A Phase 1 Open-Label, Accelerated Dose-Escalation Study of the Hypoxia-Activated Prodrug AQ4N in Patients with Advanced Malignancies

Kyriakos P. Papadopoulos,[1] Sanjay Goel,[2] Murali Beeram,[1] Alvin Wong,[3] Kavita Desai,[2] Missak Haigentz,[2] María L. Milián,[3] Sridhar Mani,[2] Anthony Tolcher,[1] Alshad S. Lalani,[3] and John Sarantopoulos[1]

Abstract

Purpose: AQ4N is a novel prodrug that is selectively bioreduced to AQ4, a topoisomerase II inhibitor, in hypoxic tumor. This study assessed the maximum tolerated dose and pharmacokinetics of AQ4N when administered weekly in patients with advanced cancers.

Experimental Design: AQ4N was administered as a 30-minute i.v. infusion on days 1, 8, and 15 of a 28-day cycle in eight dose cohorts ranging from 12 to 1,200 mg/m^2. Accelerated titration design was used and the maximum tolerated dose was defined as the highest dose at which fewer than two of six patients had a dose-limiting toxicity.

Results: Sixteen patients were treated with cumulative doses of AQ4N ranging from 61.6 through 9,099.1 mg/m^2. A single patient per cohort was treated up to 384 mg/m^2 without toxicities. At 1,200 mg/m^2, two of five patients experienced a dose-limiting toxicity (grade 5 respiratory failure and grade 3 fatigue). Five cohort assigned patients were treated without toxicity at 768 mg/m^2, establishing this dose as the maximum tolerated dose. Among the most common adverse events observed were fatigue (38%), diarrhea (31%), nausea (25%), vomiting (25%), and anorexia (13%). Anticipated blue coloration of body fluids or skin was observed in all patients. The pharmacokinetics of AQ4N were dose proportional over all doses studied. Three patients experienced stable disease, including a patient with collecting duct renal cancer stable for 25 months.

Conclusion: AQ4N is well tolerated when administered weekly on a 3-of-4-week schedule at 768 mg/m^2. Further combination studies investigating the safety and efficacy of AQ4N are ongoing.

Hypoxia is characteristic of solid tumors and mediates, in part via hypoxia-inducible factor-1, changes in transcriptional regulation of genes involved in cellular metabolism, survival, angiogenesis, and metastasis (1–6). Prolonged tumor hypoxia increases genomic instability and treatment resistance, selects for metastatic variants, and has been shown to influence disease progression in preclinical and clinical studies (1–7).

AQ4N (banoxantrone; 1,4-bis{[2-(dimethylamino)ethyl] amino}-5,8-dihydroxyanthracene-9,10-dione bis-*N*-oxide) is rationally designed as a prodrug that is preferentially converted to a potent cytotoxin in hypoxic tumor regions (8–11). The prodrug form of AQ4N is relatively nontoxic to cells until it is bioreduced in hypoxic tumor cells by a two-step enzymatic reduction to form AQ4M, a short-lived mono-*N*-oxide intermediate, and then to the ditertiary cationic amine, AQ4 (11–14). AQ4, but not AQ4N, binds DNA with high avidity and functions as a potent inhibitor of topoisomerase II (14). Bioreduction of AQ4N occurs strictly in the absence of oxygen and seems to be catalyzed by a number of cytochrome *P*450 enzymes, which are commonly up-regulated in tumors (15–20). The high-affinity DNA binding limits diffusion of AQ4 outside of the tumor microenvironment, resulting in minimal systemic toxicity. In tumor xenograft models, AQ4N undergoes dose-dependent and selective activation in tumors and results in significant inhibition of tumor growth and progression (21). Moreover, AQ4N in combination with other systemic therapies or radiation has been postulated to enhance therapeutic efficacy by facilitating the targeting of both normoxic and hypoxic regions of the tumor microenvironment. Indeed, preclinical models have shown an enhanced

Authors' Affiliations: [1]Institute for Drug Development, Cancer Therapy and Research Center, University of Texas Health Science Center at San Antonio, San Antonio, Texas; [2]Montefiore Medical Center, Albert Einstein Cancer Center, Bronx, New York; and [3]Novacea, Inc., South San Francisco, California

Received 2/25/08; revised 5/20/08; accepted 6/19/08.

Grant support: Novacea, Inc.

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.

Presented in part at the 18th European Organization for Research and Treatment of Cancer-National Cancer Institute-AACR Symposium on Molecular Targets and Cancer Therapeutics, November 7-10, 2006, Prague, Czech Republic, and at the American Society of Clinical Oncology Annual Meeting, June 2-6, 2006, Atlanta, Georgia.

Requests for reprints: Kyriakos P. Papadopoulos, South Texas Accelerated Research Therapeutics, 4319 Medical Drive, Suite 205, San Antonio, TX 78229. Phone: 210-593-5250; Fax: 210-615-1121; E-mail: kyri.papadopoulos@start.stoh.com.

doi:10.1158/1078-0432.CCR-08-0483

Translational Relevance

Hypoxia occurring in solid tumors is a key microenvironmental factor for resistance to treatment and tumor progression. Efforts are thus being actively pursued to develop therapeutic agents that target hypoxic tumor. AQ4N is rationally designed as a relatively nontoxic prodrug that is preferentially converted to a potent cytotoxin in hypoxic tumor regions. AQ4N undergoes dose-dependent and selective activation in tumors and results in significant inhibition of tumor growth and progression. This phase 1 study establishes the clinical safety of repeated weekly i.v. dosing of AQ4N at 768 mg/m^2 in patients with advanced solid tumors. AQ4N used in combination with other systemic therapies and radiation may enhance therapeutic efficacy in patients with solid malignancies by facilitating the targeting of both normoxic and hypoxic regions of the tumor.

antitumor response when AQ4N is combined with chemotherapy and/or radiation (8, 22–27).

Animal toxicology studies have established repeated doses of AQ4N at 20 mg/kg/wk in rats and 6 mg/kg/wk in monkeys as the maximum tolerated dose when given for 6 consecutive weeks. AQ4N toxicity was consistent with that of a cytotoxin targeting the lymphoid system, gastrointestinal tract, and urogenital tract, indicating differences in the conversion of AQ4N to AQ4 or differences in sensitivity in these tissues. There was also dose-related blue discoloration of skin and urine.

Single and limited repeat dosing of AQ4N have been explored previously in two clinical trials. A phase 1 trial of administration of a single dose of AQ4N (200 mg/m^2) before elective surgery in 32 cancer patients confirmed selective activation of AQ4N in hypoxic regions of tumors (28). In another phase 1 trial of 22 patients with advanced esophageal carcinoma who had been clinically assessed as being inoperable and not eligible for chemotherapy, AQ4N was administered i.v. on day 1, followed by a second dose at the same dose level on day 15 given 6 hours before the first of five daily 4-Gy fractions of radiotherapy (29). The dose of AQ4N was escalated to 447 mg/m^2 without dose-limiting toxicities (29). Although this prior clinical experience with AQ4N suggested a higher initial dose for the current weekly repeat dosing study we present here, the repeat dose toxicology studies in animals showed increased and cumulative toxicity. A more conservative starting dose of 12 mg/m^2 was thus chosen to ensure patient safety in case of cumulative toxicity. To balance the conservative starting dose with the risk of undertreatment, an accelerated dose escalation design was selected (30).

The primary objective of the present study was to determine the maximum tolerated dose of AQ4N when administered on days 1, 8, and 15 of a 28-day cycle. The secondary objectives of this study were to evaluate the safety and determine dose-limiting toxicities of this schedule, characterize the pharmacokinetics of AQ4N, and seek preliminary evidence of antitumor activity in patients with advanced solid malignancies.

Patients and Methods

Patient selection. Patients with histologically confirmed solid malignancies refractory to standard therapy or for whom no standard therapy exists were eligible. Patient entry criteria included age ≥18 y; life expectancy ≥3 mo; an Eastern Cooperative Oncology Group performance status of ≤2; no prior chemotherapy or radiation within 4 wk; adequate hematopoietic (hemoglobin ≥9 g/dL, absolute neutrophil count ≥1.5 × 10^9/L, and platelet count ≥100 × 10^9/L), hepatic (total bilirubin ≤ 1.5 mg/dL; aspartate aminotransferase and alanine aminotransferase <3 × upper limit of normal or <5 × upper limit of normal in presence of hepatic metastases), and renal (serum creatinine ≤1.5 mg/dL) functions; measurable or evaluable disease; cardiac ejection fraction (LVEF) greater than the institutional lower limit of normal; and no coexisting medical problem of sufficient severity to limit compliance with the study. Patients with symptomatic or uncontrolled brain metastases were not enrolled. All patients gave written informed consent before performing any study-related procedures according to federal and local institutional guidelines.

Treatment and dose escalation. AQ4N was administered i.v. on days 1, 8, and 15 of a 28-d treatment cycle. The starting dose of AQ4N was 12 mg/m^2. Stepwise escalation by dose doubling was done until the first drug-related grade 2 toxicity was observed; thereafter, the dose was increased by a maximum of 50%. If a drug-related grade ≥3 toxicity was observed, then the maximum increment was 33%. Each cohort consisted of one patient until a grade 2 AQ4N-related toxicity was observed and a minimum of three patients per cohort thereafter. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events, version 3.0. Dose-limiting toxicity was defined as any of the following drug-related events occurring during the first cycle: grade 4 neutropenia lasting ≥7 d; grade 4 lymphopenia lasting ≥14 d; thrombocytopenia grade ≥4; non-hematologic toxicity grade ≥3 (including nausea, vomiting, or diarrhea despite optimal supportive care); and AQ4N-related toxicities that delayed subsequent courses for >7 d or omission of ≥1 dose of AQ4N. If one dose-limiting toxicity occurred, a maximum of six patients were to be treated at that dose level. The maximum tolerated dose was defined as the highest dose at which fewer than two of six patients in a cohort experienced a dose-limiting toxicity. At least five of six patients were to be treated without a dose-limiting toxicity at the maximum tolerated dose to establish this dose as tolerable. In all but the first cohort, one dose reduction was allowed for patients who received at least one course of therapy and developed a dose-limiting toxicity or for safety reasons as determined by the investigators.

An aqueous formulation of AQ4N (100 mg) was provided in 20 mmol/L sodium phosphate buffer in 5-mL glass vials (Novacea, Inc.). AQ4N was mixed aseptically with 0.9% NaCl in i.v. saline bags and infused i.v. over ~30 min.

Study assessments. Baseline assessment included a complete medical history, physical examination, laboratory tests, urinalysis, electrocardiogram, multiple gated acquisition scan, and radiographic imaging. Toxicities were evaluated at each visit with appropriate cardiac monitoring before each odd cycle. Computed tomography scans for assessment of tumor burden were obtained at baseline, and response to study drug was assessed every two cycles according to the Response Evaluation Criteria in Solid Tumor; patients showing disease progression were discontinued from the study.

Pharmacokinetic evaluation. Serial blood samples for plasma pharmacokinetics of AQ4N and its metabolites were collected on days 1 and 15 of the first cycle at the following time points: predose and post-AQ4N infusion at 2 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, and 24 h. Urine for analysis was collected over designated intervals for 24 h after the first dose of AQ4N and frozen before analysis. AQ4N and its two bioreduced metabolites, AQ4M and AQ4, were quantified from frozen heparanized plasma samples using validated high-performance liquid chromatography with tandem mass

Table 1. Patient characteristics

Characteristics	
No. of patients	16
Median age (range), y	57 (32-80)
Sex (male/female)	8/8
Race (White/Black/Hispanic)	10/3/3
Performance status	
0	2
1	13
2	1
Prior therapies	16
Chemotherapy regimens, median (range)	3 (1-10)
Anthracyclines	4
Radiation therapy	5
Tumor types	
Lung	2
Renal cell	2
Prostate	2
Head and neck	2
Breast, cervical, colon, hepatocellular, ileum, melanoma, ovarian, sarcoma	1 each

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

spectrometric methods as previously described (31, 32). In brief, AQ4N, AQ4M, and AQ4 were extracted from plasma samples using MCX SPE well plates (Waters Corp.). Aliquots of the extracts were analyzed by reverse-phase high-performance liquid chromatography (Agilent Technologies) using an Astec Polymer C18 column maintained at 50°C. The mobile phase was nebulized using heated nitrogen in a Z-spray source/interface and the ionized compounds were detected using a tandem quadrupole Quatro II mass spectrometer (Micromass, Inc.). The lower limit of quantitation was 10 ng/mL for AQ4N and AQ4M and 2 ng/mL for AQ4 in human plasma. Noncompartmental analysis was used to evaluate the overall pharmacokinetics using WinNonlin Professional 4.1 (Pharsight, Inc.). Pharmacokinetic variables that were assessed included maximum plasma concentration (C_{max}), area under the plasma concentration versus time curve from time to infinity ($AUC_{0-\infty}$), elimination half-life ($t_{1/2}$), volume of distribution at steady-state (V_{ss}), and plasma clearance (CL_p). Pharmacokinetic variables are expressed as mean and SD except for half-life, which is presented as the harmonic mean and pseudo-SDs.

Results

Patient characteristics. Sixteen patients, whose pertinent demographic characteristics are shown in Table 1, were enrolled in the study between September 2004 and March 2006. Patients were treated with cumulative doses of AQ4N ranging from 61.6 through 9,099.1 mg/m^2, escalated through eight sequential dose level cohorts; the median number of cycles administered per patient was 2 (range, 1-27). All patients were evaluable for toxicity and response.

Dose escalation and serious adverse events. A single patient per cohort was evaluated at AQ4N doses of 12, 24, 48, 96, 192, and 384 mg/m^2, and five patients each were evaluated at doses of 768 and 1,200 mg/m^2 (Table 2). Grade 2 drug-related toxicities were observed at the 768 mg/m^2 dose level and three patients were initially evaluated. Dose-limiting toxicities of grade 3 fatigue and fatal grade 5 respiratory failure occurred respectively in two patients following treatment at 1,200 mg/m^2. The fatal respiratory failure was probably related to postmortem findings of extensive progressive metastatic sarcoma, including myocardial and pulmonary organ involvement. As a consequence of these toxicities, dose reduction to 768 mg/m^2 was done in three of the patients actively receiving therapy in the 1,200 mg/m^2 cohort, and the 768 mg/m^2 cohort was further expanded. No dose-limiting toxicities or drug-related serious adverse events were observed in any of the five cohort-assigned and three dose-reduced patients treated at the 768 mg/m^2 level, and this dose level was identified as the maximum tolerated dose.

Toxicity. Toxicities reported in at least 10% of patients and all grade 3 and 4 toxicities in all cycles are summarized in Table 3. Among the most common drug-related adverse events observed were fatigue (38%), diarrhea (31%), nausea (25%), vomiting (25%), and anorexia (13%). Nausea, vomiting, and diarrhea were grade 1 or 2 and managed with medication. Anticipated blue coloration of skin and body fluids was observed in all patients at doses ≥48 mg/m^2 or with prolonged dosage (Table 3). The duration of skin discoloration was predicated by the dose and extent of therapy, usually lasting <24 to 72 hours. Transient blurred vision occurred in two patients at 1,200 mg/m^2, and eye irritation was reported by

Table 2. AQ4N treatment by dose level

Dose level (mg/m^2)	No. of patients	No. of cycles (range)	Cumulative dose* (mg/m^2)	No. of patients with DLT	Response
12	1	1	61.6	0	—
24	1	2	144.2	0	—
48	1	27	3,621.9	0	1 SD
96	1	2	556.5	0	—
192	1	2	1,105.1	0	—
384	1	1	1,143.3	0	—
768	5 †	2 (1-2) ‡	3,815.0 ‡	0	1 SD
1,200	5	2 (1-3) ‡	4,376.9 ‡	2 [grade 3 fatigue (1); grade 5 respiratory failure (1)]	1 SD

Abbreviations: DLT, dose-limiting toxicity; SD, stable disease.
*Total includes partially completed cycles.
† Excludes three dose-reduced patients treated at the 768 mg/m^2 level.
‡ Based on a median of five patients.

Table 3. Related adverse events occurring in >10% of patients and all Common Toxicity Criteria for Adverse Events grade 3-5 in patients treated with AQ4N

Adverse event	All (n = 16)	Grade 3/4/[5]
	n (%)	
Chromaturia	16 (100)	0 (0)
Skin discoloration	13* (81)	0 (0)
Fatigue	6 (38)	1 (6)
Diarrhea	5 (31)	0 (0)
Nausea	4 (25)	0 (0)
Vomiting	4 (25)	0 (0)
Anorexia	2 (13)	0 (0)
Abdominal discomfort	2 (13)	0 (0)
Eye irritation	2 (13)	0 (0)
Blurred vision	2 (13)	0 (0)
Alopecia	2 (13)	0 (0)
Respiratory failure	1 (6)	[1 (6)]
Neutropenia	2 (13)	0 (0)

*Not reported in two patients from the <48 mg/m^2 cohorts and one patient of African American heritage from the 1,200 mg/m^{2}cohort.

two patients treated at 192 and 384 mg/m^2. Myeloid and lymphoid suppression was dose dependent, generally mild, and not associated with clinical sequelae except in one patient receiving AQ4N at 48 mg/m^2, who after 25 cycles of therapy developed persistent pancytopenia. With one exception, patients evaluated with serial electrocardiogram (n = 16) and multiple gated acquisition scan (n = 10) every two cycles of treatment or at study discontinuation showed no evidence of significant electrocardiogram changes or cardiac dysfunction. A patient with a history of diabetes, hypertension, ischemic heart disease, and previous myocardial infarction and angioplasty was treated at 192 mg/m^2 of AQ4N and had a 13% decline in ejection fraction to 43% after two cycles of therapy. This event was not attributed to AQ4N following further cardiac evaluation.

Pharmacokinetics. The first-dose pharmacokinetic variables of AQ4N in 16 patients are summarized in Table 4. Following i.v. administration, AQ4N plasma concentrations declined rapidly in a multiexponential manner and there was no drug accumulation after repeated dosing (Fig. 1A). The C_{max} and AUC incremented proportionally with increasing dose level (Fig. 1B and C). The mean clearance (CL_p) of AQ4N for all patients was 3.09 $\pm$ 0.93 L/h/m^2, and although CL_p ranged from 1.69 to 4.45 L/h/m^2 across dose groups, the overall clearance values were relatively constant. The mean volume of distribution at steady state (V_{ss}) for the 16 patients completing pharmacokinetic evaluation was 11.48 $\pm$ 3.29 L/m^2. Both variables seemed to be independent of dose. The harmonic mean terminal elimination half-life for all dose levels was 4.10 $\pm$ 2.29 hours. Following i.v. administration of AQ4N, plasma concentrations of the intermediate mono-*N*-oxide AQ4M metabolite were appreciably lower (<5%) than the parent AQ4N prodrug. Although AQ4M concentrations declined in a somewhat parallel fashion to AQ4N, there was considerable fluctuation in the declining plasma concentrations, and determination of the terminal $t_{1/2}$ was not possible for most patients. Due to the limited number of patients evaluated in each dose group and the considerable variability in data across and within doses, a dose-relationship for AQ4M C_{max} and AUC could not be assessed. Plasma concentrations for the fully bioreduced metabolite, AQ4, were below assay quantification limits (< 2.00 ng/mL) in the majority of the samples, with most quantifiable samples <5.00 ng/mL. Overall, urinary excretion of AQ4N accounted for ~70% of the administered AQ4N dose over the first 24 hours. At lower doses (12-192 mg/m^2), the percent of AQ4N excreted in urine ranged from 14% to 47% of the dose, whereas urinary excretion in the 368 to 1,200 mg/m^2 dose groups accounted for 78% to 94% of their respective doses. In all dose groups, the majority of the dose excreted in urine was recovered during the first 10 hours following dose administration. The renal clearance of AQ4N ranged from 16.52 to 108 mL/min across all dose groups and seemed to be dose dependent with clearance values >50 mL/min at doses $\geq$192 mg/m^2.

Tests for correlations of AQ4N dose and pharmacokinetic variables $AUC_{0-\infty}$ and C_{max} with toxicity were done. Except for a nonsignificant trend for cytopenia with increasing drug dose, no correlation for toxicities was apparent (data not shown).

Antitumor activity. No responses were observed, with 13 patients progressing. There were three patients with stable disease, including two patients with bronchoalveolar lung cancer and ovarian cancer. One patient with collecting duct renal cancer treated with AQ4N at 48 mg/m^2 had prolonged disease stabilization for 25 months.

Discussion

AQ4N is designed to be an inactive prodrug in normal tissues but to be selectively activated to a potent cytotoxin in hypoxic

Table 4. AQ4N noncompartmental pharmacokinetic variables on day 1 of cycle 1 following infusion

Variable	Dose (mg/m^2)							
	12	24	48	96	192	384	768	1,200
n	1	1	1	1	1	1	5	5
$t_{1/2}$ (h)	5.3	2.3	2.1	3.4	3.5	4.5	3.6 $\pm$ 0.6*	4.1 $\pm$ 1.5*
C_{max} (ng/mL)	1,720	4,382	7,374	13,457	28,440	50,660	98,415 $\pm$ 23,635	144,556 $\pm$ 37,088
$AUC_{0-\infty}$ (h ng/mL)	7,092.0	6,609	12,320	44,376	65,115	153,801	242,282 $\pm$ 70,032	485,034 $\pm$ 291,176
CL_p (L/h/m^2)	1.69	3.63	3.90	2.16	2.95	2.50	3.39 $\pm$ 0.97	2.97 $\pm$ 1.07
V_{ss} (L/m^2)	10.47	8.38	9.05	8.66	9.83	13.15	11.32 $\pm$ 2.73	13.49 $\pm$ 4.54

*Data are presented as median $\pm$ SD, except for half-life, which is expressed as harmonic mean $\pm$ pseudo-SD (34).

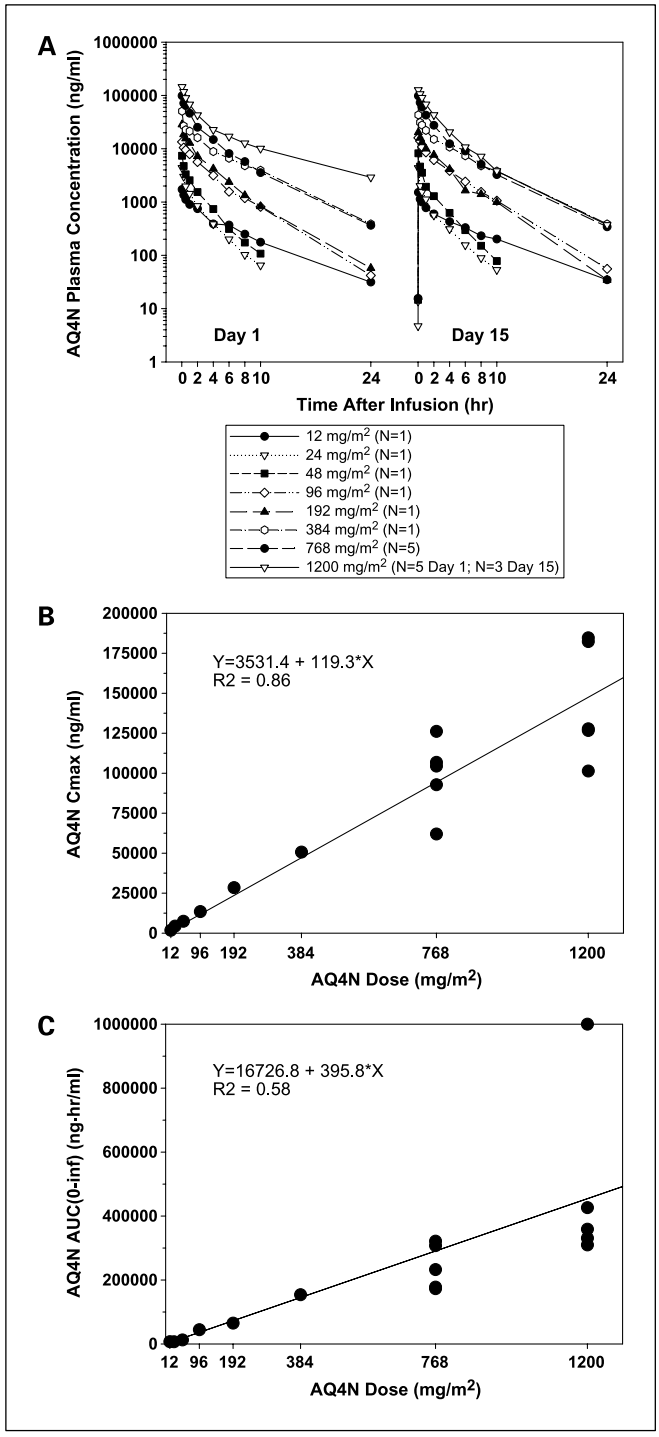

Fig. 1. AQ4N pharmacokinetics. *A,* AQ4N plasma concentration-time plots following a 12 to 1,200 mg/m^2 dose of AQ4N administered by a 30-min i.v. infusion on days 1 and 15 of cycle 1. Lines connect individual values or medians at 768 and 1,200 mg/m^2. *B* and *C,* scatterplots showing the distributions of the noncompartmental pharmacokinetic variable values reflecting C_{max} (*B*) and $AUC_{0-\infty}$ (*C*) as a function of AQ4N dose on day 1 of cycle 1.

regions that are characteristic of many solid tumors. The key objectives of this present study were to determine the maximum tolerated dose and pharmacokinetics of AQ4N following weekly administration in patients with advanced cancers.

In this phase 1 study of AQ4N, dose-limiting toxicities (i.e., fatigue and respiratory failure) were observed at 1,200 mg/m^2. The patient with fatal respiratory failure had extensive cardiac and pulmonary involvement with sarcoma that likely contributed to her death. Five cohort assigned patients were enrolled at an AQ4N dose of 768 mg/m^2 without dose-limiting toxicity, and this dose was established as the maximum tolerated dose. Aside from fatigue, nonhematologic and hematologic toxicities of grade >2 severity were uncommon and AQ4N was generally well tolerated. Noteworthy is that although anticipated skin-related toxicity occurred across all dose cohorts ≥48 mg/m^2, this was largely cosmetic. Previous dose-finding studies were limited and suggested good tolerance of AQ4N (29). Except for skin discoloration, no drug-related adverse events were noted in 22 patients following a single 22.5 to 447 mg/m^2 dose of AQ4N (29). Combination with radiation following a second dose of AQ4N made attribution of adverse events difficult in this prior study. Similarly, AQ4N-related adverse events in a single 200 mg/m^2 dose study included skin discoloration and fatigue but few other drug-related toxicities (28). One patient with ischemic heart disease in this latter study had a myocardial infarction and left ventricular failure thought to be possibly drug related. AQ4N is similar in structural to anthracenedione antineoplastic agents, which are known to result in reduced cardiac function following high cumulative dosing. There was no evidence of change in electrocardiogram, decline in ejection fraction, or myocardial dysfunction in the current study to suggest acute cardiotoxicity from higher cumulative doses of AQ4N.

Pharmacokinetic data from this phase 1 trial showed that AQ4N exhibits dose-proportional kinetics over the dose range tested. Plasma concentrations of the bioreduced active metabolites, AQ4M and AQ4, were appreciably lower (<5% for AQ4M) or undetectable (for AQ4). The lack of systemic activation of AQ4N is consistent with its prodrug mode of action. Previous preclinical and clinical studies have confirmed that AQ4N seems to be selectively metabolized into its active form, AQ4, in hypoxic regions of tumors but not in normal healthy tissues (21, 22, 27–29, 33). The majority of administered AQ4N undergoes rapid renal excretion during the first 10 hours following dose administration. Plasma levels achieved at the maximum tolerated dose with this weekly dosing are sufficient for antineoplastic activity reported in preclinical models (28). Although no objective antitumor response to AQ4N was observed in patients in this phase 1 study, three patients (collecting duct renal cell, ovarian, and bronchoalveolar lung cancer) had stable disease.

In conclusion, the maximum tolerated dose of AQ4N administered on days 1, 8, and 15 of a 28-day cycle is 768 mg/m^2. The low toxicity of this weekly dosing schedule in this patient population together with prior demonstration of tumor selectivity and hypoxia targeting of AQ4N, particularly in glioblastoma multiforme (28), provides the basis for rationally designed monotherapy and combination studies. Weekly AQ4N in combination with radiation therapy and temozolomide is currently under evaluation in patients with newly diagnosed glioblastoma multiforme.

Disclosure of Potential Conflicts of Interest

A. Wong, M. Milián, and A. Lalani are former employees of Novacae, Inc.

References

1. Harris AL. Hypoxia-a key regulatory factor in tumour growth. Nat Rev Cancer 2002;2:38–47.
2. Hockel M, Vaupel P. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst 2001;93:266–76.
3. Brown JM, Wilson WR. Exploiting tumour hypoxia in cancer treatment. Nat Rev Cancer 2004;4:437–47.
4. Le QT, Denko NC, Giaccia AJ. Hypoxic gene expression and metastasis. Cancer Metastasis Rev 2004;23:293–310.
5. Subarsky P, Hill RP. The hypoxic tumour microenvironment and metastatic progression. Clin Exp Metastasis 2003;20:237–50.
6. Shannon AM, Bouchier-Hayes DJ, Condron CM, Toomey D. Tumour hypoxia, chemotherapeutic resistance and hypoxia-related therapies. Cancer Treat Rev 2003;29:297–307.
7. Tredan O, Galmarini CM, Patel K, Tannock IF. Drug resistance and the solid tumor microenvironment. J Natl Cancer Inst 2007;99:1441–54.
8. McKeown SR, Friery OP, McIntyre IA, Hejmadi MV, Patterson LH, Hirst DG. Evidence for a therapeutic gain when AQ4N or tirapazamine is combined with radiation. Br J Cancer Suppl 1996;27:S39–42.
9. McKeown SR, Gallagher R, Albertella MR, et al. Evaluation of the bioreductive activity of AQ4N, from lab to clinic. Abstract Book 13th International Congress of Radiation Research 2007;13.
10. Patterson LH, McKeown SR. AQ4N: a new approach to hypoxia-activated cancer chemotherapy. Br J Cancer 2000;83:1589–93.
11. Patterson LH. Bioreductively activated antitumor *N*-oxides: the case of AQ4N, a unique approach to hypoxia-activated cancer chemotherapy. Drug Metab Rev 2002;34:581–92.
12. Patterson LH. Rationale for the use of aliphatic *N*-oxides of cytotoxic anthraquinones as prodrug DNA binding agents: a new class of bioreductive agent. Cancer Metastasis Rev 1993;12:119–34.
13. Patterson LH, Craven MR, Fisher GR, Teesdale-Spittle P. Aliphatic amine *N*-oxides of DNA binding agents as bioreductive drugs. Oncol Res 1994;6:533–8.
14. Smith PJ, Blunt NJ, Desnoyers R, Giles Y, Patterson LH. DNA topoisomerase II-dependent cytotoxicity of alkylaminoanthraquinones and their *N*-oxides. Cancer Chemother Pharmacol 1997;39:455–61.
15. McCarthy HO, Yakkundi A, McErlane V, et al. Bioreductive GDEPT using cytochrome *P*450 3A4 in combination with AQ4N. Cancer Gene Ther 2003;10:40–8.
16. Patterson LH, Murray GI. Tumour cytochrome *P*450 and drug activation. Curr Pharm Des 2002;8:1335–47.
17. Patterson LH, McKeown SR, Robson T, Gallagher R, Raleigh SM, Orr S. Antitumour prodrug development using cytochrome *P*450 (CYP) mediated activation. Anticancer Drug Des 1999;14:473–86.
18. Raleigh SM, Wanogho E, Burke MD, Patterson LH. Rat cytochromes P450 (CYP) specifically contribute to the reductive bioactivation of AQ4N, an alkylaminoanthraquinone-di-*N*-oxide anticancer prodrug. Xenobiotica 1999;29:1115–22.
19. Raleigh SM, Wanogho E, Burke MD, McKeown SR, Patterson LH. Involvement of human cytochromes P450 (CYP) in the reductive metabolism of AQ4N, a hypoxia activated anthraquinone di-*N*-oxide prodrug. Int J Radiat Oncol Biol Phys 1998;42:763–7.
20. Yakkundi A, McErlane V, Murray M, et al. Tumor-selective drug activation: a GDEPT approach utilizing cytochrome *P*450 1A1 and AQ4N. Cancer Gene Ther 2006;13:598–605.
21. Lalani AS, Alters SE, Wong A, Albertella MR, Cleland JL, Henner WD. Selective tumor targeting by the hypoxia-activated prodrug AQ4N blocks tumor growth and metastasis in preclinical models of pancreatic cancer. Clin Cancer Res 2007;13:2216–25.
22. Tredan O, Lalani AS, Garber AS, Tannock IF. The hypoxia-activated prodrug, AQ4N, penetrates deeply in tumor tissues and complements the limited distribution of mitoxantrone. Proc Am Assoc Cancer Res 2007;48:1356.
23. Friery OP, Gallagher R, Murray MM, et al. Enhancement of the anti-tumour effect of cyclophosphamide by the bioreductive drugs AQ4N and tirapazamine. Br J Cancer 2000;82:1469–73.
24. Gallagher R, Hughes CM, Murray MM, et al. The chemopotentiation of cisplatin by the novel bioreductive drug AQ4N. Br J Cancer 2001;85:625–9.
25. McKeown SR, Hejmadi MV, McIntyre IA, McAleer JJ, Patterson LH. AQ4N: an alkylaminoanthraquinone *N*-oxide showing bioreductive potential and positive interaction with radiation *in vivo*. Br J Cancer 1995;72:76–81.
26. Patterson LH, McKeown SR, Ruparelia K, et al. Enhancement of chemotherapy and radiotherapy of murine tumours by AQ4N, a bioreductively activated anti-tumour agent. Br J Cancer 2000;82:1984–90.
27. Albertella MR, Loadman PM, Wiliams KJ, et al. *In vivo* activation of the hypoxia-targeted cytotoxin AQ4N in human tumour xenografts. Proc Am Assoc Cancer Res 2006;47:314.
28. Albertella MR, Loadman PM, Jones PH, et al. Hypoxia-selective targeting by the bioreductive prodrug AQ4N in patients with solid tumors: results of a phase 1 study. Clin Cancer Res 2008;14:1096–104.
29. Steward WP, Middleton M, Benghiat A, et al. The use of pharmacokinetic and pharmacodynamic end points to determine the dose of AQ4N, a novel hypoxic cell cytotoxin, given with fractionated radiotherapy in a phase I study. Ann Oncol 2007;18:1098–103.
30. Simon R, Freidlin B, Rubinstein L, Arbuck SG, Collins J, Christian MC. Accelerated titration designs for phase I clinical trials in oncology. J Natl Cancer Inst 1997;89:1138–47.
31. Swaine DJ, Loadman PM, Bibby MC, Graham MA, Patterson LH. High-performance liquid chromatographic analysis of AQ4N, an alkylaminoanthraquinone *N*-oxide. J Chromatogr B Biomed Sci Appl 2000;742:239–45.
32. Loadman PM, Swaine DJ, Bibby MC, Welham KJ, Patterson LH. A preclinical pharmacokinetic study of the bioreductive drug AQ4N. Drug Metab Dispos 2001;29:422–6.
33. Atkinson SJ, Loadman PM, Sutton C, Patterson LH, Clench MR. Examination of the distribution of the bioreductive drug AQ4N and its active metabolite AQ4 in solid tumours by imaging matrix-assisted laser desorption/ionisation mass spectrometry. Rapid Commun Mass Spectrom 2007;21:1271–6.
34. Lam FC, Hung CT, Perrier DG. Estimation of variance for harmonic mean half-lives. J Pharm Sci 1985;74:229–31.