Targeting the Proapoptotic Factor Bcl-2 in Non-Hodgkin's Lymphoma

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Bcl-2 functions as a key survival factor for lymphocytes and is highly expressed in a majority of non-Hodgkin's lymphomas. The ability of oblimersen sodium (Genasense, previously known as G3139) to target bcl-2 messenger RNA and decrease Bcl-2 protein levels has the potential to enhance the activity of cytotoxic chemotherapy. Pretreatment with oblimersen followed by cyclophosphamide (Cytoxan, Neosar) markedly improved survival relative to single-agent cyclophosphamide in a murine xenograft model. Oblimersen has also enhanced the cytotoxicity of a variety of other agents against non-Hodgkin's lymphoma, including etoposide, rituximab (Rituxan), and alemtuzumab (Campath). An initial phase I study of oblimersen in non-Hodgkin's lymphoma demonstrated modest single-agent activity. Recent reports suggest that oblimersen may add to the activity of R-CHOP (rituximab-cyclophosphamide/doxorubicin/vincristine/prednisone) in previously untreated mantle cell lymphoma and to rituximab alone in a variety of subtypes of relapsed non-Hodgkin's lymphoma. Additional studies in both treatment-naive and relapsed patients will define the role of oblimersen in the treatment of non-Hodgkin's lymphoma.

Bcl-2, a member of a family of proteins primarily located between the two layers of the mitochondrial membrane,[1] has been identified as a logical therapeutic target in a variety of human cancers, given its role in regulating a major apoptotic pathway, namely, the mitochondria-mediated or intrinsic pathway of caspase activation.[2,3] Non-Hodgkin's lymphomas of B-cell phenotype are among the malignancies associated with a high level of Bcl-2 expression, seen in greater than 90% of follicular or mantle cell histologies[4,5] and in 50% of diffuse large-cell lymphomas.[6-8] No curative therapy exists for follicular and mantle cell lymphomas, and only 50% of all patients presenting with diffuse large B-cell lymphoma will be long-term failure-free survivors. Therefore, strategies that may reduce Bcl-2 expression in patients with these diseases, which make up almost two-thirds of all non-Hodgkin's lymphomas, are being evaluated for their potential to reduce resistance to therapy and improve patient outcomes. Bcl-2 Expression and Chemotherapy Drug Resistance in NHL A number of research groups have independently found that Bcl-2 overexpression is unequivocally associated with (1) more advanced disease, according to Ann Arbor criteria; (2) a constellation of poor prognostic features, as defined by the International Prognostic Index; and (3) significantly lower survival rates.[6-12] The studies in diffuse large B-cell lymphoma have implicated it as an independent prognostic factor. For example, in a British Columbia Cancer Agency study of 116 patients with uniformly staged and treated diffuse large B-cell lymphoma, the 8-year overall, disease-free, and relapse-free survival rates were significantly (P < .01) lower in patients whose tumors expressed Bcl-2 protein on initial biopsy: 34%, 32%, and 25% vs 60%, 66%, and 59%, respectively, for those with Bcl-2-negative disease.[6] Does the expression of Bcl-2 have a biologic impact on malignant cells? In an elegant series of experiments, in which lymphoma cells isolated from Eμ-myc transgenic mice were retrovirally transfected with the murine bcl-2 gene, Schmitt and colleagues[13] attempted to isolate the effect of a single-gene change on tumor behavior. Essentially, it was demonstrated that upregulating Bcl-2 results in greater cell viability and less apoptosis but no difference in the actual rate of proliferation (Figure 1), confirming that the effects are specific to the rate of cellular death. This upregulation of Bcl-2 conferred relative resistance to both cyclophosphamide (Cytoxan, Neosar) and docetaxel (Taxotere), with notable differences in the rates of tumor-free survival between animals bearing Bcl-2-positive vs Bcl-2-negative lymphomas (Figure 2).
This direct experimental evidence, taken together with the clinical data amassed over the past decade, makes it clear that strategies designed to decrease Bcl-2 expression in lymphomas and other malignancies have the potential to alter outcomes significantly. **Oblimersen Sodium Antisense**

**Oligonucleotide Therapy in Lymphoma** Oblimersen sodium (Genasense), formerly known as G3139, is an 18-mer DNA oligonucleotide that is complementary to, and thus selectively targets, the first six codons of the *bcl-2* messenger RNA (mRNA) to decrease Bcl-2 protein translation. The reverse orientation of the nucleotides to the "sense" strand of the RNA message led to the antisense designation. A sulfa group, added via a phosphorothioate linkage, protects the molecule from degradation by endonucleases and exonucleases, allowing the formulation of a drug with a favorable pharmacokinetic profile when administered systemically. The antisense molecules are believed to function as "therapeutic DNA" in that they bind to *bcl-2* mRNA in the cytoplasm to form a heteroduplex by Watson-Crick base pairing, thereby creating a biologically undesirable complex.[14,15] Various endonucleases, particularly RNase H, are subsequently engaged to cleave the mRNA portion off the DNA backbone, thus eliminating the message while leaving an intact antisense molecule that, in theory, can proceed to target additional *bcl-2* mRNA. In this way, the normal process by which the DNA code is transcribed into a mRNA that translocates into the cytoplasm, binds a ribosome, and is translated into a functional Bcl-2 protein is abrogated.

Can Bcl-2 actually be demonstrably downregulated in this way? Working with tumor cell lines, numerous groups, including our own with lymphoid tumors, have shown specific downregulation of Bcl-2 protein using oblimersen.[16] Murine SCID/human tumor xenograft models have allowed study in vivo that at least mimics the clinical therapeutic situation. In solid tumor studies that examined human melanoma-bearing mice in a subcutaneous model, Jansen and colleagues[ 17], from the University of Vienna, showed that systemically delivered oblimersen is clearly capable of downregulating Bcl-2 and improving sensitivity to a cytotoxic agent, dacarbazine (DTIC-Dome). In studies in our laboratory, oblimersen alone, somewhat to our surprise, completely eliminated DoHH2 (a transformed follicular lymphoma cell line associated with highlevel Bcl-2 expression) in a fraction of animals with systemic disease. Reverse polarity oligonucleotides used as controls had no effect, a proof in principle that such widespread disease could be targeted by oblimersen.[16] Guinness and coworkers,[18] from the Yale Comprehensive Cancer Center, examined the effect of oblimersen as a single-agent modality in a human/SCID model of Epstein-Barr virus-associated posttransplant lymphoproliferative disease. In two separate in vivo experiments, the administration of oblimersen (10 mg/m²/ d) for 12 days led to a significantly longer disease-free survival than that seen in the control group (*P* < .001), with no evidence of disease in most oblimersen-treated animals at the time of sacrifice. However, given what is known about the role of Bcl-2 in preventing the engagement of the cell's apoptotic program, it stood to reason on theoretical grounds that Bcl-2 blockade in the face of the genotoxic insult associated with the types of therapy customarily given in clinical practice might yield the greatest benefit (Figure 3). Most chemotherapeutic agents are known to cause DNA damage, which is sensed via p53 and other mechanisms, in turn, forcing malignant cells toward a
critical decision point to activate either repair or cell death programs. A reduction in Bcl-2 expression at this critical moment could shift the balance in favor of proceeding down the apoptotic pathway.

![Graph showing the effect of chemotherapies on Bcl-2 expression over time.](image)

**Figure 2: Bcl-2 Transfection Confers Multidrug Resistance in Non-Hodgkin’s Lymphoma—Murine Ep-myc transgenic lymphoma model; tumor-free survival after cyclophosphamide or docetaxel. Reproduced, with permission, from Schmitt CA, Rosenthal CT, Lowe SW: Genetic analysis of chemoresistance in primary murine lymphomas. *Nat Med* 6:1029-1035, 2000.**

**Figure 3: Rationale for Combining Bcl-2–Targeted Therapy With Chemotherapy.**

**Investigations Combining Oblimersen With Chemotherapy**

In our own studies at the British Columbia Cancer Agency, oblimersen given as a single agent was shown to improve the median survival and the 90-day survival rate in the SCID xenograft model of DoHH2.[16] At all dose levels evaluated, ranging from 2.5 mg/kg every other day to 12.5 mg/kg/d, oblimersen-treated animals had a highly significant ($P < .000001$) survival advantage over the untreated and irrelevant oligonucleotide control groups. Cyclophosphamide, given as a single agent at a dose of 35 mg/kg on days 4, 8, and 12, exerted modest effects, improving median survival to 47 days from 33 days in the control group, but with no cured animals seen (Figure 4). When oblimersen was combined with cyclophosphamide in a variety of doses and schedules, the vast majority of animals were cured. Perhaps the most striking finding was that adding a 28-day course of oblimersen (2.5 or 5 mg/kg every other day, a total of 14 doses) to a completely ineffective...
single-agent cyclophosphamide dose (15 mg/kg on days 4, 8, and 12) dramatically increased the 90-day survival rate from 0% to 50%. The specificity (use of a number of control oligonucleotides), consistency (across a large number of animal cohorts studied), and durability (cured animals were human Bcl-2 negative by polymerase chain reaction at necropsy) of this effect provided the scientific rationale for the design of subsequent clinical trials. In both preclinical and clinical settings, there has been interest in combining oblimersen with other agents, including the CD20-targeted monoclonal antibody rituximab (Rituxan). In an in vitro study by Auer et al, exposing primary chronic lymphocytic leukemia (CLL) cells to the combination of oblimersen plus rituximab dramatically increased the degree of apoptosis in a synergistic, dose-dependent (for rituximab) manner.[19]
Collaborators from Yale University reported that combining oblimersen with rituximab in a human/SCID model of Epstein-Barr virus-associated posttransplant lymphoproliferative disease increases cure rates and significantly prolongs survival compared with either agent alone. When combining the results of two identical experiments, the tumor-free survival rates were 79% (11/14) for the combination of oblimersen/rituximab, vs 7% (1/14) and 15% (2/13) for single-agent rituximab and oblimersen, respectively. Expanding on that work, this research team showed that oblimersen enhances the in vitro and in vivo cytotoxicity of etoposide in this model. Oblimersen with etoposide was associated with a significantly prolonged median survival of > 150 days, vs 98 days for oblimersen alone and 86 days for etoposide alone (Figure 5). As one further example of the synergistic interaction between oblimersen and other targeted therapies, Cotter and colleagues from Barts and the London School of Medicine found that the combination of oblimersen plus alemtuzumab (Campath) exerts more proapoptotic activity than oblimersen alone in CLL and non-Hodgkin's lymphoma cell lines. Collectively, in vitro and in vivo preclinical data support that oblimersen has a direct effect on downregulating Bcl-2 and that this effect has considerable therapeutic potential in treating lymphomas.

Clinical Studies of Single-Agent and Combination Therapy

To date, clinical data on oblimersen in lymphomas are available from one completed phase I trial of single-agent therapy and two ongoing phase II trials of rituximab-containing combination regimens (Table 1). Lymphomas are arguably the most logical target for developing oblimersen; however, until recently, clinical research efforts in hematologic malignancies have been slow to develop relative to solid tumors.

- **Phase I Trial of Single-Agent Oblimersen in Heavily Pretreated Lymphoma** - In 1997 and 2000, Waters, Webb, and colleagues from the United Kingdom published the results of a phase I trial of oblimersen in 21 patients with various types of previously treated non-Hodgkin's lymphoma, all of whom had documented Bcl-2 protein expression by tumor biopsy. Oblimersen was delivered as a 14-day continuous subcutaneous infusion with eight doses, ranging from 4.6 to 195.8 mg/m²/d. Three patients received a second cycle. Most patients had indolent lymphomas (nine with follicular and eight with small lymphocytic histology) and had received a median of four chemotherapy regimens. Toxicities generally were mild to modest, with no significant grade 3/4 toxicity up to 110 mg/m²/d. Reversible grade 2/3 thrombocytopenia was in three of five patients treated at the maximum tolerated dose of 147 mg/m²/d, which is equivalent to 4 mg/kg/d. In one patient treated at the
maximum tolerated dose, who had an unexpectedly high plasma level of oblimersen, dose-limiting toxicity consisted of grade 3 fever in conjunction with grade 3 hypotension (it resolved rapidly and did not recur on retreatment at a lower dose). At doses exceeding 37 mg/m$^2$/d, 10 patients developed transient grade 3/4 lymphopenia; of note, most of these patients had preexisting lymphopenia, and any worsening that occurred during oblimersen therapy was deemed clinically insignificant. Grade 1/2 hyperglycemia in the nonfasting state was transiently seen in 19 patients, possibly associated with the sulfur molecule that is part of the phosphorothioate backbone of oblimersen. One patient with node-positive follicular lymphoma with bone marrow involvement achieved a complete response of longer than 3 years' duration. Additionally, in this heavily pretreated population, there were two minor responses and nine patients with stable disease. Disease-related symptoms improved in 6 of 10 patients (60%) who were symptomatic prior to treatment, and Bcl-2 protein expression decreased in 7 of 16 (44%) assessable biopsy samples. After a median follow-up of nearly 1 year, median overall and disease progression-free survival times were 13.4 and 3.6 months, respectively. The feasibility, tolerability, and activity of oblimersen in this study have prompted investigators to initiate phase II trials of oblimersen as a component of cytotoxic

### Table 1

<table>
<thead>
<tr>
<th>Study</th>
<th>Phase</th>
<th>Population</th>
<th>Regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waters et al 2000[26]</td>
<td>I</td>
<td>Pretreated non-Hodgkin’s lymphoma (various types)</td>
<td>OBL alone</td>
</tr>
<tr>
<td>Leonard et al 2003[23,24]</td>
<td>I/II</td>
<td>Pretreated or chemotherapy-naive mantle cell lymphoma</td>
<td>OBL alone (if pretreated) or OBL→OBL+R-CHOP (if chemotherapy-naive)</td>
</tr>
</tbody>
</table>

OBL = oblimersen sodium; R-CHOP = rituximab-cyclophosphamide/doxorubicin/vincristine (Oncovin)/prednisone.

- **Phase I/II Trial of Oblimersen Plus Rituximab-Cyclophosphamide/Doxorubicin/Vincristine/Prednisone in Mantle Cell Lymphoma** - Preclinical work within our own group, characterizing mantle cell lymphoma cell lines and exposing them to various agents in vitro and in murine xenograft models, indicates that both rituximab and oblimersen are active agents with potentially additive/synergistic effects.[28,29] Leonard and colleagues[23,24] designed a multicenter phase I/II study of oblimersen in combination with rituximab-cyclophosphamide/doxorubicin HCl/vincristine (Oncovin)/prednisone (R-CHOP) in mantle cell lymphoma, a disease with a median survival of only 3 to 4 years associated with overexpression of both Bcl-1 and Bcl-2, the latter of which has been somewhat underappreciated. Treatment was initiated with oblimersen (3 to 5 mg/kg/d) administered as a 7-day continuous infusion every 21 days for up to six cycles or until disease progression; treating relapsed/refractory patients with an additional six cycles was permitted. In chemotherapy-naive patients failing to achieve a complete response after six cycles of oblimersen alone, subsequent therapy consisted of oblimersen (3 mg/m$^2$/d) on days 1 to 7 plus R-CHOP on day 5 for up to six cycles. Results for the first 45 enrolled patients, 27 with relapsed/refractory and 18 with chemotherapy-naive disease, have been reported in abstract form (Table 2). All six cycles of initial oblimersen therapy were completed without evidence of disease progression in seven relapsed/refractory and two chemotherapy-naive patients. Of 18 patients with relapsed/refractory disease treated at the 3-mg/kg/d dose (determined to be the maximum tolerated dose for cycle 1), 1 patient achieved a complete response and 7 patients had stable disease. No objective responses to single-agent oblimersen were seen
among the 13 evaluable patients in the chemotherapy-naive subset. However, six of eight evaluable patients had a complete response (n = 2) or partial response (n = 4) on completion of oblimersen plus R-CHOP, an objective response rate of 75%. Based on preliminary safety analysis, combination therapy has been well tolerated, and adding oblimersen to R-CHOP did not appear to increase the expected toxicity of this regimen.

**Phase I/II Trial of Oblimersen Plus Rituximab in B-Cell Non-Hodgkin's Lymphoma**—With oblimersen and rituximab demonstrating synergy against lymphoma in vitro and at least additive effects in xenografts, Pro and colleagues[25,30] from the University of Texas M.D. Anderson Cancer Center initiated a phase II trial of this combination in refractory/recurrent B-cell non-Hodgkin's lymphoma. Oblimersen (3 mg/kg/d) is given as a 7-day continuous intravenous infusion every 2 weeks (on days 1 to 7, 15 to 21, and 29 to 35) in conjunction with six doses of once-weekly rituximab (375 mg/m²) on days 3, 8, 15, 22, 29, and 36. In the absence of disease progression or unacceptable toxicity, one additional course was permitted.

### Table 2

<table>
<thead>
<tr>
<th>Population</th>
<th>Complete Response</th>
<th>Partial Response</th>
<th>Stable Disease</th>
<th>Progressive Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relapsed/refractory (n = 18)</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>10</td>
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<tr>
<td>Chemotherapy-naive</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>After OBL (n = 13)</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>After OBL + R-CHOP (n = 8)</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

*Data from Leonard et al.[23,24]

OBL = oblimersen sodium; R-CHOP = rituximab-cyclophosphamide/doxorubicin/vincristine (Oncovin)/prednisone.

Most of the first 16 enrolled patients had indolent histologies, the most common being follicular lymphoma (n = 8), and 14 patients had previously received rituximab. In the 11 evaluable patients, four responses were seen, for a preliminary response rate of 36%. One complete response was seen in a patient with stage IV mucosa-associated lymphoid tissue lymphoma with bone marrow involvement. All but one of the responders had a history of rituximab exposure. Accrual into this trial is ongoing as of March 2004, with a target enrollment of 120 patients.

**Conclusion** Bcl-2 appears to be a key antiapoptotic factor in non-Hodgkin's lymphoma. Oblimersen has demonstrated modest single-agent activity against various types of non-Hodgkin's lymphoma, and clinical research efforts are seeking to combine this antisense oligonucleotide with other forms of effective therapy. The maximum tolerated dose for oblimersen has been 3 mg/kg/d in patients with B-cell hematologic malignancies versus 7 mg/m²/d in patients with solid tumors. For reasons that are not yet fully understood, patients with non-Hodgkin's lymphoma and CLL appear to be more susceptible to developing certain types of toxicities (particularly hypotension and fever) during oblimersen therapy than those who have solid tumors. Accumulating preclinical data support the chemosensitizing potential of oblimersen, and that it can be safely administered with chemotherapeutic agents, singly and in combination, as well as with other targeted therapies such as rituximab. A number of studies combining oblimersen with various chemotherapy combinations or single drugs in a variety of histologic subtypes of non-Hodgkin's lymphoma are in progress. These phase II and III studies, modeled on the preclinical work, will determine whether the addition of an antiapoptotic agent to a variety of established as well as novel approaches used in treating non-Hodgkin's lymphoma can increase their clinical efficacy.

**Disclosures:** Dr. Klasa has consulted for and is a member of the speakers bureaus of Ortho-McNeil
**References:**


22. Cotter FE, Auer R, Corbo M, et al: Oblimersen sodium (G3139) sensitizes malignant B-cells to...


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