A pilot trial of G3139, a \textit{bcl}-2 antisense oligonucleotide, and paclitaxel in patients with chemorefractory small-cell lung cancer

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Background: Chemorefractory small-cell lung cancer (SCLC) is defined as disease that progresses during primary therapy or within 3 months of completion of primary therapy. Patients with chemorefractory SCLC have a very poor prognosis, and no treatment has been shown to be of significant clinical benefit. Elevated expression of Bcl-2 is found in the majority of SCLCs and has been associated with therapeutic resistance. Suppression of Bcl-2 levels through the use of G3139, an antisense oligonucleotide complementary to the mRNA encoding Bcl-2, might increase the antitumor efficacy of cytotoxic therapy.

Patients and methods: Twelve patients with chemorefractory SCLC participated in this pilot trial of paclitaxel combined with G3139. G3139 was given by continuous i.v. infusion over 7 days at a fixed dose of 3 mg/kg/day. Paclitaxel dose was initially 175 mg/m\textsuperscript{2} on day 6, but was decreased to 150 mg/m\textsuperscript{2} due to myelosuppression observed in two of the three patients treated in the first dose cohort.

Results: The combination of paclitaxel at 150 mg/m\textsuperscript{2} and G3139 at 3 mg/kg/day was found to be feasible and well tolerated. No objective responses were observed, but two patients had stable disease, one remaining stable on therapy for >30 weeks. Plasma G3139 levels were determined, and were found to be highest in the patient with prolonged stable disease, suggesting that individual variation in metabolism and clearance of the antisense oligonucleotide may influence activity.

Conclusions: This study demonstrates that G3139 can be combined with paclitaxel in a cytotoxic dose range, and suggests that a similar combination be tested for activity in the context of chemoresponsive disease.

Key words: apoptosis, chemoresistance, phosphorothioate, translational inhibition

Introduction

Lung cancer was diagnosed in ~164000 patients in the USA in 2000, and accounted for 157000 deaths [1]. Approximately 25\% of these patients had small-cell lung cancer (SCLC). Despite response rates to initial therapy as high as 75–85\%, SCLC is characterized by very high recurrence rates [2–4]. Recurrent SCLC is almost universally fatal. Among patients with recurrent SCLC, two distinct subsets of patients have been defined: a subset with chemosensitive SCLC, defined as disease that progresses after an interval of at least 3 months after primary platinum-based chemotherapy; and a subset with chemorefractory SCLC, defined as disease that progresses either during or within 3 months of completion of initial therapy [5]. Patients with chemorefractory SCLC have a particularly poor prognosis. No chemotherapeutic regimen has been shown to be of significant clinical benefit for patients with chemorefractory SCLC. Topotecan, which has a reported 38\% response rate in chemosensitive relapsed SCLC, has only a 2–6\% response rate in patients with chemorefractory disease [5–7].

Paclitaxel has been reported to be an active agent in SCLC, with first line efficacy comparable to cisplatin [8, 9]. Smit et al. reported that single agent paclitaxel had a response rate of 29\% in 24 patients, with residual SCLC within 3 months of primary therapy [10]. These patients may not have all met criteria of chemorefractory SCLC (residual disease versus progressive disease within 3 months). Although the response rate was encouraging, no complete responses were observed,
and the median survival was ~100 days. This study suggested that while paclitaxel had activity in residual SCLC, the clinical impact of this activity was minimal.

The oncogene bcl-2 is expressed in 83–90% of SCLC, and therefore represents a potential therapeutic target in this disease [11–13]. Several studies involving both cell culture and animal models have suggested that overproduction of Bcl-2, or related inhibitors of cell death, can confer multidrug chemotherapeutic resistance [14–22]. Conversely, suppression of Bcl-2 in tumors in which it is expressed may increase chemotherapeutic efficacy [23, 24].

G3139, or Genasense, is an 18-base antisense phosphorothioate oligonucleotide complementary to the bcl-2 mRNA in the region encoding the first six amino acids of Bcl-2 [25]. The mechanism of action of G3139, and other antisense oligonucleotides, is thought to depend on binding of the antisense oligonucleotide to the cognate sequence within the mRNA, resulting in inhibition of mRNA translation and RNase H-mediated mRNA degradation [26, 27]. Preclinical and clinical studies have demonstrated that i.v. administered G3139 can be taken up by tumor cells, and can result in reduction of Bcl-2 protein production [28–32].

Preclinical studies have demonstrated synergy between G3139 and taxane therapy [33, 34]. In a murine xenograft model of human breast cancer cell lines that overexpress Bcl-2, synergistic cytotoxicity and durable tumor regression were noted in animals treated with docetaxel together with G3139, but not in animals treated with either agent alone, or animals treated with docetaxel and control mismatch oligonucleotides [34]. Recent data suggest that paclitaxel cytotoxicity may be mediated in part through phosphorylation and functional inhibition of Bcl-2, and that up-regulation of Bcl-2 expression may be a mechanism of resistance to paclitaxel [35, 36].

This trial was designed to evaluate the safety, feasibility and toxicity profile of the combination of paclitaxel and G3139 in patients with chemoresistant SCLC.

Patients and methods

Patient population
All patients on this trial were adults with Cancer and Leukaemia Group B (CALGB) performance status (PS) ≤ 2 and a histologically confirmed diagnosis of SCLC with documented progressive disease either during or within 3 months of completing initial platinum-based chemotherapy. All patients had measurable disease. At the time of enrollment, all patients had serum creatinine ≤1.5 mg/dl, serum bilirubin ≤1.5 mg/dl, serum aspartate aminotransferase and alanine aminotransferase ≤2.5 × upper limit of normal, absolute neutrophil count >1500 cells/mm³, platelet count >100,000/mm³, hemoglobin >9.0 g/dl and a normal blood coagulation profile as assessed by prothrombin time and activated partial thromboplastin time. All patients provided written informed consent before study enrollment or performance of study-related procedures.

Drug administration
G3139, an 18-base phosphorothioate oligonucleotide (5′-TCTCCAGC-GTGCGCCAT-3′) was administered by continuous i.v. administration through a 0.22 μm in-line filter using a portable volumetric infusion pump over 7 days (day 1–8). Paclitaxel was administered by a 3-h constant rate i.v. infusion on day 6 of each cycle. This schedule was chosen, based on protein kinetic and pharmacological considerations, to permit sufficient time for the G3139 infusion to maximally suppress Bcl-2 protein levels (Bcl-2 t1/2 = 12–24 h) before paclitaxel administration on day 6, and to prolong Bcl-2 suppression for the 48 h after paclitaxel, during which time paclitaxel-induced apoptosis may be maximal. No premedication was used before initiation of G3139. Before paclitaxel administration, all patients were given 20 mg i.v. dexamethasone, 50 mg i.v. diphenhydramine, 20 mg i.v. famotidine and 24 mg oral ondansetron. Cycle length was 21 days, no therapy was planned for days 9–21.

Study design
This was a single arm study designed to establish toxicity, feasibility and a recommended dose for the combination of paclitaxel and G3139 in this patient population. G3139 was administered at a fixed dose of 3 mg/kg/day. The initial paclitaxel dose was 175 mg/m². In the event of cycle 1 dose-limiting toxicity (DLT) in at least two of six patients, the paclitaxel dose was to be decreased in 25 mg/m² increments in subsequent dose cohorts, until a dose was reached at which less than two of six patients experienced DLT. Patients not completing cycle 1 for reasons other than DLT were considered not evaluable for toxicity and were replaced to meet enrollment criteria. In the event of DLT, therapy was withheld pending resolution of toxicity. Therapy could then be resumed at the next lower dose level, at the discretion of the patient and the treating physician.

Toxicity and response evaluation
Toxicity was assessed using NCI Common Toxicity Criteria version 2.0. DLT was defined as any grade 3 or 4 toxicity with the exception of grade 3 neutropenia, alopecia, nausea or vomiting.

Response was evaluated by computerized tomography (CT) in the last week of every second cycle, using a direct comparison with a CT scan obtained in the 2 weeks before initiation of therapy. Standard two-dimensional measurement criteria were used to classify the response. Patients with stable disease were allowed to continue therapy.

Pharmacokinetic analysis
Intact plasma G3139 levels were determined by anion exchange high performance liquid chromatography in cycle 1 on day 6 (at the time of paclitaxel administration) and day 8 (before discontinuation of G3139), as previously described [32, 37]. A plasma sample was also obtained before initiation of therapy, as a negative control. This analysis was performed by Oread Inc. (Lawrence, KS, USA).

Paclitaxel pharmacokinetic analysis was not performed in this trial. It was predicted that if G3139 resulted in significant inhibition of paclitaxel clearance, this would be detected as increased paclitaxel-related toxicity, particularly peripheral neuropathy.

Western blotting and protein quantitation
Blood draws were performed on day 1, before initiation of treatment, and day 6, before paclitaxel administration. Peripheral blood mononuclear cells were isolated using Vacutainer CPT tubes (Becton-Dickinson; Franklin Lakes, NJ, USA), according to the manufacturer’s instructions.
Protein extracts were prepared by resuspension in RIPA buffer (Roche; Indianapolis, IN, USA). Western blotting was performed by electrophoresis through a 14% polyacrylamide gel, followed by electroblotting to nitrocellulose. The blot was first probed with monoclonal anti-Bcl-2 clone 124 (DAKO; Carpinteria, CA, USA), and developed using an enhanced chemiluminescence (ECL) kit (Amersham/Pharmacia; Piscataway, NJ, USA) according to the manufacturer’s instructions. The blot was then stripped and re-probed using monoclonal anti-β-actin clone AC-15 (Sigma; St Louis, MO, USA). Quantitative densitometry was assessed using the ChemiImager 5500 system (Alpha Innotech; San Leandro, CA, USA).

Results

Patient characteristics and response
A total of 12 patients (six male, six female) were enrolled on this study (Table 1). Median age was 55 years, with a range of 42–68. All patients had been treated with etoposide and either cisplatin or carboplatin, and had documented progressive disease within 3 months of completing this therapy. Five patients had been treated with at least one, and up to three, additional chemotherapeutic regimens. Four patients had progressed following prior paclitaxel therapy. Six patients had been previously treated with gamma radiation; three patients had CNS radiation for brain metastases, three patients had radiation to the chest, two patients had radiation to the vertebral column and one had radiation to the femur. One patient required a pericardial window to drain a symptomatic malignant pericardial effusion before enrollment. Three patients were judged to have a CALGB PS of 0, five had PS of 1, and four had PS of 2 before initiating therapy.

There were no objective responses. One patient had an evident hypersensitivity reaction upon initiation of G3139 and was not evaluable for response. Of the remaining 11 patients, disease stabilization after two cycles was observed in four patients. However, two of these four patients had clinical or objective disease progression within a month of that evaluation, and stopped therapy after cycle 3. One patient had progressive disease in the liver after cycle 6, and another patient discontinued therapy with persistent stable disease after a total of 10 cycles. The latter patient had an improvement in PS from 2 to 0 over the course of G3139/paclitaxel therapy, and gained back >20 kg of weight she had lost since the onset of her disease. She remained without evident progression for more than a year, and is currently alive 20 months after starting therapy.

Toxicity
The initial dose level evaluated was paclitaxel 175 mg/m² combined with G3139 3 mg/kg/day continuous i.v. infusion. Two of the first three patients treated at this dose level experienced dose-limiting hematological toxicity in cycle 1. One of these patients had completed a course of lumbosacral and pelvic radiation immediately before starting G3139, and was found to have grade 3 leukopenia and thrombocytopenia on day 6 of therapy, before administration of paclitaxel. The second of these patients experienced grade 3 leukopenia, neutropenia, thrombocytopenia and anemia on day 16 of cycle 1. Shortly thereafter she was hospitalized with a pulmonary embolus and became acutely short of breath, requiring intubation and mechanical ventilation. A chest X-ray revealed disease progression. She died 3 days later. The third patient treated with paclitaxel 175 mg/m² experienced no leukopenia or neutropenia, and only grade 1 thrombocytopenia, but had disease progression after two cycles of therapy.

Subsequent patients were treated at a paclitaxel dose of 150 mg/m², combined with G3139 of 3 mg/kg/day. Only one of eight patients at this dose level experienced any cycle 1 hematologic toxicity greater than grade 2, this patient experienced grade 3 leukopenia and thrombocytopenia on day 6 of therapy, before administration of paclitaxel. The second of these patients experienced grade 3 leukopenia, neutropenia, thrombocytopenia and anemia on day 16 of cycle 1. Shortly thereafter she was hospitalized with a pulmonary embolus and became acutely short of breath, requiring intubation and mechanical ventilation. A chest X-ray revealed disease progression. She died 3 days later. The third patient treated with paclitaxel 175 mg/m² experienced no leukopenia or neutropenia, and only grade 1 thrombocytopenia, but had disease progression after two cycles of therapy.

Non-hematological toxicity on all cycles is summarized in Table 3. Grade 3 or 4 non-hematological toxicity was observed in only one patient at the paclitaxel dose of 150 mg/m². This individual developed a post-obstructive pneumonia (grade 4 respiratory toxicity, grade 3 infection and fatigue) complicated by progressive disease during cycle 1. Fatigue was the most consistent toxicity, reported during cycle 1 in all but two patients, but was typically grade 1 or 2. In contrast to studies of

Table 1. Patient demographics

| Total enrolled | 12 |
| Gender | 6 male, 6 female |
| Age | Median 55, Range 42–68 |
| Performance status | |
| 0 | 3 |
| 1 | 5 |
| 2 | 4 |
| Number of prior chemotherapy agents | |
| 2 | 7 |
| 3 | 3 |
| 4 | 1 |
| 5 | 1 |
| Prior paclitaxel | 4 |
| Prior radiation therapy (not CNS) | 6 |
| Cycles completed | |
| 0 | 1 |
| 1 | 3 |
| 2 | 4 |
| 3 | 2 |
| 6 | 1 |
| 10 | 1 |
other phosphorothioate oligonucleotides [38], fever during antisense administration occurred in only one patient on this trial.

### Pharmacokinetic analysis

Previous pharmacokinetic studies have determined that the plasma $t_{1/2}$ of G3139 is <24 h [32], so it was expected that the G3139 level on day 6 would represent a steady state concentration. Plasma G3139 levels were determined on day 6, to evaluate antisense drug levels at the time of paclitaxel administration, and on day 8, to evaluate whether paclitaxel administration significantly altered G3139 steady state concentration. There was no consistent difference between the G3139 levels measured on day 6 and on day 8, suggesting that paclitaxel did not alter G3139 kinetics.

Previous work has also suggested that Bcl-2 protein levels are most consistently suppressed if plasma G3139 levels are >1–2 µg/ml [32, 37, 39]. Notably, the only patient on this study to have G3139 levels consistently >2 µg/ml was the patient with prolonged stable disease over 10 cycles (Figure 1). The other patient with stable disease up to cycle 6 also had relatively high plasma G3139 levels on both day 6 and 8 (averaging ~2 µg/ml).

### Bcl-2 protein levels

The relatively high levels of G3139 in the individual with prolonged stable disease suggested that Bcl-2 levels may be significantly suppressed in this patient. To examine this possibility, a western blot was performed using peripheral blood mononuclear-cell protein extracts from duplicate blood samples taken before treatment (day 1) and at the time of first paclitaxel administration (day 6). Consistent with the anticipated activity of G3139, suppression of Bcl-2, but not of a control protein ($\beta$-actin), was observed (Figure 2). The ratio of Bcl-2 protein level (day 6:day 1) was $0.70 \pm 0.05$; the $\beta$-actin level in the same samples remained constant, with a ratio of $0.99 \pm 0.06$.

### Discussion

This trial investigated the combination of paclitaxel and G3139, an antisense oligonucleotide directed against bcl-2, in patients with chemorefractory SCLC. This was an initial safety and tolerability study of this combination in a heavily pretreated population. The combination, at a G3139 dose of 3 mg/kg/day for 7 days and paclitaxel dose of 150 mg/m$^2$ over...
3 h on day 6, was found to be feasible and tolerable in this context. DLT was myelosuppression, with cycle 1 grade 3 leukopenia and thrombocytopenia in two of the three patients treated in the first dose cohort (paclitaxel dose 175 mg/m²). This toxicity may be primarily attributable to paclitaxel, and G3139 dose escalation was not performed in this study. Subsequent single agent phase I testing in patients with advanced solid tumors has demonstrated safety and tolerability of G3139 at doses up to 14 mg/kg/day. G3139 is currently being administered at 7 mg/kg/day in combination with other cytotoxic chemotherapy, including dacarbazine in patients with malignant melanoma, and irinotecan in patients with colorectal cancer [39, 40]. Clearly, it would be of interest to test higher doses of G3139 in combination with either paclitaxel or other cytotoxic therapy in this disease.

The rationale behind the combination of paclitaxel and G3139 was that Bcl-2 is commonly expressed in SCLC and has been associated with resistance to taxanes. Preclinical data suggested that suppression of Bcl-2 by G3139 can synergize with taxane therapy against solid tumors in vivo [33, 34]. Clearly, it would be of interest to test higher doses of G3139 in combination with either paclitaxel or other cytotoxic therapy in this disease.

First, the paclitaxel dose may have been below a necessary threshold. A paclitaxel dose of 150 mg/m² every 21 days may be considered relatively low by many investigators, and some prior studies have suggested a measurable benefit of increased paclitaxel dose intensity [41]. However, other studies have failed to support a clear dose–response relationship for taxane therapy in lung cancer [42]. Therefore, although a suboptimal paclitaxel dose may have contributed to the lack of objective response, we believe this is likely to be a relatively minor factor.

Secondly, the G3139 dose level chosen may have been insufficient to suppress expression of the target gene bcl-2. Prior and ongoing studies of G3139 have suggested that consistent suppression of Bcl-2 protein levels may depend on achieving a threshold G3139 concentration. The observed levels on days 6 and 8, in most cases <2 µg/ml, are consistent with the suggestion that an escalated G3139 dose should be considered. The intriguing observation of levels ≥3 µg/ml only in the patient with prolonged stable disease further supports this hypothesis. Bcl-2 protein level was only moderately suppressed even in this patient, although peripheral blood Bcl-2 levels may be an inadequate reflection of intratumoral effects. Notably, the observed extent of Bcl-2 suppression in this patient was similar to the intermediate levels of suppression (60% of baseline) associated with evident chemosensitization in patients with melanoma treated with G3139 and dacarbazine [40].

Thirdly, the Bcl-2 antisense oligonucleotide may not have been taken up by tumor cells, and therefore may not have gained access to the relevant intracellular mRNA. Data from tumor biopsies of a more readily accessible solid tumor, melanoma, have demonstrated intratumoral suppression of Bcl-2 [40]. In the design of the current trial, biopsies were permitted to assess intratumoral effects in patients with superficial lesions. However, due to poor PS and a lack of safely accessible tumors, no tumor biopsies could be obtained. Establishing

**Figure 1.** Plasma G3139 levels determined during cycle 1. (A) Data from patients with stable disease for six cycles and (B) with stable disease for 10 cycles are presented separately, with the bars indicating the day 6 and 8 values (only two data points per patient). Data from all other patients is combined; error bars represent standard deviation.

**Figure 2.** Suppression of Bcl-2 protein expression in patient with prolonged stable disease. Western blotting was performed using duplicate peripheral blood mononuclear-cell protein extracts prepared before initiation of therapy (day 1, pretreatment) and before administration of paclitaxel (day 6). The blot was probed sequentially for Bcl-2 and β-actin, and the ratios of protein levels on day 6 to that of day 1 were determined by quantitative densitometry. Error bars indicate standard deviation.
invasive tumor monitoring will be challenging in this patient population.

It has been noted that the absolute level of Bcl-2 may be less relevant than the ratio of Bcl-2 to Bax in determining apoptotic threshold [43]. Bax levels were not measured in the current trial and could have an impact on the efficacy of therapy. However, the rapid expansion of the known members of this complex gene family precludes a simple analysis of such a ratio; it is likely that the relative balance of the many active pro- and anti-apoptotic Bcl-2 family members contributes to apoptotic sensitivity. Within tumor types clearly associated with elevated Bcl-2 expression (follicular lymphoma, melanoma, small-cell lung carcinoma), both preclinical and clinical data suggest that suppression of the single target Bcl-2 can enhance both tumor cell killing and chemosensitivity [23–25, 28–31, 40].

Finally, the highly resistant nature of this subset of recurrent SCLC may not be amenable to chemosensitization by G3139. Antisense inhibition of Bcl-2 expression may increase therapeutic efficacy of cytotoxic agents given for chemoresponsive disease, as demonstrated in several preclinical models, without reversing the inherent therapeutic insensitivity of chemorefractory disease.

An ideal setting in which to evaluate the ability of G3139 to enhance responsiveness of chemosensitive SCLC would be in patients with previously untreated disease, where the response rate is high but response duration remains disappointingly short. In contrast to the heavily pretreated population here, in patients receiving first-line therapy it may also be possible to maintain a relatively high dose intensity of both the antisense oligonucleotide and the cytotoxic therapy. We have chosen to pursue this approach. Analysis of G3139 in combination with cytotoxic chemotherapy for patients with previously untreated SCLC is ongoing.

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