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13. ABSTRACT (Maximum 200 Words)

Background: The specific aims of this grant are to demonstrate (1) that *bcl-2* overexpression in prostate cancer specimens is a predictive biomarker for enhanced responsiveness to G3139, and antisense oligonucleotide targeting Bcl-2, and docetaxel; (2) that the degree of *bcl-2* downregulation in normal tissue surrogate (peripheral blood mononuclear cells [MNC]) will predict prostate cancer responsiveness to G3139 and docetaxel; and (3) whether the pharmacokinetic parameters of G3139 and docetaxel are predictive of *bcl-2* biomodulation and antitumor activity, respectively.

Results End of Year 2: The mean G3139 steady-state concentrations (Css) was significantly higher in responding patients than in those patients who did not respond (6.2 ± 0.4 versus 4.8 ± 0.3 μg/mL, $p = 0.015$), with a longer, although not statistically significant, increase in median survival for patients with Css > 5μg/mL (689 versus 595 days, $P > 0.05$). Bcl-2 levels in MNC, although decreased in the majority of patients after 5 days of G3139, were not predictive of clinical response.

Interim Conclusions: G3139 Css is a predictive marker for response and optimal concentrations for G3139 may need to be targeted above 5 μg/mL. Immunohistochemistry for Bcl-2, BAX and Bcl-X_L and the relationship to response and survival is continuing.

14. SUBJECT TERMS
Biomarkers, Bcl-2, Bax, Apoptosis, Pharmacokinetics, Clinical Research, and Hormone Refractory Prostate Cancer.

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Introduction:

The central hypothesis is that *bcl-2* protein overexpression confers intrinsic resistance to chemotherapy in patients with hormone-refractory prostate cancer (HRPC), therefore downregulation of *bcl-2* protein by G3139 will enhance the antitumor activity of docetaxel in patients HRPC. We further hypothesize and the focus of the current grant application is that patients whose tumors utilize *bcl-2* overexpression as a mechanism to escape the apoptotic events of chemotherapy will benefit from G3139 biomodulation, whereas tumors that exhibit other mechanisms for impaired apoptosis, such as diminished Bax expression, will fail to respond or fail to have a durable response. The specific aims of this project are to demonstrate (1) that *bcl-2* overexpression in prostate cancer specimens is a predictive biomarker for enhanced responsiveness to G3139 and docetaxel therapy; (2) that the degree of *bcl-2* downregulation in normal tissue surrogate (peripheral blood mononuclear cells) will predict prostate cancer responsiveness to G3139 and docetaxel; and (3) whether the pharmacokinetic parameters of G3139 and docetaxel are predictive of *bcl-2* biomodulation and antitumor activity, respectively.

Body:

Annual Report Year 2 (12-24 months)

A total of 31 patients were entered into the clinical study entitled A Phase II pharmacokinetic and biologic correlative study of G3139 (antisense oligonucleotide directed to bcl-2) and docetaxel in patients with hormone-refractory prostate cancer and accrual of new patients is complete with updated response and survival data available up to December 2003. The clinical study has been overseen by Dr. Anthony Tolcher, the principal investigator of the clinical study and the biologic correlative grant. The correlative biologic and pharmacokinetic studies are the companion to the clinical study and funded by the current Department of Defense Grant (PC010504). The accomplishments of year 2 are described below in order of the Tasks described in the original grant application and the relevant sections in Task 4.

Task 1: Immunohistochemical Detection of bcl-2, bcl-X_L, and Bax from patients entered onto phase II study of G3139 and docetaxel for HRPC

- ***a. Obtain primary tissue blocks (paraffin embedded) from each patient entered on Phase II study (30 patients total) for banking, section paraffin blocks for representative tumor, and perform immunohistochemical staining for bcl-2, bcl-X_L, and Bax staining. 24 months.***
- ***b. Pathologic scoring of all immunohistochemical stained specimens will be complete by end of year 2. 24 months.***

Task 4: Examine the predictive pharmacokinetic and biomarkers for response to bcl-2 biomodulation by G3139 and docetaxel.

- ***a. Examine relationships between the biomarkers of bcl-2, bcl-X_L, and Bax expression and clinical outcome (6-12 months, Year 3).***

This Task is not yet complete. All 28 specimens have been collected, sectioned and are awaiting immunohistochemistry. A pilot analysis of the techniques used and the addition of immunohistochemical staining for PTEN and phospho-Akt was performed based on emerging data provided by one of our subinvestigators (Dr. J Kreisberg) and included in the Year 1 interim report. The objectives of this pilot study were to determine the frequency of expression of the listed markers Bcl-2, Bcl-X_L, and BAX, as well as PTEN and Akt-p since the latter appears to be involved in aberrant apoptosis signaling (phosphorylation of BAD). Specimens were obtained from the original phase I study cohort (16 specimens of 20 patients entered) and IRB approved. No funds from the DOD grant were used to perform this pilot work although the results will be used to further select predictive markers in the DOD Phase II portion (Table 1).

Table 1: Relative Expression* of Predictive Biomarkers in Prostate Cancer Patients

Patient #	BAX	Bcl-2	Bcl-X_L	PTEN	Total Akt	p-Akt (ser473)
1	0	0	2+ (2)	0	2+ (3)	2+ (3)
2	2+ (2)	0	3+ (3)	1+	2+ (3)	2+ (3)
3	0	--	0	0	3+ (3)	3+ (3)
4	2+ (1)	0	3 (3)	0	3+ (3)	3+ (3)
5	0	0	0	--	--	--
6	3+ (3)	3 (3)	3+ (3)	1+	3+ (3)	3+ (3)
7	0	3 (3)	3+ (3)	3+	3+ (3)	0
8	0	0	0	0	2+ (3)	2+ (3)
10	3+ (3)	2+ (2)	2+ (2)	2+	3+ (3)	3+ (2)
12	0	0	3+ (1)	3+	3+ (3)	3+ (3)
13	0	3+ (3)	3+ (3)	1+	3+ (3)	2+ (2)
14	3+ (3)	2+ (2)	2+ (2)	2+	3+ (3)	2+ (3)
15	0	0	0	0	0-1+	0-1+
17	3+	3+ (3)	0	0	3+ (2)	3+ (2)
18	3 (3)	0	0	2+	3+ (3)	3+ (2)

*numbers (1-3+) reflect intensity, whereas numbers in parentheses represent distribution in tumor: 1 = 10-20%, 2 = 20-50%, 3 = >50%. Hyphen indicates insufficient tumor material

The frequency of expression of the Bcl-2 protein expression in the primary tumor specimens was 6/15 (40%), BAX 7/ 15 (47%), and Bcl-X_L 9/15 (60%). The results of this pilot study indicate that both Total Akt and phospho Akt are nearly uniformly expressed in prostate cancer specimens and therefore have insufficient diversity across patients to represent a useful predictive biomarker. Moreover, staining for these proteins, as envisioned in progress report for year 1, will not be carried out in the current study.

We anticipate the completion of the immunohistochemical staining as outlined in the grant in the next 2 months with the inclusion of PTEN to the originally proposed staining for Bcl-2, Bax, and Bcl-X_L.

Task 2: Quantification of G3139 mediated bcl-2 dowregulation in peripheral blood mononuclear cells.

- ***a. Obtain isolate blood mononuclear cells (MNCs) from all patients (30 patients) at the two time points (prior to G3139 therapy and on day 5), isolate protein 18-24 months.***
- ***b. Perform western assay for bcl-2 protein. 18-24 months.***

Task 4: Examine the predictive pharmacokinetic and biomarkers for response to bcl-2 biomodulation by G3139 and docetaxel.

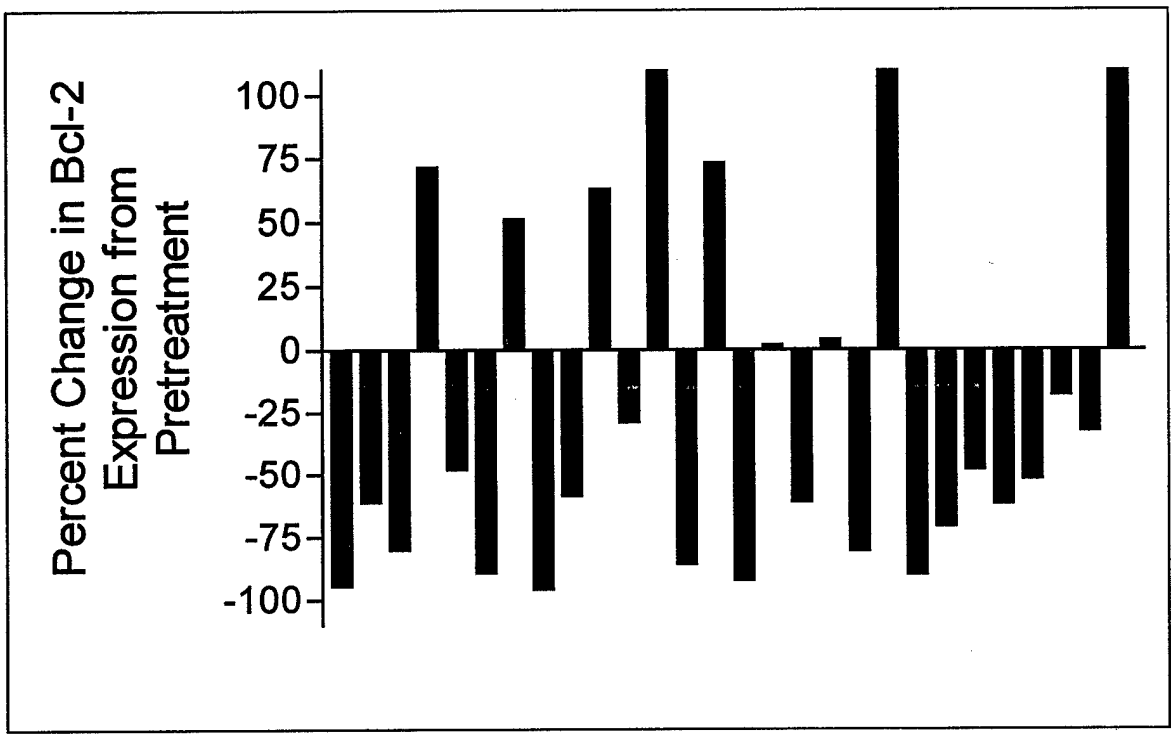
- ***b. Examine relationships between MNCs and G3139 steady-state concentrations, and patient clinical outcome (e.g. response rate, time to progression, survival). (6-12 months, Year 3).***

A total of 28 patients had paired mononuclear specimens were obtained from the 31 patients entered into this study. Peripheral blood mononuclear cells (MNCs) were obtained day 1 prior to the initiation of the antisense molecule G3139 and again following 5 days (12hours) of continuous intravenous administered G3139 (protocol day 6), immediately prior to the initiation of docetaxel. Following separation of the MNCs, protein was isolated from the samples and Bcl-2 protein quantified by Western Blot analysis as outline in the methodology section. The measured Bcl-2 values were normalized to actin expression.

There was considerable interpatient variability in the absolute value of Bcl-2 protein obtained and this was reflected in the median normalized ratio (0.286) and the wide range of ratios (0.017-15.159).

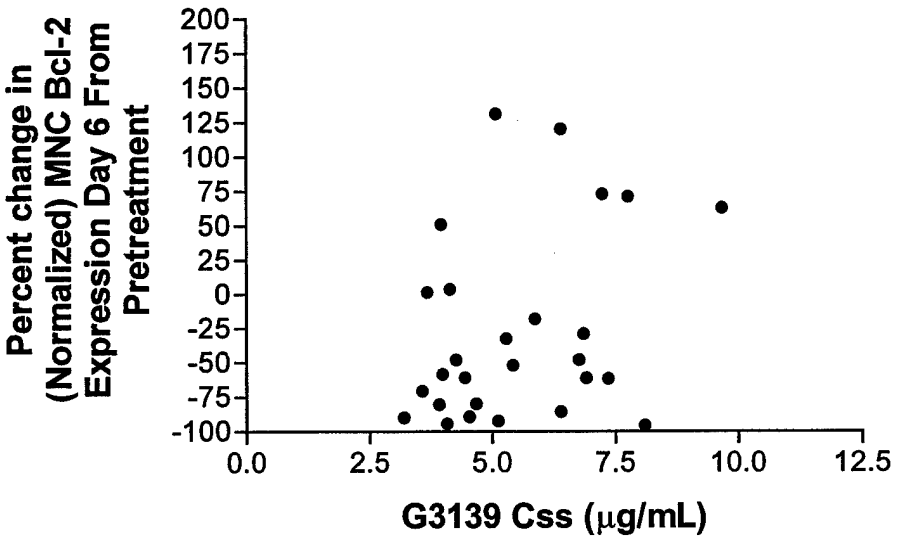
The decrement of normalized Bcl-2 from baseline to day 6 was calculated and 19 patients had a net decrease in Bcl-2 expression whereas 9 patients had a net increase in Bcl-2 expression. The median percent decrement was 49.9% with a range of 95% decrease to 444% increase. The individual patients percent decrements in normal Bcl-2 values are depicted in Figure 1.

Figure 1. The percent decrement of Bcl-2 protein expression following treatment with G3139 in peripheral blood mononuclear cells at Day 6 compared to pretreatment. The Y axis was is truncated at 100 with 3 patients having increases of 120, 131, 444%.



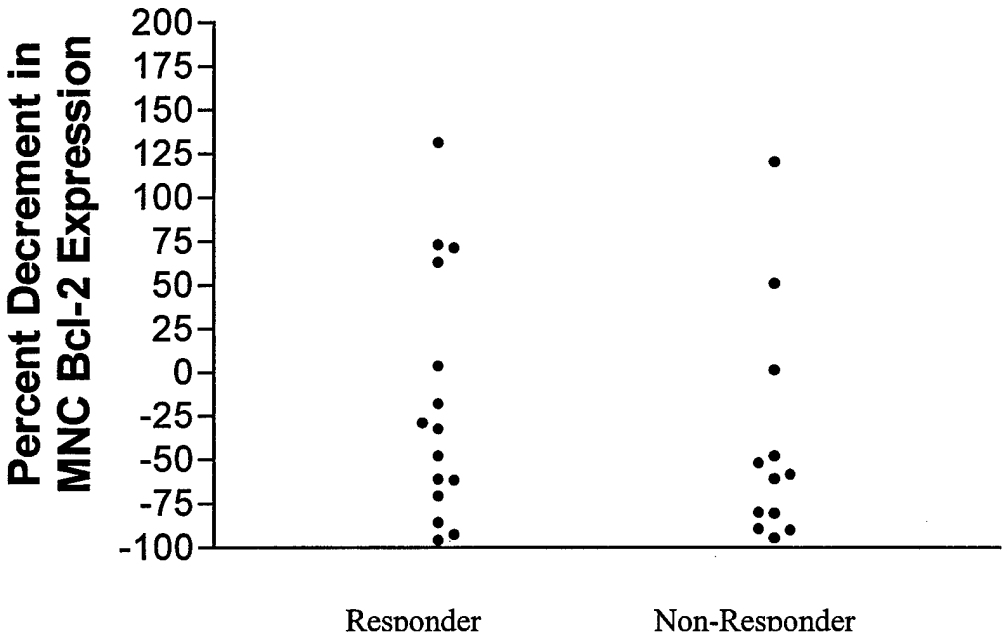
To determine if the decrement of MNC Bcl-2 protein may be a function of G3139 steady state concentration (C_{ss}), the relationships of these two parameters were evaluated. The mean C_{ss} ±SD of G3139 for the patients who had a decrease in Bcl-2 expression was 5.3 ± 1.4 versus 6.1 ± 2.0 μg/mL. The difference of these means was not statistically significant ($p = 0.24$). Furthermore, no linear or non-linear relationship could be derived for the values of C_{ss} and decrements in Bcl-2 expression (Figure 2).

Figure 2: Scatterplot of decrement of Bcl-2 expression day 6 versus pretreatment in MNC as a function of G3139 steady state concentrations. The Y-axis was limited at 200% increase therefore 1 patient at 444% not shown



To evaluate whether the decrement in Bcl-2 levels in MNCs would be a predictive biomarker for clinical outcome the percent decrement in Bcl-2 was evaluated in the context of the response to therapy (PSA response), time to progression, and survival. There was no evidence that the decrement in Bcl-2 expression predicted response to therapy as demonstrated in Figure 3 (means comparison $p > 0.05$).

Figure 3: Distribution of percent Bcl-2 expression decrements in MNC Expression in patients that responded versus no response



There was no evidence that MNC Bcl-2 expression predicted either improvement in time to progression or overall survival (data not shown).

The results of this study indicate that although the majority of patients had decrements in MNC Bcl-2 expression following G3139 treatment, the decrement in MNC Bcl-2 expression is not a function of G3139 steady-state concentrations, nor a predictive biomarker for the relevant clinical outcomes of response and survival following treatment with G3139 and docetaxel.

Task 3: Pharmacokinetic Sampling of G3139 and docetaxel from patients entered on Phase II study.

- ***a. Collect G3139 and docetaxel plasma samples for pharmacokinetic analysis from all 30 patients (18-24 months).***
- ***b. Perform high-performance liquid chromatography to determine plasma concentrations of each agent (18-24 months).***

Task 4: Examine the predictive pharmacokinetic and biomarkers for response to bcl-2 biomodulation by G3139 and docetaxel.

- ***b. Examine relationships between MNCs and G3139 steady-state concentrations, and patient clinical outcome (e.g. response rate, time to progression, survival). (6-12 months, Year 3).***
- ***c. Model docetaxel pharmacokinetic parameters, and determine relationships with clinical outcome (6-12 months, Year 3)***

We are currently ahead of schedule for the completion of Task 3 and relevant aspects of Task 4. Plasma specimens were obtained in 28 patients for the determination of G3139 and docetaxel pharmacokinetic parameters. Plasma concentrations for both G3139 and docetaxel were determined using high-performance liquid chromatography methods outlined in the grant submission.

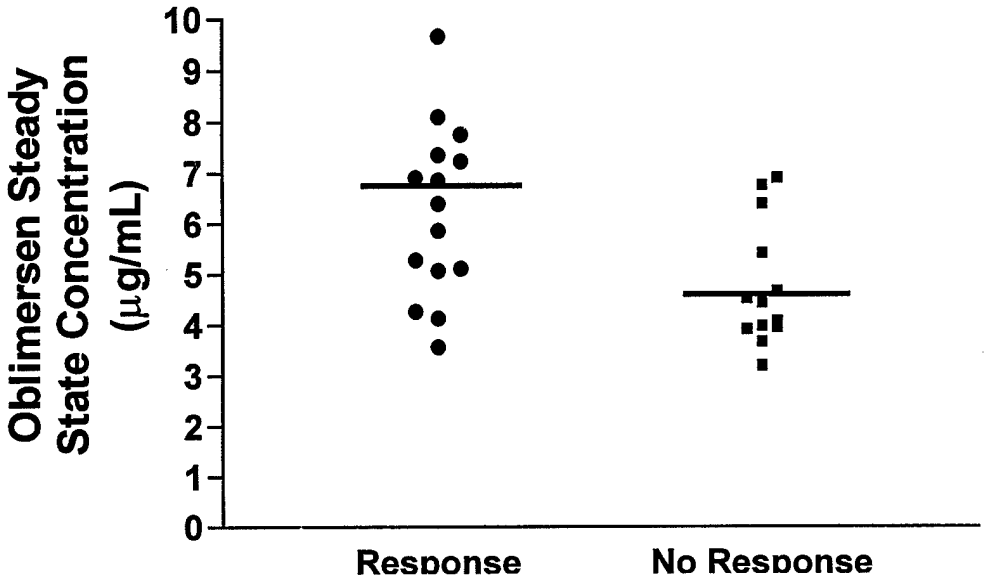
Mean (±SD) G3139 steady state concentrations for the entire group treated at 7 mg/kg/day was 5.6 (±1.6) μg/mL.

The premise of the clinical study is that Bcl-2 downregulation will enhance the antitumor efficacy of docetaxel. To determine if the C_{ss} of G3139 is a predictive of clinical outcome, the relationship between G3139 C_{ss} and antitumor response (PSA Response), time to progression, and survival were explored.

There was a significant difference between the mean C_{ss} for patients who responded compared to those patients who did not respond. The mean (±SEM) C_{ss} for patients who

responded was 6.2 ± 0.4 compared to 4.8 ± 0.3 μg/mL for patients who failed to respond to treatment (p = 0.015). The distribution of Css and response is depicted in Figure 4.

Figure 4: Distribution of Css for responding versus non-responding patients



Furthermore, if the subsets of patients are divided into those patients that achieve G3139 plasma Css ≥ 5 μg/mL, the response rate for this cohort of patients was 12/16 (75%) patients whereas in the patients whose Css was less than 5 μg/mL the response rate was 3/12 (25%). Analysis of the impact of Css on survival indicates that the group with Css ≥ 5 μg/mL had a median survival of 689 days versus 595 days for the patients with G3139 Css less than 5 μg/mL, although the difference was not statistically significant (Logrank test $p > 0.05$).

These results suggest that Css plasma concentrations may be a strong predictive marker for antitumor activity of this combination and raises some important questions regarding the selection of G3139 dosing.

- Is the current dosing at 7 mg/kg/day optimal?

- Are there confounding factors responsible for increased individual patient clearance of G3139 (e.g. total protein levels, albumin, renal function) that may predict which patients will not achieve optimal plasma concentrations?
- What are the implications of this finding on other G3139 studies including currently active or recent pivotal studies that utilize the 7 mg/kg/day dose?

Twenty-one of twenty-eight patients had sufficient blood sampling time-points for docetaxel pharmacokinetic determinations. The mean clearance values for docetaxel for the responding versus non-responding patient subgroups were not significantly different and therefore unlikely to be a predictive marker (433±69 versus 561±94 L/hr, for responders and non responders respectively).

During the remaining 12 months of the grant the remaining questions will be addressed. Moreover, there are implications for Task 1 of this study. With completion of the immunohistochemistry results for Bcl-2, BAX, and Bcl-X_L further analysis will need to be performed in the individual groups of patients based on G3139 Css values.

Key Research Accomplishments Year 2:

- Steady-State concentrations for G3139 are predictive for response to the combination therapy of G3139 and docetaxel in patients with hormone refractory prostate cancer. These results have implications for the optimal dose of the Bcl-2 targeting antisense oligonucleotide and should be further examined in a clinical study in which higher Css are achieved (e.g. using 9 mg/kg/day) to determine if the high response rate in the patient population that had Css > 5 μg/mL can be duplicated and furthermore has implications for the development of this drug in other clinical studies, including pivotal studies, using this agent.
- Bcl-2 protein levels in peripheral blood mononuclear cells are not predictive for clinical response or G3139 steady-state concentrations.

Reportable Outcomes:

KN Chi, RN Murray, ME Gleave, J Kuhn, E Izbicka, K Berg, EK Rowinsky, AW Tolcher. A Phase II study of oblimersen sodium (G3139) and docetaxel in patients with prostate cancer (HRPC) Proceedings of the American Society of Clinical Oncology 22: 2003 (Abstr 1580)

Conclusions:

For patients with hormone-refractory prostate cancer treated with 7mg/kg/day days 1-8 of G3139 (antisense oligonulcleotide to Bcl-2) and docetaxel 75 mg/kg day 6, G3139 steady-state concentration levels that equaled or exceeding 5 ug/mL was a significant predictor of PSA responsiveness. This finding has important implications: 1) at the recommended dose (7mg/kg/day) of G3139 for this and other phase II studies and the ongoing or recently completed pivotal phase III studies may not be optimal for Bcl-2 biomodulation and antitumor activity due to the marked interpatient variablility of G3139 steady state concentrations; 2) are there other factors that hasten the clearance of G3139 and may permit optimal dosing of individual patients (e.g. serum albumin,total protein levels, or renal function); 3) the basis for the recommended dose from the original phase I study may be incorrect since an MTD was not established, and therefore reinforces the need for rigorously performed phase I studies with targeted therapies[1].

Peripheral blood mononuclear cells are poor surrogates for anti-Bcl-2 activity since the decrement in Bcl-2 protein cannot be related to either G3139 concentrations, or antitumor activity.

References:

1. Morris MJ, Tong WP, Cordon-Cardo C, et al: Phase I trial of BCL-2 antisense oligonucleotide (G3139) administered by continuous intravenous infusion in patients with advanced cancer. Clinical Cancer Research. 8:679-83, 2002

Abstract # 1580

A phase II study of oblimersen sodium (G3139) and docetaxel (D) in patients (pts) with metastatic hormone-refractory prostate cancer (HRPC). K. N. Chi, R. N. Murray, M. E. Gleave, J. Kuhn, E. Izbicka, K. Berg, E. K. Rowinsky, A. W. Tolcher; British Columbia Cancer Agency, Vancouver, BC, Canada; University of Texas Health Sciences Center, San Antonio, TX; Institute for Drug Development, Cancer Therapy and Research Center, San Antonio, TX.

The mitochondrial associated protein Bcl-2 confers resistance to apoptosis and is a negative prognostic indicator in prostate cancer. Bcl-2 is overexpressed in HRPC and implicated in the development of androgen independence and treatment resistance. We conducted a phase II study to determine the antitumor activity of *bcl-2* antisense oligonucleotide, G3139, 7 mg/kg/day(d) CIVI d 1-8) given with D, (75 mg/m^{2}IV on d 6), in pts with metastatic HRPC. Therapy was repeated every 21 d until progression or toxicity. Pharmacokinetic (PK) assessments of G3139 and D, serial evaluation of Bcl-2 protein in mononuclear cells (MNC) and Bcl-2 expression in primary tumor samples were correlated with antitumor activity. Thirty-one men were enrolled. Data for analysis are available on 29 pts. Median age was 66 yrs (44-82). Prior therapy included (n pts): bilateral orchiectomy (3), chemotherapy (8), radiotherapy (RT) to the prostate (13), and RT to other sites (17). All pts failed at least 1 course of androgen blockade. Median time from initial diagnosis of PC to study entry was 5.8 yrs (range 0.6-17.4 yrs). Baseline median alkaline phosphatase was 166, hemoglobin 12.7 g/dL, and PSA 128. Median number of cycles was 4 (range 1-10), with 20 pts continuing protocol therapy. To date, 146 cycles of therapy have been administered. Partial response was achieved in 4/15 pts (27%) with measurable disease. A > 50% reduction in PSA was measured in 15/31 (48%) pts with 8 pts continuing on therapy. Grade 3-4 neutropenia occurred in 13/31 (42%) of pts and in 27/146 cycles (18%); 5 pts had grade 3-4 febrile neutropenia. Grade 3 fatigue was reported in 3 pts (10%). The most common grade 1-2 adverse events were fatigue (35% of pts) and non-neutropenic fever (31% of pts). Median decrement of normalized Bcl-2 protein in MNC by day 6 of G3139 infusion was 50% (range +72 to -97%). PK and pharmacodynamic analyses are ongoing. The combination of G3139 and D was well tolerated and is associated with encouraging decreases in PSA and clinical responses. Phase III trials are warranted to determine if G3139 adds to the efficacy of docetaxel for pts with metastatic HRPC.

Vol. 8, 679–683, March 2002

Phase I Trial of BCL-2 Antisense Oligonucleotide (G3139) Administered by Continuous Intravenous Infusion in Patients with Advanced Cancer[1]

Michael J. Morris, William P. Tong, Carlos Cordon-Cardo, Marija Drobnjak, William K. Kelly, Susan F. Slovin, Kathryn L. Terry, Karen Siedlecki, Paul Swanson, Mohmed Rafi, Robert S. DiPaola, Neal Rosen, and Howard I. Scher[2]

Genitourinary Oncology Service, Division of Solid Tumor Oncology, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York 10021 [M. J. M., W. P. T., C. C-C., M. D., W. K. K., S. F. S., K. L. T., K. S., P. S., N. R., H. I. S.]; Department of Medicine, Weill Medical College of Cornell University, New York, New York [M. J. M., W. P. T., C. C-C., M. D., W. K. K., S. F. S., P. S., N. R., H. I. S.]; and The Cancer Institute of New Jersey, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, New Brunswick, New Jersey [M. R., R. S. D.]

ABSTRACT

Purpose: **To evaluate the safety and pharmacokinetics of BCL-2 antisense oligonucleotide (G3139) administered by prolonged i.v. infusion in patients with advanced cancer.**

Experimental Design: **A total of 35 patients was treated in cohorts of 3–6 with 0.6–6.9 mg/kg/day of BCL-2 antisense oligonucleotide as a continuous infusion for 14 or 21 days. Plasma levels of intact antisense oligonucleotide were measured in all patients.**

Results: **G3139 was generally well tolerated. At the highest dose level examined in this study (6.9 mg/kg/day), fatigue and transient reversible elevations of serum transaminases (grades 2–3) became apparent after ≥7 days of treatment. Both reactions were believed to be drug related. Pharmacokinetic analyses showed that steady-state plasma concentrations of G3139 were reached ~10 h after starting the infusion and increased linearly across the range of doses administered ≤6.9 mg/kg/day. The terminal plasma half-life was ~2 h. Exploratory studies using Western blots, performed on peripheral blood mononuclear cells on selected patients, demonstrated a decline in bcl-2 protein levels during treatment. No major antitumor responses were observed.**

Conclusions: **BCL-2 antisense therapy is well tolerated. Relative to other dose-finding studies of G3139, fatigue was somewhat more prominent in this study, possibly because of the protracted i.v. infusion schedule of the antisense oligonucleotide. Current randomized trials are using the highest daily dose established in this study given by shorter infusion periods (*i.e.*, 7 mg/kg/day for 5–7 days) to enhance the antitumor activity of standard cytotoxic drugs.**

INTRODUCTION

The *BCL-2* gene product is a 239 amino acid integral-membrane mitochondrial protein that inhibits apoptosis (1–12). It has been implicated in the growth and development of a variety of solid tumors, including prostate, breast, lung, renal, ovary, and prostate cancers, as well as melanoma (6, 9–11, 13–18), and has the potential to confer chemoresistance and radioresistance to established tumors (19).

The bcl-2 protein dimerizes both with itself and with other members of the bcl-2 family, including bax, bcl-X_L, and bcl-Xs (20–22). The interaction of these protein dimers influences sensitivity to apoptotic stimuli and has been reviewed extensively elsewhere (23–25). Preclinical data demonstrate that BCL-2 antisense therapy has antitumor effects against a variety of solid tumors, including prostate, breast, and melanoma (26–28). In human testing, single agent treatment with BCL-2 antisense oligonucleotide has resulted in tumor regression in patients with relapsed non-Hodgkin's lymphoma (29). We undertook a Phase I trial to determine the safety, PKs, and preliminary efficacy of BCL-2 antisense oligonucleotide therapy, delivered as a continuous i.v. 14- or 21-day infusion, in patients with solid tumors.

The BCL-2 antisense oligonucleotide used in this study [G3139 (Genasense); Genta, Inc., Berkeley Heights, NJ] is an 18-mer phosphorothioate complementary to the first six codons of Bcl-2 mRNA.

PATIENTS AND METHODS

Patients and Patient Eligibility. Eligible patients had progressive solid tumors with no acceptable standard treatment options. Patients with prostate cancer had androgen-independent disease that had progressed despite antiandrogen withdrawal. All patients were required to have a life expectancy of ≥6 months, a total leukocyte count > 3,500/mm^3, platelet count > 100,000/mm^3, aspartate aminotransferase < 3 × the upper limit of normal, creatinine < 2 mg/dl or creatinine clearance > 60 ml/min, and prothrombin time < 14 s.

All patients underwent placement of a central venous catheter. BCL-2 antisense oligonucleotide was administered at a rate of 12 ml/min using a portable continuous infusion pump. For the

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[1] Supported in part by NIH Grants CA-80654 and CA-09207, the PepsiCo Foundation, and a grant from Genta Inc.
[2] To whom requests for reprints should be addressed, at Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021. Phone: (212) 639-2364; Fax: (212) 717-3817.

first 24 h of treatment, all patients were hospitalized for PK[3] studies. Remaining treatment was administered as an outpatient. This trial was reviewed and approved in advance by Memorial Sloan-Kettering Cancer Center's Institutional Review Board, and written informed consent was obtained from each patient.

Study Design. Cohorts of 3–6 patients each were defined by antisense dose (*i.e.*, 0.6, 1.3, 1.7, 2.3, 3.1, 4.1, 5.3, and 6.9 mg/kg/day). The starting dose of 0.6 mg/kg/day was one-half the s.c. dose at which toxicity had first been observed in a prior study, based on an assumed 60% bioavailability for the s.c. dose. All patients received an initial 2-week infusion of oligonucleotide, followed by an observation period. There were two treatment schedules in the trial:

1) 2 weeks on/4 weeks off for a total of three cycles;

2) 2 weeks on/2 weeks off for one cycle, then 3 weeks on/1 week off for two additional cycles.

Cohorts 5 (3.1 mg/kg/day) and 6 (4.1 mg/kg/day) were pre-established at 6 patients for the purpose of ensuring safety after the infusion duration was increased from 2 weeks to 3, and the duration of the breaks between cycles was reduced from 3 weeks to 1 (see Table 2).

Table 1 Patient characteristics (n = 35)

	No. of patients
Primary Tumor	
Prostate	23
Sarcoma	1
Rectal	1
Bladder	1
Renal[a]	7
Esophageal	1
Unknown	1
Median Age, years	65 (range, 41–77)
Median KPS	80 (range, 70–90)
Prior Therapy	
Surgery	21
Radiation	22
Hormones	23
Immunotherapy	6
Chemotherapy	28
1 regimen	8
2 regimens	10
3 regimens	6
>3 regimens	3

[a] Three renal patients also had stable, hormone-sensitive prostate cancer.

End Points

PK Studies. Blood was drawn at the start of each cycle at baseline, then 0.5, 2, 4, 6, 24, and 48 h after the pump was started. At termination, blood was drawn before discontinuation and at 0.5, 2, 4, 6, 24, and 48 h thereafter.

Intact oligonucleotide concentration in plasma was detected using a modular high-performance liquid chromatography system that consisted of an SP8800 (Spectra-Physics) gradient pump, an ISS100 autosampler (Perkin-Elmer), a Spectra 100 UV detector (Spectra-Physics) at 254 nm, WINner chromatographic station, and a Pharmacia HiTrap Q 1-ml (Supelco) column. Because the back pressure of this column was low, 10 feet of PEEK tubing (0.005-inch inside diameter) were added between the autosampler and the pump, which allowed the pump to work at 4–500 p.s.i. A short length of tubing (0.5-mm internal diameter) was inserted into the column outlet to reduce possible mixing with the large diameter outlet. The column was then connected to standard tubing with M6 to 10–32 union (Upchurch).

The sample was prepared by adding an equal volume of plasma to 10 mM ammonium hydroxide to denature plasma proteins. This procedure was performed to minimize the protein-bound fraction of the drug, which was not specifically assayed. With this technique, the recovery of drug from plasma samples loaded with a predetermined amount of G3139 exceeded 90%, relative to identically spiked aqueous controls.

The sample was then extracted once with chloroform, and 100 μl of the aqueous solution were injected onto the column. Two solutions were used for the elution. Solution A was composed of 25 mM disodium hydrogen phosphate, and solution B was composed of 25 mM disodium hydrogen phosphate plus 2 M sodium bromide. The sample was gradient eluted over 15 min using 100% solution A, followed by 40% solution A and 60% solution B. The column was then washed 10 min later in solution B for 10 min and was then re-equilibrated using solution A before the next sample was run. This assay has a sensitivity of 0.2 μg/ml and is linear to 10 μg/ml. Interday/intraday variation was ≤10%. PK analysis was performed with WinNonlin 2.1, model 2, one compartment with constant i.v. input, first-order output.

Safety and Response Criteria. Toxicities were assessed by the National Cancer Institute Common Toxicity Criteria version 2.0.[4] Standard response criteria were used. Patients were imaged after each cycle of treatment.

RESULTS

Patients. Thirty-five patients were registered and treated between August 1997 and September 1999. Their diagnoses, prior treatments, and other demographic characteristics are described in Table 1, and the treatments that patients received are described in Table 2. Seventeen patients received treatment on schedule 1, and 18 patients were treated on schedule 2. Of note, an additional patient was added to Cohort 6 because 1 patient died during the first cycle of treatment attributable to a post-obstructive pneumonia. The final 8 patients treated received antisense therapy alone for the first cycle, then antisense in combination with paclitaxel for cycles 2 and 3. Only the data from treatment with BCL-2 antisense alone are presented here. The data regarding combination therapy will be presented in a separate future report.

Safety. Table 3 describes the adverse events observed during treatment. At the end of the 14-day infusion, 1 patient receiving drug at 2.3 mg/kg/day developed grade 3 leukopenia that resolved spontaneously off treatment ≤48 h. Two patients who

[3] The abbreviations used are: PK, pharmacokinetic; Css, steady-state concentration; PBMC, peripheral blood mononuclear cell.

[4] Internet address: http://ctep.info.nih.gov.

Table 2 Treatment, dose escalation, and cycles given per cohort

Level	G3139 dose (mg/kg/day)	Duration of infusion & breaks per cycle	Cohort size	Mean no. of cycles completed	Range
I	0.6	2 weeks on/4 weeks off, max. 3 cycles	3	2.3	(1–3)
II	1.3	Same as above	3	1.0	1
III	1.7	Same as above	3	1.3	(1–2)
IV	2.3	Same as above	6	1.3	(1–3)
V "a"	3.1	Same as above	2		
V "b"	3.1	2 weeks on/2 weeks off for 1 cycle, then 3 weeks on/1 week off for max. 2 cycles	4		
			Total = 6	2.0	(1–3)
VI	4.1	Same as above	7	1.7	(1–3)
VII	5.3	Same as above	4	2.3	(1–3)
VIII	6.9	Same as above	3	3.0	(1–4)
Total			35		

Table 3 Adverse events (n = 35)

	Event	Grade 1	Grade 2	Grade 3	Grade 4	Total
Constitutional	Fatigue	16 (46%)	6 (17%)	1 (3%)	0 (0%)	23 (66%)
Gastrointestinal	Transaminitis	10 (29%)	3 (9%)	1 (3%)	0 (0%)	14 (40%)
	Diarrhea	10 (29%)	0 (0%)	0 (0%)	0 (0%)	10 (29%)
	Abdominal pain	6 (17%)	0 (0%)	1 (3%)	0 (0%)	7 (20%)
	Hepatic enlargement	0 (0%)	1 (3%)	1 (3%)	0 (0%)	2 (6%)
Genitourinary	Urinary retention	0 (0%)	0 (0%)	1 (3%)	0 (0%)	1 (3%)
Hematologic	Anemia	12 (34%)	4 (11%)	0 (0%)	0 (0%)	16 (46%)
	Thrombocytopenia	12 (34%)	1 (3%)	0 (0%)	0 (0%)	13 (37%)
	Leukopenia	6 (17%)	5 (14%)	1 (3%)	0 (0%)	12 (34%)
	Thrombosis	0 (0%)	0 (0%)	2 (6%)	0 (0%)	2 (6%)
Infection	Infection	0 (0%)	1 (3%)	1 (3%)	1 (3%)	3 (9%)
Chemistry	Hyperglycemia	10 (29%)	2 (6%)	1 (3%)	0 (0%)	13 (37%)
	Alkaline phosphatase	9 (26%)	2 (6%)	0 (0%)	0 (0%)	11 (31%)
Pulmonary	Dyspnea	2 (6%)	7 (20%)	0 (0%)	1 (3%)	10 (29%)
Renal	Creatinine	13 (37%)	1 (3%)	0 (0%)	0 (0%)	14 (40%)
Miscellaneous	Bone pain	0 (0%)	3 (9%)	2 (6%)	0 (0%)	5 (14%)
	Skin rash	3 (9%)	1 (3%)	1 (3%)	0 (0%)	5 (14%)
	Palpitations	0 (0%)	0 (0%)	1 (3%)	0 (0%)	1 (3%)

received antisense oligonucleotide at 6.9 mg/kg/day developed elevated serum transaminase levels, grades 2–3. One patient developed a drug-induced rash and angioedema that resolved with steroids and discontinuation of G3139. One patient died of sepsis related to a tumor-induced postobstructive pneumonia, and 1 patient was hospitalized because of grade 4 dyspnea, likely related to tumor progression. Isolated instances of grade 3 hepatomegaly, palpitations, urinary retention, pathological fracture, and abdominal pain were most likely related to progressive disease.

A subgroup of patients (66%) experienced some degree of fatigue with antisense therapy, and in only 1 patient (1.7 mg/kg/day) was it grade 3. Fatigue occurred at all doses, with no correlation between severity and dose. In addition, grade 1–2 anorexia, increased serum creatinine concentration, dyspnea on exertion, hot flashes, malaise, and gastrointestinal complaints were frequent and may have been drug related but not dose related.

Three patients were hospitalized for port-related thromboses or infections. This reaction limited therapy in 1 patient, whereas the other 2 patients were able to continue treatment. These events were possibly related to treatment.

PKs. The results of PK studies are summarized in Table 4. The maximum serum concentration and the area under the curve increased with dose. The terminal plasma half-life of drug was 2 h at all dose levels. The Css remained constant throughout the infusion. Therefore, the 24-h plasma level represents the Css. In Fig. 1, the mean Css for each cohort is plotted on a linear scale, and the plasma levels increased linearly with doses $\leq$5.3 mg/kg/day. At 6.9 mg/kg/day, the plasma level was higher than projected. The mechanism of elimination was not determined in this study, although predominantly renal excretion has been reported (30).

Effects on Bcl-2 Protein Expression. Western blots were performed on PBMCs on selected patients. These studies were exploratory in nature; a sample blot is shown in Fig. 2, illustrating the decline in bcl-2 protein levels observed in 1 patient with treatment.

Clinical Effects. No major antitumor responses were observed. Of the 35 patients, 13 (37%) patients had stable disease during treatment. Twenty (57%) patients progressed, and 2 (6%) were not evaluable for response.

DISCUSSION

The present study examined the PK profile and side effects of BCL-2 antisense oligonucleotide. Treatment administered as

Table 4 Pharmacokinetic properties[a]

Dose level	Patient	C_{max} (μg/ml)	AUC (μg/ml/h)[b]	Half-life (h)
4.1	1	2.61	880	1.52
	2	2.32	779	2.87
	3	2.93	985	1.74
	4	3	1010	2.22
	5	2.43	816	1.72
	Avg. (SD)	2.66 (0.3)	894 (102)	2 (0.54)
5.3	1	5.4	1812	2.7
	2	2.8	954	1.5
	3	4.64	1562	2.05
	Avg. (SD)	4.28 (1.34)	1443 (441)	2.08 (0.6)
6.9	1	8.2	2762	2.1
	2	8.2	2752	1.4
	3	6.35	2135	2.8
	Avg. (SD)	7.58 (1.07)	2550 (359)	2.1 (0.7)

[a] Pharmacokinetic properties were not obtainable on 2 patients treated at 4.1 mg/kg/day.
[b] AUC, area under the curve.

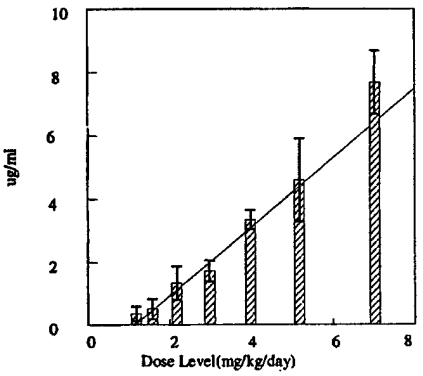

Fig. 1 Mean 24-h plasma drug levels (and SD) of each cohort. The increase in plasma level was linear with each cohort until 6.9 mg/kg/day, when the mean plasma level was somewhat higher than expected.

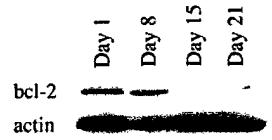

Fig. 2 Western blot of bcl-2 levels in PBMCs from a patient treated with bcl-2 antisense at 4.1 mg/kg/day for 2 weeks.

a continuous i.v. infusion was safe, well tolerated, and had a half-life of 2 h. Fatigue and elevated transaminase levels were the only adverse events likely related to treatment that were dose limiting. These effects, as well as hematological abnormalities and other adverse events not seen in this study, have been reported previously with the administration of other phosphorothioate oligonucleotides (31–33).

In our study, i.v. BCL-2 antisense oligonucleotide had a half-life of 2 h, whereas the half-life of the same agent delivered s.c. has been reported to be ~7 h (29). Steady-state serum plasma levels were achieved in ≤10 h and remained constant throughout treatment. These data contrast with s.c. delivery in which steady-state plasma levels were not seen until 48 h (29).

Css varied linearly as a function of delivered dose ≤5.3 mg/kg/day. At 6.9 mg/kg/day, the plasma levels were higher than predicted. Css > 1 μg/ml, the level at which BCL-2 antisense oligonucleotide is reported to have antitumor activity and to suppress bcl-2 protein in animal models and humans (30, 34), was achieved at doses of >2.3 mg/kg/day. These findings are consistent with those of Jansen *et al.* (35), who also tested this molecule by i.v. infusion.

A maximum tolerated cumulative dose of 147.2 mg/m^2 (4 mg/kg/day) was reported recently in a trial in which this drug was delivered by continuous s.c. infusion to patients with lymphoma. Dose-limiting reactions in that study were thrombocytopenia, fever, hypotension, and infusion site reactions. The corresponding Css was 3.16 μg/ml (range, 4.17–7.37). By contrast, we observed no such toxicities in patients treated ≤5.3 mg/kg/day, with a mean Css of 4.6 μg/ml (SD 0.33). Patients treated at 6.9 mg/kg/day achieved a mean Css 7.67 μg/ml (SD 1), which was associated with reversible transaminitis.

We explored using Western blots of PBMCs to assess changes in bcl-2 protein levels. Although our own data are preliminary, and one cannot assume that changes in PBMCs reflect events within the tumor, recent human trials have shown that treatments using this BCL-2 oligonucleotide are associated with reductions in intratumoral bcl-2 protein levels (29, 35). These studies were performed using tissue acquired through superficial tumor biopsies of melanoma patients and circulating lymphoma cells in patients with non-Hodgkin's lymphoma. Jansen *et al.* (35) found that protein levels in melanoma cells diminished by 20–70% in ≤1 week of initiating the infusion. As prolonged infusions of antisense are not required to decrease bcl-2 protein levels and as doses in the range of 4.1–6.9 mg/kg/day have been reported to have biological activity, dose escalations in our study were stopped in favor of studies involving shorter infusion times using combinations with chemotherapy. These studies are ongoing, as are determinations of the association between clinical effects, dose, and the timing and degree of bcl-2 protein reduction.

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