Bcl-2 Antisense Therapy in Multiple Myeloma

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Most malignant plasma cells overexpress Bcl-2, which contributes to resistance against apoptosis induced by dexamethasone and other anticancer agents. Oblimersen sodium (Genasense, previously known as G3139), an antisense oligonucleotide that specifically binds to bcl-2 messenger RNA, decreases production of Bcl-2 protein in both human myeloma cell lines, as well as in ex vivo purified myeloma cells, and enhances the cytotoxicity of dexamethasone and doxorubicin. Combining oblimersen with other anticancer agents represents a therapy enhancing strategy to reverse the multidrug resistance seen in multiple myeloma (MM). Phase II trials are evaluating the potential role of oblimersen in reversing resistance to standard therapies. Preliminary results from these trials in patients with refractory or relapsed MM indicate that the combination of oblimersen with dexamethasone/thalidomide (Thalomid) or vincristine/doxorubicin/dexamethasone is active and well tolerated and that oblimersen may help overcome chemotherapy resistance and restore sensitivity to MM cells. A randomized phase III clinical trial comparing dexamethasone plus oblimersen with dexamethasone alone in patients with relapsed or refractory myeloma has completed enrollment, with results expected to be available in 2004. Future studies will focus on the role of oblimersen in combination with novel biologic agents such as bortezomib (Velcade).

Multiple myeloma (MM) remains incurable with conventional chemotherapy, with a median survival between 2 and 3 years.[1] MM is typically chemosensitive earlier in its clinical course but is ultimately characterized by the development of multidrug resistance in almost all patients. There is growing evidence that the apoptosis-regulating Bcl-2 protein may play an important role in the development of multidrug resistance in patients with MM. Bcl-2 antisense therapy may therefore provide a new therapeutic approach to reverse this resistance and to potentiate antitumor effects of chemotherapy in the treatment of MM.[2] Bcl-2 Protein and Multidrug Resistance in Multiple Myeloma Bcl-2 is a conserved, ubiquitous protein associated with the inner mitochondrial membranes.[3] It exhibits a regulatory role in apoptosis by blocking the release of cytochrome c.[4] Most human myeloma cell lines and samples obtained from patients with MM overexpress Bcl-2 protein.[5,6] Various preclinical studies have demonstrated that the Bcl-2 protein plays an important role in mediating resistance of MM cells to apoptosis induced by dexamethasone and cytotoxic agents.[7-13] Tian and Gazitt[14] initially demonstrated that the extent of dexamethasone-induced apoptosis in MM cell lines in vitro was inversely correlated with intracellular levels of Bcl-2. Subsequently, these investigators transfected dexamethasone-sensitive, low Bcl-2-expressing MM cell lines with a Bcl-2-inducible gene construct expressed under the control of a lac repressor operon.[13] Activation of the inducible gene resulted in increased intracellular levels of Bcl-2, enhanced cell growth, and decreased spontaneous apoptosis, with concomitant increased resistance to dexamethasone. Conversely, inactivation of the inducible gene restored sensitivity to dexamethasone-induced apoptosis. Collectively, these seminal studies demonstrated a potential role of Bcl-2 in apoptosis regulation of MM cell lines as well as development of resistance to dexamethasone-induced apoptosis in these cells. This provides the basis for the hypothesis that Bcl-2 antisense therapy might decrease Bcl-2 protein production and thereby facilitate apoptosis in malignant myeloma cells.
Preclinical Studies With Oblimersen Sodium

Oblimersen sodium (Genasense, previously known as G3139) is a Bcl-2 antisense oligonucleotide designed to specifically bind to the bcl-2 messenger RNA. It binds to the first six codons of the human bcl-2 mRNA, forming a heterodimer. This double-stranded mRNA is perceived as aberrant and is subsequently degraded in the cell's cytoplasm. The result is a decreased production of the Bcl-2 protein by the ribosome. Recent preclinical studies have evaluated the potential of oblimersen to decrease Bcl-2 protein in MM cells and to sensitize or reverse resistance of MM cells to agents active against MM. Results from these studies indicate that oblimersen is taken up by myeloma cells and can decrease Bcl-2 protein production. Thus, the sensitivity of myeloma cells to therapeutic agents commonly used in the treatment of MM, such as dexamethasone and doxorubicin, is enhanced, providing a rationale for conducting clinical trials using oblimersen in patients with refractory MM. Liu and Gazitt[16] reported the effects of pretreatment with oblimersen on dexamethasone-, paclitaxel-, and adenovirus p53-induced apoptosis and intracellular levels of Bcl-2 in myeloma cells expressing varying levels of Bcl-2. Multiple myeloma cells were treated with oblimersen (10 μg/mL) for 3 days, followed by exposure to dexamethasone, paclitaxel, or adenovirus p53 for up to 2 additional days. In myeloma cells expressing relatively low levels of Bcl-2, oblimersen exposure resulted in substantial apoptosis with a concomitant decrease of Bcl-2 protein. This decrease in Bcl-2 protein levels and the increased apoptotic events were time and concentration-dependent, and apoptosis was noted to be mediated through activation of caspase-9 and caspase-3 and by the release of cytochrome c into the cytosol. In another experiment with high Bcl-2-expressing myeloma cell lines (ARH-77, U266), oblimersen pretreatment followed by exposure to dexamethasone or paclitaxel resulted in a substantial increase in the percentage of myeloma cells undergoing apoptosis when compared with the effects obtained with each agent with prior exposure to oblimersen. The increased apoptosis was associated with a decrease in Bcl-2 protein. Similar results were obtained when freshly isolated myeloma cells from
patients were used. In another study, van de Donk et al[17] also evaluated the effects of decreased Bcl-2 protein in ex vivo purified malignant plasma cells from patients with MM. Following incubation of the cells with oblimersen, but not with solvent or the sense oligonucleotides, a substantial reduction (> 75%) of bcl-2 mRNA levels occurred after 2 and 4 days of exposure as measured by realtime polymerase chain reaction. Exposure of the cells to oblimersen resulted in a sequence-specific reduction of Bcl-2 protein within 4 days in 10 of 11 patient samples. Significant enhancement of dexamethasone- or doxorubicin-induced apoptosis and cytotoxicity was also noted in these experiments (Figures 1 and 2). O'Connor et al[18] were the first to report on the beneficial antitumor effects of oblimersen when combined with the biologic agent bortezomib (Velcade) in non-Hodgkin's lymphoma (NHL) and MM cell lines. These authors recently shared the preliminary results of in vitro and in vivo experiments with oblimersen and bortezomib with established MM (U266, RPMI 8226, KMS-11) and NHL (Raji, SKI-DLCL-1, DOHH2) cell lines. These seminal experiments showed that oblimersen enhances the antiproliferative effect of bortezomib in MM cell lines. The augmented antiproliferative response of oblimersen was particularly evident in the NHL cells. In a xenograft experiment with SKI-DLCL-1 comparing oblimersen alone, bortezomib alone, and oblimersen prior to bortezomib administration, mice receiving either agent alone showed 20% to 25% inhibition of tumor growth, whereas animals receiving the combination experienced a reduction in tumor growth of approximately 50% at 24 days. This effect of oblimersen was noted to be sequence-specific. Based on these experiments, future studies are proposed to explore the role of oblimersen in optimizing the use of bortezomib in patients with B-cell malignant disorders.

Clinical Studies of Oblimersen

Based on the potential role of antiapoptotic Bcl-2 protein in MM and the encouraging preclinical studies with oblimersen, several clinical trials in patients with relapsed or refractory MM were initiated. These clinical studies are designed to evaluate the potential role of oblimersen to enhance sensitivity or reverse resistance to standard MM therapies, including dexamethasone, dexamethasone/thalidomide (Thalomid), and the combination of vincristine, doxorubicin (Adriamycin), and dexamethasone (VAD).

Phase II Trials

Badros et al[19,20] are conducting a phase I/II clinical trial evaluating the administration of oblimersen followed by dexamethasone/thalidomide in patients with relapsed or refractory MM. This study has been active as of May 2004, with a target accrual of up to 46 patients. To be eligible, patients may have received no more than four prior chemotherapy regimens. Oblimersen (7 mg/kg/d) is administered by continuous intravenous infusion on days 1 to 7 (the first three patients received 5 mg/kg/d), with dexamethasone (40 mg/d) given on days 4 to 7 and thalidomide (100 to 400 mg/d) starting on day 4. Treatment cycles are repeated every 3 weeks. After three induction cycles, responding patients continue oblimersen on a 5-week cycle with dexamethasone (20 mg/d) for 4 days and thalidomide at the tolerated dose for up to 1 year.
Preliminary data have been reported for the first 18 patients treated.[20] The median age was 58 years (range: 47 to 74), and 11 patients were male. Patients had received a median of 3 prior regimens (range: 2 to 4 regimens), including prior autologous (n = 15) or allogeneic (n = 1) stem cell transplantation. Eight patients had received thalidomide previously, for a median duration of 6.5 months (range: 2 to 10 months), with 6 of these patients demonstrating disease progression while receiving thalidomide. Ten patients had complex karyotype. Of the enrolled patients, 16 have completed the induction phase. Two patients had a complete response, two others had a near-complete response, and five patients had a partial response. The overall response rate of 75% is notable in a group of patients with advanced disease who had failed multiple prior therapies. At a median follow-up of 4 months (range: 1.5 to 8.5 months), 1 responding patient had relapsed and 11 continued on study therapy. Bone marrow with adequate plasma cells was available for 11 patients; in this study no significant decrease in Bcl-2 protein was observed at days 4 to 7 or day 28 of oblimersen infusion compared with baseline. In addition, there were no detectable differences between responders and nonresponders with respect to Bcl-2 protein. The combination of oblimersen, dexamethasone, and thalidomide was well-tolerated. Oblimersen toxicities included reversible increases in serum creatinine (to > 2 mg/dL) in 10 patients, which required oblimersen dose reduction to 3 to 5 mg/kg/d, and thrombocytopenia in 6 patients. No grade 4 toxicities occurred, and the majority of toxicities were reversible. In another phase II trial, the same concept of restoring chemosensitivity in patients refractory to standard chemotherapy by administration of oblimersen to decrease Bcl-2 protein production in combination with chemotherapy is being evaluated.[21] Ten patients with refractory MM, including eight patients whose disease was refractory to VAD, were treated with oblimersen (7
mg/kg/d) by continuous intravenous infusion for 7 days in combination with VAD. All patients were heavily pretreated and had received a median of 4 previous chemotherapy regimens (range: 2 to 6). Four patients had a partial response, and three patients had a minor response. Median disease progression-free survival was approximately 6 months (range: 2 to 7+ months), and median overall survival had not been reached. Oblimersen decreased the amount of Bcl-2 protein in peripheral blood myeloma cells, T cells, B cells, and monocytes. The oblimersen/VAD combination was feasible and well tolerated. The median number of treatment cycles was 2.5. Baseline Bcl-2 protein in bone marrow, Ki-67 growth fraction, and the presence of deletion of chromosome 13 were not predictive of response. These preliminary results suggest that oblimersen may help to overcome chemotherapy resistance and restore sensitivity of myeloma tumor cells to VAD chemotherapy.

**Phase III Trial**

A randomized phase III trial of dexamethasone with or without oblimersen in patients with relapsed or refractory MM (GMY302) recently completed accrual (n = 220). Patients could have had up to six prior therapies. In a 4-week induction period, oral dexamethasone (40 mg) was given every day for 4 days of weeks 1, 2, and 3 to both cohorts. Patients who were randomized to the study arm received oblimersen (7 mg/kg/d) as a continuous infusion for 7 days on weeks 1 and 3, with dexamethasone initiated on day 4 of the oblimersen infusion. Subsequent cycles in both cohorts incorporated only one 4-day dexamethasone pulse every 3 weeks. The primary end point is time to disease progression. Results of this study are expected to be presented at the Annual Session of the American Society of Hematology (ASH) in 2004. **Conclusion**

Bcl-2 protein confers a clinically relevant chemoresistant phenotype on many types of cancer cells, including multiple myeloma, making it a relevant target for MM therapy. Preclinical studies indicate that oblimersen decreases Bcl-2 protein in MM cell lines as well as in ex vivo MM cells from patients and enhances the cytotoxic potential of dexamethasone, doxorubicin, and bortezomib. Preliminary data from phase II clinical trials in patients with refractory or relapsed MM are encouraging. Clinically oblimersen appears to enhance the cytotoxic potential of antimelanoma therapies and is noted to have an acceptable safety profile in these patients; the results of a fully accrued phase III trial comparing dexamethasone plus oblimersen to dexamethasone alone in patients with refractory or relapsed disease are awaited with considerable interest. Results from these ongoing studies will determine the role of oblimersen in the treatment of patients with MM. Future studies will focus on combining oblimersen with other novel biologic agents such as bortezomib.

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**References:**


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