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Cancer Therapy: Clinical

Clinical Cancer Research

Phase I Trial of Pelvic Radiation, Weekly Cisplatin, and 3-Aminopyridine-2-Carboxaldehyde Thiosemicarbazone (3-AP, NSC #663249) for Locally Advanced Cervical Cancer

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Abstract

Purpose: This study assessed the safety/tolerability, pharmacokinetics, and clinical activity of three times weekly i.v. 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, NSC #663249) in combination with once-weekly i.v. cisplatin and daily pelvic radiation in patients with gynecologic malignancies. 3-AP is a novel small-molecule inhibitor of ribonucleotide reductase (RNR) and is being tested as a potential radiosensitizer and chemosensitizer.

Experimental Design: Patients with stage IB2 to IVB cervical cancer (n = 10) or recurrent uterine sarcoma (n = 1) were assigned to dose-finding cohorts of 2-hour 3-AP infusions during 5 weeks of cisplatin chemoradiation. Pharmacokinetic and methemoglobin samples and tumor biopsy for RNR activity were obtained on day 1 and day 10. Clinical response was assessed.

Results: The maximum tolerated 3-AP dose was 25 mg/m^2 given three times weekly during cisplatin and pelvic radiation. Two patients experienced manageable 3-AP–related grade 3 or 4 electrolyte abnormalities. 3-AP pharmacokinetics showed a 2-hour half-life, with median peak plasma concentrations of 277 ng/mL (25 mg/m^2) and 467 ng/mL (50 mg/m^2). Median methemoglobin levels peaked at 1% (25 mg/m^2) and 6% (50 mg/m^2) at 4 hours after initiating 3-AP infusions. No change in RNR activity was found on day 1 versus day 10 in six early complete responders, whereas elevated RNR activity was seen on day 10 as compared with day 1 in four late complete responders (P = 0.02). Ten (100%) patients with stage IB2 to IVB cervical cancer achieved complete clinical response and remained without disease relapse with a median 18 months of follow-up (6-32 months).

Conclusions: 3-AP was well tolerated at a three times weekly i.v. 25 mg/m^2 dose during cisplatin and pelvic radiation. *Clin Cancer Res; 16(4); 1298–306. ©2010 AACR.*

Single- and double-strand DNA breaks resulting from therapeutic ionizing radiation (IR) and replication fork blocks caused by cisplatin-induced DNA adduct formation must be effectively repaired for cell survival and replication. The rate-limiting step in the *de novo* synthesis of deoxyribonucleotide triphosphate, which is critical for DNA damage repair, is catalyzed by ribonucleotide reductase (RNR). RNR has two constitutively expressed homodimeric active-site subunits (RNR-M1) and two tightly regulated homodimeric small subunits (RNR-R2 or p53R2) that carry diferric irons stabilizing a tyrosyl free radical that is critical for catalytic function (1, 2). RNR activity correlates with tumor proliferation rate and repair of IR-induced DNA damage (3–6). Inhibiting RNR activity is not a new approach, as one of the earliest cervical cancer clinical trials targeted RNR with the chemotherapeutic hydroxyurea. The Gynecologic Oncology Group showed significant improvement in response (68% versus 49%), disease-free survival (13.6 versus 7.6 mo), and median survival (19.5 versus 10.7 mo) with hydroxyurea-radiation versus radiation treatment (7). Leukopenia became a dose-limiting toxicity (DLT) of oral daily hydroxyurea (8–11).

The investigational chemotherapeutic drug 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, Triapine, NSC#663249) is a 1,000-fold more potent irreversible inhibitor of the RNR-R2 and p53R2 subunits of RNR as compared with hydroxyurea (11–13). Preclinical studies have shown that 3-AP suppresses deoxyribonucleotide triphosphate generation required for IR-related DNA damage repair and thereby enhances IR cytotoxicity (14–16). Moreover, cervical cancer cells show a 17-fold increase in RNR-R2 protein and a 4-fold increase in RNR

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Translational Relevance

Worldwide, half a million women are diagnosed annually with cervical cancer, with 90% of new cervical cancer cases related to human papillomavirus–silenced p53. Cancer cell replication depends on ribonucleotide reductase (RNR), the rate-limiting enzyme catalyzing *de novo* deoxyribonucleotide production needed for DNA synthesis. After ionizing radiation, RNR activity increases, facilitating DNA repair and decreasing cancer cell sensitivity to this important cancer treatment. A new intravenous and oral antitumor drug, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP), potently inhibits RNR. In this study, we report the National Cancer Institute-Cancer Therapeutics Evaluation Treatment sponsored phase I safety and translational science clinical trial of radiation and cisplatin plus 3-AP in patients with locally advanced stage IB2 to IVa cervical cancer. In this 10-patient study population at high-risk for relapse and cancer-related death, a 100% complete response rate was observed and no disease progression was documented through 18 months of median follow-up.

activity 18 to 24 hours after IR (5), suggesting that therapeutic RNR targeting may affect cervical cancer treatment. Our *in vitro* data show that cervical cancer cells exposed to IR plus 3-AP experience a G_1 cell cycle block and increased IR cytotoxicty (16). Given these findings, RNR inhibition following radiation is an appealing therapeutic strategy.

In phase I solid cancer studies, single agent 3-AP was well tolerated at doses of 96 to 100 mg/m^2 (17–19). Pharmacokinetic data indicate that 3-AP concentrations peak at 1 to 10 μmol/L at 1 to 2 hours after a 2-hour i.v. infusion. In another phase I solid cancer study, 2-hour 96 mg/m^2 i.v. 3-AP (days 1-5) plus 25 or 37.5 mg/m^2 i.v. cisplatin (day 2 or day 3) given every other week or every 3 weeks showed a pooled 33% clinical response rate (20). Thus, 3-AP also seems to modify cisplatin-mediated cytotoxicity.

This study was designed to (*a*) assess the safety/tolerability of a three times weekly 2-hour i.v. 3-AP infusion during daily pelvic radiation and weekly i.v. cisplatin chemotherapy; (*b*) assess the 2-hour 3-AP infusion pharmacokinetics during radiation and cisplatin treatment; (*c*) evaluate methemoglobin levels and cancer tissue RNR activity as markers of 3-AP pharmacologic inhibition; and (*d*) assess the clinical activity of radiation and cisplatin plus 3-AP chemotherapy.

Patients and Methods

Eligibility criteria. Enrolled patients were females of ages ≥18 y with histologically confirmed primary or recurrent gynecologic malignancies not amenable to curative surgery. Patients had a Karnofsky performance status of ≥50%; life expectancy of ≥12 wk; hemoglobin concentration ≥10 g/dL, absolute granulocyte count ≥1,500/μL, platelet count ≥100,000/μL, total bilirubin ≤2.0 mg/dL, aspartate aminotransferase/alanine aminotransferase ≤2.5× and prothrombin time/activated partial thromboplastin time ≤1.5 × institutional normal limits, and plasma creatinine ≤2.0 mg/dL. Previously treated patients were off therapy for 4 wk. Patients with symptomatic cardiac and/or pulmonary disease were excluded.

The study protocol was approved by the National Cancer Institute (NCI) Cancer Therapeutics Evaluation Treatment (CTEP) committee and the institutional review board of University Hospitals of Cleveland Case Medical Center and monitored by the Case Comprehensive Cancer Center Data Safety and Monitoring Board. All patients provided a signed informed consent.

Safety assessments. Patients underwent examination and hematologic, hepatic, and renal blood testing and baseline computed tomography (CT) or 2-[^{18}F]fluoro-2-deoxy-D-glucose positron emission tomography scans ([^{18}F]FDG PET/CT) within 28 d before the first 3-AP infusion. Physical examinations, adverse event assessments (NCI Common Toxicity Criteria version 3.0), and bloodwork were repeated weekly. Post-study examinations and adverse event assessments were required at 1 and 3 mo after completing all radiation. Patients were followed every 3 mo thereafter. Optional 3-mo post-study [^{18}F]FDG PET/CT studies were recommended.

Protocol treatments. This was a dose-finding phase I study of three times weekly i.v. 3-AP (Triapine) in combination with once-weekly i.v. cisplatin chemotherapy and daily pelvic radiation therapy administered for 5 weekly cycles. 3-AP was supplied by Vion Pharmaceuticals to NCI-CTEP in 50-mg viscous liquid vials and, for 2-h i.v. infusion, was diluted in 0.9% sodium chloride to a final concentration of 0.01 to 2 mg/mL. A Fibonacci 3+3 cohort trial design was implemented for 2-h i.v. 3-AP dose escalation levels of 25, 50, 75, and 100 mg/m^2. A single observed DLT event led to an additional three patients treated at the dose level where the DLT event occurred. Dose-finding escalation continued if no additional DLTs were observed. Two observed DLTs stopped dose escalation, with the prior dose level declared the maximum tolerated dose as long as six patients had been treated with ≤1 instance of DLT.

Cisplatin (40 mg/m^2) was given i.v. before radiation therapy once every week for 5 wk with an optional week 6 dosing. Cisplatin and 3-AP were not given on the same day.

Pelvic radiation consisted of parallel-opposed anteroposterior-posterioranterior and lateral pelvic external-beam treatment fields, delivering 25 fractions of 1.8-Gy daily fractions for a total dose of 45.0 Gy using 6- to 18-MV photons (Table 1). An optional parametrial boost (n = 10) of 5 fractions of 1.8-Gy daily fractions for a total dose of 9.0 Gy was administered using parallel-opposed anteroposterior-posterioranterior fields. Brachytherapy in patients with cervical cancer (n = 10) followed pelvic radiation such that total radiation treatment time was less

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Table 1. Patient characteristics by 3-AP dose cohort

Characteristic	No. of patients	
	25 mg/m^2	50 mg/m^2
Age, y		
Median	60	62
Range	34-68	54-69
Race		
White	5	4
African American	1	1
Disease site		
Cervix		
Stage IB2	1	0
Stage IIA	1	2
Stage IIB	0	1
Stage IIIB	2	1
Stage IVA	1	0
Stage IVB	1	0
Uterus		
Stage IV	0	1

than 56 d. Intracavitary (n = 9) or interstitial (n = 1) low-dose rate brachytherapy was allowed. Brachytherapy treatment increased total point A dose to a median 80.0 Gy (median 43 h; median 62 cGy/h). No cisplatin or 3-AP infusions were given during brachytherapy.

3-AP plasma pharmacokinetic and serum methemoglobin measurements. Heparinized intravenous blood samples determined 3-AP concentrations on day 1 and day 10 before and at 2, 4, 6, and 24 h after start of 2-h infusion. Plasma was centrifuged at 3,000 rpm (15 min) in a refrigerated centrifuge and then stored (−80°C). 3-AP concentrations were measured by liquid chromatography tandem mass spectrometry, as previously described (21). The lower limit of quantification was 20 ng/mL. Median peak plasma concentrations (C_{max}) and terminal elimination constants for drug half-life were calculated by noncompartmental methods (SPSS 12.0).

Heparinized intravenous blood samples drawn into arterial blood gas syringes determined serum methemoglobin concentrations on day 1 and day 10 before and at 2, 4, 6, and 24 h after start of 2-h infusion. Methemoglobin levels were reported as a percentage of total hemoglobin observed by direct spectrophotometry (22, 23).

Sequential tumor biopsies, immunohistochemistry, and RNR assay. Tumor biopsies were obtained before radiation plus 3-AP (day 1) and again on day 10 by transvaginal punch biopsy (~500 mg, 0.5 cm^3), then snap-frozen (<30 min) and stored (−80°C). For immunohistochemistry, the distal 0.5-mm biopsy ends were sectioned and stained with H&E to confirm presence of tumor (24). Modified immunohistochemistry assays were done using RNR-R2 mouse monoclonal (0.5 mg/mL, 1:100; Abcam, Inc.) and RNR-p53R2 rabbit polyclonal (0.2 mg/mL, 1:250; Novus Biologicals) antibodies (25). Adapting previous methods and blinded to treatment and response (26), two pathologists scored immunohistochemistry specimens for RNR-R2 and p53R2 protein positivity: negative 0 (<5%), positive 1+ (5% to <25%), positive 2+ (25% to <75%), and positive 3+ (≥75%).

Tumor and stromal intracellular dCTP pools were quantified for RNR activity using a DNA polymerase extension assay (16). Tumor biopsies were thawed and homogenized by glass microbead pulverization, and intracellular deoxyribonucleotide triphosphates were extracted by ice-cold 60% methanol. The DNA polymerase extension assay template was 5′-AAAGAAAGAAAGAAAGAAAGGGCGGTGGAGGCGG-3′ and the primer was 5′-CCGCCTCCACCGCC-3′ (Integrated DNA Technologies). A liquid scintillation counter quantified radioactivity, with incorporated [^{3}H]dTTP radioactivity linearly proportional to dCTP (nmol/L/mg).

Evaluation of clinical activity and statistical methods. The study design reflected the desire to detect differential tumor response for translational biology end points (immunohistochemistry and RNR activity, day 1 and day 10) before any planned brachytherapy (i.e., after 5 wk of pelvic radiation and cisplatin plus 3-AP chemotherapy), even though complete study treatment concluded after brachytherapy. Thus, differences in tumor response could be compared among treated patients who did or did not receive brachytherapy. Early complete responders were defined as having disappearance of all active cancer after 5 wk of protocol therapy and before any brachytherapy. Late complete responders were defined as having disappearance of all active cancer after all protocol therapy and at the 1-mo follow-up assessment. Tumor response was reassessed following all protocol therapy at 1 mo by physical examination and at 3 mo by physical examination and repeat CT or [^{18}F]FDG PET/CT imaging. Patients were followed every 3 mo. T tests, ANOVA, and Wilcoxon rank sum statistics (α = 0.05) were computed (SPSS 12.0).

Results

Patient characteristics. Eleven patients were enrolled between May 2006 and August 2008 (Table 1); 10 had cervical cancer. Patients were assigned to dose-finding cohorts of 25 mg/m^2 AP and 50 mg/m^2 3-AP during radiation and cisplatin therapy (Fig. 1). Further dose escalation was not done because of DLTs. Patients included primarily women with new diagnoses, as only a single patient diagnosed with uterine stromal sarcoma had received previous three cycles of gemcitabine-docetaxel chemotherapy. No patients had received prior pelvic radiation.

Safety and tolerability. Eleven patients received 145 i.v. doses of 3-AP (median 15 doses). The 2-hour i.v. 3-AP infusion was well tolerated at the 25 mg/m^2 and 50 mg/m^2 doses, with no immediate infusion-related sequelae reported. One patient received nine of fifteen 50 mg/m^2 3-AP infusions; 3-AP was stopped for non-DLT

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leukocytopenia resulting in two delays of cisplatin administration. One patient, who had 9-cm abdominopelvic relapse of her uterine stromal sarcoma, received a single 50 mg/m^2 3-AP infusion and four pelvic radiation doses for abdominopelvic disease before symptomatic metastatic pulmonary disease progression.

Eighteen 3-AP drug-related adverse events occurred in 4 of 11 (36%) patients (Table 2). Most 3-AP drug-related adverse events (14 of 18) were mild to moderate in intensity (i.e., grade ≤3, resolving to grade 0-2 within 2 days). The four DLTs occurred in two patients. One patient at the 50 mg/m^2 3-AP dose level had grade 3 anorexia requiring hospitalization; her anorexia resolved within 4 days. The other patient enrolled at the 50 mg/m^2 3-AP dose level had grade 3 nausea and dehydration requiring hospitalization for i.v. hydration, with a grade 3 increase in blood urea nitrogen, a grade 4 lowering of serum bicarbonate, and a grade 4 increase in serum creatinine observed and attributed to cisplatin administration. Grade 4 serum bicarbonate corrected to grade 2 after 2 days and grade 3 blood urea nitrogen and grade 4 creatinine corrected to grade 2 after 8 days of i.v. hydration in this one diabetic patient.

No significant 3-AP drug-related symptomatic dyspnea or methemoglobinemia was reported in the 10 cervical cancer patients. The one uterine sarcoma patient who had pulmonary metastases and prior chemotherapy experienced grade 3 hypoxia with peak methemoglobin level of 11% requiring continuous oxygen supplementation 4 hours after her first 50 mg/m^2 3-AP 2-hour infusion. After 24-hour continuous oxygen supplementation, her room-air oxygen saturation normalized and methemoglobin levels lowered to 1%.

Clinical activity. Ten of 11 (91%) enrolled patients were assessed for tumor response, each with squamous cervical cancer. All 10 (100%) patients achieved a complete clinical response at post-treatment 1-month follow-up. Of the 10 complete responders, 6 (60%) had an early complete clinical response (i.e., no disease detected after 5 weeks of radiation and cisplatin plus 3-AP chemotherapy). These six patients had a median tumor size of 7.5 cm (range 4-8 cm). The four late complete responders had a 7-cm (range 6-8 cm) median tumor size, which decreased to a 1-cm (range 1-1.5 cm) median after 5 weeks of radiation and cisplatin plus 3-AP chemotherapy. One late complete responder had a solitary pulmonary lesion at clinical presentation, achieved complete response of her pelvic cervical cancer following all protocol therapy, and had biopsy-confirmed nonviable pulmonary metastatic disease 2 months after completing cisplatin plus 3-AP chemotherapy. With a median follow-up of 18 months (range 6-32 months), the 10 evaluable patients had no disease progression. Five of these 10 patients had [^{18}F]FDG PET/CT metabolically avid pelvic or lower (L4-L5) para-aortic lymphadenopathy before treatment; none had disease relapse as assessed by repeat CT or [^{18}F]FDG PET/CT imaging.

Tumor tissue RNR protein levels and activity. One objective of the study was to identify, using immunohistochemistry and biochemical assays, biomarkers of the targeted enzyme RNR from day 1 and day 10 tumor biopsies that might distinguish responders from nonresponders. Figure 2 shows representative immunohistochemistry tumor biopsies from day 1 (pretreatment) and day 10 in early and late complete response cervical cancer patients. p53R2 and RMR-R2 protein expression levels by immunohistochemistry on day 1 and day 10 increased in six early complete responders (Fig. 2A). Four late complete responders exhibited unchanged, elevated day 1 and day 10 p53R2 and RNR-R2 protein levels (Fig. 2B).

Figure 3 shows RNR activity using a biochemical assay from cervical cancer biopsies expressed as a ratio of day 10 to day 1 and correlated with treatment response. Our recent *in vitro* data suggest that radiation treatment significantly increases RNR activity, whereas radiation plus 3-AP treatment significantly reduces RNR activity (16). Among six early complete responders, radiation and cisplatin plus 3-AP chemotherapy resulted in no substantial change in RNR activity ratio for day 1 versus day 10, ranging between 0.37 and 1.30 (Fig. 3). Among four late complete responders, day 10 RNR activity levels were substantially higher than day 1, with ratios ranging between 2.15 and 9.55 (P =

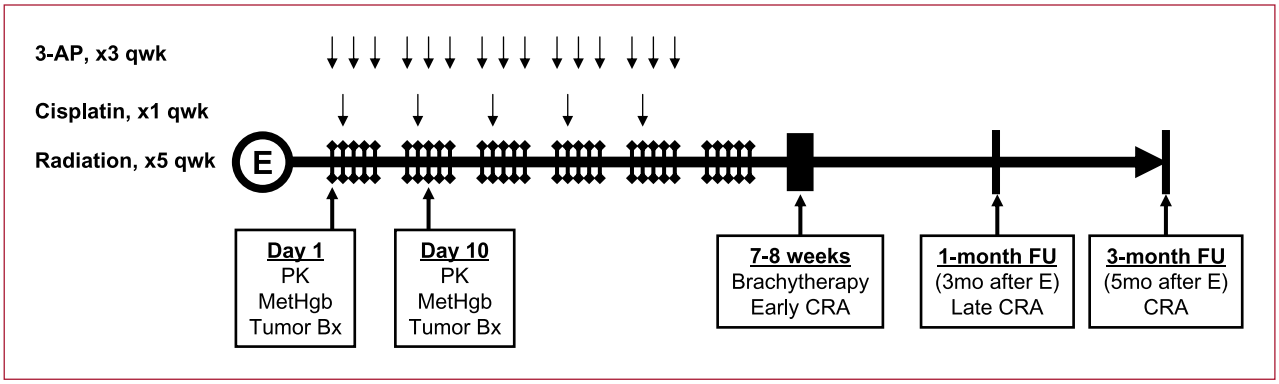

Fig. 1. Phase I clinical trial schema. E, enrollment; qwk, per week; PK, pharmacokinetic sampling; MetHgb, methemoglobin sampling; Tumor Bx, transvaginal tumor biopsy; FU, after all protocol therapy completion follow-up; CRA, clinical response assessment.

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Table 2. Number of patients with drug-related adverse events that occurred in ≥5% of patients receiving 3-AP

Adverse event	25 mg/m^2 (n = 6)	50 mg/m^2 (n = 5)	n = 11			
			Total	Grade 3	Grade 3*	Grade 4*
Cardiovascular						
Transient flushing	0	1	1	1	0	0
Electrocardiogram QTc ≥0.48 s	0	1	1	1	0	0
Pulmonary						
Hypoxia	0	1	1	1	0	0
Gastrointestinal						
Anorexia	0	1	1	1	1	0
Nausea	0	2	2	2	0	0
Dehydration	0	1	1	1	0	0
Constipation	1	0	1	1	0	0
Abdominal cramping	1	1	2	1	0	0
Metabolic/laboratory						
Bicarbonate (<11-8 mmol/L)	0	1	1	0	0	1
Blood urea nitrogen (severe elevation)	0	1	1	1	1	0
Creatinine (>3.0-6.0 × upper limit of normal)	0	2	2	0	0	1
Neurologic						
Confusion	0	2	2	1	0	0
Sensory neuropathy	0	1	1	1	0	0
Dermatologic						
Skin decubitus ulcer	0	1	1	0	0	0

NOTE: Adverse events were summarized by the dose to which the patient was initially assigned. Grade 3 adverse events were evaluated using the NCI Common Terminology Criteria for Adverse Events version 3.0.
*Grade 3 adverse event not resolving to grade 0 to 2 over 2 d is considered DLT. Grade 4 toxicity is considered DLT.

0.02; Fig. 3). On average, the four late complete responders showed a 2.5-fold elevation in dCTP pool levels on day 10. The patient that had the highest RNR activity day 10/day 1 ratio (i.e., 9.55) had a clinical 8-cm tumor shrink to 2 cm after 5 weeks of radiation and cisplatin plus 3-AP chemotherapy; the patient then achieved a late complete response after all protocol therapy including brachytherapy.

Pharmacokinetics and methemoglobin levels. Median (i.e., both day 1 and day 10) 3-AP plasma concentration profiles are shown in Fig. 4 (bottom). Plasma concentrations reached C_{max} at the completion of a 2-hour 3-AP infusion, with median peak plasma concentrations of 277 ng/mL (25 mg/m^2) and 467 ng/mL (50 mg/m^2) and a half-life of 2 hours with no change in day 1 and day 10 levels (25 mg/m^2, P = 0.18; 50 mg/m^2, P = 0.35). 3-AP plasma concentrations, on average, decayed to 2% (25 mg/m^2) and 13% (50 mg/m^2) of observed C_{max} at 6 hours after start of 2-hour infusion.

Median (i.e., both day 1 and day 10) methemoglobin percentages are shown in Fig. 4 (top). 3-AP is an iron chelator that is able to inhibit iron-dependent cytochrome b_5 and methemoglobin reductases in RBC (12). Pharmacologic inhibition of RBC reductases leads to an accumulation of ferric (III) methemoglobin through naturally occurring, spontaneous oxidation of RBC ferrous (II) hemoglobin (27). Methemoglobin is incapable of binding oxygen and manifests biologically as tissue hypoxia and clinically as dyspnea. In a prior phase I clinical trial, every-4-week infusion of 3-AP (105 mg/m^2, day 1) plus gemcitabine (600-1,000 mg/m^2, days 1, 8, and 15) resulted in dose-limiting, symptomatic methemoglobinemia (>10% MHgb) in 3 of 29 (10%) patients (28). In this study, median peak methemoglobin was 1% (range 0-2%) for the 25 mg/m^2 and 6% (range 1-11%) for the 50 mg/m^2 dosing 4 hours after start of 2-hour 3-AP infusion (P < 0.001), with no difference between day 1 and day 10 (P = 1.00).

Discussion

3-AP was well tolerated at a three times weekly i.v. dosing of 25 mg/m^2 during daily pelvic radiation and weekly cisplatin treatment. Complete clinical responses were observed in all six patients with advanced stage cervical cancer receiving 25 mg/m^2 3-AP infusions. With 25 mg/m^2 3-AP doses, toxicities were minor. Dose-limiting grade 3 gastrointestinal and grade 4 electrolyte changes were restricted to 50 mg/m^2 3-AP infusions.

Ten patients had advanced stage IB2 to IVB cervical cancer, with a median 7.5-cm tumor size often leading to

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parametrial tissue or pelvic wall muscle invasion and nephropathy. Early complete clinical responses were achieved in 6 of 10 (60%) patients after 5 weeks of daily pelvic radiation and cisplatin plus 3-AP chemotherapy and before intracavitary brachytherapy. At 1 and 3 months after completing all protocol therapy including brachytherapy, all 10 (100%) cervical cancer patients achieved complete tumor response. With a median 18 months of follow-up (range 6-32 months), there has been no documented local or distant disease relapse. Historically, pelvic radiation plus cisplatin chemotherapy followed by brachytherapy achieves a 70% complete clinical response and a 73% 18-month progression-free survival in advanced stage cervical cancer patients (29–31).

Here, 3-AP pharmacokinetics did not display a time-dependent increase in the 24-hour pharmacokinetic evaluations done on both day 1 and day 10 and no corresponding symptomatic increase in serum methemoglobin. As such, 3-AP treatment was scheduled three times weekly to provide repeated drug-induced RNR inhibition and, thereby, prolonged inhibition of on-demand deoxyribonucleotide synthesis during radiation. We observed 3-AP drug concentrations sufficient to achieve tumor responses up to 4 hours post-dose (Fig. 4), and as such, frequent 3-AP dosing seems reasonable for 3-AP–mediated radiosensitization. Using experimental cervical cancer cell models, we found that 3-AP treatment significantly enhanced IR-related cytotoxicity through a significant 3-AP–induced reduction in RNR activity, sustained IR-induced DNA damage, and a long G_1-phase cell cycle arrest perhaps indicating a p53-independent radiosensitizing mechanism (16).

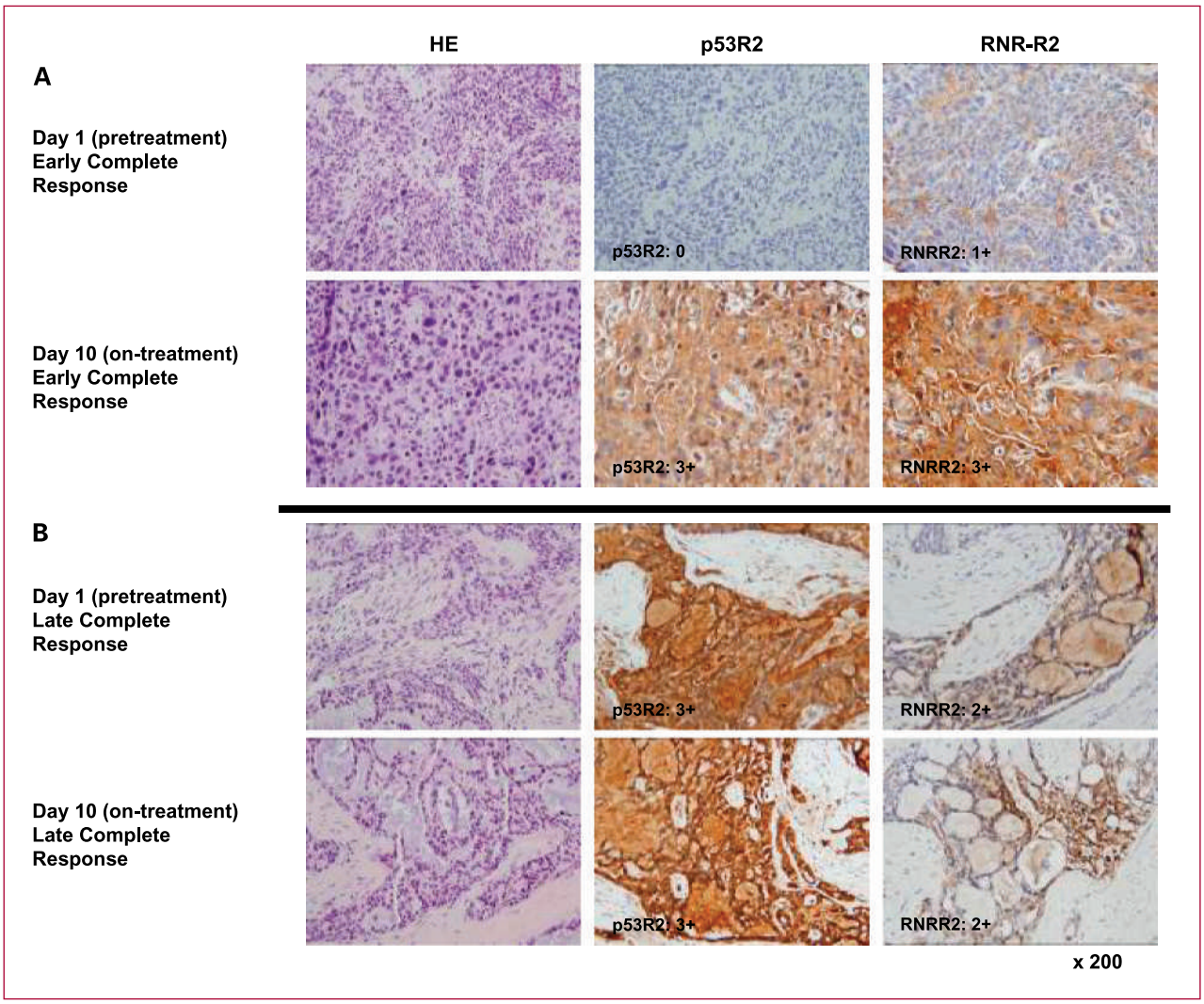

Fig. 2. Representative cervical cancer histopathology and RNR-R2 and p53R2 protein levels on day 1 and day 10 are depicted in an early complete response patient (A) and a late complete response cervical cancer patient (B) after radiation and cisplatin (40 mg/m^2) plus 3-AP (25 mg/m^2). Histopathology (H&E) shows high-grade cervical cancer with evident radiation-drug treatment effect comparing biopsy specimens pretreatment (day 1) to on-treatment (day 10) sections. Immunohistochemical staining shows increase in pretreatment day 1 to day 10 RNR-R2 and p53R2 protein levels in an early complete response patient (A) but unchanged elevated RNR-R2 and p53R2 protein levels in a late complete response patient (B).

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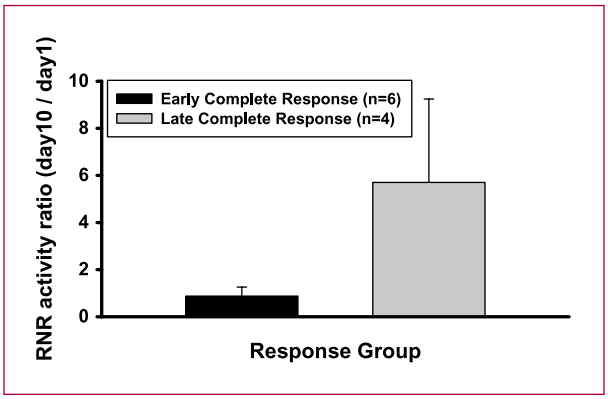

Fig. 3. RNR activity in early and late complete clinical responders after treatment with radiation and cisplatin (40 mg/m^2) plus 3-AP (25 or 50 mg/m^2) is illustrated. Mean RNR activity [dCTP (nmol/L/mg)], expressed as a ratio of day 10 to day 1 levels, is higher in late complete response patients as compared with early complete response patients (P = 0.02).

In this clinical trial, we measured the target enzyme RNR in day 1 and day 10 tumor biopsies using two different assays: an immunohistochemistry assay of RNR-R2 and RNR p53R2 protein expression levels and a biochemical assay of total RNR activity. Based on this small patient series, an early complete response (six patients) was associated with a moderate change in the immunohistochemistry assay (Fig. 2) and no change in the biochemical assay (Fig. 3) comparing day 1 to day 10 cancer biopsies. In early complete responders in whom low pretreatment RNR-R2 and RNR-p53R2 protein levels were observed, it can be argued that 3-AP treatment effectively reduced a predictably higher RNR activity level in these cervical cancers, based on our prior *in vitro* data, resulting in no change in the RNR biochemical assay (5, 6, 16). In late complete responders in whom we found no change in the elevated RNR-R2 and RNR-p53R2 protein levels by immunohistochemistry, comparing pretherapy (day 1) to on-therapy (day 10) tumor biopsy sections (Fig. 2) and actually higher RNR activity levels (Fig. 3), one could argue that the overall effect of 3-AP treatment was less than optimal. However, these late complete responders have experienced durable responses with a median follow-up of 18 months similar to early complete responders, suggesting that additional 3-AP mechanisms of radiosensitization and chemosensitization are operative. As discussed in detail in our recent publication, multiple mechanisms of radiosensitization by 3-AP may contribute to the overall tumor cytotoxicity, including an enhanced G_1-S cell cycle delay and reduced radiation-mediated DNA damage repair. Enhanced tumor cytotoxicity in these patients could also result from IR-cisplatin interactions independent of 3-AP treatment.

Durable clinical activity was observed with a median 18-month follow-up after administering i.v. 25 mg/m^2 3-AP doses given three times weekly during pelvic radiation and cisplatin chemotherapy in advanced stage cervical cancer patients. The favorable adverse event profile of this combination makes this regimen an exciting new cervical cancer treatment for women. A confirmatory phase II study of daily pelvic radiation and once-weekly cisplatin

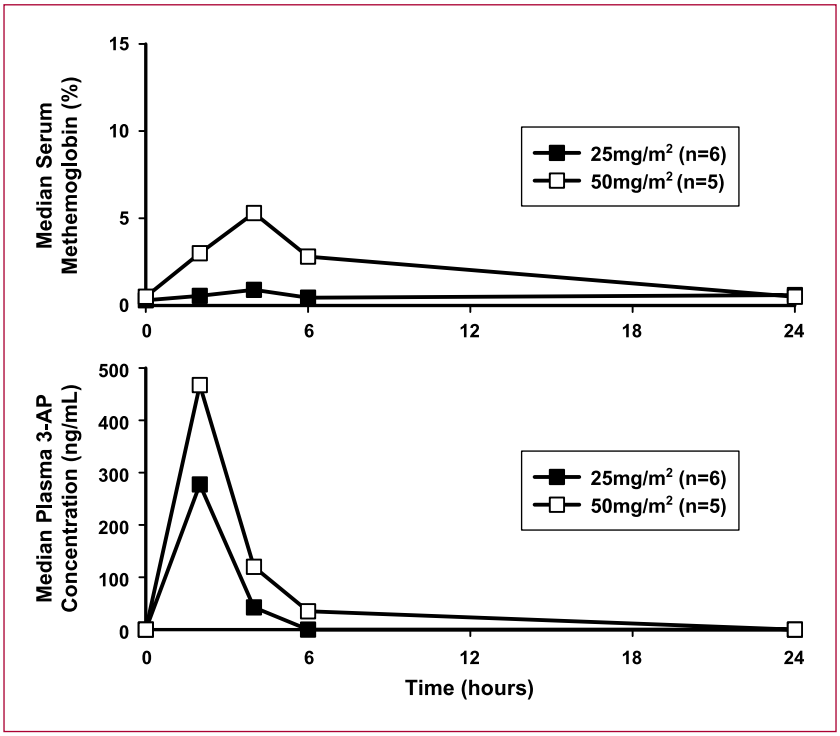

Fig. 4. Median steady-state plasma concentration profiles for each 3-AP dose group (*bottom*) and the corresponding median serum methemoglobin proportion (*top*).

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(40 mg/m^2) plus three times weekly 3-AP (25 mg/m^2) is under way.

Disclosure of Potential Conflicts of Interest

There are no potential conflicts of interest among the authors of this article. This article has been seen, read, and agreed on in its content by all designated authors. This article has not been submitted or published elsewhere.

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