Investigational New Drugs **23:** 263–269, 2005.

Randomized phase II evaluation of aprinocarsen in combination with gemcitabine and cisplatin for patients with advanced/metastatic non-small cell lung cancer

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Key words: aprinocarsen, cisplatin, gemcitabine, non-small cell lung cancer, phase II

Summary

Aprinocarsen is a specific antisense oligonucleotide inhibitor of protein kinase C-α. This study aimed to evaluate the response rate to combination therapy with aprinocarsen, gemcitabine and cisplatin, in chemonaive patients with advanced/metastatic NSCLC. Secondary objectives included comparison of response rate, time to event efficacy parameters, and toxicities on the 2 treatment arms. Patients with stage IV, or stage IIIB disease (N_3 and/or pleural/pericardial effusion), were randomized to either control or experimental arm. Patients on both arms received gemcitabine 1250 mg/m^2 on days 1 and 8, and cisplatin 80 mg/m^2 on day 1 of a 3-week cycle. Additionally, on the experimental arm, aprinocarsen was administered as 2 mg/kg continuous iv infusion on days 1–14, every 21 days. A total of 18 enrolled patients were randomized on the 2 arms. Further enrollment was terminated in March 2003 as a result of a phase III trial suggesting that aprinocarsen did not have an added survival benefit when combined with paclitaxel and carboplatin therapy in patients with NSCLC. Patients received a median of 4 cycles on control arm and 2.5 cycles on experimental arm. The response rate was 16.7% in the experimental arm and 44.4% in the control arm. Most frequent grade 3/4 toxicities were hematologic, with a higher incidence of thrombocytopenia in the experimental arm (87.5% vs. 33.3%). Despite the 14-day continuous infusion schedule, infection rate was not increased in the experimental arm. The present study did not show any advantage, in response rate or secondary endpoints, with aprinocarsen; however, the toxicity was not unduly increased, and aprinocarsen regimen was safely administered.

Introduction

Lung cancer is a leading cause of cancer deaths worldwide [1], and non-small cell lung cancer (NSCLC) is the most common form of the disease. Platinum-based combination chemotherapy has produced modest improvements in tumor response rates and survival benefit in NSCLC [2]. However, the overall survival remains grim, and efforts are being made to develop drugs to improve the treatment of unresectable NSCLC [3]. One class of novel agents to treat NSCLC is called antisense oligonucleotides (ASOs) [4]. They represent an attractive platform because of their ability to specifically inhibit the mRNA, thereby blocking the formation of the specific protein, and inhibiting tumor growth [5, 6]. One such target is the intracellular signaling protein kinase C-alpha (PKC-α) [7] against which the ASO aprinocarsen (Affinitak^{TM}, LY900003, formerly Isis 3521) was developed [8]. Aprinocarsen belongs to the first wave of ASOs developed for clinical application [6] and has undergone extensive preclinical and clinical development [9].

In preclinical studies, aprinocarsen showed sequence-specific inhibition of the mRNA coding for PKC-α and reduced production of PKC-α protein in the human NSCLC cell line A549 [8]. Single-agent aprinocarsen can be safely administered to patients and has shown activity against NSCLC, non-Hodgkin's lymphoma, and ovarian cancer [10–12].

Among the current chemotherapy options for advanced NSCLC, the combination of gemcitabine and cisplatin is

one of the most active regimens with an acceptable toxicity profile [13]. A phase I/II trial of aprinocarsen in combination with gemcitabine and cisplatin in patients with advanced NSCLC showed a response rate of 38% and stable disease in 55% among the 31 evaluable patients [14].

Therefore, we planned this randomized phase II study to evaluate gemcitabine and cisplatin with and without aprinocarsen for chemonaive patients with locally advanced or metastatic NSCLC. This study was conducted in parallel with another ongoing phase III study in which aprinocarsen was being studied in combination with paclitaxel and carboplatin for patients with locally advanced or metastatic NSCLC [15]. The primary objective of the present study was to evaluate the tumor response rate to the combination regimen of aprinocarsen, gemcitabine, and cisplatin. The secondary objectives included the determination of the differences in the response rate, time to event efficacy parameters, and toxicities on the 2 treatment arms.

Patients and methods

Eligibility criteria

Patients, aged 18 years and older, with histologically or cytologically confirmed diagnosis of NSCLC and stage IIIB (N_3 and/or pleural or pericardial effusion) or IV disease not amenable to curative surgery or radiation therapy were enrolled. Patients were not permitted to have prior chemotherapy, and for those who had received prior radiotherapy, lesions used for determination of response were not to be previously irradiated, or the lesions must have increased in size since the completion of radiotherapy. Prior to randomization, all radiation had to be completed for at least 2 weeks, and patients must have fully recovered from any radiotherapy-related toxicity. Patients were also required to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, adequate bone marrow reserve (absolute neutrophil count [ANC] $\geq 1.5 \times 10^9$/L, platelet count $\geq 100 \times 10^9$/L, and hemoglobin $\geq$9.0 g/dL); normal liver function (bilirubin $\leq$1.5 times upper limit of normal [ULN]; alkaline phosphatase, aspartate transaminase, and alanine transaminase $\leq$3 times ULN ($\leq$5 times ULN is acceptable if liver has tumor involvement), and adequate renal function as indicated by calculated creatinine clearance of 50 mL/min or more per the Cockcroft and Gault formula. Presence of at least 1 unidimensionally measurable lesion was essential. Female patients were required to be nonlactating and have a negative serum pregnancy test, and all patients with childbearing potential were required to be abstinent or use approved contraceptive methods during, and for 3 months after, the treatment period.

Patients were excluded from the study for any prior chemotherapy or biologic therapy, or treatment within the last 30 days with a drug that has not received regulatory approval. Patients could not participate if they had peripheral neuropathy of grade 2 or greater, serious concomitant disorders, or presence of central nervous system metastases other than locally treated lesions that showed no signs of progression for at least 4 weeks. Patients were also excluded for having active infection or prior malignancy (except carcinoma in situ of the cervix or nonmelanoma skin cancer treated successfully at least 5 years previously).

Signed informed consent was obtained from all patients prior to enrollment. The study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki, or the guidelines on good clinical practices, and was approved by the appropriate ethical review boards.

Treatment plan

This was an open-label, randomized, phase II study. Patients were randomized using a centralized Interactive Voice-activated Response System. The patients were balanced in the 2 arms with respect to history of brain metastases, disease stage, ECOG performance status, and investigational center.

On both arms, each treatment cycle was 21 days. On the control arm, gemcitabine 1250 mg/m^2 was administered intravenously (iv) over approximately 30 min on days 1 and 8 of each 21-day treatment cycle. On day 1, this was followed by cisplatin 80 mg/m^2 given iv (about 4 h after gemcitabine administration). On the experimental arm, aprinocarsen was administered as continuous iv infusion for the first 14 days of each treatment cycle. This drug was obtained from Isis Pharmaceuticals Inc., Carlsbad, CA. The aprinocarsen dose was dependent on the patient's weight at initial screening: If the body weight was less than 65 kg, a daily dose of 125 mg of aprinocarsen was given; if weight was between 65 and 90 kg, the daily dose was 175 mg; and if weight was more than 90 kg, a daily dose of 225 mg was administered. Preliminary data from other phase II trials suggested that pretreatment with aprinocarsen might maximize its effectiveness. Hence, after the protocol amendment, aprinocarsen was administered on days 1 to 14, and gemcitabine and cisplatin were given on day 4 (the second dose of gemcitabine was given on day 11) on the experimental arm. Except for the first 2 patients, the remaining patients received aprinocarsen treatment as per the amended protocol. Standard hydration and antiemetic procedures associated with cisplatin administration were followed. Use of erythropoietin and hematologic growth factors was allowed.

Patients on both treatment arms were eligible to receive 6 cycles, unless disease progression or unacceptable toxicity occurred. Patients could receive 2 additional cycles if a complete response (CR) was identified after cycle 6. If tumor improvement occurred between the end of cycle 4 and 6, the patient could receive an additional 2 cycles at a time until further tumor improvement was documented. If the extended therapy resulted in a CR, the patient could receive 2 cycles after the CR was first documented, provided that toxicity was not excessive. Thirty days after study completion, all patients were to be evaluated in a short-term follow-up visit for response scoring and toxicity. In long-term follow-up, patients continued to be assessed at 2, 4, and 6 months; after 6 months, follow-up occurred at 3-month intervals. Any salvage therapy received by the patients was recorded at these times, and if tumor progression had not occurred, then tumor measurements and restaging were also carried out.

Use of continuous infusion

Patients in the experimental arm received aprinocarsen as a 14-day continuous infusion via a continuous pump (Deltec, WI). The cassette containing aprinocarsen diluted with saline was changed every 7 days. Antiseptic procedures were observed for the course of the administration and the regular care of the catheter. While 3 patients received Bard Port and Tunneled central venous catheter, Port-a-Cath was adopted for the remaining patients.

Dose adjustments

Dose adjustments were based on ANC and platelet count measured on day 1 of treatment, and at weekly intervals thereafter. In addition, adjustments were also dependent on the grade and type of toxicity observed during the treatment, as graded according to the Common Toxicity Criteria (CTC) version 2.0 from the National Cancer Institute.

If on day 1 of a new cycle the ANC was $<1.5 \times 10^9$/L and/or the platelet count was $<100 \times 10^9$/L, the cycle was delayed 1 week. For subsequent cycles, if previous cycle showed febrile neutropenia, grade 4 neutropenia lasting 7 days or more, grade 4 thrombocytopenia, or grade 2 bleeding associated with grade 3 thrombocytopenia, the doses of gemcitabine and cisplatin were reduced by 25%. Aprinocarsen doses were given at full dose for neutropenia, but for thrombocytopenia, the doses were reduced by 1 step. Aprinocarsen dose reduction steps were from 225 mg/day to 175 mg/day, 125 mg/day, 100 mg/day and finally 75 mg/day. Any unresolved nonhematologic toxicity had to be less than CTC grade 2 or baseline grade in order to begin a new cycle. For any grade 3 nonhematologic toxicity (except nausea and vomiting), the gemcitabine and cisplatin doses were reduced by 25%, and aprinocarsen was given at full dose. For any grade 4 toxicities, gemcitabine and cisplatin doses were reduced by 50% or delayed, and aprinocarsen doses were reduced by 1 step or delayed. Additionally, the cisplatin dose was reduced by 50% for grade 2 peripheral neurotoxicity; for grade 3 or 4, it was delayed until it was grade 2 or less, and then it was reduced by 50%. Similarly, for calculated creatinine clearance of 35 to 49 mL/min, the cisplatin dose was reduced by 50%; for less than 35 mL/min, the dose was delayed until it reached 35, and then the dose was reduced by 50%. For tinnitus or significant hearing loss, the cisplatin dose was reduced or stopped at investigator's discretion. If a dose was delayed on day 1, the cycle was considered to start on the day the first dose was actually given to the patient.

Dose adjustments within a cycle were made on day 8 or 11. If a dose of gemcitabine was omitted on day 8 or 11, the cycle continued per protocol with 1 dose not given. For ANC of 0.5 to 0.99×10^9/L or platelets 50 to 99×10^9/L, the gemcitabine dose was reduced to 75%; for ANC $<0.5 \times 10^9$/L and platelets $>50 \times 10^9$/L, it was omitted; however, aprinocarsen was given at full dose in both these conditions. Gemcitabine dose was omitted for any platelet count $<50 \times 10^9$/L, while aprinocarsen was reduced by 1 step, unless the count dropped below 25×10^9/L, then it was omitted. For any grade 3 nonhematologic toxicity (except nausea and vomiting), gemcitabine dose on day 8 or 11 was reduced by 50% or omitted, but aprinocarsen was given at full dose. For any grade 4 toxicity, the gemcitabine dose was omitted, while the aprinocarsen dose was reduced by 1 step or omitted. If the aprinocarsen dose was omitted, it had to be omitted for the remainder of the cycle.

Baseline and treatment assessments

All baseline evaluations had to be performed no more than 28 days before randomization. Each patient was assessed for tumor measurement by a radiologic imaging study, preferably using computed tomography scan or magnetic resonance imaging, but chest X-ray and physical examination was also allowed. These assessments had to include chest and abdomen and were routinely repeated at the start of every other cycle throughout the treatment period and at follow-up visits. About 2 weeks before randomization, patients were seen for medical history, physical examination, ECOG performance status, and complete hematologic and biochemical laboratory analysis, including creatinine clearance. Additionally, patients on experimental arm had a 12-lead electrocardiogram (ECG) and were fitted with a central venous catheter for administration

of aprinocarsen about a week before starting treatment. These tests, including ECG for experimental arm, were repeated on day 1 of every treatment cycle.

All measurable lesions (10 lesions total from all involved organs and up to 5 measurable lesions in any organ site) were designated as target lesions and measured for purposes of response scoring. Responses were evaluated using the Response Evaluation Criteria In Solid Tumors (RECIST) guidelines [16]. Response was assessed every other cycle, prior to the drug administration. The CR and PR had to be confirmed at least 4 weeks after the response was first observed.

Survival was defined as the time from the date of randomization to the date of death or the date the patient was last known to be alive. Progression-free survival was measured from the date of randomization to the first date of progressive disease (PD), or death from any cause. Time-to-treatment failure was measured from the date of randomization to the date of any of the following events: discontinuation of study treatment without completion of therapy, PD, initiation of salvage therapy, or death from any cause. Response duration was measured from the date of the first objective status assessment of CR or PR to the first date of PD or death from any cause.

All randomized patients who completed at least 2 full cycles of study therapy and had tumor measurements at baseline and for at least 1 post-baseline study visit were considered protocol-qualified and were evaluated for efficacy. All patients treated with study drugs were evaluated for safety. Safety was assessed through clinical adverse events and CTC grade toxicities at the beginning of every cycle.

Statistical design

The primary objective of this study was to estimate the tumor response rate of the experimental arm. The target enrollment was 100 patients, and assuming exactly 50 protocol-qualified patients enrolled in each arm, 95% confidence intervals for response rate would have a margin of error no greater than ± 0.145. The time-to-event parameters were estimated using the Kaplan-Meier method and compared using the standard log-rank test.

Results

Patient characteristics

Between August 2002 and March 2003, 18 patients from 6 centers in Austria, Belgium, and Finland were randomized to either the control arm (gemcitabine and cisplatin) or the experimental arm (aprinocarsen, gemcitabine, and cisplatin). Further enrollment for this trial was terminated in March 2003 due to the new efficacy data from a phase III trial (Isis CS17), suggesting that aprinocarsen did not add survival benefit or improve the response rate to the combination of paclitaxel and carboplatin among patients with stage IIIB/IV NSCLC [15].

Table 1. Patient demographics and characteristics

	Control arm ($n = 9$)	Experimental arm ($n = 9$)
Gender, *n* (%)		
Male	7 (77.8)	8 (88.9)
Female	2 (22.2)	1 (11.1)
Age (years)		
Median	56.4	60.1
Range	51.7–68.1	44.2–75.7
ECOG performance status, *n* (%)		
0	1 (11.1)	0 (0)
1	8 (88.9)	9 (100)
Pathological diagnosis, *n* (%)		
Adenocarcinoma	4 (44.4)	6 (66.7)
Large cell carcinoma	2 (22.20	2 (22.2)
Squamous cell carcinoma	2 (22.2)	1 (11.1)
Others	1 (11.1)	0
Disease stage, *n* (%)		
IIIB	1 (11.1)	1 (11.1)
IV	8 (88.9)	8 (88.9)
Prior surgery, *n* (%)	1 (11.1)	1 (11.1)
Prior radiotherapy, *n* (%)	2 (22.2)	0

ECOG = Eastern Cooperative Oncology Group.

All enrolled patients were Caucasian, with a majority (83.3%) being male. Patient demographics and disease characteristics are given in Table 1. Patients had median age of 57.9 years in the 2 arms, with ECOG performance status of 0 (5.6% patients) or 1 (94.4% patients). On both treatment arms, most patients (88.9%) had stage IV disease. Only 11.1% patients in both treatment arms had undergone prior surgery for their cancer. Interestingly, 22.2% of patients on the control arm had received prior radiation therapy, while none of the patients on the experimental arm had received prior radiation therapy.

Response and time-to-event measures

Seventeen of the 18 patients received study drugs. One patient in the experimental arm did not receive study drug due to suspension of the study post-randomization, but before start of treatment. Overall, 5 patients completed all 6 pre-specified treatment cycles: 4 in the control arm and 1 in the experimental arm. All 9 patients in the control arm and 6 patients in the experimental arm were considered evaluable for efficacy. Two patients in the experimental arm were considered to be non-protocol-qualified because of either not completing 2 treatment cycles or

Table 2. Summary of response rates

	Control arm ($n = 9$) No. (%)	Experimental arm ($n = 6$) No. (%)
Partial response	4 (44.4)	1 (16.7)
Stable disease	2 (22.2)	2 (33.3)
Progressive disease	3 (33.3)	2 (33.3)
Unknown	0	1 (16.7)

not having all the tumor measurements. No CRs were reported, and PRs were observed in 44.4% of the control arm and 16.7% of the experimental arm (Table 2). Disease control rate (partial response + stable disease) was 66.6% in the control arm and 50.0% in the experimental arm. These responses should be interpreted with caution, however, due to the small number of randomized patients. Because of the early termination of the study and the resulting small sample size, no measures for formal statistical inference (ie, confidence intervals or p-values) were calculated.

Median survival was 10.0 months on the control arm and 4.7 months on the experimental arm. Median progression-free survival was 4.9 months on the control arm, and 3.8 months on the experimental arm. Due to the small sample size, median response duration and time-to-treatment failure were not reported. For time-to-event parameters, no measures for formal statistical inference (ie, confidence intervals or p-values) were calculated.

Dose administration and safety

Five patients completed the planned 6 treatment cycles: 4 (44.4%) in the control arm and 1 (12.5%) in the experimental arm. In the control arm, a median of 4 cycles (range, 2–6) was administered, while in the experimental arm, a median of 2.5 cycles (range, 2–6) was administered. In the control arm, there were 6 dose reductions of gemcitabine and 1 dose reduction of cisplatin. Four delays occurred in the administration of both gemcitabine and cisplatin. In the experimental arm, there were 5 dose reductions of gemcitabine, 3 dose reductions of cisplatin, and 3 dose reductions of aprinocarsen during the study. The aprinocarsen dose reductions that took place in 3 patients were due to various causes, including 1 case with thrombocytopenia and another due to infection. Five delays occurred in the administration of gemcitabine, cisplatin, and aprinocarsen, and 3 delays in the administration of aprinocarsen.

Of all 18 patients, 1 in the experimental arm did not receive any treatment and did not qualify for safety analysis. Table 3 lists the observed grade 3 and 4 toxicities. The most common grade 3/4 toxicities observed in these arms were hematologic, with a marginally higher incidence in the experimental arm (anemia 37.5% vs. 22.2%, neutropenia 50.0% vs. 33.3%, and thrombocytopenia 87.5% vs. 33.3%). A total of 7 hospitalizations due to drug-related adverse events occurred: 3 in the control arm and 4 in the experimental arm. None of the experimental arm hospitalizations were related to peripheral catheter sites infection. A total of 3 patients died in the experimental arm, and 2 patients died in the control arm while on study; these deaths were not considered to be treatment related.

Table 3. CTC grade 3/4 toxicity

	Control arm ($n = 9$)		Experimental arm ($n = 8$)	
Toxicity	Grade 3	Grade 4	Grade 3	Grade 4
Hematologic, *n* (%)				
Anemia	2 (22.2)	0	3 (37.5)	0
Neutropenia	1 (11.1)	2 (22.2)	4 (50.0)	0
Thrombocytopenia	2 (22.2)	1 (11.1)	5 (62.5)	2 (25.0)
Nonhematologic, *n* (%)				
Anorexia	1 (11.1)	0	2 (25.0)	0
Asthenia	1 (11.1)	0	2 (25.0)	0
Dyspnea	2 (22.2)	0	1 (12.5)	0
Fatigue	1 (11.1)	0	1 (12.5)	1 (12.5)
Febrile neutropenia	1 (11.1)	0	0	0
Hypertension	1 (11.1)	1 (11.1)	1 (12.5)	0
Nausea	0	0	2 (25.0)	0
Vomiting	1 (11.1)	0	2 (25.0)	0

Discussion

The present phase II, randomized, multicenter study found that the addition of aprinocarsen to the combination of gemcitabine and cisplatin did not appear to improve the response rate in patients with advanced or metastatic NSCLC. With only a small patient population available, however, it is not advisable to draw any definitive inferences about the effects of adding aprinocarsen. The combination aprinocarsen, gemcitabine, and cisplatin appeared to be safe and feasible but with a marginally higher thrombocytopenia than in the control arm.

Lynch et al. [15] recently reported the results of a phase III trial with the combination of paclitaxel and carboplatin with or without aprinocarsen in patients with advanced or metastatic NSCLC. In their study, 616 patients were randomized between the 2 arms, and most of the patients (87%) had stage IV disease. A median of 4 cycles (range, 1–13) was delivered in the aprinocarsen arm and 5 cycles (range, 1–13) in the control arm. The overall response rate was comparable in the 2 treatment arms with 37% in the aprinocarsen arm and 36% in the control arm. Median survival and 1-year survival were also comparable in the 2 arms of their study.

Although the numbers are small in this study and the tumor response parameters cannot be fully evaluated due to the early termination of the study, we want to point out some observations relevant to the pharmacological effect of aprinocarsen. For example, no patients on the experimental arm received prior radiotherapy, while 22.2% of the patients on the control arm received prior radiotherapy. Thus, it cannot be excluded that this pretreatment factor has influenced the tumor response rate between the 2 arms when the study was terminated.

The lack of a dramatic tumor response rate using novel targeted agents in NSCLC is not surprising. A recent example is the study with trastuzumab in NSCLC [17]. In that study, trastuzumab was added to a gemcitabine-cisplatin regimen in patients with NSCLC and was compared to a standard gemcitabine and cisplatin treatment. The response rate was similar between both arms (36% vs. 41%), and only those patients with HER2/neu expression of 3+ or fluorescence in situ hybridization (FISH) positive NSCLC showed higher response rate of 83% when treated with trastuzumab. However, they found that less than 2% of the screened patient population showed immunohistochemically 3+ and/or HER2/neu amplification (as measured by FISH). It was estimated that it might be necessary to screen 15 000 patients to recruit 200 patients with overexpressing HER2/neu to demonstrate a significant difference due to the target therapy [18]. Thus, the selection of the patient population may be critical in such targeted therapies.

As research in tumor biology progresses, newer therapies are developed to specifically block tumor-growth-associated proteins. One such strategy is to block the formation of such proteins at the mRNA level by administering ASOs [6]. Because these compounds belong to a new drug class, information about dosing schedules, tolerability, and toxicity information are important to the medical community. Thus, the present study is a contribution to provide specific and comprehensive information about this drug class and for aprinocarsen in particular.

While aprinocarsen-related toxicities appear to be manageable, treatment-related toxicity observations are of greater potential concern. Because aprinocarsen is being developed as a 14-day continuous infusion, catheter-related infections were feared to be more common in patients receiving aprinocarsen. In the course of the clinical development, we learned that continuous infusions are handled differently in various European countries. While there are general guidelines to prevent catheter-related infections [19, 20], there are no specific guidelines for continuous infusions in oncology. For aprinocarsen administration in this study, infusate cassettes were changed every 7 days. As suggested by previous studies in oncology [21, 22], changes of cassettes every 7 days appeared to be safe and did not result in increased infection rates. As a result of these observations and the lack of increased infection rate in the present study with aprinocarsen, changes of infusion cassette every 7 days appear to be acceptable. These observations are in line with earlier aprinocarsen studies but not with the recent phase III study by Lynch et al. [15], where the aprinocarsen-treated patients had a higher infection rate and catheter-related complications: infection (8.2% vs. 0.3%), catheter sepsis (4.8% vs. 0.3%), and fever/neutropenia (8.8% vs. 2.1%). Because there are no universal standards on how to best administer infusates over a prolonged time, it is possible that trial sites used different venous-access procedures in that study, perhaps accounting for the higher infection rates. In the present study, 5 out of 8 patients used a Port-a-Cath, a procedure recommended to minimize infection rate risk [23].

In conclusion, the present phase II trial combining aprinocarsen with the standard regimen of gemcitabine and cisplatin for patients with advanced or metastatic NSCLC did not show any advantage in terms of response rate or survival. However, the study confirms earlier observation that the aprinocarsen dose and dose schedule is safe, and it contributes to the growing safety database of phosphorothioate-ASOs use in humans.

Acknowledgments

The authors gratefully acknowledge and wish to thank those sites that were either opened or were ready to open for this study; *Austria*: Dr. Baumgartner (Krankenhaus der Stadt Wien Lainz), Dr. Ludwig (Wilhelminenspital, Wien), Dr. Gastl (Landeskrankenhaus Innsbruck), Dr. Aigner (Elisabethinen Krankenhaus, Linz); *Belgium*: Dr. LeGrand (Hopital Erasme, Brussels), Dr. Aumann (Private Practice, Hasselt); *Greece*: Dr. Kosmidis ("HYGEIA" Hospital of Athens), Dr. Georgoulias (University Hospital of Heraklion), Dr. Syrigos (SOTIRIA General Hospital, Athens), Dr. Foutzilas (AHEPA Hospital, Thessaloniki), Dr. Dimopoulos (Alexandra Peripheral General Hospital, Athens); *Portugal*: Dr. Pimentel (Hospital de São Sebastião, Santa Maria da Feira), Dr. Santos Dionisio (Instituto Português de Oncologia, Lisboa), Dr. José de Melo (Hospital de Pulido Valente, Lisboa), Dr. Teixeira (Hospital de Santa Maria, Lisboa), Dr. Sanches (Instituto Português de Oncologia, Porto). We also want to thank Dr. Timothy Grau and the contract research organization OmniCare Inc. for helping to conduct this trial. We thank Dr. Jon Holmlund at Isis Pharmaceuticals, Carlsbad (CA), for his valuable advice on the general clinical development for aprinocarsen.

The following Eli Lilly and Company individuals contributed to the study: Data management, validation, and technical assistance were performed by Nancy Iturria and

Julie Ashland. Ghulam Kalimi, PhD, provided support with manuscript preparation, and Peter Fairfield provided editorial assistance. This study was funded by Eli Lilly and Company, Indianapolis, IN.

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