Phase I Clinical and Pharmacokinetic Study of Protein Kinase C-α Antisense Oligonucleotide ISIS 3521 Administered in Combination with 5-Fluorouracil and Leucovorin in Patients with Advanced Cancer[1]

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ABSTRACT

The present study was designed to determine the maximum tolerated dose (MTD), toxicity profile, pharmacokinetics (PKs), and antitumor activity of the protein kinase C-α antisense oligonucleotide ISIS 3521 (ISIS Pharmaceuticals, Inc., Carlsbad, CA) when administered in combination with 5-fluorouracil (5-FU) and leucovorin (LV). Patients with refractory solid tumors received ISIS 3521 as a 21-day continuous infusion administered simultaneously with 5-FU and LV given daily for 5 days repeated every 4–5 weeks (one cycle). 5-FU and ISIS 3521 PK analysis were performed on samples taken during the first cycle in all patients. Fifteen patients received ISIS 3521 at one of three dose levels: (*a*) 1.0 ($n = 3$ patients); (*b*) 1.5 ($n = 3$ patients); and (*c*) 2.0 ($n = 9$ patients) mg/kg/day. All patients simultaneously received 5-FU (425 mg/m^2/day) and LV (20 mg/m^2/day) for 5 consecutive days. Grade 1–2 toxicities included alopecia, fatigue, mucositis, diarrhea, anorexia, nausea/vomiting, and tumor pain. One patient had grade 3 chest pain considered to be related to 5-FU therapy, another patient had dose-limiting grade 3 mucositis resolving in <7 days, and one patient with a history of gastritis had an acute upper gastrointestinal bleed thought to be 5-FU-induced toxicity. Five patients developed cycle 1 grade 4 neutropenia, which resolved without colony-stimulating factors before the next treatment cycle. There were no effects on prothrombin time and activated partial thromboplastin time. A clinically defined MTD was not reached. The character and severity of these toxicities do not seem to be dose related, and, as such, there was no classical dose-limiting toxicity defining the MTD. ISIS 3521 PKs in the presence of 5-FU was consistent with those reported previously. 5-FU PK parameters were also similar in the presence or absence of ISIS 3521. Six of 14 patients (~43%) across all dose cohorts had an improvement in measurable tumor response ranging from minor reduction in tumor size (4 patients) to objective partial response (>50% reduction in tumor size, 2 patients). ISIS 3521 is tolerable at its recommended single-agent dose when given with 5-FU and LV. There is no apparent PK interaction between ISIS 3521 and 5-FU and LV. Antitumor activity was observed with the combination; however, it is uncertain whether clinical activity is a result of enhanced drug interaction. Our study warrants further exploration of efficacy in a Phase II and/or Phase III clinical trial setting.

INTRODUCTION

Antisense therapy is a new approach to pharmacology that exploits the exquisite sensitivity of the genetic code to permit highly selective inhibition of a molecular target (1). Antisense oligonucleotides are specific nucleotide sequences in antisense orientation that selectively hybridize to target mRNA. Because of the specificity of the genetic code, it is possible to design oligonucleotide inhibitors of a single member of a closely related protein family. After hybridizing to target mRNA, antisense oligonucleotides reduce the expression of the target protein (2–5). This reduction of protein expression may occur by several mechanisms, most commonly by RNase H-mediated degradation of the hybridized mRNA. An antisense drug, fomivirsen, has been approved in the United States and Europe for AIDS-related cytomegalovirus retinitis.

PKC[3] is a family of cytoplasmic serine/threonine kinases responsible for signal transduction in response to growth factors, hormones, and neurotransmitters (6). Although many isoforms of PKC have been described with distinct tissue specificity and

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[3] The abbreviations used are: PKC, protein kinase C; PK, pharmacokinetic; 5-FU, 5-fluorouracil; LV, leucovorin; ECOG, Eastern Cooperative Oncology Group; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT, prothrombin time; aPTT, activated partial thromboplastin time; AUC, area under the curve; PR, partial response; PD, progressive disease; MTD, maximum tolerated dose; ANC, absolute neutrophil count; CL, clearance; CTC, Common Toxicity Criteria; SD, stable disease.

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intracellular localization, several lines of evidence implicate PKC-α in the malignant phenotype through altering effects on other transforming gene products. PKC-α mediates transforming effects of oncogenic *ras* in keratinocytes (7). Overexpression of PKC-α in MCF7 breast cancer cells resulted in a more aggressive neoplastic phenotype that was manifested by enhanced proliferative rate, anchorage-independent growth, and increased tumorigenicity in nude mice (8). PKC-α has been implicated in drug resistance and responsiveness of colon carcinoma cells to growth factors (9–11). Antisense down-regulation of PKC-α increases the sensitivity of colon cell lines to 5-FU (12).

ISIS 3521 is a 20-base phosphorothioate antisense oligodeoxynucleotide that hybridizes to a sequence in the 3′-untranslated region of the human PKC-α mRNA, leading to RNase H-mediated reduction in protein expression. In cell culture, ISIS 3521 specifically reduced PKC-α mRNA and protein expression in a sequence- and concentration-dependent manner, with an IC_{50} of 100–200 nM (13, 14). Preclinical antitumor activity has been observed *in vivo* in human tumor xenograft models (15, 16). Furthermore, preclinical data in animal models of human cancer show that the combination of ISIS 3521 with cisplatin, 5-FU, or paclitaxel results in greater antitumor efficacy than is observed with the individual agents (16).

A Phase I trial of ISIS 3521 demonstrated that it is feasible to administer ISIS 3521 as a 21-day continuous infusion to patients with advanced cancer (17). In that trial, fatigue and thrombocytopenia were reported to be dose-limiting at a dose of 3.0 mg/kg/day. Evidence for antitumor activity was observed in three of four patients with ovarian cancer. Recently, promising activity using the combination of ISIS 3521 with carboplatin and paclitaxel has been demonstrated in patients with non-small cell lung cancer (18). Based on the observed enhanced preclinical activity with chemotherapy and the prediction that the combination of ISIS 3521 and 5-FU would produce nonoverlapping toxicity, we initiated a Phase I therapeutic and PK study of the combination of ISIS 3521 and 5-FU and LV in patients with advanced cancer. The principal objectives were to characterize the toxicities of the combination and to determine the MTD of ISIS 3521 administered as a 21-day i.v. infusion with 5-day bolus injections of 5-FU and LV repeated every 28 days. Secondary objectives included definition of the PK behavior of ISIS 3521 and 5-FU when administered in combination and initial assessment of the potential antitumor activity of the combination. At the highest doses of ISIS 3521 administered with 5-FU and LV in this trial, these results define a regimen suitable for outpatient Phase II development in specific tumors including but not limited to gastrointestinal tract malignancies.

PATIENTS AND METHODS

Patient Population. Patients eligible for this study included those with a histological diagnosis of cancer for which no effective therapy was available or that was unresponsive to conventional therapy. Patients were ≥18 years of age with an ECOG performance status of ≤2 and a life expectancy of ≥12 weeks. Patients with measurable or evaluable disease were eligible. All patients were required to have fully recovered from toxicity of prior therapies and could not have received chemotherapy ≤28 days before study entry. Organ function and laboratory requirements included the following: ANC > 1500 cells/mm^3; platelet count > 100,000 cells/mm^3; hemoglobin concentration > 9.0 g/dl; normal blood coagulation as assessed by PT, International normalized ratio, and aPTT; serum creatinine ≤ 1.5 mg/dl; serum bilirubin ≤ 1.5 mg/dl; and serum AST and ALT < 2.5 times the upper limit of normal (or <5 times normal in the presence of hepatic metastases). Patients were specifically excluded if they (*a*) had an underlying disease state associated with active bleeding or (*b*) had a history of coagulopathy or complement abnormality or were receiving therapeutic doses of anticoagulants such as heparin or warfarin. All patients received information regarding the purpose and conduct of this study and provided written informed consent in accordance with federal and institutional guidelines.

Eligible patients were asked to undergo a standard history and physical examination, ECOG performance status assessment, monitoring of routine complete blood counts (total WBC with automated differential count; hemoglobin, hematocrit, and platelet count), serum chemistry panel (electrolytes, urea, creatinine, glucose, total protein, albumin, calcium, magnesium, phosphate, uric acid, alkaline phosphatase, total bilirubin, ALT, AST, and γ-glutamyltransferase), urinalysis, 12-lead electrocardiogram, chest X-ray, and other radiological assessments of tumor burden (*e.g.*, computed tomography scan) within 14 days of study entry. After treatment was initiated, patients underwent routine monitoring of blood counts every week and serum chemistries every 3 weeks. During cycle 1, PT and aPTT were assessed every week for 4 weeks; PT and aPTT were then assessed pre-dose and on days 7 and 21 of each subsequent cycle.

Study Drugs. ISIS 3521 is a 20-nucleotide phosphorothioate oligodeoxyribonucleotide with the nucleotide sequence 5′-GTTCTCGCTGGTGAGTTTCA-3′ and was supplied as a 10 mg/ml sterile solution in PBS. ISIS 3521 was administered by three consecutive 7-day continuous infusions on days 1–21 of each 28-day treatment cycle, using a volumetric ambulatory infusion pump through a 0.22 μm in-line filter at a rate of approximately 1.5 ml/h. The total dose given for 1 week was calculated based on the weight of the patient. The volume to be infused for 1 week was calculated and adjusted in 0.9% NaCl.

5-FU is commercially available and was administered as a bolus i.v. injection daily on days 0–4 of each 28-day treatment cycle. LV calcium, also commercially available, is a mixture of the diastereomers of 5-formyl derivative of tetrahydrofolate.

Drug Administration. Three dose cohorts (n = 3–6 patients/cohort) were studied with ascending doses of 1.0, 1.5, and 2.0 mg/kg/day ISIS 3521 as a continuous 21-day infusion repeated every 28 days. 5-FU was administered at a dose of 425 mg/m^2/day with LV at a dose of 20 mg/m^2/day as a bolus infusion for 5 consecutive days (days 0–4) repeated every 28 days. On day 0 of cycle 1, 5-FU injection was administered *before* beginning the ISIS 3521 infusion. For cycle 2 and subsequent cycles, on day 0 of treatment, ISIS 3521 infusion was initiated before 5-FU injection.

Study Design, Dose-limiting Toxicities, and Dose Modifications. Three patients per dose cohort were initially enrolled. Doses of ISIS 3521 were escalated in subsequent cohorts if there was no evidence of treatment-related dose-limiting tox-

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Table 1 Dose modifications for ISIS 3521 and 5-FU based on toxicity

Toxicity	CTC grade	Dose modification
Neutropenia	1	None
	2	None
	3	None
	4	Hold therapy until <grade 2, reduce dose of ISIS 3521 and 5-FU according to protocol[a]
Neutropenic fever		Hold therapy until fever has resolved and neutropenia ≤grade 2, reduce dose of ISIS 3521 and 5-FU according to protocol[a]
Thrombocytopenia	1	None
	2	None
	3	If with bleeding, hold therapy until ≤grade 1 and then reduce dose of ISIS 3521 and 5-FU according to protocol;[a] if without bleeding, no dose modifications required
	4	Hold therapy until ≤grade 1 and then reduce dose of ISIS 3521 and 5-FU according to protocol[a]
Diarrhea	1–2	None
	3–4	Hold 5-FU until ≤grade 2 and then reduce dose of 5-FU according to protocol[a]
Nausea/vomiting	1–3	None
	4	If on maximal antiemetic therapy, hold 5-FU dose until ≤grade 2 and then reduce dose of 5-FU according to protocol[a]
Coagulation (PT/aPTT)	1–3	None
	4	If associated with ≥grade 2 bleeding, reduce dose of ISIS 3521 according to protocol[a]
Other toxicities (Except alopecia)	1	None
	2	None
	3–4	Hold therapy until ≤grade 2 and then decrease dose of ISIS 3521 and 5-FU according to protocol[a]

[a] Decrease ISIS 3521 by 1.0 mg/kg/day to a minimum of 1.0 mg/kg/day. Decrease 5-FU from 425 mg/m^2 to 375 mg/m^2, from 375 mg/m^2 to 325 mg/m^2, and decrease thereafter in 25% decrements to a minimum of 200 mg/m^2

icity in any of the three patients after completing one cycle of treatment. Patients were replaced in that dose cohort if treatment was stopped before completion of the first cycle for reasons other than toxicity. Dose-limiting toxicity was defined as any of the following by the National Cancer Institute CTC (CTC Version 2.0): (*a*) ≥grade 4 coagulation abnormality associated with ≥grade 2 hemorrhage; (*b*) grade 4 thrombocytopenia or ≥grade 3 thrombocytopenia with any clinical evidence for bleeding; (*c*) grade 4 neutropenia associated with fever *or* lasting >5 days; (*d*) grade 4 anemia; (*e*) any prolonged drug-related toxicity requiring treatment to be interrupted for more than 2 weeks; (*f*) grade 4 diarrhea or vomiting requiring dose reduction of 5-FU below 200 mg/m^2; and (*g*) ≥grade 3 nonhematological toxicity, excluding nausea, vomiting, and alopecia. Dose escalation was stopped if two or more patients at any dose level experienced treatment-related dose-limiting side effects. A minimum of six patients were to be treated at the highest dose achievable for ISIS 3521 in combination with 5-FU and LV. Patients were allowed to continue treatment until there was evidence of disease progression or treatment-related, dose-limiting toxicities that precluded further therapy. No intrapatient dose escalation was allowed. Dose reductions of 5-FU and ISIS 3521 for toxicity were made as outlined in Table 1.

PK Sampling and Analysis. Serial plasma samples were obtained for PK assessment in cycle 1. For cycle 1 only, the ISIS 3521 infusion was started approximately 90–120 min after 5-FU and LV therapy, after collection of specimens for 5-FU PK was completed. This was done to allow characterization of 5-FU PK alone and in combination with ISIS 3521. Samples for PK assessment were drawn at the following intervals. On day 0, samples for 5-FU PK were drawn 5 min before the start of 5-FU administration and then drawn 5, 10, 20, 30, 45, 60, and 90 min after 5-FU administration was completed. On day 1, one sample for ISIS 3521 PK assessment was drawn 24 h after the start of the ISIS 3521 infusion. On day 4, samples for ISIS 3521 PK were drawn 5 min before the start of 5-FU administration and then drawn 10, 20, 30, 60, and 90 min after completion of 5-FU administration. Also on day 4, 5-FU PK samples were obtained 5 min before the start of 5-FU dose and then drawn 5, 10, 20, 30, 45, 60, and 90 min after discontinuation of 5-FU. On days 7, 14, and 21, only ISIS 3521 PK samples were collected just before discontinuation of ISIS 3521. On day 21, ISIS 3521 samples were also collected at 30, 60, 90, 120, and 180 min after completion of ISIS 3521 treatment.

Analysis of ISIS 3521 concentration was performed on aliquots of plasma samples by capillary gel electrophoresis by Covance Laboratories (Madison, WI) as described previously (19). The percentage of intact ISIS 3521 was calculated by dividing the full-length compound concentration by the sum of all measurable oligonucleotide species (full-length ISIS 3521 and its chain-shortened metabolites) and multiplying the fraction by 100. The steady-state plasma concentration (C_{ss}) of ISIS 3521 without 5-FU was determined by averaging the measured plasma concentrations at time 24 h to time 21 days during the continuous infusion. After 5-FU administration on day 4, ISIS 3521 concentrations measured at multiple time points within 90 min of 5-FU administration were averaged to obtain C_{ss} with 5-FU. Descriptive statistics for ISIS 3521 plasma concentrations

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Table 2 Dose cohorts and length of therapy

Cohort	Starting dose (mg/kg/day)	No. of patients	No. of dose reductions	Duration of therapy (no. of cycles)	Best response
1	1.0	3	0, 0, 0	11, 7, 4	PR, PR, SD
2	1.5	3	0, 0, 1 (cycle 4)	2, 8, 6	PD, SD, SD[a]
3	2.0	9	0, 1 (cycle 2), 0, 1 (cycle 2), 0, 0, 0, 0, 1 (cycle 2)	4, 3, 1, 10, 1, 1, 3, 4, 2	SD, SD, PD, SD, PD, PD, SD,[a] SD, PD

[a] Reduction in tumor size did not meet criteria for PR.

Table 3 Treatment-related worst nonhematologic toxicities[a]

	Toxicity grade 1		Toxicity grade 2		Toxicity grade 3		Toxicity grade 4	
	No. of patients	No. of cycles	No. of patients	No. of cycles	No. of patients	No. of cycles	No. of patients	No. of cycles
Fatigue	5	28	10	15	0	0	0	0
Stomatitis	6	24	3	6	1	1	0	0
Diarrhea	8	17	3	4	1	1	0	0
Nausea	10	17	1	2	0	0	0	0
Anorexia	7	12	1	1	0	0	0	0
Vomiting	5	6	2	2	1	1	0	0
Alopecia	4	5	1	1	0	0	0	0
Pain	3	6	0	0	0	0	0	0
Infection	1	1	1	1	0	0	0	0
Abdominal pain	1	1	0	0	1	1	0	0
Paresthesia	1	1	0	0	0	0	0	0

[a] National Cancer Institute CTC grade version 2.0.

measured both during and after 5-FU administration were calculated.

ISIS 5-FU PKs was analyzed using noncompartmental analysis methods (WinNonlin 3.1; Pharsight). The terminal rate constant (λ_z) was estimated by log-linear regression of the last three or more concentration time points. Plasma elimination $t_{1/2}$ was calculated by dividing 0.693 by λ_z. The AUC for 5-FU was calculated using linear trapezoidal rule. CL for 5-FU was calculated by dividing the dose by AUC. The apparent volume of distribution for 5-FU was estimated using first-moment analysis methods (20).

Antitumor Response Assessments. Tumor response was assessed before the start of the third cycle and then assessed every other cycle thereafter. Additional tumor response assessments were conducted at any point if there was clinical suspicion of PD. Complete response was defined as the disappearance of all evident disease for at least 4 weeks (28 days) when confirmed using repeat computed tomography scans. PR was defined as a ≥50% decrease from baseline in the sum of the products of the maximum perpendicular diameters of all measurable lesions with no documented progression for at least 4 weeks (28 days) and no new lesions. PD was defined as a ≥25% increase in the sum of products of the maximum perpendicular diameters of measurable lesions or the appearance of any new lesion. SD was defined as measurements that did not fit the definition of complete response, PR, or PD (21).

RESULTS

Patient Characteristics. Fifteen patients were enrolled in the study between June 1998 and January 1999. The median age was 62 years (age range, 43–75 years); there were 10 males and 5 females. Thirteen patients had an ECOG performance score of 0–1. The majority of patients (eight patients) had colorectal cancer; however, other tumor types included unknown primary adenocarcinoma (two patients) and gallbladder (one patient), ovarian (one patient), breast (one patient), pancreatic (one patient), and esophageal tumors (one patient). The majority of patients (12 patients) had received prior systemic chemotherapy (11 patients) or radiation therapy (2 patients) that included flavopiridol (5 patients), 5-FU (5 patients), CPT-11 (2 patients), chemoembolization (1 patient), and miscellaneous other agents (5 patients). The median number of prior chemotherapy regimens was 1.5 (range, 0–4 prior chemotherapy regimens). Table 2 lists the dose cohorts, starting doses, number of dose reductions/cohort, and duration of therapy. The median treatment duration was 3 cycles (range, 1–11 cycles), and 12 patients across all three dose levels received ≥2 cycles of therapy.

Toxicity. Three ISIS 3521 dose levels were studied: (*a*) 1.0; (*b*) 1.5; and (*c*) 2.0 mg/kg/day. Table 3 summarizes all nonhematological adverse events observed. Events are tabulated without regard to their association with the study treatment. The most common adverse events were anorexia, fatigue, and tumor pain, followed by nausea, vomiting, mucositis, and diarrhea. The latter four symptoms are common in 5-FU therapy, and the number of patients with these toxicities did not appear to be greater than what might be expected from 5-FU/LV alone. Fatigue was not worse than grade 2 in any cycle. Anorexia was limited to grade 1–2 in 13 treatment cycles. There was one occurrence of neurological toxicity in a patient who developed mild (grade 1) perioral numbness and finger paresthesia. This resolved spontaneously and did not recur. Finally, there was one

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Table 4 Neutropenia observed by cycle

	Cycle										
Grade	1 (n = 15)	2 (n = 12)	3 (n = 10)	4 (n = 8)	5 (n = 5)	6 (n = 5)	7 (n = 4)	8 (n = 3)	9 (n = 3)	10 (n = 2)	11 (n = 1)
1	0	0	0	0	0	0	0	0	0	0	0
2	0	3	3	2	1	2	3	0	0	0	1
3	7	4	2	2	3	2	1	1	2	2	1
4	5	3	2	1	0	0	0	1	0	0	0

Table 5 Thrombocytopenia observed by cycle

	Cycle										
Grade	1 (n = 15)	2 (n = 12)	3 (n = 10)	4 (n = 8)	5 (n = 5)	6 (n = 5)	7 (n = 4)	8 (n = 3)	9 (n = 3)	10 (n = 2)	11 (n = 1)
1	6	5	6	4	3	3	3	2	1	1	0
2	2	4	0	1	1	1	0	1	0	1	0
3	3	0	0	0	0	0	1	0	1	0	1
4	0	0	0	0	0	0	0	0	0	0	0

patient who experienced an acute upper gastrointestinal hemorrhage while on study. This patient had a diagnosis of advanced pancreatic cancer and a history of gastritis/peptic ulcer disease. Two days before this patient died at home, his hemoglobin was 9.4 g/dl, and his platelet count was 115,000/mm^3 with no change from baseline in his coagulation parameters. A postmortem was not available, but the cause of death was believed to be uncontrolled hemorrhage.

Dose-limiting toxicities included one incidence of grade 3 mucositis lasting <7 days, which did not recur with appropriate reduction in dose of the study drugs, and one incidence of grade 3 chest pain that persisted on therapy. The patient subsequently withdrew from further treatment. There were also two incidences of grade 4 febrile neutropenia. None of the dose-limiting toxicities occurred in cycle 1, and, as such, there was no classical dose-limiting toxicity defining the MTD.

Neutropenia and thrombocytopenia, summarized in Tables 4 and 5, were mild. During cycle 1, the median ANC nadir was 570 $\times$ 10^6/liter (range, 130-4030 $\times$ 10^6/liter), and the median platelet nadir was 100 $\times$ 10^9/liter (range, 34–303 $\times$ 10^9/liter). There was no evidence for cumulative neutropenia or thrombocytopenia. Neutropenia (ANC < 1000 cells/mm^3) and/or thrombocytopenia typically developed between days 7 and 14, with full recovery in most cases within 3 weeks. Five patients had nadir ANCs of $\leq$500 $\times$ 10^6/liter during cycle 1 with adequate recovery in time for retreatment. Three patients completing $\geq$2 cycles had recurrent nadir ANC values < 500 $\times$ 10^6/liter lasting <7 days without fever. One patient developed grade 4 febrile neutropenia that resolved within 4 weeks of the start of the cycle, requiring a dosing delay. 5-FU dosing delays of up to 2 weeks because of neutrophil recovery were documented in 9 cycles for five patients. PT and aPTT levels in all patients remained largely within the institutional normal range throughout treatment.

Liver function tests (total bilirubin, serum ALT, and AST) remained stable and largely within institution normal values in all patients who received >1 cycle of treatment, with the exception of one patient who developed grade 2 hyperbilirubinemia in cycle 2 (baseline bilirubin, 1.0 mg/dl; peak level, 2.1 mg/dl) that progressively increased to a peak bilirubin level of 2.4 mg/dl in cycle 4. This patient had liver metastases with documented progression of disease after cycle 4 of therapy.

PKs. The plasma concentration of ISIS 3521 increased in a dose-dependent manner over the range of doses investigated. There was no significant change in the ISIS 3521 steady-state plasma concentrations when ISIS 3521 was given with or without 5-FU (Table 6). Furthermore, there was no significant change in the percentage of intact ISIS 3521 oligonucleotide in the presence or absence of 5-FU. Because there were only two to three patient observations recorded in each dose treatment cohort, t-statistic cannot be calculated with confidence; however, on visual inspection of the data, there is considerable overlap of the ISIS 3521 C_{ss} values in the presence and absence of 5-FU. The PK parameters [AUC, $t_{1/2}$, CL, and volume at steady-state (V_{ss})] of 5-FU in the presence or absence of ISIS 3521 similarly show no evidence for PK interactions (Table 7). The summarized data suggest no significant interactions in the distribution, metabolism, or CL of 5-FU and ISIS when administered together.

Tumor Responses. Six patients had an improvement in measurable tumor. Two of the six patients had the >50% reduction in measurable tumor size required for a PR. The first patient with a PR had flavopiridol-resistant metastatic colorectal cancer with disease in the liver consisting of approximately 20 small lesions, the largest of which was 15 mm in diameter, at baseline. After 2 cycles of treatment, the 15-mm lesion had decreased to 10 mm, and the total number of lesions had been reduced to five. The measurable lesion subsequently increased to 12 mm with no increase in the number or size of other lesions. This patient discontinued therapy after completing 11 cycles (while maintaining an ECOG performance status of 1 with little toxicity from study drugs) because of personal choice. The second patient, with metastatic adenocarcinoma from an unknown primary tumor and no prior chemotherapy, had a PR that persisted until cycle 6, at which time he was found to have new

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Table 6 ISIS 3521 C_{ss} determination

Dose (mg/kg/day)	Parameter description	ISIS 3521 plasma concentration (μg/ml)	% Intact
1.0[a]	ISIS 3521 C_{ss} alone	0.348	86.11
	ISIS 3521 C_{ss} with 5-FU[b]	0.342	100
1.5[c]	ISIS 3521 C_{ss} alone	0.367 ± 0.127	75.88 ± 22.70
	ISIS 3521 C_{ss} with 5-FU[b]	0.321 ± 0.078	65.24 ± 24.65
2.0[d]	ISIS 3521 C_{ss} alone	0.717 ± 0.562	77.10 ± 17.06
	ISIS 3521 C_{ss} with 5-FU[b]	0.561 ± 0.267	74.66 ± 17/45

[a] $n = 2$ patients.
[b] Average concentration of ISIS 3521 in the presence of 5-FU (includes 10, 20, 30, 60, and 90 min after 5-FU dose).
[c] $n = 3$ patients.
[d] $n = 8$ patients without 5-FU and 7 patients with 5-FU.

Table 7 5-FU PK measurements ($N = 13$)

Estimates are presented as the average (SD) of the individual pharmacokinetic estimates.

5-FU pharmacokinetic parameter	Without ISIS 3521	With ISIS 3521	P[a]
AUC (ng*h/ml)	9342.2 (2402.5)	10637.4 (2602.4)	0.08
CL (liter/h/m^2)	48.4 (12.4)	42.9 (13.6)	0.23
$t_{1/2}$ (h)	0.17 (0.05)	0.20 (0.04)	0.06
V_{ss} (liter/m^2)	11.3 (4.9)	11.2 (3.8)	0.95

[a] Paired *t*-test (SPSS).

peritoneal metastases. This patient had a >50% reduction in tumor mass with disease mainly in abdominal soft tissues.

Four other patients had a reduction in tumor after 2 cycles of therapy, although the reduction was less than that required for a PR. One patient with flavopiridol-resistant metastatic colorectal cancer had a reduction of approximately 30% in the size of a liver metastasis. Another patient with 5-FU and flavopiridol-resistant colorectal cancer had a reduction of approximately 30% in pulmonary metastases. A third patient with flavopiridol-resistant colorectal cancer had a reduction of approximately 73% in a single hepatic metastasis without response of other measurable metastatic lesions. After the third cycle of treatment, this patient developed unstable angina (history of prior coronary artery disease) and severe arthritic pain, necessitating discontinuation of therapy. A fourth patient, who had metastatic pancreatic cancer and a history of prior 5-FU and radiation, had a decrease in the size of a liver metastasis.

DISCUSSION

This Phase I clinical and PK study demonstrates that the combination of ISIS 3521 and 5-FU/LV is tolerable at recommended Phase II doses when given separately. The most common side effects were those observed with 5-FU and LV alone, including fatigue, anorexia, mucositis, nausea, vomiting, and diarrhea. These events were not severe, and neither the severity nor character of each toxicity was related to dose or dose level. There was no classical dose-limiting event in cycle 1 defining the MTD, and these toxicities appeared to be attributable to the known effects of the individual drugs or to advanced cancer.

In this trial, neither mild prolongation of aPTT nor symptoms related to cytokine release were observed. These have been reported in trials with other schedules of ISIS 3521 (22, 23) but have not been observed with the continuous infusion schedule (16–18). Furthermore, although alterations in transaminases have been reported in studies of other phosphorothioate oligonucleotides (24, 25), these were not observed in the present study or in other trials of ISIS 3521 (16–18). Finally, complement activation, reported in monkeys receiving phosphorothioate oligonucleotides (26, 27), has not been observed in trials of ISIS 3521 by continuous infusion (16, 17). Therefore, measurements of complement were not performed in the present trial.

It appeared from this trial that there were no PK interactions between ISIS 3521 and 5-FU. The C_{ss} of ISIS 3521 appeared to be unaltered by coadministration of 5-FU. 5-FU PK parameters were also not significantly altered by coadministration of ISIS 3521.

In this trial, the dose of ISIS 3521 was not escalated beyond 2.0 mg/kg/day. The recommended Phase II dose of ISIS 3521 from the prior Phase I 21-day infusion trial was 2.0 mg/kg/day (17). This was attributable to fatigue observed at 3.0 mg/kg/day. However, it is conceivable that higher doses may also be tolerable in combination with 5-FU and LV. Subsequent to the Phase I trial, patients have received 3 mg/kg/day ISIS 3521 by 21-day infusion without significant fatigue or other toxicity.[4]

Although tumor response was not the primary end point of our Phase I trial, we have observed significant evidence of antitumor activity with the combination of ISIS 3521, 5-FU, and LV. This was observed in six patients, including four patients with previously treated colorectal cancer and one patient with previously treated pancreatic cancer.

Another recent clinical report has suggested that adding ISIS 3521 to standard chemotherapy may be promising. In that report, the median survival of 53 patients with advanced non-small cell lung cancer was 15.9 months after treatment with the combination of carboplatin, paclitaxel, and ISIS 3521 (18). A Phase III study is under way comparing the benefits of carboplatin-paclitaxel doublet *versus* carboplatin-paclitaxel-ISIS 3521 triplet therapy for patients with advanced non-small cell lung cancer.

In this trial, the combination of ISIS 3521, 5-FU, and LV was tolerable, feasible, and had significant antitumor activity. These results support further evaluation of this therapy in a

[4] J. Holmlund, personal communication.

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Phase III trial comparing outcome in patients treated with 5-FU and LV with or without ISIS 3521.

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Phase I Clinical and Pharmacokinetic Study of Protein Kinase C- α Antisense Oligonucleotide ISIS 3521 Administered in Combination with 5-Fluorouracil and Leucovorin in Patients with Advanced Cancer

Sridhar Mani, Charles M. Rudin, Katie Kunkel, et al.

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