



Published in final edited form as:

Leuk Lymphoma. 2014 September ; 55(9): 1980–1992. doi:10.3109/10428194.2013.855307.

Targeting the Apoptosis Pathway in Hematologic Malignancies

Shadia Zaman¹, Rui Wang¹, and Varsha Gandhi¹

¹Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, TX

Abstract

Apoptosis is a cell death program that is well-orchestrated for normal tissue homeostasis and for removal of damaged, old, or infected cells. It is regulated by intrinsic and extrinsic pathways. The intrinsic pathway responds to signals such as ultraviolet radiation or DNA damage and activates “executioner” caspases through a mitochondria-dependent pathway. The extrinsic pathway is activated by death signals induced, for example, by an infection that activates the immune system or receptor-mediated pathways. The extrinsic pathway signals also cascade down to executioner caspases that cleave target proteins and lead to cell death. Strict control of cellular apoptosis is important for the hematopoietic system as it has a high turnover rate. However, the apoptosis program is often deregulated in hematologic malignancies leading to the accumulation of malignant cells. Therefore, apoptosis pathways have been identified for development of anticancer therapeutics. We review here the proteins that have been targeted for anticancer drug development in hematologic malignancies. These include BCL-2 family proteins, death ligands and receptors, inhibitor of apoptosis family proteins, and caspases. Except for caspase activators, drugs that target each of these classes of proteins have advanced into clinical trials.

Keywords

Apoptosis; cancer therapeutics; hematologic malignancies

INTRODUCTION

Apoptosis is a cell death program that is important for normal tissue development and for removal of damaged, old, or infected cells. This pathway is responsible for eradication of 60 billion cells per day from the human body. During apoptosis, the nucleosomal DNA fragments and the cell undergo structural changes whereby the nucleus and cytoplasm condense and break the cell into small membrane-bound structures [1,2]. These structures are engulfed by neighboring cells and are degraded by lysosomal enzymes.

The apoptosis program is deregulated in cancer, leading to malignant cells [3]. The hematopoietic system is particularly sensitive to deregulation in the apoptosis program as

Corresponding author: Varsha Gandhi, Ph.D., Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Unit 1950, Houston, TX 77230, vgandhi@mdanderson.org, Phone: 713-794-2989, Fax: 713-794-4316.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

these cells undergo a high turnover rate. Consequently, these cells have to maintain a tight balance between cell apoptosis and proliferation. Changes in protein expression that upsets this balance lead to accumulation of malignant cells.

In hematologic malignancies, proteins that inhibit apoptosis are often upregulated. One such family of antiapoptotic proteins is BCL-2. The founding member BCL-2 was discovered through cloning of the t(14,18) translocation breakpoint in non-Hodgkins lymphoma which lead to enhanced expression of BCL2 [3,4]. High levels of *BCL2* gene expression have been observed in chronic lymphocytic leukemia (CLL), mostly associated with gene hypomethylation or due to loss of miRNAs [5]. In CLL, *BCL2* is repressed by microRNAs, miR15 and miR16, and inactivating mutations in these non-coding RNAs have been discovered in more than 70% of CLLs [6]. BCL-2 gene amplification have also been found in non-Hodgkins lymphoma [7]. Myeloma patients who have t(11;14) chromosomal abnormality have high levels of BCL-2 [8]. High BCL-2 expression is associated with poor prognosis and resistance to treatment. In large-cell non-Hodgkin lymphomas, BCL-2 rearrangements have been shown to be associated with poor prognosis [9]. High levels of BCL-2 was also associated with resistance to chemotherapy in acute myeloid leukemia patients [10]. Since the discovery of BCL-2 five additional members of this family have been recognized. All of these six proteins were discovered in heme malignancies and their aberrant expression is due to chromosomal translocation, epigenetic modifications, gene amplifications, or miRNA expression.

In addition to the BCL-2 family proteins, apoptosis is inhibited in hematologic malignancies through upregulation of inhibitor of apoptosis (IAP) family of proteins that blocks terminal caspases that are responsible for execution of cell death [11]. Changes in expression levels of IAPs are due to chromosomal translocation, mutations, amplifications, or loss of endogenous inhibitors such as SMAC. For example, in MALT lymphoma, the t(11;18) (q21;q21) translocation leads to the fusion of the BIR domains of cIAP2 with mucosa-associated lymphoid tissue (MALT) 1 with consequent upregulation of NF- κ B signaling and activation of a feed-forward loop that activates cIAP2 and cell survival [12–14]. The IAP proteins are also activated by viral oncoproteins in hematologic malignancies. For example, in adult T-cell leukemia, the human T-cell leukemia virus type 1 (HTLV-1) expresses the oncoprotein Tax that stimulates NF- κ B signaling and cIAP2 expression [15]. The expression of IAPs is correlated with poor prognosis. In newly diagnosed acute myeloid leukemia (AML) patients, a gene expression signature that included cIAP2 was able to accurately predict poor overall survival in the patient population [16]. In childhood *de novo* AML, high XIAP expression was found to be an unfavorable prognostic factor [17,18]. High XIAP expression was also associated with poor response to prednisone in pediatric T-cell acute lymphocytic leukemia (ALL) [19]. In CLL, high expression of XIAP, cIAP1 and cIAP2 is correlated with progressive disease [20]

These data suggest that targeting the apoptosis pathway directly will be an effective therapeutic strategy against hematologic malignancies. In this review, we have reviewed literature that target the BCL-2 protein family, the IAP protein family, death receptors and caspases as anticancer strategy. Research on these proteins has yielded novel therapeutic approaches to inhibit or activate these proteins. In the present review, we discuss these 4

protein families, deregulation of their expression levels in hematological malignancies and anticancer agents that have moved from preclinical application to clinical settings [21].

THE APOPTOSIS PATHWAY

The key proteins responsible for apoptosis are caspases (cysteine aspartyl-specific proteases), a class of cysteine proteases that cleave at sites carboxy terminal to aspartic acid residues in target proteins. The cell death caspases include initiator caspases (caspase-2, -8, -9, -10) and executioner caspases (caspase-3, -6, -7). These caspases are activated by intracellular signals (intrinsic pathway), such as DNA damage, or growth factor and cytokine deprivation, or by extracellular death-inducing signals (extrinsic pathway) produced by cytotoxic T cells of the immune system in response to infected or damaged cells (Figure 1). When the executioner caspases are activated, they cleave substrates such as poly-ADP ribose polymerase (PARP) and inhibitor of caspase-activated DNase, eventually leading to cell death.

The intrinsic cell death pathway is regulated by B-cell lymphoma-2 (BCL-2) family proteins, which have either antiapoptotic or proapoptotic functions (Figure 2). The antiapoptotic BCL-2 proteins inhibit apoptosis by inhibiting proapoptotic BCL-2 family proteins BAX and BAK. On the other hand, the antiapoptotic BCL-2 proteins are themselves inhibited by the BH3-only proteins (BIM, BIK, NOXA, PUMA, BID, BAD, BMF) in response to an intrinsic signal. This results in BAX and BAK oligomerization, leading to mitochondrial membrane permeabilization and release of second mitochondria-derived activator of caspase (SMAC, also called DIABLO) and cytochrome c. Cytochrome c forms a complex with apoptotic protease-activating factor-1 (APAF-1), dATP, and procaspase-9; this complex called the “apoptosome” converts procaspase-9 to active caspase-9. At the same time, SMAC inhibits the inhibitor of apoptosis (IAP) proteins, preventing them from inhibiting procaspase-9 processing and activation [22,23]. Caspase-9 then activates the executioner caspases, leading to cell death.

In the extrinsic pathway, cell death signals are initiated by receptor signaling mediated by the tumor necrosis factor (TNF) family death receptors. Signaling is activated by death ligands (TNF, FASL/CD95L/TNFSF6, TRAIL/APO2L/TNFSF10). When these ligands bind to their respective receptors (TNFR1, FAS/CD95/TNFRSF6, DR4/TNFRSF10A, DR5/TNFRSF10B), they recruit an adaptor protein such as Fas-associated protein with death domain (FADD). The adaptor protein binds the initiator procaspase (procaspase-8, -10) to form a complex called the death-inducing signaling complex (DISC). DISC activates procaspase-8 and procaspase-10, which in turn activate the executioner caspases, leading to cell death. DISC is negatively regulated by c-FLIP, a protein that is homologous to caspase-8 but lacks caspase activity [24]. In some cell types, the extrinsic pathway can also crosstalk with the intrinsic pathway through caspase-8 cleavage of BID (BH3-interacting death domain agonist) to truncated BID (tBID), which translocates to the mitochondria and activates mitochondrial outer membrane permeabilization. In both intrinsic and extrinsic pathways, the cell death signal cascades down to the executioner caspase (caspase-3, -6, and -7), which cleaves substrates, leading to orderly destruction of cells.

TARGETING THE APOPTOSIS PATHWAY

Targeting Antiapoptotic BCL-2 Proteins

BCL-2 is a prosurvival protein which was initially identified in follicular lymphoma and shown to be expressed through chromosomal translocation [4,25,26]. Since then, five other BCL-2 family members (BCL-X_L, BCL-w, MCL-1, BCL-B, and BCL2A1/BFL-1) have been identified; each is homologous to BCL-2 and has similar prosurvival functions. These prosurvival proteins are often overexpressed in hematologic malignancies and therefore, have been targeted for anticancer drug development [27–34].

The antiapoptotic BCL-2 proteins have four regions that are homologous to each other, called BCL-2 homology (BH1, BH2, BH3, and BH4) domains. Nuclear magnetic resonance (NMR) structural studies of BCL-X_L in complex with BAK or BAD revealed that the BH1, BH2, and BH3 domains of BCL-X_L form a hydrophobic groove which binds the α -helical BH3 domain of BAK or BAD [35,36]. This structural information provided the platform for designing BH3 peptidomimetics and small molecules that selectively inhibit BCL-2 family proteins. BCL-2 proteins have also been targeted with antisense oligonucleotides; one of these, oblimersen, has been tested in clinical trials and is described here.

BH3 peptidomimetics—BCL-2 has been targeted with BH3-only peptidomimetics. Since the BH3-only proteins are the natural inhibitors of BCL-2 proteins, peptides are being developed that are homologous to the BH3 domain of antiapoptotic BH3-only proteins. Information obtained from the NMR structural studies of BCL-X_L complexed with the BH3 domain of BAK and BAD facilitated the design of BH3 domain peptidomimetics [35,36]. These studies demonstrated that high affinity between BH3 domain peptides and their target proteins depends on the helicity of the peptides [35]. One approach used to address this issue was designing peptides that mimic the BH3 domain of BID using non-natural amino acids with olefinic side chains that react to form a hydrocarbon “staple” and stabilize the alpha helix. These peptides have greater protease resistance, serum stability and cell permeability than the original BH3 peptide [37,38]. However, the poor pharmacological properties of these peptides have hampered their advancement into clinical trials.

Small-molecule inhibitors—Antiapoptotic BCL-2 proteins have been targeted with small-molecule antagonists. These antagonists have been identified through *in vitro* and *in silico* screens of natural and chemical compound libraries [37]. BCL-2 antagonists function primarily by disrupting the interaction between BCL-2 proteins and BH3-only proteins. These compounds include BH3I compounds, HA14-1, compound 6, antimycin A, theaflavins and epigallocatechins found in black and green tea, respectively, chelerythrine, gossypol and purpurogallin [39–45]. Of the known small-molecule BCL-2 protein inhibitors, only three classes have entered into clinical trials: GX15-070 (obatoclox mesylate), gossypol and its derivatives, and ABT-263 and ABT-199 (Table I).

Obatoclox mesylate is a pan-BCL2 inhibitor. It was developed from a compound identified in a natural compound library that disrupted interactions between BCL-2 family proteins [46]. Preclinical studies with obatoclox demonstrated that it inhibited the growth of cells lines and primary samples in several models such as myeloma, systemic mastocytosis, mast

cell leukemia, acute lymphoblastic leukemia and acute myeloid leukemia [47–51]. Obatoclax-induced apoptosis was associated with release of cytochrome c and activation of the BAX/BAK complex [49]. However, it also induced apoptosis in the absence of BAX/BAK indicating that an apoptosis-independent pathway also contributed to its cytotoxic effects [49,52]. Obatoclax showed single agent activity in xenograft models of lymphoma [53]. It overcame drug resistance induced by the stromal microenvironment in leukemic cells [50,54]. It was also cytotoxic in cell lines resistant to drugs such as dexamethasone, melphalan and rituximab [50,55,56]. Combination studies with obatoclax have been more successful both in *in vitro* and *in vivo* models. Obatoclax has been shown to synergize with chemotherapy, NVP-BE2235 (PI3K inhibitor), PKC412 (KIT inhibitor), dexamethasone, to name a few [47,48,50,51,55].

Phase I trials have determined the toxicity profile and maximum tolerated dose for obatoclax [57,58]. The most common adverse event was transient neurotoxicity. In phase I studies for patients with refractory CLL, one of 26 patients (4%) achieved a partial response [57]. Patients also displayed activation of apoptosis characterized by activation of BAX and BAK in circulating mononuclear cells (12 of 26 patients), increase in oligonucleosomal DNA fragments in the plasma, and decrease in circulating lymphocytes (18 of 26 patients). Phase I study in patients with advanced hematologic malignancies resulted in one patient with acute myeloid leukemia achieving complete remission and 3/14 patients showed hematologic improvements [58]. However, phase II studies in 2 different trials with obatoclax mesylate in patients with myelofibrosis and relapsed or refractory classic Hodgkin Lymphoma (cHL) did not demonstrate single agent activity [59,60]. Combination treatment with obatoclax has shown more promise. Several combination trials have been initiated, which include combination with bortezomib in mantle cell lymphoma with 3/12 complete remissions, combination with bortezomib in multiple myeloma with 4/10 partial responses, and combination with fludarabine and rituximab in CLL with 54% overall response rate [61]. Obatoclax has been tested in mantle cell lymphoma, follicular lymphoma, Hodgkin's lymphomas, AML, multiple myeloma, and myelofibrosis, either as a single agent or in combination [37].

Gossypol is a natural phenol derived from the cotton plant (*Gossypium*). Gossypol, as well as its more potent R-enantiomer ((-)-gossypol, AT-101) have antiproliferative properties [62–65]. AT-101 has been further developed to increase its affinity to BCL-2, producing TW37, TM-1206, acylpyrrogallol derivative apogossypolone, and an apogossypol analog called sabutoclax [66]. These products are currently in preclinical development [37]. These compounds bind directly to BCL-2, BCL-X_L, and MCL-1 to displace BH3 peptides [44]. Preclinical studies in hematologic malignancies such as lymphoma and CLL demonstrated that gossypol and its analogs were cytotoxic in cell lines and primary patient samples [63,64,67,68]. Additional mouse studies for lymphoma also demonstrated the efficacy of gossypol analogs to reduce tumor weight [67,69]. Cytotoxicity mediated by gossypol and its analogs occurred in a BAX/BAK-dependent manner [63,64]. However, gossypol can also induce cell death in a BAX/BAK-independent manner [70]. In CLL, AT-101 abrogates microenvironment-mediated resistance [68]. Of the gossypol analogs, AT-101 has advanced into clinical trials for hematologic malignancies.

Single-agent AT-101 phase II trial for relapsed/refractory B-cell malignancies, which included follicular lymphoma, diffuse large cell lymphoma, mantle cell lymphoma, small lymphocytic leukemia, and CLL has been completed, but the results have not yet been reported. Single agent study have been followed by combination studies with rituximab in CLL and follicular lymphoma.

ABT-737 is a small molecule that was discovered in a chemical library screen using NMR structure-based computational tools to identify compounds that bind the hydrophobic BH-3 binding groove of BCL-X_L. This method identified lead compounds that were modified for cellular availability. ABT-737 binds with high affinity to BCL-X_L, BCL-2, and BCL-w but not to BCL-B, MCL-1, or BCL2A1 [71]. It is considered a true BCL-2 inhibitor because it cannot induce cell death in BAX/BAK double-knockout mouse embryonic fibroblasts [72]. It has been further developed to produce a clinical candidate, ABT-263 (navitoclax), which is orally bioavailable with more favorable pharmacological properties than ABT-737. Navitoclax targets BCL-2, BCL-X_L, and BCL-w and functions by disrupting their interaction with pro-death proteins such as BIM [73]. It is active as a single agent in *in vitro* models of hematologic malignancies such as CLL and multiple myeloma. However, in mouse studies, ABT-263 was active as a single agent only in mouse model of acute lymphocytic leukemia but not in diffuse large B-cell lymphoma, mantle cell lymphoma or multiple myeloma. In these models, ABT-263 was more effective in combination with rituximab (for diffuse large B-cell lymphoma), R-CHOP (combination of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone for mantle cell lymphoma) and bortezomib (for multiple myeloma). These preclinical studies also revealed that ABT-263 reduced the platelet count predicting thrombocytopenia in patients. Two phase I studies in lymphoid malignancies have been conducted and reported [74,75]. In the initial phase I study in relapsed/refractory lymphoid malignancies which established the safety, pharmacokinetics and pharmacodynamics of navitoclax, the maximum tolerated dose was determined. In this study, 10 out of 46 evaluable patients achieved partial response. In phase I trials in CLL, nine of 26 patients had a partial response and seven showed stable disease for over 6 months [74]. Moreover, 19 of 21 patients showed more than 50% reduction in lymphocytosis. Even patients with fludarabine-refractory disease, bulky adenopathy, and a del17p chromosomal abnormality showed activity. Even though ABT-263 showed response in lymphoid malignancies, its activity was limited as a single agent, but these initial studies have supported the initiation of phase II combination studies [61]. A major dose-limiting toxicity with ABT-263 is thrombocytopenia, which is induced due to inhibition of BCL-X_L in megakaryocytes resulting in diminished production of platelets [76]. Navitoclax has been developed further to produce ABT-199 that does not inhibit BCL-X_L and therefore, does not result in thrombocytopenia [77]. Preclinical studies demonstrated that ABT-199 was cytotoxic for cell lines that were dependent on BCL-2 for survival but not on BCL-X_L. It selectively disrupted BCL-2-BIM complexes but was less effective on BCL-X_L-BCL-X_S or MCL-1-NOXA complexes. In xenograft models, ABT-199 inhibited tumor growth in acute lymphoblastic leukemia and lymphoma mouse models. Combination treatment with rituximab or rituximab and bendamustine was more efficacious than the single agent. While a clinical trial for CLL patients is ongoing, results for 3 patients have been reported. There was reduction in lymphadenopathy within 24 hours of drug administration and 2 patients

achieved > 95% reduction in peripheral blood lymphocytes. All 3 patients demonstrated tumor lysis syndrome. There was no major organ dysfunction. However, one patient did develop mild disseminated intravascular coagulation due to tumor lysis. In general, the effect on platelet count was minor compared to navitoclax. However, in an update on the study in CLL patients at ASCO 2013 indicated there was one fatality due to tumor lysis syndrome. Therefore, dosing and scheduling of ABT-199 need to be modified to reduce tumor lysis syndrome.

One of the BCL-2 proteins, MCL-1, plays an important role in defective cell death in B-cell malignancies, such as CLL and multiple myeloma, and is a source of resistance to chemotherapeutic agents [78]. *In vitro* studies in CLL demonstrated that decreasing MCL-1 levels with chemical inhibitors such as dasatinib or roscovitine increased the antitumor activity of ABT-737 [79]. This highlights the need to develop drugs that directly target MCL-1. However, none of the current agents binds MCL-1 with high affinity [80]. A few compounds in preclinical studies designed from the BH3 domain of BIM or MCL-1 show MCL-1-dependent proapoptotic activity and have the potential to be developed as MCL-1 inhibitors [81,82].

Drugs targeting BCL-2 mRNA—BCL-2 has been targeted with oblimersen, an antisense oligonucleotide that reduces the level of *BCL-2* mRNA. Oblimersen is an 18-base, single-stranded phosphorothioate oligonucleotide designed to target the first six codons of the open reading frame of *BCL-2* mRNA. The first clinical use of oblimersen was in patients with non-Hodgkin lymphoma (NHL), in whom it was well tolerated and showed antitumor activity [83]. Some of these patients demonstrated down-regulation of BCL-2 protein. Down-regulation of BCL-2 mRNA and protein levels were also observed in patients in a phase I study involving untreated older patients with acute myeloid leukemia. In this trial, patients who achieved complete response demonstrated a decrease in BCL-2 mRNA and protein levels [84]. In CLL patients with advanced disease, a phase I/II trial with oblimersen showed modest single-agent activity: 8% of patients (2 of 26) achieved partial response [85]. However, high doses induced a cytokine and CpG-mediated immune response suggesting non-targeted effect. This trial established the maximum tolerated dose at 3 mg/kg/day. Oblimersen advanced to phase III trial in CLL patients with relapsed/refractory disease [86]. In this trial, the investigators examined the primary end-point of complete response (CR) or nodal partial response (nPR) comparing oblimersen in combination with fludarabine and cyclophosphamide (FCO) to FC only. In this trial, the FCO group achieved 17% CR+nPR versus 7% in the FC group. However, in fludarabine-sensitive patients, 25% achieved CR/nPR in the FCO regimen versus 6% in the FC group. For those patients who achieved CR/nPR, the 3 years overall survival was 70% in FCO versus 38% in FC group. The 5 year overall survival was 47% in FCO arm versus 24% in FC arm [87]. Oblimersen has also been tested in chronic myelogenous leukemia, acute lymphoblastic leukemia, and multiple myeloma. In myeloma, the initial phase II combination of oblimersen with dexamethasone and thalidomide was well tolerated and exhibited clinical response in patients with relapsed disease [88,89]. Phase II studies in VAD (vincristine, doxorubicin, and dexamethasone)–refractory myeloma also demonstrated clinical response [90]. In a phase III study, however, a combination of oblimersen with dexamethasone showed no significant advantage over

dexamethasone alone in patients with advanced myeloma [91]. Phase III trials in multiple myeloma, acute myeloid leukemia, CLL, and melanoma did not demonstrate overall survival. The drug has not been approved by the Food and Drug Administration, however, these studies set precedence for targeting BCL-2 protein in hematologic malignancies.

Targeting Death Ligands and Death Receptors

The extrinsic pathway is a promising target for anticancer drug development. It induces cell death independent of p53 and therefore is active in p53-mutated cells. Overexpression of BCL-2 proteins is common in B-cell malignancies and can result in resistance to agents that induce apoptosis through the intrinsic pathway. Targeting the extrinsic pathway will therefore bypass mutations in the intrinsic pathway. Moreover, oncogenes such as *MYC* and *RAS* increase tumor sensitivity to the extrinsic pathway [92].

The extrinsic pathway became a focus for anticancer therapeutics after the discovery that TNF induces cell suicide [93]. This discovery led to various attempts to stimulate apoptosis in cancer cells by activating TNF signaling. However, TNF also activates the NF- κ B pathway and induces expression of genes that regulate host defenses and immune responses. Therefore, initial attempts to activate the pathway by designing agents that activate through TNF met with failure because of the inflammatory response induced by activation of the NF- κ B pathway. Both FAS and TNF induced toxic effects in preclinical studies, limiting their development as anticancer therapeutics [94]. Current efforts are focusing on targeting TRAIL because of its relatively low toxicity profile [95,96].

TRAIL binds to receptors DR4, DR5, DcR1 (TRID), and DcR2 [97–99]. Of these, DR4 and DR5 mediate death signals to activate apoptosis. DcR1 and DcR2 are decoy receptors that do not have an intracellular death domain and inhibit TRAIL signaling. The TRAIL pathway has been targeted with proapoptotic receptor agonists, which comprise two types: recombinant human (rh)Apo2L/TRAIL, which activates both DR4 and DR5, and monoclonal agonistic antibodies that target either DR4 or DR5. Proapoptotic receptor agonists that target DR4 or DR5 have been well tolerated and have entered into clinical trials [92].

Recombinant human Apo2L/TRAIL has been tested in leukemia and lymphoma models and demonstrated cytotoxicity against cancer cells without being toxic to normal cells [100–102]. Phase I studies of rhApo2L/TRAIL (dulanermin) in advanced solid tumors and NHL (n = 71) indicated a favorable toxicity profile [103]. Two patients, both with chondrosarcoma, achieved partial response. Biomarker analysis confirmed that dulanermin activated apoptosis, as reflected by statistically significant increases in serum caspase-3 and -7 levels, in patients with colorectal cancer or sarcoma [104]. A phase Ib trial of a combination of dulanermin and rituximab in relapsed, low-grade NHL was well tolerated with three complete responses and three partial responses (out of 11 patients) [92]. Dulanermin was moved into phase II trials in non-small cell lung cancer (NSCLC) after phase Ib studies showed favorable toxicity and an overall response rate of 58% when combined with paclitaxel, carboplatin, and bevacizumab [105]. However, these phase II studies showed that adding dulanermin to this regimen did not yield any benefit for patients [106].

Some of the agonist antibodies targeting DR4 or DR5 have advanced into clinical trials. For example, mapatumumab targets DR4, while lexatumumab, drozitumab, AMG655, CS-1008, and LBY-135 target DR5 (Table I). AMG655 (conatumumab), a fully human monoclonal agonist antibody to DR5, demonstrated antitumor activity in *in vitro* models of colon, pancreatic, and lung cancers [107]. In phase I studies in advanced solid tumors, AMG655 was well tolerated [108]. One patient with NSCLC achieved partial response with 48% reduction in tumor size, and an additional 14 patients achieved stable disease. Phase II combination studies have been completed with conatumumab. Combination studies were in soft tissue sarcoma (combined with doxorubicin), in metastatic pancreatic cancer (combined with gemcitabine), in NSCLC (combined with carboplatin and paclitaxel) and in metastatic colorectal carcinoma with FOLFIRI (combination of folinic acid, fluorouracil, and irinotecan) [109–112]. Even though combination with gemcitabine showed some activity in 6-month survival rate, and combination with FOLFIRI showed a trend towards progression free survival, these studies did not demonstrate any significant improvement in disease outcome. AMG655 was combined with bortezomib or vorinostat for lymphoma, but these studies were suspended.

Mapatumumab targets DR4 and exhibited a favorable toxicity profile in phase I clinical trials for advanced solid tumors [113]. It has shown promise in lymphoma. In phase Ib/II studies in relapsed/refractory NHL, two patients achieved complete response and one patient achieved partial response [114]. A phase II study of mapatumumab in combination with bortezomib in multiple myeloma has been completed, but the results have not yet been reported. Lexatumumab, another agonist monoclonal antibody, targets DR5. Phase I studies in advanced solid tumors and in children with solid tumors have been completed, and some patients achieved stable disease [115–117].

Drozitumab (PRO95780/ Apomab) is an agonistic monoclonal antibody against DR5 [118]. This agent is in phase I clinical trials in patients with advanced malignancies. Drozitumab was found to have a good safety profile and showed antitumor activity in patients with colorectal cancer, granulosa cell ovarian cancer, or chondrosarcoma [92,119]. It is being tested in combination with rituximab in a phase II trial in NHL. Other antibodies against DR5 include LBY-135, a chimeric (mouse/human) monoclonal antibody, and CS-1008 (tigatuzumab), a humanized monoclonal antibody [92]. Phase I studies indicated that both are well tolerated. Seven of 17 patients (16 with advanced solid tumor and one with lymphoma) who received CS-1008 showed stable disease [120]. In phase I studies with single agent LBY-135, one sarcoma patient showed minor response and two patients (one with NSCLC, one with prostate cancer) showed decrease in antitumor markers [121].

TAS266 is a tetrameric DR5 targeted agonist that uses nanobody technology [122]. This technology is superior to current dimeric agonist that activate DR5 because dimeric agonist have to cross-link to Fc α receptors on immune cells in the tumor microenvironment to cluster DR5 receptors into the active state. Because immune cell content is heterogeneous in the tumor microenvironment the potency of these dimeric agonists that depend on cross-linking is variable. However, TAS266 uses nanobodies that have single, high affinity heavy chain domain (V_{HH}) that can form multivalent structures. In preclinical studies, it activated caspases and was more cytotoxic than TRAIL and cross-linked DR5 agonist. It induced

tumor regression in xenograft model and in patient-derived primary pancreatic tumor model. First in-man human trials began in 2012 in advanced solid tumors. However, the trial has been terminated and the results have not yet been reported.

Targeting Inhibitor of Apoptosis Proteins

The IAP proteins contribute to cell survival. There are eight IAP proteins: cIAP1 (cellular IAP1), cIAP2, XIAP (X chromosome-linked IAP), NAIP (neuronal apoptosis inhibitory protein), ML-IAP (melanoma IAP), survivin, Apollon, and ILP2 (IAP-like protein 2) (Figure 3). These proteins have a 70- to 80-amino-acid zinc-binding domain called baculovirus IAP repeat (BIR), which mediates protein-protein interactions and is important for inhibition of apoptosis. Some members of the IAP protein family (cIAP1, cIAP2, ML-IAP, XIAP) have a RING (really interesting new gene) domain, which has E3 ubiquitin ligase activity. Each of the IAPs has specialized functions. XIAP is a direct inhibitor of caspase-3, -7 and -9. IAPs with a RING domain can regulate protein stability. cIAPs also inhibit SMAC, preventing it from inhibiting XIAP. cIAP1 and cIAP2 stimulate cell survival by activating NF- κ B signaling.

Studies have demonstrated that high expression of cIAP1, cIAP2, XIAP or survivin mRNA or protein is associated with shorter survival or poor clinical outcome [16,18,123,124]. High expression of XIAP or cIAP1 results in poor response to chemotherapeutic agents and resistance to apoptosis [17,19,125]. Genetic mutations have been identified that deregulate the expression of the IAP proteins. cIAP2 is often found to be fused to MALT1 in lymphoma, resulting in activation of NF- κ B signaling [126,127]. IAP protein activity has been inhibited with antisense oligonucleotides and small-molecule inhibitors or SMAC mimetics (Table I).

Targeting IAP mRNA—AEG35156 is an antisense oligonucleotide designed to selectively target XIAP, the only IAP family member that directly inhibits caspase-3, -7, and -9. Phase I studies of AEG35156 in advanced refractory cancer demonstrated that it was well tolerated [128]. It has also been tested in a phase I/II clinical trial in combination with induction therapy with idarubicin and cytarabine for relapsed or refractory AML. In this trial, 47% of patients receiving high-dose AEG35156 achieved complete response or complete response with incomplete platelet count recovery [129]. Administration of AEG35156 was correlated with decrease in XIAP mRNA and protein levels and induction of apoptosis in CD34⁺38⁻ AML stem cells [129,130]. However, a subsequent randomized phase II study of AEG35156 in combination with re-induction chemotherapy with cytarabine and idarubicin in AML showed no improvement in remission rate compared to treatment with cytarabine and idarubicin alone and the study was terminated early due to lack of benefit [131,132]. There are currently no active trials with AEG35156.

Survivin is another good target for drug development. It is differentially expressed between normal and cancerous tissues [133]. Studies have indicated high survivin expression in tumors associated with Hodgkin's lymphoma, non-Hodgkin's lymphoma and leukemia [133]. It is both a prognostic and predictive tumor marker [134,135]. It has been targeted with antisense oligonucleotides (LY2181308) and, transcriptionally, with YM155, an

inhibitor of survivin promoter activity. In phase I clinical studies with YM155 involving 41 patients with advanced solid malignancies or lymphoma, one complete response and two partial responses were observed in patients with NHL [134]. A phase II study in diffuse large B-cell lymphoma patients with refractory disease has also been completed. However, low response rate (one patient out of 41 (2.4%) with complete remission) lead to early termination of the study [136]. However, preclinical studies suggested that YM155 might be efficacious in combination with rituximab and based on these data a phase II trial of YM155 in combination with rituximab is currently ongoing in NHL patients [136]. The antisense oligonucleotide LY2181308 (Gataparsen) was demonstrated to be well tolerated in advanced solid tumors and phase II trials as a single agent and in combination with cytarabine and idarubicin for AML have recently been completed [135,137–139]. In this study, LY2181308 was found to be well tolerated in AML patients and survivin expression was reduced in patients expressing high levels of survivin. In the combination arm, 4/16 patients achieved complete remission; these results therefore warrant further investigation of the drug.

As another approach, spontaneous anti-survivin T-cell reactivity has been observed in patients with solid tumors, leukemia, or lymphoma. Therefore, it has been used as a target for vaccine development against cancer, which is in phase I/II trial in myeloma patients [135,140].

SMAC mimetics—SMAC inhibits IAP proteins and functions as a proapoptotic protein. SMAC has been a target for drug development because expression of SMAC at low levels has been correlated with poor prognosis in solid tumors [141–143]. In AML, high expression of SMAC has been correlated with longer overall survival and higher complete remission rates [144]. The crystal structure of SMAC complexed with XIAP has been solved [145–147]. These studies demonstrated that SMAC inhibits XIAP through interaction of a four-amino-acid peptide motif (AVPI) on SMAC with the BIR3 domain of XIAP. This interaction between SMAC and XIAP has been exploited through design of SMAC peptidomimetics or small molecules that inhibit SMAC and IAP interaction. SMAC peptidomimetics have antitumor activity in cell culture models, but their poor pharmacological properties prevented their development for therapeutic applications [148,149]. Small-molecule SMAC mimetics that have better pharmacological properties are now being developed.

The crystal structure of the XIAP BIR3 domain in complex with the SMAC AVPI peptide has been determined and is being used to design SMAC mimetics that potently inhibit XIAP, cIAP1, cIAP2 and ML-IAP [145,147]. Several monovalent and bivalent SMAC mimetics have been designed that are cytotoxic in antitumor assays [150–153]. Mechanistic studies have demonstrated that these monovalent and bivalent SMAC mimetics activate the E3 ubiquitin ligase activity of cIAP1 and cIAP2 that induce autoubiquitylation and degradation of cIAPs [154,155]. The E3 ubiquitin ligase activity of cIAPs activates both the canonical and non-canonical NF- κ B signaling pathways. cIAPs ubiquitylates RIP1 and activates the canonical NF- κ B pathway which induces TNF α expression and TNF-R1 signaling. At the same time, when the cIAP proteins are degraded, RIP1 can no longer be ubiquitylated and it forms a complex with FADD and caspase-8 to activate apoptosis. Therefore, the E3 ubiquitin ligase activity of cIAP proteins converts the prosurvival TNF

signaling to a pro-apoptotic signaling. cIAP degradation also leads to the stabilization of NIK (NF- κ B-inducing kinase) and activation of the non-canonical NF- κ B pathway.

The SMAC mimetic that has moved furthest in clinical trials is AT-406, which inhibits XIAP, cIAP1, and cIAP2 at nanomolar concentrations [156]. In preclinical studies, AT-406 showed favorable pharmacokinetic properties in mice, rats, nonhuman primates, and dogs, and was orally bioavailable. It decreased tumor volume without decreasing body weight in a breast cancer model and improved survival rates in a mouse ovarian cancer model [156,157]. It is currently in phase I clinical trials in advanced solid tumors and lymphomas.

TL32711 is a bivalent small-molecule SMAC mimetic that inhibits IAPs [158]. It was well tolerated in a phase I clinical trial in advanced solid tumors and lymphomas. Administration of TL32711 at doses 1.44 mg/m² decreased cIAP1 levels >80% within 24 hours. Cleaved CK-18 and activated caspase-3/7 was observed in serum of patients treated at doses of 2.88 mg/m². Lysate prepared from tumor biopsy of a melanoma patient treated with TL32711 demonstrated >90% cIAP1 suppression, high levels of activated caspase-8 and PARP cleavage. A phase I/II study in AML, myelodysplastic syndrome and acute lymphoblastic leukemia is currently ongoing.

GDC-0917 is a monovalent SMAC mimetic for which a phase I clinical trial in advanced solid tumors or lymphoma has recently been completed (NCT01226277). LCL161 is a SMAC mimetic that showed antitumor activity in preclinical studies in leukemia [159,160]. It is currently in phase I studies in solid tumors. HGS1029 is a bivalent SMAC mimetic that is currently in phase I clinical trials for solid tumors. Reports from phase I studies have indicated that HGS1029 is well tolerated [161].

Activating Caspases

Apoptosis occurs through a cascade of events involving members of the caspase family of cysteine proteases. Caspases are synthesized as zymogens and are activated by proteolysis. Of particular importance is procaspase-3, which serves as an “executioner” procaspase in both the intrinsic and extrinsic cell death pathways. Procaspase-3 is expressed at elevated levels in colon and liver cancers, neuroblastomas, lymphomas, leukemias, and melanomas [162]. In colon cancer, the cancerous cells expressed higher levels of procaspase-3 than surrounding noncancerous cells [163]. This indicates that there is a therapeutic index for targeting procaspase-3. Targeting procaspase-3 can also overcome resistance to apoptosis through mutations or altered expression of key upstream proteins in the apoptotic cascade.

Caspases have been activated in cancer cells by various strategies. In one approach, a chimeric protein in which anti-erbB2/HER2 antibody was fused to a C-terminal constitutively active caspase-3 was designed to selectively kill cancer cells overexpressing HER2 [164]. This chimeric protein, called immunocasp3, showed antitumor activity and improved the survival rate of mice transplanted with SKBR-3 HER2-positive human tumor cells. Fusion of active caspase-6 to anti-erbB2/HER2 antibody also induced apoptosis in HER2-positive tumor cells [165]. Caspase-3 has been targeted with PEF-F8-CP3, a caspase-3 fusion construct with a single chain antibody [166]. Specific activation of caspases in tumor cells has been achieved with inducible caspase-3 (Ad-G/iCasp3) and

inducible caspase-9 (FKBP12/Casp-9 or iCasp9), which are regulated by cell-permeable chemical inducers of dimerization to selectively kill cancer cells [167,168]. Besides these, several small-molecule activators of caspase and RGD peptides are being developed by Maxim, Gemin X, and Merck-Frosst [169]. All of these agents are in preclinical studies.

Among the small-molecule activators, two agents have shown promise in inducing apoptosis: compound 1541 and procaspase-activating compound (PAC-1), both of which target procaspase-3. Compound 1541 enhanced susceptibility of procaspase-3 to proteolysis by stabilizing a conformation that exposes the internal cleavage site and makes it more accessible to proteolysis [170]. In *in vitro* assays, compound 1541 activated procaspase-3 at a concentration of 2.4 μ M. It was active against a number of cell lines, such as BT549, HCC1954, MB361, 600MPE, HeLa, and HEK293. In contrast, it was not active in MCF7, a cell line with caspase-3 mutation, indicating the specificity of its action. It also induced apoptosis in BAX and BAK double-knockout mouse embryonic fibroblasts, highlighting that it can bypass defects in the apoptosis cascade and still induce cell death.

PAC-1 is another agent that is being developed as a procaspase-3 activator [162]. It activates procaspase-3 by chelating inhibitory zinc ions from procaspase-3, allowing it to autoactivate to caspase-3 [171]. *In vivo* studies with PAC-1 in mice demonstrated that it was well tolerated; it exhibited antitumor activity in mice transplanted with ACHN renal cancer cell line or NCI-H226 lung cancer cell line [162]. A small clinical trial with a PAC-1 analog, S-PAC-1, in dogs with spontaneous lymphoma demonstrated that it was well tolerated, and either partial response or stable disease was achieved in four of the six dogs treated [172]. This indicates that PAC-1 and its analogs have potential as anticancer agents.

CONCLUSIONS

Hematologic malignancies are characterized by deregulation of the apoptosis program that leads to the accumulation of malignant cells. Therefore, targeting the apoptosis pathway is an effective anticancer strategy. Cell death pathways include extrinsic and intrinsic routes with involvement of proteins that either facilitate or prevent apoptosis (Figure 1). Therefore, key families of proteins have been identified and targeted in hematologic malignancies. Four major nodes that have resulted in design of specific chemotherapeutic agents include BCL-2 family antiapoptotic protein antagonists, cell death pathway-activating ligand and receptor agonists, IAP and SMAC mimetics, and executioner caspase activators. Many of these strategies are being tested in clinical investigations and are providing encouraging data.

Acknowledgments

This work was supported in part by grants CLL PO1 CA81534 from the National Cancer Institute, Department of Health and Human Services and a Global Research Foundation European Alliance award. We thank Kathryn L. Hale from the Department of Scientific Publications at The University of Texas MD Anderson Cancer Center for editorial help in preparing this manuscript.

REFERENCES

1. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer*. 1972; 26:239–257. [PubMed: 4561027]

2. Nagata S. Apoptotic DNA fragmentation. *Exp Cell Res.* 2000; 256:12–18. [PubMed: 10739646]
3. Reed JC. Bcl-2-family proteins and hematologic malignancies: history and future prospects. *Blood.* 2008; 111:3322–3330. [PubMed: 18362212]
4. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. Involvement of the bcl-2 gene in human follicular lymphoma. *Science.* 1985; 228:1440–1443. [PubMed: 3874430]
5. Hanada M, Delia D, Aiello A, Stadtmauer E, Reed JC. bcl-2 gene hypomethylation and high-level expression in B-cell chronic lymphocytic leukemia. *Blood.* 1993; 82:1820–1828. [PubMed: 8104532]
6. Cimmino A, Calin GA, Fabbri M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci U S A.* 2005; 102:13944–13949. [PubMed: 16166262]
7. Monni O, Joensuu H, Franssila K, Klefstrom J, Alitalo K, Knuutila S. BCL2 overexpression associated with chromosomal amplification in diffuse large B-cell lymphoma. *Blood.* 1997; 90:1168–1174. [PubMed: 9242549]
8. Bodet L, Gomez-Bougie P, Touzeau C, et al. ABT-737 is highly effective against molecular subgroups of multiple myeloma. *Blood.* 2011; 118:3901–3910. [PubMed: 21835956]
9. Yunis JJ, Mayer MG, Arnesen MA, Aeppli DP, Oken MM, Frizzera G. bcl-2 and other genomic alterations in the prognosis of large-cell lymphoma. *N Engl J Med.* 1989; 320:1047–1054. [PubMed: 2648153]
10. Campos L, Rouault JP, Sabido O, et al. High expression of bcl-2 protein in acute myeloid leukemia cells is associated with poor response to chemotherapy. *Blood.* 1993; 81:3091–3096. [PubMed: 7684624]
11. Fulda S, Vucic D. Targeting IAP proteins for therapeutic intervention in cancer. *Nat Rev Drug Discov.* 2012; 11:109–124. [PubMed: 22293567]
12. Akagi T, Motegi M, Tamura A, et al. A novel gene, MALT1 at 18q21, is involved in t(11;18)(q21;q21) found in low-grade B-cell lymphoma of mucosa-associated lymphoid tissue. *Oncogene.* 1999; 18:5785–5794. [PubMed: 10523859]
13. Dierlamm J, Baens M, Wlodarska I, et al. The apoptosis inhibitor gene API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21) associated with mucosa-associated lymphoid tissue lymphomas. *Blood.* 1999; 93:3601–3609. [PubMed: 10339464]
14. Morgan JA, Yin Y, Borowsky AD, et al. Breakpoints of the t(11;18)(q21;q21) in mucosa-associated lymphoid tissue (MALT) lymphoma lie within or near the previously undescribed gene MALT1 in chromosome 18. *Cancer Res.* 1999; 59:6205–6213. [PubMed: 10626814]
15. Waldele K, Silbermann K, Schneider G, Ruckes T, Cullen BR, Grassmann R. Requirement of the human T-cell leukemia virus (HTLV-1) tax-stimulated HIAP-1 gene for the survival of transformed lymphocytes. *Blood.* 2006; 107:4491–4499. [PubMed: 16467195]
16. Hess CJ, Berkhof J, Denkers F, et al. Activated intrinsic apoptosis pathway is a key related prognostic parameter in acute myeloid leukemia. *J Clin Oncol.* 2007; 25:1209–1215. [PubMed: 17401010]
17. Sung KW, Choi J, Hwang YK, et al. Overexpression of X-linked inhibitor of apoptosis protein (XIAP) is an independent unfavorable prognostic factor in childhood de novo acute myeloid leukemia. *J Korean Med Sci.* 2009; 24:605–613. [PubMed: 19654940]
18. Tamm I, Richter S, Oltersdorf D, et al. High expression levels of x-linked inhibitor of apoptosis protein and survivin correlate with poor overall survival in childhood de novo acute myeloid leukemia. *Clin Cancer Res.* 2004; 10:3737–3744. [PubMed: 15173080]
19. Hundsdoerfer P, Dietrich I, Schmelz K, Eckert C, Henze G. XIAP expression is post-transcriptionally upregulated in childhood ALL and is associated with glucocorticoid response in T-cell ALL. *Pediatr Blood Cancer.* 2010; 55:260–266. [PubMed: 20582956]
20. Grzybowska-Izydorczyk O, Cebula B, Robak T, Smolewski P. Expression and prognostic significance of the inhibitor of apoptosis protein (IAP) family and its antagonists in chronic lymphocytic leukaemia. *Eur J Cancer.* 2010; 46:800–810. [PubMed: 20045309]
21. Thomas S, Quinn BA, Das SK, et al. Targeting the Bcl-2 family for cancer therapy. *Expert Opin Ther Targets.* 2013; 17:61–75. [PubMed: 23173842]

22. Deveraux QL, Roy N, Stennicke HR, et al. IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. *EMBO J.* 1998; 17:2215–2223. [PubMed: 9545235]
23. Verhagen AM, Ekert PG, Pakusch M, et al. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. *Cell.* 2000; 102:43–53. [PubMed: 10929712]
24. Safa AR. c-FLIP, a master anti-apoptotic regulator. *Exp Oncol.* 2012; 34:176–184. [PubMed: 23070002]
25. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. *Science.* 1984; 226:1097–1099. [PubMed: 6093263]
26. Vaux DL, Cory S, Adams JM. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature.* 1988; 335:440–442. [PubMed: 3262202]
27. Gauthier ER, Piche L, Lemieux G, Lemieux R. Role of bcl-X(L) in the control of apoptosis in murine myeloma cells. *Cancer Res.* 1996; 56:1451–1456. [PubMed: 8640839]
28. Jourdan M, Veyrune JL, De Vos J, Redal N, Couderc G, Klein B. A major role for Mcl-1 antiapoptotic protein in the IL-6-induced survival of human myeloma cells. *Oncogene.* 2003; 22:2950–2959. [PubMed: 12771946]
29. Kitada S, Pedersen IM, Schimmer AD, Reed JC. Dysregulation of apoptosis genes in hematopoietic malignancies. *Oncogene.* 2002; 21:3459–3474. [PubMed: 12032782]
30. Moshynska O, Sankaran K, Pahwa P, Saxena A. Prognostic significance of a short sequence insertion in the MCL-1 promoter in chronic lymphocytic leukemia. *J Natl Cancer Inst.* 2004; 96:673–682. [PubMed: 15126604]
31. Kaufmann SH, Karp JE, Svingen PA, et al. Elevated expression of the apoptotic regulator Mcl-1 at the time of leukemic relapse. *Blood.* 1998; 91:991–1000. [PubMed: 9446661]
32. Kitada S, Andersen J, Akar S, et al. Expression of apoptosis-regulating proteins in chronic lymphocytic leukemia: correlations with In vitro and In vivo chemoresponses. *Blood.* 1998; 91:3379–3389. [PubMed: 9558396]
33. Pepper C, Bentley P, Hoy T. Regulation of clinical chemoresistance by bcl-2 and bax oncoproteins in B-cell chronic lymphocytic leukaemia. *Br J Haematol.* 1996; 95:513–517. [PubMed: 8943893]
34. Saxena A, Viswanathan S, Moshynska O, Tandon P, Sankaran K, Sheridan DP. Mcl-1 and Bcl-2/Bax ratio are associated with treatment response but not with Rai stage in B-cell chronic lymphocytic leukemia. *Am J Hematol.* 2004; 75:22–33. [PubMed: 14695629]
35. Petros AM, Nettessheim DG, Wang Y, et al. Rationale for Bcl-xL/Bad peptide complex formation from structure, mutagenesis, and biophysical studies. *Protein Sci.* 2000; 9:2528–2534. [PubMed: 11206074]
36. Sattler M, Liang H, Nettessheim D, et al. Structure of Bcl-xL-Bak peptide complex: recognition between regulators of apoptosis. *Science.* 1997; 275:983–986. [PubMed: 9020082]
37. Lessene G, Czabotar PE, Colman PM. BCL-2 family antagonists for cancer therapy. *Nat Rev Drug Discov.* 2008; 7:989–1000. [PubMed: 19043450]
38. Walensky LD, Kung AL, Escher I, et al. Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix. *Science.* 2004; 305:1466–1470. [PubMed: 15353804]
39. Degterev A, Lugovskoy A, Cardone M, et al. Identification of small-molecule inhibitors of interaction between the BH3 domain and Bcl-xL. *Nat Cell Biol.* 2001; 3:173–182. [PubMed: 11175750]
40. Wang JL, Liu D, Zhang ZJ, et al. Structure-based discovery of an organic compound that binds Bcl-2 protein and induces apoptosis of tumor cells. *Proc Natl Acad Sci U S A.* 2000; 97:7124–7129. [PubMed: 10860979]
41. Enyedy IJ, Ling Y, Nacro K, et al. Discovery of small-molecule inhibitors of Bcl-2 through structure-based computer screening. *J Med Chem.* 2001; 44:4313–4324. [PubMed: 11728179]
42. Tzung SP, Kim KM, Basanez G, et al. Antimycin A mimics a cell-death-inducing Bcl-2 homology domain 3. *Nat Cell Biol.* 2001; 3:183–191. [PubMed: 11175751]
43. Chan SL, Lee MC, Tan KO, et al. Identification of chelerythrine as an inhibitor of BclXL function. *J Biol Chem.* 2003; 278:20453–20456. [PubMed: 12702731]

44. Kitada S, Leone M, Sareth S, Zhai D, Reed JC, Pellecchia M. Discovery, characterization, and structure-activity relationships studies of proapoptotic polyphenols targeting B-cell lymphocyte/leukemia-2 proteins. *J Med Chem.* 2003; 46:4259–4264. [PubMed: 13678404]
45. Leone M, Zhai D, Sareth S, Kitada S, Reed JC, Pellecchia M. Cancer prevention by tea polyphenols is linked to their direct inhibition of antiapoptotic Bcl-2-family proteins. *Cancer Res.* 2003; 63:8118–8121. [PubMed: 14678963]
46. Shore GC, Viallet J. Modulating the bcl-2 family of apoptosis suppressors for potential therapeutic benefit in cancer. *Hematology Am Soc Hematol Educ Program.* 2005:226–230. [PubMed: 16304385]
47. Aichberger KJ, Gleixner KV, Mirkina I, et al. Identification of proapoptotic Bim as a tumor suppressor in neoplastic mast cells: role of KIT D816V and effects of various targeted drugs. *Blood.* 2009; 114:5342–5351. [PubMed: 19850739]
48. Buet D, Gallais I, Lauret E, et al. Cotargeting signaling pathways driving survival and cell cycle circumvents resistance to Kit inhibitors in leukemia. *Blood.* 2012; 119:4228–4241. [PubMed: 22438255]
49. Konopleva M, Watt J, Contractor R, et al. Mechanisms of antileukemic activity of the novel Bcl-2 homology domain-3 mimetic GX15-070 (obatoclax). *Cancer Res.* 2008; 68:3413–3420. [PubMed: 18451169]
50. Trudel S, Li ZH, Rauw J, Tiedemann RE, Wen XY, Stewart AK. Preclinical studies of the pan-Bcl inhibitor obatoclax (GX015-070) in multiple myeloma. *Blood.* 2007; 109:5430–5438. [PubMed: 17332241]
51. Urtishak KA, Edwards AY, Wang LS, et al. Potent obatoclax cytotoxicity and activation of triple death mode killing across infant acute lymphoblastic leukemia. *Blood.* 2013; 121:2689–2703. [PubMed: 23393050]
52. Vogler M, Weber K, Dinsdale D, et al. Different forms of cell death induced by putative BCL2 inhibitors. *Cell Death Differ.* 2009; 16:1030–1039. [PubMed: 19390557]
53. Samuel S, Tumilasci VF, Oliere S, et al. VSV oncolysis in combination with the BCL-2 inhibitor obatoclax overcomes apoptosis resistance in chronic lymphocytic leukemia. *Mol Ther.* 2010; 18:2094–2103. [PubMed: 20842105]
54. Herishanu Y, Gibellini F, Njuguna N, et al. Activation of CD44, a receptor for extracellular matrix components, protects chronic lymphocytic leukemia cells from spontaneous and drug induced apoptosis through MCL-1. *Leuk Lymphoma.* 2011; 52:1758–1769. [PubMed: 21649540]
55. Bonapace L, Bornhauser BC, Schmitz M, et al. Induction of autophagy-dependent necroptosis is required for childhood acute lymphoblastic leukemia cells to overcome glucocorticoid resistance. *J Clin Invest.* 2010; 120:1310–1323. [PubMed: 20200450]
56. Brem EA, Thudium K, Khubchandani S, et al. Distinct cellular and therapeutic effects of obatoclax in rituximab-sensitive and -resistant lymphomas. *Br J Haematol.* 2011; 153:599–611. [PubMed: 21492126]
57. O'Brien SM, Claxton DF, Crump M, et al. Phase I study of obatoclax mesylate (GX15-070), a small molecule pan-Bcl-2 family antagonist, in patients with advanced chronic lymphocytic leukemia. *Blood.* 2009; 113:299–305. [PubMed: 18931344]
58. Schimmer AD, O'Brien S, Kantarjian H, et al. A phase I study of the pan bcl-2 family inhibitor obatoclax mesylate in patients with advanced hematologic malignancies. *Clin Cancer Res.* 2008; 14:8295–8301. [PubMed: 19088047]
59. Parikh SA, Kantarjian H, Schimmer A, et al. Phase II study of obatoclax mesylate (GX15-070), a small-molecule BCL-2 family antagonist, for patients with myelofibrosis. *Clin Lymphoma Myeloma Leuk.* 2010; 10:285–289. [PubMed: 20709666]
60. Oki Y, Copeland A, Hagemester F, et al. Experience with obatoclax mesylate (GX15-070), a small molecule pan-Bcl-2 family antagonist in patients with relapsed or refractory classical Hodgkin lymphoma. *Blood.* 2012; 119:2171–2172. [PubMed: 22383790]
61. Goard CA, Schimmer AD. An evidence-based review of obatoclax mesylate in the treatment of hematological malignancies. *Core Evid.* 2013; 8:15–26. [PubMed: 23515850]
62. Qiu J, Levin LR, Buck J, Reidenberg MM. Different pathways of cell killing by gossypol enantiomers. *Exp Biol Med (Maywood).* 2002; 227:398–401. [PubMed: 12037129]

63. Balakrishnan K, Aggarwal S, Wierda W, Gandhi V. Bax and Bak are required for apogossypolone, a BH3-mimetic, induced apoptosis in chronic lymphocytic leukemia cells. *Leuk Lymphoma*. 2012
64. Balakrishnan K, Wierda WG, Keating MJ, Gandhi V. Gossypol, a BH3 mimetic, induces apoptosis in chronic lymphocytic leukemia cells. *Blood*. 2008; 112:1971–1980. [PubMed: 18566329]
65. Kitada S, Kress CL, Krajewska M, Jia L, Pellecchia M, Reed JC. Bcl-2 antagonist apogossypol (NSC736630) displays single-agent activity in Bcl-2-transgenic mice and has superior efficacy with less toxicity compared with gossypol (NSC19048). *Blood*. 2008; 111:3211–3219. [PubMed: 18202226]
66. Wei J, Stebbins JL, Kitada S, et al. BI-97C1, an optically pure Apogossypol derivative as pan-active inhibitor of antiapoptotic B-cell lymphoma/leukemia-2 (Bcl-2) family proteins. *J Med Chem*. 2010; 53:4166–4176. [PubMed: 20443627]
67. Mohammad RM, Wang S, Aboukameel A, et al. Preclinical studies of a nonpeptidic small-molecule inhibitor of Bcl-2 and Bcl-X(L) [(-)-gossypol] against diffuse large cell lymphoma. *Mol Cancer Ther*. 2005; 4:13–21. [PubMed: 15657349]
68. Balakrishnan K, Burger JA, Wierda WG, Gandhi V. AT-101 induces apoptosis in CLL B cells and overcomes stromal cell-mediated Mcl-1 induction and drug resistance. *Blood*. 2009; 113:149–153. [PubMed: 18836097]
69. Sun Y, Wu J, Aboukameel A, et al. Apogossypolone, a nonpeptidic small molecule inhibitor targeting Bcl-2 family proteins, effectively inhibits growth of diffuse large cell lymphoma cells in vitro and in vivo. *Cancer Biol Ther*. 2008; 7:1418–1426. [PubMed: 18769131]
70. Lei X, Chen Y, Du G, et al. Gossypol induces Bax/Bak-independent activation of apoptosis and cytochrome c release via a conformational change in Bcl-2. *FASEB J*. 2006; 20:2147–2149. [PubMed: 16935937]
71. Oltersdorf T, Elmore SW, Shoemaker AR, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature*. 2005; 435:677–681. [PubMed: 15902208]
72. van Delft MF, Wei AH, Mason KD, et al. The BH3 mimetic ABT-737 targets selective Bcl-2 proteins and efficiently induces apoptosis via Bak/Bax if Mcl-1 is neutralized. *Cancer Cell*. 2006; 10:389–399. [PubMed: 17097561]
73. Tse C, Shoemaker AR, Adickes J, et al. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. *Cancer Res*. 2008; 68:3421–3428. [PubMed: 18451170]
74. Roberts AW, Seymour JF, Brown JR, et al. Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. *J Clin Oncol*. 2012; 30:488–496. [PubMed: 22184378]
75. Wilson WH, O'Connor OA, Czuczman MS, et al. Navitoclax, a targeted high-affinity inhibitor of BCL-2, in lymphoid malignancies: a phase 1 dose-escalation study of safety, pharmacokinetics, pharmacodynamics, and antitumour activity. *Lancet Oncol*. 2010; 11:1149–1159. [PubMed: 21094089]
76. Mason KD, Carpinelli MR, Fletcher JI, et al. Programmed anuclear cell death delimits platelet life span. *Cell*. 2007; 128:1173–1186. [PubMed: 17382885]
77. Souers AJ, Levenson JD, Boghaert ER, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat Med*. 2013; 19:202–208. [PubMed: 23291630]
78. Chen LS, Balakrishnan K, Gandhi V. Inflammation and survival pathways: chronic lymphocytic leukemia as a model system. *Biochem Pharmacol*. 2010; 80:1936–1945. [PubMed: 20696142]
79. Tromp JM, Geest CR, Breij EC, et al. Tipping the Noxa/Mcl-1 balance overcomes ABT-737 resistance in chronic lymphocytic leukemia. *Clin Cancer Res*. 2012; 18:487–498. [PubMed: 22128299]
80. Billard C. Development of Noxa-like BH3 mimetics for apoptosis-based therapeutic strategy in chronic lymphocytic leukemia. *Mol Cancer Res*. 2012; 10:673–676. [PubMed: 22466256]
81. Lee EF, Czabotar PE, van Delft MF, et al. A novel BH3 ligand that selectively targets Mcl-1 reveals that apoptosis can proceed without Mcl-1 degradation. *J Cell Biol*. 2008; 180:341–355. [PubMed: 18209102]
82. Stewart ML, Fire E, Keating AE, Walensky LD. The MCL-1 BH3 helix is an exclusive MCL-1 inhibitor and apoptosis sensitizer. *Nat Chem Biol*. 2010; 6:595–601. [PubMed: 20562877]

83. Webb A, Cunningham D, Cotter F, et al. BCL-2 antisense therapy in patients with non-Hodgkin lymphoma. *Lancet*. 1997; 349:1137–1141. [PubMed: 9113013]
84. Marcucci G, Stock W, Dai G, et al. Phase I study of oblimersen sodium, an antisense to Bcl-2, in untreated older patients with acute myeloid leukemia: pharmacokinetics, pharmacodynamics, and clinical activity. *J Clin Oncol*. 2005; 23:3404–3411. [PubMed: 15824414]
85. O'Brien SM, Cunningham CC, Golenkov AK, Turkina AG, Novick SC, Rai KR. Phase I to II multicenter study of oblimersen sodium, a Bcl-2 antisense oligonucleotide, in patients with advanced chronic lymphocytic leukemia. *J Clin Oncol*. 2005; 23:7697–7702. [PubMed: 16186597]
86. O'Brien S, Moore JO, Boyd TE, et al. Randomized phase III trial of fludarabine plus cyclophosphamide with or without oblimersen sodium (Bcl-2 antisense) in patients with relapsed or refractory chronic lymphocytic leukemia. *J Clin Oncol*. 2007; 25:1114–1120. [PubMed: 17296974]
87. O'Brien S, Moore JO, Boyd TE, et al. 5-year survival in patients with relapsed or refractory chronic lymphocytic leukemia in a randomized, phase III trial of fludarabine plus cyclophosphamide with or without oblimersen. *J Clin Oncol*. 2009; 27:5208–5212. [PubMed: 19738118]
88. Badros AZ, Golubeva O, Rapoport AP, et al. Phase II study of G3139, a Bcl-2 antisense oligonucleotide, in combination with dexamethasone and thalidomide in relapsed multiple myeloma patients. *J Clin Oncol*. 2005; 23:4089–4099. [PubMed: 15867202]
89. Chanan-Khan AA. Bcl-2 antisense therapy in multiple myeloma. *Oncology (Williston Park)*. 2004; 18:21–24. [PubMed: 15651173]
90. van de Donk NW, de Weerd O, Veth G, et al. G3139, a Bcl-2 antisense oligodeoxynucleotide, induces clinical responses in VAD refractory myeloma. *Leukemia*. 2004; 18:1078–1084. [PubMed: 15085157]
91. Chanan-Khan AA, Niesvizky R, Hohl RJ, et al. Phase III randomised study of dexamethasone with or without oblimersen sodium for patients with advanced multiple myeloma. *Leuk Lymphoma*. 2009; 50:559–565. [PubMed: 19373653]
92. Ashkenazi A. Directing cancer cells to self-destruct with pro-apoptotic receptor agonists. *Nat Rev Drug Discov*. 2008; 7:1001–1012. [PubMed: 18989337]
93. Liu CC, Detmers PA, Jiang SB, Young JD. Identification and characterization of a membrane-bound cytotoxin of murine cytolytic lymphocytes that is related to tumor necrosis factor/cachectin. *Proc Natl Acad Sci U S A*. 1989; 86:3286–3290. [PubMed: 2654942]
94. Tanaka M, Suda T, Yatomi T, Nakamura N, Nagata S. Lethal effect of recombinant human Fas ligand in mice pretreated with *Propionibacterium acnes*. *J Immunol*. 1997; 158:2303–2309. [PubMed: 9036978]
95. Walczak H, Miller RE, Ariail K, et al. Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. *Nat Med*. 1999; 5:157–163. [PubMed: 9930862]
96. Ashkenazi A, Pai RC, Fong S, et al. Safety and antitumor activity of recombinant soluble Apo2 ligand. *J Clin Invest*. 1999; 104:155–162. [PubMed: 10411544]
97. Marsters SA, Sheridan JP, Pitti RM, et al. A novel receptor for Apo2L/TRAIL contains a truncated death domain. *Curr Biol*. 1997; 7:1003–1006. [PubMed: 9382840]
98. Pan G, Ni J, Wei YF, Yu G, Gentz R, Dixit VM. An antagonist decoy receptor and a death domain-containing receptor for TRAIL. *Science*. 1997; 277:815–818. [PubMed: 9242610]
99. Pan G, O'Rourke K, Chinnaiyan AM, et al. The receptor for the cytotoxic ligand TRAIL. *Science*. 1997; 276:111–113. [PubMed: 9082980]
100. Daniel D, Yang B, Lawrence DA, et al. Cooperation of the proapoptotic receptor agonist rhApo2L/TRAIL with the CD20 antibody rituximab against non-Hodgkin lymphoma xenografts. *Blood*. 2007; 110:4037–4046. [PubMed: 17724141]
101. Mitsiades CS, Treon SP, Mitsiades N, et al. TRAIL/Apo2L ligand selectively induces apoptosis and overcomes drug resistance in multiple myeloma: therapeutic applications. *Blood*. 2001; 98:795–804. [PubMed: 11468181]
102. Yao GH, Ling LJ, Luan JF, Ye D, Zhu PY, Lei QH. Induction of apoptosis by recombinant soluble human TRAIL in Jurkat cells. *Biomed Environ Sci*. 2007; 20:470–477. [PubMed: 18348405]

103. Herbst RS, Eckhardt SG, Kurzrock R, et al. Phase I dose-escalation study of recombinant human Apo2L/TRAIL, a dual proapoptotic receptor agonist, in patients with advanced cancer. *J Clin Oncol.* 2010; 28:2839–2846. [PubMed: 20458040]
104. Pan Y, Xu R, Peach M, et al. Evaluation of pharmacodynamic biomarkers in a Phase 1a trial of dulanermin (rhApo2L/TRAIL) in patients with advanced tumours. *Br J Cancer.* 2011; 105:1830–1838. [PubMed: 22033270]
105. Soria JC, Smit E, Khayat D, et al. Phase 1b study of dulanermin (recombinant human Apo2L/TRAIL) in combination with paclitaxel, carboplatin, and bevacizumab in patients with advanced non-squamous non-small-cell lung cancer. *J Clin Oncol.* 2010; 28:1527–1533. [PubMed: 20159815]
106. Soria JC, Mark Z, Zatloukal P, et al. Randomized phase II study of dulanermin in combination with paclitaxel, carboplatin, and bevacizumab in advanced non-small-cell lung cancer. *J Clin Oncol.* 2011; 29:4442–4451. [PubMed: 22010015]
107. Kaplan-Lefko PJ, Graves JD, Zoog SJ, et al. Conatumumab, a fully human agonist antibody to death receptor 5, induces apoptosis via caspase activation in multiple tumor types. *Cancer Biol Ther.* 2010; 9:618–631. [PubMed: 20150762]
108. Herbst RS, Kurzrock R, Hong DS, et al. A first-in-human study of conatumumab in adult patients with advanced solid tumors. *Clin Cancer Res.* 2010; 16:5883–5891. [PubMed: 20947515]
109. Demetri GD, Le Cesne A, Chawla SP, et al. First-line treatment of metastatic or locally advanced unresectable soft tissue sarcomas with conatumumab in combination with doxorubicin or doxorubicin alone: a phase I/II open-label and double-blind study. *Eur J Cancer.* 2012; 48:547–563. [PubMed: 22240283]
110. Kindler HL, Richards DA, Garbo LE, et al. A randomized, placebo-controlled phase 2 study of ganitumab (AMG 479) or conatumumab (AMG 655) in combination with gemcitabine in patients with metastatic pancreatic cancer. *Ann Oncol.* 2012; 23:2834–2842. [PubMed: 22700995]
111. Cohn AL, Tabernero J, Maurel J, et al. A randomized, placebo-controlled phase 2 study of ganitumab or conatumumab in combination with FOLFIRI for second-line treatment of mutant KRAS metastatic colorectal cancer. *Ann Oncol.* 2013
112. Paz-Ares L, Balint B, de Boer RH, et al. A randomized phase 2 study of paclitaxel and carboplatin with or without conatumumab for first-line treatment of advanced non-small-cell lung cancer. *J Thorac Oncol.* 2013; 8:329–337. [PubMed: 23370314]
113. Moretto P, Hotte SJ. Targeting apoptosis: preclinical and early clinical experience with mapatumumab, an agonist monoclonal antibody targeting TRAIL-R1. *Expert Opin Investig Drugs.* 2009; 18:311–325.
114. Younes A, Vose JM, Zelenetz AD, et al. A Phase 1b/2 trial of mapatumumab in patients with relapsed/refractory non-Hodgkin's lymphoma. *Br J Cancer.* 2010; 103:1783–1787. [PubMed: 21081929]
115. Merchant MS, Geller JI, Baird K, et al. Phase I trial and pharmacokinetic study of lexatumumab in pediatric patients with solid tumors. *J Clin Oncol.* 2012; 30:4141–4147. [PubMed: 23071222]
116. Plummer R, Attard G, Pacey S, et al. Phase 1 and pharmacokinetic study of lexatumumab in patients with advanced cancers. *Clin Cancer Res.* 2007; 13:6187–6194. [PubMed: 17947486]
117. Wakelee HA, Patnaik A, Sikic BI, et al. Phase I and pharmacokinetic study of lexatumumab (HGS-ETR2) given every 2 weeks in patients with advanced solid tumors. *Ann Oncol.* 2010; 21:376–381. [PubMed: 19633048]
118. Adams C, Totpal K, Lawrence D, et al. Structural and functional analysis of the interaction between the agonistic monoclonal antibody Apomab and the proapoptotic receptor DR5. *Cell Death Differ.* 2008; 15:751–761. [PubMed: 18219321]
119. Camidge DR, Herbst RS, Gordon MS, et al. A phase I safety and pharmacokinetic study of the death receptor 5 agonistic antibody PRO95780 in patients with advanced malignancies. *Clin Cancer Res.* 2010; 16:1256–1263. [PubMed: 20145186]
120. Forero-Torres A, Shah J, Wood T, et al. Phase I trial of weekly tigatuzumab, an agonistic humanized monoclonal antibody targeting death receptor 5 (DR5). *Cancer Biother Radiopharm.* 2010; 25:13–19. [PubMed: 20187792]

121. Sharma, S.; De Vries, EG.; Infante, JR., et al. Phase I trial of LBY135, a monoclonal antibody agonist to DR5, alone and in combination with capecitabine in advanced solid tumors. Vol. 26. Chicago, IL: 2008. p. 3538
122. Huet, H.; Schuller, A.; Li, J., et al. TAS266, a novel tetrameric nanobody agonist targeting death receptor 5 (DR5), elicits superior antitumor efficacy than conventional DR5-targeted approaches. Chicago, IL: 2012. p. 3853
123. Hussain AR, Uddin S, Ahmed M, et al. Prognostic significance of XIAP expression in DLBCL and effect of its inhibition on AKT signalling. *J Pathol.* 2010; 222:180–190. [PubMed: 20632385]
124. Nakagawa Y, Abe S, Kurata M, et al. IAP family protein expression correlates with poor outcome of multiple myeloma patients in association with chemotherapy-induced overexpression of multidrug resistance genes. *Am J Hematol.* 2006; 81:824–831. [PubMed: 16929535]
125. Vallat L, Magdelenat H, Merle-Beral H, et al. The resistance of B-CLL cells to DNA damage-induced apoptosis defined by DNA microarrays. *Blood.* 2003; 101:4598–4606. [PubMed: 12586635]
126. Varfolomeev E, Wayson SM, Dixit VM, Fairbrother WJ, Vucic D. The inhibitor of apoptosis protein fusion c-IAP2.MALT1 stimulates NF-kappaB activation independently of TRAF1 AND TRAF2. *J Biol Chem.* 2006; 281:29022–29029. [PubMed: 16891304]
127. Zhou H, Du MQ, Dixit VM. Constitutive NF-kappaB activation by the t(11;18)(q21;q21) product in MALT lymphoma is linked to deregulated ubiquitin ligase activity. *Cancer Cell.* 2005; 7:425–431. [PubMed: 15894263]
128. Dean E, Jodrell D, Connolly K, et al. Phase I trial of AEG35156 administered as a 7-day and 3-day continuous intravenous infusion in patients with advanced refractory cancer. *J Clin Oncol.* 2009; 27:1660–1666. [PubMed: 19237630]
129. Schimmer AD, Estey EH, Borthakur G, et al. Phase I/II trial of AEG35156 X-linked inhibitor of apoptosis protein antisense oligonucleotide combined with idarubicin and cytarabine in patients with relapsed or primary refractory acute myeloid leukemia. *J Clin Oncol.* 2009; 27:4741–4746. [PubMed: 19652057]
130. Carter BZ, Mak DH, Morris SJ, et al. XIAP antisense oligonucleotide (AEG35156) achieves target knockdown and induces apoptosis preferentially in CD34+38– cells in a phase 1/2 study of patients with relapsed/refractory AML. *Apoptosis.* 2011; 16:67–74. [PubMed: 20938744]
131. Schimmer AD, Herr W, Hanel M, et al. Addition of AEG35156 XIAP antisense oligonucleotide in reinduction chemotherapy does not improve remission rates in patients with primary refractory acute myeloid leukemia in a randomized phase II study. *Clin Lymphoma Myeloma Leuk.* 2011; 11:433–438. [PubMed: 21729686]
132. Katragadda L, Carter BZ, Borthakur G. XIAP antisense therapy with AEG 35156 in acute myeloid leukemia. *Expert Opin Investig Drugs.* 2013; 22:663–670.
133. Altieri DC. Validating survivin as a cancer therapeutic target. *Nat Rev Cancer.* 2003; 3:46–54. [PubMed: 12509766]
134. Tolcher AW, Mita A, Lewis LD, et al. Phase I and pharmacokinetic study of YM155, a small-molecule inhibitor of survivin. *J Clin Oncol.* 2008; 26:5198–5203. [PubMed: 18824702]
135. Rodel F, Sprenger T, Kaina B, et al. Survivin as a prognostic/predictive marker and molecular target in cancer therapy. *Curr Med Chem.* 2012; 19:3679–3688. [PubMed: 22680927]
136. Cheson BD, Bartlett NL, Vose JM, et al. A phase II study of the survivin suppressant YM155 in patients with refractory diffuse large B-cell lymphoma. *Cancer.* 2012; 118:3128–3134. [PubMed: 22006123]
137. Talbot DC, Ranson M, Davies J, et al. Tumor survivin is downregulated by the antisense oligonucleotide LY2181308: a proof-of-concept, first-in-human dose study. *Clin Cancer Res.* 2010; 16:6150–6158. [PubMed: 21041181]
138. Tanioka M, Nokihara H, Yamamoto N, et al. Phase I study of LY2181308, an antisense oligonucleotide against survivin, in patients with advanced solid tumors. *Cancer Chemother Pharmacol.* 2011; 68:505–511. [PubMed: 21079959]

139. Erba HP, Sayar H, Juckett M, et al. Safety and pharmacokinetics of the antisense oligonucleotide (ASO) LY2181308 as a single-agent or in combination with idarubicin and cytarabine in patients with refractory or relapsed acute myeloid leukemia (AML). *Invest New Drugs*. 2013
140. Rapoport AP, Aqui NA, Stadtmauer EA, et al. Combination immunotherapy using adoptive T-cell transfer and tumor antigen vaccination on the basis of hTERT and survivin after ASCT for myeloma. *Blood*. 2011; 117:788–797. [PubMed: 21030558]
141. Kempkensteffen C, Hinz S, Christoph F, et al. Expression levels of the mitochondrial IAP antagonists Smac/DIABLO and Omi/HtrA2 in clear-cell renal cell carcinomas and their prognostic value. *J Cancer Res Clin Oncol*. 2008; 134:543–550. [PubMed: 17922292]
142. Mizutani Y, Nakanishi H, Yamamoto K, et al. Downregulation of Smac/DIABLO expression in renal cell carcinoma and its prognostic significance. *J Clin Oncol*. 2005; 23:448–454. [PubMed: 15572731]
143. Endo K, Kohnoe S, Watanabe A, et al. Clinical significance of Smac/DIABLO expression in colorectal cancer. *Oncol Rep*. 2009; 21:351–355. [PubMed: 19148507]
144. Pluta A, Wrzesien-Kus A, Cebula-Obrzut B, et al. Influence of high expression of Smac/DIABLO protein on the clinical outcome in acute myeloid leukemia patients. *Leuk Res*. 2010; 34:1308–1313. [PubMed: 20061022]
145. Chai J, Du C, Wu JW, Kyin S, Wang X, Shi Y. Structural and biochemical basis of apoptotic activation by Smac/DIABLO. *Nature*. 2000; 406:855–862. [PubMed: 10972280]
146. Liu Z, Sun C, Olejniczak ET, et al. Structural basis for binding of Smac/DIABLO to the XIAP BIR3 domain. *Nature*. 2000; 408:1004–1008. [PubMed: 11140637]
147. Wu G, Chai J, Suber TL, et al. Structural basis of IAP recognition by Smac/DIABLO. *Nature*. 2000; 408:1008–1012. [PubMed: 11140638]
148. Fulda S, Wick W, Weller M, Debatin KM. Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma in vivo. *Nat Med*. 2002; 8:808–815. [PubMed: 12118245]
149. Vucic D, Deshayes K, Ackerly H, et al. SMAC negatively regulates the anti-apoptotic activity of melanoma inhibitor of apoptosis (ML-IAP). *J Biol Chem*. 2002; 277:12275–12279. [PubMed: 11801603]
150. Gaither A, Porter D, Yao Y, et al. A Smac mimetic rescue screen reveals roles for inhibitor of apoptosis proteins in tumor necrosis factor-alpha signaling. *Cancer Res*. 2007; 67:11493–11498. [PubMed: 18089776]
151. Petersen SL, Wang L, Yalcin-Chin A, et al. Autocrine TNFalpha signaling renders human cancer cells susceptible to Smac-mimetic-induced apoptosis. *Cancer Cell*. 2007; 12:445–456. [PubMed: 17996648]
152. Sun H, Nikolovska-Coleska Z, Yang CY, et al. Design of small-molecule peptidic and nonpeptidic Smac mimetics. *Acc Chem Res*. 2008; 41:1264–1277. [PubMed: 18937395]
153. Sun H, Nikolovska-Coleska Z, Yang CY, et al. Structure-based design, synthesis, and evaluation of conformationally constrained mimetics of the second mitochondria-derived activator of caspase that target the X-linked inhibitor of apoptosis protein/caspase-9 interaction site. *J Med Chem*. 2004; 47:4147–4150. [PubMed: 15293984]
154. Varfolomeev E, Blankenship JW, Wayson SM, et al. IAP antagonists induce autoubiquitination of c-IAPs, NF-kappaB activation, and TNFalpha-dependent apoptosis. *Cell*. 2007; 131:669–681. [PubMed: 18022362]
155. Vince JE, Wong WW, Khan N, et al. IAP antagonists target cIAP1 to induce TNFalpha-dependent apoptosis. *Cell*. 2007; 131:682–693. [PubMed: 18022363]
156. Cai Q, Sun H, Peng Y, et al. A potent and orally active antagonist (SM-406/AT-406) of multiple inhibitor of apoptosis proteins (IAPs) in clinical development for cancer treatment. *J Med Chem*. 2011; 54:2714–2726. [PubMed: 21443232]
157. Brunckhorst MK, Lerner D, Wang S, Yu Q. AT-406, an orally active antagonist of multiple inhibitor of apoptosis proteins, inhibits progression of human ovarian cancer. *Cancer Biol Ther*. 2012; 13:804–811. [PubMed: 22669575]

158. Amaravadi, RK.; Schilder, RJ.; Dy, GK., et al. Phase 1 study of the Smac mimetic TL32711 in adult subjects with advanced solid tumors and lymphoma to evaluate safety, pharmacokinetics, pharmacodynamics, and antitumor activity. Orlando, FL: 2011. p. LB-406
159. Houghton PJ, Kang MH, Reynolds CP, et al. Initial testing (stage 1) of LCL161, a SMAC mimetic, by the Pediatric Preclinical Testing Program. *Pediatr Blood Cancer*. 2012; 58:636–639. [PubMed: 21681929]
160. Weisberg E, Ray A, Barrett R, et al. Smac mimetics: implications for enhancement of targeted therapies in leukemia. *Leukemia*. 2010; 24:2100–2109. [PubMed: 20844561]
161. Eckhardt, SG.; Gallant, G.; Sikic, BI., et al. Phase I study evaluating the safety, tolerability, and pharmacokinetics (PK) of HGS1029, a small-molecule inhibitor of apoptosis protein (IAP), in patients (pts) with advanced solid tumors. Chicago, IL: 2010. p. 2580
162. Putt KS, Chen GW, Pearson JM, et al. Small-molecule activation of procaspase-3 to caspase-3 as a personalized anticancer strategy. *Nat Chem Biol*. 2006; 2:543–550. [PubMed: 16936720]
163. Roy S, Bayly CI, Gareau Y, et al. Maintenance of caspase-3 proenzyme dormancy by an intrinsic "safety catch" regulatory tripeptide. *Proc Natl Acad Sci U S A*. 2001; 98:6132–6137. [PubMed: 11353841]
164. Jia LT, Zhang LH, Yu CJ, et al. Specific tumoricidal activity of a secreted proapoptotic protein consisting of HER2 antibody and constitutively active caspase-3. *Cancer Res*. 2003; 63:3257–3262. [PubMed: 12810656]
165. Xu YM, Wang LF, Jia LT, et al. A caspase-6 and anti-human epidermal growth factor receptor-2 (HER2) antibody chimeric molecule suppresses the growth of HER2-overexpressing tumors. *J Immunol*. 2004; 173:61–67. [PubMed: 15210759]
166. Tse E, Rabbitts TH. Intracellular antibody-caspase-mediated cell killing: an approach for application in cancer therapy. *Proc Natl Acad Sci U S A*. 2000; 97:12266–12271. [PubMed: 11050246]
167. Xie X, Zhao X, Liu Y, et al. Adenovirus-mediated tissue-targeted expression of a caspase-9-based artificial death switch for the treatment of prostate cancer. *Cancer Res*. 2001; 61:6795–6804. [PubMed: 11559553]
168. Shariat SF, Desai S, Song W, et al. Adenovirus-mediated transfer of inducible caspases: a novel "death switch" gene therapeutic approach to prostate cancer. *Cancer Res*. 2001; 61:2562–2571. [PubMed: 11289132]
169. Fischer U, Schulze-Osthoff K. Apoptosis-based therapies and drug targets. *Cell Death Differ*. 2005; 12(Suppl 1):942–961. [PubMed: 15665817]
170. Wolan DW, Zorn JA, Gray DC, Wells JA. Small-molecule activators of a proenzyme. *Science*. 2009; 326:853–858. [PubMed: 19892984]
171. Peterson QP, Goode DR, West DC, Ramsey KN, Lee JJ, Hergenrother PJ. PAC-1 activates procaspase-3 in vitro through relief of zinc-mediated inhibition. *J Mol Biol*. 2009; 388:144–158. [PubMed: 19281821]
172. Peterson QP, Hsu DC, Novotny CJ, et al. Discovery and canine preclinical assessment of a nontoxic procaspase-3-activating compound. *Cancer Res*. 2010; 70:7232–7241. [PubMed: 20823163]

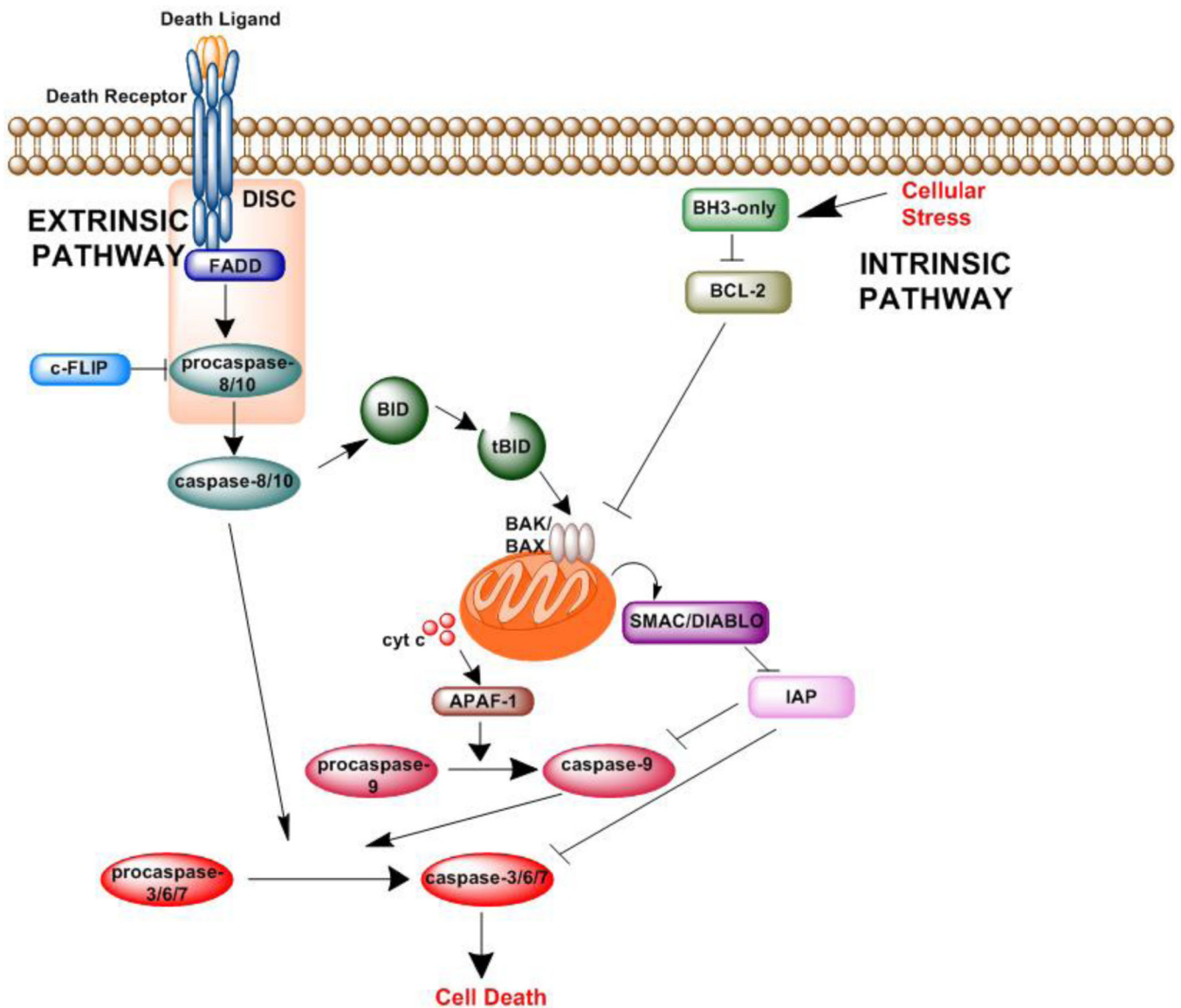


Figure 1. The apoptosis pathway

There are two apoptosis pathways, intrinsic and extrinsic. The intrinsic pathway is activated by cellular stress. When activated, the BH3-only proteins inhibit antiapoptotic BCL-2 proteins and induce BAK/BAX oligomerization and permeabilization of the mitochondria and release of cytochrome c and SMAC/DIABLO. Cytochrome c forms a complex with caspase-9 and APAF-1, leading to activation of caspase-9. Caspase-9 activates the executioner caspases (caspase-3, -6, and -7) and induces cell death. The extrinsic pathway is activated by death signals mediated by death ligands. The death ligand activates the death receptor, which forms a complex called the death-inducing signaling complex (DISC) and sends a signal to activate caspase-8 and -10. Caspase-8 and -10 activate caspase-3. In some cells, the extrinsic pathway crosstalks with the intrinsic pathway through caspase-8-mediated truncation of BID to tBID. tBID activates BAK/BAX oligomerization and induces apoptosis through the mitochondrial pathway.

Anti-apoptotic:

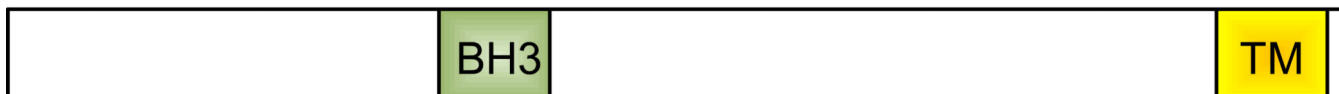
BCL-2 (239 aa), BCL-X_L (233 aa), BCL-w (193 aa), MCL-1 (350 aa), BCL2A1 (175 aa), BCL-B (194 aa)

**Pro-apoptotic:**

BAK (211 aa), BAX (192 aa), BOK (212 aa)

**Pro-apoptotic:**

BIM (198 aa), BID (195 aa), PUMA (261 aa), NOXA (54 aa), BAD (168 aa), BMF (184 aa), BIK (160 aa), HRK (91 aa)

**Figure 2. The BCL-2 protein family**

The BCL-2 proteins share a BCL-2 homology (BH) domain. The antiapoptotic BCL-2 proteins (BCL-2, BCL-X_L, BCL-w, MCL-1, BCL2A1 and BCL-B) have four BH domains and the proapoptotic BAK, BAX and BOK proteins have three BH domains, while the proapoptotic BIM, BID, PUMA, NOXA, BAD, BMF, BIK, and HRK proteins have only BH3 domain. The location of the transmembrane (TM) domain is indicated in the figure. Of the BH3-only proteins, only BIK and HRK have a TM domain. The panels representing the three groups of proteins are not drawn to scale, and the amino acid lengths shown represent the canonical size.

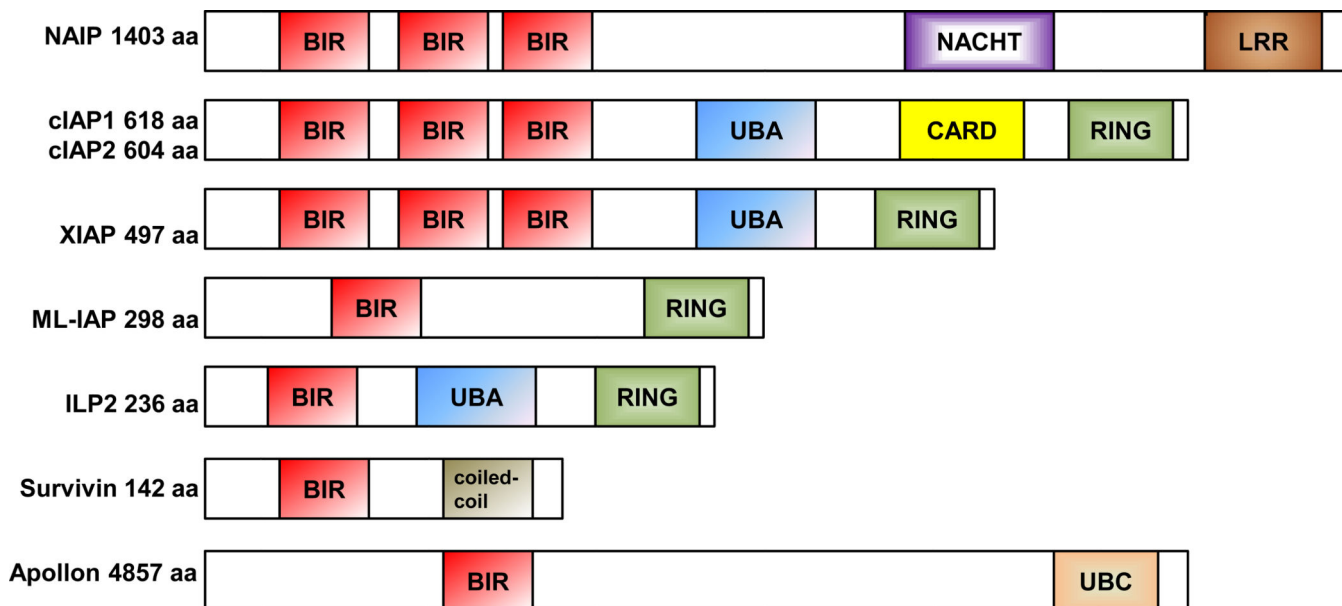


Figure 3. The IAP protein family

The IAP family has eight members: NAIP, cIAP1, cIAP2, XIAP, ML-IAP, ILP2, survivin, and Apollon. All of the IAP proteins have a baculoviral IAP repeat (BIR) domain, which mediates protein-protein interactions. The RING domain mediates E3 ubiquitin ligase activity. Ubiquitin-associated domain (UBA) binds monoubiquitin and polyubiquitin chains. CARD is a caspase recruitment domain. UBC is an ubiquitin-conjugating domain. LRR is a leucine-rich domain. NACHT, a predicted nucleoside-triphosphatase domain, is named for its components: NAIP, CIITA (MHC class II transactivator), HET-E (20-hydroxyeicosatetraenoic acid synthase), and TP1 (transition protein 1). The panels representing the proteins are not drawn to scale, and the amino acid lengths shown represent the canonical size. Figure is modified from [11]. Permission to reprint modified figure was obtained from Nature Publishing Group.

Table 1

Apoptosis pathway–targeting drugs in clinical trials.

Approach/Drug	Other names	Target	Type	Clinical Trial Status	Company
Targeting Antiapoptotic BCL-2 Proteins					
Oblimersen sodium	G3139, Genasense, Augmerosen	BCL-2	Antisense oligonucleotide	Phase 3	Genta Incorporated
Obatocicax mesylate	GXI15-070	BCL-2, BCL-X _L , BCL-w and MCL-1	Small-molecule inhibitor	Phase 1/2	Gemin X/Teva Pharmaceutical Industries
AT-101	(-)-gossypol	BCL-2, BCL-X _L and MCL-1	Small-molecule inhibitor	Phase 2	Ascenta Therapeutics
Navitoclax	ABT-263	BCL-X _L , BCL-2 and BCL-w	Small-molecule inhibitor	Phase 2	AbbVie and Genentech
GDC-0199	ABT-199	BCL-2	Small-molecule inhibitor	Phase 1	AbbVie and Genentech
Targeting IAPs					
AEG35156	GEM640	XIAP	Antisense oligonucleotide	Phase 1/2	Aegera Therapeutics
YMI155		survivin	Small-molecule antagonist	Phase 2	Astellas Pharma
AT-406	Debio 1143	XIAP, cIAP1 and cIAP2	SMAC mimetic	Phase 1	Ascenta Therapeutics and Debiopharm S.A.
TL32711	Birinapant	cIAP1	SMAC mimetic	Phase 1/2	TetraLogic Pharma
GDC-0917		IAPs	SMAC mimetic	Phase 1	Genentech
LCL161		IAPs	SMAC mimetic	Phase 1/2	Novartis
AEG40826-2HCl	HGS1029	IAPs	SMAC mimetic	Phase 1	Aegera Therapeutics and GlaxoSmithKline/Human Genome Science?
LY2181308	Gataparsen	survivin	Antisense oligonucleotide	Phase 2	Eli Lilly and Company
Targeting Death Receptors					
rhApo2L/TRAIL	Dulanermin	DR4, DR5	Soluble ligand	Phase 2	Genentech and Amgen
Mapatumumab	HGS-ETR1, TRM-1	DR4	Monoclonal antibody	Phase 2	GlaxoSmithKline/Human Genome Sciences
Lexatumumab	HGS-ETR2	DR5	Monoclonal antibody	Phase 1	GlaxoSmithKline/Human Genome Sciences
Drozitumab	PRO95780, Apomab	DR5	Monoclonal antibody	Phase 2	Genentech
AMG655	Conatumumab	DR5	Monoclonal antibody	Phase 2	Amgen
CS-1008	Tigatuzumab	DR5	Monoclonal antibody	Phase 2	Daiichi
LBY-135		DR5	Monoclonal antibody	Phase 2	Novartis
TAS266		DR5	Nanobody	Phase 1	Novartis

IAP, inhibitor of apoptosis proteins; SMAC, second mitochondria-derived activator of caspase

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript