

Phase I Trial of ISIS 5132, an Antisense Oligonucleotide Inhibitor of *c-raf-1*, Administered by 24-hour Weekly Infusion to Patients with Advanced Cancer¹

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ABSTRACT

Raf-1 is a serine/threonine kinase that functions as a critical effector of Ras-mediated signal transduction via the mitogen-activated protein kinase pathway. Constitutive activation of this pathway directly contributes to malignant transformation in many human tumors. A 20-base phosphorothioate oligonucleotide complementary to *c-raf-1* mRNA (ISIS 5132; CGP 69846A) has been shown to specifically suppress Raf-1 expression both *in vitro* and *in vivo*. This Phase I trial, involving 22 patients with advanced cancer, was designed to evaluate the safety, feasibility, and maximum tolerated dose of ISIS 5132 administration as a weekly 24-h i.v. infusion. Pharmacokinetic analysis was performed, and *c-raf-1* mRNA levels in peripheral blood mononuclear cells were assessed using quantitative reverse transcription-PCR. This trial defined a maximum tolerated dose of 24 mg/kg/week on this schedule. Two of four patients treated at 30 mg/kg/week had serious adverse events after the first dose of ISIS 5132, including acute hemolytic anemia and acute renal failure and anasarca. There were no major responses documented. Dose-dependent complement activation was demonstrated on this schedule, but not on previously evaluated schedules, of ISIS 5132 administration. In contrast to other trials of ISIS 5132, there appeared to be no

consistent suppression of peripheral blood mononuclear cell *c-raf-1* mRNA level on this schedule at any of the dose levels analyzed. These data suggest that the efficacy and toxicity profiles of antisense oligonucleotides may be highly dependent on the schedule of administration and support the analysis of the putative molecular target in the evaluation of novel therapeutics.

INTRODUCTION

Over the past decade there has been increasing interest in the development of specific molecular target-based anticancer therapeutics (1, 2). Antisense oligonucleotides represent one such approach (3–5). Short synthetic oligonucleotides, complementary to a mRNA of interest, are taken up by cells *in vivo* through mechanisms that are still unclear. Intracellularly, these oligonucleotides hybridize with their cognate mRNA and can lead to its clearance, reducing synthesis of the encoded protein. This approach to gene-specific suppression has potential utility not only as an anticancer strategy but also for a variety of human diseases characterized by inappropriate gene expression (6).

Raf-1 is one of a number of potential therapeutic targets for antisense suppression that are currently being explored (7). Raf-1 is a serine/threonine kinase that occupies a critical position in the MAP³ kinase pathway (8–10). Ligand interaction with a variety of cell surface growth factor receptors promotes activation of the critical oncoprotein Ras. In turn, Ras mediates its effects through a network of intracellular signaling pathways, one of the most important of which appears to be the MAP kinase pathway. Activated Ras interacts directly with the NH₂-terminal regulatory domain of Raf-1, resulting in the activation and recruitment of Raf-1 to the plasma membrane (11–14). Activated Raf-1 initiates a cascade of reactions by direct activation of mitogen-activated protein kinase kinase, a dual specificity kinase that in turn catalyzes the phosphorylation of MAP kinase. MAP kinase activation ultimately modulates the function of a host of factors involved in cellular differentiation and proliferation.

Inappropriate activation of the MAP kinase pathway can occur through tumor cell synthesis of autostimulatory growth factors, through constitutively active growth factor receptors, or through activating mutations of Ras and Raf-1 (15, 16). Deregulated activation of this pathway has been extensively documented in cancer cells and is thought to be a critical contributor

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³ The abbreviations used are: MAP, mitogen-activated protein; aPTT, activated partial thromboplastin time; MTD, maximal tolerated dose; PBMC, peripheral blood mononuclear cells; DLT, dose-limiting toxicity; RT-PCR, reverse transcription-PCR; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; ECOG, Eastern Cooperative Oncology Group; TNF, tumor necrosis factor; IL, interleukin.

to the transformed phenotype and growth pattern of malignant cells (17, 18).

ISIS 5132 is a 20-base phosphorothioate oligonucleotide complementary to a sequence within the 3' untranslated region of the *c-raf-1* mRNA. It was initially identified through screening of multiple anti-*c-raf-1* oligonucleotides as a highly active suppressor of *c-raf-1* mRNA and protein levels in cell culture assays (19). Binding of ISIS 5132 to the *c-raf-1* mRNA appears to promote RNase H-mediated mRNA degradation (20). Nucleotide-specific and concentration-dependent suppression of Raf-1 by ISIS 5132 has been demonstrated in A549 (lung), T-24 (bladder), and SW480 (colon) carcinoma cells (21, 22). Similar sequence-specific inhibition of cellular growth occurs in tissue culture, with concentration dependence parallel to that observed for suppression of Raf-1.

The phosphorothioate backbone has been used in the majority of the clinically evaluated antisense oligonucleotides. It significantly increases resistance of oligonucleotides to nuclease digestion, thus increasing stability and potential practical utility *in vivo* (23). A number of sequence-independent effects of antisense compounds have been ascribed to the phosphorothioate backbone itself. These include constitutional symptoms such as fever and fatigue, as well as transient prolongation of the aPTT. Activation of the alternative complement pathway has been observed in nonclinical primate studies but not previously in clinical trials of antisense compounds.

This Phase I trial examined a weekly 24-h continuous *i.v.* infusion schedule of ISIS 5132 as a single agent in patients with advanced malignancy. In addition to determining MTD and analyzing the toxicity of this regimen, the trial examined oligonucleotide pharmacokinetics, complement activation, effects on coagulation, and suppression of the molecular target of ISIS 5132, the *c-raf-1* mRNA, in PBMC.

MATERIALS AND METHODS

Patient Population. A total of 22 patients were enrolled in this study. The trial was limited to adults of ECOG performance status of ≤ 2 and a life expectancy of ≥ 12 weeks, with a histologically confirmed diagnosis of cancer that was unresponsive to conventional therapy or that had no effective therapy available. Measurable or evaluable disease was required. Eligible patients had received no chemotherapy, radiotherapy, biological therapy, hormonal treatment, or investigational drugs within 28 days before study entry. Patients were required to have serum creatinine ≤ 1.5 mg/dl, serum bilirubin ≤ 1.5 mg/dl, serum aspartate aminotransferases and alanine aminotransferases ≤ 2.5 times upper limit of normal, absolute neutrophil count > 1500 cells/mm³, platelet count $> 100,000$ /mm³, hemoglobin > 9.0 g/dl, and normal blood coagulation profile as assessed by prothrombin time and aPTT. All of the patients provided written informed consent before study enrollment or performance of study-related procedures in accordance with institutional and federal guidelines.

Drug Administration. ISIS 5132, a 20-base phosphorothioate oligonucleotide (5'TCCCGCCTGTGACATGCATT3'), was administered in 500 ml of 0.9% sodium chloride as a 24-h *i.v.* continuous infusion (21 ml/h) through a 0.22 μ m in-line filter using a portable volumetric infusion pump. Dosing was

repeated on days 8 and 15 of each 3-week treatment cycle. After fever was identified as a common toxicity, patients were given 650 mg of acetaminophen before and up to every 4 h during ISIS 5132 infusion. Prophylactic antiemetics were not routinely administered. In the event of dose-limiting toxicity, therapy was held pending resolution of toxicity. Therapy could then be resumed at the next lower dose level, at the discretion of the patient and treating physician.

Study Design. ISIS 5132 was to be administered weekly without planned interruption, and a treatment cycle was defined as 3 weeks. Patients not completing cycle 1 for reasons other than DLT were considered inevaluable for toxicity and were replaced to meet enrollment criteria. The starting dose was 6 mg/kg over 24 h. A trial design of early accelerated dose escalation was used, with only one patient at the first dose level, if no significant toxicity was observed, and an initial doubling of dose. Subsequent dose levels consisted of a minimum of three evaluable patients. Dose escalation was permitted after completion of the first cycle (3 weeks) by three patients without evidence of DLT. If one of the initial three patients experienced DLT in cycle 1, the dose cohort was expanded to six evaluable patients. MTD was defined as the highest dose level at which no more than one in six patients experienced DLT in cycle 1.

Toxicity and Response Evaluation. Toxicity was assessed using National Cancer Institute Common Toxicity Criteria (version 2.0). DLT was defined as: (a) grade 4 coagulopathy (as defined by prothrombin time and aPTT values), associated with \geq grade 2 thrombocytopenia or \geq grade 1 hemorrhage-clinical; (b) grade 4 neutropenia lasting ≥ 5 days or associated with fever; (c) grade 4 thrombocytopenia or grade 3 thrombocytopenia, associated with \geq grade 1 hemorrhage-clinical; (d) grade 4 anemia; (e) grade 4 nausea/vomiting despite maximal antiemetic therapy; or (f) other \geq grade 3 toxicity (except lymphopenia). Complement split products were assessed on all of the patients immediately before and after infusion of ISIS 5132 on each of the three doses of cycle 1 (*i.e.*, on days 1, 2, 8, 9, 15, and 16).

Response was evaluated after the first three cycles (9 weeks) of therapy and every two cycles (6 weeks) thereafter. Two-dimensional measurement criteria were used to classify response using standard criteria. Patients with stable disease were allowed to continue therapy.

Pharmacokinetic Analysis. This trial evaluated plasma ISIS 5132 concentrations at dose levels of 6–30 mg/kg/wk. Plasma concentrations of ISIS 5132 and chain-shortened metabolites were determined by capillary gel electrophoresis before and 4, 8, and 24 h after initiation of infusion and 15, 30, and 60 min and 2, 3, and 4 h after end of infusion on cycle 1 for each patient.

Measurement of mRNA Levels. Blood sampling for determination of PBMC *c-raf-1* mRNA levels was performed in all of the patients before and at the conclusion of the first 24-h infusion of ISIS 5132, as well immediately before initiation of the subsequent dose (6 days later). PBMCs were isolated by Ficoll-Hypaque separation, and total mRNA was prepared using Trizol reagent (Life Technologies, Inc.) according to the manufacturer's instructions. *c-raf-1* mRNA analysis was performed using quantitative RT-PCR, with normalization to mRNA level of G3PDH, determined in parallel in the same reaction. All of the RT-PCR determinations were made in duplicate or triplicate.

Table 1 Patient demographics

Total enrolled	22
Gender (male/female)	13/9
Age	
Median	57
Range	40–80
ECOG performance status	
0–1	20
2	2
Number of prior chemotherapy regimens	
0	2
2	11
>2	9
Primary tumor site	
Colon	6
Kidney/bladder	4
Gynecologic	4
Lung	3
Pancreas	2
Liver	1
Breast	1
Sarcoma	1
Cycles ISIS 5132 completed	
<1	4
1 or 2	5
3	11
>3	2

Measurement of Complement Split Products and Cytokine Levels. Serum C3a and Bb levels were measured immediately before and after ISIS 5132 infusion. In addition, serum levels of TNF- α and IL-1R α were assessed in patients at 24 mg/kg and 30 mg/kg before and 24 h after initiation of the first 24-h infusion.

Statistical Analysis of Complement Activation. Regression models were fit to each measure of complement activation, using treatment (before infusion *versus* after infusion), dose level, and day as covariates. An interaction term between treatment and dose level was used to measure the dose-response relationship. On the basis of visual inspection of the data, we chose to fit a generalized linear model with log link and variance function proportional to the square of the mean. To accommodate the correlation among observations from the same individual, we fit the models using the generalized estimating equations approach of Liang and Zeger (24). An exchangeable correlation structure was assumed (*i.e.*, constant correlation within individuals), and the robust variance estimator was used to obtain SEs for the estimates. All of the calculations were performed using Stata 6.0 (25).

RESULTS

Patient Characteristics and Response. A total of 22 patients were enrolled in the trial, of which 20 were evaluable for toxicity and 13 were evaluable for response (*i.e.*, completed three cycles or 9 weeks of therapy). Patient demographics and dose levels are indicated in Table 1. Most of the patients had started ECOG performance status 0 or 1. Neither of the two patients with a baseline performance status of 2 was able to complete the first three cycles. Of the four patients who completed less than one cycle of therapy, two withdrew because of

Table 2 Clinical toxicity summary (cycle 1)

Dose level	N	Toxicity	CTC grade (no. of patients)
6	1	Fever	1 (1)
		Fatigue	1 (1)
12	3	Fever	2 (2); 1 (1)
		Fatigue	2 (1); 1 (1)
		Nausea/vomiting	1 (1)
18	7	Fever	3 (1); 2 (1); 1 (3)
		Fatigue	2 (1); 1 (2)
		Nausea/vomiting	3 (1, with grade-3 fever)
24	7	Fever	2 (1); 1 (5)
		Fatigue	2 (2); 1 (2)
		Nausea/vomiting	2 (2); 1 (2)
30	4	Hemolytic anemia	3 (1)
		Acute renal failure/anasarca	3 (1)
		Fever	1 (1)
		Nausea/vomiting	2 (1)

Table 3 Serum cytokine levels

Dose	TNF- α (pg/ml)			IL1R- α (pg/ml)		
	Start	EOI ^a	Fold increase	Start	EOI ^a	Fold increase
24 mg/kg	33	138	4.2	450	2425	5.4
	34	110	3.2	769	1611	2.1
	14.7	39.8	2.7	790	>3000	>3.8
	6.7	104.6	15.6	409	>3000	>7.3
30 mg/kg	8	96	12.0	2123	5641	2.7
	18	57	3.2	679	5971	8.8
	147	369	2.5	2274	5552	2.4

^aEOI, end of infusion (24 h).

evident progressive disease, and two, both treated at 30 mg/kg, suffered adverse events as described below.

No objective responses were seen. Five of 13 evaluable patients (after cycle 3) had stable disease, including one with small cell lung cancer who had progressed after two previous chemotherapy regimens; this patient also noted symptomatic improvement over 15 weeks on ISIS 5132.

Toxicity. Dose levels evaluated included 6 mg/kg, 12 mg/kg, 18 mg/kg, 24 mg/kg, and 30 mg/kg. Clinical toxicity included fever and fatigue at all of the dose levels and mild to moderate nausea and vomiting at all of the dose levels above 6 mg/kg (Table 2). After grade-3 fever was noted in a patient treated at 18 mg/kg, the decision was made to incorporate prophylactic acetaminophen before and during ISIS 5132 infusion. No other incidents of dose-limiting fever were observed.

Because of the noted frequency of fever and constitutional symptoms, serum cytokine levels were analyzed before and after the first infusion in a subset of patients at 24 and 30 mg/kg (Table 3). Serum TNF- α levels in all of the seven patients increased 2.5–15.6-fold over the 24-h infusion ($P = 0.016$; sign test), and IL-1R- α levels increased 2.1–8.8-fold over the same interval ($P = 0.016$).

Two of four patients at dose level 30 mg/kg manifested serious adverse events in the first week of therapy. The first was a 63-year-old woman with adenocarcinoma of the fallopian tube who, upon completion of initial ISIS 5132 infusion, was noted

to be icteric. Laboratory evaluation revealed a Coombs' positive hemolytic anemia, with an acute fall in hematocrit from 32% to 22% and a concomitant rise in serum bilirubin from 0.8 mg/dl to 7.8 mg/dl (5.6 mg/dl unconjugated). No renal insufficiency ensued, and hemolysis and hyperbilirubinemia resolved over the subsequent week. The patient was not rechallenged with ISIS 5132. The second was a 60-year-old man with ampullary pancreatic cancer, large hepatic masses, and marked elevations in alkaline phosphatase (1061 IU/liter) and GGT (1400 IU/liter) before initiation of therapy. This patient developed acute onset renal failure and anasarca 48–72 h after first ISIS 5132 infusion. The patient withdrew from the study and requested palliative care measures only; the etiology of the acute renal failure and the relationship to ISIS 5132 remains unclear.

Because of these adverse events, the dose level of 30 mg/kg was considered to have exceeded MTD, and the 24 mg/kg dose level was expanded. A total of seven patients were treated at this dose level. One patient withdrew before completing cycle 1 because of disease progression. All of the remaining six patients completed at least one cycle of therapy with clinical adverse effects limited to grade 1–2 fever, fatigue, nausea, and vomiting. No grade 3 toxicity was observed at this dose level, which represents the suggested MTD on this schedule.

Effects on Coagulation and Complement. Because of the previously defined potential effects of phosphorothioate oligonucleotides on blood coagulation and complement activation, these parameters were also analyzed. Transient prolongations of aPTT ($1.25\text{--}1.75 \times$ baseline) were noted at the end of infusion in patients treated at or above 18 mg/kg. These elevations were not associated with any clinical sequelae. Transient grade 1 thrombocytopenia, with recovery before the next scheduled dose, was observed in cycle 1 in two of three patients at 12 mg/kg, in three of seven patients at 18 mg/kg, in five of seven patients at 24 mg/kg, and in two of four patients at 30 mg/kg. The transient thrombocytopenia noted was generally not progressive in subsequent cycles. One patient with cervical cancer, treated at 18 mg/kg, had grade-2 thrombocytopenia (platelet count, $61,000/\text{mm}^3$) in cycle 3, associated with vaginal bleeding. She received a fourth cycle without thrombocytopenia and was subsequently found to have progressive disease in the bladder.

Measures of complement activation analyzed included C3a and Bb levels (Fig. 1). In contrast to previous trials with ISIS 5132 on other schedules where complement activation was not seen, dose-dependent transient elevations in both C3a and Bb were consistently noted. No clinical sequelae were clearly associated with these sometimes-marked elevations. The effect was greatest for C3a, where a dose of 12 mg/kg increased the mean level of activation by an estimated 95%, whereas a dose of 30 mg/kg increased the mean level by 852%. With each 6 mg/kg increase in dose level, there was a 70% increase in the treatment effect ($P < 0.001$), where treatment effect is measured as the ratio of the post-infusion mean:the pre-infusion mean. Similarly for Bb, each increase in dose level yielded a 19% increase in the treatment effect ($P = 0.016$). C5a was assessed in patients at 30 mg/kg; in contrast to C3a and Bb, no significant alterations were seen.

We also examined the effects of repeated ISIS 5132 administration on complement activation. There was no evidence

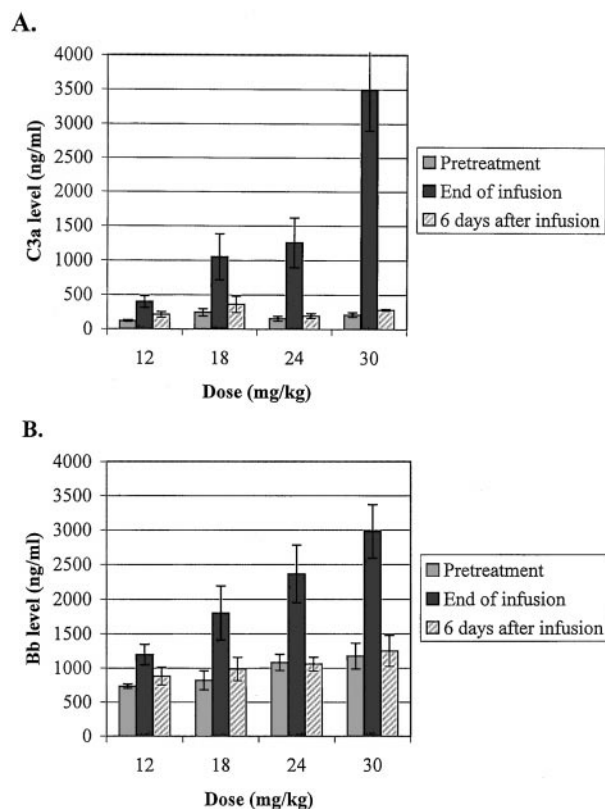


Fig. 1 Serum complement activation by ISIS 5132. All of the data from the first 4 weeks are included. C3a (A) and Bb (B) levels at each dose level are indicated at baseline (before initiating therapy on day 1), at the end of 24-h infusion (days 2, 9, 16, and 23), and at 6 days after infusion (*i.e.*, immediately before subsequent doses; days 8, 15, 22, and 29). Values shown are means; error bars, SD.

(for C3a or Bb) of a change over time in the mean level of complement activation after infusion (data not shown). However, the mean level of C3a 6 days after infusion, *i.e.*, pretreatment “baseline” level on this schedule, did increase an estimated 10%/week ($P < 0.001$). There was no evidence of a change over time in Bb.

Pharmacokinetic Analysis. Plasma levels of intact ISIS 5132 and of oligonucleotide metabolites were determined at the start of cycle 1, after 4 and 8 h, at the end of infusion, and hourly for 4 h after end of infusion. These data were used to assess steady state levels, area under the curve, clearance, and $t_{1/2}$ of ISIS 5132 on this schedule (Table 4 and Fig. 2). Clearance was found to be essentially constant across the dose range, and both steady state concentration and area under the curve appeared to vary linearly with dose. Over 50% of the oligonucleotide detected at the end of infusion was intact ISIS 5132 in all of the dose cohorts. An apparent dose-related increase in $t_{1/2}$ was observed, as has been noted previously (26, 27) with other phosphorothioate oligonucleotides of similar length, consistent with known saturable distribution to major tissues of distribution including liver and kidney.

PBMC *c-raf-1* mRNA Levels. As a measure of biological efficacy, *c-raf-1* mRNA levels in PBMCs were analyzed by

Table 4 Summary of pharmacokinetic parameters

Dose level (mg/kg)	6	12	18	24	30
C_{ss} ($\mu\text{g/ml}$)	2.88 ± 1.04	7.19 ± 3.12	9.89 ± 2.21	13.38 ± 4.24	19.11 ± 3.41
AUC_{inf} (mg/h/ml) ^a	67.0	172 ± 79	242 ± 43	331 ± 109	421 ± 138
Cl (ml/min/kg)	1.49	1.32 ± 0.46	1.28 ± 0.25	1.35 ± 0.53	1.37 ± 0.70
$t_{1/2}$ (min)	69.6	79.7 ± 19.0	79.2 ± 16.2	100 ± 19.0	144 ± 22.2
% Intact at EOI ^b	53.1	56.7 ± 3.0	56.0 ± 3.8	55.3 ± 3.6	58.2 ± 0.8

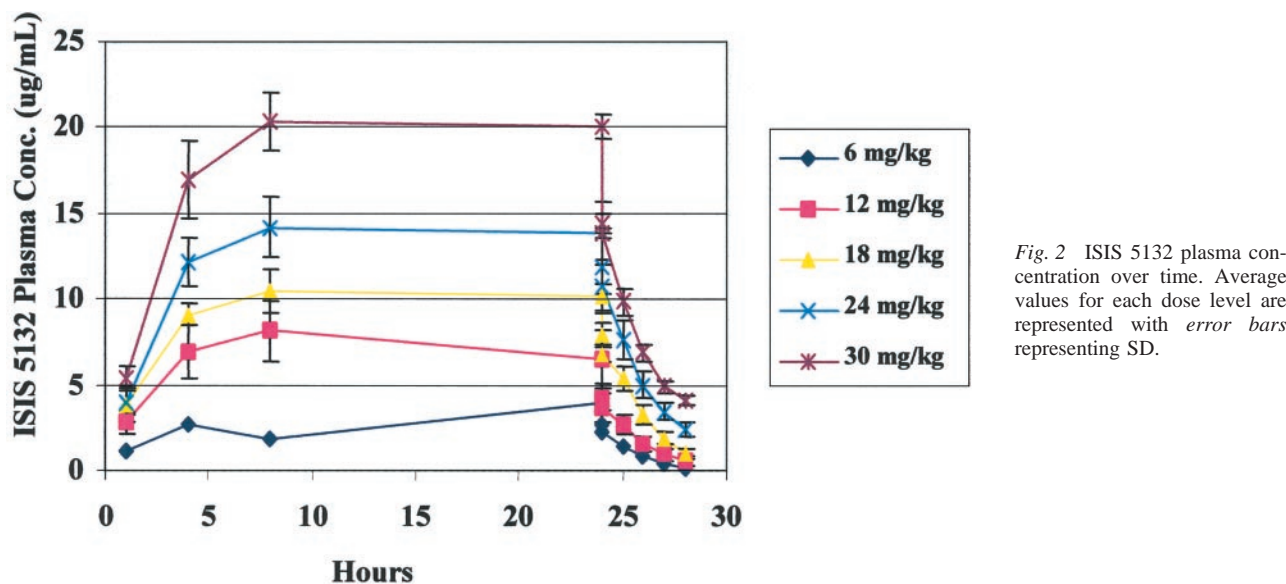
^a EOI, end of infusion (24 h).^b % Intact, $100 \times [ISIS\ 5132]/([ISIS\ 5132] + (\text{oligonucleotide metabolites}))$.

Fig. 2 ISIS 5132 plasma concentration over time. Average values for each dose level are represented with error bars representing SD.

quantitative RT-PCR, normalized to G3PDH mRNA analyzed in parallel. There was no evidence of suppression of cellular *c-raf-1* mRNA at any of the ISIS 5132 dose levels evaluated (Fig. 3).

DISCUSSION

This trial evaluated the administration of ISIS 5132 as a weekly 24-h infusion. This schedule was found to be feasible up to a dose level of 24 mg/kg/week, with clinical adverse effects limited primarily to grade 1–2 fatigue, fever (despite acetaminophen), nausea, and vomiting. Cytokine induction, minor aPTT elevation, transient thrombocytopenia, and complement activation were notable at or near MTD. No significant antitumor activity was observed, and no significant suppression of PBMC *c-raf-1* mRNA level was evident by quantitative RT-PCR.

Several observations on this trial are in marked contrast to other published Phase I trials of ISIS 5132, and the comparison suggests that clinically relevant effects of this class of compounds may be highly schedule-dependent. Previous trials include 21-day continuous i.v. infusion on a 28-day cycle (28) and a 2-h infusion three times/week \times 3 weeks, again on a 28-day cycle (29). MTD was not reached on either of these two trials, which escalated to doses of 4 mg/kg/day \times 21 days and to 6 mg/kg three times/week. These doses achieved on these trials deliver a total amount of ISIS 5132 over 28 days comparable

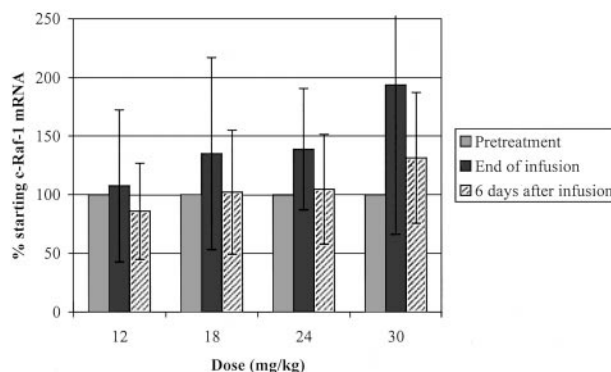


Fig. 3 Relative *c-raf-1* mRNA levels in cycle 1. Levels are normalized to G3PDH mRNA determined in parallel; day-1 level for each patient is arbitrarily set at 100%. Data are at the end of each infusion and immediately before subsequent infusions (6 days after completion of infusion). Each data point represents the mean of 10 or more independent samples, analyzed in duplicate or triplicate. Error bars, SD.

with amounts delivered at 18 mg/kg to 24 mg/kg on the current trial.

The rapid infusion on the latter of these two trials resulted in relatively high plasma concentrations ($C_{max} = 22.7 \mu\text{g/ml}$ at 6 mg/kg). In association with this higher serum concentration,

there was consistent reduction in *c-raf-1* mRNA expression with treatment. Thirteen of 14 patients evaluated demonstrated suppression of *c-raf-1* mRNA as assessed by similar RT-PCR analysis in PBMC. No DLT was noted, and prolonged stable disease was noted in two patients. The 21-day continuous i.v. infusion schedule similarly resulted in no DLT and prolonged stable disease in three patients. *c-raf-1* mRNA levels were not assayed.

It is difficult to correlate the plasma levels reached in this and other similar antisense oligonucleotide trials to an *in vitro* IC₅₀ of these agents. Intracellular uptake of ISIS 5132 and other phosphorothioate oligonucleotides in cell culture has required addition of cationic lipids, which are not required for oligonucleotide uptake and suppression of target genes *in vivo*, for reasons that are not fully understood. The *in vitro* IC₅₀ of ISIS 5132 in A549 lung cancer cells in the presence of a cationic lipid has been defined as 50–100 nM (0.34–0.67 μg/ml) for cell proliferation, *c-raf-1* mRNA expression, and protein expression. This concentration was exceeded in the plasma on all of the dose levels, but a correlation with intratumoral levels cannot be made. The best evidence that the concentrations of ISIS 5132 reached on this trial would have been expected to be bioactive may be comparison with the prior human Phase I trials involving similar dose intensity, on one of which a similar C_{max} was reached and suppression of the relevant target gene was demonstrated.

Interestingly, no complement activation was found on either of the two alternative schedules, in contrast to the marked complement activation noted with 24-h infusion. This represents an unanticipated schedule dependence of potential toxicity for phosphorothioate oligonucleotides. Complement activation by ISIS 5132 may require a combination of threshold level and minimal duration not met by either rapid and brief infusion (6 mg/kg over 2 h) or slow and prolonged infusion (4 mg/kg/day over 21 days). This finding warrants additional study before firm conclusions can be made.

The lack of evident *c-raf-1* mRNA suppression on this trial, in contrast to the data using a 2-h infusion, was surprising, and the explanation for this apparent disparity of results is not clear. One contributing factor may be lower peak concentrations achieved on the more prolonged 24-h infusion, but technical differences related to the timing or method of quantitative mRNA determination cannot be definitively ruled out. *c-raf-1* mRNA level was not determined at time points 1–5 days after completion of infusion. It is uncertain to what extent lack of suppression of *c-raf-1* mRNA levels in circulating mononuclear cells reflects lack of suppression of intratumoral *c-raf-1* mRNA. Despite these caveats, we believe that the reported consistent suppression of *c-raf-1* mRNA level using a 2-h infusion argues in favor of the short duration infusion. Data are unfortunately lacking for suppression of *c-raf-1* mRNA in patients on prolonged (21-day) infusion. This information would be of utility in the further evaluation of this agent and may be forthcoming from an ongoing Phase II trial.

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