Apoptosis-based Therapies for Hematological Malignancies

John C. Reed\textsuperscript{1*} and Maurizio Pellecchia\textsuperscript{1}

\textsuperscript{1}The Burnham Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037, U.S.A.

*To whom correspondence should be addressed (e-mail: reedoffice@burnham.org).

Running Title: Apoptosis-based therapies for hematological malignancies.

Key words: Apoptosis, Drug Discovery, Bcl-2, IAP, TNF
ABSTRACT

Apoptosis is an intrinsic cell death program that plays critical roles in tissue homeostasis, especially in organs where high rates of daily cell production are offset by rapid cell turnover. The hematopoietic system provides numerous examples attesting to the importance of cell death mechanisms for achieving homeostatic control. Much has been learned about the mechanisms of apoptosis of lymphoid and hematopoietic cells since the seminal observation in 1980 that glucocorticoids induce DNA fragmentation and apoptosis of thymocytes and the demonstration in 1990 that depriving Colony Stimulating Factors (CSFs) from factor-dependent hematopoietic cells causes programmed cell death. From an understanding of the core components of the apoptosis machinery at the molecular and structural levels, many potential new therapies for leukemia and lymphoma are emerging. In this review, we introduce some of the drug discovery targets thus far identified within the core apoptotic machinery, and describe some of the progress to date towards translating our growing knowledge about these targets into new therapies for cancer and leukemia.

Apoptosis inducers and effectors

Apoptosis is caused by the activation of intracellular proteases, known as caspases. The human genome encodes 11 or 12 caspases, depending on certain hereditary polymorphisms. Numerous cellular targets of caspases have been identified, which in aggregate produce the characteristic morphology we call “apoptosis” when cleaved. Several pathways for triggering caspase activation exist, though two have been elucidated in great
detail and have been the center of much attention in recent years. These two pathways for apoptosis are commonly referred to as the Intrinsic and Extrinsic pathways (Figure 1)  

The intrinsic pathway centers on mitochondria as initiators of cell death. Multiple signals converge on mitochondria, including DNA damage, hypoxia, and oxidative stress, causing these organelles to release cytochrome c (cyt c) and other apoptogenic proteins into cytosol. In the cytosol, cyt c binds caspase-activating protein Apaf1, triggering its oligomerization into a heptameric complex that binds pro-caspase-9, forming a multi-protein structure known as the “apoptosome”  

Physical binding of Apaf1 to pro-caspase-9 is mediated by their Caspase Recruitment Domains (CARDs), through homotypic CARD-CARD binding. Activation of apoptosome-associated cell death protease caspase-9 then initiates a proteolytic cascade, where activated caspase-9 cleaves and activates downstream effector proteases, such as pro-caspase-3.

In contrast, the extrinsic apoptotic pathway relies on TNF (Tumor Necrosis Factor)-family death receptors for triggering apoptosis. A subgroup of the TNF-family receptors contain a cytosolic Death Domain, that enables their intracellular interaction with downstream adapter proteins, linking these receptors to specific caspases. Upon ligand binding, TNF-family receptors containing cytosolic Death Domains cluster in membranes, recruiting caspase-binding adaptor proteins, including the bipartite adapter FADD that contains both a Death Domain (DD) and a Death Effector Domain (DED)  

The DED of FADD binds DED-containing pro-caspases (e.g. caspases-8 and -10), forming a “death inducing signaling complex” (DICS) and resulting in caspase activation by an “induced proximity” mechanism  

---

For personal use only.on October 28, 2017. By guest
A delicate balance between pro-apoptotic and anti-apoptotic regulators of apoptosis pathways is at play on a continual basis, ensuring the survival of long-lived cells and the proper turnover of short-lived cells in a variety of tissues, including the bone marrow, thymus, and peripheral lymphoid tissues. However, imbalances in this delicate dance of pro-and anti-apoptotic proteins occur in disease scenarios, including cancer where the scales tip in favor of anti-apoptotic proteins, endowing cells with a selective survival advantage that promotes neoplasia and malignancy.

**Drug discovery strategies directed at core components of the apoptosis machinery**

The apoptosis-blocking proteins that thwart signaling through specific apoptosis pathways provide targets for possible drug discovery, as do agents that stimulate pro-apoptotic proteins within these cell death pathways. Theoretically, all drug targets can be addressed in multiple ways, modulating the activity of the target at the levels of the DNA (gene expression), mRNA, or protein. A summary of some of the more promising strategies for altering the activity of apoptosis genes and proteins for cancer therapy is provided below, according to the apoptosis pathways they modulate.

**Intrinsic pathway**

Bcl-2-family proteins are the most prominent of the intrinsic pathway targets for cancer drug discovery. Though the human genome contains 25 members of this gene family, only six are anti-apoptotic and thus represent logical targets for therapy: Bcl-2, Bcl-XL, McI-
1, Bcl-W, Bfl-1, and Bcl-B. Over-expression of several of these anti-apoptotic Bcl-2-family proteins has been documented in various hematopoietic malignancies, though data are most extensive for the founding member of the family Bcl-2 (reviewed in 6,7). For example, elevated Bcl-2 protein as a result of t(14;18) translocations involving the \textit{BCL-2} gene occurs in 80-90% of low-grade follicular non-Hodgkin’s lymphomas 6. Approximately one-third of diffuse large cell lymphomas (DLCL) have pathologically elevated Bcl-2 (often in association with t(14;18) translocations or gene amplification), correlating with shorter patient survival when treated with combination chemotherapy, unless supplemented with anti-CD20 antibody (Rituximab) 8. Most chronic lymphocytic leukemias (CLLs) contain elevated Bcl-2, associated with hypomethylation of the \textit{BCL-2} gene 9. In addition, elevated Mcl-1 protein expression occurs in nearly half of CLL B-cells, correlating with failure to achieve complete remission after treatment with chemotherapy (reviewed in Kitada, 2004 #12450). In some cases, increased Mcl-1 protein expression is associated with mutations in the promoter of the \textit{MCL-1} gene 10. In contrast to genetic alterations that activate anti-apoptotic genes such as \textit{BCL-2} and \textit{MCL-1}, leukemias and lymphomas with microsatellite instability commonly develop inactivating mutations in pro-apoptotic gene \textit{BAX} 11.

Attempts to overcome the cytoprotective effects of Bcl-2 or related anti-apoptotic proteins in cancer and leukemia include three strategies: 1) shutting off gene transcription; 2) inducing mRNA degradation with antisense oligonucleotides; and 3) directly attacking the proteins with small-molecule drugs. A fourth strategy attempts to invoke endogenous antagonists of anti-apoptotic Bcl-2-family proteins.
**Drugs regulating BCL-2 gene expression.** Some synthetic retinoids reduce levels of BCL-2 or BCL-X<sub>L</sub> mRNA in leukemia cells, suggesting a potential explanation for the pro-apoptotic effect of these agents already approved for clinical use<sup>12</sup>. Compounds that inhibit histone deactylases (HDACs), chromatin-modifying enzymes, also reduce the expression of BCL-2, BCL-X, or MCL-1 at a transcriptional level in some leukemia cell lines and primary cultured myeloma and leukemia cells<sup>13-15</sup>. Clinical trials of HDAC inhibitors are progressing, with hints of activity documented for lymphoma and some solid tumors<sup>16</sup>. PPARγ-modulating drugs also reduce expression of BCL-2 or other anti-apoptotic BCL-2 family genes, at least in preclinical studies of human leukemia cell lines<sup>17</sup>.

**Drugs attacking BCL-2 mRNA.** Antisense oligodeoxynucleotides (ODNs) targeting the BCL-2 mRNA are undergoing clinical evaluation, with Phase III trials underway for relapsed or refractory CLL, AML, and myeloma<sup>18</sup>. The molecules in clinical trials are comprised of nuclease-resistant phosphorothioates, which hybridize via Watson-Crick base-pairing to the first 18 nucleotides within the open reading frame of BCL-2 mRNAs and induce RNaseH-mediated degradation<sup>19</sup>. This DNA-based drug (G3139) (Oblimersen sodium) (Genasense<sup>TM</sup>) has been delivered in combination with cytotoxic chemotherapy, in an attempt to exploit the chemosensitizing properties of Bel-2-based therapies. The first reported Phase I trial of BCL-2 antisense therapy showed promising activity against chemorefractory non-Hodgkin’s lymphoma (NHL)<sup>20</sup>. Uncontrolled Phase II data also suggest that this BCL-2 antisense molecule (G3139) (Oblimersen sodium) (Genasense<sup>TM</sup>) has promising bioactivity against B-cell malignancies and adult AML. A recently completed
trial of Oblimersen sodium for advanced melanoma (in combination with Dacarbazine) demonstrated improved response rates and time to progression, but failed to meet its primary end-point of prolonging survival.

Demonstration of a correlation between clinical responses and antisense-mediated knock-down of BCL-2 mRNA or protein levels in patients undergoing treatment with G3139 has been documented in some but not all studies, raising questions about the mechanism of this novel targeted therapy \(^{21,22}\). However, it has been argued that cells with successful antisense-mediated reductions in BCL-2 mRNA are difficult to recover from patients, because of their rapid in vivo clearance induced by apoptosis. The Bcl-2 protein also exhibits a slow degradation rate (~12-36 hrs), suggesting that prolong suppression of mRNA accumulation is necessary to produce a decline in Bcl-2 protein \(^{23}\). A proinflammatory effect of G3139, seen particularly at higher doses, suggests an additional non-antisense bioactivity, which may or may not be relevant to tumor responses. In this regard, CpG motifs in synthetic DNA molecules trigger inflammatory responses by engaging Toll-like Receptor-9 (TLR9), tapping into a mechanism of innate immunity intended to sense the presence of unmethylated bacterial DNA \(^{24}\). Interestingly, mismatched ODNs containing CpG motifs reduce BCL-2 expression and promote apoptosis of prostate cancer cell lines through an antisense-independent mechanism \(^{25}\). Nevertheless, tumor xenograft studies of G3139 in immunocompromised mice provide strong support for an antisense-based mechanism, whereby sequence-specific reductions in BCL-2 expression either directly trigger tumor apoptosis or sensitize malignant cells to traditional cytotoxic anticancer drugs \(^{26-28}\).
Aside from G3139, no nucleic acid-based inhibitors of Bcl-2 or its anti-apoptotic relatives are presently in clinical testing, but preclinical studies have been reported Bcl-XL\textsuperscript{29}. Dual Bcl-2/Bcl-XL-suppressing antisense oligonucleotides may overcome some of the potential problems of redundancy caused by simultaneous over-expression of multiple anti-apoptotic members of the Bcl-2 family in malignant cells\textsuperscript{30}.

**Drugs attacking Bcl-2 proteins.** Small-molecule inhibitors directly binding Bcl-2 or related anti-apoptotic proteins have also entered clinical trials for cancer. The most advanced is a natural product, Gossypol, whose ability to bind and inhibit Bcl-2 was unknown when initial clinical testing began\textsuperscript{31}. Gossypol is a compound found in cottonseeds originally used as an herbal medicine in China\textsuperscript{32}. Gossypol binds a hydrophobic pocket found on the surface of anti-apoptotic Bcl-2-family proteins (Figure 2). This binding pocket represents a regulatory site, where endogenous antagonists dock onto Bcl-2 and related anti-apoptotic proteins, negating their cytoprotective activity\textsuperscript{33}. The endogenous antagonists bind via a conserved 16 amino-acid motif called a Bcl-2 Homology-3 (BH3) domain. Proof of concept experiments using BH3 peptides have suggested that compounds docking at this regulatory site on Bcl-2 and Bcl-XL effectively promote apoptosis of lymphoma and leukemial cells *in vivo* in mice.\textsuperscript{34,35}

Gossypol interacts with the BH3-binding pockets of four anti-apoptotic Bcl-2-family proteins tested to date, Bcl-2, Bcl-XL, Bcl-B, and Bfl-1, displacing BH3 peptides with an IC\textsubscript{50} of \(~0.5\) uM. A Gossypol enantiomer may have superior activity compared to the naturally occurring racemer, and is undergoing late steps of preclinical development in preparation for
clinical trials 36. However, Gossypol is a highly reactive compound, containing two aldehydes, probably explaining some of the toxicities originally seen in Phase I trials of this natural product, in addition to producing unfavorable pharmacological properties. For this reason, attempts to produce semi-synthetic analogs that retain activity against Bcl-2 have begun. The most advanced is the compound Apogossypol, in which the two aldehydes were eliminated 37.

Several other chemical inhibitors of Bcl-2, Bcl-XL, and Mcl-1 have been reported, all of which are currently in preclinical evaluation, including: (a) HA14-1, a chromene derivative identified by a computational modeling approach 38; (b) BH3I-1 and -2, a thiazolidin derivative and benzene sulfonyl derivative, respectfully, identified by screening using a BH3 peptide displacement assay 39; (c) antimycin analogs, identified by computational modeling 40; (d) certain Theaflavins and Epigallocatechin (EGCG), natural products abundant in black and green tea, respectively, discovered by NMR-based methods 41; (e) A-779024, a synthetic small-molecule inhibitor produced by NMR-guided, structure-based drug design (Abbott), and others 32(Table 1). Side-by-side comparisons of these chemical inhibitors of anti-apoptotic Bcl-2 proteins have not been reported, but their approximate rank-order potency with respect to affinity for the BH3 pocket of Bcl-2 or Bcl-XL appears to be A-779024 > EGCG > Theaflavins > Gossypol > Apogossypol > HA14-1 and Antimycin. Also, it is unclear which of the six anti-apoptotic Bcl-2-family proteins are targeted by these compounds, though Bcl-XL and Bcl-2 define a minimal subset. Several issues will be encountered as these chemical antagonists of Bcl-2 move forward with their
preclinical and clinical development, including: (a) compound stability and formulation; (b) pharmacokinetics and metabolism; (c) toxicity; and (d) off-target mechanisms.

**Activating endogenous antagonists of Bcl-2.** A fourth route to addressing the problem of pathological over-expression of Bcl-2 in malignant cells seeks to induce expression or activation of endogenous proteins that bind and negate the cytoprotective action of Bcl-2. For example, an orphan member of the retinoid/steroid family of nuclear receptors, TR3 (Nur77) translocates from nucleus to cytosol in response to certain cell death stimuli. In the cytosol, TR3 binds a regulatory domain within the Bcl-2 protein, thus accounting for accumulation of TR3 on mitochondria. TR3 induces a profound conformational change of Bcl-2, causing BH3 domain exposure and converting Bcl-2 from a protector to a killer. The BH3 domain of Bcl-2 binds pro-apoptotic Bcl-2-family members Bax and Bak, as well as anti-apoptotic Bcl-2-family proteins, probably activating the pro-apoptotic while inactivating the anti-apoptotic (Figure 3).

Recently, a class of compounds representing analogs of the retinoid AHPN has been identified that induce TR3 expression and translocation into the cytosol. The prototype compound, 3Cl-AHPC, lacks activity against retinoid receptors, though having evolved from attempts to synthesize highly selective retinoid ligands. 3Cl-AHPC has demonstrated activity against cultured leukemia cells and in preclinical animal models of AML, retaining activity even against retinoid refractory leukemia cell lines. 3Cl-AHPC appears to induce phosphorylation of TR3, possibly via a Jun N-terminal kinase (JNK) pathway, correlating with its exodus from the nucleus. This compound may therefore provide a
mechanism to invoke the TR3 pathway, converting Bcl-2 from a protector to a killer, and thus making Bcl-2 a liability rather than an advantage for malignant cells.

Opposing this TR3-mediated pathway for apoptosis is Akt/PKB, which appears to nullify pro-apoptotic actions of TR3 by phosphorylation at Serine 530⁴⁸,⁴⁹. Thus, TR3 joins a long list of apoptosis-relevant substrates of Akt, which includes Bcl-2 antagonist BID, human Caspase-9, pro-apoptotic kinase Ask1, p53 antagonist Mdm2, and others⁵⁰. Akt is activated downstream of several oncogenes of relevance to leukemia and lymphoma, including BCR/ABL of CML, Tcl 1 of T cell CLL, NPM-ACK of anaplastic large cell lymphomas, FOP-FGFR1 of stem cell myeloproliferative disorder, LMP1 and LMP2A of Epstein Bar Virus, and the K1 protein of Karposi’s sarcoma associated Herpes Virus implicated in some lymphomas. It remains to be determined what effect the Tcl-1 oncoprotein has on nuclear export of TR3, but it may be of interest that this protein over expressed in CLLs promotes nuclear targeting of Akt and regulates the transcriptional activity of TR3⁴⁸. Small wonder then that Akt has emerged as a prominent target for cancer drug discovery⁵¹. Relatively selective but fairly weak inhibitors of Akt such as 1L-Hydroxymethyl-chiro-inositol-2-([(R)-2-methyl-3-O-octadecylcarbonate) have been described, which has preclinical activity against multiple myeloma cells and sensitizes established myeloid leukemia cell lines to chemotherapeutic drugs, retinoids, radiation, and TRAIL⁵¹. Interestingly, 3Cl-AHPC may also reduce Akt activity in tumor cells⁵², further promoting the TR3 pathway for cell death AND HSP90 inhibitors such as 17-AAG [17-allylamino-17-demethoxygeldanamycin] destabilize and cause reductions in Akt levels⁵³.
The concept of invoking endogenous antagonists of Bcl-2 is not novel. Anti-apoptotic Bcl-2-family proteins are normally held in-check by endogenous proteins that contain a BH3 domain, especially the so-called BH3-only proteins (BOPs). Several BOPs are linked to pathways that explain how currently available cytotoxic drugs trigger apoptosis, including: (a) the genes encoding Puma, Noxa, and Bid, whose expression is directly induced by p53 in response to DNA damage; (b) Bim proteins, which are normally sequestered on microtubules; and (c) Bax, which is held in an inactive conformation by the regulatory subunit (Ku70) of the DNA-dependent kinase, becoming released during DNA damage responses \(^{54,55}\). Unfortunately, defects in pathways involving BOPs and pathological over-expression of Bcl-2 all too frequently create roadblocks to apoptosis that must be overcome by alternative means. The observation that commonly used cytotoxic anticancer drugs are capable of modulating endogenous pathways impinging on Bcl-2 underscores why Bcl-2-targeted therapies are often synergistic with conventional cytotoxic agents. Knowledge of the molecular details of mechanisms by which traditional cytotoxic anticancer drugs affect Bcl-2-family proteins thus may help in selecting optimal combination therapies for clinical trials of Bcl-2 antagonists. Underscoring the importance of this knowledge-base are recent studies suggesting that alkylating agents induce cell death via necrosis rather than apoptosis, independent of Bcl-2/Bax-family proteins \(^{56}\). It is therefore unfortunate that many early clinical trials of Bcl-2 antagonists (e.g. G3139 [Genasense\textsuperscript{TM}]) have involved combination with alkylating agents, which may not provide a synergistic combination. New targets for drug discovery are also emerging from studies of these endogenous pathways, such as compounds that enhance wild type P53 by blocking interactions with Mdm2 (Table 3) \(^{57}\).
**Extrinsic pathway**

The extrinsic pathway is activated by TNF-family ligands that engage TNF-family death-receptors, resulting in activation of caspases and apoptosis. Interest has emerged in therapeutics that kill cancer cells via the extrinsic pathway, particularly since chemorefractory cells often have defects in their intrinsic pathway, given the predominant reliance of cytotoxic drugs and x-irradiation on the mitochondrial cell death pathway. In this regard, the TNF cytokine family consists of 18 members in humans, with 29 counter receptors. Some of these TNF-family receptors transduce signals predominantly for cell survival, particularly those that bind intracellular TRAF-family adapter proteins that link to downstream protein kinases. Blocking these receptors represents a potentially attractive strategy for a variety of lymphoid malignancies, but will not be discussed here. Other members of the TNF-family directly trigger apoptosis, particularly those that contain DDs within their cytosolic tails (n = 8 in humans). Of these receptors and their corresponding ligands, four receptors (three ligands) have been studied in detail for use as cancer therapeutics—namely, TNF (TNFR1), FasL (Fas), and TRAIL (DR4; DR5). Strategies for exploiting apoptosis-inducing TNF-family ligands and receptors for cancer therapy mostly focus on: (a) recombinant ligands (biologics), expressing only the extracellular domain of these type II integral membrane proteins, or (b) agonistic monoclonal antibodies that bind the receptors and trigger apoptosis. In addition, however, emerging knowledge about intracellular modulators of apoptosis pathways utilized by TNF-family death receptors has
suggested opportunities for engaging the extrinsic pathway at other levels, improving sensitivity of malignant cells to biologicals that activate the extrinsic pathway.

**TNF-family ligands and receptor-targeting antibodies.** Over 3 decades ago, TNF was reported to selectively kill tumor but not normal cells, raising hopes that this cytokine might be exploited for cancer treatment. Unfortunately, the pro-inflammatory actions of TNF precluded its systemic administration, and squelched efforts to apply it clinically. Then, we knew little about how TNF transduces signals into cells making it difficult to propose alternative strategies. Today, we have an in-depth knowledge of the pathways that TNF and related cytokines activate, and from this knowledge has come several potential targets for drug discovery. We also understand far better how TNF-family cytokines regulate pathways that control apoptosis, and we can begin to envision ways of exploiting that knowledge for sensitizing tumors to these cytokines that immune cells employ for battling cancer.

Binding of TNF to its cellular receptor, TNFR1 triggers two parallel signaling pathways (Figure 4). These pathways bifurcate at the adapter protein TRADD. One of these pathways results in activation of caspase-family proteases, triggering apoptosis. The other parallel pathway triggers activation of NFκB-family transcription factors. NF-κB influences the expression of many target genes involved in host defenses and immune regulation, but also genes that suppress apoptosis. As a result, this NFκB pathway nullifies the caspase pathway, in addition to producing untoward inflammatory side-effects.

The TNF-family cytokines Fas-Ligand (FasL) and TRAIL (Apo2 Ligand) trigger activation of the Caspase pathway without concomitant induction of NFκB. These death
ligands are expressed on cytolytic T cells (CTLs), NK cells, and other types of immune cells and are used as weapons for eradication of virus-infected and transformed cells \(^{59,62,63}\). Various studies using mice with ablation of genes encoding these death ligands or their receptors, as well as neutralizing antibodies and Fc-fusion proteins, have provided evidence that FasL and TRAIL play important roles in tumor suppression by cellular immune mechanisms. For example, FasL, is important for CTL-mediated killing of some tumor targets, and TRAIL is critical for NK-mediated tumor suppression \(^{64,65}\).

The absence of proinflammatory effects of FasL and TRAIL has raised hopes that they might be successfully applied for cancer therapy, where TNF failed due to toxicity. While agonistic antibodies that trigger the Fas (CD95) are unfortunately highly toxic to liver \(^{66}\), TRAIL and agonistic antibodies that bind TRAIL-receptors appear to be well tolerated in vivo. Indeed, Phase I clinical trials have recently been completed using an agonistic monoclonal antibody ETR1 (Human Genome Sciences), directed against TRAIL-Receptor-1 (TRAIL-R1) [DR4], revealing little toxicity \(^{67}\). In mouse xenograft models using selected tumor human cells lines, TRAIL and agonistic antibodies directed against TRAIL-receptors exhibit potent antitumor activity and often synergize with chemotherapy \(^{68}\), raising hopes of using these biological agents as a novel approach to cancer treatment and thereby mimicking some of the effector mechanisms employed by the immune system in its defense against transformed cells.

*Intracellular modifiers of extrinsic pathway – drug targets.* Among the prominent modulators of sensitivity to TNF-family death receptors are NFκB-family transcription
factors and FLIP, a DED-containing protein that binds the apical proteases in the extrinsic pathway, pro-caspases-8 and 10. Both of these drug targets have garnered the attention of the researchers seeking ways of increasing sensitivity of malignant cell to TNF-family cytokines.

**NFκB.** Abnormal elevations in NFκB activity occur in many tumors, including many lymphoid malignancies. In fact, the first NFκB-family member identified, c-REL, is the cellular counterpart of a viral transforming gene, v-rel, discovered in the avian leukosis virus. The importance of NFκB for suppression of TNF-induced apoptosis is well established, and several anti-apoptotic genes are among the direct transcriptional targets of Rel-family proteins, including the genes encoding c-FLIP, cIAP2, Bcl-XL, and Bfl-1. Consequently, interfering with signal transduction events that generate active NFκB has emerged as an attractive strategy for sensitizing cancer cells to TNF and related cytokines.

Studies of signaling events responsible for NFκB activation by TNF have revealed several candidate targets potentially amenable to small-molecule drug discovery. For example, though alternative pathways exist, a evolutionarily conserved pathway for NFκB activation has been elucidated involving various upstream kinases funneling signals into a pivotal kinase complex that consists of two homologous serine-kinases, IKKα and IKKβ, and a scaffold protein, IKKγ (NEMO). This IKK complex is responsible for phosphorylating endogenous inhibitors of NFκB, the IκB proteins, thus targeting them for ubiquitination and subsequent proteasome-dependent degradation. Degradation of IκB
releases NFκB, allowing translocation into the nucleus. Thus, the protein kinases involved in this pathway have emerged as promising drug targets, as well as the proteasome (Figure 4).

Chemical inhibitors of IKKs have been described, which display pro-apoptotic activity against cultured malignant cells, including BMS-345541, Bay 11-7082, SC-514, Sulfasalazine, Silibinin, a dietary flavonoid, pyrrolidine dithiocarbamate, natural product Wedelolactone, semi-synthetic derivatives of B-carboline natural products, and others. The specificity of these inhibitors is questionable, but toxicology studies will determine whether they might nevertheless be clinically useful. Proteasome inhibitors include Bortezomib, recently approved for treatment of refractory myeloma and in clinical testing for several types of cancer and leukemia (Millennium Pharmaceuticals, Inc.). Bortezomib (PS-341) (Velcade™) inhibits the protease activity of the proteasome. Pharmacological inhibition of proteasome activity suppresses IκB degradation, but also has effects on stability of many cellular proteins, and thus the anti-tumor activity of proteasome inhibitors may only partly be related to NFκB activity.

**FLIP.** Many leukemias and lymphomas exhibit intrinsic resistance to TNF, TRAIL, and FasL, despite expressing the necessary cell-surface receptors. Multiple antagonists of the extrinsic pathway have been identified, including several DED-containing proteins that compete for binding to the adapter proteins or pro-caspases that participate in TNF-family death receptor signaling. Among these, FLIP has received the most attention for its role in producing Fas- and TRAIL-resistant states in tumor cells, including Reed-Steinberg cells.
of Hodgkin’s disease, EBV-positive Burkitt lymphomas, KSHV (HHV8)-associated B-cell lymphomas, and other types of B-cell NHLs.

The c-FLIP protein is highly similar in sequence to pro-caspase-8 and –10, containing tandem DEDs, followed by a pseudo-caspase domain lacking enzymatic activity. Two isoforms of c-FLIP are produced from a single gene, including the long form described above and a shorter isoform that consists only of DED domains. The shorter FLIP thus resembles analogous proteins encoded in genomes of animal viruses. FLIP-S is exclusively anti-apoptotic while FLIP-L can be either pro- or anti-apoptotic, depending on its levels of expression relative to pro-caspase-8 and –10. FLIP proteins form complexes with pro-caspase-8 and 10, preventing their effective activation, as well as competing for binding to adapter proteins required for caspase recruitment to death receptor complexes. Over-expression of FLIP occurs commonly in a variety of B-cell malignancies, and some AMLs and CLLs. FLIP promotes tumor progression in immunocompetent mouse models of lymphoma, implying in vivo selection for high FLIP expression as a result of confrontations of neoplastic cells with the immune system.

Though no compounds have been described that directly bind FLIPs, experimental agents have been reported that reduce FLIP protein expression. Among these are synthetic triterpenoids 2-cyano-3,12-Dioxooolean-1,9-Bien-28-Oic Acid (CDDO) and CDDO-methyl ester (CDDO-me), which induce ubiquitination and proteasome-dependent destruction of FLIPs in cultured cancer cells, sensitizing solid tumor lines to apoptosis induction by TNF, Fas, and TRAIL. Moreover, CDDO analogs exhibit single agent activity in terms of inducing apoptosis in vitro of cultured leukemia cells, including chemorefractory CLLs,
AMLs, and myelomas \textsuperscript{89,90}. The mechanism of CDDO and related compounds is under investigation, but most studies report caspase-8 activation as a proximal event, indicative of extrinsic pathway stimulation \textsuperscript{89,91}. As such, CDDO and its analogs may provide an option for bypassing blocks to apoptosis typically arising within the mitochondrial apoptosis pathway in chemorefractory tumors. CDDO and CDDO-me are undergoing final preclinical development steps in preparation for clinical trials. Additional possible routes to FLIP suppression include NF\textsubscript{k}B pathway inhibitors \textit{(see above)} and HDAC inhibitors \textsuperscript{92}.

\textbf{Convergence pathway}

The intrinsic and extrinsic pathways for apoptosis converge on downstream effector caspases. Certain effector caspases are targets of suppression by an endogenous family of anti-apoptotic proteins called IAPs (Inhibitor of Apoptosis Proteins). IAPs contain one or more copies of a domain called the baculoviral IAP repeat (BIR) \textsuperscript{93}. BIRs are sometimes accompanied by other domains, including RING and ubiquiting-conjugating enzyme domains, endowing them with additional properties such as ability to target themselves and associated proteins for ubiquitination and proteasome-dependent degradation. The human genome encodes eight IAP-family members: XIAP, cIAP1, cIAP2, Naip, Survivin, ML-IAP (Livin; K-IAP), ILP2 (Ts-IAP), and Apollon (Bruce) \textsuperscript{2}.

Pathological over-expression of IAPs has been documented in cancer and leukemia \textsuperscript{94-96}. The functional importance of IAPs for apoptosis suppression in cancers has been documented by antisense experiments, in which knocking-down expression of \textit{SURVIVIN}, \textit{XIAP}, or others
induced apoptosis of tumor cell lines in culture or sensitized to apoptosis induced by anticancer drugs \(^{97-100}\).

Routes to small-molecule drug discovery have been revealed through knowledge of the structural and molecular details of how IAPs inhibit caspases, enlightened by insights gained from studies of endogenous inhibitors of IAPs. For instance, specific segments within IAP-family proteins have been identified that bind certain caspases, providing structural information useful for generating compounds that disrupt these protein interactions, freeing caspases to induce apoptosis \(^{101,102}\).

**Compounds targeting IAP-family proteins.** Using an enzyme derepression assay in which XIAP-mediated suppression of caspase-3 is overcome by chemical compounds, two classes of XIAP antagonists were identified that target in the vicinity of the second BIR domain (BIR2), freeing caspase-3, including di/tri-phenylureas and benzenesulfonamide derivatives \(^{103,104}\) (Table 2). The phenylureas exhibit broad-spectrum apoptosis-inducing activity against cultured tumor cell lines and primary leukemias, and suppress growth of tumor xenografts in mice without apparent toxicity to normal tissues \(^{103}\). Importantly, these compounds induce apoptosis through a Bcl-2/Bcl-X\(_L\)-independent mechanism, suggesting they may prove useful for chemorefractory cancers and leukemias.

Mimicking endogenous antagonists of IAPs defines another strategy for identifying chemical inhibitors. Endogenous proteins SMAC and OMI (HtrA2) bind and suppress IAPs, releasing caspases \(^{101}\). A 4'mer peptide corresponding to the N-terminus of the mature SMAC protein is sufficient to bind IAPs and block their association with caspases, analogous
to similar mechanisms previously defined in Drosophila where IAPs are suppressed by endogenous antagonists Reaper, Hid, Grim, and Sickle \(^{105}\). By fusing membrane-penetrating peptides onto SMAC, OMI, Hid or Reaper peptides, apoptosis of human cancer cell lines can be induced in culture, and tumor growth suppression obtained in xenograft models in mice \(^{106-108}\). These data thus provide proof-of-concept evidence that compounds mimicking effects of IAP-binding peptides could potentially be exploited as drugs for cancer treatment.

The 3-dimensional structure of the BIR3 domain of XIAP complexed with SMAC reveals the N-terminal 4 amino-acids of mature SMAC bind in the same crevice normally occupied by the N-terminus of the small-subunit of processed caspase-9, thus suggesting competition for binding \(^{101}\) (Figure 5). Consequently, compounds that mimic the SMAC 4’mer peptide should dislodge active caspase-9 from BIR3, promoting apoptosis. Several groups have initiated preclinical studies of SMAC-mimics, though few details are available currently. Reported antagonists targeting the SMAC binding site include natural product, embelin \(^{109}\), various peptidiomimetics and nonpeptidic small molecules \(^{110-112}\) (Table 2).

Besides SMAC and OMI, additional endogenous antagonists of IAPs have been described: ARTS, XAF1, and NRAGE \(^{113-115}\) (Figure 6). Structural details of how these proteins interact with IAP targets are unknown, but could provide additional strategies for generation of small-molecule antagonists that work through non-SMAC mechanisms. Given that the reported phenylurea-base antagonists of XIAP target a non-SMAC site on XIAP \(^{103}\), it will be interesting to determine whether those compounds mimic ARTS, NRAGE or XAF1 in terms of their docking site on XIAP.
Finally, various indirect strategies for negating IAP activity are emerging. For example, phosphorylation of Survivin on threonine 34 is required for cell division and suppression of apoptosis. The kinases predominantly responsible are Cdc2 or related cyclin-dependent kinases. Compounds inhibiting Cdc2-family kinases include Flavopiridol (Aventis), currently in clinical trials.

**Drugs Inhibiting IAP-family gene expression.** Preclinical efforts to reduce expression using antisense-based drugs are well underway. Antisense ODNs targeting SURVIVIN mRNA have been evaluated, and designated for clinical development, using second-generation antisense chemistry (ISIS Pharmaceuticals/Lilly Inc). An antisense-based approach to Survivin is particularly attractive, because the mechanism by which this protein suppresses caspases differs from most other IAPs, and a clear path forward for compound discovery remains uncertain based on protein structure data. In addition, a phosphorothioate-based antisense ODN targeting XIAP (AEG35156/GEM640™) has been designated for human clinical trials (Agera Pharmaceuticals). It remains to be determined whether a highly selective (antisense) versus broad-spectrum (small-molecule) approach to attacking IAPs will yield the optimal therapeutic index.

**Conclusions**
Emerging knowledge about molecular mechanism of apoptosis dysregulation in cancer and leukemia has revealed a plethora of potential drug discovery targets. Structural analysis of apoptosis proteins and studies of their biochemical mechanisms have suggested strategies for
lead generation, resulting in numerous novel chemical entities with mechanism-based activity. Though providing encouraging proof of principle data that validate these targets, much work lies ahead in terms of optimizing the spectrum of activity of compounds that interact with multiple members of apoptosis protein families, improving their pharmacological properties, establishing optimal formulations for stability and delivery, and elucidating rate-limiting toxicities. Many of the most logical targets for promoting apoptosis of cancer and leukemia cells are technically challenging, often involving disrupting protein interactions or altering gene expression, as opposed to traditional pharmaceuticals that attack enzymes. Modern techniques of structure-based drug optimization render this task feasible, but still challenging. Such targets require long-term commitments, often outstripping the usual drug discovery and development cycle time practiced at pharmaceutical companies. For those with the stamina, the rewards are likely to be great, creating a new era in cancer therapy where the intrinsic or acquired resistance of malignant cells to apoptosis can be pharmacologically reversed, reinstating natural pathways for cell suicide.

**Acknowledgements.** We thank I. Pedersen and S. Kitada for helpful information, A. Godzik, X. Zhang and G. Salvesen for suggestions about figures, and M. LaMie and J. Valois for manuscript preparation. Our apologies to the many authors whose work we could not cite due to manuscript length limitations.
REFERENCES:


52. Farhana L, Dawson MI, Huang Y, Zhang Y, Rishi AK, Reddy KB, Freeman RS, Fontana JA. Apoptosis signaling by the novel compound 3-Cl-AHPC involves increased EGFR proteolysis and accompanying decreased phosphatidylinositol 3-kinase and AKT kinase activities. Oncogene. 2004;23:1874-1884


69. Tschopp J, Irmler M, Thome M. Inhibition of Fas death signals by FLIPs. Curr Opin Immunol. 1998;10:552-558
87. Chang DW, Xing Z, Pan Y, Algeciras-Schimnich A, Barnhart BC, Yaish-Ohad S, Peter ME, Yang X. c-FLIP_L is a dual function regulator for caspase-8 activation and CD95-mediated apoptosis. EMBO J. 2002;21:3704-3714
95. Altieri DC, Marchisio PC, Marchisio PC. Survivin apoptosis: An interloper between cell death and cell proliferation in cancer. Lab Invest. 1999;79:1327-1333
96. Liston P, Fong WG, Korneluk RG. The inhibitors of apoptosis: there is more to life than Bcl2. Oncogene. 2003;22:8568-8580
Smac/DIABLO peptide potentiates epothilone B derivative-(BMS 247550) and Apo-2L/TRAIL-induced apoptosis. Blood. 2002;99:3419-3426
Table 1. Bcl-2 antagonists. The structures of non-peptidyl antagonists of Bcl-2 and Bcl-XL are shown.

Table 2. IAP antagonists. The structures of non-peptidyl chemical inhibitors are shown.

Table 3. Summary of chemical leads addressing apoptosis-relevant targets. Chemical leads against targets are listed. The approximate stage of clinical development is indicated. It should be noted that some of the chemical leads interact with additional protein targets not listed here. Some targets such as the proteasome, NFκB, and HSP90 operate on many other proteins besides the apoptosis-relevant proteins. Only antisense and protein compounds that are in clinical development are listed. Gene therapy is excluded.

Figure 1. Caspase-activation pathways. See text for details.

Figure 2. Bcl-XL with a chemical inhibitor obtained via docking studies. A) Ribbon model of the NMR structure of Bcl-XL with the docked inhibitor epigallocatechin gallate (EGCG; ball-and-stick). B) The BH3 binding pocket of Bcl-XL is shown and color coded according to cavity depth: blue, shallow; yellow, deep. The docked conformation of the compound EGCG is also shown (ball-and-stick). The figures were generated with MOLMOL (A) and MOLCAD (B) as implemented in Sybyl (Tripos, St. Louis).

Figure 3. Model for TR3-mediated conversion from protector to killer. Bcl-2 can assume two conformational states, including an anti-apoptotic conformation in which its BH3 domain is buried and a pro-apoptotic state in which its BH3 domain is exposed. The pro-apoptotic form can either activate pro-apoptotic proteins Bax and Bak, or inactivate anti-apoptotic proteins such as Bcl-XL.

Figure 4. Dual pathway activation by TNF. TNFR1 (as well as DR3 and DR6) recruits TRADD, which mediates interactions with two opposing pathways. Targets for drug discovery are indicated. See text for details.

Figure 5. BIR domain and its inhibitory SMAC peptide derived from X-ray crystallography. A) X-ray structure of the third BIR domain of XIAP (ribbon drawing) in complex with the tetra-peptide AVPI derived from SMAC (ball-and-stick). B) The surface of the third BIR domain of XIAP is shown and color coded according to cavity depth: blue, shallow; yellow, deep. The AVPI tetra-peptide is shown in ball-and-stick representation. The figures were generated with MOLMOL (A) and MOLCAD (B) as implemented in Sybyl (Tripos, St. Louis).

Figure 6. Endogenous antagonists of IAPs. The known mammalian inhibitors of IAPs are indicated. ARTS, SMAC, and OMI normally reside inside mitochondria. Only selected members of the IAP family are targeted by these endogenous antagonists.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA14-1</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>(2-Amino-6-bromo-4-(cyano-ethoxy carbonyl-methyl)-4H-chromene-3-carboxylic acid ethyl ester) Chromene derivative</td>
</tr>
<tr>
<td>BH3I-1</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>(2-[5-(4-Bromo-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-3-methyl-butyric acid) Thiazolidin derivative</td>
</tr>
<tr>
<td>BH3I-2</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>(5-Chloro-N-[2-chloro-4-(4-chloro-benzenesulfonyl)-phenyl]-2-hydroxy-3-iodo-benzamide) Benzenesulfonyl derivative</td>
</tr>
<tr>
<td>Antimycin A3</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>3-(3-Formylamino-2-hydroxy-benzoylamino)-2,6-dimethyl-4,9-dioxo-8-pentyl[1,5]dioxonane-7-carboxylic acid isopropyl ester</td>
</tr>
<tr>
<td>Compound 6</td>
<td><img src="image5.png" alt="Structure" /></td>
<td>(2,9-Dimethoxy-11,12-dihydro-dibenzo[c,g]1,2)diazocine 5,6-dioxide) Dibenzodiazocine derivative</td>
</tr>
<tr>
<td>Apogossypol</td>
<td><img src="image6.png" alt="Structure" /></td>
<td>(5,5'-Disopropyl-3,3'-dimethyl-[2,2]binaphthalenyl-8,8'-dicarbaldehyde)</td>
</tr>
<tr>
<td>Gossypol</td>
<td><img src="image7.png" alt="Structure" /></td>
<td>(1,6,7,1',6',7'-Hexahydroxy-5,5'-disopropyl-3,3'-dimethyl-[2,2]binaphthalenyl-8,8'-dicarbaldehyde)</td>
</tr>
<tr>
<td>Terphenyl derivative</td>
<td><img src="image8.png" alt="Structure" /></td>
<td>3-(3-Formylamino-2-hydroxy-benzoylamino)-2,6-dimethyl-4,9-dioxo-8-pentyl[1,5]dioxonane-7-carboxylic acid isopropyl ester</td>
</tr>
<tr>
<td>Theaflavin</td>
<td><img src="image9.png" alt="Structure" /></td>
<td>(3,4,6-Trihydroxy-1-(3,5,7-trihydroxy-chroman-2-yl)-benzocyclohepten-5-one)</td>
</tr>
<tr>
<td>EGCG</td>
<td><img src="image10.png" alt="Structure" /></td>
<td>(3,4,5-Trihydroxy-benzoic acid 5,7-dihydroxy-2-(3,4,5-trihydroxy-phenyl)-chroman-3-yl ester) Epigallocatechingallate</td>
</tr>
</tbody>
</table>

**Table 1**
Table 2
### Chemical lead Developing company or organization Target Stage

<table>
<thead>
<tr>
<th>Compound/Target</th>
<th>Developing company/organization</th>
<th>Target</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BCL2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genasense (antisense)</td>
<td>Genta Inc. (Aventis)</td>
<td>BCL2</td>
<td>Phase III</td>
</tr>
<tr>
<td>Gossypol</td>
<td>NCI</td>
<td>BCL2</td>
<td>Phase II</td>
</tr>
<tr>
<td>Polyphenol E</td>
<td>Mayo (Mitsui Norin)</td>
<td>BCL2</td>
<td>Phase I</td>
</tr>
<tr>
<td>HA14-1 analogues</td>
<td>Raylight Chemokine Pharmaceuticals Inc.</td>
<td>BCL2</td>
<td>Preclinical</td>
</tr>
<tr>
<td>BH3I-1</td>
<td>Harvard</td>
<td>BCL2</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Antimycin A3</td>
<td>U. Washington</td>
<td>BCL2</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Compound 6</td>
<td>U. Michigan</td>
<td>BCL2</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Terphenyl derivative</td>
<td>Yale</td>
<td>BCL2</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Apogossypol</td>
<td>Burnham/NCI</td>
<td>BCL2</td>
<td>Preclinical</td>
</tr>
<tr>
<td>SAHBs</td>
<td>Harvard University</td>
<td>BCL2</td>
<td>Preclinical</td>
</tr>
<tr>
<td>A-779024 (ABT 737)</td>
<td>Abbott Laboratories</td>
<td>BCL2</td>
<td>Preclinical</td>
</tr>
<tr>
<td>3Cl-AHPC/MM11453</td>
<td>Burnham</td>
<td>NUR77/TR3</td>
<td>Preclinical</td>
</tr>
<tr>
<td><strong>IAP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzenesulphonamide derivatives</td>
<td>Novartis Genome Foundation</td>
<td>IAP</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Tripeptides</td>
<td>Abbott Laboratories</td>
<td>IAP</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Constrained peptideomimetic</td>
<td>U Michigan</td>
<td>IAP</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Embeline</td>
<td>Michigan</td>
<td>IAP</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Di/tri phenylureas (1396-11,12,34)</td>
<td>The Burnham Institute/TPIMS</td>
<td>IAP</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Compound 3</td>
<td>University of Texas Southwestern Medical Center</td>
<td>IAP</td>
<td>Preclinical</td>
</tr>
<tr>
<td>XIAP antisense (AEG35156/GEM640)</td>
<td>Agera Therapeutics Inc.</td>
<td>XIAP</td>
<td>Phase I</td>
</tr>
<tr>
<td>Survivin antisense (ISIS 23722)</td>
<td>ISIS Pharmaceuticals Inc. (Lilly)</td>
<td>Survivin</td>
<td>Phase I</td>
</tr>
<tr>
<td><strong>P53</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP-31398</td>
<td>Pfizer Inc.</td>
<td>p53</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Chlorofusin</td>
<td>University Chemical Laboratory, UK</td>
<td>MDM2</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Sulphonamidic compound 1</td>
<td>Virginia Commonwealth University</td>
<td>MDM2</td>
<td>Preclinical</td>
</tr>
<tr>
<td>2-phenoxystyril-tryptophan derivatives</td>
<td>Schering-Plough Research Institute</td>
<td>MDM2</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Nutlins</td>
<td>Hoffmann-La Roche Inc.</td>
<td>MDM2</td>
<td>Preclinical</td>
</tr>
</tbody>
</table>
## Death Receptor

<table>
<thead>
<tr>
<th>Death Receptor</th>
<th>Genentech Inc./Amgen Inc.</th>
<th>pan-TRAILR</th>
<th>Phase I</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAIL</td>
<td>Human Genome Sciences Inc.</td>
<td>TRAILR1</td>
<td>Phase I</td>
</tr>
<tr>
<td>HGS-ETR1/HGS-TR2J</td>
<td>Dartmouth College/Reata Discovery Inc.</td>
<td>FLIP</td>
<td>Preclinical</td>
</tr>
<tr>
<td>2-cyano-3,12-dioxoolean-1,9-bien-28-oic acid (CDDO)</td>
<td>NCI</td>
<td>PKC (TNF)</td>
<td>Phase II</td>
</tr>
<tr>
<td>Bryostatin</td>
<td>Ligand</td>
<td>RAR (TRAIL)</td>
<td>Approved</td>
</tr>
<tr>
<td>ATRA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## AKT

<table>
<thead>
<tr>
<th>AKT</th>
<th>University of Arizona</th>
<th>AKT/PKB</th>
<th>Preclinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIs</td>
<td>University of Bologna</td>
<td>AKT/PKB</td>
<td>Preclinical</td>
</tr>
<tr>
<td>11-6-hydroxymethyl-chiro-inositol 2(R)-2-O-methyl-3-O-octadecylcarbonate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>API-2</td>
<td>University of South Florida</td>
<td>AKT/PKB</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Wortmannin</td>
<td>University of Fribourg, Switzerland</td>
<td>PI3K</td>
<td>Preclinical</td>
</tr>
<tr>
<td>LY294002</td>
<td>Lilly Research Laboratories</td>
<td>PI3K</td>
<td>Preclinical</td>
</tr>
<tr>
<td>PX866</td>
<td>University of Arizona</td>
<td>PI3K</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Geldanamycin</td>
<td>University of Arizona</td>
<td>HSP90</td>
<td>Phase II</td>
</tr>
<tr>
<td>PU24FCI</td>
<td>Memorial Sloan-Kettering Cancer Center</td>
<td>HSP90</td>
<td>Preclinical</td>
</tr>
<tr>
<td>ADD70</td>
<td>Institut National de la Santé et de la Recherche Médicale U-517, France</td>
<td>HSP70</td>
<td>Preclinical</td>
</tr>
</tbody>
</table>

## IKK/NFκB

<table>
<thead>
<tr>
<th>IKK/NFκB</th>
<th>Millennium Pharmaceuticals Inc.</th>
<th>26S proteasome</th>
<th>Approved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bortezomib (Velcade)</td>
<td>Tokyo Metropolitan Institute of Medical Science</td>
<td>26S proteasome</td>
<td>Preclinical</td>
</tr>
<tr>
<td>MG-115, MG-132</td>
<td>University of Tokyo</td>
<td>26S proteasome</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Lactacystin</td>
<td>Yale University</td>
<td>26S proteasome</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Epoxomicin</td>
<td>SPC-839 Signal Pharmaceuticals Inc.</td>
<td>IKK</td>
<td>Preclinical</td>
</tr>
<tr>
<td>SC-514</td>
<td>Pfizer Inc.</td>
<td>IKK</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Pyridooxazinone derivative</td>
<td>Bayer AG</td>
<td>IKK</td>
<td>Preclinical</td>
</tr>
<tr>
<td>BMS-345541</td>
<td>Bristol-Myers Squibb Pharmaceutical Research Institute</td>
<td>IKK</td>
<td>Preclinical</td>
</tr>
<tr>
<td>b-carboline</td>
<td>Millennium Pharmaceuticals Inc.</td>
<td>IKK</td>
<td>Preclinical</td>
</tr>
<tr>
<td>2-amino-6-[2-(cyclopropylmethoxy)-6-hydroxyphenyl]-4-piperidin-4-yl nicotinonitri le</td>
<td>Bayer Yakuhin Ltd.</td>
<td>IKK</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Ureido-thiophene carboxamide derivative</td>
<td>SmithKline Beecham Corp./AstraZeneca</td>
<td>IKK</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Indole carboxamide derivative</td>
<td>Aventis Pharma</td>
<td>IKK</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Compound Type</td>
<td>Company</td>
<td>Inhibitor</td>
<td>Stage</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>-------------------------------</td>
<td>-----------</td>
<td>--------</td>
</tr>
<tr>
<td>Benzo-imidazole carboxamide derivative</td>
<td>Aventis Pharma</td>
<td>IKK</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Amino-imidazole carboxamide derivative</td>
<td>SmithKline Beecham Corp.</td>
<td>IKK</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Pyridyl cyanoguanidine derivative</td>
<td>LEO Pharma A/S</td>
<td>IKK</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Anilino-pyrimidine derivative</td>
<td>Signal Pharmaceuticals Inc.</td>
<td>IKK</td>
<td>Preclinical</td>
</tr>
</tbody>
</table>
Figure 3
Figure 4
Figure 5
Figure 6
Apoptosis-based therapies for hematological malignancies

John C Reed and Maurizio Pellecchia