mcl-1. *Clin. Cancer Res.* 13, 4934–4942
101. Wickenden, J.A. *et al.* (2008) Colorectal cancer cells with the BRAF(V600E) mutation are addicted to the ERK1/2 pathway for growth factor-independent survival and repression of BIM. *Oncogene* 27, 7150–7161
102. Nangia, V. *et al.* (2018) Exploiting MCL1 dependency with combination MEK + MCL1 inhibitors leads to induction of apoptosis and tumor regression in KRAS-mutant non-small cell lung cancer. *Cancer Discov.* 8, 1598–1613
103. Wood, K.C. (2020) Overcoming MCL-1-driven adaptive resistance to targeted therapies. *Nat. Commun.* 11, 531
104. Akiyama, T. and Tanaka, S. (2011) Bim: Guardian of tissue homeostasis and critical regulator of the immune system, tumorigenesis and bone biology. *Arch. Immunol. Ther. Exp. (Warsz)* 59, 277–287
105. Okamoto, T. *et al.* (2014) Enhanced stability of Mcl1, a prosurvival Bcl2 relative, blunts stress-induced apoptosis, causes male sterility, and promotes tumorigenesis. *Proc. Natl. Acad. Sci. U. S. A.* 111, 261–266
106. Kamer, I. *et al.* (2005) Proapoptotic BID is an ATM effector in the DNA-damage response. *Cell* 122, 593–603
107. Zinkel, S.S. *et al.* (2005) A role for proapoptotic BID in the DNA-damage response. *Cell* 122, 579–591
108. Kutuk, O. and Letai, A. (2008) Regulation of Bcl-2 family proteins by posttranslational modifications. *Curr. Mol. Med.* 8, 102–118
109. Craxton, A. *et al.* (2012) NOXA, a sensor of proteasome integrity, is degraded by 26S proteasomes by an ubiquitin-independent pathway that is blocked by MCL-1. *Cell Death Differ.* 19, 1424–1434
110. Fraser, C.S. *et al.* (2022) Exploiting endogenous and therapy-induced apoptotic vulnerabilities in immunoglobulin light chain amyloidosis with BH3 mimetics. *Nat. Commun.* 13, 5789
111. Paek, A.L. *et al.* (2016) Cell-to-cell variation in p53 dynamics leads to fractional killing. *Cell* 165, 631–642
112. Montero, J. and Haq, R. (2022) Adapted to survive: Targeting cancer cells with BH3 mimetics. *Cancer Discov.* 12, 1217–1232
113. Letai, A. *et al.* (2022) Functional precision oncology: Testing tumors with drugs to identify vulnerabilities and novel combinations. *Cancer Cell* 40, 26–35
114. Mason, K.D. *et al.* (2007) Programmed anuclear cell death delimits platelet life span. *Cell* 128, 1173–1186
115. Wang, H. *et al.* (2021) Targeting MCL-1 in cancer: Current status and perspectives. *J. Hematol. Oncol.* 14, 67
116. Wang, X. *et al.* (2013) Deletion of MCL-1 causes lethal cardiac failure and mitochondrial dysfunction. *Genes Dev.* 27, 1351–1364
117. Kumar, S.K. *et al.* (2020) Venetoclax or placebo in combination with bortezomib and dexamethasone in patients with relapsed or refractory multiple myeloma (BELLINI): A randomised, double-blind, multicentre, phase 3 trial. *Lancet Oncol.* 21, 1630–1642

118. Spitz, A.Z. and Gavathiotis, E. (2022) Physiological and pharmacological modulation of BAX. *Trends Pharmacol. Sci.* 43, 206–220
119. Khan, S. *et al.* (2019) A selective BCL-XL PROTAC degrader achieves safe and potent antitumor activity. *Nat. Med.* 25, 1938–1947
120. Ichim, G. and Tait, S.W. (2016) A fate worse than death: Apoptosis as an oncogenic process. *Nat. Rev. Cancer* 16, 539–548
121. Ichim, G. *et al.* (2015) Limited mitochondrial permeabilization causes DNA damage and genomic instability in the absence of cell death. *Mol. Cell* 57, 860–872
122. Kohlhapp, F.J. *et al.* (2021) Venetoclax increases intratumoral effector T cells and antitumor efficacy in combination with immune checkpoint blockade. *Cancer Discov.* 11, 68–79
123. Pan, R. *et al.* (2022) Augmenting NK cell-based immunotherapy by targeting mitochondrial apoptosis. *Cell* 185, 1521–1538 e18
124. White, M.J. *et al.* (2014) Apoptotic caspases suppress mtDNA-induced STING-mediated type I IFN production. *Cell* 159, 1549–1562
125. Rongvaux, A. *et al.* (2014) Apoptotic caspases prevent the induction of type I interferons by mitochondrial DNA. *Cell* 159, 1563–1577
126. Giampazolias, E. *et al.* (2017) Mitochondrial permeabilization engages NF-kappaB-dependent anti-tumour activity under caspase deficiency. *Nat. Cell Biol.* 19, 1116–1129
127. Han, C. *et al.* (2020) Tumor cells suppress radiation-induced immunity by hijacking caspase 9 signaling. *Nat. Immunol.* 21, 546–554