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Efficacy and toxicity of the antisense oligonucleotide aprinocarsen directed against protein kinase C-α delivered as a 21-day continuous intravenous infusion in patients with recurrent high-grade astrocytomas[1,2]

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Protein kinase C alpha (PKC-α) is a cytoplasmic serine threonine kinase involved in regulating cell differentiation and proliferation. Aprinocarsen is an antisense oligonucleotide against PKC-α that reduces PKC-α in human cell lines and inhibits a human glioblastoma tumor cell line in athymic mice. In this phase 2 study, aprinocarsen was administered to patients with recurrent high-grade gliomas by continuous intravenous infusion (2.0 mg/kg/day for 21 days per month). Twenty-one patients entered this trial. Their median age was 46 years (range, 28–68 years), median Karnofsky performance status was 80 (range, 60–100), median tumor volume was 58 cm^3 (range, 16–254 cm^3), and histology included glioblastoma multiforme (n = 16), anaplastic oligodendroglioma (n = 4), and anaplastic astrocytoma (n = 1). The number of prior chemotherapy regimens included none (n = 3), one (n = 10), and two (n = 8). No tumor responses were observed. Patients on this therapy rapidly developed symptoms of increased intracranial pressure with increased edema, enhancement, and mass effect on neuroimaging. The median time to progression was 36 days, and median survival was 3.4 months. The observed toxicities were mild, reversible, and uncommon (grade 3 thrombocytopenia [n = 3] and grade 4 AST [n = 1]), and no coagulopathy or CNS bleeding resulted from this therapy. Plasma concentrations of aprinocarsen during the infusion exhibited significant interpatient variability (mean = 1.06 μg/ml; range, 0.34–6.08 μg/ml). This is the first study to use an antisense oligonucleotide or a specific PKC-α inhibitor in patients with high-grade gliomas. No clinical benefit was seen. The rapid deterioration seen in these patients could result from tumor growth or an effect of aprinocarsen on blood-brain barrier integrity. *Neuro-Oncology 6, 32–40, 2004 (Posted to Neuro-Oncology [serial online], Doc. 04-035, October 28, 2004. URL http://neuro-oncology.mc.duke.edu; DOI: 10.1215/S1152851703000353)*

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[2] Aprinocarsen was initially developed as Isis 3521 by Isis Pharmaceuticals, Inc. (Carlsbad, Calif.), which supplied the drug for this trial. After completion of this study, this agent was licensed to Eli Lilly, and its name was changed to LY900003 (Affinitak).

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[4] Abbreviations used are as follows: AQP4, Aquaporin 4; BBB, blood-brain barrier; C_{ss}, steady-state concentration; CI, confidence interval; CV, coefficient of variation; EIASD, enzyme-inducing antiseizure drugs; KPS, Karnofsky performance status; MR, magnetic resonance; NABTT, New Approaches to Brain Tumor Therapy; NCI, National Cancer Institute; PKC, protein kinase C; PT, prothrombin time; PTT, partial thromboplastin time.

Protein kinase C (PKC)[4] is a phospholipid-dependent, cytoplasmic serine threonine kinase responsible for signal transduction in response to growth factors, hormones, and neurotransmitters. Gene cloning studies have demonstrated that PKC exists as a family of proteins consisting of multiple discrete isozymes (Nishizuka, 1988). The PKC isozymes are products of distinct genes and differ in their biochemical properties, tissue-specific expression, and intracellular localization (Basu, 1993; Nishizuka, 1988). They are classified in three groups: Group A (classical) PKC-α, βI, βII, and γ; Group B (new) PKC-δ, ϵ, η, θ, and μ; and Group C (atypical) PKC-λ and ζ. It is believed that the various isozymes carry out distinct cellular functions (Gescher, 1992; Nishizuka, 1988).

The role of PKC has been extensively studied in human tumors. Its activity has been shown to be increased or decreased in solid tumors, depending on the tumor type and the PKC isozyme quantitated. Breast tumors have been found to contain more than 3 times the normal amount of PKC activity when compared to normal tissue (Basu, 1993). Additional studies have demonstrated that several antineoplastic agents, such as tamoxifen and the anthracyclines, are also inhibitors of PKC (Basu, 1993; Bignon et al., 1989; Palayoor et al., 1987; Rotenberg et al., 1990). The inhibition of PKC may be one of the mechanisms by which these compounds exert their antitumor activity. PKC-α is widely expressed in tissues and in many transformed cell lines (Basu, 1993; Gescher, 1992).

Aprinocarsen is a phosphorothioate oligonucleotide that hybridizes to the 3'-untranslated region of human PKC-α mRNA and inhibits PKC-α expression through RNase H–mediated cleavage of hybridized PKC-α mRNA (Dean and McKay, 1994). Considerable in vitro and in vivo evidence suggests that aprinocarsen may be useful for the treatment of patients with cancer (Dean et al., 1994; O'Brian et al., 1989). One relevant example demonstrated that mice with U-87 (glioblastoma) tumors implanted subcutaneously and intracranially had significant growth delay and tumor reduction after receiving this antisense oligonucleotide (Yazaki et al., 1996).

This manuscript reports the first study using antisense oligonucleotides as a novel therapeutic approach to the treatment of recurrent primary brain tumors. The primary objective of this study was to determine the response rate to aprinocarsen in patients with recurrent high-grade astrocytomas. Secondary objectives were to determine time to tumor progression and to characterize the safety profile and pharmacokinetics of aprinocarsen when given as a 21-day continuous infusion.

Materials and Methods

Aprinocarsen was administered to outpatients as a 21-day infusion at a rate of 2.0 mg/kg/day. One treatment cycle was defined as 21 consecutive days of aprinocarsen followed by a 7-day, treatment-free interval. This dose and schedule were the result of discussions between the National Cancer Institute (NCI), the pharmaceutical company, and the New Approaches to Brain Tumor Therapy (NABTT) CNS Consortium investigators and were based on information from phase 1 studies conducted in other patient populations. Treatment of an individual patient was to continue until there was objective evidence of disease progression or drug-related, dose-limiting toxicity or until a patient decided to discontinue treatment.

This phase 2 trial was conducted by using a two-stage design. Twenty-two patients were to be accrued to the first stage, and if two or fewer responses were seen, the study was to be stopped. If more than two patients had a complete or partial response, then an additional 11 patients were to be accrued. If six or fewer of the 33 patients responded, we would be 90% confident that the response rate was $<30\%$.

This trial was approved by the Cancer Therapy Evaluation Program at the NCI, Isis Pharmaceuticals, Inc. (Carlsbad, Calif.), and the Institutional Review Board of each NABTT CNS Consortium institution participating in this study. These institutions included the University of Alabama, Emory University, Henry Ford Hospital, the Johns Hopkins University, Massachusetts General Hospital, Moffitt Cancer Center, the University of Pennsylvania, and Wake Forest University.

Patient Eligibility Criteria

Eligible patients were at least 18 years of age with histologically proven malignant glioma (anaplastic astrocytoma, anaplastic oligodendroglioma, or glioblastoma multiforme) that had progressed or recurred following radiation therapy. Patients were required to have measurable disease on magnetic resonance (MR) or CT imaging and to be on a stable dose of glucocorticoids when they began therapy. Other eligibility requirements included the following: ⩾3 months since completion of radiation; ⩾3 weeks since non-nitrosourea chemotherapy or ⩾6 weeks since a nitrosourea chemotherapy; ⩽2 prior chemotherapy regimens; adequate hematologic (absolute neutrophil count ⩾1500/mm^3, platelets ⩾100,000/mm^3, hemoglobin concentration ⩾9.0 g/dl), renal (creatinine ⩽1.5 mg/dl), and hepatic (total bilirubin ⩽1.5 mg/dl, transaminases ⩽4 times the upper limits of the institutional normal) function; and a Karnofsky performance status (KPS) of ⩾60. Additionally, patients needed to have an estimated life expectancy of ⩾2 months, to be able to give informed consent, and to not be pregnant or breast feeding.

Patients were excluded from this study if they had received treatment with an investigational drug or a biological or therapeutic device within 28 days of starting aprinocarsen, if they had prior therapy with a PKC inhibitor, or if they had a serious concurrent infection or other medical illness that would jeopardize their ability to receive this experimental agent. They were also ineligible if they were receiving therapeutic doses of heparin. Patients with prior malignancies must have been disease free for more than five years or have curatively treated basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix or breast. All patients with the

potential for pregnancy were counseled on the need and methods to avoid conception.

Patients were enrolled and treated as soon after craniotomy or biopsy as was deemed medically appropriate. They received treatment cycles four weeks apart, with volumetric scans performed before every other treatment cycle. The neuropathologic diagnosis of malignant glioma was made at the treating institution. Histologic slides of all responding patients were to be centrally reviewed by the NABTT CNS Consortium.

Drug Supply

Aprinocarsen is the sodium salt of a 20 unit phosphorothioate deoxyribooligonucleotide that was manufactured and supplied by Isis Pharmaceuticals (Nemunaitis et al., 1999; Yuen et al., 1999). It was formulated as a 10-mg/ml sterile solution for intravenous administration in phosphate-buffered saline (pH 7.4). Vials of the drug were stored between 2°C and 8°C and protected from light. These were permitted to warm to room temperature prior to use and diluted with normal saline for patient administration. The formulated drug was stable for up to seven days and compatible with polyvinyl chloride and non–polyvinyl chloride i.v. infusion bags and tubing.

Study Drug Administration

Aprinocarsen was given as a continuous i.v. infusion over 21 days, followed by a seven-day treatment-free interval. The total dose of aprinocarsen to be administered for seven consecutive days was added to 250 ml of normal saline and infused with a portable Verifuse infusion pump (I-Flow Corp., Lake Forest, Calif.) through a 0.22-μm in-line filter at a rate of 1.5 ml/h. Aprinocarsen was administered at 2 mg/kg/day, with an option of increasing the infusion rate to 3 mg/kg/day after cycle 2.

Dose-Limiting Toxicity

The NCI's Common Toxicity Criteria were used for all toxicity reporting. For purposes of this study, the following toxicities were considered dose-limiting: absolute neutrophil count $\leq 500/mm^3$ (grade 4) for 3 days or longer, thrombocytopenia $\leq 25{,}000/mm^3$ (grade 4), febrile neutropenia, grade 3 or 4 thrombocytopenia associated with grade 2 or greater of bleeding, grade 4 coagulation abnormality associated with grade 2 or greater bleeding, grade 4 nausea or vomiting on maximal antiemetic therapy, and other treatment-related grade 3 or grade 4 toxicity. The following were not considered dose-limiting for this study unless the investigator felt the event was attributed to the study drug and not the underlying CNS malignancy: seizures, deep vein thrombosis, pulmonary emboli, and neurologic abnormalities attributed to the CNS malignancy by the investigator.

Dose Reductions Due to Dose-Limiting Toxicity

Treatment with aprinocarsen was held until any treatment-related, dose-limiting toxicity had resolved. Patients could continue on treatment at a 50% dose reduction if the toxicity was hematologic and the counts returned to normal. Patients who experienced other dose-limiting toxicity could be retreated at a 50% dose reduction when toxicity resolved to grade 0 or 1. However, patients who experienced dose-limiting cardiac or neurologic toxicity (NCI grade 3–4) or who had toxicity that did not resolve within two weeks of their last day of dose were removed from the study.

Concomitant Medications

Glucocorticoids were administered at a dose required to control each patient's brain edema. An effort was made to keep the patient on a stable steroid dose during the first two treatment cycles, as alterations in steroid doses can complicate the radiologic interpretation of response. Glucocorticoids were not used as antiemetic agents, and granulocyte colony–stimulating factor was to be used only with life-threatening infection and neutropenia. Other medications were prescribed as deemed clinically appropriate by the investigator. However, heparin was not to be administered at any time during the study. If a patient developed a deep venous thrombosis or pulmonary embolus while on study, treatment with aprinocarsen was interrupted while heparin was required. The experimental therapy could resume once the patient was anticoagulated with Coumadin (DuPont Pharmaceuticals Co., Wilmington, Del.).

Monitoring of Patients

Prior to study entry, patients were required to have a volumetric MR/CT, history and physical exam, KPS, Mini-Mental State Examination, complete blood count, chemistry panel, electrolytes, urinalysis, prothrombin time (PT), partial thromboplastin time (PTT), electrocardiogram, and chest X ray. The KPS, complete blood count, and anticonvulsant levels were monitored weekly, and PT/PTT was monitored every one to two weeks. Scans and exams were repeated every two months.

Response Assessment

All eligible patients were required to have measurable contrast-enhancing disease evident on a baseline pretreatment MRI or CT scan. The standard NABTT response criteria utilizing MR/CT scans with volumetric analysis, neurologic examinations, and glucocorticoid doses were used to determine the response to therapy. A complete response was defined as complete disappearance of all tumor on MR/CT scan, on no glucocorticoids, with a stable or improving neurologic examination for at least four weeks. A partial response was defined as $\geq 50\%$ reduction in tumor size on volumetric MR/CT scan, on a stable or decreasing dose of glucocorticoids, with a stable or improving neurologic examination for at least

four weeks. Patients with complete or partial responses were to undergo a confirmatory MRI or CT scan in four weeks. Progressive disease was characterized by progressive neurologic abnormalities not explained by causes unrelated to tumor progression (e.g., anticonvulsant or corticosteroid toxicity, electrolyte abnormalities, hyperglycemia) or ⩾25% increase in the volume of the tumor by MR/CT scan. If neurologic status deteriorated on a stable or increasing dose of steroids, or if new lesions appeared on serial MR/CT, the patient was considered to have progressive disease, and aprinocarsen was discontinued. Patients whose clinical status and MR/CT volumetrics did not meet the criteria for partial response or progressive disease were considered to have stable disease.

Reasons for Premature Discontinuation

Aprinocarsen was discontinued with evidence of disease progression, excessive toxicity, failure to recover from treatment-related toxicity within two weeks of the last dose of drug, or development of an intercurrent illness or condition that would interfere with the patient's continued participation. In addition, the patient was withdrawn from the study if the investigator felt this was in the best interest of the patient or if the patient declined further participation in this research study.

Pharmacokinetics

Sampling to determine the steady-state concentration (C_{ss}) of aprinocarsen during the 21-day continuous infusion was performed during the first cycle of therapy. Blood specimens (2 ml) were collected from a peripheral arm vein into tubes containing EDTA as an anticoagulant shortly before treatment and 5 to 10 min before stopping the infusion pump on days 8, 15, and 22. Sample tubes were mixed by inversion and immediately placed on wet ice until centrifuged. The plasma was removed and maintained at ⩽–20°C until assayed. Concentrations of aprinocarsen and phosphorothioate oligonucleotide metabolites up to four nucleotides shorter than the parent drug were determined with a validated assay based upon capillary gel electrophoresis by ClinTrials BioResearch, Ltd. (Senneville, Que., Canada), as previously described (Leeds et al., 1996). The lower limit of quantitation of the assay was 30 nM for each compound, corresponding to a plasma concentration of 0.21 μg/ml for aprinocarsen.

The apparent C_{ss} of aprinocarsen in plasma was calculated for each patient as the mean of the determinations made during the 21-day continuous i.v. infusion. Total body clearance of the drug was calculated by dividing the infusion rate by the C_{ss}. The C_{ss} was also expressed as a percentage of the sum of the steady-state molar concentrations of the parent drug and all measurable nucleotide-shortened drug metabolites in plasma. The mean and standard deviation of the drug concentration at each sample time and other pharmacokinetic variables were calculated for the entire cohort of patients. Mean values were calculated as the geometric mean, with standard deviation estimated by the jackknife technique (Lacey et al., 1997; Lam et al., 1985; Mizuta and Tsubotani, 1985).

Statistical Considerations

The primary end point of this study was complete or partial response. The primary efficacy analysis included all eligible patients, and analyses were based on intention to treat. A two-staged minimax design to detect a 20% improvement over a background rate of 10% with type I error rate of 5% and type II error rate of 10% was used to assess efficacy (Simon, 1989).

Secondary objectives of this trial included estimating overall survival and time to progression with this treatment regimen. Survival time was calculated from time of entry to the study until death from any cause. Event times were censored if the patient was alive at last follow-up. Survival distributions were estimated by using the product limit method (Kaplan and Meier, 1958) and compared by using the Wilcoxon and log-rank tests (Kalbfleisch and Prentice, 1980). The hazard (risk) ratio was calculated by using Cox proportional hazards regression analysis (Cox, 1972). Confidence intervals were calculated by standard methods.

In addition, the frequency of toxicity associated with aprinocarsen treatment was estimated by using the proportion of patients with serious or life-threatening toxicities. Differences in patient characteristics between groups were tested for statistical significance by using chi-square and Student *t* tests. SAS software version 8.2 (SAS Institute, Cary, N.C.) was used to perform analyses. All reported *P*-values are two-sided.

Results

Patient Characteristics

Between March and November 1998, 22 patients with supratentorial recurrent malignant glioma were enrolled in this study by investigators at eight participating NABTT institutions. One patient was later found to be ineligible for this study on pathology review, which left 21 eligible and evaluable patients. The median age of these patients was 46 years (range, 28–68 years), median pretreatment KPS was 80 (range, 60–100), and the median tumor volume on the pretreatment imaging scan was 58 cm^3 (range, 16–254 cm^3). All patients were Caucasian, and 15 (71%) were male. Four patients had recurrent anaplastic oligodendroglioma, one had anaplastic astrocytoma, and 16 had glioblastoma multiforme. Three patients had received no prior chemotherapy, 10 had one prior regimen, and eight had two prior regimens. Sixteen of these patients (76%) were taking enzyme-inducing antiseizure drugs (EIASDs).

Outcome

Accrual to this protocol was temporarily halted after four of the first six patients required early hospitaliza-

tions for "tumor progression" as defined above by worsening scans or neurologic function. The median time to progression of these patients was 51 days, with an event rate of 0.21 per person-day (95% confidence interval [CI], 0.008–0.47). This was informally compared with the event rate from another NABTT study with similar eligibility criteria (median time to progression, 112 days; event rate, 0.009 per person-day [95% CI, 0.005–0.015]). The NABTT biostatisticians recommended that the study be reopened to complete accrual because the early results were not convincing enough to warrant terminating the trial.

No complete or partial responses were noted in the 21 eligible patients entered into the first stage of the trial, and further accrual was thus stopped. The ineligible patient was not replaced because the criterion of more than two responses could not be met. The patients had a median time to progression of 36 days (range, 22–112 days) and a median survival of 3.4 months (range, 1.7–>47.9 months) after entering this trial. The number of cycles of aprinocarsen administered ranged from 0.66 to 4 with a median of 1. Clinically, patients developed altered mental status and signs and symptoms of increased intracranial pressure. Increased brain edema, contrast enhancement, and mass effect were found on follow-up MRI scans. Although 90% of patients died within 1 year of entering the protocol, two patients remain alive 3 years after study entry.

Survival for patients on this trial was compared to that of patients on nine other NABTT studies with similar eligibility criteria where the experimental therapies have not proven efficacious (Fig. 1). There were no statistical differences in age, race, gender, or baseline KPS between the patients in these studies and the aprinocarsen study patients (Table 1). Patients receiving aprinocarsen had significantly worse survival at early time points (Wilcoxon test, $P = 0.01$) compared to similar patients receiving other experimental therapies at NABTT institutions. However, because two patients on this trial have survived longer than 3 years, the log-rank test and the unadjusted Cox proportional hazards risk ratio were not significant (log-rank, $P = 0.11$; risk ratio = 1.47, 95% CI, 0.92–2.36, $P = 0.11$).

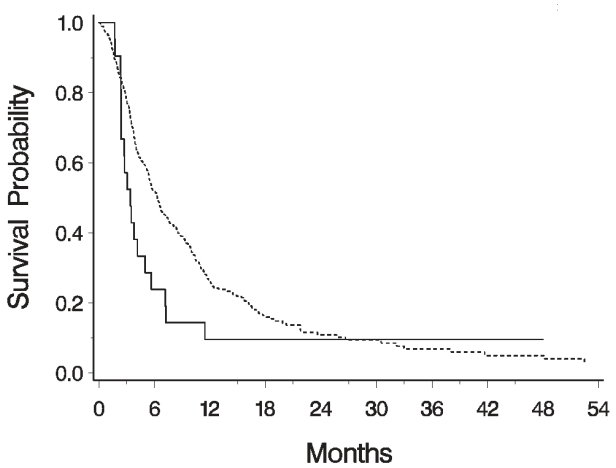

Fig. 1. Kaplan-Meier estimates of overall survival for the aprinocarsen phase 2 study (solid line; N = 21) and patients from nine prior NABTT clinical trials of chemotherapeutic agents with similar eligibility criteria (broken line; N = 285)

Table 1. Baseline demographic and clinical characteristics. Number of patients, with percentages in parentheses, except as noted

Characteristic	Aprinocarsen Study N = 21	Other NABTT Study N = 285	*P*-value
Sex, male	15 (71)	183 (64)	0.50
Race, white	21 (100)	259 (91)	0.24
Age, in years, mean [SD]	49.7 [11.4]	49.7 [12.3]	0.98
Karnofsky performance scale, mean [SD]	78 [12]	82 [12][a]	0.17
Histology			0.87
GBM	16 (76)	205 (72)	
Other	5 (24)	80 (28)	

[a]n = 267 because of missing values.

One case suggested that aprinocarsen could have contributed to the early neurological deterioration seen in patients treated on this research protocol. A 42-year-old who had previously presented with headaches, nausea, and vomiting was found to have hydrocephalus and a left frontal mass. A ventriculoperitoneal shunt was placed, the left frontal mass was partially resected, and the pathology revealed malignant glioma with oligodendroglial features. The patient returned to full-time work in April 1998 after completing radiation therapy and two cycles of procarbazine, CCNU (lomustine), and vincristine. Four months later an MRI scan revealed an increase in the left frontal mass and new bilateral contrast-enhancing lesions. He was not taking dexamethasone and complained of gradually worsening short-term memory. He joined this research study with a KPS of 80, a slightly unstable gait, some left-hand clumsiness, the ability to remember two of three objects at 5 min, and a pretreatment MRI that is shown in Fig. 2A. His condition rapidly deteriorated after beginning aprinocarsen, with progressive difficulties with speech and gait. Dexamethasone was initiated and increased to 32 mg per day, but he progressively declined, becoming unable to walk or care for himself, with worsening mentation and increasing headaches. An MRI was obtained (Fig. 2B) that demonstrated marked progression, and the infusion of aprinocarsen was stopped in the first week of the second cycle of therapy. The patient was placed on hospice care and subsequently developed bilateral deep venous thrombosis as well as a pulmonary embolism. Over the ensuing months, the patient's condition gradually improved to the point where he could function independently. He remained clinically stable without further therapy for his brain tumor until early in 2003, when he developed neurologic progression and subsequently died of his brain tumor.

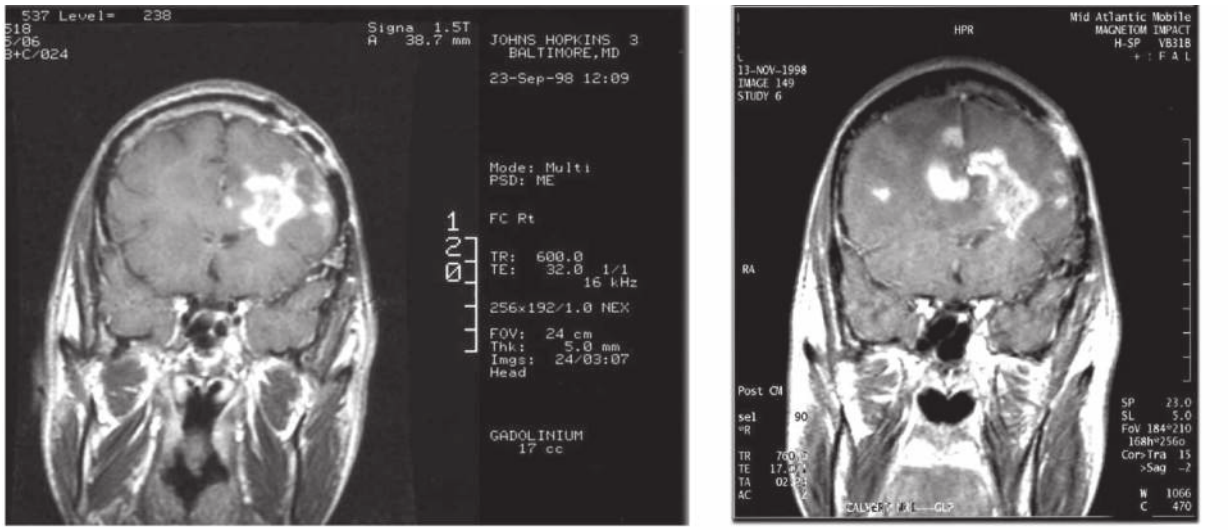

Fig. 2. Increased blood-brain barrier dysfunction following treatment with aprinocarsen. A. Pretreatment (9/98) MRI from patient. B. MRI after treatment (11/98) with aprinocarsen

Toxicities

The hematologic toxicities attributed to aprinocarsen were mild, reversible, and infrequent. Grade 3 thrombocytopenia was seen in five patients (24%), and one patient (5%) experienced elevations of ALT (grade 4) and AST (grade 3), which may have been related to concomitant anticonvulsant use. Other drug-related toxicities included nausea (grade 3), vomiting (grade 3), and fatigue (grade 3), which were each seen in one patient. There was no evidence of CNS or systemic bleeding, and the only elevations of PT and PTT occurred in one patient who was on anticoagulants for a deep venous thrombosis. The other significant adverse events noted were related to progression of the underlying brain tumor, bone flap infection, line infections, or deep venous thrombosis.

Pharmacokinetics

Data on the plasma concentration of aprinocarsen and its metabolites achieved during the infusion was available from 16 of the 21 evaluable patients. The full-length 20-mer parent drug was the predominant phosphorothioate oligonucleotide in plasma, accounting for 80.0 ± 17.6% of the measurable drug-related species. Mean values of the aprinocarsen plasma concentration at the end of the first (0.81 ± 0.70 μg/ml), second (1.11 ± 1.07 μg/ml), and third (1.06 ± 0.90 μg/ml) weeks of the 21-day continuous i.v. infusion were not significantly different ($P = 0.73$). This suggested that steady-state conditions were achieved during the first week of the infusion. The grand mean C_{ss} of aprinocarsen for the entire group of patients was 1.06 ± 0.99 μg/ml. Interpatient variability in the C_{ss} was extremely high, spanning a 19-fold range from 0.32 to 6.07 μg/ml in individual patients, with a coefficient of variation (CV) of 93.7%. The magnitude of interpatient variability was comparable for clearance when calculated either directly (6.04 liters/h; CV, 95.0%) or with normalization to patient weight (1.26 ml/min/kg; CV, 96.5%).

Discussion

Antisense oligonucleotides provide a novel approach to cancer therapy. They have been the focus of evaluation in multiple preclinical model systems. In tissue culture, aprinocarsen inhibits PKC-α mRNA expression in A549 (lung) and T-24 (bladder) carcinoma cells and reduces the concentration of PKC-α protein in A549 carcinoma cells, in a nucleotide sequence–dependent and concentration-dependent manner as compared with control. This compound also showed activity in a human glioblastoma cell line implanted subcutaneously and intracerebrally in nude mice. The U-87 tumor cells were implanted subcutaneously into the flank of 10-week-old, female BALB/c athymic, nude mice. Treatment with intraperitoneal aprinocarsen was initiated when the tumor reached a volume of approximately 100 mm^3. A range of different oligonucleotide doses were administered daily to determine the effect of treatment on tumor growth. Aprinocarsen demonstrated a significant reduction in tumor growth at intraperitoneal doses of 2.0 and 20 mg/kg administered once daily, in comparison with animals treated with a control antisense compound with a scrambled sequence or saline (Rotenberg et al., 1990). When aprinocarsen was discontinued, tumor growth returned, and the increase in tumor volume as a func-

tion of time was comparable to that observed in control animals. The concentration of intact aprinocarsen in subcutaneously implanted tumor cells measured 24 h after the last dose of 20 mg/kg of aprinocarsen was approximately 2.0 μM, which is 10- to 20-fold greater than the in vitro IC_{50} (Yazaki et al., 1996). Mice with U-87 carcinoma cells implanted intracranially were also treated with intraperitoneal aprinocarsen at a dose of 20 mg/kg administered once daily. This resulted in a significant reduction in mortality at 42 days versus animals receiving the antisense control (Palayoor et al., 1987). Histological examination of the brains from aprinocarsen-treated animals revealed a marked reduction in tumor size and no evidence of drug-related toxicity. In addition, an immunohistochemical study of the distribution of antisense oligonucleotide suggests that this compound is able to enter brain tumors with a disrupted blood-brain barrier (BBB) (Portnow et al., 2000).

The maximum tolerated dose of aprinocarsen in cancer patients has been determined from multiple phase 1 clinical trials (Nemunaitis et al., 1999; Yuen et al., 1999). Dosing schedules were designed to avoid high peak plasma concentrations in excess of 40 μg/ml of intact oligonucleotide, which have been associated with complement activation in monkeys. Two patients with refractory low-grade lymphoma responded to this therapy. One patient with ovarian cancer had a partial response to treatment. No neurologic toxicities were noted in either of these phase 1 studies. Multiple phase 2 trials of aprinocarsen have been conducted for a number of different types of cancer types, including colon, breast, non–small cell lung, prostate, brain, ovarian, and melanoma, in which a dose of 2 mg/kg has been given as a three-week continuous infusion every four weeks, but very limited responses have been observed with aprinocarsen administered as a single agent (Cripps et al., 2002; Mani et al., 2002). Recent preclinical data suggest that PKC could play a significant role in the modulation of drug resistance and in synergy with conventional cytotoxic drugs (Goekjian and Jirousek, 2001; Swannie and Kaye, 2002).

The study reported herein describes the first clinical study to use a specific PKC inhibitor in patients with high-grade astrocytomas and the first to use antisense oligonucleotides in this setting. The results indicate that there is no clinical benefit from aprinocarsen, as patients who were treated rapidly developed clinical and radiographic evidence consistent with tumor progression. As noted above, the timing of the tumor "progression" was more rapid than that seen in any other study of the same patient population conducted by the NABTT CNS Consortium. These observations could be a reflection of the aggressive nature of the underlying disease and the particular patients entering this trial or even of a growth stimulatory effect of this experimental agent. However, the dramatic neurologic and radiologic deterioration of one patient receiving aprinocarsen, his recovery when this agent was discontinued, and his long survival without other antineoplastic therapy suggest that his deterioration could be the result of a drug-related toxicity. Drug-related toxicity could be the result of excess exposure to this new agent or a specific toxicity related to its mechanism of action.

Many recent studies of patients with high-grade astrocytomas have reported striking changes in the pharmacokinetics of chemotherapeutic agents related to EIASDs (Ciordia et al., 2000; Fetell et al., 1997; Gilbert et al., 2003; Grossman et al., 1998; Vecht et al., 2003). The study described in this report was not designed to assess the potential for an alteration in the pharmacokinetics of aprinocarsen because catabolism by exonuclease-mediated base deletion appeared to be the principal route of elimination of phosphorothioate oligonucleotides, not hepatic metabolism. The pharmacokinetic data that were obtained demonstrated an extremely high degree of interpatient variability in the steady-state plasma concentration of aprinocarsen, as observed in prior clinical trials of the drug in patients with systemic cancers (Nemunaitis et al., 1999; Yuen et al., 1999). Only four of the 16 patients for whom pharmacokinetic data were determined did not receive EIASD, precluding a meaningful statistical comparison of the pharmacokinetic data with the non-EIASD cohort. However, mean values of the steady-state plasma concentration for this infusion rate and clearance of the intact oligonucleotide were comparable to those reported in clinical trials of aprinocarsen in patients with non-CNS-involved tumors, who were presumably not receiving stable doses of glucocorticoids and EIASD (Cripps et al., 2002; Mani et al., 2002; Swannie and Kaye, 2002; Yuen et al., 1999). Therefore, the neurological deterioration observed in this study is unlikely to be related to an increased systemic exposure to the drug.

Another potential explanation for the rapid deterioration seen in these patients is that aprinocarsen could result in worsening of BBB dysfunction in patients with primary brain tumors. Although neurologic deterioration from aprinocarsen has not been noted in other phase 1 or phase 2 studies of this agent, data suggest a possible link between the function of PKC and BBB function. Aquaporins are a family of water-selective transporting proteins that increase plasma membrane water permeability in secretory and absorptive cells (Venero et al., 2001). Aquaporin 4 (AQP4) is a predominant water channel protein in mammalian brains, localized in the astrocyte plasma membrane. In addition, AQP4 in cerebral microvessels appears to be important in the control of water transport between blood and brain (Kobayashi et al., 2001). The regulation of AQP4 is believed important for homeostasis of water in the brain, but its regulatory mechanisms are unknown. Several investigators have noted that the function of AQP4 is significantly affected by PKC. PKC may regulate the activity of AQP4 through a mechanism involving protein phosphorylation (Han et al., 1998). AQP4 may have an important role in several clinical disorders of rapid water transport, such as glaucoma, brain edema, and swelling of premature infant lungs. Protein kinase C and dopamine may affect water permeability through AQP4 (Zelenina et al., 2002). In addition, AQP4 mRNA appears to be inhibited by phorbol ester 12-0-tetradecanoylphorbol 13-acetate via PKC activation (Nakahama et al., 1999). A PKC acti-

vator has also been shown to decrease AQP4 and AQP9 mRNAs and proteins in a time- and concentration-dependent manner. These results suggest that signal transduction via PKC may be critical in regulating the expression of AQP4 and 9 (Yamamoto et al., 2001).

Anatomic studies have shown that the water channel protein AQP4 is normally expressed in astrocyte foot processes around cerebral microvessels (Davies, 2002). Its expression is upregulated in high-grade astrocytomas and around metastatic adenocarcinoma. There is a significant correlation between AQP4 expression and the degree of cerebral edema and BBB disruption as judged by contrast-enhanced CT (Saadoun et al., 2002). However, it is not clear whether increased AQP4 expression enhances edema formation or clearance. Mice lacking AQP4 in the CNS are partially protected from brain edema in water intoxication and ischemic models of brain injury (Verkman, 2002). The cerebral AQP4 water channel is thought to be involved in genesis of brain edema after traumatic brain injury and is associated with a reduction in AQP4 expression (Kiening et al., 2002).

The potential relationship between BBB dysfunction and aprinocarsen treatment warrants further evaluation. Although symptomatic brain edema has not been seen in patients with an intact BBB treated with this compound, patients with primary and metastatic brain tumors in whom BBB function is already somewhat compromised may be particularly sensitive to this PKC inhibitor. In addition, if there is a relationship between PKC inhibition and BBB dysfunction, this could be exploited to develop novel agents to manipulate the integrity of the BBB. Agents that temporarily disrupt the BBB have been sought to allow therapeutic agents to penetrate into the central nervous system (Prados et al., 2003). Alternatively, this relationship might provide insight into the development of agents to restore BBB integrity. This could lead to a much-needed alternative to glucocorticoids, which are associated with significant toxicities in this patient population.

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