Phase I Study of an Antisense Oligonucleotide to Protein Kinase C-α (ISIS 3521/CGP 64128A) in Patients with Cancer


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Abstract

Protein kinase C (PKC) is an attractive target in cancer therapy. It is overexpressed in a variety of cancers, and nonspecific inhibitors of PKC have demonstrated antitumor activity. Antisense oligonucleotides targeted against PKC-α, which have high specificity, can inhibit mRNA and protein expression as well as the growth of tumors in vitro and in vivo. This Phase I study sought to characterize the safety profile and to determine the maximum tolerated dose of antisense to PKC-α when administered by continuous infusion in patients. Patients with incurable malignancies received ISIS 3521, a 20-length phosphorothioate oligodeoxynucleotide specific for PKC-α. Treatment was delivered over a period of 21 days by continuous i.v. infusion followed by a 7-day rest period. Doses were increased from 0.5 to 3.0 mg/kg/day. Patients continued on the study until evidence of disease progression or unacceptable toxicity was detected. Between August 1996 and September 1997, 21 patients were treated in five patient cohorts. The maximum tolerated dose was 2.0 mg/kg/day. The dose-limiting toxicities were thrombocytopenia and fatigue at a dose of 3.0 mg/kg/day. Pharmacokinetic measurements showed rapid plasma clearance and dose-dependent steady-state concentrations of ISIS 3521. Evidence of tumor response lasting up to 11 months was observed in three of four patients with ovarian cancer. The recommended dose of ISIS 3521 for Phase II studies is 2.0 mg/kg/day when given over a period of 21 days. Side effects are modest and consist of thrombocytopenia and fatigue. Evidence of antitumor activity provides the rationale for Phase II studies in ovarian cancer and other malignancies.

Introduction

Antisense oligonucleotides can inhibit the expression of specific genes by binding to mRNA transcripts. Specific antisense activity has been demonstrated in vitro and in preclinical animal models. Cancer has been an attractive disease for developing antisense therapies because of the identification of a number of genes that play a key role in the development and maintenance of tumors. However, few studies have explored the safety or efficacy of antisense compounds in patients with cancer. Some of the earliest phase I studies have used antisense oligonucleotides targeted to genes such as c-myb (1), p53 (2), and bcl-2 (3). Many of these studies were purposely limited in dose and duration of therapy by toxicology concerns. In addition, little evidence of antitumor activity was observed.

PKC-α is an attractive target in cancer therapy. It is involved in the signaling pathway that controls cellular proliferation (4), and perturbations of PKC expression have been implicated in the growth and progression of some human tumors (5–7). Experiments in keratinocytes have suggested that PKC-α is involved in mediating the transforming effects of oncogenic ras (8). Overexpression of PKC-α in MCF-7 breast cancer cells led to a more aggressive neoplastic phenotype (9). Several compounds that inhibit PKC expression have demonstrated promising antitumor activity in vitro and in preclinical animal models (10).

ISIS 3521 (CGP 64128A) is a phosphorothioate oligodeoxynucleotide, 20 nucleotides in length. The phosphorothioate backbone provides resistance to exonucleases and increases the stability of the oligodeoxynucleotide in serum and in tissue compared with compounds with unmodified phosphodiester linkages. This modified DNA hybridizes to the 3'-untranslated region of human PKC-α mRNA, resulting in a substrate amenable to degradation by RNase H. Preclinical evidence of antitumor activity and PKC-α inhibition by an antisense mechanism was demonstrated in a variety of in vitro and in vivo experiments. In tissue culture, ISIS 3521 inhibited the expression of PKC-α mRNA in T24 bladder carcinoma cells and A549 lung carcinoma cells (11). Additional studies in A549 cells demonstrated this effect to be oligonucleotide sequence-specific, highly specific for the PKC-α isozyme, reversible, and correlated with a reduction in PKC-α protein expression (11).

Evaluation of ISIS 3521 in human tumor xenograft models in nude mice demonstrated the inhibition of the growth of s.c. and orthotopically transplanted U-87 glioblastoma (12) as well as a significant reduction in the growth of MDA-MB-231 (breast) and Calu-1 (lung) models. The inhibition of PKC-α protein expression was also demonstrated by immunohisto-

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⁴The abbreviations used are: PKC, protein kinase C; aPTT, activated partial thromboplastin time; C ss , steady-state (plasma) concentration; CI, (plasma) clearance; CT, computed tomography.

⁵ISIS Pharmaceuticals, unpublished data.
chemical analysis of A549 tumors recovered from nude mice after treatment with ISIS 3521.

Toxicology studies in animals revealed chemistry-related effects but no apparent sequence- or target-specific effects. In primates, the principal toxic effects of ISIS 3521 were complement activation and prolongation of aPTT. These effects have been observed with other phosphorothioates and seem to be attributable to the backbone chemistry. These effects are correlated with plasma concentration, particularly in excess of 40 μg/ml (approximately 6 μM; Ref. 13).

Because of the preclinical evidence showing antitumor activity and a favorable safety profile, ISIS 3521 was selected for clinical development. The primary objective of this study was to characterize the safety profile of this compound in patients with cancer. In addition, patients were followed for evidence of response to treatment. A continuous i.v. infusion schedule was selected for this study to avoid high peak plasma drug concentrations and to maintain prolonged inhibition of PKC-α mRNA expression.

Patients and Methods

Patient Selection. Eligible patients included those for whom no efficacious therapy was available or who were unresponsive to conventional therapy. Patients must have had measurable lesions or disease that was evaluable with tumor markers. Patients were required to be 18 years of age or older and to have a Southwest Oncology Group performance status ≤ 2. Evidence of adequate hematological, renal, and hepatic organ function was required and included the following parameters: (a) serum creatinine <1.5 mg/dl; (b) bilirubin < 2.0 mg/dl; (c) aspartate aminotransferase < twice the upper limit of normal in the absence of hepatic metastases and < five times normal if hepatic metastases were present; (d) prothrombin time and aPTT within the normal range; (e) absolute neutrophil count > 1500 cells/mm³; (f) hemoglobin > 9.0 g/dl; and (g) platelet count > 100,000 cells/mm³. Patients were excluded if they had (a) an underlying disease state associated with active bleeding; (b) a past medical history of coagulopathy; or (c) complement abnormality, or if patients were receiving therapeutic doses of anti-coagulants such as heparin or warfarin. Patients must not have had New York Heart Association Class IV congestive heart failure. Patients could not have received chemotherapy, an investigational new drug, or a biological or therapeutic device within 28 days of starting treatment. Life expectancy was required to be greater than 12 weeks. Informed and signed consent was obtained from all of the patients.

Study Drug. ISIS 3521 consists of a 20-nucleotide phosphorothioate deoxyribooligonucleotide with the following sequence: GTTCTCGCTGGTGAGTTTCA. The antisense was supplied by Isis Pharmaceuticals, Inc. (Carlsbad, CA) in 2-ml or 11-ml vials containing 1.1 or 10 ml, respectively, of ISIS 3521 as a sterile solution of 10 mg/ml in PBS (pH 7.31–7.36). The percentage (area-percent) of full-length oligonucleotide, determined by capillary gel electrophoresis, was 89.9–93.4%, with the major impurity consisting of 5.6–8.8% “shortmers” (n-1, n-2, n-3, and so forth, deletion sequences). The product was 88.5–90.7% fully thioated, with the rare occurrence of more than one nonthioated (phosphodiester) linkage in a single molecule. ISIS 3521 is essentially a racemic mixture, with an opportunity for chirality at each of its 19 phosphorothioate linkages (14).

The trial drug was administered in three consecutive 7-day continuous infusions per cycle. The total dose for a given week was calculated using a given patient’s weight (in kg) prior to that treatment cycle. A volume of 0.9% NaCl equivalent to the volume of the weekly dose of study drug was removed from 250 ml of 0.9% NaCl, and the study drug added to the remaining NaCl using a sterile technique. The resulting solution was infused with a portable volumetric infusion pump (Verifuse, I-FLOW Corporation), through a 0.22 μm in-line filter at approximately 1.5 ml/h.

Drug Administration. Ascending doses of oligonucleotide were given via continuous i.v. infusion over 21 days. ISIS 3521 was supplied by Isis Pharmaceuticals, Inc. Cycles were repeated after a 7-day rest period. Cohorts of three patients were evaluated at each dose level of ISIS 3521. Doses of the drug were escalated in subsequent cohorts if there was no evidence of treatment-related dose-limiting toxicity in any of the three patients after one complete treatment cycle. Dose-limiting toxicity was defined as WHO toxicity grade 4 for coagulation abnormalities associated with clinically significant bleeding, or WHO grade 3, or greater, kidney/bladder abnormalities, or WHO grade 4 hematological abnormalities, or WHO grade 3, or greater, for all of the other abnormalities excluding alopecia and nausea/vomiting in the presence of antiemetic therapy.

Doses of ISIS 3521 were increased according to the following schedule: 0.5, 1.0, 1.5, 2.0, or 3.0 mg/kg/day. If one of three patients treated at any dose level demonstrated evidence of unacceptable toxicity, three additional patients were enrolled at that level before dose escalation in the subsequent cohort. Dose escalation was stopped if two or more patients (i.e., two of three or two of six) at any dose level experienced treatment-related dose-limiting toxicity. A total of six patients received ISIS 3521 at a dose immediately below the dose-level that resulted in unacceptable toxicity. Individual patients continued on treatment until there was evidence of either disease progression or treatment-related dose-limiting toxicity. Patients continuing on study could receive increasing doses of ISIS 3521 if that dose had already been studied in a cohort of three patients with acceptable toxicities.

Pharmacokinetic Sampling. Plasma and urine samples were obtained for pharmacokinetic assay. Samples for pharmacokinetic analysis were taken at the following time intervals: (a) before the start of infusion on day 1; (b) 4 and 24 h after the start of infusion; (c) during follow-up on days 7 and 14; (d) on day 21 immediately before the discontinuation of i.v. infusion; and (e) at 10, 20, 30, 60, 90, 120, and 180 min and 4 h after discontinuing the infusion. Twenty-four-h urine collections were obtained on days 0, 6, and 21 on cycle 1 and on day 6 and 21 on cycles 2 and 3.

Analysis of ISIS 3521 in Blood and Urine. Drug analysis was performed on aliquots of plasma and urine samples by capillary gel electrophoresis (CGE) by Covance Laboratories (Madison, WI) using a previously described method (15).

Pharmacokinetic Analysis. Descriptive statistics for plasma concentrations measured during infusion were calculated. A noncompartmental analysis was conducted using Win-
Nonlin software. The terminal plasma elimination half-life was calculated at \( t_{1/2}\beta = \ln(2)/K \), where \( K \) is the rate constant for the terminal decline in plasma ISIS 3521 concentration estimated by log-linear regression. \( C_{ss} \) was determined by averaging the measured plasma concentrations at time 24 h to time 21 days during the infusion. \( CL \) was determined by dividing the zero-order dose rate (\( ko \)) by the calculated \( C_{ss} \). Area under the plasma concentration-time curve at steady state (\( AUC_{ss} \)) was then calculated by dividing the daily dose by \( CL \). The apparent volume of distribution was calculated using the following equation: 

\[
V_{infusion} = CL/K.
\]

Response Assessment. Tumor response was assessed before the institution of the third cycle of treatment and every other cycle thereafter. Earlier assessment of tumor activity was conducted if symptoms suggested tumor progression. A complete response was defined as no evidence of disease for at least 28 days as confirmed by repeat tumor measurements. A partial response was defined at a 50% or greater decrease from baseline in the sum of the products of the maximum perpendicular diameters of indicator lesions, no progressive disease for at least 28 days, and no new lesions. Progressive disease was defined as a 25% or greater increase in the sum of the products of the maximum perpendicular diameters for indicator lesions or the appearance of a new lesion. Stable disease was defined as disease that did not meet criteria for either partial or complete response or progressive disease.

Results

Patient Characteristics. Between August 1996 and September 1997, 21 patients were enrolled in the study. The median age was 63 years (range, 40–74 years); there were 10 men and 11 women. The majority of patients had ovarian (4), colon (3), pancreatic (3), or lung cancer (2). Other tumor histologies included granulosa cell, gastric, esophageal, breast, and lymphoma. All of the patients had received prior chemotherapy, ranging from one to six regimens (median, 2). Twelve patients had received radiotherapy. Table 1 summarizes the starting dose of the drug administered, the number of patients treated at each level, and the duration of treatment of patients in the study. The longest treatment duration was 11 cycles.

Toxicity. Transient thrombocytopenia (grade 2) was observed in two patients at doses of ISIS 3521 ranging from 0.5 to 1.5 mg/kg/day. In both cases, thrombocytopenia became apparent on day 14 of the infusion but resolved (platelet count > 100,000 cells/mm\(^3\)), while treatment continued, by day 21. One patient, treated at 2.0 mg/kg/day, developed grade 3 thrombocytopenia by day 7. A bone marrow biopsy obtained at that time showed decreased numbers of megakaryocytes. A pretreatment bone marrow biopsy for comparison, however, had not been done. The patient continued with the 21-day infusion with grade 2 thrombocytopenia on days 14 and 21. However, the platelet count returned to normal by the start of the next cycle 7 days later and remained above 100,000 cell/mm\(^3\) during the next cycle of treatment.

Dose-limiting toxicity was observed in four of six patients treated at 3.0 mg/kg/day. The dose-limiting toxicities included grade 4 thrombocytopenia in one patient and grade 2 thrombocytopenia with grade 4 bleeding in another. Four patients experienced grade 3 fatigue. The patient with grade 4 bleeding had a uterine leiomyosarcoma with a large pulmonary metastasis. She had not required transfusions before starting therapy and had a normal platelet count and aPTT. She experienced a life-threatening hemotherax on day 11 of her first cycle. At that time, the platelet count was 57,000 cells/mm\(^3\), and the aPTT was slightly elevated at 38.4 (normal 36) s. The patient recovered after a thoracentesis and RBC transfusions. The platelet count returned to 98,000 cell/mm\(^3\) spontaneously by the fifth day after admission. The patient with grade 4 thrombocytopenia had a platelet count of 52,000 cells/mm\(^3\) at day 7, which dropped to 24,000 cells/mm\(^3\) by day 14. However, by day 28, the start of the next cycle, the platelet count returned to 116,000 cells/mm\(^3\) and dipped to no lower than 57,000 cells/mm\(^3\) on day 14 of the next cycle. The grade 3 fatigue experienced by four patients appeared generally in the middle of the 21-day infusion. Patients described the fatigue as a general lack of energy that led to spending most of the day in bed. These symptoms, however, improved markedly during the 7-day period between cycles.

Six patients received initial treatment at 2.0 mg/kg/day. Other than fatigue in three of the patients, treatment was well tolerated. An additional patient who had fatigue at the higher dose of 3.0 mg/kg/day also experienced similar symptoms at a lower dose of 2.0 mg/kg/day. As at the higher dose, fatigue usually appeared midway through the 21-day cycle, and often remitted during the 7 days between cycles.

Effects on coagulation and complement were minimal. No increases in PT or aPTT were noted in association with ISIS 3521 except for the patient with the leiomyosarcoma. She had a slightly prolonged PT and aPTT on one occasion. Isolated increases of C3a were observed, but nearly all of the patients began with elevated levels of this complement split product (>100 ng/ml). Isolated increases in C5a were also noted at the 0.5 mg/kg dose level only, where two of three patients began with an elevated C5a level >10 ng/ml. No treatment-related effects on the Bb complement split product were noted. There were no adverse events suggestive of complement activation.

Because of a suggestion of increased bleeding in conjunction with thrombocytopenia, three patients, for whom these tests could be arranged, had in vitro studies of platelet function before and during the antisense infusion of the first treatment cycle. One patient, treated at 3.0 mg/kg/day, showed normal platelet aggregation with all of the agonists tested before oligonucleotide infusion (ADP, epinephrine, ristocetin, and collagen). On day 14, aggregation was abnormal with ADP (2 \( \mu \)M) and epinephrine (2 and 5 \( \mu \)M). A second patient treated at 3.0 mg/kg/day displayed abnormal

| Table 1 | Dosing schema and treatment duration |
|---|---|---|---|
| Cohort | Starting dose (mg/kg/day) | No. of patients | Duration (treatment cycles) |
| 1 | 0.5 | 3 | 0.8, 11, 4 |
| 2 | 1.0 | 3 | 1.7, 2, 2 |
| 3 | 1.5 | 3 | 1, 1.7, 2 |
| 4 | 2.0 | 6 | 1.3, 1.8, 2, 2, 4, 5 |
| 5 | 3.0 | 6 | 0.3, 0.5, 1.7, 2, 2, 7 |

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Phase I Study of ISIS 3521

Table 2  Pharmacokinetics

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<th>Parameter</th>
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<th>1.5</th>
<th>2.0</th>
<th>3.0</th>
</tr>
</thead>
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<td>3</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>C24-h, µg/ml</td>
<td>0.11 ± 0.02</td>
<td>0.28 ± 0.08</td>
<td>0.44 ± 0.06</td>
<td>0.49 ± 0.07</td>
<td>1.07 ± 0.04</td>
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<tr>
<td>C7-day, µg/ml</td>
<td>0.49 ± 0.31</td>
<td>0.19 ± 0.08</td>
<td>0.51 ± 0.30</td>
<td>0.33 ± 0.23</td>
<td>0.99 ± 0.83</td>
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<tr>
<td>C14-day, µg/ml</td>
<td>0.28 ± 0.24</td>
<td>0.23 ± 0.07</td>
<td>0.45 ± 0.11</td>
<td>0.68 ± 0.16</td>
<td>1.86 ± 0.60</td>
</tr>
<tr>
<td>CEO, µg/ml</td>
<td>0.08c</td>
<td>0.25 ± 0.06</td>
<td>0.38 ± 0.16</td>
<td>0.53 ± 0.24</td>
<td>0.89 ± 0.30</td>
</tr>
<tr>
<td>CSS, µg/ml</td>
<td>0.18 ± 0.09</td>
<td>0.24 ± 0.02</td>
<td>0.41 ± 0.13</td>
<td>0.50 ± 0.13</td>
<td>1.26 ± 0.15</td>
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<tr>
<td>AUCSS, µg·min/ml</td>
<td>253 ± 123</td>
<td>349 ± 22.5</td>
<td>596 ± 192</td>
<td>723 ± 181</td>
<td>1821 ± 215</td>
</tr>
<tr>
<td>Cl, ml/min/kg</td>
<td>2.30 ± 1.04</td>
<td>2.87 ± 0.18</td>
<td>2.67 ± 0.73</td>
<td>2.88 ± 0.65</td>
<td>1.66 ± 0.21</td>
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<tr>
<td>Vinf, ml/kg</td>
<td>nc</td>
<td>186 ± 12</td>
<td>96 ± 26</td>
<td>254 ± 57</td>
<td>146 ± 19</td>
</tr>
<tr>
<td>t1/2, min</td>
<td>nc</td>
<td>45 ± 18</td>
<td>25 ± 13</td>
<td>61 ± 14</td>
<td>61 ± 5.4</td>
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<tr>
<td>% intact 24 h</td>
<td>nc</td>
<td>75.6 ± 4.8</td>
<td>65 ± 13.1</td>
<td>54 ± 8.1</td>
<td>52.4 ± 10.5</td>
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<tr>
<td>% intact EOI</td>
<td>nc</td>
<td>66.1 ± 3.6</td>
<td>50.7 ± 7.2</td>
<td>52.9 ± 10.9</td>
<td>45 ± 3.7</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD.

C, concentration in plasma; EOI, end of infusion; ss, steady state; AUC, area under the curve; Vinf, apparent volume of infusion; nc, not calculated due to lack of measurable plasma concentrations postinfusion; % intact, % of intact antisense oligonucleotide.

n = 2.

Platelet aggregation to ADP and epinephrine before starting therapy. However, on day 7 of infusion, aggregation was abnormal to all of the agonists tested including ristocetin and collagen. The third patient who was treated at 2.0 mg/kg/day had normal platelet aggregation studies before infusion and when tested at the end of the 21-day infusion.

Pharmacokinetics. Plasma concentrations of ISIS 3521 increased in a dose-dependent manner over the range of doses investigated (Table 2). AUCss and plasma Css values increased in a manner suggestive of nonlinear kinetics with saturable Cl (Fig. 1). Plasma concentrations of ISIS 3521 were similar at 24 h after the start of infusion and at day 21, which suggests that Css was achieved by 24 h (Table 2). The maximum plasma concentrations achieved during continuous infusion were well below the plasma concentrations associated with acute toxicological effects in nonclinical primate studies (13).

Plasma half-life and Cl values were consistent with estimates calculated after a shorter, 2-h infusion in both monkey and man (16). The half-life of ISIS 3521 in plasma ranged from about 40 min after the 1.0 mg/kg/day to about 60 min after a dose of 3.0 mg/kg/day. Cl of ISIS 3521 from plasma was similar across doses through the 2.0 mg/kg/day dose (range, 2.3–2.9 ml/min/kg) and decreased slightly at the 3.0 mg/kg/day dose to approximately 1.7 ml/min/kg.

The primary metabolite seen in plasma was ISIS 3521 shortened from the 3’ end by one nucleotide. Other shortened metabolites were measurable and appeared in decreasing concentrations in order of the number of oligonucleotides deleted. The full-length oligonucleotide was the predominant species at all of the time points and represented 50–75% of the total measurable oligonucleotide at the end of the 21-day infusion (Table 2). This metabolite profile in plasma was also consistent with previous data from monkeys in preclinical safety studies.

Tumor Response. Three of the four patients with ovarian cancer showed evidence of tumor response with ISIS 3521. The disease course of one patient with rapidly growing clear cell carcinoma of the ovary is illustrated in Fig. 2. After 2 cycles of treatment at 0.5 mg/kg/day, CT scans showed a decrease in the size of the tumor mass (Fig. 3) with a delayed, but corresponding decrement, in serum tumor markers. The patient received 7 cycles at 0.5 mg/kg/day, one cycle each at 1.0 and 1.5 mg/kg/day, and two cycles at 2.0 mg/kg/day. She achieved a maximum tumor reduction of 60% and remained in the study until repeat CT scans showed new disease in the pelvis. Two other patients with ovarian cancer and elevated CA-125 tumor markers (the only measurable or evaluable disease in these patients) had a decline in CA-125 levels of 40% and 76% lasting 5 and 7 months, respectively. All four of the patients with ovarian cancer had received prior platinum-based therapy, with two patients having achieved a remission longer than six months after therapy (“platinum sensitive”). Both of the platinum-sensitive patients had a decline in tumor markers or measurable disease on ISIS 3521 treatment, and one of the platinum-resistant patients had such a response.
Discussion

Antisense therapy holds the promise that targeting individual genes may allow the inhibition of tumor growth with less toxicity than that associated with less specific therapies. The first step in developing such treatments for cancer patients is to characterize safety and determine a dose for additional clinical studies. This Phase I study of an antisense oligonucleotide to PKC-α established a recommended Phase II dose of 2.0 mg/kg/day when given over a period of 21 days by continuous i.v. infusion. The dose-limiting toxicities consisted of fatigue and thrombocytopenia.

The clinical experience in this study showed little overlap with adverse effects observed in preclinical toxicology studies. Patients did not experience complement activation, the most prominent toxic effect that might have been expected from primate studies. In addition, there was no evidence of nephrotoxicity from the deposition of oligonucleotides in proximal renal tubules observed in primates or hepatic inflammation seen in mice. Thrombocytopenia, observed primarily at higher doses, recovered spontaneously in most patients while therapy continued. In preclinical studies, thrombocytopenia was observed only at antisense doses significantly higher than in the current study (80 mg/kg) and was attributed to decreased numbers of megakaryocytes. The etiology of the decreased platelets in patients is unclear. The quickly reversible recovery of platelets argues against an antibody-mediated process. Most likely, the thrombocytopenia relates to class effects of the phosphorothioate-substituted backbone rather than to the specific target; similar effects have been observed with other phosphorothioate antisense compounds such as ISIS 5132, which targets C-raf kinase mRNA (17).

The pharmacokinetics of ISIS 3521 were predictable and similar to that expected from preclinical studies. Plasma levels showed a dose-dependent rise in C0 at all of the doses of infusion. Terminal half-life after the cessation of infusion was very rapid. The consistent metabolite pattern over the dosing period suggested that there was no inhibition or induction of metabolism of ISIS 3521. Plasma pharmacokinetics were consistent between patients and remained consistent over the 21 days of infusion in the treatment dose range.

Inhibitors of PKC have shown promising antitumor activity in vitro and in human tumor xenograft models (10). This preclinical activity has encouraged the clinical development of a number of PKC inhibitors that now represent a growing and promising class of anticancer agents. Staurosporine, for example, is one of the most potent inhibitors of PKC. However, its activity is relatively nonspecific because it inhibits other protein kinases as well (10). Analogues to Staurosporine, such as UCN-01 and CGP 41251, have shown greater selectivity for PKC. Early-phase clinical studies of these compounds are ongoing, and preliminary results have shown some antitumor activity (18, 19). However, as with traditional cytotoxics, the relatively nonspecific targeting of PKC was associated with a variety of toxicities (pulmonary toxicity, transaminitis, hypotension, nausea and vomiting, and leukopenia) that may be lessened with a more specific inhibitor.

In this study of ISIS 3521, antitumor activity was observed in three of four patients with ovarian cancer. The changes in CA-125 are postulated to reflect changes in tumor amount inasmuch as the measurement of CA-125 is not affected by the presence of the oligonucleotide.3 Interestingly, the tumor mass in the patient with ovarian cancer stabilized for several months before shrinking. This delayed response suggests that the time frame for assessing activity may differ from that of traditional cytotoxics; prolonged therapy may be needed to assess activity accurately. A companion study to the current protocol admin-
istered the antisense oligonucleotide three times weekly. Complete remissions were observed in two patients with low-grade lymphoma (20). The activity in ovarian cancer and lymphoma suggests that ISIS 3521 warrants further testing in a variety of tumors.

In summary, the relatively mild side effects, predictable pharmacokinetics, and evidence of antitumor activity support the continued development of this antisense oligonucleotide to PKC-α. Ongoing investigations include a Phase II study in ovarian cancer and a Phase I study of ISIS 3521 combined with carboplatin and paclitaxel chemotherapy. Upcoming Phase II studies include trials in cancer of the prostate, colon, breast, lung, and other solid tumors.

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