Apoptosis is one of the major mechanisms of cell death in response to cancer therapies (1). Alterations in susceptibility to apoptosis not only contribute to neoplastic development (2) but also can enhance resistance to conventional anticancer therapies, such as radiation and cytotoxic agents (3). One of the suggested mechanisms of resistance to cytotoxic antineoplastic drugs is the alteration in expression of B-cell lymphoma-2 (Bcl-2) family members. The Bcl-2 family of proteins consists of 25 pro- and anti-apoptotic members, which interact to maintain a balance between newly forming cells and old dying cells. When anti-apoptotic Bcl-2 family members are overexpressed, the ratio of pro- and anti-apoptotic Bcl-2 family members is disturbed and apoptotic cell death can be prevented. Targeting the anti-apoptotic Bcl-2 family of proteins can improve apoptosis and thus overcome drug resistance to cancer chemotherapy (4–6).

The key players that execute the apoptotic cascade are the initiator and the effector caspases, which are activated by cleavage early in apoptosis (3, 7). Two major pathways of apoptosis converge on the effector caspases, the intrinsic and extrinsic cell-death pathways. The intrinsic cell death pathway, also known as the mitochondrial apoptotic pathway, is activated by a wide range of signals, including radiation, cytotoxic drugs, cellular stress, and growth factor withdrawal, and involves the release of proteins (including cytochrome c) from the mitochondrial membrane space (8). Cytochrome c combines with an adaptor molecule, apoptosis protease-activating factor 1, and also with an inactive initiator caspase, procaspase-9, within a multiprotein complex called the apoptosome (9). This leads to the activation of caspase-9, which then triggers a cascade of caspase (caspase-3, caspase-6, caspase-7) activation, resulting in the morphologic and biochemical changes associated with apoptosis. By contrast, the extrinsic cell-death pathway can function independently of mitochondria and is activated by cell-surface death receptors, such as Fas and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors, directly activating the caspase cascade via an “initiator” caspase (caspase-8) within a death-inducing signaling complex (10).

The intrinsic pathway (via mitochondria) plays a key role in regulating cell death in response to various stimuli (11). Mitochondrial outer membrane permeabilization is considered the “point of no return” for apoptotic cell death, triggering release into the cytoplasm of proteins that mediate cell death, such as cytochrome c (12). Outer membrane permeabilization is mediated by certain Bcl-2 family members that coordinately regulate apoptosis among a panoply of interacting pro- and anti-apoptotic proteins (13). Inner membrane permeabilization can be altered by the redox status of mitochondrial protein vicinal thiols (14) and through opening of the mitochondrial permeability transition pore (15). Although the mitochondrial permeability transition pore complex contains proteins on the outer and inner mitochondrial membranes, there is no clear involvement of inner membrane permeabilization in mitochondrial apoptosis and there is no compelling evidence that inner membrane permeabilization is necessary for outer membrane permeabilization (16).
The p53 tumor suppressor protein can induce the expression of numerous pro-apoptotic gene products that can initiate the extrinsic and intrinsic apoptotic pathways. Although the mechanisms through which p53 contributes to apoptosis remains to be clarified (and may act in a cell type–specific manner), current evidence points toward p53 functioning through transcriptional activation of pro-apoptotic target genes that contain p53-binding sites within their regulatory regions (17). Of the several apoptotic genes that p53 can directly target, NOXA and PUMA (pro-apoptotic Bcl-2 family) are induced by p53 in cell types that express pro-apoptotic Bcl-2 family members, although Noxa and Puma can also be induced by p53-independent mechanisms (18). In addition to Noxa and Puma, p53 causes transcriptional (17) and transcription-independent (13) activation of Bax, a pro-apoptotic member of the Bcl-2 family that is capable of directly triggering apoptosis.

**Bcl-2 Family of Proteins**

To date, 25 members of the Bcl-2 family of proteins have been identified (8). These proteins are localized to mitochondria, smooth endoplasmic reticulum, and perinuclear membranes in hematopoietic cells. Overexpression of several anti-apoptotic Bcl-2 family proteins has been reported in hematologic malignancies (19). Bcl-2 proteins are characterized by the presence of up to four relatively short sequence motifs, which are <20 amino acid residues in length, termed Bcl-2 homology domains (8). Bcl-2 family members can be divided into three subfamilies based on structural and functional features. The anti-apoptotic subfamily contains the Bcl-2, Bcl-XL, Bcl-w, Mcl-1, Bfl1/A-1, and Bcl-B proteins, which suppress apoptosis and contain all four Bcl-2 homology domains, designated Bcl-2 homology 1-4. Some pro-apoptotic proteins, such as Bax, Bak, and Bok, contain Bcl-2 homology 1-3 domains and are termed “multidomain proteins,” whereas other pro-apoptotic proteins, such as Bim, Bad, and Bid, contain only the BH3 domain and are termed “BH3-only” proteins (20). Figure 1 illustrates the role of the Bcl-2 family of proteins in apoptosis.

How Bcl-2 family proteins interact to permeabilize the mitochondrial membrane remains controversial. There are two tentative models explaining how BH3-only proteins can interact with other Bcl-2 family proteins to induce apoptosis. The “direct activation model” suggested by Letai et al. (21) divides BH3-only domain proteins into groups, which are “sensitizers” and “activators.” The activator BH3-only molecules (Bim, Bid) directly bind and oligomerize Bax/Bak, leading to release of cytochrome c. A study supporting the latter theory showed that a Bid mutant that lacks the ability to interact with Bcl-2 (but maintains interaction with Bax) is still potently pro-apoptotic, suggesting that the interaction with Bax and/or Bak is important in the function of Bid (22). The sensitizers, such as Bad, Bik, and Noxa, cannot directly activate Bax/Bak, but they inhibit anti-apoptotic Bcl-2 proteins from engaging with activator proteins or Bax/Bak, thereby sequestering activators and Bax/Bak. The engagement of BH3-only proteins with anti-apoptotic Bcl-2 members is common between the two models.

The second model (indirect activation model) challenges the direct activation model with data showing that Bax did not bind...
to any of the BH3-only proteins and that Bax and Bak can mediate apoptosis without discernable association with the putative BH3-only activators (Bim, Bid), even in cells with no Bim or Bid and reduced Puma (23). Although further work is needed to fully elucidate the mechanisms regulating Bcl-2 family protein–induced apoptosis and to fully understand the biology of the Bcl-2 pathway, agents targeting the Bcl-2 family proteins are already entering oncology clinical trials.

Unlike most oncogenes that promote proliferation, Bcl-2 functions by preventing programmed cell death (24). As the anti-apoptotic Bcl-2 family proteins promote cancer cell survival by antagonizing apoptosis (25), they provide therapeutic targets, and inhibition of anti-apoptotic Bcl-2 family proteins is expected to predominantly induce apoptosis in cancer cells (26). Bcl-2 was identified because of a characteristic chromosomal translocation t(14;18) present in 85% of follicular lymphomas and 20% of diffuse B-cell lymphomas (27), which results in deregulated BCL-2 gene expression at the transcriptional level. The in vivo effects of Bcl-2 were initially investigated in BCL-2 transgenic mice in which Bcl-2 overexpression was targeted to B and T lymphocytes, which lead to follicular hyperplasia or T-cell lymphomas (28).

Constitutively high levels of Bcl-2 or Bcl-Xl have been associated with a more aggressive malignant phenotype and/or drug resistance to various categories of chemotherapeutic agents in hematologic malignancies and solid tumors (5, 6, 19, 29). As an example, high Bcl-2 levels in primary prostate cancer were associated with high Gleason scores and a high rate of cancer recurrence after radical prostatectomy (6). Expression of BCL-Xl in the NCI 60 cell line panel strongly correlated with resistance to most chemotherapy agents (30). Overexpression of Bcl-2 RNA and/or protein has been observed in acute myelogenous leukemia (AML) and in acute lymphoblastic leukemia (31, 32), and the Bax/Bcl-2 ratio inversely correlates with prognosis of AML and acute lymphoblastic leukemia (4, 33). Bcl-2 overexpression is commonly observed but is highly variable in diagnosis in acute lymphoblastic leukemia (31, 32, 34, 35). Despite observations that Bcl-2 modulation can increase the sensitivity to chemotherapeutic agents in vitro, the level of Bcl-2 expression has not been observed to impact event-free survival or aggressiveness of acute lymphoblastic leukemia (34, 35), possibly owing to the complexity of interactions among Bcl-2 family members. Thus, it has been speculated that the balance between anti- and pro-apoptotic Bcl-2 family members, rather than mere overexpression of Bcl-2, regulates the death of cancer cells (36).

### Table 1. Agents targeting anti-apoptotic Bcl-2 family proteins

<table>
<thead>
<tr>
<th>Agents</th>
<th>Target proteins</th>
<th>Sponsor</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apogossypol</td>
<td>Bcl-2, Bcl-XL, Mcl-1</td>
<td>Burnham (NCI)</td>
<td>Preclinical</td>
</tr>
<tr>
<td>HA-14</td>
<td>Bcl-2</td>
<td>Maybridge Chem</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Antimycin A</td>
<td>Bcl-2, Bcl-XL</td>
<td>U of Washington</td>
<td>Preclinical</td>
</tr>
<tr>
<td>BH3Is</td>
<td>Bcl-XL</td>
<td>Harvard U</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Oblimersen sodium</td>
<td>Bcl-2</td>
<td>Genta</td>
<td>Phase III</td>
</tr>
<tr>
<td>Gossypol (AT-101)</td>
<td>Bcl-2, Bcl-XL, Bcl-w, Mcl-1</td>
<td>Ascenta (NCI)</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>ABT-737 (ABT-263)</td>
<td>Bcl-2, Bcl-XL, Bcl-w</td>
<td>Abbott</td>
<td>Phase I</td>
</tr>
<tr>
<td>GX15-070</td>
<td>Bcl-2, Bcl-XL, Bcl-w, Mcl-1</td>
<td>Gemin X</td>
<td>Phase I</td>
</tr>
</tbody>
</table>

Abbreviations: BH3Is, BH3 inhibitors; NCI, National Cancer Institute; Maybridge Chem, Maybridge Chemical Co. Ltd.; U, University.

### Clinical and Translational Advances in Bcl-2 Inhibitors

Since the molecular cloning of Bcl-2 by Korsmeyer et al. (36), there has been tremendous progress in identifying Bcl-2 family members as targets for drug development. In the last 20 years, the anti-apoptotic properties of Bcl-2 were discovered, the overexpression of Bcl-2 conferring chemoresistance was reported, and the 3-dimensional protein structure of Bcl-Xl was determined, which contributed to the development of protein inhibitors (37). The first agent targeting Bcl-2 that entered clinical trials is a Bcl-2 antisense (oblimersen sodium), which has shown chemosensitizing effects when combined with conventional chemotherapy drugs in chronic lymphocytic leukemia (CLL) patients, leading to improved survival (38). More recent advances include the discovery of small molecule inhibitors of the Bcl-2 family proteins. They are designed to bind the hydrophobic groove of anti-apoptotic Bcl-2 proteins in place of BH3-only proteins (i.e., BH3-mimetics). They can oligomerize Bax or Bak, which can subsequently depolarize mitochondrial membrane potential to release cytochrome c. Several agents targeting the Bcl-2 family of proteins have been developed, and three of these have entered clinical trials. Table 1 summarizes the various Bcl-2 inhibitors currently in preclinical and clinical development.

#### BCL-2 Antisense Effect on Cell Death

Oblimersen sodium (G3139, Genasense) is a phosphorothioate BCL2 antisense oligodeoxynucleotide that targets BCL2 mRNA. The cell death mechanisms of BCL-2 antisense can be classified in two categories exerting either an apoptotic or a nonapoptotic effect based on their involvement in cell death pathways. The apoptotic effect of G3139 antisense occurs via an increase in Bax and PARP, releasing cytochrome c to activate caspas and ultimately releasing Smac/DIABLO to antagonize inhibitors of apoptosis proteins or releasing apoptosis-inducing factors from mitochondria to induce DNA fragmentation (39). Inhibitors of apoptosis protein inhibition result in activation of caspase-3 and caspase-9, which initiates apoptosis, whereas apoptosis-inducing factor release induces necrosis of cells.

Autophagy is another cell death pathway that Bcl-2 interferes with via inhibition of Beclin-1, an autophagy-inducible gene in mammalian cells (40). Autophagy maintains cellular homeostasis by causing cell death or cell survival, depending on the nutritional status of cells (41). Autophagic (self-eating) cell...
death is considered an intracellular degradation of organelles by lysosomes for digestion or reuse of cellular components (41). Recent observations suggested that the BH3 domain of Beclin-1 interacts with Bcl-2 or Bcl-XL, and that pharmacologic disruption of the interaction between Bcl-2 and Beclin-1 can stimulate autophagy (42). Bcl-2 down-regulation by BCL-2 antisense has been reported to induce autophagic cell death (a nonapoptotic cell death) in HL-60 cells, possibly via release of Beclin-1 (43). However, the contribution of apoptosis versus autophagy to cell death in cancer from various cytotoxic agents remains to be determined.

Substantial data are available on the nonantisense effects of oblimersen. CpG-motifs present in Bcl-2 antisense are recognized by toll-like receptor 9 expressed on subsets of B cells and lymphoid dendritic cells, which may result in production of polyclonal antibodies and promotion of dendritic-cell maturation, leading to secretion of multiple cytokines, including interleukin 6 and interferon-α (44). Immature plasmacytoid dendritic cells may be recruited to tumor sites from the bone marrow through a stromal-derived factor-1/CXCR4 interaction, wherein they are biochemically and functionally modulated into tumor-associated immature plasmacytoid dendritic cells that can weaken antitumor immunity by inhibiting dendritic-cell and T-cell activation (43, 45). Activation of immature plasmacytoid dendritic cells with Bcl-2 antisense may account for some of its activity due to stimulation of tumor antigen-presenting plasmacytoid dendritic cells to promote tumor immunity, a non-specific effect (observed with other nucleic acids) that has been attributed to the two CpG motifs in the antisense molecule (43).

Oblimersen has been clinically tested in combination with other anticancer chemotherapeutic agents in CLL (46, 47), AML (48), multiple myeloma, small cell lung cancer (49), non-Hodgkin’s lymphoma, and melanoma (50). A summary of current phase II and III clinical trials of oblimersen is provided in Table 2. A randomized phase III study on dacarbazine +/- oblimersen in patients with advanced melanoma led to filing with the FDA of an unsuccessful new drug application (50). A randomized phase III trial comparing fludarabine/cyclophosphamide versus fludarabine/cyclophosphamide + oblimersen in relapse or refractory CLL was conducted seeking registration of oblimersen (47), but the new drug application was deemed nonapprovable by the Food and Drug Administration. However, because long-term follow-up data showed a significant increase in overall survival for patients who received oblimersen plus chemotherapy compared with patients treated with chemotherapy alone (38), an amended new drug application was submitted for oblimersen and is currently under review by the Food and Drug Administration.

### Table 2. Phase II and III clinical trials with oblimersen sodium

<table>
<thead>
<tr>
<th>Disease (group)</th>
<th>Phase</th>
<th>Regimen</th>
<th>n</th>
<th>Response/outcome</th>
<th>Survival benefit</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>III</td>
<td>Dacarbazine ± oblimersen</td>
<td>771</td>
<td>7.5% vs 13.5% overall</td>
<td>9.7 vs 11.4 mos</td>
<td>50</td>
</tr>
<tr>
<td>CLL</td>
<td>III</td>
<td>FC ± oblimersen</td>
<td>241</td>
<td>7% vs 17% CR/nPR</td>
<td>At 40 mos (P = 0.05)</td>
<td>38, 47</td>
</tr>
<tr>
<td>MM</td>
<td>III</td>
<td>Dexamethasone ± oblimersen</td>
<td>Ongoing</td>
<td>Ongoing</td>
<td>To be determined</td>
<td></td>
</tr>
<tr>
<td>NSCLC or SCLC</td>
<td>III</td>
<td>Docetaxel ± oblimersen</td>
<td>Ongoing</td>
<td>Ongoing</td>
<td>To be determined</td>
<td></td>
</tr>
<tr>
<td>AML (CALGB)</td>
<td>III</td>
<td>Ara-C/daunorubicin + HiDAC + oblimersen</td>
<td>503</td>
<td>No outcome benefit</td>
<td>No survival benefit</td>
<td>48</td>
</tr>
<tr>
<td>SCLC (CALGB)</td>
<td>III</td>
<td>Carboplatin/etoposide ± oblimersen</td>
<td>56</td>
<td>61% vs 60%</td>
<td>No survival benefit</td>
<td>49</td>
</tr>
<tr>
<td>AML</td>
<td>(nR)</td>
<td>Gemtuzumab ± oblimersen</td>
<td>48</td>
<td>5 CR, 7 PR</td>
<td>nR</td>
<td>87</td>
</tr>
<tr>
<td>HRPca (EORTC)</td>
<td>(nR)</td>
<td>Doxetaxel ± oblimersen</td>
<td>28</td>
<td>4 of 12 (33%)</td>
<td>Objective response</td>
<td>88</td>
</tr>
<tr>
<td>Renal Ca</td>
<td>(nR)</td>
<td>IFN-α + oblimersen</td>
<td>23</td>
<td>1 PR</td>
<td>nR</td>
<td>89</td>
</tr>
<tr>
<td>MM</td>
<td>(nR)</td>
<td>Dexamethasone/thalidomide + oblimersen</td>
<td>33</td>
<td>2 CR, 4 near CR, 12 PR</td>
<td>nR</td>
<td>90</td>
</tr>
<tr>
<td>CLL</td>
<td>(nR)</td>
<td>Oblimersen</td>
<td>40 (26)*</td>
<td>2 of 26 PR</td>
<td>nR</td>
<td>46</td>
</tr>
<tr>
<td>Hepatocellular</td>
<td>(nR)</td>
<td>Doxorubicin + oblimersen</td>
<td>27 (19)*</td>
<td>6 of 19 SD</td>
<td>nR</td>
<td>91</td>
</tr>
</tbody>
</table>

Abbreviations: MM, multiple myeloma; NSCLC, non–small cell lung cancer; SCLC, small cell lung cancer; HRPca, hormone-refractory prostate cancer; Renal Ca, renal cell cancer; HiDAC, high-dose cytosine arabinoside; CR, complete response; nPR, nodal partial response; PR, partial response; SD, stable disease; nR, not randomized; CALGB, Cancer and Leukemia Group B; EORTC, European Organization of Research and Treatment of Cancer; IFN, interferon; FC, fludarabine/cyclophosphamide.

*Number of patients whose responses were assessed is marked in parenthesis.

### Small Molecule Inhibitors of the Bcl-2 Family of Proteins

**Molecules affecting gene or protein expression.** Several classes of drugs have been found to regulate gene expression of anti-apoptotic Bcl-2 members. Sodium butyrate is a histone deacetylase inhibitor that decreases Bcl-XL protein levels by down-regulating its RNA expression in mesothelioma cell lines (51). Another histone deacetylase inhibitor, depsipeptide, decreases the expression of Bcl-2, Bcl-XL, and Mcl-1 in multiple myeloma cells (52). Fenretinide, a synthetic cytotoxic retinoid, down-regulates Bcl-2, Bcl-XL, and Mcl-1 in leukemia cells (53, 54). Flavopiridol, a cyclin-dependent kinase inhibitor, reduces Mcl-1 levels in lung cancer cells (55). These agents may achieve cytotoxicity for cancer cells in part by affecting Bcl-2 family members. Alternatively, the observed effects on Bcl-2 proteins could be secondary to other cytotoxic actions of the drugs. Although the specificity for the anti-apoptotic Bcl-2 family members for drugs such as histone deacetylase inhibitors, fenretinide, or flavopiridol is lower than for agents that are designed to directly target anti-apoptotic Bcl-2 family members, the ability of such agents to modulate drug resistance mechanisms via the Bcl-2 family proteins provides opportunities for potentially synergistic drug combination studies.

**Molecules acting on proteins.** Recently, small molecules have been developed that directly interact with anti-apoptotic Bcl-2
proteins. These agents mimic the action of BH3 proteins and interact with anti-apoptotic Bcl-2 proteins at their BH3-binding groove.

Gossypol is the first compound that showed inhibition of Bcl-2, Bcl-XL, and Mcl-1. Gossypol is a potentially toxic phenolic pigment found in the seed, stem, and root of the cotton plant, and was initially identified as an antifertility agent in China during the 1950s (56). Natural gossypol is a racemic mixture, and levo gossypol (AT-101, Ascenta) phase II clinical trials are ongoing in CLL (in combination with rituximab) and in hormone refractory prostate cancer (in combination with docetaxel). AT-101 exhibits submicromolar binding affinity for Bcl-2 and Mcl-1. Gastrointestinal toxicity was dose limiting in a phase I/II clinical trial in prostate cancer patients (57). A gossypol analog, apogossypol (Burnham Institute), is in preclinical development. Apogossypol seems to better target Bcl-2 and Mcl-1 and may decrease the systemic toxicities observed with gossypol.

ABT-737 (A-779024, Abbott Laboratories) is a small molecule that targets anti-apoptotic Bcl-2 family proteins (Bcl-2, Bcl-XL, and Bcl-w), thereby sequestering pro-apoptotic BH3 domain proteins, promoting Bax and Bak oligomerization and ultimately programmed cell death of malignant cells (58). ABT-737 was developed using nuclear magnetic resonance–based screening and structure-based design to target the Bcl-2 family of proteins, with linking of two different molecules of modest affinity into a single molecule of high affinity. ABT-737 binds the anti-apoptotic Bcl-2 family of proteins with an affinity two or three orders of magnitude more potent than previously reported compounds ($K_i \leq 1$ nmol/L for Bcl-2, Bcl-XL, and Bcl-w; $K_i \cong 0.46$ nmol/L for Bcl-B, Mcl-1, Bfl1/A-1).

ABT-737 markedly increased the response to radiation as well as multiple chemotherapy agents in vitro and showed good activity as a single agent in two small cell lung cancer xenograft models (58). Other studies have shown preclinical activity of ABT-737 as a single agent or in combination with various cytotoxic agents against AML (59, 60), multiple myeloma (61), lymphoma (62), CLL (63), acute lymphoblastic leukemia (64), and small cell lung cancer (65, 66). Consistent with the low affinity of ABT-737 for Mcl-1, multiple reports have suggested that high basal levels of Mcl-1 expression are associated with resistance to ABT-737 (60, 62, 63, 67). Combining ABT-737 with a cyclin-dependent kinase inhibitor ( flavopiridol), arsenic trioxide, or fenretinide have achieved synergy via inactivation of Mcl-1 by the second agent (54, 68), paving the way for future combination chemotherapy strategies targeting the Bcl-2 family of proteins.

ABT-263, an oral version of ABT-737, shares similar biological properties with ABT-737. ABT-263 is active as a single agent in small cell lung cancer xenografts, and it enhanced the activity of other chemotherapy agents in preclinical studies of B-cell lymphoma and multiple myeloma (69). Although dose-dependent transient thrombocytopenia (possibly due to Bcl-XL inhibition) was observed (69), ABT-263 is non-myelosuppressive and has shown activity as a single agent against refractory or relapsed lymphoid malignancies with minimal systemic toxicities (70, 71). ABT-263 is also under clinical investigation in adults with chronic myelogenous leukemia and small cell lung cancer (72).

Another pan-Bcl-2 inhibitor, GX15-070 (obatoclax, Gemin X), is being tested in a phase I clinical trial for CLL. The agent has been granted orphan drug status by the U.S. Food and Drug Administration (FDA) for the treatment of CLL. It is a small molecule indole bipyrrole compound that inhibits most anti-apoptotic Bcl-2 family of proteins. Compared with ABT-737, GX15-070 has a relatively low affinity for Bcl-2, Bcl-XL, Bcl-w, and Mcl-1 ($K_i = 220$ nmol/L for Bcl-2 and ~0.5 $\mu$mol/L for Bcl-XL, Bcl-w, and Mcl-1; ref. 73). However, interaction between Mcl-1 and Bak was inhibited by GX15-070 relative to vehicle controls with an apparent IC$_{50}$ of about 1.5 $\mu$mol/L (73). A more recent report suggested that the cell death induced by GX15-070 and gossypol is in part independent of Bax and Bak, by showing that the compounds exhibited a similar cell killing spectrum in wild-type and Bcl-X–, Mcl-1–, and Bax/Bak–deficient mouse embryonic fibroblasts (72). Preclinical experiments showed that GX15-070 has single agent activity and also enhanced the in vitro cytotoxicity of bortezomib against human multiple myeloma (74) and mantle cell lymphoma cell lines (75). A phase I study of GX15-070 has established a dose for GX15-070 in combination with docetaxel (in non–small cell lung cancer patients) that did not aggravate neutropenia of docetaxel (76).

HA14-1 (in preclinical development) is a small-molecule inhibitor of the anti-apoptotic Bcl-2 family of proteins that was identified by structure-based screening (77). HA14-1 induced apoptosis in various cancer cells, including leukemia, lymphoma, glioblastoma, neuroblastoma, and colon cancer (78, 79). It enhanced the cytotoxicity of doxorubicin, TRAIL ligand, dexamethasone, bortezomib, and flavopiridol, and caused a growth delay in glioblastoma xenograft s.c. model (78). However, the binding affinity of HA14-1 to Bcl-2 (IC$_{50}$ ~ 9 $\mu$mol/L) is relatively high compared with other inhibitors of anti-apoptotic Bcl-2 proteins (77). A recent study suggested that HA14-1 decomposes rapidly in solution, but a chemical modification has improved its stability (80).

Other small molecules of anti-apoptotic Bcl-2 proteins that are in preclinical development include BH3 inhibitor-1 and -2 and antimycin A. Antimycin A generates reactive oxygen species and inhibits mitochondrial electron transport, which results in mitochondrial membrane depolarization, Bcl-2 down-regulation, and Bax up-regulation (81). However, a limitation of using antimycin A in vivo is that it requires a high concentration to inhibit the growth of cancer cells (82). BH3 inhibitors are small molecule cell-permeable inhibitors of BH3 domain–mediated dimerization, which interfere with cell-intrinsic signaling pathways. The BH3 inhibitors (BH3I) are reported to sensitize TRAIL-induced apoptosis in leukemia (83) and prostate cancer cells (84) and to enhance radiation sensitivity in non–small cell lung cancer (85). The BH3Is also require high concentrations (~ 20 $\mu$mol/L) to exhibit sensitizing activity in cancer cells (86), which may limit their clinical application.

**Therapeutic Implications**

Many agents have been identified or designed to target the Bcl-2 family at the mRNA or protein level. Pharmacologic and cellular aspects of agents targeting the Bcl-2 family should be considered when exploring their potential application as chemotherapy. The binding affinity for inhibiting
the anti-apoptotic Bcl-2 family members, a large and redundant family of proteins, should optimally be in the clinically achievable concentrations for each agent. Agents with high specificity provide ready opportunities for cancer cell drug resistance, whereas broader acting agents may contribute to drug resistance in cancer cells (8), suggesting that inhibition of multiple Bcl-2 family members will be necessary to achieve optimal therapeutic effect. One approach to enhancing the therapeutic effect of anti-apoptotic Bcl-2 family members would be to use a combination of drugs that target different Bcl-2 family members.

To date, one Bcl-2 antisense and three small molecule Bcl-2 protein inhibitors are being tested in clinical trials. Preclinical studies seem promising, especially in combination with additional chemotherapy agents. Ongoing and planned phase II clinical trials to define the activity of single agents and drug combinations will determine the direction of future clinical development of the Bcl-2 inhibitors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

Molecular Pathways

Khan SB, Maududi T, Barton K, Ayers J, Alkan S. 52.


Oltersdorf T, Elmore SW, Shoemaker AR, et al. An

van Delft MF, Wei AH, Mason KD, et al. The BH3

Konopleva M, Contractor R, Tsao T, et al. Mecha-

van Delft MF, Wei AH, Mason KD, et al. The BH3


Bcl-2 Inhibitors: Targeting Mitochondrial Apoptotic Pathways in Cancer Therapy

Min H. Kang and C. Patrick Reynolds


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