Targeting Bcl-2 Protein Expression in Solid Tumors and Hematologic Malignancies With Antisense Oligonucleotides

Anthony W. Tolcher, MD, FRCPC

Dr. Tolcher is Director of Clinical Research at the Institute for Drug Development, Cancer Therapy and Research Center, and Clinical Professor of Medicine at the University of Texas Health Sciences Center, both located in San Antonio, Tex.

Address correspondence to:
Anthony W. Tolcher, MD, FRCPC, 7979 Wurzbach, San Antonio, TX 78229;
E-mail: atolcher@idd.org.

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Abstract: This review article focuses on the intrinsic (mitochondrial) pathway of apoptosis, the function of the Bcl-2 protein family that regulates this pathway, the background of the antisense oligonucleotide oblimersen, which targets Bcl-2 protein synthesis, and the implications for this agent in hematologic malignancies.

The precise balance of tissue proliferation and apoptosis not only maintains tissue homeostasis but also preserves genomic integrity. The governance of both proliferation and apoptosis involves a highly evolved and varied set of processes that intertwines signals of proliferation that are frequently proapoptotic in nature with those of apoptosis that permit cell division only in the event of preserved genomic integrity. Diminished or aberrant apoptosis is a hallmark of malignant transformation and the mechanisms by which cells escape normal apoptotic forces are now recognized as diverse.

Regulation of Caspase Activation by the Bcl-2 Family of Proteins

Cellular viability is dependent upon the tight regulation of caspases. Caspases (cysteine aspartyl–specific proteases) are proteases that exist largely as inactive zymogens and which, once activated, are the biochemical effectors that methodically lead to the cellular proteolysis that characterizes apoptosis. Caspases are organized hierarchically into both early and late proteases. Once activated by cytochrome C release, early initiator caspases further activate late effector caspases and initiate the cascade of proteolysis that degrades a restricted set of housekeeping proteins selected by the presence of aspartate residues and specific adjoining amino acid sequences.[1] Although not all caspases are involved in apoptosis, caspases 3, 6, 7, 8, and 9 have well-described functions in cell-death pathways. Early caspase activation is further regulated by inhibitors of apoptosis proteins, such as survivin and livin, that inhibit the activation of the caspase cascade. Once downstream effector caspases are activated (eg, caspases 6 and 7) rapid and irreversible systematic degradation of proteins essential for cellular viability occurs, including proteolysis of protein kinases and other signal transduction proteins, cytoskeletal proteins, chromatin-modifying proteins, DNA repair proteins, inhibitors of

apoptosis (eg, Bcl-2 protein), and inhibitory subunits of endonucleases (CIDE family of proteins).[1,2]

Although late-stage caspase activation is common amongst both the extrinsic (tumor necrosis factor superfamily of receptors and ligands) and intrinsic (Bcl-2 superfamily) pathways that govern apoptosis, the separation of these 2 pathways is largely arbitrary, with important crosstalk and coregulation described.[3,4] Each pathway utilizes a separate upstream caspase family member to activate a common protease, caspase 3, that subsequently activates common downstream effector caspases.

The Bcl-2 Family of Proteins

The *bcl-2* gene and protein product were originally described following the identification of the t(14;18) chromosome translocation in follicular B-cell non-Hodgkin lymphoma (NHL) cells.[5-7] Bcl-2 protein is recognized as the prototypic antiapoptotic protein of the superfamily of proteins that includes both death antagonists (eg, Bcl-2, Bcl-XL, Bcl-w, Bfl-1, A1, and Mcl-1) and death agonists (Bax, Bak, Bok, Bad, Bcl-XS, Bid, Bmf, Bik, BNIP3, Noxa, and Puma). Most Bcl-2 family members, including the apoptosis agonists and antagonists, have Bcl-2 homologous (BH) regions, designated BH1–BH4, which determine their capacity to interact with other Bcl-2 family members and the mitochondrial membrane. Structure and function studies indicate that the presence of regions BH1 and BH2 on Bcl-2 is required for the antiparallel homodimerization of Bcl-2 proteins, whereas the BH3 domain on the death agonists Bax and Bak is required for both heterodimerization with Bcl-2 and a functional proapoptotic effect.[8,9] The majority of proteins encoded by genes of the Bcl-2 family localize to the outer mitochondrial membrane and, with the exception of Bid and Bad, possess a carboxyl-terminal transmembrane region that is responsible for subcellular localization and interaction with the mitochondria.[10]

The homeostatic control of the intrinsic pathway of cell death occurs through the competitive dimerization between selective pairs of antagonists and agonists.[11] Bax is the prototypic proapoptotic protein capable of inducing apoptosis in the intrinsic pathway. Although the exact mechanism whereby an apoptotic stimuli initiates Bax localization to the mitochondrial membrane is not currently known, the events appear to be mediated by DNA damage, cell cycle arrest, failed DNA repair, and p53 function.[12] Several members of the protein family, including Bax and Bid, share structural similarity to pore-forming bacterial proteins.[13-15] These proapoptotic proteins interact with the mitochondrial membrane followed by the initial manifestations of the apoptotic process, including disruption of mitochondrial membrane integrity, loss of the transmembrane potential and the release of cytochrome C, protease activators (including caspases 2, 3, and 9), and apoptosis-inducing factors.[1] A critical mediator of caspase activation external to the mitochondria membrane, apoptotic protease–activating factor-1, exists as an inactive monomeric protein that binds mitochondrial-released cytochrome C in conjunction with adenosine triphosphate, and undergoes conformational changes that include oligomerization to form a functional “hub” for biochemical activation of caspase 9. These activated oligomers have been termed the apoptosome. Caspase recruitment domains (CARDs) at the center of the apoptosome bind CARDs of procaspase 9 molecules and activate caspase 9, resulting in cleavage of procaspase 3, caspase 3 activity, and the initiation of downstream proapoptotic caspase effectors.

The mitochondrial membrane alteration and biochemical trigger therefore initiates and commits the cell to the apoptotic process through to the activation of caspase 9 and executes the active process of cellular destruction. Bcl-2 has a central role in the inhibition of this process through competitive heterodimerization with Bax and other apoptosis-agonist proteins (eg, Bid), sequestering and thereby restricting the interaction of these proapoptotic proteins at the mitochondrial membrane.[8]

The Bcl-2 protein function is further regulated, at least in part, by the dynamic equilibrium of phosphorylated (inactivated) and unphosphorylated (active) serine and threonine residues on the Bcl-2 protein. Furthermore, the dynamic balance of phosphorylated and unphosphorylated Bcl-2 protein appears to be a normal physiologic process during cell division. Phosphorylation of serine and threonine residues between the BH3 and BH4 regions of the Bcl-2 protein inhibits successful dimerization with Bax protein, thereby promoting apoptosis. A hierarchy may exist with respect to specific serine or threonine sites within these BH regions and their relative impact on Bcl-2 function. Mutations, particularly of threonine 56 and serine 87, appear to markedly impair the ability of Bcl-2 to inhibit antineoplastic-induced apoptosis, whereas mutations of serine 70 and threonine 74 appear to have less impact.[16-21] Regulation of selective phosphorylation of serine and threonine residues occurs normally during cellular proliferation. During G_2M, Jun N-terminal kinase/stress-activated protein kinase that associate with microtubules phosphorylate and inhibit Bcl-2 function leading to, at least transiently, a proapoptotic state.[22] This has led to the hypothesis that the proapoptotic state during cell division ensures genomic integrity since cellular and DNA abnormalities that lead to cell cycle arrest in G_2M result in a proapoptotic state that favors cell destruction. Moreover, this appears to be one mechanism by which antimicrotubule chemotherapeutic agents mediate their

antitumor therapeutic effect. Paclitaxel and docetaxel both exert their maximal antimitotic activity during the G_2M phase, resulting in altered microtubule dynamics and G_2M arrest. The hyperphosphorylated Bcl-2 protein during G_2M growth arrest following taxane exposure not only elicits a proapoptotic state, but also appears to enhance antitumor effect. This observation may explain, at least in part, the observation that apoptosis induction occurs at concentrations of taxanes below those necessary for microtubule polymerization and aster formation, and this was secondary to prolonged G_2M transit, Bcl-2 hyperphsophorylation, and the resultant shift in equilibrium towards an apoptotic state.[23] Nonetheless, alternate mechanisms may also be operative and responsible for Bcl-2 phosphorylation; investigators have described inhibition of cytosolic phosphatases by antimicrotubule agents that shift the equilibrium to favor Bcl-2 in the inactivated phosphorylated state.[24,25] In either case, inactivation of a proportion of the total Bcl-2 protein pool characterizes cellular proliferation at the G_2M phase and the result of antimicrotubule therapy.

Dysregulation of the intrinsic pathway, either mediated through overexpression of apoptosis antagonists or by diminished function or expression of apoptosis agonists, contributes to the malignant transformation of human cells. In many normal epithelial tissues Bcl-2 protein is ubiquitously expressed at low levels in the basal proliferative cell population but diminished in fully differentiated cells.[26-28] Bcl-2 protein is also normally expressed in both pre–B cells and quiescent memory B cells, but is downregulated in differentiated B cells.[29] However, overexpression of Bcl-2 protein, secondarily to the t(14;18) chromosomal translocation, is associated with abnormal and extended cellular survival with follicular lymphoproliferation in transgenic murine models and an indolent clonal proliferation of B cells. This association appears to be similar in nature to follicular NHL.[6,30]

Several hematologic malignancies exhibit overexpression of Bcl-2 protein (Table 1). These include the B-cell malignancies (NHL, chronic lymphocytic leukemia [CLL], and multiple myeloma) and acute and chronic myelogenous leukemias.[27,31,32] Furthermore, Bcl-2 overexpression appears to be a molecular determinant of poor prognosis in intermediate-grade NHL independent of the presence of a 14;18 translocation, predicting both early relapse and/or decreased survival.[33]

In experimental models, Bcl-2 protein expression confers resistance to a diverse array of cytotoxic chemotherapeutic agents and corticosteroids. This includes the alkylating agents, topoisomerase I and II inhibitors, mitomycin C, antimetabolites including cytosine arabinoside, methotrexate, 5-fluorouracil, cisplatin, vincristine, and dexamethasone.[38-42] These studies indicate that the relative expression of Bcl-2 protein to proapoptotic proteins has a critical role in the execution of cellular apoptosis irrespective of the therapeutic strategy (eg, chemotherapy or hormone therapy) used to induce cell death. Based on its central role in the regulation of cellular apoptosis in a diverse spectrum of malignancies, as well as its ability to confer resistance to a broad array of chemotherapeutic and hormone therapies, Bcl-2 protein represents an attractive therapeutic target in the treatment of malignant diseases.

Table 1. Frequency of Bcl-2 Expression in Selected Hematologic Malignancies

Hematologic Malignancy	Prevalence, %	Reference
Acute myelogenous leukemia	34	Campos et al[34]
Chronic myelogenous leukemia	57	Ravandi et al[35]
Chronic lymphocytic leukemia	100*	Zutter et al[36]
Myeloma	71	Harada et al[37]
Non-Hodgkin lymphoma	89	Krajewski et al[32]

* Small sample size.

Therapeutic Antisense Oligonucleotides

Antisense oligonucleotides were originally used as experimental tools to inhibit specific gene expression.[43-46] Antisense oligonucleotides are short strands of RNA or DNA that are synthesized to hybridize by Watson and Crick pairing to the complementary sequence within the mRNA of a specific gene. Hybridization results in RNAse H–mediated scission of the mRNA/oligonucleotide pairing, thereby inhibiting translation and protein expression.[43-47] Based on the marked selectivity antisense oligonucleotides possess to inhibit gene expression, their therapeutic potential was quickly recognized.[48] However, several physiochemical barriers associated with both oligonucleotide chemistry and the target mRNA/oligonucleotide hybridization impeded the clinical development of antisense oligonucleotide therapeutics.

Unmodified oligonucleotides are unsuitable for therapeutic purposes. Rapid degradation of the phosphodiester linkage occurs in vivo by ubiquitous plasma and cellular nucleases. To overcome this obstacle, the phosphodiester backbone is modified to incorporate sulfur substitutions for nonbridging oxygen atoms.[49] Since these modifications also reduce the affinity of antisense oligonucleotides to hybridize with the target mRNA, strands of 16–20 bases

are used to optimize binding kinetics and target specificity.[49] Not all regions within mRNA molecules are suitable for antisense-mediated scission since the conformational structure of mRNA is not linear but exists as a 3-dimensional structure with sequence-specific folding. These conformations sterically hinder successful mRNA-antisense oligonucleotide hybridization, thereby diminishing the potency of mRNA scission between different sequences of antisense Bcl-2 oligonucleotides, even though they may target close or even partially overlapping mRNA regions.[50] The diversity of response between antisense molecules with seemingly similar or partially overlapping sequences necessitates the screening of numerous oligonucleotide sequences, essentially "gene treadmilling," to determine an optimal candidate for therapeutic use.[49] For the *bcl-2* mRNA, several regions exhibit superior sensitivity to antisense oligonucleotide–mediated cytotoxicity, including codons 1–6, codons 141–147, and the termination region.[50]

Oblimersen

Oblimersen sodium (Genasense, Genta) is an 18-mer phosphorothioate antisense deoxynucleotide directed to the first 6 codons of the open reading frame of *bcl-2* mRNA. Extensive preclinical studies across a broad spectrum of human tumor cell lines in vitro have demonstrated that oblimersen induces nucleotide sequence–specific degradation of *bcl-2* mRNA that subsequently inhibits Bcl-2 protein expression.[51-54] In contrast, no reduction in Bcl-2 expression was observed with either a 2–base pair mismatch control oligonucleotide or a reverse-polarity control oligonucleotide.[51,52,55] Furthermore, the inhibitory effects are dose-dependent, with concentrations of 1–5 μg/mL necessary for Bcl-2 protein expression inhibition in vitro.[55] In experimental time-course studies in human tumor cell lines, oblimersen reduced Bcl-2 protein expression to undetectable levels within 24 hours, although this effect was reversible and protein expression recovered to baseline values 48 hours after washout.[55]

Oblimersen has demonstrated dose-dependent tumor growth inhibition of several human tumor cell lines and implanted xenograft models, including myeloid leukemia cell lines (KG1a and HL-60), chronic leukemia cell lines, myeloma, and transformed and nontransformed lymphoma cell lines.[53,56] Because Bcl-2 overexpression confers intrinsic resistance to the apoptotic effects of cytotoxic agents in experimental models, oblimersen has been combined with several chemotherapeutic agents to determine if inhibition of Bcl-2 expression enhances the apoptotic and therapeutic effectiveness of these agents. Various chemotherapeutic agents had superior activity when combined with oblimersen in several human xenograft models, including cyclophosphamide in a lymphoma cell line (DoHH2).[57] The enhancement of therapeutic effect by oblimersen extends to combinations with nonchemotherapeutic strategies such as dexamethasone and rituximab in an Epstein-Barr virus–transformed lymphoblastoid cell line.[58,59]

Cell proliferation and cell death pathways are inherently interrelated and Bcl-2 protein is an attractive target in malignancies in which there is a direct link between the aberrant signal transduction pathway mediating proliferation and Bcl-2 expression. In BCR-ABL transformed cells, the BCR-ABL tyrosine kinase mediates clonal proliferation and inhibits apoptosis by increased Bcl-2 protein expression.[60] Consistent with these findings, inhibition of Bcl-2 by oblimersen has been shown to induce single-agent tumor regressions in an imatinib-resistant BCR-ABL transformed cell line.[61] The clinical implications of these results indicate that oblimersen may have a role as a single agent in the treatment of imatinib-resistant chronic myelogenous leukemia (CML) as well as first-line treatment of CML to enhance the effectiveness of imatinib therapy.

The preclinical evaluation of oblimersen's distribution, metabolism, and excretion was performed in mice. Following a single intravenous bolus injection of ^{35}S-labeled oblimersen sodium, the radioactivity rapidly distributed from the plasma to the tissues.[62] Initially, the majority of oblimersen sodium (98%) was plasma protein–bound, although at 24 hours only a minority of radioactivity (23%) was associated with plasma proteins. The plasma concentration-time curve fit to a 3-compartment model with a terminal elimination half-life averaging 11 hours. Parent compound represented the vast majority of radioactivity detected initially (98% at 15 minutes) but was rapidly reduced at 6 hours to 36% and at 48 hours to 25%. Accumulation of oblimersen in the kidney, liver, spleen, gut, heart, and lung was observed without detectable oblimersen in the brain. Although most of the radioactivity detected in the kidney was parent compound, only trace amounts of parent drug were detected in the urine. Continuous subcutaneous administration for 7 days led to rapid distribution into tissues, and radioactivity accumulated in the previously described organs between days 3 and 7, followed by undetectable radioactivity at day 14 (7 days after discontinuation of infusion). Subcutaneous administration was also associated with a prolonged elimination half-life averaging 22 hours. Two main metabolites were detected, both of which appeared to be shortened oligonucleotide fragments presumably derived from ubiquitous plasma and tissue exonucleases, and these were excreted in the urine and feces.

Oblimersen distributes preferentially in tumor tissue xenotransplanted into immunocompromised mice compared to the normal murine host tissue and the ratio

of oblimersen distributed to implanted tumor tissue versus normal tissue ranged from 2.5- to 3.8-fold.[63] Taken together these studies indicate that oblimersen distributes widely and accumulates preferentially in the kidney, liver, and tumor tissues at concentrations that portend biologic activity.

Phase I Studies with Oblimersen

Oblimersen has been examined in 3 single-agent phase I studies. The initial clinical study administered oblimersen by continuous subcutaneous 14-day infusion at doses that ranged from 4.6 to 195.8 mg/m^2/day in heavily pretreated patients with low-grade and diffuse large-cell NHL.[64,65] The principal toxicities consisted of hyperglycemia, transient hepatic transaminase elevations, fever, fatigue, and local skin inflammation at the infusion site. Thrombocytopenia accompanied by both fever and fatigue was dose-limiting in 2 of 3 patients treated at the highest dose level, with the maximum tolerated dose (MTD) determined to be 147.2 mg/m^2/day (approximately 4.1 mg/kg/day). Steady-state concentrations (Css) were reached within 48 hours and were proportional to dose. There was considerable interpatient variability with respect to Css, with a range of 0.96–6.67 µg/mL at the MTD. In a multivariate analysis of covariates that potentially influenced Css, only dose and renal function were found to be significant.[65] Thrombocytopenia was the predominant dose-limiting toxicity and was related to both dose and Css. The plasma elimination half-life averaged 7.46 hours using this continuous subcutaneous infusion route of administration. One patient achieved a durable complete response that persists today beyond 10 years, whereas 2 other patients experienced minor responses.[64,65]

An intravenous administration schedule was examined in the second single-agent dose-escalation study performed in patients with advanced solid tumor malignancies (largely hormone-refractory prostate or renal carcinomas).[66] Doses up to and including 10 mg/kg/day could be feasibly administered for 14 days with severe dose-limiting toxicity being rarely observed. However, dose escalation to an MTD was not performed. The principal toxicities included fatigue, hepatic transaminitis, diarrhea, hyperglycemia, and modest (grade 1 and 2) thrombocytopenia and leukopenia. Steady-state concentrations were attained within 10 hours and were dose-proportional. The mean plasma elimination half-life was 2.1 hours. Average Css were 4.28 and 7.58 µg/mL at the 5.3 and 6.9 mg/kg/day dose levels, respectively, comparable to those achieved with subcutaneous administration.[62,65] Analysis of Bcl-2 quantification assays performed on peripheral blood mononuclear cells obtained from patients treated with oblimersen indicate that a marked reduction of Bcl-2 protein expression occurs by day 8 and Bcl-2 protein was undetectable at days 15 and 21. No objective responses were observed in this study.

A third phase I study performed in patients with CLL elicited a constellation of side effects including fever, fatigue, and—unexpectedly at the time—tumor lysis syndrome.[67] In this study, dose escalation beyond 3 mg/kg/day was not feasible in patients with CLL during the first course. However, for those patients who did not experience evidence of dose-limiting toxicity during course 1, dose escalation to 4 mg/kg/day was possible. Responses in nodal disease as well as remission in circulating leukemic and bone marrow cells were observed.

The divergence in the tolerability of oblimersen in patients with solid tumors versus those with lymphoproliferative malignancies (CLL and NHL) may be attributable, at least in part, to the release of inflammatory cytokines as part of the antilymphocytic effects of oblimersen and, in the case of CLL, byproducts of tumor cell death leading to tumor lysis syndrome.[67] Because of these effects, oblimersen therapy in CLL and NHL patients should be administered with close observation, specifically for the cytokine release syndrome. Simple measures such as antipyretics and anti-inflammatory agents can be utilized to reduce or prevent this syndrome.

The antilymphoma and antileukemic responses seen with single-agent oblimersen are presumably due, at least in part, to the pivotal role Bcl-2 expression plays in the etiology of these diseases. The responses observed in NHL and CLL provide important clinical "proof of concept" of Bcl-2 inhibition restoring failed apoptosis.[6,68] However, for most other malignancies, Bcl-2 protein expression may be part of a larger number of molecular genetic changes associated with cellular proliferation and apoptosis, and Bcl-2 protein in these patients may not be fundamental to cell survival. Rather, targeting Bcl-2 may enhance apoptosis associated with chemotherapy.

The role Bcl-2 expression has in the inhibition of the apoptotic effects of chemotherapy in experimental models, combined with evidence that Bcl-2 inhibition enhances the effectiveness of several chemotherapeutic agents in vitro and in vivo, provide a strong impetus for the evaluation of oblimersen in combination with antineoplastic agents. Disease-specific studies that combine oblimersen with a broad array of chemotherapeutic agents have been initiated or completed in solid tumors (Table 2) and hematologic malignancies (Table 3). The results indicate that oblimersen can be feasibly administered with full or near-full doses of the commonly indicated chemotherapeutic agents to patients with a wide variety of malignancies.[69-72] In some of these studies, particularly those in melanoma, acute myelogenous leukemia, and multiple myeloma, responses have been observed following the reintroduction of previously used

Table 2. Selected Studies of Oblimersen in Combination With Cytotoxic Agents for Solid Tumors

Malignancy	Oblimersen Dose	Chemotherapy Dose	Reference
Melanoma	6.5 mg/kg/d CIVI × 14	DTIC 200 mg/m²/day IV × 5 days	Jansen et al[73]
	7.7 mg/kg/day CIVI × 5 d	DTIC 1000 mg/m² day 6	Jansen et al[73]
Prostate cancer	5 mg/kg/day CIVI × 14 d	Mitoxantrone 12mg/m² IV d 8	Chi et al[77]
	7 mg/kg/day CIVI × 5 d	Docetaxel 75 mg/m² IV d 6	Tolcher et al[78]
Small-cell lung cancer	3 mg/kg/day CIVI × 7 d*	Paclitaxel 150 mg/m² IV d 6	Rudin et al[79]
Breast cancer	4 mg/kg/day CIVI × 21 d*	Docetaxel 35 mg/m² IV qwk	Chen et al[80]
Colorectal cancer	7 mg/kg/day CIVI × 7 d	Irinotecan 280 mg/m² IV d 6	Ochoa et al[81]
	5 mg/kg/day CIVI × 5	FOLFOX4	Ochoa et al[81]

* Maximum tolerated dose not defined.

CIVI = continuous intravenous infusion; DTIC = dacarbazine; IV = intravenous; FOLFOX4 = oxaliplatin plus 5-fluorouracil/leucovorin.

Table 3. Studies of Oblimersen in Hematologic Malignancies Alone or in Combination With Antineoplastic Agents

Malignancy	Oblimersen Dose	Other Agents	Reference
Myeloma	7 mg/kg × 7 d	Dexamethasone	Chanan-Khan et al[75]
	7 mg/kg × 7 d	Vincristine, doxorubicin, decadron	van de Donk et al[82]
	7 mg/kg × 7 d	Dexamethasone, thalidomide	Badros et al[83]
Waldenström macroglobulinemia	7 mg/kg × 7 d		Gertz et al[84]
Chronic lymphocytic leukemia	3 mg/kg × 5 or 7 d		Rai et al[85]
	3 mg/kg × 7 d	Fludarabine, cyclophosphamide	Rai et al[76]
Acute myelogenous leukemia	7 mg/kg/d × 1–10 d	Daunorubicin, cytarabine	Marcucci et al[86]
	7 mg/kg/d × 7 d	Gemtuzumab ozogamycin	Moore et al[87]
Non-Hodgkin lymphoma	4 mg/kg/d SC × 14 d		Waters et al[65]
	3 mg/kg/d × 7 d	Rituximab	Pro et al[88]
	3 mg/kg/d × 7 d	Cyclophosphamide, doxorubicin, vincristine, prednisone plus rituximab	Leonard et al[89]

SC = subcutaneously.

cytotoxic chemotherapeutic agents.[69,73,74] The results demonstrate that Bcl-2 expression may be responsible for both intrinsic and acquired chemotherapy resistance, and inhibition of Bcl-2 by oblimersen may result in reversal of clinical resistance.

Randomized Studies of Oblimersen

Preliminary results were recently reported for 3 randomized studies that address whether oblimersen can improve the efficacy of current chemotherapeutic agents. In the first study, patients with metastatic melanoma were randomized to receive dacarbazine with or without oblimersen administered as a 5 day continuous intravenous infusion at 7 mg/kg/day. The primary endpoint for this study was overall survival with secondary endpoints of progression-free survival and response rate. As of the last update there was a non–statistically significant difference in overall survival (9.1 vs 7.9 months) in favor of oblimersen combined with dacarbazine, with statistically significant differences in progression-free survival (78 vs 49 days, $P<.05$) as well as superior response rates (11.7% vs 6.9%, $P<.05$). The failure to reach a significant difference in the primary endpoint prevented this agent from achieving regulatory approval. An increased rate of complete responses as well as the observation of durable responses may ultimately result in reanalysis of the data with longer follow up to ascertain if a survival difference is apparent.

In a randomized study of oblimersen combined with dexamethasone versus dexamethasone alone for the treatment of patients with multiple myeloma refractory to first-line therapy, the preliminary results indicate no improvement in outcome or primary endpoints with combination therapy.[75]

The results of a randomized phase III study of fludarabine and cyclophosphamide therapy with or without oblimersen in patients with relapsed or refractory CLL were recently reported.[76] In this study, fludarabine (25 mg/m^2/day) and cyclophosphamide (250 mg/m^2/day) were administered on days 5–7 and oblimersen was administered in the experimental arm at 3 mg/kg/day by continuous intravenous infusion on days 1–7. All patients had received prior fludarabine therapy and the patient population was stratified for fludarabine-refractory versus -nonrefractory, number of prior regimens (1 vs 2 or 3), and response to prior therapy. Eligible patients had acceptable levels of hematologic, hepatic, and renal function. The primary endpoint was response (complete response and nodular partial response), with secondary endpoints of overall response, response duration, time to progression, and overall survival. A total of 241 patients were randomized and were equally balanced amongst the 2 arms for major prognostic factors. The addition of oblimersen to fludarabine and cyclophosphamide was superior to fludarabine and cyclophosphamide alone in terms of the overall response rate (16% vs 7%, P=.04) and complete response rate (11% vs 3%). The differences were noted across all patient stratification variables. In addition, a reduced time to response was noted, suggesting that oblimersen accelerated lymph node regression. Follow-up is too brief to assess differences in either survival or response duration. The hematologic toxicity profile of the combination regimen did not appear to be significantly different from that of fludarabine and cyclophosphamide alone.

At this point in its development, oblimersen has demonstrated superior antitumor activity, although only in response rate endpoints, in 2 randomized studies that have reported results and no improvement in second-line therapy for myeloma. Longer follow-up will be necessary in both of these studies to determine if oblimersen ultimately improves survival. However, there are several challenges for the successful clinical development of targeted agents whose mechanism of action is largely confined to the enhancement of currently available chemotherapy. Historically, the only successful regulatory approval of such an agent has been for folinic acid, used in combination with 5-fluorouracil to enhance the activity of the latter agent for the treatment of colorectal carcinoma. Single-arm phase II studies have several limitations with regard to the true assessment of an agent such as oblimersen. The small sample sizes of these studies do not permit sufficient statistical confidence that the response rate observed is actually different from the single-agent chemotherapeutic agent alone. Our current level of understanding of biomarkers that are predictive of response remains poor, yet it is critically necessary to identify the patient population that will experience maximal therapeutic gain from Bcl-2 inhibition and to improve patient selection for randomized studies accordingly. Given the diverse mechanisms by which apoptosis is inhibited in malignancies, Bcl-2 may represent only one potential apoptotic target and therefore patient selection is necessary to enrich the patient population most likely to gain from therapy.

Future Studies

The randomized studies do indicate that patients with CLL and melanoma appear to respond more briskly as well as with cell kill (higher complete response rates) with Bcl-2 inhibition compared to chemotherapy alone. Potential strategies for further development and registration include combinations with imatinib for the treatment of CML and, by implication, gastrointestinal stromal tumors, where highly effective therapy exists but is not curative, and for the treatment of intermediate-grade NHL and acute myelogenous leukemia, where current therapy fails to cure a substantial proportion of patients. These

studies, although comparative in nature, may ultimately elucidate the benefit associated with Bcl-2 targeting by antisense oligonucleotides.

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