

A Phase I Trial of ISIS 2503, an Antisense Inhibitor of H-ras, in Combination with Gemcitabine in Patients with Advanced Cancer¹

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ABSTRACT

Purpose: The purpose of this study was to define the toxicity, pharmacokinetics, and clinical activity of the combination of ISIS 2503, an oligodeoxynucleotide antisense inhibitor of H-ras, and gemcitabine in patients with advanced solid tumors.

Experimental Design: The target dose of ISIS 2503 on this study was 6 mg/kg/day. Twenty-seven patients (16 male, 11 female) received 97 treatment courses (median, 2; range, 1–13). Nineteen patients were treated with a fixed gemcitabine dose of 1000 mg/m² on days 1 and 8 and two escalating doses of ISIS 2503 (4 and 6 mg/kg/day) as a 14-day continuous infusion starting on day 1. In addition, 8 patients (5 male, 3 female) received a flat dose of ISIS 2503 based on ideal body weight. Cycles were repeated every 3 weeks. Toxicities, graded according to the National Cancer Institute Common Toxicity Criteria, were recorded as maximum grade/patient for all treatment cycles. Pharmacokinetic analyses were performed to evaluate any interaction between these two agents.

Results: The most common nondose-limiting toxicity was hematological, manifested as neutropenia (5 grade 2, 7 grade 3, and 1 grade 4) and thrombocytopenia (10 grade 1, 5 grade 2, 5 grade 3, and 1 grade 4). Nonhematological toxicities included anorexia (7 grade 1, 3 grade 2, and 1

grade 3), nausea (10 grade 1 and 1 grade 3), fatigue (6 grade 1, 5 grade 2, and 3 grade 3), fever (6 grade 1, 2 grade 2, 1 and grade 3), and thrombosis associated with central lines (5). The plasma concentration of gemcitabine at the end of infusion was altered in the presence of ISIS 2503, leading to alterations on other pharmacokinetic parameters, but the observed differences were not clinically relevant. The plasma disposition of ISIS 2503 was not altered by gemcitabine coadministration. One partial response was documented in a heavily pretreated patient with metastatic breast cancer. Disease stabilization for greater than six cycles of treatment was observed in 5 patients.

Conclusions: The combination of gemcitabine and ISIS 2503 was well tolerated and clinically active in this group of heavily pretreated patients. The recommended Phase II dose of gemcitabine (1000 mg/m²) and ISIS 2503 (6 mg/kg/day) warrants additional evaluation.

INTRODUCTION

Four human *ras* genes, H-*ras*, N-*ras* and the splice variants, K-*ras* A and K-*ras* B, have been implicated in the etiology and maintenance of the malignant phenotype. Activating mutations in these genes lead to sustained mitogenic signaling (1). Human cancers demonstrate multiple abnormalities involving *ras* gene products directly or indirectly. These include (a) activating mutation in at least one of the three *ras* genes at an early stage of tumor progression in 20–25% of all human tumors (2–4), the best known of which is the K-*ras* mutation in up to 90% of pancreatic cancers; (b) overexpression of *ras* proteins, with H-*ras* mRNA overexpression identified in colorectal cancer specimens reported to be associated with poor prognosis (5–8); (c) or overexpression of growth factors and their receptors, whose downstream signaling is mediated by *ras* pathways (9–12). H-*ras* itself has been implicated as an important growth promoter in pancreatic cell lines with mutations of K-*ras*. One strategy of novel cancer drug development has therefore focused on *ras* as a rational target.

The antisense approach in cancer therapy involves targeting specific RNA sequences to reduce translation of the mRNA message into protein, hence inhibiting gene and ultimately protein expression (13). ISIS 2503 is a 20-mer phosphorothioate oligodeoxynucleotide that hybridizes to the 5'-untranslated region of human H-*ras* mRNA and reduces H-*ras* mRNA expression through RNase H-mediated cleavage of the hybridized H-*ras* mRNA (14–16). Tissue culture studies have demonstrated that ISIS 2503 not only reduces H-*ras* mRNA levels but also inhibits the expression of H-*ras* protein in, and the proliferation of, T-24 bladder carcinoma cells in a dose- and sequence-dependent manner. ISIS 2503 can specifically discriminate between H-*ras* and its closely related family members, K-*ras* and N-*ras* (15, 16).

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In a recently completed Phase I trial, the dose and schedule of ISIS 2503 recommended as safe, potentially effective and feasible for additional studies was 6 mg/kg administered as a continuous 14-day infusion, repeated every 21 days (17). Interim results indicated a tolerable toxicity profile, with moderate thrombocytopenia, nausea, and fatigue as the only adverse events. No objective tumor responses were observed. However, an indication of biological activity was seen with disease stabilization in 4 patients (with liposarcoma, mesothelioma, colon cancer, and pancreatic cancer). Although doses as high as 10 mg/kg/day were tolerable in this trial, 6 mg/kg/day appeared minimally toxic and produced steady-state plasma levels of ~ 4 $\mu\text{g/ml}$, well below the >40 $\mu\text{g/ml}$ level associated with complement activation in primates. Prior preclinical and clinical studies with phosphorothioate oligonucleotides have demonstrated that the pharmacokinetic behavior of these agents in preclinical models is highly predictive for results in humans (18). These models predicted that a dose of 6 mg/kg/day would lead to tumor concentrations of oligonucleotide well in excess of the *in vitro* IC_{50} , which is on average 100 nM from multiple *in vitro* assays with different cell lines. Phase II single-agent studies of ISIS 2503 are ongoing.

Gemcitabine³ is a novel pyrimidine analogue of deoxycytidine that is primarily S-phase specific. It is anabolized sequentially to the nucleoside mono-, di-, and triphosphate intracellularly. The triphosphate, difluoro deoxycytidine triphosphate, is incorporated into DNA, resulting in chain termination. Moreover, the diphosphate derivative inhibits ribonucleotide reductase thus depleting intracellular pools of dCTP for incorporation into DNA. Gemcitabine has been approved for the treatment of NSCLC (19–22) and pancreatic cancer (23). The principal toxicities of gemcitabine at the approved dose and schedule are hematological.

Because the *ras* signaling pathway has been implicated in a variety of tumors, it is reasonable to hypothesize that combining ISIS 2503 with gemcitabine may result in a more effective treatment regimen for several cancers, particularly pancreatic and lung cancers. The activity of ISIS 2503 in xenograft models with and without *ras* mutations suggests that ISIS 2503 may be active in a number of tumor types. Furthermore, previous studies have demonstrated that inhibition of *ras* farnesylation combined with gemcitabine administration leads to additive cytotoxicity *in vitro* (24). On the basis of these reasons, a Phase I trial to evaluate the safety and pharmacokinetic profile, define the MTDs as well as any antitumor activity, of the combination of ISIS 2503 and gemcitabine was undertaken.

PATIENTS AND METHODS

Patient Selection

Patients with histological or cytological evidence of metastatic or locally advanced cancer for which there was no established life-prolonging therapy available, or who were un-

responsive to conventional therapy and had measurable or evaluable disease were eligible for this study. Eligibility criteria included age ≥ 18 years; Eastern Cooperative Oncology Group performance status ≤ 2 ; adequate bone marrow (platelets $\geq 100 \times 10^9$ cells/liter, ANC $\geq 1.5 \times 10^9$ cells/liter), hepatic (total bilirubin ≤ 2.0 mg/dl), and renal (serum creatinine $\leq 1.5 \times$ the upper limit of normal) functions; no chemotherapy, radiotherapy, biological, hormonal, or investigational drug therapy within 28 days before study entry and no prior nitrosourea or mitomycin C chemotherapy. Excluded from this study were patients who had a diagnosis of leukemia; radiation therapy to $>25\%$ of the bone marrow; brain metastasis, unless disease had been resected by surgery or radiosurgery and patient had been stable for 4 weeks; underlying coagulopathy; prior treatment with gemcitabine; presence of an active infection requiring therapy; and ongoing therapeutic anticoagulation with heparin or low-molecular weight heparin. Written informed consent was obtained according to federal and institutional guidelines.

Dosage and Administration

ISIS 2503 was supplied by Isis Pharmaceuticals, Inc. (Carlsbad, CA). It is formulated as a 10 mg/ml sterile solution for i.v. administration in a phosphate-buffered solution. The formulated product is sterile, preservative-free and packed in vials containing ~ 1 or 10 ml of the solution. The drug was reconstituted with normal saline to a final volume of no >500 ml. Gemcitabine was supplied in vials of either 200 mg or 1 g and formulated with mannitol and sodium acetate as a sterile lyophilized powder. This was reconstituted with sterile normal saline to make a solution containing 10 mg gemcitabine/ml.

Two escalating dose levels of ISIS 2503 (4 and 6 mg/kg/day) in combination with a fixed dose of gemcitabine (1000 mg/m²) were studied in 19 patients. ISIS 2503 pharmacokinetic data from single-agent studies (17) suggested that it may not be necessary to calculate drug doses based on actual measured body weight. The apparent volume of distribution for oligonucleotides does not increase proportionately to body weight in obese patients, who may therefore receive a disproportionately higher dose of drug. Conversely, patients who weigh substantially less than their ideal body weight appeared to be somewhat underdosed as compared with patients of normal body weight.

Eight patients were assigned to an additional cohort to evaluate the safety of an empirically derived fixed dosing schedule. In these patients, ISIS 2503 was administered as follows: 2400 mg total over 7 days for ideal body weight of 46–63 kg (corresponding to 6 mg/kg/day for a presumed body weight of 57 kg); 3500 mg over 7 days for ideal body weight of 64–89 kg (corresponding to 6 mg/kg/day for a presumed body weight of 83 kg); and 6 mg/kg of ideal body weight/day for patients >89 kg. One out of the 8 patients had an ideal body weight >89 kg.

ISIS 2503 was administered as a continuous i.v. infusion through a central line over 14 days. On the first day of the first cycle only, the ISIS 2503 infusion began 4 h after gemcitabine infusion for the purpose of characterization of gemcitabine pharmacokinetics alone and in combination with ISIS 2503. In all other cycles, the ISIS 2503 infusion began ~ 60 min before gemcitabine. The 7-day ISIS 2503 infusion cassette was changed on day 8 of each cycle. Compatibility of gemcitabine with ISIS 2503 in solution has not been established, thus gem-

³ The abbreviations used are: gemcitabine, 2',2'-difluorodeoxycytidine; ECOG, Eastern Cooperative Oncology Group; MTD, maximum-tolerated dose; ANC, absolute neutrophil count; NSCLC, non-small cell lung cancer; HPLC, high-performance liquid chromatography.

Table 1 Patient characteristics (n = 27)

	Value	Count
No. of courses (fully evaluable)		97 (91)
Median courses/patient (range)		2 (1, 13)
Median age (range)		63 (43, 79)
Gender	M/F	16/11
ECOG performance status	0	13
	1	13
	2	1
Prior chemotherapy	N ^a	5
	Y	22
Prior radiation	N	17
	Y	10
Tumor type	Bladder	1
	Breast	1
	Colorectal	10
	Leiomyosarcoma	2
	Lung	5
	Mesothelium	2
	Renal	2
	Pancreas	1
Upper gastrointestinal	3	

^a N, no; Y, yes.

citabine was administered through a separate IV access line from ISIS 2503. Gemcitabine was administered i.v. over 30 min on days 1 and 8 of each 21-day treatment cycle. At least 3 new patients were entered at each dose level in a standard cohorts of three Phase I design. Dose escalation was not allowed in individual patients.

Dose-limiting Toxicities

All toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0). The MTD was defined as one dose level below the dose that induced dose-limiting toxicities in one-third or more of patients (at least 2 of a maximum of 6 new patients). MTD was defined based on toxicities documented in the first cycle of treatment only. Severe or life-threatening nonhematological toxicity (grade 3 or 4), with the exception of nausea, vomiting, or diarrhea, was considered dose limiting. Nausea was not considered dose limiting. Grade 4 vomiting or diarrhea that persisted despite maximal prophylaxis and treatment with antiemetic or antidiarrheal therapy, respectively, was considered dose limiting. Grade 4 neutropenia associated with fever or lasting for ≥ 5 days and grade 4 thrombocytopenia or grade 3 thrombocytopenia with \geq grade 2 hemorrhage were likewise deemed dose limiting.

Pretreatment and Follow-up Studies

Complete patient histories, physical examinations, complete blood counts, serum electrolytes, chemistries, and coagulation profile were obtained at baseline and before each course of treatment. Laboratory studies were performed weekly while patients were on study. Radiological studies (roentgenograms, computed axial tomographic scans, and magnetic resonance imaging) were performed at baseline and at the end of every third cycle to assess tumor response. A partial response required at least a 50% reduction in the sum of the products of bidimensional measurements, separated by at least 4 weeks. A complete response was defined as the disappearance of all evidence of

Table 2 Dose escalation scheme

Dose level	ISIS 2503 (mg/kg)	Gemcitabine (mg/m ²)	No. of patients	Courses of treatment
1	4	1000	5	9
2	6	1000	14	56
3 ^a	6	1000	8	32

^a Fixed dose of 6 mg/kg/day based on ideal body weight.

tumor on two measurements separated by a minimum of 4 weeks. Progressive disease was the appearance of new lesion(s) or an increase in the sum of the bidimensional products of all known disease by at least 25%. Stable disease was documented when there was persistence of disease without meeting the criteria for progression, partial response, or complete response.

Pharmacokinetic Analyses

Pharmacokinetic studies were performed on the first cycle only. Blood samples for gemcitabine pharmacokinetics were collected on the first and eighth day in heparinized tubes containing tetrahydrouridine. ISIS 2503 pharmacokinetic samples were collected on the eighth and fifteenth day in EDTA tubes for ISIS 2503 assays. On day 1, blood was obtained 5 min before and 5, 15, 30, 60, 120, 180, 240 min after the end of gemcitabine infusion. ISIS 2503 infusion was started 4 h after the end of gemcitabine infusion on day 1. On day 8, samples were drawn 15 min before stopping ISIS 2503 and just before the start of gemcitabine infusion. Postgemcitabine infusion samples were obtained in the same schedule as aforementioned, whereas the ISIS 2503 infusion was restarted 15 min after the end of gemcitabine infusion. On day 14, blood specimens were drawn 15 min before and 30, 60, 90, 120, 180 min after termination of ISIS 2503 infusion. Immediately after collection, each sample was chilled in an ice-water slurry and centrifuged. The plasma layer then was transferred to polypropylene tubes, capped, frozen immediately and stored at -70°C . All samples were sent via express overnight priority delivery to Southwest Research Institute (San Antonio, TX) for analysis.

Assay Methodology

Gemcitabine. Gemcitabine was measured in plasma by a HPLC method using a Waters LC Module equipped with a Supelcosil LC-NH₂ HPLC column and a UV detector set at 272 nm. The mobile phase consisted of cyclohexane, 1,2-dichloroethane, methanol, water, glacial acetic acid, and triethylamine (6.3:1.5:2.2:0.01:0.005:0.01 by volume) and was pumped at a rate of 1.5 ml/min. The HPLC method was validated to specifications for precision and accuracy. Aliquots of plasma samples were thawed, and an internal standard (5-fluoro-2'-deoxyuridine) was added before extraction. One ml of isopropanol was added to 200 μl of plasma and mixed vigorously by vortexing. After mixing, 2.5 ml of ethyl acetate was added, and the sample mixture was once again vortexed. The sample was then centrifuged at ~ 3000 rpm for 10 min to pellet the precipitate. The supernatant was transferred to a clean tube and taken to dryness under nitrogen flow. The samples were then reconstituted with 250 μl of mobile phase and injected directly onto the HPLC.

Table 3 Neutropenia

A. Course 1 per patient nadirs						
ISIS dose (mg/kg)	<i>n</i>	Median ANC nadir (range)	Grade 1	Grade 2	Grade 3	Grade 4
4	5	3.36 (0.59, 5.67)	0	0	2	0
6	14	1.92 (0.9, 4.26)	1	3	2	0
6 ^a	8	1.29 (0.47, 2.4)	0	3	1	1

B. Overall per patient nadirs						
ISIS dose (mg/kg)	<i>n</i>	Median ANC nadir (range)	Grade 1	Grade 2	Grade 3	Grade 4
4	5	3.36 (0.59, 5.67)	0	0	2	0
6	14	1.75 (0.05, 4.26)	0	3	3	1
6 ^a	8	1.24 (0.47, 2.1)	0	2	2	1

^a Fixed dose of 6 mg/kg/day based on ideal body weight.

The linear range for this assay was 0.15–2.5 µg/ml. The limit of quantitation for this assay was determined to be 0.15 µg/ml.

ISIS 2503 and Metabolites. ISIS 2503 was measured in plasma using capillary gel electrophoresis. The assay methods have been described previously (25). Plasma samples were stored frozen until assayed. Upon thawing, a phosphorothioate oligodeoxynucleotide internal standard composed of 27 thymidine nucleotides (T₂₇) was added to an aliquot of each plasma sample before extraction. Plasma samples were prepared for analysis by strong anion exchange solid-phase extraction, followed by two desalting steps: (a) elution from a reverse-phase solid-phase extraction column followed by (b) membrane dialysis. Capillary gel electrophoresis was performed with a Beckman MDQ CE instrument (Beckman, Fullerton, CA) with a 27-cm capillary column. Oligonucleotides eluting from the column were detected by UV absorption at a wavelength of 260 nm. The linear range of the assay was 20 nM (~140 ng/ml) to 20 µM (~140 µg/ml) in plasma. Plasma assay methods were validated to meet acceptance criteria for precision and accuracy. The limit of quantitation for this assay was determined to be 20 nM for ISIS 2503 (~140 ng/ml). Concentrations for full-length ISIS 2503 and its major oligonucleotide metabolites (shortened oligonucleotides of 19, 18, 17, and 16 nucleotides in length) were also measured.

Data Analysis

Noncompartmental pharmacokinetic analysis methods were applied to both gemcitabine and ISIS 2503 (WinNonlin, Version 2.2, Pharsight Corp.). For gemcitabine, concentration of gemcitabine at the end of infusion (C_{EOI}) was summarized by observation. Area under the plasma concentration-time curve ($AUC_{0-\infty}$) was calculated using the linear trapezoidal method. Plasma clearance (CL) was calculated by dividing dose (1000 mg/m²) by AUC . The terminal phase rate constant (λ_z) was calculated as the negative of the slope of the log-linear terminal portion of the plasma concentration-time curve using regression. Terminal phase half-life was calculated as $0.693/\lambda_z$. Apparent steady-state volume of distribution (V_{ss}) was calculated using standard noncompartmental first-moment theory method (26). Data were compared between 4 and 6 mg/kg and fixed dosing ISIS 2503 cohorts.

The plasma pharmacokinetics for ISIS 2503 were assessed by summarizing the concentration of ISIS 2503 measured just before the end of the continuous infusion on days 8 and 15. The percentage of intact ISIS 2503 (percentage intact) was calculated by dividing the concentration of the parent oligonucleotide (20-mer oligonucleotide) by the total concentration of all measurable oligonucleotide peaks (sum of parent and metabolites) assayed in the plasma sample. Area under the plasma concentration-time curve (AUC_{ss}) was calculated by multiplying the average C_{ss} (combining both day 8 and day 15 data) by 24 h. CL was calculated by dividing the total daily dose by AUC_{ss} . The terminal phase rate constant (λ_z) was calculated as the negative of the slope of the log-linear terminal portion of the plasma concentration-time curve using regression on concentrations measured in plasma samples collected on day 15 after the infusion was stopped. The terminal phase half-life was calculated as $0.693/\lambda_z$. Data were summarized by dose cohort: 4 mg/kg, 6 mg/kg, and fixed dose, separately.

RESULTS

Patient Demographics. A total of 27 patients (Table 1) received 97 assessable courses of therapy through two dose levels (Table 2). One patient received one course of therapy that was not fully assessable. This patient developed a left upper extremity deep venous thrombosis associated with a peripheral i.v. central catheter and went off study subsequently. The median number of courses administered/patient was 2 (range, 1–13). The median age of the study participants was 63 years (range, 43–79 years). There were 16 males and 11 females enrolled. Patients were of good performance status, with all patients, except one, having an Eastern Cooperative Oncology Group performance status ≤ 1 . Twenty-two patients had received prior chemotherapy and 10 had received prior radiation therapy. The most common tumor type was colorectal cancer, with 10 patients. There were 5 patients who had NSCLC, 3 who had gastrointestinal tract cancers other than colorectal and pancreatic cancer, 2 each with mesothelioma and leiomyosarcoma, and a variety of other tumors.

Hematologic Toxicity. Table 3, A and B, lists the median and range for the nadir of the ANCs and the grades of neutropenia observed in cycle 1 only, and in all cycles of treatment,

Table 4 Thrombocytopenia

A. Course 1 per patient nadirs						
ISIS dose (mg/kg)	<i>n</i>	Median PLT Nadir (range)	Grade 1	Grade 2	Grade 3	Grade 4
4	5	96 (27, 268)	1	0	2	0
6	14	97 (58, 154)	5	3	0	0
6 ^a	8	89.5 (30, 128)	4	1	2	0
B. Overall per patient nadirs						
ISIS dose (mg/kg)	<i>n</i>	Median Platelet Nadir (range)	Grade 1	Grade 2	Grade 3	Grade 4
4	5	96 (23, 262)	1	0	2	0
6	14	87 (5, 153)	6	4	0	1
6 ^a	8	68 (29, 102)	3	1	3	0

^a Fixed dose of 6 mg/kg/day based on ideal body weight.

respectively. The severity and incidence of neutropenia seemed to be independent of the dose of ISIS 2503, either initial or cumulative. Seven patients had grade 3 neutropenia and 2 patients had nondose-limiting grade 4 neutropenia.

Table 4, A and B, lists the median and range for the nadir and grades of thrombocytopenia. Similar to the results obtained for the ANC, 5 patients had grade 3 thrombocytopenia and only one episode of grade 4 thrombocytopenia occurred in a later cycle. However, the overall effect on the platelet count appeared to be dose dependent. Nevertheless, this was modest, with most affected patients sustaining grades 1–2 thrombocytopenia. The fixed dose cohort appeared to have lower neutrophil and platelet nadirs, as well as more frequent grade 2/3 toxicity.

Nonhematological Toxicity. The nonhematological side effects of this combination were mostly mild to moderate. The common toxicities noted were nausea, anorexia, fatigue, and fever. Fig. 1 displays treatment related toxicities for all cycles of treatment.

Antitumor Activity. Twenty of 27 patients were assessable for antitumor activity. Seven patients discontinued therapy before the evaluation for tumor response, 5 of these were secondary to development of central line-associated deep vein thrombosis. Pulmonary embolus occurred in one patient and seizures were noted in another patient with a known history of seizure disorder. Six patients had prolonged disease stabilization on therapy (5, 7, 8, 11, 12, 13 cycles). Of these 6 patients, 3 patients discontinued treatment for toxicity or other intercurrent illness but not for reasons of disease progression. One patient with peritoneal mesothelioma received 13 cycles of therapy. The tumor types for the other patients include esophageal cancer, NSCLC, leiomyosarcoma, and pancreatic cancer. A partial response was seen (at dose level 2) in one breast cancer patient who had been extensively treated previously (including stem cell transplantation). s.c. metastases decreased by 80%, and liver metastases shrank by 50% in this patient. Prolonged disease stabilization was noted at all dose levels.

Pharmacokinetics. Steady-state plasma concentrations (C_{ss}) of ISIS 2503 increased as dose increased from 4 to 6 mg/kg (Tables 5 and 6). The fixed dose regimen based on ideal body weight yielded approximately equivalent ISIS 2503 C_{ss} when compared with the 6 mg/kg treatment. CL and $t_{1/2}$ were unchanged at the various ISIS 2503 dose regimens, confirming dose independence and linearity of ISIS 2503 pharmacokinetics over this dose range.

Regardless of the ISIS 2503 dose, plasma concentrations of ISIS 2503 achieved during infusion were not altered by administration of gemcitabine (Table 5). Similarly, the fraction of total oligonucleotide comprised of parent oligonucleotide (percentage of intact – 20-mer ISIS 2503) was also unchanged when dosed with gemcitabine, suggesting a lack of induction or inhibition of ISIS 2503 metabolism.

There was a consistent decrease in the gemcitabine plasma concentration observed at the end of the 30-min gemcitabine infusion (Table 7). Although alteration of gemcitabine concentration at this single time point resulted in a decrease in AUC and other related pharmacokinetic parameter estimates, the changes did not reach the level of statistical significance. Also, the elimination $t_{1/2}$ for gemcitabine was not altered, and postinfusion plasma concentrations were superimposable across the groups (Fig. 2).

DISCUSSION

The combination of ISIS 2503 and gemcitabine is well tolerated. The recommended Phase II dose on this schedule is ISIS 2503, 6 mg/kg/day as a 14-day continuous infusion and gemcitabine 1000 mg/m² on the first and eighth day of each cycle. Although neutropenia and thrombocytopenia were frequently encountered with this combination, no dose-limiting toxicity was incurred in this study. The recommended Phase II dose was based on the preselected target dose of 6 mg/kg/24 h as a dose adequate to inhibit H-ras mRNA synthesis from preclinical and early clinical data (18). The gemcitabine dose was fixed at 1000 mg/m² as the currently approved therapeutic dose. Thrombocytopenia, which was mild, appeared to be dose related with regard to ISIS 2503. Most likely, this is a sequence-independent class effect because similar events have been observed with other phosphorothioate antisense compounds such as ISIS 5132, which targets C-raf kinase mRNA (27) and ISIS 3512, which targets protein kinase C- α (28). No cumulative effects were evident, however, as the predose values were similar between the first and subsequent doses. The mechanism of thrombocytopenia has yet to be established. Treatment with ISIS 2503 in mice, but not in monkeys, produced immunoproliferative effects characterized by dose-dependent hepatosplenomegaly and lymphoid hyperplasia. Sequestration or immune destruction are, hence, plausible explanations. Additional investigations in animal models regarding this pathophysiology are

NON-HEMATOLOGIC TOXICITIES BY DOSE – All Cycles

*Fixed dose of 6/mg/kg/day, based on ideal body weight

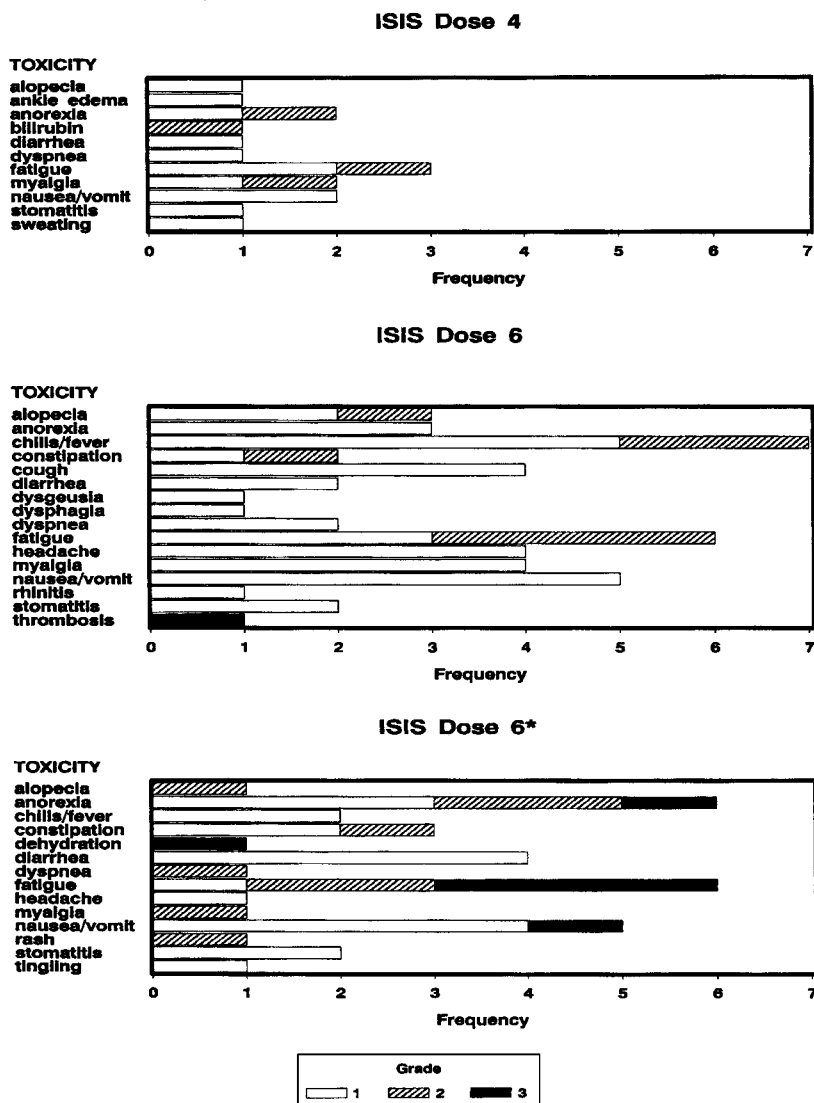


Fig. 1 Nonhematological toxicities for all cycles of treatment graded as maximum severity/patient.

being conducted. Neutropenia, on the other hand, seemed to be independent of the dose escalation schemes for ISIS 2503; thus, most probably reflecting the response to the fixed dose of gemcitabine.

It has clearly been shown *in vitro* that ISIS 2503 can specifically inhibit H-ras mRNA expression (15, 16). Because of technical difficulties inherent in human *ex vivo* assays, the effect of this antisense oligonucleotide on its target has not yet been demonstrated in the context of human clinical trials.

The pharmacokinetics of ISIS 2503 in this study have been extensively studied in animal models and previous single-agent Phase I studies in man (17). Tissue distribution and metabolism, through a succession of exonuclease-mediated cleavage, are the principal mechanisms by which ISIS 2503 is cleared from

plasma. In monkeys, the maximum plasma concentration and *AUC* values increased with dose. However, clearance of intact drug from plasma was dose dependent and showed a saturation phenomenon. This dose dependence was shown with drug infusion rates far greater than what is being clinically applied in this study. With only two dosing schedules they use a minimal linear increment, these responses were not replicated.

Although there was a decrease in the concentration of gemcitabine observed at the end of the infusion, the *t*_{1/2} was not altered. This apparent alteration of gemcitabine concentration at the end of infusion on day 8 may be artifactual because of the sensitivity in the timing of collection for that sample. In any case, an alteration at a single time point in the pharmacokinetic profile is unlikely to be clinically relevant. In addition, the

Table 5 Plasma pharmacokinetic summary for ISIS 2503 with and without gemcitabine coadministration
Only patients with ISIS 2503 plasma concentration data both with and without gemcitabine were included in the statistical summary.

Parameter	ISIS 2503 dose cohort	<i>n</i>	Day 8 (with gemcitabine)	Day 8 (no gemcitabine)
C_{SS} ($\mu\text{g}/\text{ml}$) ^a	4 mg/kg/day	2	1.93	2.39
	6 mg/kg/day	8	3.71 \pm 1.07	3.88 \pm 0.83
	Fixed dose	5	3.74 \pm 1.30	3.80 \pm 1.50
Percentage intact at C_{SS}	4 mg/kg/day	2	79.6	76.8
	6 mg/kg/day	8	71.8 \pm 4.0	71.1 \pm 4.6
	Fixed dose	5	67.8 \pm 1.3	69.7 \pm 4.3

^a Formulas: percentage intact at $C_{SS} = [(\text{ISIS 2503}) / ((\text{ISIS 2503} + \text{chain-shortened oligo metabolites}))] \times 100$; C_{SS} = observed plasma concentration during constant infusion of ISIS 2503 before and i.v. infusion of gemcitabine on day 7.

Table 6 Plasma pharmacokinetic summary for ISIS 2503^a

Cohort	<i>n</i>	C_{SS} , day 8 ($\mu\text{g}/\text{ml}$)	C_{SS} , day 15 ($\mu\text{g}/\text{ml}$)	Percentage intact (Day 8)	Percentage intact (Day 15)	AUC_{SS}^b ($\mu\text{g}^*\text{h}/\text{ml}$)	CL^c (ml/h)	$t_{1/2}$ (min)
4 mg/kg	3	2.43 (0.11)	2.51 (0.33)	76.0 (2.1)	78.6 (5.6)	59.2 (1.9)	5127 (395)	54.3
6 mg/kg	12	4.05 (0.82)	4.09 (1.21)	70.4 (4.5)	72.2 (6.2)	98.9 (23.5)	4807 (1415)	70.1 (27.3)
Fixed dose ^d	7	3.70 (1.41)	3.02 (0.76)	69.3 (3.9)	65.2 (3.5)	84.0 (25.1)	5543 (1704)	70.0 (20.5)

^a Average (SD).

^b $AUC_{SS} = C_{SS} \times 24$ h.

^c $CL = \text{total daily dose}/AUC_{SS}$.

^d Patients treated with a fixed dose of ISIS 2503.

Table 7 Plasma pharmacokinetic summary for gemcitabine (1000 mg/m², 30 min i.v. infusion) before (no ISIS 2503) and during ISIS 2503 (with ISIS 2503) treatment

Patients that did not have complete gemcitabine profiles (both with and without ISIS 2503 treatment) were not included in the statistical comparison.

Parameter	ISIS 2503 dose cohort	<i>n</i>	Day 1 (no ISIS 2503)	Day 8 (with ISIS 2503)
C_{EOI} ($\mu\text{g}/\text{ml}$)	1 ^a	2	12.7	11.4
	2 ^b	7	30.8 \pm 17.2	13.3 \pm 6.2
	3 ^c	7	31.2 \pm 13.3	16.0 \pm 5.9
$AUC_{0-\infty}$ ($\mu\text{g} \times \text{h}/\text{ml}$)	1 ^a	2	7.36	6.49
	2 ^b	7	12.4 \pm 6.0	6.5 \pm 3.3
	3 ^c	7	12.2 \pm 5.9	8.4 \pm 3.1
CL^d (liter/h/m ²)	1 ^a	2	158.4	154.4
	2 ^b	7	107.8 \pm 75.2	188.2 \pm 85.2
	3 ^c	7	103.4 \pm 55.6	136.2 \pm 55.2
V_{SS}^e (liter/m ²)	1 ^a	2	134.8	173.1
	2 ^b	7	45.2 \pm 34.7	77.0 \pm 31.8
	3 ^c	6	31.7 \pm 13.7	68.4 \pm 35.0
$t_{1/2}$ (min)	1 ^a	2	82	65
	2 ^b	7	16 \pm 10	15 \pm 10
	3 ^c	6	14 \pm 3	16 \pm 8

^a Cohort 1, patients treated with 4 mg/kg/day ISIS 2503.

^b Cohort 2, patients treated with 6 mg/kg/day.

^c Cohort 3, patients treated with a fixed dose of ISIS 2503.

^d $CL = \text{dose} / AUC_{0-\infty}$.

^e $V_{SS} = [(k_0T \times \text{AUMC}) / \text{AUC}^2] - k_0T^2 / (2 \times \text{AUC})$.

changes in some of the pharmacokinetic parameters were not statistically significant across dose levels.

Five patients developed central line-associated deep vein thrombosis in this study. After the initial thrombotic event, all patients subsequently received prophylaxis with oral low-dose (1 mg) Coumadin. Because malignancy confers a prothrombotic

effect, it is unclear whether these events are related to the study drug(s). In addition, it is known that the antisense oligonucleotides, with regard to the coagulation profile, are associated with asymptomatic activated partial time thromboplastin prolongation instead of procoagulation (27, 28). The incidence of venous thrombosis was not related to the dose of ISIS 2503, the occur-

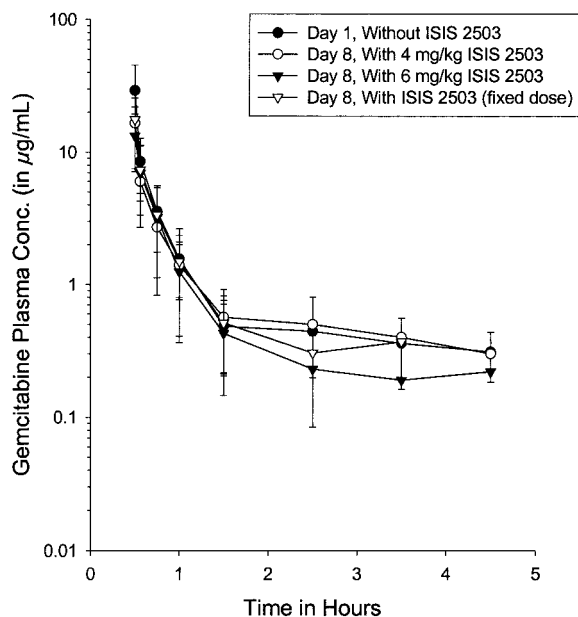


Fig. 2 Plasma concentration versus time curves of gemcitabine on day 1, day 8 with 4 mg/kg ISIS 2503, day 8 with 6 mg/kg ISIS 2503, and day 8 with a fixed dose of ISIS 2503.

rence of thrombocytopenia or any abnormalities in the coagulation profile. Additional studies with this drug combination will be needed to determine whether procoagulability could be a toxic manifestation.

There has been preliminary evidence of antitumor activity in Phase II trials of ISIS 2503 (6 mg/kg/day) in stabilizing disease in patients with pancreatic and NSCLC. In a Phase I study, evaluating clinical activity of an investigational regimen on a wide variety of tumor types and at different dose levels is difficult. However, the clinical activity seen across a broad range of tumor types suggests that this regimen warrants additional testing in Phase II trials. Consistent with this conclusion, the recommended doses of this combination are currently undergoing Phase II studies in pancreatic carcinoma through the North Central Cancer Treatment Group.

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