

A Phase I Dose-finding Study of Combined Treatment with an Antisense Bcl-2 Oligonucleotide (Genasense) and Mitoxantrone in Patients with Metastatic Hormone-refractory Prostate Cancer¹

Kim N. Chi,² Martin E. Gleave, Richard Klasa, Nevin Murray, Cicely Bryce, Daniel E. Lopes de Menezes, Susan D'Aloisio, and Anthony W. Tolcher

Vancouver Cancer Centre, British Columbia Cancer Agency, Vancouver, British Columbia, V5Z 4E6 Canada [K. N. C., M. E. G., R. K., N. M., C. B., D. E. L. d. M., S. D.]; The Prostate Centre, Vancouver General Hospital, Vancouver, British Columbia, V6H 3Z6 Canada [K. N. C., M. E. G.]; and Institute for Drug Development, Cancer Therapy and Research Center, San Antonio, Texas 78229 [A. W. T.]

ABSTRACT

Purpose: Bcl-2 is a negative prognostic indicator in prostate cancer, implicated in the development of androgen independence and treatment resistance, and is overexpressed in hormone-refractory prostate cancer (HRPC). Genasense is a phosphorothioate antisense oligonucleotide complementary to the *bcl-2* mRNA open reading frame that in preclinical studies has shown significant activity in inhibiting expression of Bcl-2, delaying androgen independence, and improving chemosensitivity in prostate and other cancer models. In this dose escalation study, we evaluated the combination of Genasense and mitoxantrone, a standard chemotherapy for patients with HRPC.

Design: Twenty-six patients with HRPC were treated at seven dose levels receiving Genasense at a dose ranging from 0.6 to 5.0 mg/kg/day and mitoxantrone from 4 mg/m² to 12 mg/m². Genasense was administered as a 14-day i.v. continuous infusion every 28 days with mitoxantrone given as an i.v. bolus on day 8.

Results: No dose-limiting toxicities were observed. Hematological toxicities were transient and included neutropenia, thrombocytopenia, and lymphopenia. Nonhematological toxicities included fatigue, fever, nausea, arthralgias, myalgias, and transient elevations in serum creatinine, none of which were severe. Two patients had >50% reductions in

prostate-specific antigen. One patient, who received six cycles of Genasense at 1.2 mg/kg/day and a low dose (4 mg/m²) of mitoxantrone, also had symptomatic improvement in bone pain. Peripheral blood lymphocyte Bcl-2 protein expression decreased in five of five patients given Genasense at 5mg/kg/day (mean change from baseline, -12.8%; SD, 16.4%) as assessed by flow cytometry. Serum concentrations of Genasense given at doses of 3 mg/kg/day and greater, exceeded 1 µg/ml.

Conclusions: Genasense and mitoxantrone are well tolerated in combination, and mitoxantrone can be delivered at a standard dose with biologically active doses of Genasense without significant additional toxicity. This observation allays concerns about trials that combine Genasense with full doses of other cytotoxic agents seeking greater evidence of activity.

INTRODUCTION

Prostate cancer is the most common cancer diagnosis and the second most common cause of cancer death in men in North America (1). Initially, prostate cancer responds very well to castration therapy; however, this response is usually brief, lasting ~18 months in the metastatic setting (2). A rising PSA³, and recurrence of symptoms herald the development of androgen independence. At this point, standard treatment options are limited, consisting of palliative radiotherapy or chemotherapy (3). Mitoxantrone has been approved by the United States Food and Drug Administration (FDA) for use in HRPC based on two randomized controlled trials demonstrating an improved palliative response rate as compared with corticosteroids alone (4, 5). The objective and PSA response rates with mitoxantrone were low, and there was no survival benefit seen with chemotherapy (4, 5), emphasizing the need for more effective treatment for this disease.

The *bcl-2* gene is the prototype of a novel class of oncogenes that contributes to neoplastic progression by enhancing tumor cell survival through the inhibition of apoptosis (6). Bcl-2 belongs to a growing family of apoptosis-regulatory gene products that act as either death antagonists or death agonists. The selective and competitive dimerization between pairs of these antagonists and agonists determines how a cell responds to an apoptotic signal (7) such as with chemotherapy. Furthermore,

Received 7/6/01; revised 9/18/01; accepted 9/19/01.

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¹ Supported in part by Genta Incorporated.

² To whom requests for reprints should be addressed, at Vancouver Cancer Centre, British Columbia Cancer Agency, 600 West 10th Avenue, Vancouver, Canada, V5Z 4E6. Phone: (604) 877-6000; Fax: (604) 877-0585; E-mail: kchi@bccancer.bc.ca.

³ The abbreviations used are: PSA, prostate-specific antigen; HRPC, hormone-refractory prostate cancer; ECOG, Eastern Cooperative Oncology Group; MTD, maximum tolerated dose; CIVI, continuous i.v. infusion; DLT, dose-limiting toxicity; AUC, area under the curve.

several lines of evidence have implicated overexpression of Bcl-2 with treatment resistance (8, 9). In prostate cancer, Bcl-2 has been found to be overexpressed in the majority of clinical samples of androgen-independent disease (10, 11), and experimental and clinical studies report that increased expression of Bcl-2 confers, or is associated with, the development of androgen independence and treatment resistance (12–15). Thus, Bcl-2 is an attractive target to improve the efficacy of treatment of patients with prostate cancer by enhancing chemotherapy-induced apoptosis.

Genasense (Genta Inc., Berkeley Heights, NJ) is an 18-mer phosphorothioate antisense oligonucleotide complementary to the first six codons of the initiating sequence of the human *bcl-2* mRNA. Antisense oligonucleotides are chemically modified strands of DNA that form RNA-DNA duplexes by Watson-Crick binding, resulting in RNase H-mediated cleavage of the target mRNA, thereby inhibiting gene expression (16). In pre-clinical prostate cancer models, Genasense and *bcl-2* antisense oligonucleotides have shown significant activity in inhibiting expression of Bcl-2, delaying time to the development of androgen independence, and enhancing the effects of chemotherapy by increased apoptosis (17–21). A Phase I study of single-agent Genasense in patients with lymphoma reported dose-limiting toxicities of thrombocytopenia, hypotension, fever, and asthenia with a MTD of 147.2 mg/m²/day (~4.1 mg/kg/day) given for 14 days by a continuous s.c. infusion (22). A Phase I/II trial of Genasense in combination with dacarbazine in patients with melanoma has shown promising activity and a Phase III multicenter trial is under way.

In this study, we sought to evaluate the combination of Genasense and mitoxantrone in patients with HRPC to define quantitatively and qualitatively the toxicities, MTD, and pharmacokinetic and biological effect, and to determine preliminary antitumor activity. Genasense was given as a 14-day CIVI with mitoxantrone delivered on day 8 to theoretically allow sufficient time for Bcl-2 expression to decrease. Dose escalation of both mitoxantrone and Genasense was performed, based on the risk of possible additive or synergistic toxicity from Bcl-2 inhibition in normal tissues.

PATIENTS AND METHODS

Patients with histologically confirmed evidence of prostate cancer and documented evidence of progression while receiving androgen ablation therapy were eligible for this study. All of the patients were required to have discontinued peripheral antiandrogens for at least 6 weeks prior to study entry. Patients must have had a PSA \geq 20 that was rising after discontinuation of peripheral antiandrogen, defined as at least two consecutive increases not less than 2 weeks apart. The most recent PSA assessment must have been done \leq 14 days before study entry. Therapies with leuteinizing hormone-releasing hormone agonists were to be continued for the duration of study treatment in those patients not surgically castrated. Patients were required to have radiological or clinical evidence of metastatic disease documented within 14 days before enrollment. Eligibility criteria also included ECOG performance status of 0 or 1; life expectancy of 12 or more weeks; and age \geq 18 years. Prior chemotherapy or strontium was not permitted. External beam

radiation was permitted if a minimum of 4 weeks had elapsed since the last dose before enrollment to the trial. Adequate hematopoietic (absolute granulocytes count, $\geq 1.5 \times 10^9$ /liter and a platelet count, $\geq 100 \times 10^9$ /liter), hepatic (bilirubin, $\leq 2 \times$ upper limits of normal) and renal (serum creatinine, $\leq 2 \times$ upper normal limit) function and normal coagulation parameters (partial thromboplastin time, international normalized ratio) were also required. Patients with a known bleeding disorder, on anticoagulant therapy, or with significant cardiac dysfunction were excluded. All of the patients gave written informed consent, and the protocol was approved by the institutional review board of the University of British Columbia.

This trial was designed as an open-label, nonrandomized, Phase I dose-finding study of Genasense given as a CIVI on days 1 through 14, in combination with mitoxantrone given on day 8 in a 28-day treatment cycle. The MTD of Genasense administered s.c. in advanced lymphoma patients was ~4 mg/kg/day for 14 days, which resulted in blood levels in the range of 0.96–6.67 μ g/ml. Because experimental animal data had indicated a 60% bioavailability of Genasense for s.c. versus i.v. administration, the MTD of 4 mg/kg/day by a s.c. route would extrapolate to an MTD of 2.4 mg/kg/day when administered i.v. The starting dose of Genasense, therefore, was 25% of the predicted MTD, or 0.6 mg/kg/day. The starting dose of mitoxantrone was 4 mg/m², one-third the standard dose used in HRPC. Mitoxantrone was given i.v. on day 8. One patient was to be treated at each dose level until National Cancer Institute of Canada common toxicity criteria (NCIC CTC) toxicity ≥ 2 (except alopecia) was observed, at which point, a minimum of three patients would be entered to additional dose levels. DLT was defined as any NCIC CTC grade 3 or 4 nonhematological toxicity (except nausea or vomiting), or hematological toxicity defined as any grade 4 thrombocytopenia, or neutropenia defined as grade 4 toxicity lasting for >5 days duration, or febrile neutropenia. DLT was defined on the first cycle only. If one patient at any level experienced DLT, additional patients were entered at that level to a maximum of six patients. If two patients experienced DLT, then accrual would stop at that level, and the next lower dose level would be declared the MTD. Additional patients would be entered at the MTD level to reach six patients if necessary to further characterize the toxicities at that level.

Genasense was supplied by Genta Incorporated (Berkeley Heights, NJ) and provided as a sterile concentrated solution containing the drug substance in sterile isotonic normal saline (pH 5.5–8). Each vial contained 300 mg of Genasense in 10 ml of solution for a concentration of 30 mg/ml. Infusion pump cassettes were filled with the total dose of Genasense concentrate to be delivered over 7 days diluted with sterile normal saline (0.9% Sodium Chloride Injection USP) delivered via a portable volumetric infusion pump through a central venous catheter.

Pretreatment investigations included history and physical exams, complete blood count, differential WBC count, partial thromboplastin time, INR, bilirubin, serum creatinine, total protein, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, PSA, and testosterone. Baseline studies also included an assessment of left ventricular function, bone scan, chest X-ray, and computed tomography scan of abdomen and pelvis. During protocol therapy, histories that included

Table 1 Baseline patient characteristics

Characteristic	
No. of patients	26
Median age, yr (range)	69 (52–80)
ECOG PS ^a	
0	10
1	16
Median PSA $\mu\text{g/liter}$ (range)	135 (30–860)
Median ALP ^b (range)	139 (68–1388)
Median hemoglobin g/liter (range)	129 (71–148)
Site of metastases	
Bone	23
Lymph nodes	8

^a ECOG PS, ECOG performance status.

^b ALP, alkaline phosphatase (normal range, 40–160 units/liter).

performance status and physical exams were conducted weekly. Hematology (CBC, WBC differential, platelets) was performed twice weekly for two cycles then on day 1 and 8 of subsequent cycles. Biochemistry (bilirubin, creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase) and PSA were performed on day 1 of each cycle. Imaging studies and left ventricular function were assessed every three cycles. Standard WHO criteria were used to assess measurable disease response (23). PSA response was defined as a greater than 50% decrease from baseline maintained for at least 4 weeks. Treatment failure was defined as new or worsening disease symptoms requiring change in management, fall in ECOG performance status by 2 levels, new or objective progressive disease, and/or continued rise in PSA for 12 weeks in asymptomatic patients.

Lymphocytes from peripheral blood were collected from patients enrolled on dose levels 6 and 7 on days 1 (pretreatment), 5, and 8 (premitoxantrone). Bcl-2 protein level was assessed using quantitative fluorescence cytometry as described previously (24). This method correlates well with Western blot analysis of Bcl-2 and allows comparisons of values expressed as molecules of equivalent soluble fluorochrome.

Plasma samples from patients on dose levels 6 and 7 (mitoxantrone dose of 12 mg/m²) were collected for Genasense and mitoxantrone pharmacokinetic studies. For Genasense, plasma samples were obtained on days 1 (predose, 1 h, 2 h, 4 h, 6 h), 2, 3, 4, 8, and 15. Plasma concentrations of Genasense were assayed by anion exchange high-performance liquid chromatography as described previously (25). Genasense steady state concentration (C_{ss}) in patient samples was determined from the average Genasense plasma concentration achieved at steady state after continuous drug infusion. For mitoxantrone, plasma samples were obtained on day 8 before the infusion, then after the infusion at 0.5, 1, 1.5, 2, 3, 6, 8, 24, and 48 h. Mitoxantrone was extracted from 0.10 ml of patient serum and spiked serum standards with 0.1% ammonium hydroxide in methanol using Oasis HLB Extraction Cartridges (Waters Ltd., Milford, MA), dried, and resuspended in an equal volume of mobile phase. Quantitation was carried out by high-performance liquid chromatography using an Alliance 2690 system with a PhotoDiode Array detector, extracted wavelength 658 nm, and a Millennium Chromatography Workstation (all Waters Ltd., Milford, MA). The column was a Symmetry C18, 3.9 mm \times 150

Table 2 Treatments administered: summary of dose escalation scheme, number of patients treated at each dose level, and completed courses

Dose level	Genasense mg/kg/day (day 1–14)	Mitoxantrone mg/m ² (day 8)	No. of patients	Median no. of courses (total)
1	0.6	4	3	3 (9)
2	1.2	4	3	3 (11)
3	2.0	4	3	2 (7)
4	3.1	4	3	1 (4)
5	3.1	8	4	3 (12)
6	3.1	12	4	2.5 (9)
7	5.0	12	6	3 (25)

mm (Waters) and the mobile phase was isocratic aqueous 18 mm formate buffer (pH 2.7) containing 34 mm hexane sulfonic acid (71%) and acetonitrile (29%) with a flow rate of 1 ml/min and injection volume of 0.05 ml. System suitability was checked by resolution of mitoxantrone from USP mitoxantrone-related compound. The assay was found to be reproducible and precise with a linear range of 1–1000 ng/ml. The mitoxantrone plasma concentration versus time data sets were fitted to two-compartment model using WinNONLIN version 1.5 pharmacokinetic software (Pharsight Corp., Mountainview, CA). Pharmacokinetic parameters, *i.e.*, AUC, peak plasma concentration (C_p), volume of distribution (V), plasma clearance (Cl) and half life ($t_{1/2}$) were calculated from model parameters using standard pharmacokinetic equations.

Student's *t* test was used to measure statistical significance between means of two treatment groups. Statistical significance was set at $P < 0.05$. Survival was calculated from the time of first treatment until death from any cause or last follow-up visit. Time-to-event analysis was done by the Kaplan-Meier method. Statistical analysis was performed using SPSS v9.0 for Windows.

RESULTS

Baseline Characteristics. Twenty-six patients were enrolled in the study from December 1998 to February 2000. The median age was 69 years (range, 52–80 years). Ten patients had an ECOG performance status of 0 and 16 had a performance status of 1. Baseline median PSA was 135 $\mu\text{g/liter}$ (range, 30–860 $\mu\text{g/liter}$). Alkaline phosphatase was elevated in nine patients (median, 139 units/liter; range, 68–1388 units/liter; institutional upper limit of normal, 160 units/liter). The median hemoglobin was 129 g/liter (range, 71–148 g/liter). The majority of patients (18 patients) had bone-only disease, 5 patients had bone and lymph node metastases, and 3 patients had lymph node metastases only. Ten patients had had previous palliative radiotherapy for symptomatic bony metastases. The relevant patient demographics are summarized in Table 1.

Treatments Administered. Twenty-five patients completed at least 1 cycle of therapy, receiving a total of 77 cycles. Patients were treated with Genasense at doses ranging from 0.6 mg/kg/d to 5.0 mg/kg/d (twice the expected MTD) and mitoxantrone at doses ranging from 4 mg/m² to 12 mg/m² (standard dose). The dose escalation scheme, numbers of patients treated at each dose level, and numbers of cycles delivered are listed in

Table 3 Hematological toxicity: number of patients with hematological adverse events during treatment with Genasense and mitoxantrone

Dose level	Genasense (mg/kg/d)	Mitoxantrone (mg/m ²)	No. of patients (courses)	No. of patients with toxicity grade first course (all courses)					
				Neutrophils		Platelets		Lymphocytes	
				1 or 2	3 or 4	1 or 2	3 or 4	1 or 2	3
1	0.6	4	3 (9)	1 (2)	0	1 (1)	0	3 (4)	1 (3)
2	1.2	4	3 (11)	1 (3)	0	1 (1)	0	3 (10)	1 (1)
3	2.0	4	3 (7)	1 (1)	0	0	0	2 (5)	0
4	3.1	4	3 (4)	1 (1)	0	1 (1)	0	2 (2)	0
5	3.1	8	4 (12)	3 (6)	1 (5)	1 (1)	0	1 (3)	1 (1)
6	3.1	12	4 (9)	2 (2)	4 (5)	2 (4)	0	1 (6)	3 (4)
7	5.0	12	6 (25)	4 (15)	2 (4)	0 (4)	0	3 (15)	4 (7)

Table 2. The median number of cycles administered was three (range, 1–8). Six patients were entered at the last planned dose level.

Hematological Toxicity. Dose-limiting hematological toxicity was not observed at any of the dose levels evaluated. First-cycle hematological toxicity is summarized in Table 3. Thrombocytopenia or neutropenia was not observed in any patient during the first 7 days of Genasense infusion on cycle 1. Patient 1 developed grade 2 neutropenia with a nadir by day 22 (14 days after mitoxantrone at 4 mg/m²), and recurred but did not worsen with subsequent cycles. Grade 3 or 4 neutropenia occurred by day 15–22 in four of four patients and two of six patients at dose levels 6 and 7, respectively. Thrombocytopenia greater than grade 1 was not seen in any patient during the first cycle of treatment. Patient 23 developed grade 2 thrombocytopenia (64×10^9 /liter) on cycle 9 but was coincident with a documented central venous catheter infection and bacteremia. Lymphopenia was observed at all of the dose levels, which worsened by grade as dose increased. Decreases in lymphocytes were noted to occur within the 1st week, with a nadir by days 10–15. Grade 3 lymphopenia occurred in three of four patients and four of six patients on dose levels 6 and 7, respectively, but was not clinically significant in any patient.

Nonhematological Toxicity. Nonhematological toxicity was mild or moderate only, and not dose-limiting (Table 4). First cycle grade 2 toxicities included elevations in creatinine in one patient each at dose levels 6 and 7. Both resolved and the patient on dose level 7 received a second cycle without incident. Grade 1 and 2 fevers were also observed at dose level 7 after initiation of Genasense but were transient and resolved within 24 h despite continuation of the infusion. Grade 1 arthralgias and myalgias during the Genasense infusion occurred in patients at several dose levels and did not appear dose related. Three patients had asymptomatic declines of 10–20% in cardiac left ventricular ejection fraction after three cycles, and in one patient after six cycles; however, all of the patients' ejection fractions remained greater than 50%. Serious adverse events related to protocol therapy included a catheter-related thrombosis and a catheter-related bacteremia.

Antitumor Activity. Two patients had >50% reductions in PSA lasting >4 weeks (Fig. 1). One patient received Genasense at 1.2 mg/kg/day and a low dose of mitoxantrone (4 mg/m²) and also had symptomatic improvement in bone pain, receiving a total of six cycles before symptomatic and biochemical progression. The other patient received Genasense at 5

Table 4 Nonhematological toxicity: summary of nonhematological adverse events during treatment with Genasense and mitoxantrone

Adverse event	No. of patients with toxicity grade first course (all courses)			
	1	2	3	4
Fatigue	13 (35)	0 (1)	0	0
Nausea	7 (21)	0 (1)	0	0
Anorexia	5 (15)	1 (2)	0	0
Elevated creatinine	2 (8)	2 (2)	0	0
Fever	2 (3)	1 (1)	0	0
Taste change	4 (17)	0	0	0
Arthralgia/Myalgia	4 (8)	0	0	0
Elevated ALP ^a	2 (2)	0	0	0
Cardiac dysfunction	0 (4)	0	0	0
Weight loss	1 (2)	0	0	0
Headache	1 (1)	0	0	0
Alopecia	1 (1)	0	0	0
Vomiting	0	1 (1)	0	0
Diarrhea	0	0 (1)	0	0
Dry mouth	0 (5)	0	0	0

^a ALP, alkaline phosphatase.

mg/kg/day and mitoxantrone at 12 mg/m² and received a total of eight complete cycles before discontinuing therapy because of catheter-related bacteremia. One patient had a PSA reduction of 25–50%, and five others had stable biochemical disease. Seven patients had measurable disease, and, of these, one had a partial response. Kaplan-Meier estimate of median overall survival was 18.7 months (95% confidence interval, 10.85–26.51).

Flow Cytometry Analysis. Bcl-2 protein levels in lymphocytes from peripheral blood were assessed in patients enrolled on dose levels 6 and 7. By day 8 (prior to mitoxantrone infusion), Bcl-2 protein decreased in one of four patients on dose level 6 and in five of five patients at dose levels 7 (Fig. 2). The mean percentage change from baseline (\pm SD) of Bcl-2 protein at dose level 6 and 7 were $+11.6 \pm 24.4\%$ and $-12.8 \pm 16.4\%$, respectively ($P = 0.11$).

Pharmacokinetic Studies. Plasma samples from patients on dose levels 6 and 7 (mitoxantrone dose of 12 mg/m²) were collected for Genasense and mitoxantrone pharmacokinetic studies. Genasense serum concentrations at dose levels 6 and 7 exceeded the targeted biologically relevant plasma levels of 1 μ g/ml by 24 h after the start of the infusion. Mean C_{ss} (\pm SD) for dose level 6 (Genasense, 3 mg/kg/day) were 2.82 ± 0.66 μ g/ml and for dose level 7 (5 mg/kg/day) were 4.29 ± 0.52

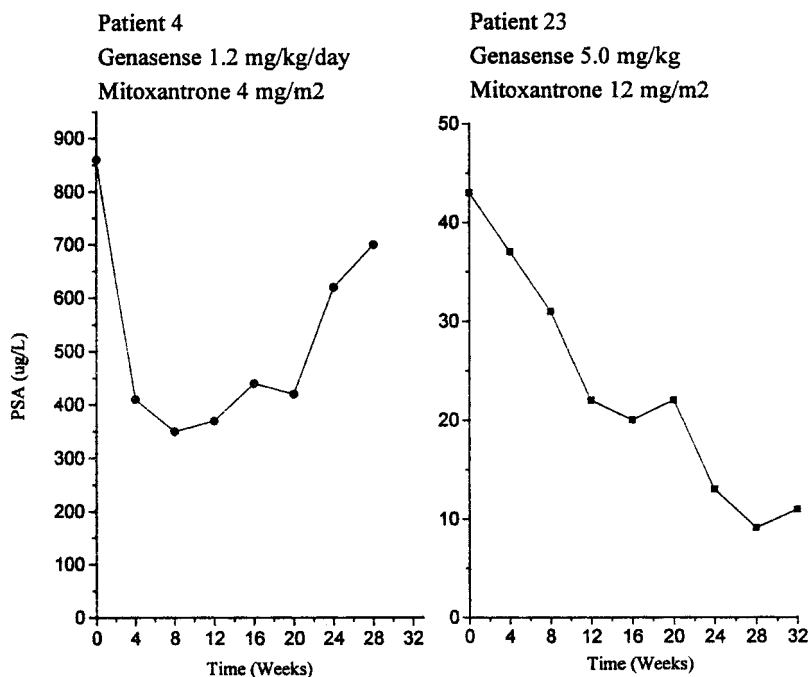


Fig. 1 PSA responses.

$\mu\text{g/ml}$. Mitoxantrone pharmacokinetic parameters at dose levels 6 and 7 are presented in Table 5. Mean AUC at dose levels 6 and 7 were $328.9 \pm 52.6 \text{ ng}\cdot\text{h/ml}$ and $579.6 \pm 172.7 \text{ ng}\cdot\text{h/ml}$, respectively ($P = 0.008$). At dose levels 6 and 7, mean C_p were $77.8 \pm 11.0 \text{ liters/h}$ and $38.4 \pm 11.51 \text{ liters/h}$, respectively ($P = 0.0003$).

DISCUSSION

This is the first study evaluating the use of Genasense, an antisense oligonucleotide complementary to *bcl-2* mRNA, in combination with chemotherapy in patients with metastatic HRPC. Our results demonstrate that Genasense is well tolerated at doses of up to 5 mg/kg/day by CIVI for 14 days in combination with mitoxantrone chemotherapy at a standard dose of 12 mg/m² in this group of patients. No DLTs were seen at these doses, and patients on this trial were able to tolerate plasma concentrations of Genasense exceeding those achieved at MTD in a previously reported Phase I study (22). Furthermore, standard doses of mitoxantrone could be given to patients in whom Genasense plasma concentrations were above the biologically targeted concentration of 1 $\mu\text{g/ml}$ (22, 26) with no significant excess toxicity being observed over and above what would be expected from mitoxantrone alone except for lymphopenia. There was no evidence to suggest cumulative toxicity with several patients receiving more than three cycles of therapy. Other trials evaluating combined therapy of Genasense with a cytotoxic agent have also reported good tolerability and MTDs that exceeded those in the initially reported Phase I trial of single-agent Genasense (27, 28).

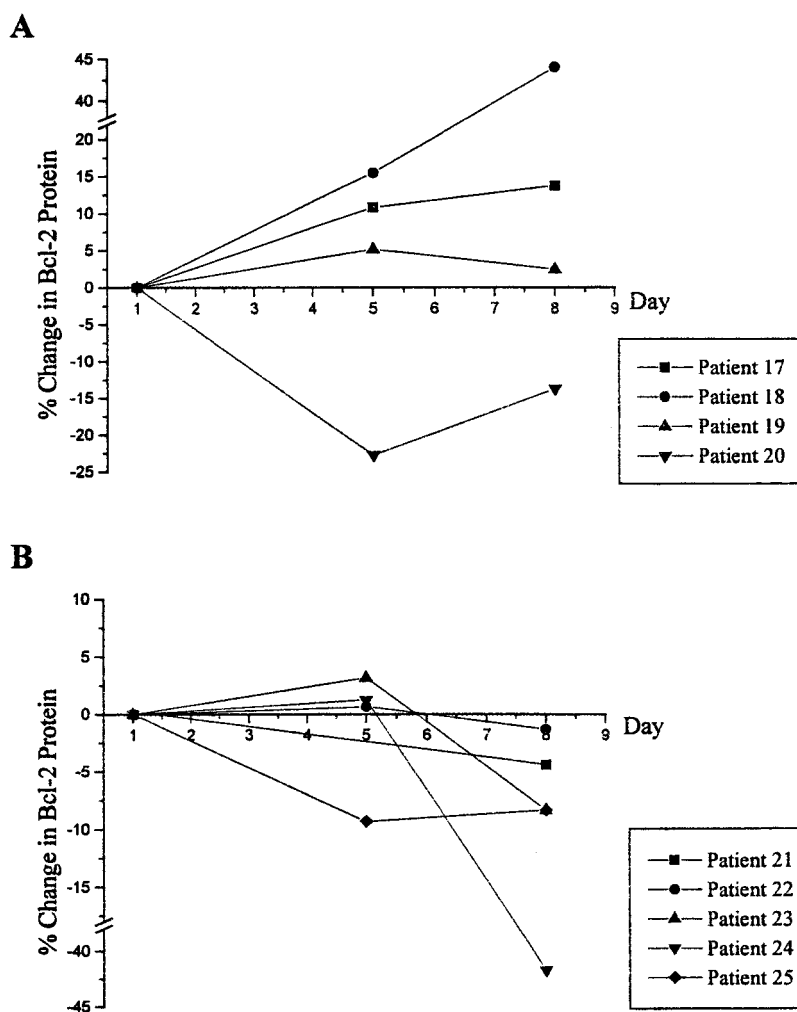
Lymphopenia has been noted in other trials with Genasense (22, 27). This is consistent with an antisense effect, because mature lymphocytes seem to be dependent on Bcl-2 for survival (29). As in previous studies, the lymphopenia observed here was

not of clinical significance with no increased occurrence of opportunistic infections. Significant thrombocytopenia, which was a DLT observed in a previous Phase I trial of Genasense as a single agent (22), was not seen in this study. Most toxicities that have been observed in other studies using antisense oligonucleotides have been non-sequence specific and have been attributed to the phosphorothioate backbone of these molecules (22, 27, 28, 30–32). Non-sequence specific toxicities have included fatigue and fever, which occurred in this study. Other reported toxicities with phosphorothioate oligonucleotides, such as elevations in transaminases, hyperglycemia, and alterations in coagulation parameters, were not observed in the present study.

We attempted to assess the biological effects of Genasense on its molecular target, Bcl-2. Ideally, an evaluation of a target effect should be made from neoplastic tissue; however with prostate cancer, patients present predominantly with bone-only disease and rarely present with nodal or visceral metastases, which would be easily amenable to repeated biopsy. Therefore, in this trial, we evaluated Bcl-2 expression in PBLs before and during the Genasense infusion, using a quantitative flow cytometric method as a possible surrogate tissue marker of biological activity. From our results, although minor changes did occur in the Bcl-2 expression that appeared dose related, an antisense effect could not be reliably described as being observed in the patient lymphocyte samples. Other investigators have recently found that Western analysis may be more sensitive in detecting decreases in PBL Bcl-2 expression than flow cytometry techniques (33). Further validation of tests for markers of target effect, accounting for normal baseline variation and indicating what constitutes a biologically meaningful decrease, and whether a surrogate tissue marker consistently reflects target tissue effect based on preclinical models, is required for future studies before they can be used as evidence of biological activity.

It can be speculated that a more pronounced target down-

Fig. 2 Change in Bcl-2 protein expression in patient PBLs. PBLs were obtained from patients on dose level 6 (A) and 7 (B) pretreatment, on day 5 during Genasense infusion and on day 8 prior to mitoxantrone. Bcl-2 protein expression determined by quantitative fluorescence cytometry.



regulation was not observed because lymphopenia occurred in most patients, and the antisense effect on Bcl-2 may have been missed because of a shift in the population of lymphocytes. Assuming that the effect of the antisense molecule is likely heterogeneous within a cell population, those lymphocytes that were affected had a decrease in Bcl-2 expression, underwent apoptosis, were cleared from the circulation, and, thus, could not be identified. Another possibility is that a significant antisense effect may be occurring only within neoplastic tissues and not within normal tissues as has been observed in some animal models (21). Lack of a greater observed biological effect might also be attributable to low intracellular uptake of the oligonucleotide. Lipid carriers are necessary for efficient uptake of antisense oligonucleotides *in vitro* (17–21); however, in *in vivo* models, this has not been necessary (17–21), and previously reported clinical trials using Genasense at similar dosing have documented decreases in Bcl-2 expression in neoplastic tissue (22, 27).

Some differences in the pharmacokinetic parameters of mitoxantrone were apparent at the higher dose of Genasense characterized by an increase in AUC and a decrease in plasma

clearance. Alterations in the serum and tissue pharmacokinetics of an anthracycline when delivered in combination with Genasense have also been observed in preclinical models (34); however, the mechanisms for these changes are not understood. Increased toxicity attributable to mitoxantrone was not observed between the dose levels despite the change in mean AUC. Furthermore, large interpatient variability in the pharmacokinetic parameters of mitoxantrone has also been previously reported (35), and, therefore, the differences seen here are likely attributable to chance alone from the small sample size.

Determining activity was not a primary end point in this study, but preliminary data here does not suggest a synergistic therapeutic effect of combining Genasense with mitoxantrone in patients with metastatic HRP. Although we were initially encouraged by one patient having had a dramatic PSA response after one cycle of Genasense and low-dose mitoxantrone, this effect was not observed in any other patient on the study except for one treated at the standard dose of mitoxantrone. Mitoxantrone was selected as the chemotherapeutic agent in this trial because it is considered a standard chemotherapy for HRP. It may, however, be difficult to enhance the activity of a drug with

Table 5 Plasma pharmacokinetic parameters of mitoxantrone following Genasense

Dose level	No. of patients	Genasense			Mitoxantrone						
		Dose (mg/kg/day)	C _{ss} (μg/ml)	Dose (mg/m ²)	[AUC] _{0-α} (ng·h/ml)	C _p (ng/ml)	t _{1/2α} (h)	t _{1/2β} (h)	V _c (liters)	V _{ss} (liters)	Cl _t (liters/h)
6	4	3.0	2.82 ± 0.66	12.0	328.9 ± 52.6	816.1 ± 202.2	0.21 ± 0.02	3.83 ± 3.1	32.8 ± 8.9	173.3 ± 198.4	77.8 ± 11.0
7	6	5.0	4.29 ± 0.52	12.0	579.6 ± 172.7	1473.4 ± 886.5	0.19 ± 0.06	5.48 ± 2.7	19.3 ± 10.2	144.9 ± 103.1	38.4 ± 11.51
P			0.0001		0.008	ns ^a	ns	ns	0.02	ns	0.0003

^a [AUC]_{0-α}, area under the concentration-time curve; C_p, peak plasma concentration; C_{ss}, concentration in plasma at steady state; t_{1/2α}, distribution half-life; t_{1/2β}, elimination half-life; V_c, central compartment volume of distribution; V_{ss}, volume of distribution at steady state; Cl_t, plasma clearance; ns, not significant.

low objective activity used for primarily palliative purposes (4, 5). Another possible explanation for the lack of a more pronounced clinical effect is that, for the reasons presented above, we did not sample patient tumors to assess for baseline overexpression of Bcl-2, and it is conceivable that only cancers that overexpress Bcl-2 are amenable to chemosensitization with Genasense. *In vitro* data however, suggests that this might not be the case and that an antisense effect can still be seen in cells that have either higher or lower expression of Bcl-2 (36). The median overall survival of the group was 18.7 months, which is significantly longer than the survival for patients with metastatic HRPC reported from randomized trials (4, 5). This certainly reflects patient selection, emphasizing that caution must be used when extrapolating survival data from early-phase clinical trials.

Recent published studies report that antimicrotubule regimens, most notably docetaxel, may have significantly more activity in prostate cancer, with response rates in the range of 30% for objective disease and 50% for PSA response in Phase II trials (37). A mechanism of action of antimicrotubule agents may be through the phosphorylation and, hence, the inactivation of Bcl-2 (38), and, therefore, the combination of Genasense with an antimicrotubule agent may be a more promising regimen for synergistic activity, as demonstrated in prostate xenograft studies (20, 21). Clinical trials that use this combination in HRPC are currently underway at our center and elsewhere.

REFERENCES

1. Wingo, P. A., Landis, S., and Ries, L. A. G. An adjustment to the 1997 estimate for new prostate cancer cases. *CA Cancer J. Clin.*, 47: 239–242, 1997.
2. Crawford, E. D., Eisenberger, M. A., McLeod, D. G., Spaulding, J. T., Benson, R., Dorr, F. A., Blumenstein, B. A., Davis, M. A., and Goodman, P. J. A controlled trial of leuprolide with and without flutamide in prostatic carcinoma. *N. Engl. J. Med.*, 321: 419–424, 1989.
3. Tannock, I. F. Is there evidence that chemotherapy is of benefit to patients with carcinoma of the prostate? *J. Clin. Oncol.*, 3: 1013–1021, 1985.
4. Tannock, I. F., Osoba, D., Stockler, M. R., Ernst, D. S., Neville, A. J., Moore, M. J., Armitage, G. R., Wilson, J. J., Venner, P. M., Coppin, C. M., and Murphy, K. C. Chemotherapy with mitoxantrone plus prednisone or prednisone alone for symptomatic hormone-resistant prostate cancer: a Canadian randomized trial with palliative end points. *J. Clin. Oncol.*, 14: 1756–1764, 1996.
5. Kantoff, P. W., Halabi, S., Conaway, M., Picus, J., Kirshner, J., Hars, V., Trump, D., Winer, E. P., and Vogelzang, N. J. Hydrocortisone with and without mitoxantrone in men with hormone-refractory prostate cancer: Results of the Cancer and Leukemia Group B 9182 study. *J. Clin. Oncol.*, 17: 2506–2513, 1999.
6. Tsujimoto, Y., and Croce, C. M. Analysis and structure, transcripts, and protein products of *bcl-2*, the gene involved in human follicular lymphoma. *Proc. Natl. Acad. Sci. USA*, 83: 5314–5218, 1986.
7. Reed, J. C. Bcl-2 and the regulation of programmed cell death. *J. Cell. Biol.*, 124: 1–6, 1994.
8. Miyashita, T., and Reed, J. C. bcl-2 oncoprotein blocks chemotherapy-induced apoptosis in a human leukemia cell line. *Blood*, 81: 151–157, 1993.
9. Kyprianou, N., King, E. D., Bradbury, D., and Rhee, J. G. bcl-2 overexpression delays radiation induced apoptosis without affecting the clonogenic survival of human prostate cancer cells. *Int. J. Cancer*, 70: 341–348, 1997.
10. McDonnell, T. J., Navone, N. M., Troncoso, P., Pisters, L. L., Conti, C., von Eschenbach, A. C., Brisbay, S., and Logothetis, C. J. Expression of bcl-2 oncoprotein and p53 protein accumulation in bone

marrow metastases of androgen-independent prostate cancer. *J. Urol.*, 157: 569–574, 1997.

11. Colombel, M., Symmans, F., Gil, S., O'Toole, K. M., Chopin, D., Benson, M., Olsson, C. A., Korsmeyer, S., and Buttyan, R. Detection of the apoptosis-suppressing oncoprotein bcl-2 in hormone-refractory human prostate cancers. *Am. J. Pathol.*, 143: 390–400, 1993.
12. McDonnell, T. J., Troncoso, P., Brisbay, S., Logothetis, C. J., Chung, L. W., Hsieh, J. T., Tu, S. M., and Campbell, M. L. Expression of the protooncogene *Bcl-2* in the prostate and its association with emergence of androgen-independent prostate cancer. *Cancer Res.*, 52: 6940–6944, 1992.
13. Raffo, A. J., Perlman, H., Chen, M. W., Streitman, J. S., and Buttyan, R. Overexpression of bcl-2 protects prostate cancer cells from apoptosis *in vitro* and confers resistance to androgen depletion *in vivo*. *Cancer Res.*, 55: 4438–4445, 1995.
14. Bubendorf, L., Sauter, G., Moch, H., Jordan, P., Blohlinger, A., Gasser, T. C., and Mihatsch, M. J. Prognostic significance of Bcl-2 in clinically localized prostate cancer. *Am. J. Pathol.*, 148: 1557–1565, 1996.
15. Scherr, D. S., Vaughan, E. D. Jr., Wei, J., Chung, M., Felsen, D., Allbright, R., Knudsen, B. S. BCL-2, and p53 expression in clinically localized prostate cancer predicts response to external beam radiotherapy. *J. Urol.*, 162: 12–16, 1999.
16. Crooke, S. T. Therapeutic application of oligonucleotides. *Annu. Rev. Pharmacol. Toxicol.*, 32: 329–376, 1992.
17. Miyake, H., Tolcher, A., and Gleave, M. E. Antisense Bcl-2 oligodeoxynucleotides inhibit expression to androgen-independence after castration in the Shionogi tumor model. *Cancer Res.*, 59: 4030–4034, 1999.
18. Gleave, M. E., Tolcher, A., Miyake, H., Nelson, C., Brown, B., Beraldi, E., and Goldie, J. Progression to androgen independence is delayed by adjuvant treatment with antisense Bcl-2 oligodeoxynucleotides after castration in the LNCaP prostate tumor model. *Clin. Cancer Res.*, 5: 2891–2898, 1999.
19. Miyake, H., Monia, B. P., and Gleave, M. E. Inhibition of progression to androgen-independence by combined adjuvant treatment with antisense BCL-xL and antisense Bcl-2 oligonucleotides plus Taxol after castration in the shionogi tumor model. *Int. J. Cancer*, 86: 855–862, 2000.
20. Leung, S., Miyake, H., Zellweger, T., Tolcher, A., and Gleave, M. E. Synergistic chemosensitization and inhibition of progression to androgen-independence by antisense Bcl-2 oligodeoxynucleotide and paclitaxel in the LNCaP prostate tumor model. *Int. J. Cancer*, 91: 846–850, 2001.
21. Miyake, H., Tolcher, A., and Gleave, M. E. Chemosensitization and delayed androgen-independent recurrence of prostate cancer with the use of antisense Bcl-2 oligonucleotides. *J. Natl. Cancer Inst. (Bethesda)*, 92: 34–41, 2000.
22. Waters, J. S., Webb, A., Cunningham, D., Clarke, P. A., Raynaud, F., di Stefano, F., and Cotter, F. E. Phase I clinical and pharmacokinetic study of Bcl-2 antisense oligonucleotide therapy in patients with non-Hodgkin's lymphoma. *J. Clin. Oncol.*, 18: 1812–1823, 2000.
23. Miller, A. B., Hoogstraten, B., Staquet, M., and Winkler, A. Reporting results of cancer treatment. *Cancer (Phila.)*, 47: 207–214, 1981.
24. Dragowska, W. H., Lopes de Menezes, D. E., Sartor, J., and Mayer, L. D. Quantitative fluorescence cytometric analysis of Bcl-2 levels in tumor cells exhibiting a wide range of inherent Bcl-2 protein expression: correlation with Western blot analysis. *Cytometry*, 40: 346–352, 2000.
25. Chen, S. H., Qian, M., Brennan, J. M., and Gallo, J. M. Determination of antisense phosphorothioate oligonucleotides and catabolites in

biological fluids and tissue extracts using anion-exchange high-performance liquid chromatography and capillary gel electrophoresis. *J. Chromatogr. B. Biomed. Sci. Appl.*, 692: 43–51, 1997.

26. Raynaud, F. I., Orr, R. M., Goddard, P. M., Lacey, H. A., Lancashire, H., Judson, I. R., Beck, T., Bryan, B., and Cotter, F. E. Pharmacokinetics of G3139, a phosphorothioate oligodeoxynucleotide antisense to bcl-2, after intravenous administration or continuous subcutaneous infusion to mice. *J. Pharmacol. Exp. Ther.*, 281: 420–427, 1997.
27. Jansen, B., Wacheck, V., Heere-Ress, E., Schlagbauer-Wedl, H., Hoeller, C., Lucas, T., Hoermann, M., Hollenstein, U., Wolff, K., and Pehamberger, H. Chemosensitization of malignant melanoma by BCL-2 antisense therapy. *Lancet*, 356: 1728–1733, 2000.
28. Scher, H. I., Morris, M. J., Tong, W. P., Cordon-Cardo, C., Drobniak, M., Kelly, W. K., Slovin, S. F., Terry, K. L., DiPaola, R. S., Rafi, M., and Rosen, N. A Phase I trial of G3139 (Genta, Inc.), a BCL2 antisense drug, by continuous infusion (CI) as a single agent and with weekly Taxol (T). *Proc. Am. Soc. Clin. Oncol.*, 19: 199, 2000.
29. Veis, D. J., Sorenson, C. M., Shutter, J. R., and Korsmeyer, S. J. Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. *Cell*, 75: 229–240, 1993.
30. Glover, J. M., Leeds, J. M., Mant, T. G., Amin, D., Kisner, D. L., Zuckerman, J. E., Geary, R. S., Levin, A. A., and Shanahan, W. R. Jr. Phase I safety and pharmacokinetic profile of an intercellular adhesion molecule-1 antisense oligodeoxynucleotide (ISIS 2302). *J. Pharmacol. Exp. Ther.*, 282: 1173–1180, 1997.
31. Bishop, M. R., Iversen, P. L., Bayever, E., Sharp, J. G., Greiner, T. C., Copple, B. L., Ruddon, R., Zon, G., Spinolo, J., Arneson, M., Armitage, J. O., and Kessinger, A. Phase I trial of an antisense oligonucleotide OL(1)p53 in hematologic malignancies. *J. Clin. Oncol.*, 14: 1320–1326, 1996.
32. O'Dwyer, P. J., Stevenson, J. P., Gallagher, M., Mitchell, E., Friedland, D., Rose, L., Cassella, A., Holmlund, J., Dean, N., Dorr, A., Geary, R., and Yao, K-S. Phase I/pharmacokinetic/pharmacodynamic trial of Raf-1 antisense ODN (ISIS 5132, CGP 69846A). *Proc. Am. Soc. Clin. Oncol.*, 17: 210, 1998.
33. de Bono, J. S., Rowinsky, E. K., Kuhn, J., Basler, J., Ochoa, L., Schwartz, G., Patnaik, A., Hammond, L. A., Smetzer, L., Smith, L., Fingert, H., Weitman, S., Thompson, L., Tolcher, A. W. Phase I pharmacokinetic (PK) and pharmacodynamic (PD) trial of bcl-2 antisense (Genasense) and docetaxel (D) in hormone refractory prostate cancer. *Proc. Am. Soc. Clin. Oncol.*, 20: 119, 2001.
34. Lopes de Menezes, D. E., Hudon, N., McIntosh, N., and Mayer, L. D. Molecular and pharmacokinetic properties associated with the therapeutics of Bcl-2 antisense oligonucleotide G3139 combined with free and liposomal doxorubicin. *Clin. Cancer Res.*, 6: 2891–2902, 2000.
35. Hu, O. Y., Chang, S. P., Law, C. K., Jian, J. M., and Chen, K. Y. Pharmacokinetic and pharmacodynamic studies with mitoxantrone in the treatment of patients with nasopharyngeal carcinoma. *Cancer (Phila.)*, 69: 847–853, 1992.
36. Chi, K. N., Wallis, A. E., Lee, C. H., De Menezes, D. L., Sartor, J., Dragowska, W. H., and Mayer, L. D. Effects of Bcl-2 modulation with G3139 antisense oligonucleotide on human breast cancer cells are independent of inherent Bcl-2 protein expression. *Breast Cancer Res. Treat.*, 63: 199–212, 2000.
37. Picus, J., and Schultz, M. A Phase II trial of docetaxel in patients with hormone refractory prostate cancer: long term results. *Proc. Am. Soc. Clin. Oncol.*, 18: 314, 1999.
38. Haldar, S., Basu, A., and Croce, C. M. Bcl-2 is the guardian of microtubule integrity. *Cancer Res.*, 57: 229–233, 1997.

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Kim N. Chi, Martin E. Gleave, Richard Klasa, et al.

Clin Cancer Res 2001;7:3920-3927.

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