

A Phase I Pharmacokinetic and Biological Correlative Study of Oblimersen Sodium (Genasense, G3139), an Antisense Oligonucleotide to the Bcl-2 mRNA, and of Docetaxel in Patients with Hormone-Refractory Prostate Cancer

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ABSTRACT

Purpose: To assess the feasibility of administering oblimersen sodium, a phosphorothioate antisense oligonucleotide directed to the Bcl-2 mRNA, with docetaxel to patients with hormone-refractory prostate cancer; to characterize the pertinent pharmacokinetic parameters, Bcl-2 protein inhibition in peripheral blood mononuclear cell(s) (PBMC) and tumor; and to seek preliminary evidence of antitumor activity.

Experimental Design: Patients were treated with increasing doses of oblimersen sodium administered by continuous i.v. infusion on days 1 to 6 and docetaxel administered i.v. over 1 h on day 6 every 3 weeks. Plasma was sampled to characterize the pharmacokinetic parameters of both oblimersen and docetaxel, and Bcl-2 protein expression was measured from paired collections of PBMCs pretreatment and post-treatment.

Results: Twenty patients received 124 courses of the oblimersen and docetaxel combination at doses ranging from 5 to 7 mg/kg/day oblimersen and 60 to 100 mg/m² docetaxel. The rate of severe fatigue accompanied by severe

neutropenia was unacceptably high at doses exceeding 7 mg/kg/day oblimersen and 75 mg/m² docetaxel. Nausea, vomiting, and fever were common, but rarely severe. Oblimersen mean steady-state concentrations were 3.44 ± 1.31 and 5.32 ± 2.34 at the 5- and 7-mg/kg dose levels, respectively. Prostate-specific antigen responses were observed in 7 of 12 taxane-naïve patients, but in taxane-refractory patients no responses were observed. Preliminary evaluation of Bcl-2 expression in diagnostic tumor specimens was not predictive of response to this therapy.

Conclusions: The recommended Phase II doses for oblimersen and docetaxel on this schedule are 7 mg/kg/day continuous i.v. infusion days 1 to 6, and 75 mg/m² i.v. day 6, respectively, once every 3 weeks. The absence of severe toxicities at this recommended dose, evidence of Bcl-2 protein inhibition in PBMC and tumor tissue, and encouraging antitumor activity in HPRC patients warrant further clinical evaluation of this combination.

INTRODUCTION

Apoptosis is a biochemical process of serial cysteine-protease (caspase) activation and recruitment of proteolytic effector caspases that ultimately results in cell death (1). Effector caspases induce selective cleavage at specific aspartate residues leading to the degradation of critical cellular housekeeping proteins required for cellular viability including signal transduction protein kinases, cytoskeletal proteins, chromatin-modifying proteins, and DNA repair proteins (2). Because the activation of effector caspases irreversibly commits the cell to apoptosis, the early caspase regulation is a tightly regulated process mediated by at least two interrelated pathways of apoptosis: the extrinsic pathway (the tumor necrosis factor receptor protein family) and the intrinsic pathway (Bcl-2 protein family; Ref. 3, 4).

The Bcl-2 family includes both proapoptotic and antiapoptotic proteins that regulate caspase-9 and -3 activation after a diverse array of apoptotic stimuli including DNA damage, chemo- and hormonal therapy, and irradiation (5, 6). Bax, the prototypic proapoptotic protein, shares structural homology with bacterial pore-forming proteins (7). After an apoptotic stimulus, Bax undergoes homodimerization and localizes to the outer mitochondrial membrane, resulting in loss of mitochondrial membrane integrity, release of cytochrome *c*, activation of caspase-9, and initiation of caspase-mediated cell death. Bcl-2 protein inhibits apoptosis through the competitive dimerization with proapoptotic protein molecules (e.g., Bax), thereby preventing homodimerization and loss of mitochondrial membrane integrity (8, 9).

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Bcl-2 protein overexpression is a common manifestation of malignancies and represents an attractive molecular target for therapy. In experimental prostate cancer models, increased expression of Bcl-2 protein mediates, at least in part, the transition from androgen-dependent growth to androgen-independent growth (10–12). Furthermore, in several human tumor cell lines, Bcl-2 protein expression mediates resistance to the cytotoxic effects of a diverse spectrum of hormone and cytotoxic chemotherapeutic agents (11, 13–18). In advanced hormone-refractory prostate cancer (HRPC) pathological specimens, the frequency and intensity of Bcl-2 protein overexpression is markedly increased compared with hormone-sensitive disease (19–21). Taken together, these findings raise the intriguing question of whether Bcl-2 overexpression mediates, at least in part, both HRPC resistance to androgen-ablation therapy and chemotherapy.

Oblimersen (G3139, Genasense) is an 18-base synthetic oligodeoxyribonucleotide strand (sequence 5'tctccagcgtgcgccat-3') that hybridizes to the first six codons of the *bcl-2* mRNA. The oligodeoxyribonucleotide-mRNA hybrid recruits endogenous RNase H, mediates scission of the *bcl-2* mRNA, and thereby depletes Bcl-2 protein. Oblimersen resists cleavage by intracellular and extracellular nucleases and exhibits greater *in vivo* stability compared with native oligonucleotides through substitutions of sulfur for non-bridging oxygen molecules at the phosphate backbone. In lymphoma, melanoma, breast, colon, ovarian, and prostate carcinoma cell lines, oblimersen led to sequence-specific and dose-dependent inhibition of both *bcl-2* mRNA and protein expression (22–24). Furthermore, in mice bearing human androgen-independent prostate cancer (PC3) tumor xenograft, oblimersen markedly enhanced the antitumor activity of docetaxel resulting in both increased rates of complete tumor regressions and cures compared with control docetaxel-treated animals at docetaxel concentrations known to phosphorylate Bcl-2 protein (25).

Oblimersen was evaluated in single-agent Phase I studies using continuous s.c. and i.v. infusion routes of administration. The maximum-tolerated doses were determined to be 147.2 mg/m²/day (approximately 4.1 mg/kg/day) for 14 days and 6.9 mg/kg/day for s.c. and i.v. administration, respectively (26–28). The principal toxicities included hyperglycemia, transient hepatic transaminase elevations, and local infusion site inflammation, with thrombocytopenia accompanied by fever and fatigue being dose limiting with s.c. administration. Antitumor activity including one durable complete response was observed in non-Hodgkin's lymphoma patients (27, 28).

The impetus for pursuing the clinical development of oblimersen sodium combined with docetaxel included the prevalence of Bcl-2 protein expression in HRPC, the intrinsic resistance of HRPC to chemotherapeutic agents, and the marked enhancement of docetaxel anticancer activity in preclinical models when combined with oblimersen. The principal objectives of this Phase I, pharmacokinetic, and biological correlative study were to (a) determine the maximum-tolerated dose of oblimersen sodium administered continuous i.v. infusion on days 1 to 6 combined with docetaxel administered i.v. over 1 h on day 6, every 3 weeks; (b) characterize the toxicities of this regimen; (c) describe the pharmacokinetic behavior of oblimersen and docetaxel when combined; (d) assess the effects

of oblimersen on Bcl-2 protein expression in paired collections of peripheral blood mononuclear cell(s) (PBMCs) and tumor biopsies; and (e) seek preliminary evidence of antitumor activity in patients with HRPC.

PATIENTS AND METHODS

Patient Selection. Patients with both histological evidence of prostate cancer and radiological evidence of metastatic disease were eligible. All patients required two or more consecutive elevations in prostate-specific antigen (PSA) values not <14 days apart in a state of surgical or chemical castration and at least ≥ 20 ng/ml; age ≥ 18 years; life-expectancy of at least 12 weeks; an Eastern Cooperative Oncology Group performance status of 0 to 2; chemotherapy completion at least 4 weeks prior (6 weeks for prior mitomycin C or a nitrosourea); discontinuation of nonsteroidal antiandrogens at least 4 weeks before study entry; adequate hematopoietic [hemoglobin ≥ 9 g/dl, absolute neutrophil count (ANC) $\geq 1500/\mu\text{l}$, platelet count $\geq 100,000/\mu\text{l}$], hepatic function [bilirubin value within institutional upper limit of normal, aspartate serum transferase, and alanine serum transferase $< 1.5 \times$ upper limit of normal, and alkaline phosphatase $\leq 2.5 \times$ upper limit of normal], renal function (serum creatinine $\leq 1.5 \times$ upper limit of normal); measurable or evaluable disease; and no coexisting medical problems of sufficient severity to limit compliance with the study. Patients treated previously with strontium or samarium were ineligible, as well as those patients with biochemical (PSA) evidence of disease without radiological confirmation of metastases. Patients gave written informed consent for all clinical and research aspects of the study according to federal and institutional guidelines before treatment. The clinical study was approved by the appropriate institutional review boards.

Drug Administration. The starting dose was 5 mg/kg/day oblimersen administered continuous i.v. infusion on days 1 to 6 in combination with 60 mg/m² docetaxel as a 1-h i.v. infusion on day 6. The starting dose and schedule of oblimersen was based on the tolerability noted of this agent in previous studies, and the sequence of administration was based on the postulated goal of maximal Bcl-2 reduction by oblimersen before docetaxel exposure. Oblimersen was escalated to a maximum of 7 mg/kg/day whereas docetaxel was serially escalated to a maximum-tolerated dose. All toxicities were graded according to the National Cancer Institute Common Toxicity Criteria version 2. Cohorts of three patients were entered at each dose level, and dose escalation was permitted only if no dose-limiting toxicities (DLT) were encountered. If one patient experienced a DLT at a given dose level, a total of six patients were entered at the dose level. If two of six patients experienced DLT, then dose escalation ceased, and additional patients were entered at the next lower dose to fully characterize the toxicities at the maximum-tolerated dose. The maximum-tolerated dose was defined as the highest dose at which less than two of six new patients experienced DLT that was defined as any grade ≥ 3 nonhematological toxicity (including grade ≥ 3 nausea or vomiting despite optimal antiemetics), thrombocytopenia ($< 25,000/\mu\text{l}$), and grade 4 neutropenia (ANC $< 500/\mu\text{l}$) lasting for at least 5 days or accompanied by fever. A patient who experienced DLT could continue on treatment with a 1 dose-level reduction.

Oblimersen was supplied as a sterile solution by Genta Inc. (Berkeley Heights, NJ) in 10-ml vials containing 300 mg. The appropriate dose of the drug was diluted with 0.9% saline solution, USP, to a total volume of 100 ml for the infusion pump drug cassette. The final solution was infused continuous i.v. infusion for 120 h.

Docetaxel was purchased commercially in 15-ml vials containing 2.36 ml of 40 mg/ml docetaxel for a total of 94.4 mg of docetaxel. To each 15-ml vial of docetaxel was added 7.33 ml of a 13% w/w solution of ethanol in water solvent, mixed by gentle rotation for 15 s, further diluted in 250 ml of 5% dextrose solution or 0.9% saline, and infused i.v. over 1 h.

A course of therapy was defined as 21 days from the initiation of oblimersen treatment, and there was no limit to the number of courses that could be administered to a patient who was both tolerating and benefiting from therapy.

Pretreatment and Follow-up Studies. Complete medical history, physical examination, and routine laboratory studies were performed pretreatment and weekly. Routine laboratory studies included a complete blood count, differential white blood count, prothrombin and partial thromboplastin times, electrolytes, blood urea nitrogen, serum creatinine, uric acid, glucose, alkaline phosphatase, lactate dehydrogenase, alanine serum transferase, aspartate serum transferase, total bilirubin, calcium, total protein, albumin, and urinalysis. Pretreatment studies also included an electrocardiogram, PSA, and relevant radiological studies for the evaluation of all measurable and evaluable sites of disease. Radiological evaluation for disease and PSA were repeated after every other course. Patients continued treatment in the absence of progressive disease or experienced intolerable toxicity. A PSA response was defined as a 50% fall in PSA from baseline maintained for ≥ 4 weeks. For patients with measurable disease, WHO-response criteria were used.

Quantification of Bcl-2 in PBMCs and Single-Paired Tumor Biopsy. Peripheral blood specimens were collected from patients before oblimersen initiation and day 6 before the initiation of docetaxel and mononuclear cell separation were performed with Histopaque 1077 (Sigma, St. Louis, MO). Briefly, the PBMCs were washed with RPMI 1640 containing 5% FCS, centrifuged, resuspended in 5 ml of PBS on ice, and immediately frozen to -70°C . Pellets were lysed in NP40 lysis buffer (Sigma), and the protein content was determined (Bradford Reagent; Bio-Rad, Hercules, CA). Protein (20 μg) was loaded per lane and fractionated in 12% SDS-polyacrylamide gels. The gels were transferred to polyvinylidene difluoride membranes, and Bcl-2 protein detection was performed using anti-Bcl-2 antibody (Dako Corp., Carpinteria, CA) and a goat antimouse horseradish peroxidase-conjugated secondary antibody (Cell Signaling, Berkeley, CA). Visualization of Bcl-2 bands was performed using enhanced chemoluminescence and normalized to β -actin levels. The change in Bcl-2 protein expression was determined using the following formula: $100\% \times [1 - (\text{day 6 normalized Bcl-2 value}/\text{pretreatment normalized Bcl-2 value})]$.

Immunohistochemistry for Bcl-2 Expression. Paraffin-embedded tumor specimens from the time of diagnosis were obtained to determine whether the expression of Bcl-2 expression was predictive of later response to treatment. Briefly,

unstained slide sections obtained from the paraffin-embedded prostate cancer tissue obtained at diagnosis were heated to 60°C , rehydrated in xylene and graded alcohols, and then sequentially rinsed in PBS and TBS-T [Tris HCl 0.5 M (pH 7.6), NaCl 0.15 M, Tween 20 0.15%]. Endogenous peroxidase activity was quenched, and slides were incubated in primary Bcl-2 antibody (Dako Corp.), biotinylated secondary antibody, followed by peroxidase-labeled streptavidin for 15 min (LSAB-2; Dako Corp.), diaminobenzidine, and hydrogen peroxide chromogen substrate (Dako Corp.) along with 3,3'-diaminobenzidine enhancer (Signet). Slides were counter-stained with hematoxylin. Antihuman monoclonal mouse IgG was used for negative controls. Scoring for Bcl-2 expression was evaluated for intensity of staining (0, +1, +2, and +3) and for percentage of cells positive (0, 10, 25, 50, 75, 100%).

Plasma Pharmacokinetic Sampling and Assay. Blood samples were collected into heparinized tubes through an indwelling venous catheter placed in the arm contralateral to the oblimersen or docetaxel infusion. Sampling was performed for oblimersen pharmacokinetic studies pretreatment; 2, 24, and 48 h after the start of the infusion; immediately before the end of infusion; and at 24 and 48 h after discontinuation. Docetaxel pharmacokinetic sampling occurred immediately before docetaxel on day 6 and at 1, 2, 4, 6, 12, 24, and 48 h after the end of the docetaxel infusion. All blood samples were centrifuged at 1,200 g for 15 min at 4°C immediately after collection and plasma stored at -20°C .

Docetaxel concentrations in plasma were measured by high-performance liquid chromatography as described previously (29). Plasma concentrations of oblimersen were measured by adding 20 μl of 1% (w/v) IPEGAL CA-630 (Sigma-Aldrich, St. Louis, MO); 0.9% (w/v) sodium chloride/PBS solution, which was vortexed and microcentrifuged for 7 min at 10,000 rpm. Supernatant (180 μl) was injected onto a temperature controlled (30°C) DNA PAC PA-100 (13 $\mu\text{m} \times 2 \times 10$ mm) alkyl quaternary amine column (Dionex, Houston, TX). An equilibrating solution [100 mM Na perchlorate, 17.5 mM Tris (pH 7.0), 0.7 mM EDTA, 30% formamide] was pumped at 1 ml/min across the column. UV detection was at 267 nm. Calibration curves were linear ($R^2 > 0.99$) from 0.2 to 20 $\mu\text{g}/\text{ml}$.

Pharmacokinetic and Pharmacodynamic Analyses. Individual oblimersen and docetaxel plasma concentration data sets were analyzed by standard non-compartmental methods. Oblimersen mean steady-state concentrations (C_{ss}) were determined by averaging the plasma concentrations at the 24-, 48-, and 120-h time points. The clearance (CL) for oblimersen was calculated as $\text{CL} = \text{drug infusion rate}/C_{ss}$.

Docetaxel peak concentrations were determined by inspection of each individual patient's plasma concentration-time curve. Elimination rate constants were estimated using linear regression of the last three data points on the terminal log linear portion of the concentration-time curves. Terminal half-lives were calculated by dividing 0.693 by the elimination rate constants. The area under the concentration *versus* time curve (AUC) was calculated using the linear trapezoidal rule up to the last measurable data point (for AUC_{0-t}) then extrapolated to infinity ($\text{AUC}_{0-\infty}$). Docetaxel CL was determined by dividing the dose (in milligrams docetaxel per m^2) by the AUC. The apparent volume of distribution at steady-state (Vd_{ss}) was de-

Table 1 Patient characteristics

Characteristic	No. of patients
No. of patients	20
Median number of courses/patient (range)	4 (1–25)
Median age (range)	64 (49–79)
Performance status	
0	6
1	12
2	2
Previous therapy	
Androgen-ablation therapy	20
Chemotherapy	10
Previous taxane chemotherapy	3
Radiation therapy	14
Median number of prior chemotherapy regimens (range)	1 (0–6)
Median PSA value at study entry (range)	116 (21–3378)

terminated by the following relationships: $Vd_{ss} = (\text{dose} \times \text{AUMC}/\text{AUC}^2) - (\text{dose} \times \text{duration of infusion})/(2 \times \text{AUC})$, where AUMC is the area under the moment curve extrapolated to infinity.

The relationships between pertinent pharmacokinetic parameters that reflected drug exposure (AUC, C_{ss} , and estimate of $C_{ss} \times$ number of days administered) for docetaxel and oblimersen and indices reflecting the degree of first course myelosuppression [ANC, absolute lymphocyte count (ALC), platelets] were explored. The percentage decrement in the ANC, ALC, and platelet counts were calculated as follows: $100\% \times [(\text{pretreatment counts} - \text{nadir counts})/\text{pretreatment counts}]$. The sigmoidal E_{max} model of drug action (*i.e.*, percentage of change in hematological parameter = $E_{max} \times \text{AUC}_{\gamma 0}/\text{AUC}_{50\gamma} +$

AUC_{γ}) assessed the relationships among dose, C_{ss} and exposure, and the observed hematological toxicity. The coefficient of determination (R^2) and the SEs for the estimated parameters measured goodness of fit for the pharmacodynamics model. Parameter values were expressed as means and SD values. Mean $\text{AUC}_{0-\infty}$ values of patients who did and did not experience severe hematological toxicity were compared using the Student's t test (two-sided).

RESULTS

General

Twenty patients, whose pertinent demographic characteristics are displayed in Table 1, received a total of 124 courses at doses ranging from 5 mg/kg/day oblimersen with 60 mg/m² docetaxel to 7 mg/kg/day oblimersen with 100 mg/m² docetaxel. The total number of new patients and courses at each dose level, as well as the overall dose escalation scheme, are depicted in Table 2. The median number of courses administered per patient was four (range, 1–25), whereas doses were reduced because of DLT in eight patients. Two patients discontinued oblimersen before receiving docetaxel. One patient developed urinary outflow obstruction related to local disease at course 1 on day 3 whereas the second patient developed an exacerbation of pre-existing ataxia during the first oblimersen infusion.

After no or negligible drug-related effects were noted at the first two dose levels consisting of 5 mg/kg/day oblimersen with either 60 mg/m² or 75 mg/m² docetaxel, the oblimersen dose was increased to 7 mg/kg/day combined with 75 mg/m² docetaxel. One of nine patients treated at this dose level experienced DLT (febrile neutropenia). Dose-escalation continued to 7 mg/kg/day oblimersen and 100 mg/m² docetaxel; however, two

Table 2 Dose escalation scheme

Oblimersen (mg/kg/day)	Docetaxel (mg/m ²)	No. of patients			No. of courses	Patients with DLT	
		New	Modified to level*	Total		First course	All courses
5	60	3	2	5	18	0/3	0/5
5	75	3	0	3	15	0/3	1/3
7†	60	0	5	5	26		
7	75	9	3	12	56	1/9	3/9
7	100	5	0	5	9	2/5	3/5
Total		20			124		

* Patients whose doses were reduced to the next lowest dose of docetaxel for DLT.

† Intermediate dose level as a result of docetaxel dose reduction from previous dose level.

Table 3 Hematologic toxicities of oblimersen and docetaxel

Oblimersen (mg/kg/day)	Docetaxel (mg/m ²)	No. of patients (courses*)	No. of first courses (all courses) with toxicity							
			Neutropenia				Lymphopenia			
			Grade 1–2	Grade 3	Grade 4	Grade 4 >5 days	Grade 4 with fever	Grade 1	Grade 2	Grade 3
5	60	3 (11)	0 (4)	0 (2)	0 (0)	0	0 (0)	0	1 (4)	1 (5)
5	75	3 (22)	0 (1)	2 (6)	1 (6)	0	0 (1)	0	1 (14)	2 (4)
7	75	9 (67)	3 (22)	2 (19)	3 (23)	0	1 (2)	0	4 (35)	4 (10)
7	100	5 (25)	0 (1)	0 (6)	4 (14)	0	1 (1)	0	1 (13)	4 (6)

* Total number of courses for patients entered at this dose level.

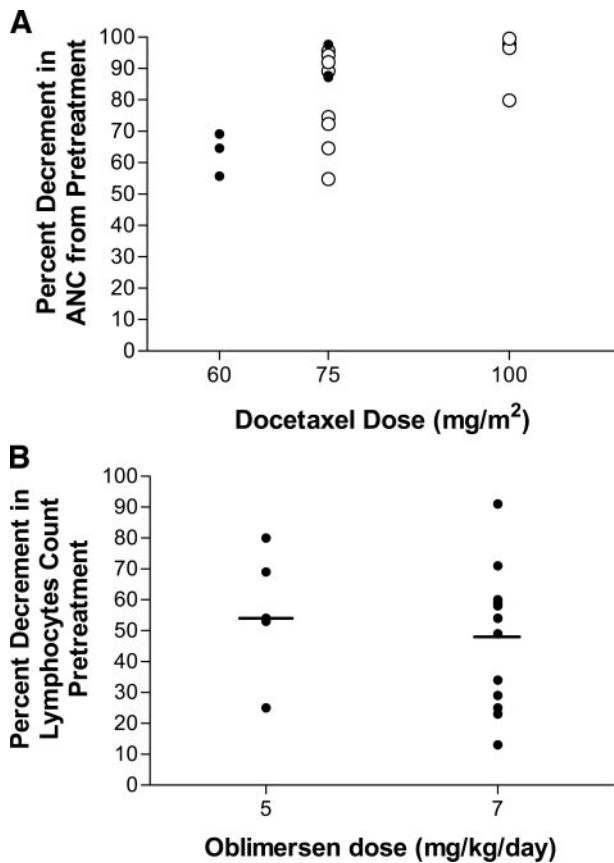


Fig. 1 A, scatterplots depicting the relationships between the percentage of decrease in ANC during the first course and dose of docetaxel and oblimersen at 5 mg/kg (●) or 7 mg/kg (○); B, the relationship between the percentage of decrease in lymphocyte count during the first course and oblimersen dose.

of five patients experienced dose-limiting fatigue (grade 3) during course 1, and in one of these patients, the fatigue was accompanied by febrile neutropenia. On the basis of these results, the recommended dose for future Phase II studies is 7

mg/kg/day oblimersen continuous i.v. infusion on days 1 to 6 with 75 mg/m² docetaxel i.v. on day 6, every 3 weeks.

Toxicity

Hematological Toxicity. The distributions and the relevant grades of neutropenia and lymphopenia as a function of dose are listed in Table 3. Myelosuppression, particularly neutropenia, was the principal hematological toxicity of the combination of oblimersen and docetaxel. The median time to ANC nadir in the first course was 15 days (range, 13–20). Despite the short interval between the administration of docetaxel and the initiation of subsequent courses of oblimersen (e.g., 16 days), patients commonly had ANC recovery by the start of the next oblimersen infusion (median ANC 2300/μl range, 190–10,300). The initiation of oblimersen therapy on course 2 and all subsequent courses was not delayed in the event of a low ANC with recovery of ANC (>1500 cells/μl) universal by day 6 for course 2.

Scatterplots of the percent decrement in ANC as a function of dose are shown in Fig. 1A. Neutropenia was docetaxel-dose-dependent with no discernible influence from the two doses of oblimersen (Fig. 1A).

Transient moderate and severe lymphopenia (grade 2 or 3) attributable to oblimersen was observed in 19 of 20 patients during the 1st course of oblimersen. The median ALC nadir was 410/μl (range, 104–1009), and the nadir occurred before docetaxel infusion at a median of 5 days from the initiation of oblimersen (range, days 2–15). The ALC decrement from pretreatment values spanned a wide distribution with an average of 56% and 47% at the 5 and 7 mg/kg/day dose levels, respectively (Fig. 1B). Despite the early onset of lymphopenia before docetaxel administration, there was no relationship between the dose of oblimersen (over the dose range examined) and the magnitude of ALC decrement.

Thrombocytopenia was uncommon and never exceeded grade 1. Drug-related anemia was also generally mild (grade 1) or moderate (grade 2), whereas severe (grade ≥3) drug-related anemia was rare and occurred in only 1 of 20 patients and 1 of 125 courses.

Nonhematological Toxicity. The most common nonhematological toxicities were fatigue, nausea, vomiting, diarrhea,

Table 4 Nonhematologic toxicity

		No. of patients experiencing toxicity during first course (all courses)											
Dose level (mg/m ²)	No. of patients (courses)	Nausea/vomiting			Diarrhea			Mucositis			Arthralgia or myalgia		
		1	2	3	1	2	3	1	2	3	1	2	3
5/60	3 (11)	0	0	0	0	0	0	1 (4)	0 (2)	0	0	1 (1)	0
5/75	3 (22)	1 (2)	0 (1)	0	0 (2)	0 (1)	0	0	1 (2)	0	0 (2)	0	0
7/75	9 (67)	2 (12)	0 (1)	0	3 (8)	1 (3)	0	1 (3)	0	0	0 (2)	1 (2)	0
7/100	5 (25)	3 (6)	2 (4)	0	0 (7)	0 (1)	0	1 (3)	1 (1)	0	1 (3)	0 (2)	1 (1)

Dose level (mg/m ²)	No. of patients (courses)	Fatigue			Fever			Peripheral neuropathy			Polyuria			
		1	2	3	1	2	3	1	2	3	1	2	3	
5/60	3 (11)	1 (1)	1 (1)	0	0 (1)	0	0	0 (3)	0	0	0	0	0 (1)	0
5/75	3 (22)	2 (4)	0 (1)	0	0	0	0 (2)	2 (4)	0	0	0	0	0 (1)	0
7/75	9 (67)	3 (19)	1 (2)	0	0 (5)	1 (6)	0	0 (3)	0	0	0	1 (2)	1 (1)	0
7/100	5 (25)	1 (4)	0 (3)	2 (3)	1 (4)	1 (4)	1 (1)	1 (4)	0 (2)	0	1 (1)	0 (1)	0	

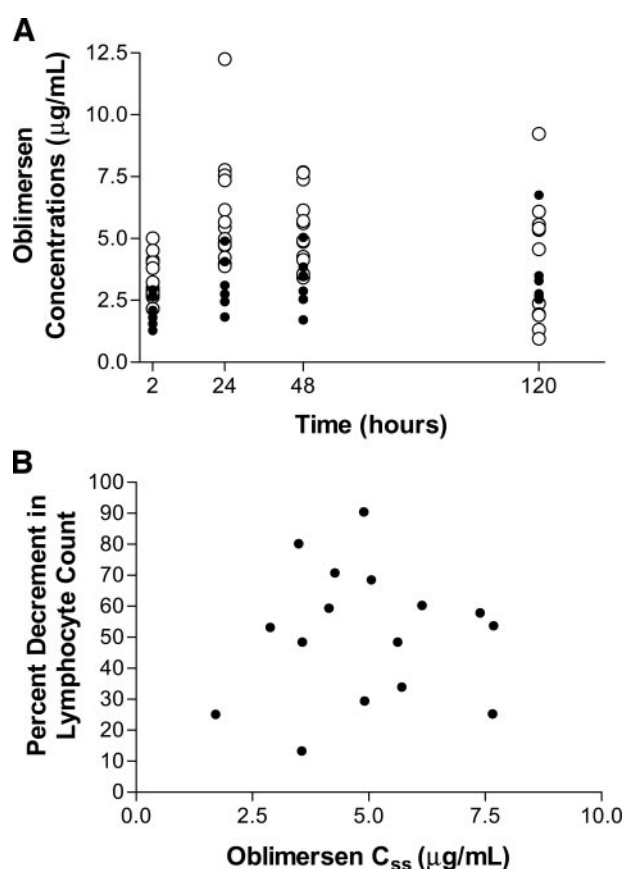


Fig. 2 Scatterplots of individual oblimersen plasma concentrations as a function of time during infusion at 5 mg/kg (●) or 7 mg/kg (○).

stomatitis, fever, and peripheral neuropathy. The distributions of these toxicities as a function of dose level and course are depicted in Table 4.

Toxicities attributed to oblimersen alone included modest (grade 1 or 2) fever that generally began on day 2 or 3 of oblimersen treatment, and despite close examination, an infectious etiology could not be ascribed. This pyrexia was treated successfully and prevented on subsequent courses with either acetaminophen or nonsteroidal anti-inflammatory agents. Polyuria was reported at least once by 35% of patients during the 5-day oblimersen infusion. Although the symptoms of nocturia and urinary frequency are common in this patient population, several patients reported symptoms of large-volume urination

during the oblimersen infusion, distinctly different from pretreatment symptoms. Marked grade 2 elevation of aspartate serum transferase was observed during the first infusion of oblimersen before docetaxel treatment in one patient. The oblimersen infusion was discontinued, the transaminase values declined to pretreatment levels, and treatment was reinitiated without significant elevation for this and two subsequent courses of oblimersen and docetaxel.

Although fatigue, fever, nausea, vomiting, mucositis, and diarrhea occurred frequently, severe nonhematological toxicity was uncommon. Fatigue was observed in 80% of patients treated with this combination but was dose limiting (grade 3) in only three patients, all of whom were at the highest dose level. Nausea and vomiting occurred frequently, were never dose limiting, and treatment with prochlorperazine and/or serotonin 5HT₃ receptor antagonists generally resulted in successful management or prevention of these toxicities. Mild or moderate (grade 1 or 2) drug-related diarrhea occurred in 10 (50%) patients during treatment. Other mild to moderate (grade 1 or 2) nonhematological toxicities that were possibly related to the oblimersen and docetaxel regimen included malaise (20% of patients), alopecia (35%), anorexia (45%), and hepatic transaminase elevation (15%). Elevations in blood glucose were noted in 70% of patients, but were attributed, at least in part, to the use of dexamethasone as premedication for docetaxel.

Cumulative toxicities associated with docetaxel included nail bed changes and onycholysis (25% of patients), excessive lacrimation (15% of patients), and peripheral neuropathy (50%).

Pharmacokinetics and Pharmacodynamics

Twenty patients had plasma sampling performed to determine oblimersen C_{ss}, and 18 patients had sampling for docetaxel pharmacokinetic parameters. Although the mean oblimersen C_{ss} increased as the oblimersen dose increased from 5 to 7 mg/kg/day, there was substantial overlap of individual C_{ss} values between the two dose levels. Scatterplots of individual C_{ss} values as a function of dose and time are depicted in Fig. 2A whereas the mean values are summarized in Table 5. Oblimersen C_{ss} were reached within 24 h of the infusion initiation and undetectable 24 h after infusion discontinuation. The mean (±SD) C_{ss} values were 3.44 ± 1.31 and 5.32 ± 2.34 µg/ml at the 5 and 7 mg/kg doses levels, respectively, and oblimersen CL averaged 0.057 ± 0.013 liter/h/kg.

The patient with the highest oblimersen concentration (12.25 µg/ml) was the aforementioned patient who experienced an exacerbation of cerebellar ataxia. The potential contribution

Table 5 Mean oblimersen concentration as a function of dose and infusion length

Oblimersen (mg/kg/day)	Docetaxel (mg/m ²)	No. of patients	2 h (µg/ml)	24 h (µg/ml)	48 h (µg/ml)	120 h (ml/m ²)
5	60	3	2.03 (0.71)	2.89 (1.12)	3.02 (1.15)	3.74 (1.25)
5	75	3	2.10 (0.72)	3.49 (1.27)	3.49 (1.36)	3.99 (2.39)
Mean (SD)			2.06 (0.64)	3.19 (1.12)	3.26 (1.15)	3.87 (1.71)
7	75	9	3.20 (0.77)	5.40 (1.39)	5.14 (1.67)	3.89 (2.13)
7	100	5	3.56 (0.99)	7.00 (3.19)	5.51 (1.25)	6.11 (4.62)
Mean (SD)			3.33 (0.83)	5.97 (2.22)	5.27 (1.49)	4.63 (3.15)

Table 6 Noncompartmental pharmacokinetic parameters of docetaxel

Dose level docetaxel/oblimersen (mg/m ²)/(mg/kg/day)	No. of patients	C _{max} (mg/ml)	t _{1/2} (h)	AUC _{0-∞} (μg/ml·h)	CL (l/h)	Vd _{ss} (liter)
60/5	3	0.84 (0.40)	0.26 (0.08)	0.87 (0.41)	162.5 (80.6)	87.7 (51.9)
75/5	3	1.72 (1.160)	2.48 (0.5)	2.00 (1.11)	85.6 (38.2)	169.0 (75.6)
75/7	8	1.56 (0.43)	4.3 (5.2)	1.96 (0.61)	87.3 (34.1)	129.3 (103.3)
100/7	4	1.4 (0.16)	1.5 (1.18)	1.64 (0.16)	133.0 (22.7)	116.0 (44.8)
Mean (±SD)			2.69 (3.57)		109.7 (49.8)	117.3 (73.6)

NOTE. All values represent means (±SD).

Abbreviations: C_{max}, peak plasma concentration observed; t_{1/2}, half-life.

Table 7 Tumor immunohistochemistry for Bcl-2

Staining distribution*	No. of intensity†	No. of patients	Responses‡
Homogeneous	3+	5	1
	2+	0	
	1+	1	
Heterogeneous	3+	1	1
	2+	1	
	1+	0	
Focal	3+	0	3
	2+	0	
	1+	9	
Total no. of evaluable specimens		17	5

* Distribution: homogeneous >75% of all cells stained; heterogeneous 25–75% of all cells stained; focal <25% of all cells stained.

† Intensity scale is as follows: 0 = equal to background; 1 = weak; 2 = moderate; 3 = strong.

‡ Of the 7 taxane naïve PSA responding patients only 5 had tumor evaluable.

of the high oblimersen plasma concentration to the ataxia is not known.

Lymphopenia was the predominant hematological effect attributable to oblimersen, and therefore the relationships of oblimersen C_{ss} and oblimersen exposure and the percent decrement of lymphocytes from pretreatment levels were examined. Despite the attribution of lymphopenia to oblimersen, neither linear nor nonlinear models could adequately describe the relationship between oblimersen C_{ss} or exposure and the percentage of decrement in lymphocytes ($r = 0.01$, $P > 0.05$, Fig. 2B).

The mean pharmacokinetic parameter estimates of docetaxel at each dose level, as well as overall mean values, are listed in Table 6. The Vd_{ss} of docetaxel was large, averaging 117.3 ± 73.6 liters; the mean plasma clearance was 109.7 ± 49.8 liter/h; and the terminal half-life of elimination was brief, averaging 2.69 ± 3.57 h. Patients who experienced severe neutropenia (grade 3 or 4) had greater AUC_(0-∞) mean values compared with those without toxicity; however, this difference was not statistically significant (AUC_(0-∞), 1492 ± 325 ng/ml·h versus 1,825 ± 190 ng/ml·h; $P = 0.36$). Furthermore, the relationship between docetaxel AUC_(0-∞) and the percent decrement in ANC could not be adequately described by either linear or nonlinear models ($R^2 = 0.05$; $P > 0.05$).

Bcl-2 Expression in Tumor Tissue and Paired PBMC and Tumor Biopsies.

Seventeen primary tumor specimens were evaluable for Bcl-2 expression, and the majority of patients (53%) exhibited

weak (1+ focal) Bcl-2 expression (Table 7). Five of 19 (29%) patient specimens exhibited strong (3+ homogenous) and three patients exhibited heterogeneous expression of Bcl-2. The limited patient sample size prevented meaningful determination of a relationship between PSA response and Bcl-2 expression.

In four-paired evaluable PBMC specimens, Bcl-2 protein decrements of 92, 93, 97, and 98% occurred from pretreatment to day 6. Fig. 3A demonstrates a representative-paired specimen. One patient with a supraclavicular lymph node metastases consented to serial biopsy pretreatment on day 6 before docetaxel therapy. The Bcl-2 protein content, normalized for β-actin expression, was reduced by 40% by day 6 (Fig. 3B).

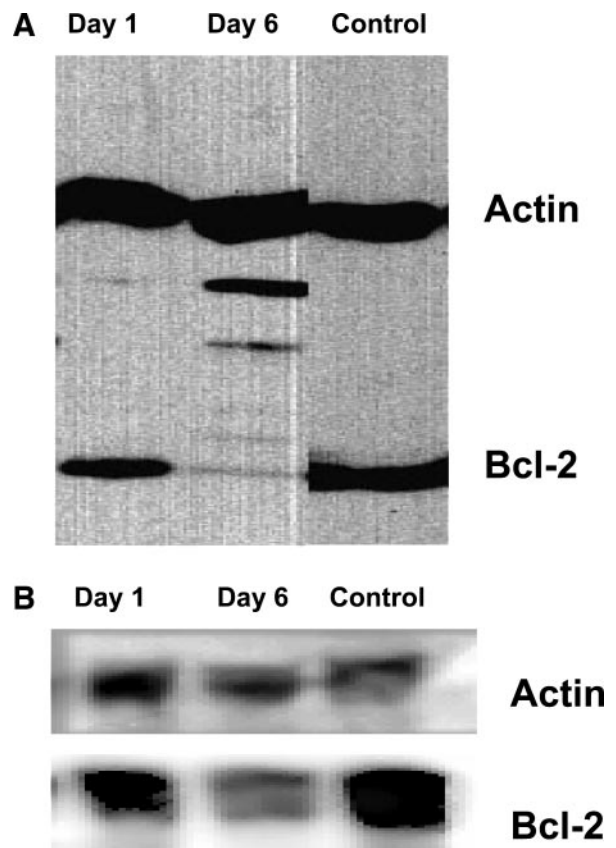


Fig. 3 Expression of Bcl-2 and Actin protein as determined by Western blot analysis from peripheral blood mononuclear cells (A) or tumor biopsies (B) on days 1 and 6 of oblimersen infusion.

Antitumor Activity

PSA responses were observed at all dose levels. Three patients were refractory to docetaxel or paclitaxel before study entry, and none of these patients responded to treatment with docetaxel or with oblimersen. Five of 17 taxane-naïve patients were not considered evaluable for response assessment including two patients who never received docetaxel treatment; one patient with evidence of PSA decline on day 1 compared with the documented elevations 2 and 4 weeks earlier; one patient with concomitant metastatic melanoma who had a progression of pulmonary lesions; and one patient found to have noncastrate levels of testosterone. This patient later responded to chemical castration. Twelve of the 17 taxane-naïve patients were considered evaluable for response. Seven of these 12 patients had a $\geq 50\%$ reduction in PSA lasting at least 4 weeks, including two patients whose PSA values normalized from pretreatment values of 32 and 82 ng/ml. The median PSA decrement for the seven responding patients was 78% (range, 52–99%). Two of the five nonresponding patients demonstrated primary progression, whereas the three remaining patients had maximal PSA decrements of 49, 29, and 12% from pretreatment. One patient with PSA progression had brain metastases diagnosed after three courses and was discontinued from treatment. One responding patient continued on therapy for 25 courses. Two of four patients with bidimensionally measurable disease had evidence of a partial response.

DISCUSSION

The regulatory proteins that govern apoptosis are important strategic targets for drug development. Several agents are currently undergoing preclinical and clinical evaluations to exploit the critical function these proteins possess in regulating tumor cell viability and to enhance the effectiveness of current therapies (30, 31). Diminished apoptosis mediated by a number of mechanisms is a common feature of malignant cell proliferation. Aberrant signal transduction pathways inhibit apoptosis through phosphorylation of proapoptotic proteins concurrent with mitogenic growth (32–35). Decreased function of Bax protein has been demonstrated in colorectal, breast, and ovarian carcinoma patients (36–40). Moreover, overexpression of Bcl-2 protein has been demonstrated in tumors from patients with malignant melanoma, non-Hodgkin's lymphoma, and carcinomas of the prostate (hormone-refractory), colon, small cell and non-small cell lung, and breast (10, 41–47). Based on these findings and preclinical evidence of Bcl-2 protein inhibition by oblimersen-increased apoptosis induction, cytotoxicity *in vitro* and tumor regressions *in vivo* in combination with diverse classes of antineoplastics, oblimersen is undergoing broad clinical development.

As predicted, myelosuppression was the predominant hematological toxicity in this Phase I study. Although grade 3 and 4 neutropenia and grade 3 lymphopenia were commonly observed, the incidence of dose-limiting infections was low. Overall, the rate of dose-limiting neutropenia complicated by infection at docetaxel doses of 75 mg/m² was acceptable with febrile neutropenia observed in only 2 of 67 courses. Attempts to further escalate the dose of docetaxel above 75 mg/m² resulted in unacceptably high rates of DLT, particularly severe (grade 3)

fatigue in two of five patients during course 1, which was accompanied by febrile neutropenia in one patient. On the basis of these results, oblimersen administered at 7 mg/kg/day on days 1 to 6 with 75 mg/m² docetaxel i.v. on day 6 is recommended for patients participating in subsequent disease-directed evaluations.

Marked lymphopenia accompanying the oblimersen infusion was observed commonly (in 95% of patients) and was the predominant hematological toxicity ascribed to oblimersen alone. The magnitude of lymphopenia could not be related to oblimersen dose or pharmacokinetic parameters that reflected oblimersen exposure; however, the oblimersen dose range in this study was limited. Although this study was not specifically designed to determine whether oblimersen altered the clearance of docetaxel, the mean pharmacokinetic values are within the range reported in previous studies, indicating that a significant drug-drug interaction is unlikely (48).

The combination of oblimersen with docetaxel may represent a multi-targeted approach for Bcl-2 protein function inhibition. In addition to caspase regulation governed by the relative expression of proapoptotic and antiapoptotic family members, apoptosis is further regulated by the extent of Bcl-2 protein phosphorylation at serine and threonine residues that interact with Bax protein. Hyperphosphorylation of Bcl-2 protein decreases the affinity of Bcl-2 to Bax heterodimerization, thereby favoring apoptosis induction. Paclitaxel, docetaxel, and estramustine inhibit cytosolic phosphatases, increase the relative extent to which Bcl-2 protein exists in a phosphorylated and inactive state, and thereby enhance the propensity for apoptosis (49, 50). However, antimicrotubule agents only partially phosphorylate Bcl-2 protein, and inhibition by oblimersen with docetaxel leads to additive and synergistic antitumor activity in preclinical models (25).

The absence of a response in the three taxane-refractory patients, albeit a small sample, suggests that Bcl-2 inhibition by oblimersen alone may not reverse acquired taxane resistance. Resistance to docetaxel is multifactorial and includes both mechanisms present intrinsically in malignant cells as well as mechanisms that are acquired through selection during treatment. The mechanisms by which aberrant pro- and antiapoptotic Bcl-2 protein family members mediate resistance are distinct from other mechanisms commonly ascribed to treatment resistance. Bcl-2 overexpression abrogates caspase downstream to a successful apoptotic stimuli caused by antineoplastic agents. Failure to initiate a successful apoptotic signal upstream to the Bcl-2 family of proteins, by mechanisms such as altered target binding (*e.g.*, tubulin), reduced cellular uptake or active efflux (MDR1 phenotype), or repair of target damage, will not be overcome by reductions in Bcl-2 protein. The greatest impact of oblimersen therapy may therefore be in tumors that are potentially sensitive to docetaxel but in which the magnitude of cell kill is suboptimal. The antitumor activity in taxane naïve HRPC patients entered into this study is encouraging and suggests, albeit preliminarily, that additional studies be performed to determine whether oblimersen may enhance the ability of docetaxel to induce apoptosis in HRPC cells. Because oblimersen may enhance the depth of response by increasing the rate of cells undergoing apoptosis attributable to docetaxel, time to progression and survival, rather than response rate, may repre-

sent the appropriate end points to judge the value of this agent in clinical studies. The rational next step for the development of this combination will be the evaluation in a broad array of docetaxel-sensitive disease-directed Phase II and III studies to ascertain whether the activity and effectiveness of docetaxel can be significantly enhanced.

A marked reduction in Bcl-2 protein expression in four-paired PBMCs and one-paired tumor biopsy observed in this study further confirms the mechanism of action of oblimersen. The 40% decrement in the paired tumor specimen, although less than the decrement in PBMCs, is consistent with other investigators using a similar methodology (51). For Bcl-2 expression in the original tumor blocks, only a minority of patients (26%) were positive (strong Bcl-2 expression) for Bcl-2 by immunohistochemistry. This finding may not reflect the extent of Bcl-2 expression in metastatic disease sites at study entry because previous investigators have demonstrated markedly increased Bcl-2 protein expression in prostate cancer specimens after androgen-ablation therapy (10, 19). Taken together this may limit both the predictive value of Bcl-2 protein determination in the original tumor specimens or the use of Bcl-2 expression in the original tumor blocks as a patient selection criteria in future oblimersen HRPC clinical studies.

Although Bcl-2 protein overexpression has been well described in HRPC, it may represent one of many molecular mechanisms associated with aberrant apoptosis, androgen-independent prostate carcinoma growth, and resistance to chemotherapy (33, 52, 53). Patients with tumors that use dysregulated Bcl-2 to mediate resistance to docetaxel, and thereby vulnerable to Bcl-2 inhibition by oblimersen, would presumably be the optimal population selected for randomized studies to determine the efficacy of this combination. The identification of predictive biomarkers for response to oblimersen-based combination therapy may be critical to the successful clinical development of this agent. To further address this issue in a homogeneous patient population undergoing uniform treatment, a Phase II study of this combination has been initiated in HRPC patients incorporating concurrent clinical and biological correlative studies to identify the relevant predictive biomarkers of response.

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