A Phase II, Pharmacokinetic, and Biological Correlative Study of Oblimersen Sodium and Docetaxel in Patients with Hormone-Refractory Prostate Cancer

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Abstract

Purpose: To determine the antitumor activity and safety of oblimersen sodium, a phosphorothioate antisense oligonucleotide directed to the *bcl-2* mRNA, with docetaxel in patients with hormone-refractory prostate cancer (HRPC) and to determine if relevant pharmacokinetic and pharmacodynamic variables of oblimersen or docetaxel influence response to this therapy.

Experimental Design: Patients with HRPC were treated with oblimersen sodium by continuous i.v. infusion on days 1 to 8 with docetaxel given i.v. over 1 hour on day 6 every 3 weeks. Plasma samples were analyzed to characterize the pharmacokinetic variables of both oblimersen and docetaxel, and paired collections of peripheral blood mononuclear cells were collected to determine Bcl-2 protein expression pretreatment and post-treatment.

Results: Twenty-eight patients received 173 courses of oblimersen (7 mg/kg/d continuous i.v. infusion on days 1-8) and docetaxel (75 mg/m^2 i.v. on day 6). Prostate-specific antigen responses were observed in 14 of 27 (52%) patients, whereas 4 of 12 (33%) patients with bidimensionally measurable disease had objective responses. The mean oblimersen steady-state concentration (C_{ss}) was a significant determinant of antitumor activity; mean C_{ss} values were higher in responders compared with nonresponders (6.24 ± 1.68 versus 4.27 ± 1.22; P = 0.008). The median survival of all patients was 19.8 months. Bcl-2 protein expression decreased a median of 49.9% in peripheral blood mononuclear cells post-treatment, but the individual incremental change did not correlate with either oblimersen C_{ss} or response.

Conclusions: Oblimersen combined with docetaxel is an active combination in HRPC patients demonstrating both an encouraging response rate and an overall median survival. The absence of severe toxicities at this recommended dose, evidence of Bcl-2 protein inhibition, and encouraging antitumor activity in HPRC patients warrant further clinical evaluation of this combination, including studies to optimize oblimersen C_{ss}.

The antiapoptotic regulatory protein, Bcl-2, represents an attractive molecular target in the treatment of hormone-refractory prostate cancer (HRPC). In human prostate carcinoma cell lines as well as in clinical prostate cancer specimens, increased Bcl-2 protein expression portends the transition to androgen-independent growth and may promote the development of androgen-independent growth (1–9). Moreover, in several experimental carcinoma cell lines, Bcl-2 protein overexpression confers resistance to a broad spectrum of antineoplastic agents (10–12). Taken together, these findings raise the intriguing question as to whether Bcl-2 overexpression mediates, at least in part, clinical resistance to both androgen deprivation and chemotherapy in prostate carcinoma patients.

Oblimersen (Genasense, G3139) is an 18-base synthetic oligodeoxyribonucleotide strand (5′-TCTCCCAGCGTGCGC-CAT-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 inhibits Bcl-2 protein expression. Oblimersen is resistant to cleavage by intracellular and extracellular nucleases and exhibits greater *in vivo* stability compared with native oligonucleotides through the substitution of sulfur for nonbridging oxygen molecules in the phosphate backbone. Treatment of lymphoma, melanoma, breast, colon, ovarian, and prostate carcinoma cell lines with oblimersen produced sequence-specific and dose-dependent inhibition of both *bcl-2* mRNA and protein expression (13–15). Furthermore, in mice bearing xenograft tumors from breast, non–small cell lung, and

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androgen-independent prostate human cancer cell lines, oblimersen markedly enhanced the antitumor activity of docetaxel resulting in both increased rates of complete tumor regression and cure compared with control animals treated with docetaxel alone (16–18). Notably, enhancement of antitumor activity occurred, although docetaxel partially phosphorylates and inactivates Bcl-2 protein (17, 19, 20). The implication of these results indicate that docetaxel only partially inactivates Bcl-2, oblimersen further inhibits of Bcl-2 leading to enhanced antitumor activity, and this multipronged approach to Bcl-2 inhibition was a rational strategy for therapeutic gain.

Several phase I studies have evaluated oblimersen as a single agent as well as combined with docetaxel. A dose-escalation study of single-agent i.v. given oblimersen to patients with solid tumors attained doses of 6.9 mg/kg/d for 14 days (21). The rapid clearance of oblimersen shown by a plasma elimination half-life approximated 2 hours, implied that prolonged infusions were necessary for optimum exposure. In the combination studies, docetaxel given at a dose of 35 mg/m^2/wk i.v. was feasible with oblimersen as a 5-day continuous i.v. infusion at doses up to 9 mg/kg/d, and a maximum tolerated dose was not defined (22). However, dose-limiting toxicity was encountered when oblimersen was given at 4 mg/kg/d using a protracted (14- to 21-day) infusion schedule. Using docetaxel administration every 3 weeks, the recommended dose for the combination was 7 mg/kg/d oblimersen continuous i.v. infusion on days 1 to 6 with docetaxel 75 mg/m^2 i.v. on day 6 (23). The principal toxicities included fatigue, fever during the oblimersen infusion, hepatic transaminitis, and lymphopenia and neutropenia; in taxane naïve patients, an encouraging rate of antitumor activity [prostate-specific antigen (PSA) response 7 of 12 (55%) patients] was observed.

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, the marked enhancement of docetaxel anticancer activity in preclinical models when combined with oblimersen, and the encouraging, albeit preliminary, antitumor activity observed in the patients with HRPC during the phase I study. In addition, a homogenously treated phase II population represented an important opportunity to examine the biomarkers that may be predictive for response to Bcl-2 targeting therapy. The principal objectives of this phase II, pharmacokinetic, and biological correlative study were to (*a*) determine the antitumor activity of oblimersen sodium given as a 7-day continuous i.v. infusion combined with docetaxel given over 1-hour i.v. every 3 weeks, (*b*) characterize the toxicities of this regimen, (*c*) describe the pharmacokinetic behaviors of oblimersen and docetaxel and relate these relevant pharmacokinetic and pharmacodynamic variables to clinical outcome, and (*d*) assess the effects of oblimersen on Bcl-2 protein expression in peripheral blood mononuclear cells (PBMC) collected pretreatment and post-treatment.

Patients and Methods

Patient selection. Patients with both histologic evidence of prostate cancer and clinical and radiological evidence of metastatic disease were eligible. Study entry criteria included two or more consecutive elevations in PSA values not <14 days apart in a state of surgical or chemical castration; 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 anti-androgens at least 4 weeks before study entry; adequate hematopoietic (hemoglobin ≥9 g/dL, absolute neutrophil count ≥1,500/μL, platelet count ≥100,000/μL), hepatic function (total bilirubin within institutional upper limit of normal, aspartate serum transferase, and alanine serum transferase <1.5 times upper limit of normal and alkaline phosphatase <2.5 times upper limit of normal), renal function (serum creatinine <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 may have received prior chemotherapy but not prior taxane therapy. Patients treated previously with strontium or samarium as well as those patients with only biochemical (PSA) evidence of disease without radiological confirmation of metastases were ineligible. The study was approved by the institutional review board at each institution and patients gave written informed consent for all clinical and research aspects of the study according to federal and institutional guidelines before treatment.

Drug administration. The doses of both oblimersen and docetaxel were those recommended in the previously reported phase I study, although the oblimersen infusion was extended from 5 to 7 days to allow for overlapping administration of the two agents (24). The patients in the current phase II study were uniformly treated with oblimersen given at a dose of 7 mg/kg/d given continuous i.v. infusion on days 1 to 8 in combination with docetaxel 75 mg/m^2 as a 1-hour i.v. infusion on day 6. All toxicities were graded according to the National Cancer Institute Common Toxicity Criteria version 2.

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.

Docetaxel was purchased commercially in 15-mL vials containing 2.36 mL of 40 mg/mL docetaxel for a total of 94.4 mg 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 seconds, further diluted in 250 mL of 5% dextrose solution or 0.9% saline, and infused i.v. over 1 hour.

Pretreatment and follow-up studies. A complete medical history, physical examination, and routine laboratory studies were done pretreatment and weekly. Routine laboratory studies included a complete blood count, differential white blood count, prothrombin and partial thromboplastin times, routine electrolytes and chemistries, 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 was repeated after every other course, whereas PSA was repeated every course. Patients could continue on study up to a maximum of 12 courses of the combination in the absence of progressive disease or intolerable toxicity. A PSA response was defined as a 50% decrease in PSA from pretreatment sustained for ≥1 course. For patients with measurable disease, WHO response criteria were used. Because the study was written before the Working Group criteria were published, progression was determined by investigator using PSA or measurable disease.

Quantification of Bcl-2 protein in peripheral blood mononuclear cells. Bcl-2 protein quantification was done on PBMCs isolated from blood specimens obtained before initiation of oblimersen treatment and again on day 6 before docetaxel. Western blot assays were used to quantify Bcl-2 protein and the methodology has been described previously (23). The percent change in Bcl-2 protein expression was determined using the following formula:

$$\frac{(\text{Pretreatment normalized Bcl–2 value} - \text{day 6 normalized Bcl–2 value}) \times 100}{\text{Pretreatment normalized Bcl–2 value}}$$

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 done for oblimersen pharmacokinetic studies pretreatment, at 2, 24, and 48 hours following the start of the infusion, immediately before the end of infusion, and at 24 and 48 hours post-treatment. To assess docetaxel pharmacokinetics, sampling was done before docetaxel on day 6 and at 1, 2, 4, 6, 12, 24, and 48 hours post-treatment. All blood samples were centrifuged at 1,200 × g for 15 minutes at 4°C immediately after collection and the plasma was stored at −20°C.

Oblimersen and docetaxel plasma concentrations were determined using high-performance liquid chromatography as described previously (23).

Pharmacokinetic and pharmacodynamic analyses. Individual oblimersen and docetaxel plasma concentration data sets were analyzed by standard noncompartmental methods. Mean steady-state oblimersen concentrations (C_{ss}) were the average of plasma concentrations at the 24-, 48-, and 120-hour time points. The clearance was calculated as follows: clearance = 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}). Docetaxel clearance was determined by dividing the dose given (mg) by the AUC. The apparent volume of distribution at steady-state was determined by the following relationships: Vd_{ss} = (Dose × AUMC / AUC^2) − (Dose × Duration of infusion) / (2 × AUC), where Vd_{ss} is the apparent volume of distribution at steady-state and AUMC is the area under the moment curve extrapolated to infinity.

The relationships between pertinent pharmacokinetic variables that reflected drug exposure (AUC and C_{ss}) for docetaxel and oblimersen and indices reflecting PSA decrement and the degree of first course myelosuppression (absolute neutrophil count) were explored. The percent decrement in the PSA and absolute neutrophil count were calculated as follows: 100% × [(Pretreatment count − Nadir count) / Pretreatment count]. The sigmoidal E_{max} model of drug action (i.e., PSA decrement = $E_{max} \times AUC_{0\gamma} / AUC_{50\gamma} + AUC_{\gamma}$) assessed the relationships between C_{ss} and change in PSA for the first course. The coefficient of determination (R^2) and the SEs for the estimated variables measured goodness of fit for the pharmacodynamics model. Variable values were expressed as means and SDs. Mean C_{ss} and $AUC_{0-\infty}$ values for those patients who had a 50% decrement in PSA versus with lesser decrements as well as patients who did and did not experience severe hematologic toxicity were compared using the Student's t test (two-sided).

Results

General. Twenty-eight patients, whose pertinent demographics are displayed in Table 1, received a total of 173 courses of oblimersen and docetaxel. Twenty-seven patients had PSA evaluable disease, 12 patients had bidimensionally measurable disease, and 11 had both. Five patients had received prior chemotherapy before study entry. The median age was 65 years (range, 44-82 years) and the median PSA at the onset of therapy was 133 ng/mL (range, 0.1-1,100 ng/mL). The median number of courses given per patient in the current study was 5 (range, 1-12) and the dose of docetaxel was reduced in 22 patients; 11 patients requiring a single docetaxel reduction to 60 mg/m^2 and another 11 patients having two dose reductions to 50 mg/m^2. Treatment delays due to toxicity occurred in 38 of 173 (22%) courses.

Table 1. Patient characteristics

Characteristics	No.
No. patients	28
Median no. courses/patient (range)	5 (1-12)
Median age (range)	65 (44-82)
Performance status	
0	8
1	17
2	3
Previous therapy	
Androgen-ablation therapy	28
Chemotherapy	5
Radiation therapy	25
No. patients evaluable for PSA	27
No. patients with measurable disease	12
Median PSA value at study entry (range)	133.8 (0.1-1,100)

Antitumor activity. Twenty-seven patients were evaluable for a PSA response and 12 patients had bidimensionally measurable disease evaluable for objective response. Fourteen of 27 patients had a PSA response for an overall response rate of 52%. Six patients (22%) had more than an 80% decrement in PSA from pretreatment levels. The median maximal PSA decrement from pretreatment values in the responding patient population was 71.1%, whereas in the 13 patients that did not meet the criteria for a PSA response 4 patients had no PSA decrement whatsoever and 9 patients had less than a 50% decrease or were discontinued due to toxicity before confirmatory PSA response assessment (range, −19.8% to −54.5%). Nine of 27 patients discontinued study drug while still experiencing a response due to completion of the mandated 12 courses (1 patient), patient choice (2 patients), or toxicity (6 patients). The median response duration for all 14 PSA responders was 3 months (range, 1-8.5+ months). In patients with measurable disease, 4 of 12 (33%) patients had evidence of a partial response. The median time to PSA progression for the entire group was 5.3 months (range, 0.7-9.1+ months). The median survival for all 28 patients was 595 days (19.8 months).

Pharmacokinetic and pharmacodynamic analyses. Twenty-eight patients had plasma sampling done to determine relevant oblimersen and docetaxel pharmacokinetic variables. Because the premise of this clinical study was that inhibition of Bcl-2 protein by oblimersen would enhance the antitumor activity of docetaxel, relationships between oblimersen pharmacokinetic variables that reflect drug exposure (C_{ss} and clearance) and PSA decrements were evaluated. Although substantial interpatient variability was observed, the mean ± SD oblimersen C_{ss} for all 28 patients was 5.56 ± 1.64 µg/mL. The mean C_{ss} of oblimersen was significantly greater in the patient population that had a PSA decrement equal to >50% compared with the patients that failed to meet this criteria. The mean plasma C_{ss} for oblimersen were 6.24 ± 1.68 versus 4.27 ± 1.11 µg/mL ($P = 0.008$) for patients that experienced a 50% decrement in PSA and those who did not, respectively. A scatter plot depicting individual mean steady-state oblimersen concentration values as a function of PSA decrement is

depicted in Fig. 1. The calculated clearance of oblimersen in patients was significantly greater in patients who failed to meet a 50% PSA decrement with a mean clearance value of 5.77 ± 0.32 versus 4.24 ± 0.31 L/h for nonresponders compared with responders, respectively ($P = 0.002$).

Because the optimal oblimersen C_{ss} is not currently known and the patient population treated in the current study had marked interpatient variability with a range of 3.2 to 9.7 µg/mL and a median of 5.2 µg/mL, a subset analysis of patients who had an oblimersen $C_{ss} \geq 5$ µg/mL was done. In the subset of patients who had $C_{ss} \geq 5$ µg/mL, 12 of 15 (80%) patients had a PSA decrement $\geq 50\%$, whereas in the subset of patients with mean oblimersen $C_{ss} < 5$ µg/mL the PSA decrement <50% was 3 of 12 (25%) patients.

Because both PSA fluctuations and C_{ss} are continuous variables, the influence of C_{ss} on the change in PSA after one course of oblimersen and docetaxel was examined. Figure 2 shows that an excess number of patients fail to have a decline in their PSA values during the first course at lower oblimersen C_{ss} compared with patients with higher C_{ss}. A sigmoidal drug concentration-effect equation seemed to adequately describe the relationship between the percent change in PSA and C_{ss} ($R^2 = 0.375$).

The effect of oblimersen C_{ss} on survival was also evaluated. Although there was a trend to improved median survival of patients with higher oblimersen C_{ss} (23 versus 19.8 months for patients with $C_{ss} \geq 5$ versus <5 µg/mL, respectively), the difference was not statistically significant ($P > 0.05$, log-rank test).

Because oblimersen is highly protein bound, degraded by ubiquitous plasma exonucleases, and the nucleotides undergo urinary excretion, the relationship of several variables, including total protein, albumin, α_1-acid glycoprotein, and renal function (calculated creatinine clearance) that reflected these processes on the clearance of oblimersen, was examined. No relationship between oblimersen clearance and these four variables could be determined (Fig. 3).

The pharmacokinetic variables for docetaxel were estimated in 25 of 28 patients. The apparent volume of distribution at steady state of docetaxel was large, averaging $2{,}574 \pm 1{,}548$ L, whereas the mean $\pm$ SD values that estimated drug exposure, C_{max}, $AUC_{0-\infty}$, and plasma clearance were 409 ± 784 ng/mL, 728 ± 905 ng/mL h, and 475 ± 339 L/h,
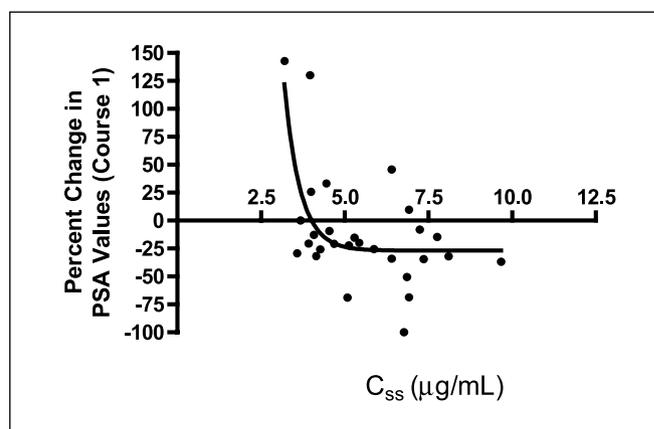

Fig. 2. Scatter plot of individual patients percent change in PSA from baseline after one course of treatment as a function of oblimersen C_{ss}. The curve represents the best fit for a sigmoidal dose-response equation ($R^2 = 0.375$).

respectively. The terminal elimination half-life was brief averaging 4.4 ± 2.5 hours. The mean clearance of docetaxel was not different for the two defined groups of patients with higher (≥ 5 µg/mL) or lower (<5 µg/mL) oblimersen C_{ss}.

Twenty-two of these 25 patients experienced severe neutropenia (grade 3 or 4) during course 1. There was no significant difference between the mean docetaxel $AUC_{0-\infty}$ for the patients with severe (grade 3 or 4) neutropenia compared with those with mild or modest (grade 1 or 2) toxicity ($AUC_{0-\infty}$, 483 ± 109 versus $1{,}044 \pm 791$ ng/mL h; $P = 0.16$). The mean docetaxel C_{max}, $AUC_{0-\infty}$, and clearance values were not different between patients who did and did not experience a PSA response.

Bcl-2 expression in peripheral blood mononuclear cells. All 28 patients had paired PBMC specimens collected for Bcl-2 protein quantification pretreatment on day 1 and again on day 6 immediately before docetaxel administration. There was considerable interpatient variability in Bcl-2 protein levels as well as the normalized values (Bcl-2: actin). Nineteen of 28 (69%) patients had net decrements in normalized Bcl-2 levels, whereas 2 patients had no change and 7 patients had increases in Bcl-2 expression. The median change Bcl-2 values for the entire population was 49.9% (range, −95% to +444%). The changes in normalized Bcl-2 values for each patient between days 1 and 6, as a percent, are depicted in Fig. 4.

The relationship between the change of PBMC Bcl-2 protein levels and oblimersen C_{ss} was examined. There was no correlation between the change in Bcl-2 protein level in PBMCs between days 1 and 6 and oblimersen C_{ss}. Moreover, there was no significant difference in oblimersen C_{ss} for the patients who had decrements in PBMC Bcl-2 protein levels compared with those patients who had increments (5.3 ± 1.4 versus 6.1 ± 2.0 µg/mL, respectively).

To evaluate whether the decrement in a patient's Bcl-2 protein levels in PBMCs correlated with antitumor activity, the percent changes in Bcl-2 values were evaluated in relation to PSA response, time to progression, and survival. There was no evidence that the decrement (or increase) in Bcl-2 value between days 1 and 6 predicted response to therapy, time to progression, or survival.

Toxicity. The distributions and the relevant grades of both nonhematologic and hematologic toxicities are listed in Table 2. Myelosuppression, particularly neutropenia, was the principal
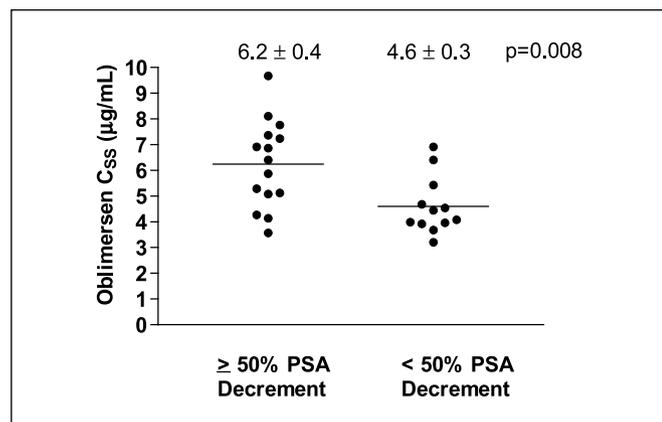

Fig. 1. Scatter plot depicting PSA decrement as a function of mean C_{ss}. Horizontal bars, mean C_{ss} values.

hematologic toxicity of the combination of oblimersen and docetaxel and led to dose reductions in 22 patients. Nineteen (68%) patients experienced grade 4 neutropenia at least once during treatment with this combination. Febrile neutropenia occurred in 4 patients during their first course of therapy and in a total of 7 patients during all courses. Due, in large part, to the hematologic toxicity encountered in this study, the median dose of docetaxel given to the study population was 60 mg/m^2. Only 1 patient had severe (grade 3) thrombocytopenia. 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 28 patients.

Nonhematologic toxicity. The most common nonhematologic toxicities were alopecia, fatigue, diarrhea, nausea, and vomiting. The frequencies of these toxicities over all courses are listed in Table 2.

The most common toxicity attributed to the oblimersen component of the regimen was modest (grade 1 or 2) pyrexia that generally began on day 2 or 3 of oblimersen treatment in the absence of manifestations suggesting infection. This pyrexia was successfully treated and prevented on subsequent courses with either acetaminophen or nonsteroidal anti-inflammatory agents. In addition, two patients experienced reversible grade 3 elevation of aspartate serum transferase during the first infusion
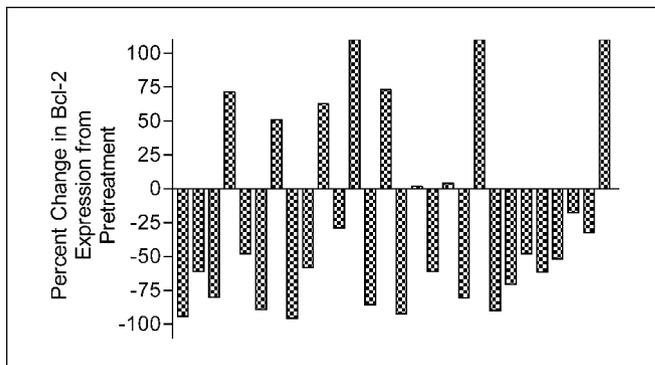

Fig. 4. Individual percent decrements and increments in Bcl-2 protein expression in PBMC at day 6 compared with pretreatment values.

that was attributed to oblimersen. Hypophosphatemia, which lacked clinical relevance, was common (28% of patients) and was attributed to oblimersen. Deep vein thrombosis was uncommon despite the presence of a central venous catheter and was observed in only 3 patients.

With the exception of alopecia, cumulative toxicities related to docetaxel were uncommon. Sensory neuropathy occurred in only 2 (7%) patients, whereas peripheral edema and
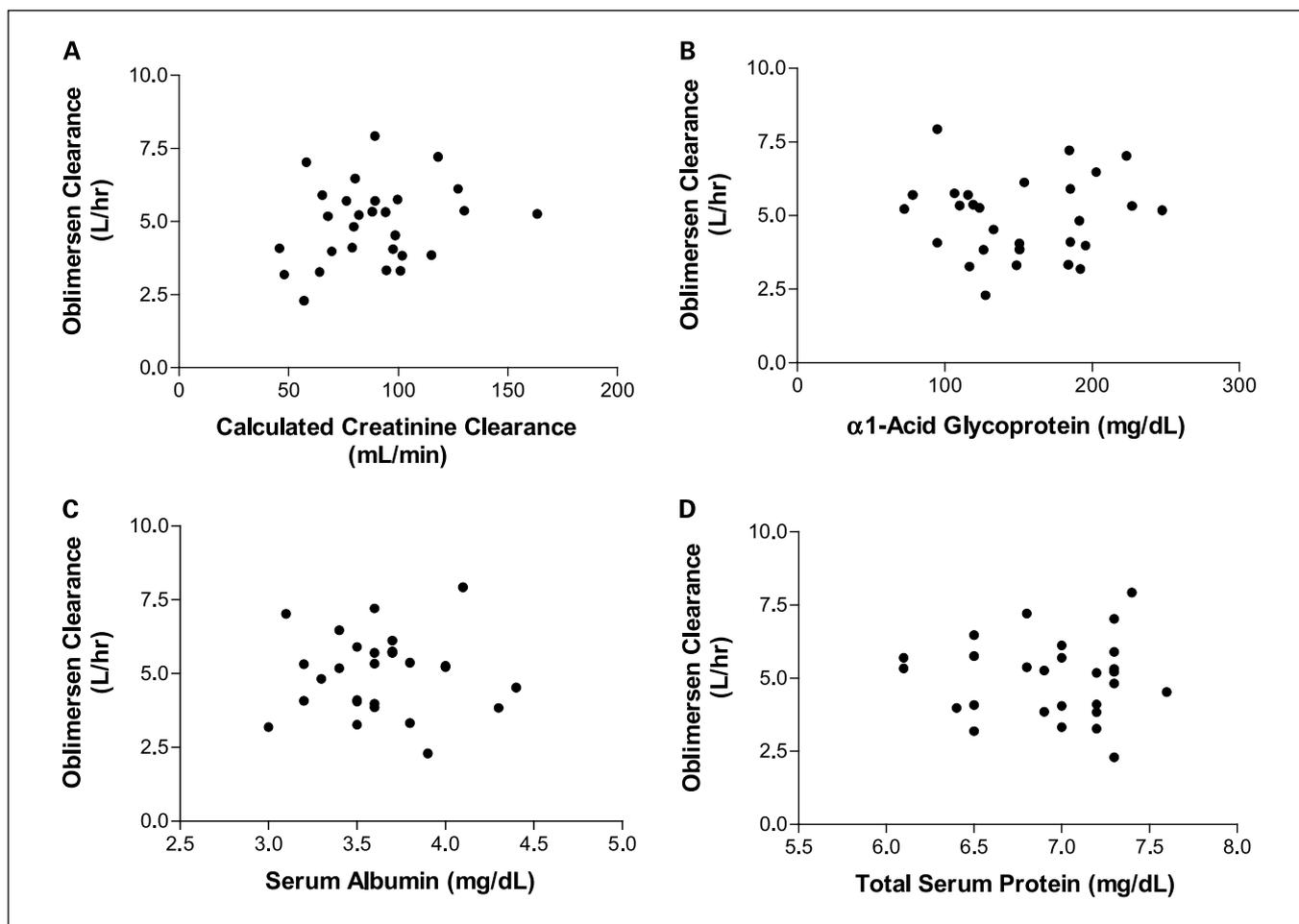

Fig. 3. Scatter plots of individual values of oblimersen clearance as a function of (*A*) calculated creatinine clearance, (*B*) α$_1$-acid glycoprotein, (*C*) serum albumin, and (*D*) total serum protein.

Table 2. Maximum selected nonhematologic and hematologic toxicities by grade ($n = 28$)

Toxicity	No. patients experiencing toxicity all courses (%)			
	Grade I	Grade II	Grade III	Grade IV
Alopecia	15 (53.6)	8 (28.6)		
Anorexia	12 (42.9)	6 (21.4)	1 (3.6)	
Aspartate serum transferase increase	1 (3.6)		2 (7.1)	
Creatinine increase	6 (21.4)	2 (7.1)	1 (3.6)	
Deep vein thrombosis	1 (3.6)	1 (3.6)	1 (3.6)	
Diarrhea	14 (50)	2 (7.1)	5 (17.9)	
Fatigue	6 (21.4)	10 (35.7)	5 (17.9)	
Febrile neutropenia		1 (3.6)	5 (17.9)	1 (3.6)
Headache	9 (32.1)			
Hypophosphatemia		2 (7.1)	6 (21.4)	
Hyperlacrimation	3 (10.7)			
Myalgia	12 (42.9)			
Limb edema	4 (14.3)	1 (3.6)		
Pyrexia	17 (60.7)	4 (14.7)		
Sensory neuropathy	2 (7.1)			
Stomatitis	3 (10.7)	1 (3.6)		
Vomiting	10 (35.7)			
Nausea	9 (32.1)	7 (25)	1 (3.6)	
Anemia	3 (10.7)	2 (7.1)	1 (3.6)	
Neutropenia			7 (25)	19 (67.9)
Thrombocytopenia			1 (3.6)	

hyperlacrimation was observed in 5 (18%) and 3 (11%) patients, respectively.

Because oblimersen C_{ss} seems to be a determinant of the effectiveness of this combination, oblimersen could also concomitantly affect the overall toxicity experienced and influence the dose of docetaxel given. To evaluate this, the mean docetaxel dose was calculated for the two previously identified patient oblimersen groups. The mean docetaxel dose delivered was significantly greater at 66.5 versus 60.6 mg/m^2 ($P < 0.0001$) for patients that had oblimersen C_{ss} <5 versus ≥5 µg/mL, respectively.

Discussion

The number of active chemotherapeutic agents, as well as their overall effect on the treatment of HRPC, continues to be modest. Patients treated with mitoxantrone and corticosteroids derive only symptomatic palliative benefit without perturbing the natural history of the disease, whereas docetaxel, albeit more active, has only a modest (2-month) survival benefit (25–27). Evidence that the transition to HRPC is accompanied both by increased Bcl-2 protein expression and by evidence that increased Bcl-2 expression diminishes the effectiveness of a many chemotherapeutic agents implicates Bcl-2 protein an attractive target for inhibition. To this end, the current study examined the antitumor activity of the combination of the Bcl-2 inhibitory antisense oligonucleotide oblimersen with docetaxel in patients with HRPC.

The overall PSA response rate of 52%, an objective response rate of 33%, and median survival of 19.8 months for this combination therapy are comparable with the results of single-agent studies for docetaxel in the treatment of HRPC (26, 27).

However, in the current study, the observation that the C_{ss} of oblimersen is a significant determinant of PSA response is intriguing. Only 15 of 28 patients had oblimersen C_{ss} that equaled or exceeded 5 µg/mL, yet ultimately 12 of these 15 (80%) patients had a PSA response compared with 3 of 12 (25%) patients with oblimersen C_{ss} <5 µg/mL. Although the patients were uniformly treated with oblimersen at the 7 mg/kg/d dose level, the marked interpatient variability of oblimersen clearance resulted in C_{ss} values that ranged from 3.2 to 9.7 µg/mL. Taken together, these results indicate that the recommended oblimersen dose for clinical studies in HRPC patients (7 mg/kg/d for 7 days) may not yield an optimal C_{ss} for a significant proportion of patients, and this may ultimately adversely affect efficacy.

The mechanism of antisense oligonucleotide action, a stoichiometric process involving antisense hybridization and degradation of Bcl-2 mRNA, diminished Bcl-2 protein synthesis, and failure to replenish Bcl-2 protein that undergoes metabolic degradation is both time and concentration dependent. Published studies indicate that the cellular uptake of oblimersen by myeloma cells *ex vivo*, as well as Bcl-2 mRNA and protein inhibition, is a function of both concentration and duration of exposure (28). Therefore, the plasma concentration, the duration of oblimersen infusion, and the magnitude of the biological target (the expression of Bcl-2 protein is widely diverse among different malignancies and within tumors) will all affect the effectiveness of oblimersen. Although in the current study greater C_{ss} yielded improved antitumor effectiveness, this may not be universally applied to other tumors. Certainly, lower doses of oblimersen (2-3 mg/kg/d) have induced single-agent tumor responses in patients with chronic lymphocytic leukemia and follicular non-Hodgkin's lymphomas (29). However, in

these two diseases, Bcl-2 expression may have a fundamental role in the early neoplastic transformation of these indolent malignancies that may render them more sensitive to oblimersen, and there may also be a significant pharmacologic advantage in these blood-borne malignancies, where oblimersen plasma C_{ss} more closely reflects cellular exposure oblimersen, to achieve optimal concentrations compared with solid tumors (30–32).

Several strategies may be employed in the attempt to clinically optimize the exposure of HRPC tumor cells to oblimersen. Single-agent phase I studies of weekly docetaxel and oblimersen in solid tumor patients did not define a maximum tolerated dose using the shorter (5- or 7-day) schedules, whereas higher doses of oblimersen (9 mg/kg/d) could feasibly and safely be given for 5 days every 3 weeks. Because the central hypothesis to the current clinical study is that Bcl-2 protein confers resistance to docetaxel-mediated apoptosis and because oblimersen can markedly enhance even suboptimally given docetaxel in preclinical models, the exploration of higher and perhaps optimal doses of oblimersen combined with 60 mg/m^2 docetaxel, a dose both feasibly and repetitively given in this study, may be a rational next step in the development of this regimen. Alternatively, the identification of variables that may predict for increased individual oblimersen clearance may permit individualized dosing for those patients who will fail to achieve a target C_{ss}.

In the majority of paired patient PBMC samples, Bcl-2 protein expression was decreased following treatment with oblimersen, thereby supporting the principal mechanism of action for oblimersen. However, decrements did not occur in all individuals and there was marked interpatient variability in the magnitude of Bcl-2 inhibition. The ability to detect Bcl-2 protein decrements in PBMC as a measure of drug effect has been inconsistent in several clinical studies of oblimersen, which may relate to the diverse analytic methodologies used (29, 33). In the current study, Bcl-2 protein content in PBMC was quantified using Western blot analysis, which has been more robust than earlier attempts to use flow cytometry (33). Bcl-2 protein is an intracellular protein; therefore, sample preparation, cellular membrane solubilization, and penetration of fluorescent-labeled antibody are important for the performance of Bcl-2 quantification by the flow cytometry. Nonetheless, despite the use of Western blot analysis in the current study, the magnitude of Bcl-2 reduction in PBMC was not a surrogate end point that correlated with either oblimersen C_{ss} or PSA response and should not yet be considered a validated biological end point to quantify inhibition of Bcl-2 protein by oblimersen.

The combination of oblimersen and docetaxel could be given over multiple courses without evidence of cumulative toxicity. However, hematologic toxicity ultimately led to at least a single dose reduction in 22 of 28 (79%) patients at sometime during their treatment, resulting in a median docetaxel dose of 60 mg/m^2. The high rate of dose reduction in the current study contrasts with the results of the prior phase I study of this regimen, which used a shorter oblimersen infusion schedule of 5 days (days 1-6) with docetaxel delivered at the end of the infusion on day 6 (23). The selection of the prolonged and overlapping schedule of oblimersen was based on preclinical evidence of improved antitumor activity with a prolonged and overlapping schedule (24). This modification may have contributed, at least in part, to the increased hematologic toxicity observed. Yet, despite both the frequent dose reductions and the lower median dose of docetaxel (60 mg/m^2) given, the PSA response rate observed in the entire HRPC population is encouraging at 52% and in the subset population with oblimersen $C_{ss} \geq 5$ μg/mL of 80%, despite a low mean docetaxel dose of 60.6 mg/m^2. A relevant but unanswered question from the current study is whether the subset of patients with suboptimal oblimersen C_{ss} required docetaxel dose reductions due to toxicity from the combination (mean docetaxel dose of 66.5 mg/m^2) that ultimately led to an inferior PSA response rate (25%) due to suboptimal docetaxel dosing.

In addition to quantifying antitumor activity, phase II studies can incorporate pharmacodynamic evaluations to optimize the dose and schedule and to define optimal patient populations for future evaluations of targeted therapies. Based on the results of the current study, oblimersen combined with docetaxel is both an active and a feasible combination in patients with HRPC. To accurately determine whether Bcl-2 inhibition by oblimersen will significantly enhance the effectiveness of docetaxel, comparative studies will need to be done. However, before the initiation of these comparative studies, the value of oblimersen C_{ss} and the effect this has on the effectiveness of this regimen as identified in the current study and the optimal dose and schedule of oblimersen and docetaxel used should be confirmed.

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