

## A phase I trial of aprinocarsen (ISIS 3521/LY900003), an antisense inhibitor of protein kinase C- $\alpha$ administered as a 24-hour weekly infusion schedule in patients with advanced cancer

Ranjana Advani<sup>1</sup>, Bert L. Lum<sup>1</sup>, George A. Fisher<sup>1</sup>, Joanne Halsey<sup>1</sup>, Richard S. Geary<sup>2</sup>, Jon T. Holmlund<sup>2</sup>, T. Jesse Kwoh<sup>2</sup>, F. Andrew Dorr<sup>2</sup> and Branimir I. Sikic<sup>1</sup>

<sup>1</sup>Oncology Division, Stanford University School of Medicine, Stanford, CA 94305-5151; <sup>2</sup>ISIS Pharmaceuticals, Inc., Carlsbad, CA 92008

**Key words:** Antisense therapy, protein kinase C, phase I trial, pharmacokinetics, pharmacodynamics.

### Summary

**Purpose:** A phase I study was performed to determine the maximum tolerated dose (MTD), safety profile and pharmacology of aprinocarsen (ISIS 3521), an antisense oligonucleotide to protein kinase C- $\alpha$ , in patients with refractory solid tumors. **Experimental design:** Fourteen patients were treated in sequential cohorts of aprinocarsen by 24-hour continuous infusion (CIV), weekly, at doses of 6, 12, 18 and 24 mg/kg. **Results:** One grade 4 toxicity was observed, transient grade 4 neutropenia at 18 mg/kg. Grade 3 toxicities included neutropenia at 12 mg/kg, fever and hemorrhage at 18 mg/kg, and neutropenia, nausea, and chills at 24 mg/kg. Grade 2 toxicities included thrombocytopenia myalgias, chills, headache, fatigue, fever and nausea/vomiting. Mean prothrombin times and activated partial thromboplastin times (aPTT) increased by 10% and 29% from baseline ( $p = 0.006$  and  $0.005$ ). Mean complement split products (Bb and C3a) increased 1.6-fold and 3.6-fold (from  $p = 0.014$  and  $0.004$ , respectively). These changes correlated with dose and were transient with recovery to baseline by day 7. Steady state plasma concentrations ( $C_{ss}$ ) of aprinocarsen were achieved within four hours.  $C_{ss}$  better described changes in aPTT than dose. Clinical evidence of complement activation was not observed. **Conclusions:** In contrast to 21-day protracted infusion schedules, delivery of aprinocarsen over a 24-hour infusion schedule showed concentration-dependent effects on coagulation and complement, which are consistent with nonclinical toxicology studies performed in the phosphorothioate DNA antisense drug class. These coagulation and complement changes resulted in a maximum tolerated dose 24 mg/kg.

### Introduction

Antisense oligonucleotides are designed selectively to inhibit the expression of a protein by binding in a sequence-specific manner to the corresponding mRNA, leading to RNase-H-mediated degradation of the bound mRNA. These agents represent a novel class of molecular targeted anticancer drugs, with toxicities different from traditional chemotherapeutic agents. The overexpression of protein kinase C- $\alpha$  (PKC- $\alpha$ ), a cytoplasmic serine/threonine kinase involved in signal transduction may play a role in the development of several tumor types [1, 2].

Aprinocarsen (ISIS 3521) is a 20-base phosphorothioate oligonucleotide complementary to the 3' untranslated region of mRNA for human PKC- $\alpha$ . The phosphorothioate backbone provides resistance to exonucle-

ases and increases the stability of the oligodeoxynucleotide in serum and tissues compared with compounds with unmodified phosphodiester linkages [3]. This compound has demonstrated antitumor activity in human tumor xenograft models [4, 5].

We previously have reported the safety of aprinocarsen administered as a 21-day continuous intravenous infusion (CIV) [6]. Pharmacokinetic studies in non-human primates suggest that a 24-hour infusion of aprinocarsen at 24 mg/kg result in  $C_{max}$  values of 18–26 mcg/ml (Geary R, Isis Pharmaceuticals, Inc., personal communication). These concentrations were well tolerated in primates, and are approximately 50% lower than those shown to produce complement activation [7]. In animal models various phosphorothioate oligonucleotides have resulted in concentration dependent effects on the coagulation and

complement systems. Thus, these effects are considered to be independent of the selected nucleotide sequence and molecular target, and are attributed to the phosphorothioate backbone chemical structure of the compounds.

The present study explored the feasibility of a 24-hour CIV of aprinocarsen administered on a weekly schedule. The objectives of the study were: (1) to determine the maximum tolerated dose (MTD) by this schedule, and (2) to characterize the safety profile, pharmacokinetic, and pharmacodynamic behavior of aprinocarsen.

## Patients and methods

### *Patient selection*

The Panel on Medical Human Subjects of Stanford University approved the protocol. Informed consent according to Stanford University and Federal Guidelines was obtained from all patients. Patients  $\geq 18$  years with histologically confirmed diagnoses of incurable solid tumors were eligible for this protocol. Female patients were required to have a negative serum pregnancy prior to enrollment, and patients of either gender were required to use an adequate contraceptive method. Measurable disease or disease that was assessable with tumor markers and an Eastern Cooperative Oncology Group (ECOG) performance status  $\leq 2$  was required. No chemotherapy, radiotherapy, biologic therapy, hormonal therapy or investigational drugs were permitted within 28 days prior to study entry (6 weeks for nitrosoureas). Evidence of adequate hematological, renal, and hepatic organ function was required and included the following laboratory parameters: creatinine  $\leq 1.5$  mg/dL, bilirubin  $\leq 1.3$  mg/dL, aspartate aminotransferase (AST) or alanine aminotransferase (ALT) concentration  $\leq 2.5$  times the upper limit of normal, an absolute neutrophil count (ANC)  $\geq 1500$  cells/mm<sup>3</sup>, platelet count  $\leq 100,000$  cells/mm<sup>3</sup>, hemoglobin  $> 9$  gm/dL, and prothrombin time (PT) and activated partial thromboplastin time (aPTT) within the normal range. Exclusion criteria included the following: central nervous system (CNS) metastases or a diagnosis of acute leukemia; underlying complement abnormality or disease state associated with active bleeding; patients receiving therapeutic doses of anticoagulants such as heparin or warfarin; or life expectancy  $< 12$  weeks.

### *Drug administration*

Aprinocarsen is a 20-nucleotide phosphorothioate deoxyribooligonucleotide with the following sequence: 5'-GTTCTCGCTGGTGAGTTTCA-3'. Aprinocarsen was supplied by Isis Pharmaceuticals, Inc. (Carlsbad, CA) as a sterile solution of 10 mg/ml in phosphate buffered solution (pH 7.31–7.36). The trial drug was administered as

a 24 hour CIV given weekly. A cycle consisted of 3 consecutive weeks of treatment. A volume of 0.9% Sodium Chloride (NaCl), USP, equivalent to the volume of the 24-hour dose of study drug was removed from 500 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  $\mu$ m in-line filter at approximately 21 ml/h.

### *Dose-limiting toxicity*

DLT was defined as any of the following: (1) NCI common toxicity criteria 2.0 (Common Toxicity Criteria, Version 2.0, 1999, National Cancer Institute, Cancer Therapy Evaluation Program, Bethesda, MD), grade 4 coagulation abnormality and either a grade 2 thrombocytopenia or grade 1 hemorrhage; (2) grade 4 neutropenia lasting 5 days or more or associated with fever; or a grade 4 thrombocytopenia or a grade  $\geq 3$  thrombocytopenia associated with a  $\geq$  grade 1 hemorrhage or grade  $\geq 2$  thrombocytopenia associated with a grade 4 coagulation abnormality; (3) grade 4 anemia; (4) grade 4 nausea/vomiting while on maximal antiemetic therapy; or (5) grade  $\geq 3$  for all other abnormalities except lymphopenia, which was not considered to be dose-limiting.

### *Dose escalation, cohort size, and maximum tolerated dose*

Ascending doses of oligonucleotide were given via CIV over 24 hours administered once weekly.

Cohorts of three patients were evaluated at each dose level. The starting dose level was 6 mg/kg with escalations to 12, 18 and 24 mg/kg as described below. One treatment cycle was defined as three consecutive weeks of treatment. If one of three patients treated at any dose level demonstrated evidence of a DLT, 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 a treatment-related DLT.

In the event of a DLT, treatment with aprinocarsen was held until the toxicity had resolved. Therapy was then resumed at the next lower dose level. A total of six patients were to receive aprinocarsen at a dose immediately below the dose-level that resulted in unacceptable toxicity. The maximum tolerated dose (MTD) was to be defined as the highest dose level studied which results in DLT in fewer than two patients. Individual patients continued on treatment until there was evidence of either disease progression or treatment-related dose-limiting toxicity.

### Response assessment

Tumor response was assessed per WHO criteria 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.

### Pharmacokinetic sampling

Samples for pharmacokinetic analysis were taken at the following time intervals during week 1: before the start of infusion on day 0, just prior to the end of infusion, and at 0.25, 0.5, 2, 4, 8 and 24 hours after the end of infusion. End of infusion plasma samples also were obtained on days 8 and 15 of the cycle. Drug analysis was performed on aliquots of plasma and urine samples by capillary gel electrophoresis (Covance Laboratories, Madison, WI) using a previously described method [8].

### Pharmacokinetic analysis

Descriptive statistics for plasma concentrations measured during infusion were calculated. A noncompartmental analysis was conducted using WinNonlin (Pharsight Corporation, Mountain View, CA). The terminal plasma elimination half-life was calculated as  $T_{1/2} = 0.693/K$ , where  $K$  is the rate constant for the terminal decline in plasma aprinocarsen concentration estimated by log-linear regression. The terminal half-life was estimated using the equation:  $T_{1/2} = 0.693/Kz$ . The concentration at steady state ( $C_{ss}$ ) was determined by averaging the concentrations at 4, 8, and 24 hours. Apparent volume of distribution ( $V_{infusion}$ ) and clearance ( $CL$ ) were derived from traditional equations, where  $CL = ko / C_{ss}$  and  $V_{infusion} = (CL/K)$ . Area under the curve at steady state ( $AUC_{ss}$ ) was calculated using the equation:  $AUC_{ss} = \text{dose}/CL$ .

### Pharmacodynamic and statistical analysis

Baseline (days), end of infusion at 24<sup>o</sup> (day 1 and day 7) samples were obtained to assess effects on complement activation (C3a and Bb levels), PT, and aPTT. Blood for immunoassays of plasma levels of interleukin-1 beta (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-alpha) were drawn at baseline and 3, 6, 24, and 168 hours after the start of infusion of the first dose of aprinocarsen.

Linear regression was used to assess the relationship of dose to pharmacokinetic parameters and response of the pharmacodynamic parameters (i.e. complement and coagulation). A sigmoid maximum response model ( $E_{max}$ , modified Hill equation), was used to describe the relationship between the change in aPTT (effect) and drug

concentration (C), where:

$$\text{Effect} = E_{\max} \times C^H / EC_{50} + C^H$$

Where  $E_{\max}$  is the maximal effect, H is the Hill constant, which describes the sigmoidicity (steepness and shape) of the slope of the curve,  $EC_{50}$  is the concentration predicted to result in 50% of the  $E_{\max}$  [9]. Due to the limited number of observations,  $E_{\max}$  was fixed at the maximum effect observed in the study and the estimated parameters were  $EC_{50}$  and H. Model fits were evaluated by R-squared values, sum of squared deviations, and model selection criterion (a derivation of the Akaike information criterion) [9]. Pharmacodynamic modeling was performed using Scientist for Windows (version 2.0, Micromath, Inc., Salt Lake City, UT).

Differences between baseline and follow-up values in the same individuals were compared using the Wilcoxon sign rank test. Data were reported as means and standard deviation (S.D.). Statistical analyses and linear regressions were performed using Statistix for Windows (version 2.0, Analytical Software, Tallahassee, FL).

## Results

### Patient characteristics

Between January 1998 and October 1999, 14 patients were enrolled in the study. There were 7 females and 7 males with a median age of 49 years (range 31–71 years). Specific tumor types were: ovarian ( $n = 3$ ), colon ( $n = 2$ ), GI stromal ( $n = 2$ ), and one each for soft tissue sarcoma, lung, parotid, schwannoma, thymoma, adrenocortical, and unknown primary site tumors. Twelve patients had received prior chemotherapy, ranging from 1–4 regimens (median 2). The number of patients treated at each dose level and the median and range of weekly treatments delivered are summarized in Table 1. Two patients received a dose escalation to a higher dose level. The median duration of treatment was 6 weeks (range 2–15).

Table 1. Dose levels and number of weekly treatments of aprinocarsen administered

Dose level (mg/kg)	Number of patients	Number of weekly treatments delivered Median (range)
6	3	6 (2–6)
12	3	6 (2–15)
18	6	8 (5–9)
24	4	7.5 (5–9)

Table 2. Number of patients experiencing toxicity during cycle 1 or 2 according to dose level

Dose level	6mg/kg			12 mg/kg			18 mg/kg			24 mg/kg		
	Number of patients*			3			6			3		
Grade	1	2	3	1	2	3	1	2	3	1	2	3
Fever	1	1			2			5	1		2	
Chills			1					5				3
Myalgias		1					3	1			1	
Headache	2							1				
Fatigue		1		1	1		3	1			1	
Nausea/Vomiting							2	1		2	2	
Diarrhea							2					
Arthralgia								1				
Sweating		1						1				
Hemoglobin	1			2	1		1	1	1			1
Platelets	1				1			2		1	1	
Proteinurea												1
Hematuria										1		
↑ Alkaline Phosphatase				1			1					
↑ Liver Enzymes				2			4			2		

\*NCI Common toxicity criteria 2.0. no patients experienced grade 4 toxicity and no patient experienced cumulative toxicity.

### Toxicity

Assessment during the first 2 cycles showed no grade 4 toxicities. Transient grade 4 neutropenia was observed in one patient at 18 mg/kg during the third cycle. One patient each in the 12 mg/kg and 24 mg/kg dose levels experienced transient grade 3 neutropenia. Grade 2 toxicities at the 24 mg/kg dose level consisted of anemia ( $n = 1$ ), nausea ( $n = 1$ ) and chills ( $n = 1$ ). At the 18 mg/kg dose level, one patient experienced grade 3 fever, one experienced grade 3 anemia secondary to menorrhagia. Other transient grade 1 or 2 toxicities included: thrombocytopenia ( $n = 8$ ), anemia ( $n = 11$ ) myalgias ( $n = 6$ ), elevated ALT ( $n = 7$ ) or AST ( $n = 4$ ), chills ( $n = 6$ ), headaches ( $n = 3$ ), fatigue ( $n = 8$ ), fever ( $n = 12$ ) and nausea/vomiting ( $n = 7$ ).

The frequency of the toxicities encountered at each dose level is shown in Table 2. Flu like symptoms were transient and were ameliorated or prevented with premedication consisting of antipyretics and antihistamines (acetaminophen and diphenhydramine) on subsequent cycles. No DLT was observed. Dose escalation beyond 24 mg/kg was not done because of the investigators' judgment, due to constitutional symptoms experienced by the patients, and the investigators' concerns for laboratory abnormalities noted in complement split products (see below).

### Effect of aprinocarsen on coagulation

Linear regression analyses (Figure 1) indicated a correlation between aprinocarsen dose and percent prolongation

of PT and aPTT ( $p$ -values of 0.005 and 0.008, respectively). There was a 10% and a 29% increase in mean PT and aPTT from baseline values at day 1 ( $p = 0.006$  and 0.005, respectively, Table 3). These changes were transient with no clinical significance and, with recovery to baseline values by day 7 (Table 3). For PT, the mean  $\pm$  S.D. day 0 and day 7 values were  $11.9 \pm 0.6$  and  $13.1 \pm 0.8$  seconds, and for aPTT, the mean  $\pm$  S.D. day 0 and day 7 values were  $27.8 \pm 3.2$  and  $30.2 \pm 10.9$ . Comparisons of day 0 to day 7 values indicated no statistical significance ( $p$ -values for PT = 0.67 and aPTT = 0.72). A sigmoidal  $E_{\max}$  model was used to describe the relationship of concentration to change in aPTT following aprinocarsen delivered as a 24-hour infusion, indicated an  $EC_{50}$  of 8.13  $\mu\text{g/ml}$ , with  $E_{\max}$  occurring at 17  $\mu\text{g/ml}$ .

During week 6 one patient developed hemorrhage (grade 3) due to menorrhagia after receiving aprinocarsen at the 18 mg/kg/day dose level. This was associated with grade 1 thrombocytopenia and normal coagulation parameters (PT and aPTT). Her pelvic ultrasound was found to be normal. She was transfused with packed red cells and given hormonal therapy with estrogen with resolution of symptoms.

### Effect of aprinocarsen on cytokine and TNF- $\alpha$ release

Cytokine (IL-6, IL-1RA, IL-1- $\alpha$ , and TNF- $\alpha$ ) measurements were performed on 4 of the 14 patients enrolled in the study and are presented in Table 3. The time course of these changes in one representative patient is depicted in Figure 3. All values were maximally elevated at the end

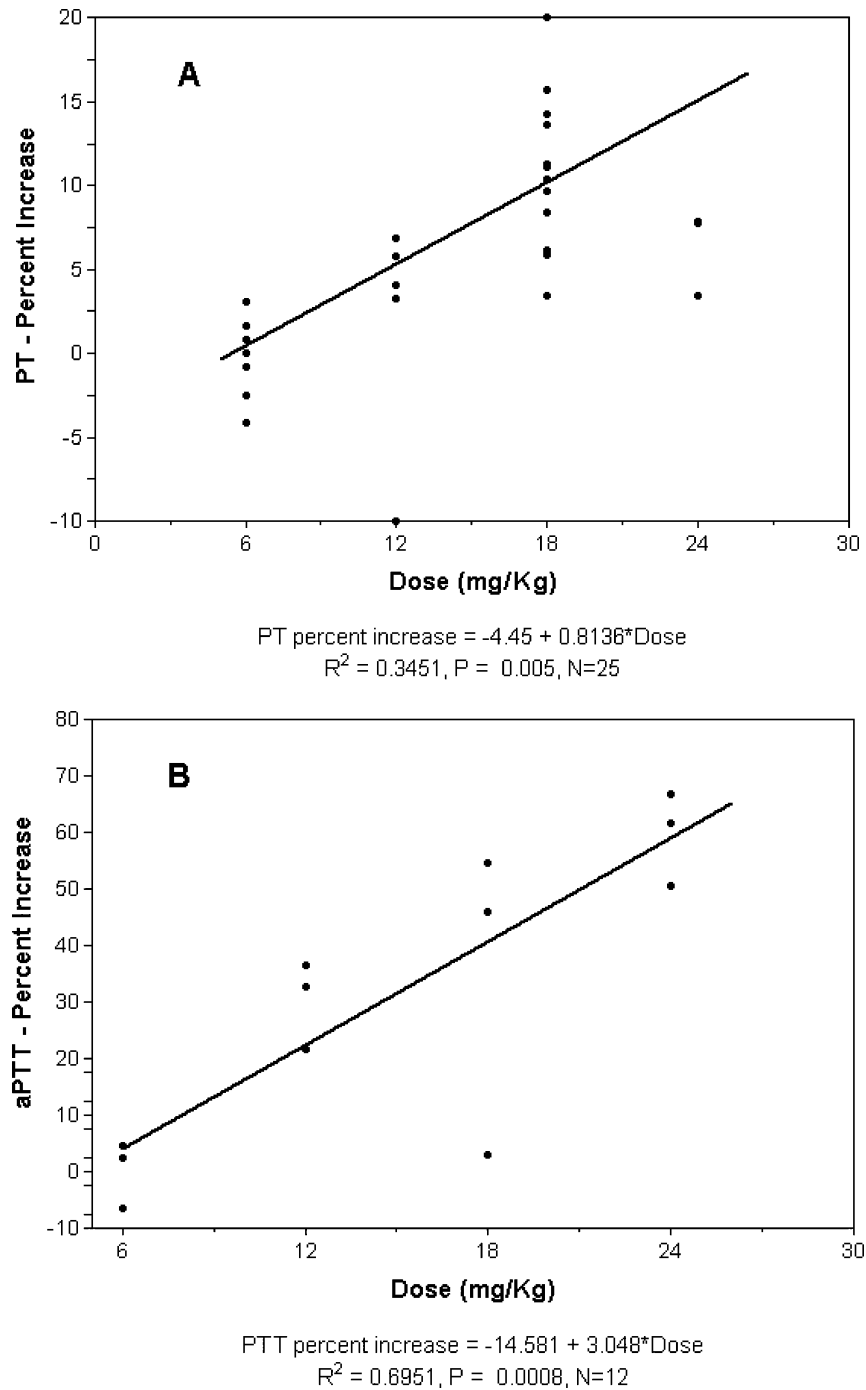


Figure 1. Linear regression plot of aprinocarsen dose to percent change in (A) prothrombin time and (B) activated partial thromboplastin time.

of the infusion and returned to day 0 pre-dose baseline values by the next assessment time point (day 7 pre-dose). At the end of infusion, IL-1- $\alpha$  and IL-1RA reached maximal values, and were found to be increased by approximately 2.0-fold and 17-fold. Maximal observed values for IL-6, and TNF- $\alpha$  were reached earlier, increasing approximately 5-fold, 8 hours following the start of infusion. At

the highest dose level studied, 24 mg/kg, grade 3 chills were experienced by all 3 patients.

#### *Effect of aprinocarsen on complement activation*

Elevations of complement proteins C3a and Bb were transient and associated with dose level. A linear correlation

Table 3. Summary of cytokine and TNF alpha values over time (data are presented as means  $\pm$  S.E.)

	IL-1 alpha		IL-1RA		IL-6		TNF-alpha	
	<i>n</i>	Concentration (pg/mL)	<i>n</i>	Concentration (pg/mL)	<i>n</i>	Concentration (pg/mL)	<i>n</i>	Concentration (pg/mL)
Cycle 1, Day 0								
Pre-dose	2	0.7 $\pm$ 0.5	4	339.8 $\pm$ 21.3	2	12.9 $\pm$ 11.7	4	34.0 $\pm$ 29.1
1 Hour	1	0.1 $\pm$ NA	1	220.0 $\pm$ NA	1	27.7 $\pm$ NA	1	13.4 $\pm$ NA
post-start of infusion								
4 hours	2	0.8 $\pm$ 0.6	4	298.3 $\pm$ 35.1	3	15.7 $\pm$ 15.1	4	42.0 $\pm$ 27.4
post-start of infusion								
8 Hours	2	0.9 $\pm$ 0.6	4	3741.0 $\pm$ 920.9	4	67.5 $\pm$ 58.7	4	167.9 $\pm$ 45.0
post-start of infusion								
Cycle 1, Day 1	2	1.5 $\square$ 1.1	3	5719.0 $\pm$ 172.0	3	4.3 $\pm$ 1.9	4	103.5 $\square$ 34.0
end of infusion								
Cycle 1, Day	2	0.5 $\pm$ 0.3	4	287.5 $\pm$ 33.8	3	11.0 $\pm$ 10.2	4	54.9 $\pm$ 39.1
7 Pre Dose								

Definitions: NA = not applicable, insufficient N for calculation of value; EOI = end of infusion; SE = standard error.

between aprinocarsen dose and fold-increase of C3a and Bb from baseline values were observed (Figure 2,  $p = 0.007$  and  $<0.001$ , respectively). Overall there was an 1.6-fold and a 3.6-fold increase in Bb and C3a complement proteins from baseline at day 1 ( $p = 0.014$  and  $0.004$ , respectively, Table 4). Despite these changes no clinical evidence of complement activation (periorbital edema, hypotension or renal failure) was observed.

The changes observed in complement proteins C3a and Bb were transient, with recovery occurring by day 7 (Table 4). The day 0 and day 7 values for C3a were  $197 \pm 85$  and  $258 \pm 95$  seconds, and for Bb, day 0 and day 7 values were  $0.84 \pm 0.21$  and  $0.96 \pm 0.17$  (means  $\pm$  S.D.), with  $p$ -values of 0.09 and 0.097, respectively.

#### Pharmacokinetics and pharmacodynamics

The full-length oligonucleotide was the predominant species at all time points ranging from 43–57% of the total measurable oligonucleotide at the end of the 24-hour infusion (Table 5). The primary metabolite in plasma was aprinocarsen shortened from the 3' end by one nucleotide. Other shortened metabolites appeared in decreasing concentrations in order of the number of nucleotides deleted.

Aprinocarsen tended to reach maximal concentrations at later times with increasing dose (Table 6). Plasma steady-state concentrations increased proportionally over the range of doses investigated (Table 6). Linear regression analyses indicated dose and mean C<sub>ss</sub> were significantly correlated ( $R^2 = 0.9967$ ,  $p = 0.0016$ ). Aprinocarsen clearance appeared to be more rapid in the lowest dose group, however small patient numbers and

variability in clearance precluded determination a nonlinear dose-clearance relationship (Table 6).

The pharmacokinetic/pharmacodynamic relationship between plasma steady-state concentrations of aprinocarsen and change in aPTT is illustrated in Figure 4. C<sub>ss</sub> described the relationship between changes in aPTT better than the dose per se, when R-square values (0.933 vs 0.869) and sum of squared deviations (86.2 vs 168.3) were compared.

#### Antitumor efficacy

No regressions of tumors were observed in this Phase I study.

#### Discussion

In this trial with aprinocarsen administered as a once weekly 24-hour continuous infusion a correlation between aprinocarsen dose and percent prolongation of PT and aPTT was observed (Figure 1), which resulted in a transient increases in mean PT and aPTT with recovery by day 7 (Table 3). Elevation of aPTT was also reported in Phase 1 clinical trials of ISIS 5132, a phosphorothioate oligonucleotide that targets C-raf kinase, delivered by a 2-hr infusion three times a week [11, 12]. However, these effects were not observed in a Phase I study of aprinocarsen administered as a 21 day CIV [6]. The mechanism of aPTT prolongation has been reported to be due to the selective inhibition of the intrinsic tenase activity [13, 14] and is a nucleotide sequence independent effect. The aPTT elevations observed in this study and from primate studies,

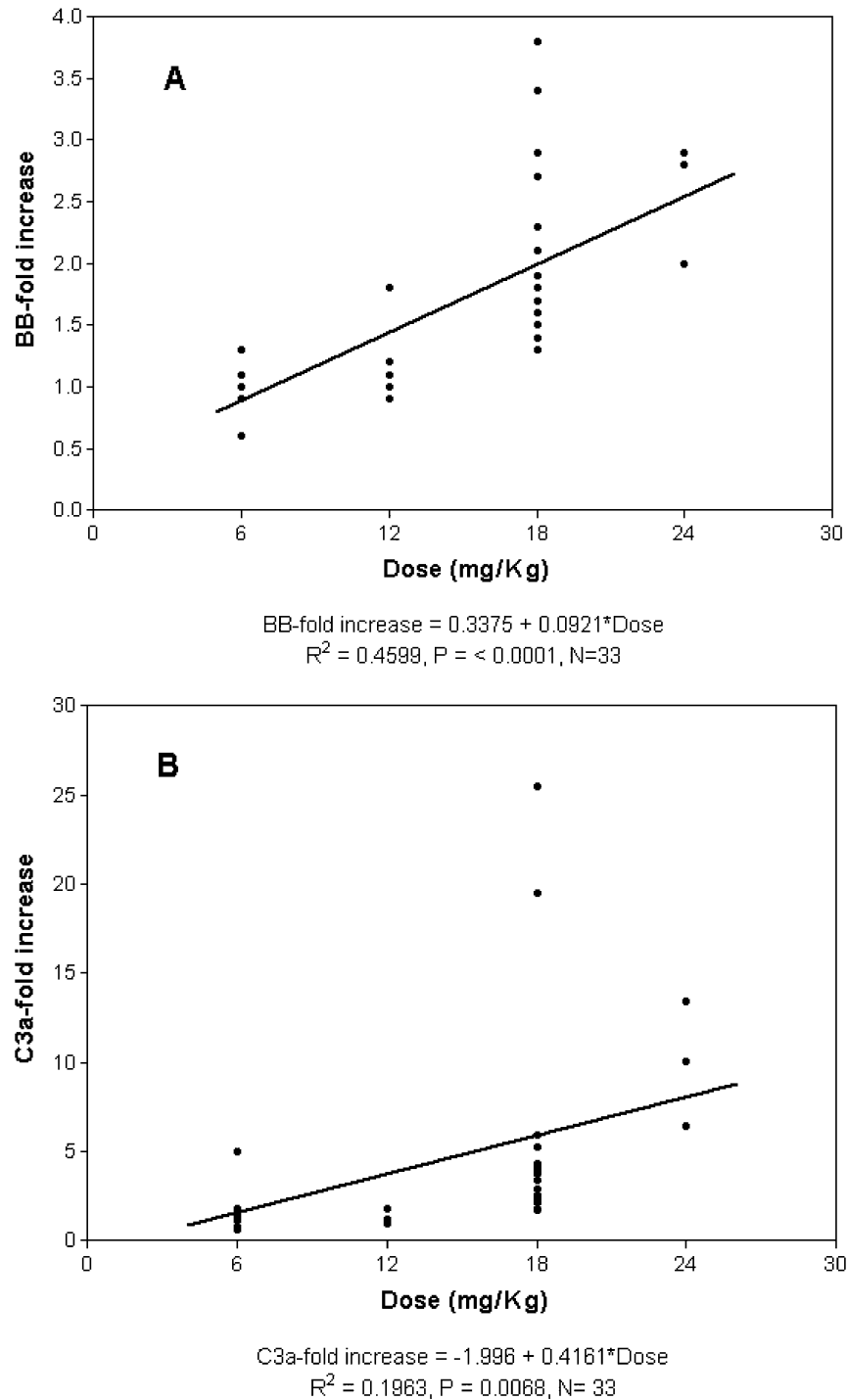


Figure 2. Linear regression plot of aprinocarsen dose to fold-increase in complement activation, (A) complement protein Bb and (B) complement protein C3a.

and the absence of these effects with a continuous infusion schedule, where lower plasma concentrations of aprinocarsen are achieved, suggest a concentration dependent effect.

Elevations of complement proteins C3a and Bb were transient and a linear correlation between effect and dose was observed (Figure 2A and B). These changes were transient, with recovery occurring by day 7 (Table 4)

Table 4. Time-related effect of aprinocarsen on coagulation and complement activation (data are presented as means  $\pm$  S.D)

Parameter	Day 0*	Day 1	Day 7	P-value day 0 vs. 1	P-value day 0 vs. 7
PT (sec)	11.9 $\pm$ 0.6	13.1 $\pm$ 0.8	11.9 $\pm$ 0.8	0.006	0.666
aPTT (sec)	27.9 $\pm$ 3.2	35.9 $\pm$ 5.0	30.1 $\pm$ 11.0	0.005	0.724
Bb (pg/ml)	0.84 $\pm$ 0.22	1.36 $\pm$ 0.64	0.96 $\pm$ 0.17	0.014	0.092
C3a (pg/ml)	197 $\pm$ 85	700 $\pm$ 507	258 $\pm$ 95	0.004	0.094

\* P-values are Wilcoxon sign rank, two sided values.

Table 5. Comparison of mean observed plasma concentrations 24 hours after starting a new dose level ( $C_{24\text{ h}}$ ) and at steady-state ( $C_{ss,24\text{ h}}$ ) for aprinocarsen and total oligonucleotide

Dose (mg/kg/day)	$C_{24\text{ h}}^1$			$C_{ss,24\text{ h}}^2$		
	3521 $\mu\text{g/mL}$ (SD, N)	Total Oligo $\mu\text{g/mL}$ (SD, N)	Intact	3521 $\mu\text{g/mL}$ (SD, N)	Total Oligo $\mu\text{g/mL}$ (SD, N)	Intact
6.0	1.5 (0.4, 3)	3.4 (1.0, 3)	44%	2.0 (0.7, 12)	4.4 (1.4, 12)	45%
12.0	4.1 (0.8, 4)	8.9 (2.2, 4)	46%	5.2 (1.5, 18)	11.1 (2.9, 18)	47%
18.0	7.6 (2.9, 6)	17.8 (7.4, 6)	43%	9.6 (4.8, 41)	21.7 (14.0, 41)	44%
24.0	10.2 (2.5, 4)	23.7 (6.4, 4)	43%	13.2 (5.1, 31)	32.8 (14.0, 31)	40%

Abbreviations: SD = standard deviation, N = number of samples.

1. Represent the average of 24-hour (nominal time) end of infusion samples measured from the first administration only of a new dose level.

2. Represent the average of 24-hour (nominal time) end of infusion samples measured after every administered dose (including first and subsequent administrations) of a particular dose level.

Table 6. Noncompartmental analysis of aprinocarsen administered by IV infusion to advanced cancer patients (data are presented as means  $\pm$  SD, N)

Parameter	Nominal Dose			
	6.0 mg/kg/day (SD, N*)	12.0 mg/kg/day (SD, N*)	18.0 mg/kg/day (SD, N*)	24.0 mg/kg/day (SD, N*)
$t_{\text{max}}$ (h)	4.0 (0, 3)	8.0 (0, 3)	12.6 (8.8, 6)	12.1 (8.0, 4)
$C_{\text{max}}$ ( $\mu\text{g/mL}$ )	2.8 (1.1, 3)	6.9 (1.5, 3)	11.1 (1.8, 6)	11.4 (3.1, 4)
$C_{ss,24\text{ h}}$ ( $\mu\text{g/mL}$ ) <sup>2</sup>	1.9 (0.6, 3)	5.1 (0.5, 3)	9.6 (0.6, 6)	12.9 (4.5, 4)
$\text{AUC}_{0-\text{inf}}$ ( $\mu\text{gh/mL}$ )	51 (15, 3)	138 (16, 3)	215 (39, 6)	272 (77, 4)
CL (mL/h/kg)	116 (30, 3)	81 (9, 3)	78 (14, 6)	92 (34, 4)
$t_{1/2z}$ (h)	0.94 (0.10, 3)	0.89 (0.16, 3)	1.71 (0.40, 6)	2.41 (0.47, 4)

Abbreviations: Std = standard deviation; N = number of patients. \*The N for these analyses take into account dose adjustments. Overall, 4 patients received 12.0 mg/kg/day; however, only 3 patients were receiving 12.0 mg/kg/day at the time of analysis. Six patients received 18.0 mg/kg/day.

Mean  $C_{ss,24\text{ h}}$  values reflect the overall mean (per dose level) of the individual mean  $C_{ss,24\text{ h}}$  values observed in each patient at a particular dose level.

without any clinical sequela. Activation of the alternative pathway of complement has been observed in primates after administration of a number of phosphorothioate oligonucleotides [7]. This phenomenon is at-

tributable to nonspecific binding and inactivation of inhibitory factors, such as complement factor H, by the oligonucleotide, due to the phosphorothioate backbone, and is not sequence-specific [3]. Interactions with factor

H leading to activation of the alternate complement pathway have also been reported at concentrations of >50 pg/ml in mice [15]. In animals these effects correlated with plasma concentrations of intact oligonucleotide in excess of 40  $\mu\text{g/ml}$  after a 2-hour intravenous infusion of oligonucleotide [3]. It is noteworthy that, in this study,

elevation of complement split products was observed at steady-state concentrations of aprinocarsen significantly less than 40  $\mu\text{g/ml}$ .

The pharmacokinetics of aprinocarsen in this study was predictable and similar to those expected from preclinical studies. Plasma levels showed a dose-dependent rise in

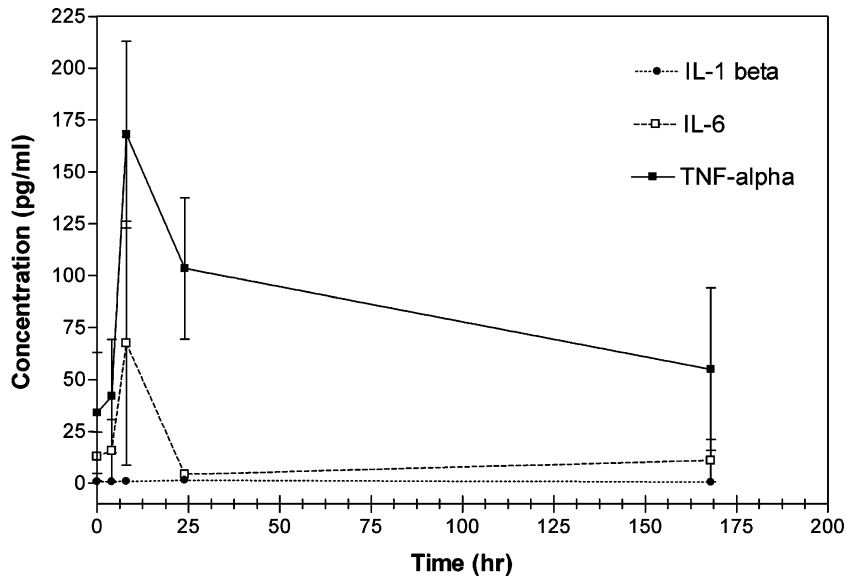


Figure 3. Time course of cytokine level elevation following aprinocarsen administration at a dose of 24 mg/kg administered as a 24-hour infusion. Data are presented as means  $\pm$  standard error.

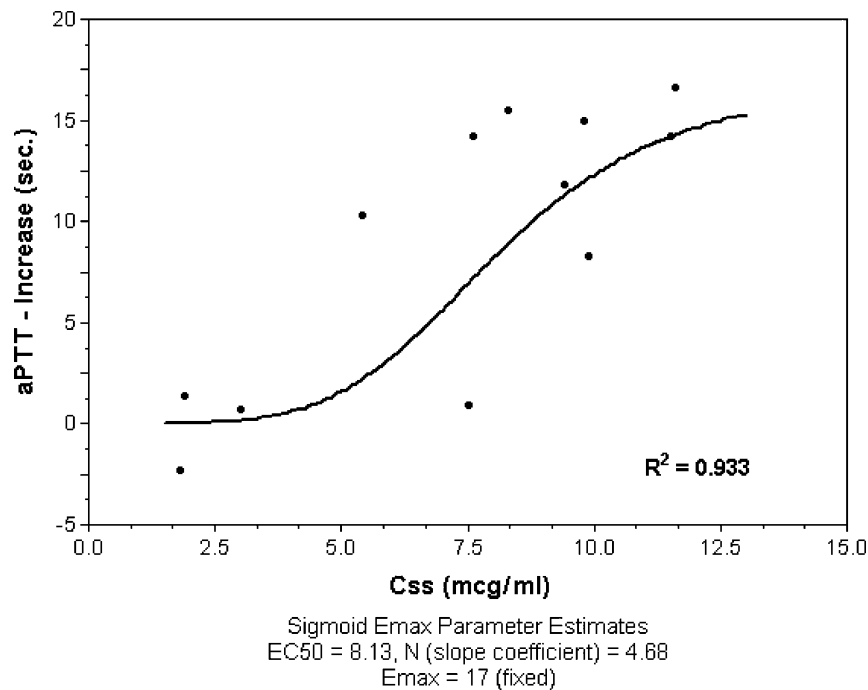


Figure 4. Pharmacokinetic/pharmacodynamic relationship between plasma steady-state concentrations of aprinocarsen and change in aPTT.

$C_{ss}$  in the dose range studied with clearance in a linear range at doses around 18 mg/kg/day. This is in contrast to the previous observations of a nonlinear (saturable) clearance with increasing doses with the 21-day infusion schedule [16]. The consistent metabolite pattern over the dosing period, with intact oligonucleotide accounting for 40–47% of the total oligonucleotide concentration at the end of infusion, suggested that there was no inhibition or induction of metabolism of aprinocarsen with escalating doses.

With this schedule, no grade 4 toxicity was observed in the first two cycles. The main side effects, although not dose limiting, were fever and chills and caused sufficient morbidity in patients to require treatment with antihistamines and antipyretics. These effects are thought to be due to cytokine release as illustrated in Figure 3. At the highest dose level studied, 24 mg/kg, grade 3 chills were experienced in all 3 patients.

Despite no grade 4 toxicities in the first two cycles, due to the significant laboratory abnormalities and poor tolerance of the drug we elected not to pursue dose escalation beyond 24 mg/kg, even though an MTD had not been reached by traditional phase I criteria.

In summary, a 24-hour infusion schedule of aprinocarsen, a phosphorothioate antisense oligonucleotide to protein kinase C- $\alpha$ , produced dose and concentration-dependent laboratory abnormalities in coagulation and complement pathways. These observations are important for the design and testing of future generations of phosphorothioate antisense oligonucleotides.

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### Disclosure

This work was presented in part at the annual meeting of the American Society of Clinical Oncology in New Orleans, LA, May 2000. Dr. Sikic was a paid consultant to Isis Pharmaceuticals, Inc. Drs. Geary and Kwoh are cur-

rently, and Drs. Dorr and Holmlund were formerly employees of ISIS Pharmaceuticals, Inc. Dr. Lum is now an employee of Genentech, Inc., South San Francisco, CA. This study was performed under a now-concluded development agreement between Isis Pharmaceuticals, Inc., and Novartis Pharma AG, Basel, Switzerland. Since completion of the trial, Isis Pharmaceuticals, Inc. has licensed ISIS 3521 to Eli Lilly and Company, which has designated the compound "LY900003."

### References

1. Basu A: The potential of protein kinase C as a target for anticancer treatment. *Pharmacol Ther* 59: 257–280, 1993
2. Blobe GC, Obeid LM, Hannun YA: Regulation of protein kinase C and role in cancer biology. *Cancer Metastasis Rev* 13: 411–431, 1994
3. Henry SP, Montheith D, Bennett F, Levin AA: Toxicological and pharmacokinetic properties of chemically modified antisense oligonucleotide inhibitors of PKC- $\alpha$  and C-raf kinase. *Anticancer Drug Des* 12: 409–420, 1997
4. Dean NM, McKay R: Inhibition of protein kinase C- $\alpha$  expression in mice after systemic administration of phosphorothioate antisense oligodeoxynucleotides. *Proc Natl Acad Sci USA* 91: 11762–11766, 1994
5. Yazaki T, Ahmad S, Chahlavi A, Zylber-Katz E, Dean NM, Rabkin SD, Martuza RL, Glazer RI: Treatment of glioblastoma U-87 by systemic administration of an antisense protein kinase C- $\alpha$  phosphorothioate oligodeoxynucleotide. *Mol Pharmacol* 50: 236–242, 1996
6. Yuen AR, Halsey J, Fisher GA, Holmlund JT, Geary RS, Kwoh TJ, Dorr A, Sikic BI: Phase I study of an antisense oligonucleotide to protein kinase C- $\alpha$  (ISIS 3521/CGP 64128A) in patients with cancer. *Clin Cancer Res* 5: 3357–3363, 1999
7. Galbraith WM, Hobson WC, Giclas PC, Schechter PJ, Agrawal S: Complement activation and hemodynamic changes following intravenous administration of phosphorothioate oligonucleotides in the monkey. *Antisense Res Dev* 4: 201–206, 1994
8. Leeds JM, Graham MJ, Truong L, Cummins LL: Quantitation of phosphorothioate oligonucleotides in human plasma. *Anal Biochem* 235: 36–43, 1996
9. Mick R, Ratain MJ: Statistical approaches to pharmacodynamic modeling: Motivations, methods, and misperceptions. *Cancer Chemother Pharmacol* 33: 1–9, 1993
10. Crooke ST, Bennett CF: Progress in antisense oligonucleotide therapeutics. *Annu Rev Pharmacol Toxicol* 36: 107–129, 1996
11. Nemunaitis J, Holmlund JT, Kravynak M, Richards D, Bruce J, Ognoskie N, Kwoh TJ, Geary R, Dorr A, Von Hoff D, Eckhardt SG: Phase I evaluation of ISIS 3521, an antisense oligodeoxynucleotide to protein kinase C- $\alpha$ , in patients with advanced cancer. *J Clin Oncol* 17: 3586–3595, 1999
12. Holmlund J, Nemunaitis J, Schiller J, Dorr A, Kisner D: Phase I trial of C-raf antisense oligonucleotide ISIS 5132 (CGP 69846A) by 21-day continuous intravenous infusion (CIV) in patients with advanced cancer (Abstract). *Proc Am Soc Clin Oncol* 17: 811a, 1998
13. Sheehan JP, Lan HC: Phosphorothioate Oligonucleotides inhibit the intrinsic tenase complex. *Blood* 92: 1617–1625, 1998
14. Henry SP, Novotny W, Leeds J, Auletta C, Kornbrust DJ: Inhibition of coagulation by a phosphorothioate oligonucleotide. *Antisense Nucleic Acid Drug Dev* 7: 503–510, 1997

15. Henry SP, Giclas PC, Leeds J, Pangburn M, Auletta C, Levin AA, Kornbrust DJ: Activation of the alternative pathway of complement by a phosphorothioate oligonucleotide: Potential mechanism of action. *J Pharmacol Exp Ther* 281: 810–816, 1997
16. Geary RS, Leeds JM, Henry SP, Monteith DK, Levin AA: Antisense oligonucleotide inhibitors for the treatment of cancer: Pharmacoki-

netic properties of phosphorothioate oligodeoxynucleotides. *Anti-cancer Drug Des* 12: 383–393, 1997

*Address for offprints:* Ranjana Advani, Oncology Division, Stanford Cancer Center, Room 2336, Stanford CA 94305-5821, USA. Tel.: 650-724-8372; Fax: 650-736-1640; E-mail: radvani@stanford.edu