Phase I Trial of BCL-2 Antisense Oligonucleotide (G3139) Administered by Continuous Intravenous Infusion in Patients with Advanced Cancer¹


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-XL, 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/mm³, platelet count > 100,000/mm³, 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.
first 24 h of treatment, all patients were hospitalized for PK³ 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**  
<table>
<thead>
<tr>
<th>Primary Tumor</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>23</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Rectal</td>
<td>1</td>
</tr>
<tr>
<td>Bladder</td>
<td>1</td>
</tr>
<tr>
<td>Renal†</td>
<td>7</td>
</tr>
<tr>
<td>Esophageal</td>
<td>1</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
</tr>
<tr>
<td>Median Age, years</td>
<td>65 (range, 41–77)</td>
</tr>
<tr>
<td>Median KPS</td>
<td>80 (range, 70–90)</td>
</tr>
</tbody>
</table>

³ 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) was 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 mM 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. 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
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. TheCss 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.

**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
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 phosphothioate 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).

$C_{\text{ss}}$ varied linearly as a function of delivered dose $\leq 5.3$ mg/kg/day. At 6.9 mg/kg/day, the plasma levels were higher than predicted. $C_{\text{ss}} > 1 \mu g/ml$, 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 $C_{\text{ss}}$ was 3.16 $\mu g/ml$ (range, 4.17–7.37). By contrast, we observed no such toxicities in patients treated at 6.9 mg/kg/day, with a mean $C_{\text{ss}}$ of 7.67 $\mu g/ml$ (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 $\leq 1$ 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.

**Table 4** Pharmacokinetic properties $^a$

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Patient</th>
<th>$C_{\text{max}}$ (µg/ml)</th>
<th>AUC (µg/ml/h)$^b$</th>
<th>Half-life (h)</th>
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<tbody>
<tr>
<td>4.1</td>
<td>1</td>
<td>2.61</td>
<td>880</td>
<td>1.52</td>
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<tr>
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<td>2</td>
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<tr>
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<td>3</td>
<td>2.93</td>
<td>985</td>
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</tr>
<tr>
<td></td>
<td>4</td>
<td>3</td>
<td>1010</td>
<td>2.22</td>
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<tr>
<td></td>
<td>5</td>
<td>2.43</td>
<td>816</td>
<td>1.72</td>
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<tr>
<td>Avg. (SD)</td>
<td></td>
<td>2.66 (0.3)</td>
<td>894 (102)</td>
<td>2 (0.54)</td>
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<tr>
<td>5.3</td>
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<td></td>
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<tr>
<td>Avg. (SD)</td>
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<td>1443 (441)</td>
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<tr>
<td>6.9</td>
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</tr>
<tr>
<td></td>
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<td>6.35</td>
<td>2135</td>
<td>2.8</td>
</tr>
<tr>
<td>Avg. (SD)</td>
<td></td>
<td>7.58 (1.07)</td>
<td>2550 (359)</td>
<td>2.1 (0.7)</td>
</tr>
</tbody>
</table>

$^a$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.
REFERENCES


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