A Randomized Phase II and Pharmacokinetic Study of the Antisense Oligonucleotides ISIS 3521 and ISIS 5132 in Patients with Hormone-refractory Prostate Cancer[1]

Anthony W. Tolcher,[2] Leonard Reyno, Peter M. Venner, Scott D. Ernst, Malcolm Moore, Richard S. Geary, Kim Chi, Sean Hall, Wendy Walsh, Andrew Dorr, and Elizabeth Eisenhauer

British Columbia Cancer Agency, Vancouver, British Columbia, V5Z 4E6 Canada [A. W. T., K. C.]; Hamilton Regional Cancer Center, Hamilton, Ontario, L8V 1C3 Canada [L. R.]; Tom Baker Cancer Center, Calgary, Alberta, T2N 4N2 Canada [P. M. V.]; Cross Cancer Institute, Edmonton, Alberta, T6G 1Z2 Canada [S. D. E.]; Princess Margaret Hospital, Toronto, Ontario, M5G 2M9 Canada [M. M.]; ISIS Pharmaceuticals Inc. Carlsbad, California [R. S. G., A. D.]; and National Cancer Institute of Canada, Kingston, Ontario, K7L 3N6 Canada [S. H., W. W., E. E.]

ABSTRACT

Purpose: **Protein kinase C (PKC)-α and Raf-1 are important elements of proliferative signal transduction pathways in both normal and malignant cells. Abrogation of either Raf-1 or PKC-α function can both inhibit cellular proliferation and induce apoptosis in several experimental cancer models including prostate cancer cell lines. ISIS 3521 and ISIS 5132 are antisense phosphorothioate oligonucleotides that inhibit PKC-α and Raf-1 expression, respectively, and induce a broad spectrum of antiproliferative and antitumor effects in several human tumor cell lines. In Phase I evaluation both ISIS 3521 and ISIS 5132 could be safely administered on 21-day i.v. infusion schedules and demonstrated preliminary evidence of antitumor activity. On the basis of these findings, a randomized Phase II study of ISIS 3521 and ISIS 5132 was performed in two comparable cohorts of patients who had chemotherapy-naïve, hormone-refractory prostate cancer (HRPC).**

Patients and Methods: **Patients with documented evidence of metastatic HRPC and a prostate-specific antigen (PSA) value ≥20 ng/ml were randomized to receive treatment with either ISIS 3521 or ISIS 5132 as a continuous i.v. infusion for 21 days repeated every 4 weeks. Patients were stratified according to the presence or absence of bidimensionally measurable disease at the time of randomization. The principal endpoints included PSA response, objective response in patients with bidimensionally measurable disease, and treatment failure defined as new or worsening symptoms; a fall in performance status of 2 levels; new or objective progression of disease; or a rise in PSA for 12 weeks without symptom improvement. Plasma samples were collected to assess individual steady-state concentrations and to relate this pharmacokinetic parameter to observed toxicities and responses.**

Results: **Thirty-one patients were randomized in this study; 15 patients received 43 courses of ISIS 3521 and 16 patients received 48 courses of ISIS 5132. The most common toxicities observed were mild to moderate (grade 1 or 2) fatigue and lethargy in 21% and 56% of patients treated with ISIS 3521 and ISIS 5132, respectively. Although no objective or PSA responses were observed in any patient treated with ISIS 3521 or ISIS 5132, persistent stable disease was observed in 3 patients for 5 or more months, and in 5 patients the PSA values did not rise >25% for 120 days or longer.**

Conclusions: **The antisense oligonucleotides ISIS 3521 and ISIS 5132, at these doses and on this schedule, do not possess clinically significant single-agent antitumor activity in HRPC. Protracted stable disease in some patients may indicate a cytostatic effect. Additional work is required to define the optimal role of PKC-α or Raf-1 inhibition in the treatment of HRPC.**

INTRODUCTION

HRPC[3] represents an intrinsically chemoresistant malignancy. Currently approved cytotoxic agents have had a limited impact on this disease with modest palliative rather than survival benefits observed in randomized studies (1, 2). The absence of effective chemotherapy that prolongs life provides a strong clinical impetus for the evaluation of new therapeutic approaches for the treatment of this disease.

Activated members of the epidermal growth factor receptor and insulin-like growth factor receptor families have been implicated in prostate carcinogenesis, and prostate cancer cell growth and metastases (3). Cell proliferation signals from growth factor receptor tyrosine kinases are transmitted to the nucleus via a cascade of downstream phosphorylation and dephosphorylation steps within the cytoplasm commonly referred to as signal transduction (3). Several of these growth factor receptors share a common signal transduction pathway, the mitogen-activated protein kinase path-

Received 9/4/01; revised 4/29/02; accepted 5/2/02.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported by grants from the National Cancer Institute of Canada and ISIS Pharmaceuticals.
[2] To whom requests for reprints should be addressed at current address: Institute for Drug Development, Cancer Therapy and Research Center, 8122 Datapoint Drive, Suite #250, San Antonio, TX 78229. Phone: (210) 616-5914; Fax: (210) 949-5009; E-mail: atolcher@saci.org.

[3] The abbreviations used are: HRPC, hormone-refractory prostate cancer; PSA, prostate-specific antigen; PKC-α, protein kinase C-α; CIVI, continuous i.v. infusion; Css, steady-state concentrations; ANC, absolute neutrophil count; PT, prothrombin; PTT, partial thromboplastin; CVC, central venous catheter; PD, progressive disease.

way (4). Because of the diverse number of growth factor receptor tyrosine kinases associated with tumor growth, selective inhibition of the specific signal transduction pathways represents an attractive therapeutic target because the antiproliferative effects may be independent of the specific growth receptor family responsible for malignant growth (5, 6).

The *raf* kinase gene family encodes specific serine/threonine protein kinases that mediate critical signal processing by connecting upstream growth factor-mediated tyrosine kinase stimulation with downstream activation of serine threonine kinases and the mitogenic signaling pathways (7). Therefore, *raf*-1 kinase is a critical element of the mitogen-activated protein kinase signal transduction pathway downstream both to epidermal growth factor receptor and insulin-like growth factor receptor family members, and is consitutively activated in several tumors that posses either *ras* or *raf* mutations (8, 9). *Raf* is also activated by PKC-α independent of ras and is regulated by the antiapoptotic protein bcl-2, which mediates, at least in part, androgen-independent growth of prostate cancer (10–13).

PKC-α also has an important role in tumor growth and proliferation independent of *raf* interactions, and has been implicated in the transformation and growth of breast, colorectal, and prostate tumors (14–16). In experimental prostate cancer models, increased expression of PKC-α enhanced tumor growth and invasiveness (16). The degree of PKC-α expression represented may also represent a biomarker of malignant transformation in the prostate gland with pathologic evidence that enhanced PKC-α expression is detectable in early prostate carcinoma specimens but not in adjacent benign prostatic epithelium (17). Several lines of experimental evidence indicate that PKC-α inhibition from a diverse array of strategies promotes apoptosis in androgen-independent prostate cell lines (18–21). Therefore, inhibition of PKC-α and *raf*-1 appears to be a valid therapeutic strategy for androgen-independent prostate cancer (22).

Antisense oligonucleotides are modified strands of deoxynucleotides that hybridize to mRNA in a nucleotide sequence-specific manner (23). The bound mRNA undergoes ribonucleotidase H-mediated degradation with subsequent inhibition of protein expression and function (23). Both *raf*-1 and PKC-α protein expression can be potently inhibited *in vitro* by specific antisense oligonucleotides in a concentration-dependent and sequence-dependent manner (24–26). Furthermore, inhibition of PKC-α or Raf-1 by antisense oligonucleotides has demonstrated broad antitumor activity in several human tumors tested *in vitro* and *in vivo* (24–26).

ISIS 3521 is a 20-nucleotide base phosphorothioate antisense oligonucleotide directed to PKC-α mRNA. Several different schedules of ISIS 3521 administration were evaluated in various Phase I studies with the principal dose-limiting toxicities being thrombocytopenia and fatigue (27, 28). On the basis of rapid systemic clearance and a brief plasma elimination half-life ($t_{1/2}$) averaging 60 min observed in the Phase I studies, a 21-day CIVI schedule was chosen for Phase II studies to optimize tumor cell exposure to ISIS 3521. At the recommended dose of 2 mg/kg/day, ISIS 3521 was well tolerated over multiple administered courses, and 1 ovarian cancer patient on this schedule had an objective response whereas 2 other ovarian cancer patients had marked decrements in CA 125 (40 and 76%; Ref. 28).

ISIS 5132 is a 20-nucleotide phosphorothioate 2′-deoxynucleotide directed to the human c-*raf* kinase gene. During the Phase I evaluation of ISIS 5132, a 21-day CIVI schedule was also examined (29). Dose escalation from 0.5 to 5.0 mg/kg/day was achieved without the observation of dose-limiting toxicity. Modest (grade 2 and 3) thrombocytopenia was observed across several dose levels but was transient and nonrecurring. Css were reached within 24 h, and ISIS 5132 was rapidly cleared after discontinuation of the infusion (91 ± 56 min). Preliminary antitumor activity was observed in 1 ovarian cancer patient who experienced a marked CA 125 decrement (97%), whereas 2 patients (1 renal and 1 pancreatic carcinoma) had prolonged disease stabilization (29).

The critical role that PKC-α and Raf-1 have in cell proliferation, the marked growth inhibitory properties of ISIS 3521 and ISIS 5132 in experimental models, and the encouraging preliminary activity observed in Phase I clinical studies of ISIS 3521 and 5132 provided a strong impetus to initiate Phase II studies of ISIS 3521 and ISIS 5132 in patients with hormone-refractory, chemotherapy-naïve prostate cancer. The objectives of this study were to: (*a*) determine the antitumor activity of ISIS 3521 and ISIS 5132 using objective and biochemical criteria; (*b*) determine the rate of treatment failure to these antiproliferative agents; and (*c*) examine the steady-state plasma concentrations of these agents, and relate these to observed toxicity and efficacy. The randomized Phase II design was chosen to examine these two agents using identical study parameters and comparable populations of men with HRPC.

PATIENTS AND METHODS

Patient Selection. Patients who had histologically or cytologically confirmed prostate cancer with documented evidence of progression while receiving androgen ablative therapy (chemical or surgical castration) and clinical or radiological evidence of metastatic disease were eligible. Patient entry criteria also included: age ≥18 years; life-expectancy of at least 12 weeks; a Eastern Cooperative Oncology Group performance status of 0 or 1; no prior chemotherapy; discontinuation of nonsteroidal antiandrogens at least 6 weeks before study entry with two documented, consecutive rises in PSA value not <14 days apart; PSA value ≥20 ng/ml at the time of study entry; ANC ≥1500/μL; platelet count ≥100,000/μL; bilirubin ≤2-times upper institutional limit of normal; serum creatinine ≥2 times upper institutional limit of normal; normal PT and PTT times; measurable or evaluable disease; and no coexisting medical problem of sufficient severity to limit compliance with the study. Patients were not eligible if they had tumor marker elevation without clinical or radiological evidence of metastatic disease; pain requiring narcotic use (codeine excepted); chronic corticosteroids use; anticoagulant therapy or a known bleeding disorder; or a history of prior malignancy unless definitively treated 5 years before study entry and without evidence of recurrence.

Study Design

Patient Entry and Randomization. Patients who met eligibility criteria were randomized to one of two therapy arms (ISIS 3521 or ISIS 5132) by the National Cancer Institute of Canada Clinical Trials Group headquarters. Before randomization, patients were stratified by the presence or absence of bidimensionally measurable disease to balance the number of patients evaluable for objective response. Protocol treatment was initiated within 5 working days of randomization.

Drug Administration. The starting dose for both ISIS 3521 and ISIS 5132 was 2 mg/kg/day administered as a CIVI for 21 days repeated every 4 weeks.

ISIS 3521 was provided by ISIS Pharmaceuticals Inc. and supplied in 10-ml glass vials containing 10 mg/ml of active agent. Each 10-ml vial contained 10 mg/ml of ISIS 3521, 14.33 mg/ml dibasic sodium phosphate heptahydrate, USP, 1.73 monobasic sodium phosphate, monohydrate, USP, and 4.4 mg/ml sodium chloride for injection, USP.

ISIS 5132 was also provided by ISIS Pharmaceuticals Inc. and supplied as a sterile solution for i.v. administration in PBS (pH 7.4). Each 10-ml vial contained ISIS 5132 10 mg/ml, dibasic sodium phosphate heptahydrate, USP, 1.73 monobasic sodium phosphate, monohydrate, USP, and 4.4 mg/ml sodium chloride for injection, USP.

Both ISIS 3521 and ISIS 5132 were infused with a portable volumetric infusion pump through a 0.22-μm in-line filter at a rate of ~1.5 ml/h. The infusion was changed every 7 days during the 21-day infusion. All of the infusions were administered through a CVC or peripherally inserted CVC.

Toxicity Evaluation and Dose Modification. During treatment patients underwent weekly physical examination, assessment of performance status, weight, and vital signs. All of the toxicities were graded according to the National Cancer Institute of Canada Expanded Common Toxicity Criteria. A complete blood count, prothrombin time, or partial thromboplastin time, and international normalized ratio were performed weekly for two courses, then on days 1 and 22 of each subsequent course. In the event of grade 3 or greater hematologic toxicity, the CBC, PT/INR, and PTT were performed twice weekly until the toxicity resolved to grade 2 or better. Dose modifications were based on both hematologic and nonhematologic toxicity assessments. Dose adjustments for all of the subsequent courses included a 50% dose reduction for an ANC nadir value $< 0.5 \times 10^9$/liter, platelet nadir $< 25 \times 10^9$/liter, or any grade 3 or 4 nonhematologic toxicity. Patients were discontinued from protocol treatment if the toxicity experienced required two dose reductions or if 2 weeks elapsed without recovery.

Response Criteria. PSA response was defined as a 50% fall in PSA from baseline maintained for ≥4 weeks. Patients with bidimensionally measurable disease were considered evaluable for objective tumor response. A complete response was defined as the complete disappearance of all of the clinical and radiological evidence of tumor, as determined by two observations not <4 weeks apart and free of all of the tumor-related symptoms. A partial response was defined as a 50% or greater decrease in the sum of all of the measurable lesions determined by two observations not <4 weeks apart. No simultaneous increase in any lesion or the appearance of new lesions may occur. Stable disease was defined as a steady state of disease documented to be present for at least 4 weeks from the start of therapy that did not meet the criteria for either a partial response or PD. PD was an unequivocal increase of at least 25% in the overall sum of the measurable lesions compared with baseline or the appearance of new lesions.

Treatment failure was defined as one or more of the following: (*a*) new or worsening symptoms requiring a change in management; (*b*) a fall in performance status by 2 levels; (*c*) new disease or objective PD in measurable disease; (*d*) a continued rise in PSA for 12 weeks in initially asymptomatic patients or in initially symptomatic patients a continued rise in PSA for 12 weeks without an improvement in symptoms (patients with a rise in PSA but with symptom improvement could continue therapy).

All of the patients who received one course of therapy were considered evaluable for toxicity, PSA response, objective tumor response, or treatment failure.

Pharmacokinetic Analysis. Blood specimens were collected for steady-state pharmacokinetic analysis at baseline preinfusion, and during infusion on days 8, 15, and 22. Blood samples were then centrifuged at 1200 rpm, the plasma separated, and then stored frozen at −70°C until analyzed.

The pharmacokinetic assay has been described in detail previously (30). Briefly, an aliquot of plasma (100 μL) for each sample was spiked with a known concentration of internal standard (T_{27}, a 27-mer phosphorothioate oligodeoxythymidine) and extracted using solid-phase extraction and analyzed by capillary gel electrophoresis. Extracted samples were analyzed using a Beckman P/ACE capillary electrophoresis instrument (Beckman Instruments, Irvine, CA) with UV detection at 260 nm. The limit of quantitation for this assay was ~0.10 μg/ml in plasma.

Statistical Analysis. This Phase II study used a two-stage design for each arm that permitted 15 patients to be entered into each arm for stage I and a total of 30 patients/arm for stage II. A multivariate stopping rule for the proportions of both response and early treatment failure was used in this study and designed to detect an active treatment with fewer number of responses if the treatment failure rate is significantly lower than expected (31). In stage I, the early stopping rule would be invoked and the drug considered inactive if 9 or more treatment failures were observed regardless of the number of responses. In stage II, the drug would be considered active if the following observations were made: (*a*) 1 or more responses and 13 or less failures; (*b*) 2 or more responses and 15 or less failures; or (*c*) 3 or more responses regardless of the number of failures. The multivariate procedure tests the null hypothesis that the response rate is 20% and the early progression rate of 60% *versus* an alternative hypothesis that the response rate is 10% and an early progression rate of 40%. The significance level is 0.053 with a power of 0.816. The expected sample size when the null hypothesis is true (EN_0) was 20.99, and the alternative hypothesis being true (EN_1) was 28.60.

RESULTS

Thirty-one patients were entered into the study; 15 patients were randomized to ISIS 3521 and 16 patients were randomized to ISIS 5132. Thirty patients were evaluable for response and toxicity. One patient randomized to ISIS 3521 had an elevated PSA without documented metastatic disease and was deemed ineligible. The median age for all of the patients was 69 years (range 52–81). The median performance status at study entry was 0 and 1 for ISIS 3521 and ISIS 5132, respectively. Ten patients (33%) had bidimensionally measurable disease (lymph node disease), whereas 26 patients had evidence of distant bone metastases. The relevant patient demographics for each treatment arm are summarized in Table 1.

Ninety-one courses of therapy were administered; 43 courses were ISIS 3521 and 48 courses were ISIS 5132. Patients treated with ISIS 3521 and ISIS 5132 received a median of 3 courses of therapy (ranges 1–7 and 2–6, respectively).

Table 1 Patient characteristics

Characteristic	ISIS 3521	ISIS 5132	Total
Number of patients	14	16	30
Median age (range)	72 (58–78)	66 (52–81)	69 (52–81)
Performance status			
0	10	7	17
1	4	9	13
Previous therapy			
Radiation therapy	9	10	19
Surgical castration			
LHRH agonist			
Time from diagnosis to study entry			
12–24 months	3	0	3
>24 months	11	16	27
Sites of disease			
Bone	12	14	26
Lymph nodes	4	6	10
Prostate	1	1	2
Measurable disease			
Bidimensional	4	6	10
Nonmeasurable	10	10	20
Baseline PSA value (ng/ml)			
<50	1	3	4
50–99	4	4	8
100–199	2	3	5
200–399	3	4	7
≥400	4	2	6

Table 2 Worst hematologic toxicity by patient

Toxicity	ISIS 3521	ISIS 5132
Number of evaluable patients	14	16
Median nadir (range)		
ANC ($\times 10^9$/liter)	2.50 (1.7–3.8)	2.65 (1.7–4.0)
WBC ($\times 10^9$/liter)	4.2 (2.8–5.5)	4.6 (2.6–5.9)
Platelet ($\times 10^9$/liter)	98 (9–149)	129 (48–195)
Hemoglobin (g/liter)	109 (76–131)	122 (80–145)
Median number of days to nadir (range)		
ANC	43 (8–162)	42 (15–83)
WBC	50 (15–162)	29 (15–83)
Platelet	22 (14–162)	42 (15–136)
Hemoglobin	53 (28–162)	50 (8–92)

Toxicity Data. The most common treatment-related toxicity observed with either agent was fatigue and lethargy. Three of 14 (21%) patients on the ISIS 3521 arm reported moderate (grade 2) lethargy during treatment. A total of 9 of 16 (56%) patients treated with ISIS 5132 experienced modest (6 patients grade 1), moderate (2 patients grade 2), or severe (1 patient grade 3) treatment-related lethargy. Mild or moderate nausea occurred in 21 and 31% of patients treated with ISIS 3521 and ISIS 5132, respectively, whereas emesis rarely occurred. Nonhematologic treatment-related toxicities are summarized in Table 2.

Because of the protracted i.v. infusion schedule used in this study, central venous access devices were mandated for all of the patients. Three patients (10%) subsequently experienced CVC infections, whereas a single patient experienced a superior vena cava thrombosis extending from the tip of the CVC.

Thrombocytopenia was the predominant hematologic toxicity observed in both treatment arms. The median platelet nadir was 98×10^9/liter (range $9–149 \times 10^9$/liter) and 129×10^9/liter (range $48–195 \times 10^9$/liter) for ISIS 3521 and ISIS 5132, respectively. Neutropenia was both uncommon and of modest magnitude (grade 1 ANC nadir), and occurred in only 3 patients of each treatment arm.

There were 2 deaths on study. A 64-year-old man with bone metastases from prostate cancer was treated with ISIS 5132 and experienced a severe headache, marked gait impairment, and a decreased level of consciousness on day 6 of course 2. At presentation the patient was found to have had a cerebral hemorrhage with evidence of disseminated intravascular coagulopathy. The patient had a markedly diminished fibrinogen level and platelet count (grade 3), and elevated PTT, INR, and fibrin degradation products, and the etiology of the disseminated intravascular coagulopathy was believed to be related to PD. A second patient with a history of pre-existing renal impairment experienced computed tomography contrast-induced renal failure after the completion of 1 course of ISIS 3521 and expired 13 days from the complications of renal insufficiency.

Pharmacokinetic Analysis. Total and parent (20 mer) steady-state plasma concentrations were determined for both ISIS 3521 and ISIS 5132, and are summarized in Table 3. Mean (±SD) Css for ISIS 3521 and ISIS 5132 were 0.89 (± 0.43) μg/ml and 0.63 (± 0.31) μg/ml, respectively. The percentage of intact parent antisense oligonucleotide (20 mer) to total oligonucleotide detected remained stable throughout the infusion period and ranged between 54 and 56% for ISIS 3521, and 57 and 62% for ISIS 5132.

Pharmacodynamic Analysis. The relationship between the degree of thrombocytopenia and the Css values for ISIS 3521 and ISIS 5132 was examined. No statistically significant correlation could be determined between individual patient Css values and decrement in platelet counts ($r = 0.1$, $P = 0.798$ and $r = -0.16$, $P = 0.568$ for ISIS 3521 and ISIS 5132, respectively).

Antitumor Activity. There were no objective responses in the subset of patients with measurable disease nor were PSA responses observed. Three patients experienced protracted periods of disease stability that lasted for 5 (ISIS 3521), 6 (ISIS 5132), and 7 (ISIS 3521) months. However, treatment failure, as defined in this protocol, occurred in >9 patients in each arm, thus terminating additional accrual as defined in the protocol (Table 4). Because these two agents may have antiproliferative rather than cytotoxic effects on tumors, the rate at which a patient PSA increased by 25% or more in both treatment arms as a function of time is depicted in Table 5. Three of 14 ISIS 3521- and 2 of 16 ISIS 5132-treated patients did not experience a PSA increase of 25% or more 120 days after commencing therapy.

DISCUSSION

Inhibition of signal transduction represents a novel approach to cancer therapy. On the basis of the antitumor activity of ISIS 3521 and ISIS 5132 in several human tumor models and the encouraging, albeit preliminary, antitumor activity observed in Phase I studies, the principal objective of this study was to determine whether either of these agents had single agent activity in two comparable HRPC populations. Unfortunately, neither treatment with ISIS 3521 nor ISIS 5132 at these doses and on this schedule demonstrated objective or biochemical (PSA) responses in these patients with HRPC.

The failure of ISIS 3521 and ISIS 5132 to produce antitumor responses in this population deserves additional

Table 3 Summary of steady-state concentrations of ISIS 3521 and ISIS 5132[a]

Agent	Sample day	No. patients	Parent oligonucleotide (μg/ml)	Total oligonucleotide (μg/ml)	% Intact
ISIS 3521	8	7	0.92 ± 0.37	1.70 ± 0.74	54.27 ± 4.07
	15	8	0.98 ± 0.61	1.78 ± 1.00	54.51 ± 6.37
	22	7	0.76 ± 0.27	1.42 ± 0.66	56.05 ± 9.79
Mean Css[b]			0.89 ± 0.43	1.65 ± 0.79	
ISIS 5132	8	7	0.73 ± 0.34	1.20 ± 0.64	61.88 ± 7.94
	15	11	0.61 ± 0.33	1.08 ± 0.56	57.46 ± 10.31
	22	11	0.60 ± 0.30	1.05 ± 0.57	58.14 ± 11.48
Mean Css[b]			0.63 ± 0.31	1.10 ± 0.57	

[a] Values represent means (± SD).
[b] Mean Css, average concentration of days 8, 15, and 22.

Table 4 Best response to therapy

Criteria	ISIS 3521	ISIS 5132
PSA Response Criteria		
Number of evaluable patients	13	16
Responders	0	0
Nonresponders	13	16
Measurable Disease		
Number with measurable disease	4	5
CR/PR	0	0
Stable Disease	1	2
Progressive Disease	3	3

Table 5 The number of patients with a 25% increase in PSA as a function of time

Time (days)	ISIS 3521 (n = 14)	ISIS 5132 (n = 16)
<30	4	11
30–60	6	1
61–120	0	2
121–240	2	2
>240	1	–
Discontinued without 25% rise	1	

scrutiny. Css values for both agents in the current study were comparable with or exceeded those reported from the Phase I studies. The steady-state plasma concentrations also exceeded the *in vitro* concentrations necessary for down-regulation of these two respective targets (25, 26, 32). O'Dwyer *et al.* (33) also documented *raf*-1 protein down-regulation in PBMCs collected from patients entered in the Phase I study of three times weekly bolus ISIS 5132 indicating successful inhibition of gene expression clinically (34). The absence of tumor regressions in the current study may also indicate the limited importance of *raf* and PKC-α for tumor cell viability in unselected HRPC patients. Patients were not selected for either the overexpression of PKC-α or *raf*-1. The absence of tumor tissue samples available for analysis in the current study contributed to the uncertainty as to the underlying cause of treatment failure and additionally emphasizes the importance of obtaining and examining tumor tissue for target validation in future antisense oligonucleotide trials. Nevertheless, the absence of responses at doses that have induced objective responses in other studies indicates that PKC-α and *raf*-1 inhibition alone is insufficient for significant tumor regressions in unselected HRPC patients.

PKC-α and *raf*-1 inhibition by ISIS 3521 and 5132, respectively, may result in a cytostatic rather than cytotoxic therapeutic outcome. In the current study, 3 of 31 (10%) patients experienced protracted stable disease for 5 (ISIS 3521), 6 (ISIS 5132), and 7 (ISIS 3521) months, and 3 of 14 (21%) patients treated with ISIS 3521 did not experience a 25% increase in serum PSA for >120 days. Taken together these findings may represent growth-inhibitory properties observed in the preclinical studies. Although the current study is one of the first Phase II studies to incorporate a multivariate stopping rule, this trial design cannot detect purely cytostatic or growth inhibitory activity. The multivariate stopping rule for Phase II studies uses proportions of response and early treatment failure to detect an active treatment when lower than conventional response rates accompany a low rate of treatment failure (31). However, in the absence of any responses, the low rate of treatment failures (or stable disease) does not prevent early termination of the study (31). This finding illustrates the need for additional clinical trial designs to appropriately evaluate cytostatic rather than cytotoxic agents.

The absence of single agent activity should not entirely exclude additional development of these agents in HRPC. Inhibitors of *raf* and PKC-α function may have greater utility when used in combination with cytotoxic chemotherapy. Preclinically, supra-additive antitumor activity was demonstrated when these agents were combined with either antimicrotubule agents or cisplatin (35, 36). Furthermore, preliminary clinical results of ISIS 3521 combined with paclitaxel and carboplatin has demonstrated a greater than anticipated response rate (48%) and median survival (15.9 months) in non-small cell lung cancer patients (37). These findings taken together with early indications in the current study of protracted stable disease in some patients provides a rationale for re-examination of ISIS 3521 and ISIS 5132 in combination with chemotherapy agents in patients with HRPC.

REFERENCES

1. Tannock, I. F., Osoba, D., Stockler, M. R., Ernst, D. S., Neville, A. J., Moore, M. J., Armitage, G. R., Wilson, J. J., Venner, P. M., Coppin, C. M., *et al.* Chemotherapy with mitoxantrone plus prednisone or prednisone alone for symptomatic hormone-resistant prostate cancer: a Canadian randomized trial with palliative end points. J. Clin. Oncol., *14:* 1756–1764, 1996.

2. Kantoff, P. W., Halabi, S., Conaway, M., Picus, J., Kirshner, J., Hars, V., Trump, D., Winer, E. P., and Vogelzang, N. J. Hydrocortisone with or without mitoxantrone in men with hormone-refractory prostate cancer: results of the cancer and leukemia group B 9182 study. J. Clin. Oncol., *17:* 2506–2513, 1999.

3. Porter, A. C., and Vaillancourt, R. R. Tyrosine kinase receptor-activated signal transduction pathways which lead to oncogenesis. Oncogene, *17:* 1343–1352, 1998.

4. Skolnik, E. Y., Batzer, A., Li, N., Lee, C. H., Lowenstein, E., Mohammadi, M., Margolis, B., and Schlessinger, J. The function of GRB2 in linking the insulin receptor to Ras signaling pathways. Science (Wash. DC), *260:* 1953–1955, 1993.

5. Margolis, B., and Skolnik, E. Y. Activation of Ras by receptor tyrosine kinases. J. Am. Soc. Nephrol., *5:* 1288–1299, 1994.

6. Schonwasser, D. C., Marais, R. M., Marshall, C. J., and Parker, P. J. Activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway by conventional, novel, and atypical protein kinase C isotypes. Mol. Cell. Biol., *18:* 790–798, 1998.

7. Fu, H., Xia, K., Pallas, D. C., Cui, C., Conroy, K., Narsimhan, R. P., Mamon, H., Collier, R. J., and Roberts, T. M. Interaction of the protein kinase Raf-1 with 14–3-3 proteins. Science (Wash. DC), *266:* 126–129, 1994.

8. Stanton, V. P., Jr., and Cooper, G. M. Activation of human raf transforming genes by deletion of normal amino-terminal coding sequences. Mol. Cell. Biol., *7:* 1171–1179, 1987.

9. Bos, J. L. ras oncogenes in human cancer: a review. Cancer Res., *49:* 4682–4689, 1989.

10. Kolch, W., Heidecker, G., Kochs, G., Hummel, R., Vahidi, H., Mischak, H., Finkenzeller, G., Marme, D., and Rapp, U. R. Protein kinase C α activates RAF-1 by direct phosphorylation. Nature (Lond.), *364:* 249–252, 1993.

11. Wang, H. G., Rapp, U. R., and Reed, J. C. Bcl-2 targets the protein kinase Raf-1 to mitochondria. Cell, *87:* 629–638, 1996.

12. Wang, H. G., Takayama, S., Rapp, U. R., and Reed, J. C. Bcl-2 interacting protein, BAG-1, binds to and activates the kinase Raf-1. Proc. Natl. Acad. Sci. USA, *93:* 7063–7068, 1996.

13. Apakama, I., Robinson, M. C., Walter, N. M., Charlton, R. G., Royds, J. A., Fuller, C. E., Neal, D. E., and Hamdy, F. C. bcl-2 overexpression combined with p53 protein accumulation correlates with hormone-refractory prostate cancer. Br. J. Cancer, *74:* 1258–1262, 1258.

14. O'Brian, C., Vogel, V. G., Singletary, S. E., and Ward, N. E. Elevated protein kinase C expression in human breast tumor biopsies relative to normal breast tissue. Cancer Res., *49:* 3215–3217, 1989.

15. Kopp, R., Noelke, B., Sauter, G., Schildberg, F. W., Paumgartner, G., and Pfeiffer, A. Altered protein kinase C activity in biopsies of human colonic adenomas and carcinomas. Cancer Res., *51:* 205–210, 1991.

16. Liu, B., Maher, R. J., Hannun, Y. A., Porter, A. T., and Honn, K. V. 12(S)-HETE enhancement of prostate tumor cell invasion: selective role of PKC α. J. Natl. Cancer Inst., *86:* 1145–1151, 1994.

17. Cornford, P., Evans, J., Dodson, A., Parsons, K., Woolfenden, A., Neoptolemos, J., and Foster, C. S. Protein kinase C isoenzyme patterns characteristically modulated in early prostate cancer. Am. J. Pathol., *154:* 137–144, 1999.

18. Henttu, P., and Vihko, P. The protein kinase C activator, phorbol ester, elicits disparate functional responses in androgen-sensitive and androgen-independent human prostatic cancer cells. Biochem. Biophys. Res. Commun., *244:* 167–171, 1998.

19. Krongrad, A., and Bai, G. c-fos promoter insensitivity to phorbol ester and possible role of protein kinase C in androgen-independent cancer cells. Cancer Res., *54:* 6073–6077, 1994.

20. Lamm, M. L., Long, D. D., Goodwin, S. M., and Lee, C. Transforming growth factor-β1 inhibits membrane association of protein kinase C α in a human prostate cancer cell line, PC3. Endocrinology, *138:* 4657–4664, 1997.

21. Gschwend, J. E., Fair, W. R., and Powell, C. T. Bryostatin 1 induces prolonged activation of extracellular regulated protein kinases in and apoptosis of LNCaP human prostate cancer cells overexpressing protein kinase cα. Mol. Pharmacol., *57:* 1224–1234, 2000.

22. Basu, A. The potential of protein kinase C as a target for anticancer treatment. Pharmacol. Ther., *59:* 257–280, 1993.

23. Dean, N. M., McKay, R., Miraglia, L., Geiger, T., Muller, M., Fabbro, D., and Bennett, C. F. Antisense oligonucleotides as inhibitors of signal transduction: development from research tools to therapeutic agents. Biochem. Soc. Trans., *24:* 623–629, 1996.

24. Dean, N. M., and McKay, R. Inhibition of protein kinase C-α expression in mice after systemic administration of phosphorothioate antisense oligodeoxynucleotides. Proc. Natl. Acad. Sci. USA, *91:* 11762–11766, 1994.

25. Monia, B. P., Sasmor, H., Johnston, J. F., Freier, S. M., Lesnik, E. A., Muller, M., Geiger, T., Altmann, K. H., Moser, H., and Fabbro, D. Sequence-specific antitumor activity of a phosphorothioate oligodeoxyribonucleotide targeted to human C-raf kinase supports an antisense mechanism of action *in vivo*. Proc. Natl. Acad. Sci. USA, *93:* 15481–4, 1996.

26. Monia, B. P., Johnston, J. F., Geiger, T., Muller, M., and Fabbro, D. Antitumor activity of a phosphorothioate antisense oligodeoxynucleotide targeted against C-raf kinase. Nat. Med., *2:* 668–675, 1996.

27. Nemunaitis, J., Holmlund, J. T., Kraynak, M., Richards, D., Bruce, J., Ognoskie, N., Kwoh, T. J., Geary, R., Dorr, A., Von Hoff, D., *et al*. Phase I evaluation of ISIS 3521, an antisense oligodeoxynucleotide to protein kinase C-α, in patients with advanced cancer. J. Clin. Oncol., *17:* 3586–3595, 1999.

28. Yuen, A. R., Halsey, J., Fisher, G. A., Holmlund, J. T., Geary, R. S., Kwoh, T. J., Dorr, A., Sikic, B. I. Phase I study of an antisense oligonucleotide to protein kinase C-α (ISIS 3521/CGP 64128A) in patients with cancer. Clin. Cancer Res., *5:* 3357–3363, 1999.

29. Cunningham, C. C., Holmlund, J. T., Schiller, J. H., Geary, R. S., Kwoh, T. J., Dorr, A., and Nemunaitis, J. A phase I trial of c-Raf kinase antisense oligonucleotide ISIS 5132 administered as a continuous intravenous infusion in patients with advanced cancer. Clin. Cancer Res., *6:* 1626–1631., 2000.

30. Leeds, J. M., Graham, M. J., Truong, L., and Cummins, L. L. Quantitation of phosphorothioate oligonucleotides in human plasma. Anal. Biochem., *235:* 36–43, 1996.

31. Zee, B., Melnychuk, D., Dancey, J., and Eisenhauer, E. Multinomial phase II cancer trials incorporating response and early progression. J. Biopharm. Stat., *9:* 351–363, 1999.

32. McKay, R. A., Miraglia, L. J., Cummins, L. L., Owens, S. R., Sasmor, H., and Dean, N. M. Characterization of a potent and specific class of antisense oligonucleotide inhibitor of human protein kinase C-α expression. J. Biol. Chem., *274:* 1715–1722, 1999.

33. O'Dwyer, P. J., Stevenson, J. P., Gallagher, M., Cassella, A., Vasilevskaya, I., Monia, B. P., Holmlund, J., Dorr, F. A., Yao, K. S. c-raf-1 depletion and tumor responses in patients treated with the c-raf-1 antisense oligodeoxynucleotide ISIS 5132 (CGP 69846A). Clin. Cancer Res., *5:* 3977–3982, 1999.

34. Stevenson, J. P., Yao, K. S., Gallagher, M., Friedland, D., Mitchell, E. P., Cassella, A., Monia, B., Kwoh, T. J., Yu, R., Holmlund, J., *et al*. Phase I clinical/pharmacokinetic and pharmacodynamic trial of the c-raf-1 antisense oligonucleotide ISIS 5132 (CGP 69846A). J. Clin. Oncol., *17:* 2227–2236, 1999.

35. Geiger, T., Muller, M., Dean, N. M., and Fabbro, D. Antitumor activity of a PKC-α antisense oligonucleotide in combination with standard chemotherapeutic agents against various human tumors transplanted into nude mice. Anticancer Drug Des., *13:* 35–45, 1998.

36. Geiger, T., Muller, M., Monia, B. P., and Fabbro, D. Antitumor activity of a C-raf antisense oligonucleotide in combination with standard chemotherapeutic agents against various human tumors transplanted subcutaneously into nude mice. Clin. Cancer Res., *3:* 1179–1185, 1997.

37. Yuen, A., Halsey, J., Lum, B., Fisher, G., Advani, R., Moore, M., Saleh, M., Ritch, P., Harker, G., Ahmed, F., *et al*. Phase I/II trial of ISIS 3521, and antisense inhibitor of PKC-α with carboplatin and paclitaxel in non-small cell lung cancer. Clin Cancer Res., *7:* 3681s, 2001.