A Phase I Trial of c-Raf Kinase Antisense Oligonucleotide ISIS 5132 Administered as a Continuous Intravenous Infusion in Patients with Advanced Cancer

C. Casey Cunningham,1 Jon T. Holmlund, Joan H. Schiller, Richard S. Geary, T. Jesse Kwoh, Andrew Dorr, and John Nemunaitis

US Oncology, Dallas, Texas 75246 [C. C. C., J. N.]; Baylor University Medical Center, Sammons Cancer Center, Dallas, Texas [C. C. C., J. N.]; ISIS Pharmaceuticals, Carlsbad, California [J. T. H., R. S. G., T. J. K., A. D.]; and University of Wisconsin, Madison, Wisconsin [J. H. S.]

Abstract

Raf proteins play a central role in the mitogen-activated protein kinase signaling pathway and hence are involved in oncogenic transformation and tumor cell proliferation. ISIS 5132 is a 20-base antisense phosphorothioate oligodeoxyribonucleotide that specifically down-regulates c-raf expression. We report here an initial study of the safety and tolerability of an i.v. infusion of ISIS 5132 in patients with advanced cancer. A continuous i.v. infusion of ISIS 5132 was administered for 21 days every 4 weeks to 34 patients with a variety of solid tumors refractory to standard therapy. The dose of ISIS 5132 was increased in sequential cohorts of patients, as toxicity allowed, until a final dose of 5.0 mg/kg body weight was reached. Toxicity was scored by common toxicity criteria, and tumor response was monitored. Pharmacokinetic studies were performed for 30 patients treated at doses of ≤4.0 mg/kg/day. The initial dose of ISIS 5132 was 0.5 mg/kg body weight and was successfully increased incrementally to 5.0 mg/kg body weight. Toxicities through the 4.0 mg/kg dose level were not dose limiting. Side effects were minimal and could not be specifically related to ISIS 5132. Two patients had prolonged stabilization of their disease, and one patient with ovarian carcinoma had a significant response with a 97% reduction in CA-125 levels. ISIS 5132, an antisense oligonucleotide against c-raf, was well tolerated at doses up to and including 4.0 mg/kg/day by 21-day continuous i.v. infusion and demonstrated antitumor activity at the doses tested.

Introduction

During gene transcription, the duplex strand of DNA partially uncoils into two complementary strands, “sense” and “antisense.” The antisense strand is used as a template to generate sense mRNA that can be translated to produce the final protein product. Translation of individual mRNAs can be prevented by antisense oligonucleotides as short as 15–20 bases long, which bind by complementary Watson-Crick base pairing, resulting in destruction of the target mRNA by RNase H or steric inhibition of translation (reviewed in Ref. 1). This technique allows highly selective inhibition of the expression of specific proteins and is being increasingly investigated as a therapeutic modality in a variety of human diseases (2).

Raf kinases [of which three are presently known; A-, B-, and C-Raf (3)] are attractive targets for antisense therapy. These proteins are serine/threonine kinases that regulate mitotic signaling pathways, most notably the mitogen-activated protein kinase pathway (4, 5) that transmits signals from Ras. C-Raf also associates with Bcl-2, possibly playing a role in the regulation of apoptosis (6). Mutations in raf and ras (common in human tumors) may then contribute to oncogenic transformation (7–9). Inhibition of c-raf translation with an antisense oligonucleotide inhibits the growth of a human leukemia cell line (10). Finally, increased raf activity apparently correlates with the metastatic behavior of experimental cancer and is a poor prognostic factor in human breast cancer (9, 11).

Because the phosphodiester backbone of native oligonucleotides is susceptible to rapid degradation by nucleases in human serum, systemic administration of antisense oligonucleotides for therapy requires chemical modifications conferring relative resistance to nuclease activity to enhance stability in vivo. One modification substitutes a sulfur for an oxygen in the phosphodiester linkage between nucleotides, creating a phosphorothioate (7). One modification substitutes a sulfur for an oxygen in the phosphodiester linkage between nucleotides, creating a phosphorothioate (7). Antisense oligonucleotides with this backbone have been shown to be resistant to rapid degradation by serum nucleases in vitro. In cell culture, ISIS 5132 specifically reduced the expression of c-raf kinase mRNA and protein and inhibited cell proliferation in A549 human lung cancer cells. The IC50 for these effects was approximately 100 nM (12, 13). Furthermore, after repeated administration, ISIS 5132 reduced the expression of c-raf mRNA in xenografted A549 tumors (13). Finally, ISIS 5132 administered daily to mice at doses of 6–25 mg/kg/day suppressed the growth of several human tumor xenografts (12).

Therefore, we began a Phase I study to determine the safety...
Patients and Methods

Eligible patients had histologically documented solid malignancies of measurable or evaluable status refractory to standard therapy or for which no effective therapy existed. The patients also were at least 18 years of age or greater, had been untreated for at least 4 weeks before the study, had a Southwest Oncology Group performance status of ≤2, and demonstrated adequate hematopoietic, renal, and liver functions. Finally, the patients could not have any condition requiring the administration of therapeutic doses of anticoagulants, a history of coagulopathy or complement abnormality, or any coexisting medical problem of sufficient severity to limit full compliance with the study. Informed consent was obtained according to federal and institutional guidelines.

Cohorts of patients received sequential, ascending, multiple doses of ISIS 5132 administered as a continuous i.v. infusion for 21 consecutive days, followed by 1 week of rest (one cycle). The initial dose of ISIS 5132 was 0.5 mg/kg body weight, which was chosen because it was below dose levels observed to be nontoxic in primate and human studies of ISIS 5132 (12, 18). Subsequent doses were 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 mg/kg. Cohorts of three patients were evaluated at each dose level. If one of the three patients demonstrated evidence of treatment-related toxicity, an additional three patients were studied at the dose level before dose escalation. Dose escalation was stopped if two patients at any dose level developed evidence of treatment-related, dose-limiting toxicity.

ISIS 5132 was supplied by Isis Pharmaceuticals, Inc. (Carlsbad, CA) as a sterile solution. The total dose of ISIS 5132 administered over 7 consecutive days (168 h) was added to 250 ml of normal saline and infused via a portable volumetric infusion pump at a rate of 1.5 ml/h. This process was repeated three times over 21 consecutive days.

A complete medical history, physical examination, and blood studies, including routine laboratory studies, coagulation studies, and complement split products (Bb, C3a, and C5a), were performed before the first infusion of ISIS 5132 and repeated on days 7, 14, and 21. Routine laboratory studies included a complete blood count, differential WBC count, electrolytes, urea, creatinine, glucose, total protein, albumin, calcium, magnesium, phosphate, alkaline phosphatase, total bilirubin, alanine aminotransferase, AST, GGT, urinalysis, and clotting time (PT and aPTT). Complement split products were also measured 4 and 24 h after starting the infusion in cycle 1.

Assessment of measurable or evaluable disease was performed at baseline and after every other course. A complete response was defined as resolution of all measurable and evaluable disease for at least 4 weeks without worsening of disease-related symptoms or performance status. A partial response required at least a 50% reduction in the sum of the products of the maximum perpendicular diameters of measurable lesions and at least a 50% improvement in evaluable disease for at least 4 weeks. Progressive disease was defined as a 25% or greater increase in measurable disease or the appearance of new tumor lesions. Stable disease was defined as disease status failing to meet the criteria for response or progressive disease. Patients could continue treatment in the absence of disease progression or intolerable toxicity.

Pharmacokinetic studies of ISIS 5132 were obtained during the first three cycles of treatment. Whole blood samples were obtained for pharmacokinetic analysis at baseline (preinfusion) and just before the end of infusion on days 7 and 14. In cycle 1, specimens were also obtained 4 and 24 h after the start of infusion on day 0. On day 21, pharmacokinetic samples were obtained immediately before discontinuation of the i.v. infusion and 90, 120, 180, and 240 min after discontinuing the infusion. Urine was collected over a 48-h period, beginning with the infusion on day 1; an 8-ml aliquot was removed and stored immediately at −20°C for later analysis.

Drug analysis was performed by capillary gel electrophoresis by Covance Laboratories (Madison, WI) using a previously described method (23) on aliquots of each sample of plasma and

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*The abbreviations used are: aPTT, activated partial thromboplastin time; AST, aspartate aminotransferase; Cl, plasma clearance; C∞, steady-state plasma concentration.*

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**Table 1 Summary of patient characteristics**

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median patient age (range) (yrs)</td>
<td>56.2 (31–77)</td>
</tr>
<tr>
<td>Male:female</td>
<td>18:16</td>
</tr>
<tr>
<td>Tumor type</td>
<td></td>
</tr>
<tr>
<td>NSCLC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6</td>
</tr>
<tr>
<td>Colon</td>
<td>4</td>
</tr>
<tr>
<td>Breast</td>
<td>4</td>
</tr>
<tr>
<td>Renal</td>
<td>3</td>
</tr>
<tr>
<td>Ovarian</td>
<td>3</td>
</tr>
<tr>
<td>Rectal</td>
<td>2</td>
</tr>
<tr>
<td>Hepatocellular</td>
<td>2</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>2</td>
</tr>
<tr>
<td>Other&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup> NSCLC, non-small cell lung cancer.

<sup>b</sup> One each of the following tumor types: pancreas, melanoma, sarcoma, small cell lung cancer, prostate, esophageal, head and neck, and undifferentiated.
urine. Concentrations of full-length ISIS 5132 and of major metabolites (shortened oligonucleotides of 19, 18, and 17 nucleotides in length) were calculated by standard techniques.

The terminal plasma disposition half-life was calculated as $t_{1/2b} = \ln(2)/\beta$, where $\beta$ is the rate constant for the terminal decline in plasma ISIS 5321 concentration estimated by log-linear regression. $C_{av}$ values were determined by averaging the measured plasma concentrations at 24 h to 21 days during the infusion. CI was determined by dividing the zero order dose rate ($k_0$) by the calculated $C_{av}$. Area under the plasma concentration-time curve at steady state (AUC$_{ss}$) was then calculated by dividing the daily dose by CI. The apparent volume of distribution was calculated using the following equation: $V_{infusion} = CI/\beta$.

**Results**

Thirty-four patients were entered into the study (Table 1). Twenty-nine patients had received chemotherapy, immunotherapy, or some combination of these therapies, and 13 patients had also received prior radiotherapy. The dose escalation schema, as well as the number of patients and courses administered as a function of dose level, is depicted in Table 2. All patients received at least a single dose and were evaluable for toxicity. Six patients received $\geq 3$ cycles (range, 3–10 cycles).

**Toxic Effects of Treatment.** Toxicity was modest in all respects. Specifically, we found no relationship between ISIS 5132 infusion and activation of the complement system. Modest elevations in C3a, C5a, and Bb levels were sometimes present either at baseline or at inconsistent time points during the infusion, but these did not correlate with dose level. In all cases, no clear increase was seen with infusion of ISIS 5132. There were also no adverse events clinically suggestive of complement activation in any patient.

Similarly, there was no clear relationship between elevation of aPTT and oligonucleotide infusion. One patient did develop a grade 4 elevation of aPTT on day 7 of cycle 2 at the 2.0 mg/kg dose level. However, that patient had received a previous complete cycle without any elevation of aPTT. Five patients developed grade 1 elevations by the end of a cycle, but these occurred at all dose levels. All other elevations of aPTT occurred before infusion of ISIS 5132 began and did not increase during oligonucleotide infusion.

Grade 3 fever, associated with grade 4 hypotension, was observed in a patient with non-Hodgkin’s lymphoma treated at the 5.0 mg/kg dose level after 1 day of infusion. The fever recurred (grade 2) without hypotension when the patient was rechallenged at 50% dose reduction. A patient with renal cell carcinoma treated at the 1.5 mg/kg dose level also had fever in six separate cycles with fevers of grades 1, 2, and 3 occurring in two cycles each. Another patient with renal cell carcinoma treated at the 0.5 mg/kg dose level experienced a grade 1 fever during the first week of infusion in the first cycle, but this was in the setting of tumor progression, and the patient was withdrawn from the study.

ISIS 5132 had minimal effect on hematological parameters. One patient developed a grade 3 thrombocytopenia with the first cycle of the 1.5 mg/kg dose level but went on to receive a second cycle without any decrease. One patient each at the 2.0, 3.0, and 4.0 mg/kg dose levels developed grade 2 thrombocytopenia toward the end of a cycle. One patient developed grade 3 thrombocytopenia in the setting of sepsis occurring during week 3 of cycle 3 of the 1.0 mg/kg dose level. This patient had had two previous cycles without any decrease in platelet count or other significant event. Another
patient treated at the 1.0 mg/kg dose level developed sepsis at the start of therapy and, while septic, manifested grade 4 thrombocytopenia, grade 4 hyperbilirubinemia, and a grade 3 elevation in AST. One patient at the 5.0 mg/kg dose level had fever as a dose-limiting toxicity. Leukopenia was mild and, notably, no patient had neutropenia. Mild anemia was common, but most patients with anemia had it as a preexisting condition that did not worsen during the course of this study. Mild fatigue was also common. A summary of clinical toxicities is shown in Table 3.

**Pharmacokinetics.** Pharmacokinetic data from cycle 1 were available for all patients in cohorts receiving dose levels from 0.5–4.0 mg/kg (n = 30). A summary of pharmacokinetic parameters by dose is shown in Table 4 and Fig. 1. Consistent with rapid clearance from plasma, constant plasma concentrations of oligonucleotide (steady state) were achieved early in the infusion (by 24 h). The principal oligonucleotide species detected in the plasma was full-length intact ISIS 5132, which predominated at all time points and represented between 50% and 64% of the total measurable oligonucleotide (Table 4). The $C_{ss}$ values of intact ISIS 5132 given in Table 3 at 2.0 and 4.0 mg/kg/day correspond to approximately 110 and 420 nM, respectively. There was no difference in pharmacokinetic parameters between cycle 1 and subsequent cycles in 12 patients for whom pharmacokinetic data after cycle 1 were available (data not shown).

**Antitumor Activity.** No patients achieved a complete response. Of these, two patients had prolonged stabilization; one patient (treated at 1.5 mg/kg/day) with renal cell carcinoma remained stable for 9 months, and the other patient (treated at 4.0 mg/kg/day), who had pancreatic cancer, remained stable for 10 months.

**Discussion**

By virtue of their selectivity, antisense oligonucleotides offer the potential for achieving therapeutic effect while minimizing toxicity due to nonspecific interactions. The results of the present trial are consistent with that expectation. Although primate toxicology studies had identified complement activation and coagulopathy as possible toxic effects of ISIS 5132, neither was observed in the patients treated in this trial. This is likely due to the use of a 21-day continuous infusion dosing schedule, avoiding the high peak plasma oligonucleotide concentrations associated with complement activation and prolongation of aPTT in monkeys. Additionally, no toxic effects potentially referable to suppression of c-raf expression were observed, a result anticipated from the preclinical studies. Theoretically, suppression of Raf kinase activity, which has been reported to be required for growth factor stimulation of hematopoietic progenitor cells (24), could have deleterious effects on hematopoiesis. However, in preclinical studies, hematological toxicities (thrombocytopenia, reduction in bone marrow megakaryocytes, and erythroid hyperplasia) have been limited to rodents and, like other toxicities, were common to phosphorothioate oligonucleotides of different sequences (15, 25). Moreover, a phosphorothioate oligonucleotide directed against the murine C-raf sequence that was shown to reduce c-raf mRNA expression in murine liver had identical hematological effects to those of

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>$C_{ss}$ (µg/ml)</th>
<th>$C_{ss}$ (nm)</th>
<th>AUC (µg·min/ml)*</th>
<th>CI (ml/min/kg)</th>
<th>$t_{1/2}$ (min)</th>
<th>% intact</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.21 ± 0.13</td>
<td>31.0</td>
<td>302 ± 187</td>
<td>1.65 ± 1.02</td>
<td>ND*</td>
<td>ND</td>
</tr>
<tr>
<td>1</td>
<td>0.28 ± 0.12</td>
<td>41.4</td>
<td>403 ± 173</td>
<td>2.48 ± 1.06</td>
<td>61</td>
<td>50</td>
</tr>
<tr>
<td>1.5</td>
<td>0.64 ± 0.37</td>
<td>94.6</td>
<td>922 ± 533</td>
<td>1.63 ± 0.94</td>
<td>76 ± 38</td>
<td>61</td>
</tr>
<tr>
<td>2</td>
<td>0.76 ± 0.42</td>
<td>112</td>
<td>1094 ± 605</td>
<td>1.83 ± 1.01</td>
<td>91 ± 56</td>
<td>59</td>
</tr>
<tr>
<td>3</td>
<td>1.12 ± 0.72</td>
<td>166</td>
<td>1613 ± 1037</td>
<td>1.86 ± 1.20</td>
<td>68 ± 27</td>
<td>61</td>
</tr>
<tr>
<td>4</td>
<td>2.84 ± 1.49</td>
<td>420</td>
<td>4090 ± 2146</td>
<td>0.98 ± 0.51</td>
<td>72 ± 6</td>
<td>64</td>
</tr>
</tbody>
</table>

* AUC, area under the plasma concentration-time curve.

**Fig. 1** Average plasma pharmacokinetic profiles as a function of dose for ISIS 5132: mean plasma concentration versus time. Concentrations for intact ISIS 5132 in plasma during and after the start of the infusion are shown. Points, the mean ± SD for each dose level.

A summary of clinical toxicities is shown in Table 3.
ISIS 5132 in mice, indicating that the observed effects were not due to suppression of the target mRNA (12). In the present study, as in primate studies, there was no clear hematological toxicity of ISIS 5132; the patients treated had only inconsistent thrombocytopenia that lacked a clear dose relationship.

At the minimally toxic doses used in this trial, we also observed evidence of antitumor activity. Although this evidence was limited, it was encouraging in the context of a Phase I trial whose primary goal was to determine the safety of ISIS 5132. Two patients had no disease progression for 9 and 10 months with tumors that normally have a more aggressive course (renal cell and pancreatic carcinoma, respectively). Most notable was a 97% decrease in the CA-125 levels of a patient with ovarian cancer. Although not conclusive evidence of antitumor activity, CA-125 levels are a recognized marker of activity in that disease (26), and we note that smaller decreases in CA-125 level had been observed in this patient with several previous cytotoxic therapies.

The study was terminated before a true maximum tolerated dose level was reached for several reasons. First, tissue exposure models based on data from animals suggest that human doses of 2.0–4.0 mg/kg are comparable to doses of 12–24 mg/kg in mice, a dose range at which activity was observed in human tumor xenograft models. Furthermore, doses of 2.0–4.0 mg/kg/day in the present trial resulted in plasma concentrations of intact ISIS 5132 greater than or equal to the in vitro IC₅₀. Finally, results of a concurrent Phase I trial of ISIS 5132 given as an intermittent 2-h infusion demonstrated reductions of c-raf expression even at low doses of the oligonucleotide (18, 27). This study also found evidence of antitumor activity over a dose range (0.5–6.0 mg/kg) similar to that used in this study. Based on these findings and drug supply considerations, Phase II trials of ISIS 5132 have been instituted in this dose range, which appears appropriate for further study of this agent.

In conclusion, this study demonstrates the safety and mild toxicity of ISIS 5132 at the doses and schedule studied. The antitumor activity and prolonged stable disease observed argue for further study of ISIS 5132. The possibility that ISIS 5132 may act primarily by delaying tumor progression should be examined in such studies. In addition, evidence from preclinical studies supports studying ISIS 5132 in combination with certain chemotherapeutic agents and/or radiotherapy. Elevated c-raf expression is associated with resistance of tumor cell lines to radiation or paclitaxel (28–30), but not with resistance to cisplatinum (31), and antisense inhibition of c-raf (with a transfected sequence or with liposomally encapsulated or unmodified ISIS 5132) has been reported to reverse radioresistance of a human laryngeal squamous cell carcinoma cell line in vitro and in vivo (32, 33). Data from ongoing single-agent Phase II trials and from Phase I trials in combination with chemotherapy (34) will provide important data about the use of ISIS 5132 in the treatment of human cancer.

References
5. Skolnik, E. Y., Batzer, A., Li, N., Lee, C. H., Lowenstein, E., Mohammadi, M., Margolis, B., and Schlessinger, J. The function of...

Figure 2: Graph of CA-125 levels over time for patient 219, with the timing of the various therapies as indicated.
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