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 intravenous 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, NSC #663249) in combination with once weekly intravenous 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-IVB cervical cancer (n=10) or recurrent uterine sarcoma (n=1) were assigned to dose-finding cohorts of 2-hour 3-AP infusions during five weeks of cisplatin chemoradiation. Pharmacokinetic and methemoglobin samples and tumor biopsy for RNR activity were obtained on days 1 and 10. Clinical response was assessed.

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Results—The maximum tolerated 3-AP dose is $25mg/m^2$ 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 277ng/mL ($25mg/m^2$) and 467ng/mL ($50mg/m^2$). Median methemoglobin levels peaked at 1% ($25mg/m^2$) and 6% ($50mg/m^2$) at 4 hours after initiating 3-AP infusions. No change in RNR activity was found on day 1 versus 10 in six early complete responders, while elevated RNR activity was seen on day 10 as compared to day 1 in four late complete responders ($P =0.02$). Ten (100%) patients with stage IB2-IVB cervical cancer achieved complete clinical response and remain 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 intravenous $25mg/m^2$ dose during cisplatin and pelvic radiation.

Keywords

Introduction

Single- and double-strand DNA breaks resulting from therapeutic ionizing radiation (IR) and replication fork blocks resulting from cisplatin-induced DNA adduct formation must be effectively repaired for cell survival and replication. The rate-limiting step in *de novo* deoxyribonucleotide triphosphates (dNTPs) synthesis 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) which carry diferric irons stabilizing a tyrosyl free radical 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% *v.* 49%), disease-free (13.6mo *v.* 7.6mo) and median survival (19.5mo *v.* 10.7mo) with hydroxyurea-radiation versus radiation treatment.(7) Leukopenia became a dose-limiting toxicity of oral daily hydroxyurea.(8-11)

The investigational chemotherapeutic drug 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, Triapine®, NSC#663249) is a 1000-fold more potent irreversible inhibitor of the RNR-R2 and p53R2 subunits of RNR as compared to hydroxyurea.(11-13) Preclinical studies have shown that 3-AP suppresses dNTP generation required for IR-related DNA damage repair, and thereby, enhances IR cytotoxicity.(14-16) Moreover, cervical cancer cells demonstrate a 17-fold increase in RNR-R2 protein and 4-fold rise in RNR activity 18 to 24 hours after IR,(5) suggesting that therapeutic RNR targeting may impact cervical cancer treatment. Our *in vitro* data shows cervical cancer cells exposed to IR plus 3-AP experience a G1 cell cycle block and increased IR cytotoxicty.(16) Given these findings, RNR inhibition following radiation is an appealing therapeutic strategy.

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

This study was designed to (1) assess safety/tolerability of three-times weekly 2-hour intravenous 3-AP infusion during daily pelvic radiation and weekly intravenous cisplatin

chemotherapy; (2) assess 2-hour 3-AP infusion pharmacokinetics during radiation and cisplatin treatment; (3) evaluate methemoglobin levels and cancer tissue RNR activity as markers of 3-AP pharmacological inhibition; and (4) assess the clinical activity of radiation and cisplatin plus 3-AP chemotherapy.

Patients and Methods

Eligibility Criteria

Enrolled patients were females age ≥18 years 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 weeks; hemoglobin concentration ≥10g/dL, absolute granulocyte count ≥1,500/μL, platelet count ≥100,000/μL, total bilirubin ≤2.0mg/dL, AST(SGOT)/ALT(SGPT) ≤2.5X and PT/aPTT ≤1.5X institutional normal limits, and plasma creatinine ≤2.0mg/dL. Previously treated patients were off therapy for four weeks. 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 days 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-months after completing all radiation. Patients were followed every 3-months thereafter. Optional 3-month post-study ^{18}F-FDG PET/CT studies were recommended.

Protocol Treatments

This was a dose-finding phase 1 study of three-times weekly intravenous 3-AP (Triapine®) in combination with once weekly intravenous cisplatin chemotherapy and daily pelvic radiation therapy administered for five weekly cycles. 3-AP was supplied by Vion Pharmaceuticals (New Haven, CT) to NCI-CTEP (Bethesda, MD) in 50 mg viscous liquid vials, and for 2-hour intravenous infusion was diluted in 0.9% sodium chloride to a final concentration of 0.01 to 2mg/mL. A Fibonacci 3+3 cohort trial design was implemented for 2-hour intravenous 3-AP dose escalation levels of 25, 50, 75, and 100mg/m^2. A single observed dose limiting toxicity (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 (MTD) as long as 6 patients had been treated with ≤1 instance of DLT.

Cisplatin (40mg/m^2) was given intravenously prior to radiation therapy on a once every week for five weeks with an optional week six dosing. Cisplatin and 3-AP were not given on the same day.

Pelvic radiation consisted of parallel-opposed anteroposterior-posterioranterior (AP/PA) and lateral pelvic external-beam treatment fields, delivering 25 fractions of 1.8Gy daily fractions for a total dose of 45.0Gy using 6-MV to 18-MV photons (Table 1). An optional parametrial boost (n=10) of 5 fractions of 1.8Gy daily fractions for a total dose of 9.0Gy was administered

using parallel-opposed AP/PA fields. Brachytherapy in patients with cervical cancer (n=10) followed pelvic radiation such that total radiation treatment time was less than 56 days. Intracavitary (n=9) or interstitial (n=1) low-dose rate brachytherapy was allowed. Brachytherapy treatment increased total point A dose to a median 80.0Gy (median 43 hours; median 62cGy/hour). No cisplatin nor 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 hours after start of 2-hour infusion. Plasma was centrifuged at 3,000rpm (15min) 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 20ng/mL. Median peak plasma concentrations (C_{max}) and terminal elimination constants for drug half-life were calculated by noncompartmental methods (SPSS 12.0, Chicago, IL).

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

Sequential Tumor Biopsies, Immunohistochemistry, and Ribonucleotide Reductase Assay

Tumor biopsies were obtained before radiation plus 3-AP (day 1) and again on day 10 by transvaginal punch biopsy (~500mg, 0.5cm^3), then snap-frozen (<30min) and stored (−80°C). For immunohistochemistry (IHC), the distal 0.5mm biopsy ends were sectioned and stained with hematoxylin and eosin to confirm presence of tumor.(24) Modified IHC assays were performed using RNR-R2 mouse monoclonal (0.5mg/mL, 1:100, Abcam, Inc. [Cambridge, MA]) and RNR-p53R2 rabbit polyclonal (0.2mg/mL, 1:250; Novus Biologicals [Littleton, CO]) antibodies.(25) Adapting previous methods and blinded to treatment and response,(26) two pathologists scored IHC 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 deoxycytidine (dCTP) pools were quantified for RNR activity using a DNA-polymerase extension assay.(16) Tumor biopsies were thawed, homogenized by glass micro-bead pulverization, and intracellular dNTPs 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, Coralville, IA). A liquid scintillation counter quantified radioactivity, with incorporated H^3-dTTP radioactivity linearly proportional to dCTP (nM/mg).

Evaluation of Clinical Activity and Statistical Methods

The study design reflected the desire to detect differential tumor response for translational biology endpoints (IHC and RNR activity, day 1 and 10) before any planned brachytherapy (i.e., after 5 weeks 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 whom did or did not receive brachytherapy. Early complete responders were defined as having disappearance of all active cancer after 5 weeks 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-month follow-up assessment. Tumor response was reassessed following all protocol therapy at 1-month by physical examination and at 3-months by physical examination and repeat CT or ^{18}F-FDG PET/CT imaging. Patients were followed every three months. T-tests, analysis of variance

(ANOVA), and Wilcoxon rank sum statistics ($\alpha = 0.05$) were computed (SPSS 12.0, Chicago, IL).

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 $25mg/m^2$ and $50mg/m^2$ 3-AP during radiation and cisplatin therapy (Fig. 1). Further dose escalation was not performed because of dose-limiting toxicities. Patients included primarily women with new diagnoses, as only a single patient diagnosed with uterine stromal sarcoma had received previous 3 cycles of gemcitabine-docetaxel chemotherapy. No patients had received prior pelvic radiation.

Safety and Tolerability

Eleven patients received 145 intravenous doses of 3-AP (median 15 doses). The 2-hour intravenous 3-AP infusion was well tolerated at the $25mg/m^2$ and $50mg/m^2$ doses, with no immediate infusion-related sequelae reported. One patient received nine of 15 $50mg/m^2$ 3-AP infusions; 3-AP was stopped for non-DLT leukocytopenia resulting in two delays of cisplatin administration. One patient, who had 9cm abdominopelvic relapse of her uterine stromal sarcoma, received a single $50mg/m^2$ 3-AP infusion and four pelvic radiation doses for abdominopelvic disease prior to symptomatic metastatic pulmonary disease progression.

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

No significant 3-AP drug-related symptomatic dyspnea or methemoglobinemia were 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 four hours after her first $50mg/m^2$ 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 (91%) of 11 enrolled patients were assessed for tumor response, each with squamous cervical cancer. All 10 (100%) patients achieved a complete clinical response at post-treatment one-month follow-up. Of the 10 complete responders, six (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 7.5cm (range 4-8cm). The four late complete responders had a 7cm (range 6-8cm) median tumor size, which decreased to a 1cm (range 1-1.5cm) 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 non-viable pulmonary metastatic disease two months after completing cisplatin plus

3-AP chemotherapy. With a median follow-up of 18 months (range 6-32 months), the 10 evaluable patients have 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 have had disease relapse as assessed by repeat CT or ^{18}F-FDG PET/CT imaging.

Tumor tissue ribonucleotide reductase protein levels and activity

One study objective was to identify biomarkers of the targeted enzyme RNR from day 1 and day 10 tumor biopsies using IHC and biochemical assays that might distinguish responders from nonresponders. Figure 2 shows representative IHC 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 IHC 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 suggests that radiation treatment significantly increases RNR activity, while 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 RNR activity ratio change day 1 to 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 ($P = 0.02$; Fig. 3). On average, the four late complete responders demonstrated a 2.5-fold elevation in dCTP pool levels on day 10. The patient that had the highest RNR activity day10:day1 ratio (i.e., 9.55) had a clinical 8cm tumor shrink to 2cm after five 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 Figure 4 (lower panel). Plasma concentrations reached C_{max} at the completion of a 2-hour 3-AP infusion, with median peak plasma concentrations of 277ng/mL (25mg/m^2) and 467ng/mL (50mg/m^2) and a half-life of 2 hours with no change in day 1 and 10 levels (25mg/m^2, $P = 0.18$; 50mg/m^2, $P = 0.35$). 3-AP plasma concentrations on average decayed to 2% (25mg/m^2) and 13% (50mg/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 Figure 4 (upper panel). 3-AP is an iron chelator, able to inhibit iron-dependent cytochrome b_5 and methemoglobin reductases in red blood cells.(12) Pharmacologic inhibition of red blood cell reductases leads to an accumulation of ferric (III) methemoglobin through naturally-occurring, spontaneous oxidation of red blood cell ferrous (II) hemoglobin.(27) Methemoglobin is incapable of binding oxygen and manifests biologically as tissue hypoxia and clinically as dyspnea. In a prior phase 1 clinical trial, every 4-week infusion of 3-AP (105mg/m^2, day 1) plus gemcitabine (600-1000mg/m^2, day 1, 8, 15) resulted in dose-limiting, symptomatic methemoglobinemia (>10% MHgb) in 3 (10%) of 29 patients.(28) In this study, median peak methemoglobin was 1% (range 0-2%) for the 25mg/m^2 and 6% (range 1-11%) for the 50mg/ m^2 dosing four hours after start of 2-hour 3-AP infusion ($P < 0.001$) and no difference in day 1 and day 10 ($P = 1.00$).

Discussion

3-AP was well tolerated at a three-times weekly intravenous dosing of 25mg/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 25mg/m^2 3-AP infusions. With 25mg/m^2 3-AP doses, toxicities were minor. Dose-limiting grade 3 gastrointestinal and grade 4 electrolyte changes were restricted to 50mg/m^2 3-AP infusions.

Ten patients had advanced stage IB2-IVB cervical cancer, with a median 7.5cm tumor size often leading to parametrial tissue or pelvic wall muscle invasion and nephropathy. Early complete clinical responses were achieved in 6 of 10 patients (60%) after 5 weeks of daily pelvic radiation and cisplatin plus 3-AP chemotherapy and prior to 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 achieve a 70% complete clinical response and 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 performed on both day 1 and day 10 and no corresponding symptomatic rise 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 four hours postdose (Fig. 4), and as such, frequent 3-AP dosing appears 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 G1-phase cell cycle arrest perhaps indicating a p53-independent radiosensitizing mechanism.(16)

In this clinical trial, we measured the target enzyme RNR in day 1 and day 10 tumor biopsies using two different assays, an IHC 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 moderate change in the IHC 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, where 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 G1/S cell cycle delay and reduced radiation-mediated DNA damage reapir. Enahnced tumor cytotoxicity in these patients could also result from ionizing radiation-cisplatin interactions, independent of 3-AP treatment.

Durable clinical activity was observed with a median 18-month follow-up after administering intravenous 25mg/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 2 study of daily pelvic radiation and once weekly cisplatin (40mg/m^2) plus three-times weekly 3-AP (25mg/m^2) is underway.

Acknowledgments

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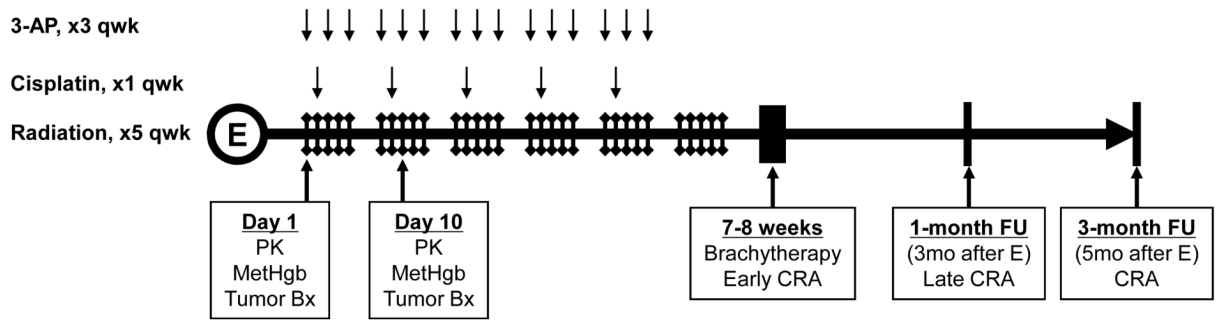
Figure 1.
Phase 1 clinical trial schema. Abbreviations: E = enrollment, qwk = per week, PK = pharmacokinetic sampling, MetHgb = methemoglobin sampling, Tumor Bx = transvaginal tumor biopsy, FU = after all protocol therapy completion follow-up, CRA = clinical response assessment.
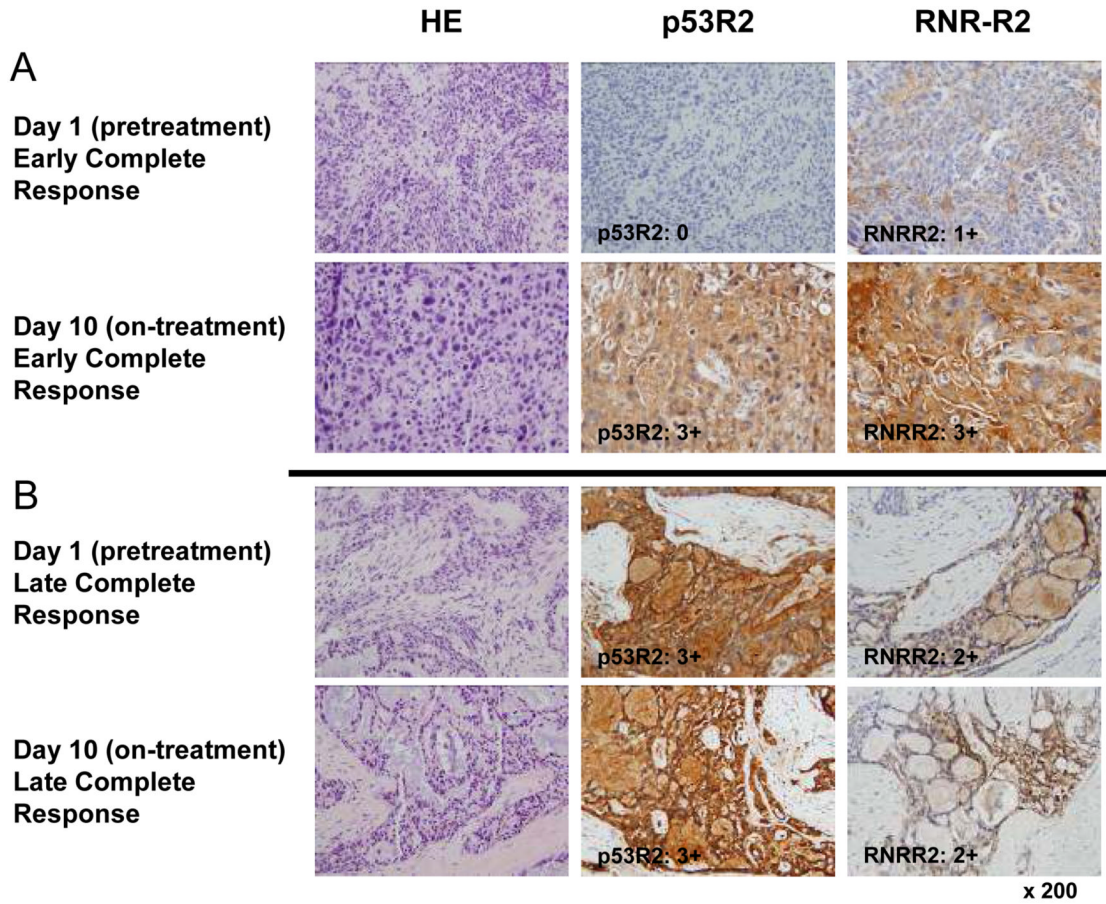
Figure 2.
Representative cervical cancer histopathology and ribonucleotide reductase (RNR) R2 and p53R2 protein levels day 1 and day 10 are depicted in (A) a early complete response and (B) an late complete response cervical cancer patient after radiation and cisplatin (40mg/m^2) plus 3-AP (25mg/m^2). Histopathology (hematoxalin eosin: HE) demonstrates high grade cervical cancer with evident radiation-drug treatment effect comparing biopsy specimens pretreatment (day 1) to on-treatment (day 10) sections. Immunohistochemistry (IHC) staining shows rise 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).
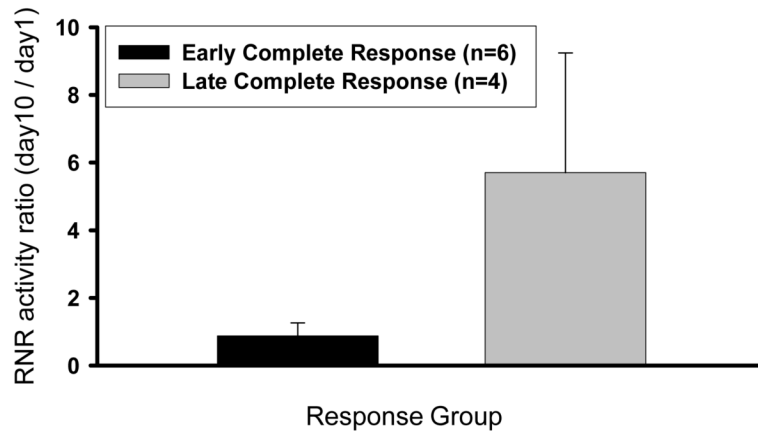
Figure 3.
Ribonucleotide reductase (RNR) activity in early and late complete clinical responders after radiation and cisplatin ($40mg/m^2$) plus 3-AP (25 or $50mg/m^2$) is illustrated. Mean RNR activity (dCTP [nM/mg]), expressed as a ratio of day 10 to day 1 levels, is higher in late complete response as compared to early complete response patients ($P =0.02$).
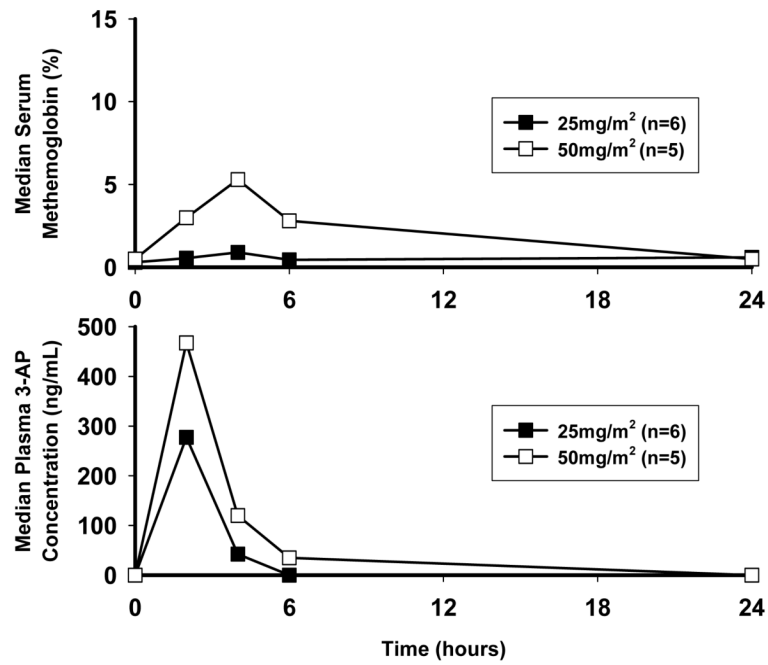
Figure 4.
Median steady-state plasma concentration profiles for each 3-AP dose group (lower panel) and corresponding median serum methemoglobin proportion (upper panel).

Table 1

Patient Characteristics by 3-AP Dose Cohort

	Number of Patients	
Characteristic	**25mg/m^2**	**50mg/m^2**
Age, years		
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

Table 2

Number of patients with Drug-related Adverse Events that occurred in ≥ 5% of patients receiving 3-AP*

Adverse Event	25mg/m² (n = 6)	50mg/m² (n = 5)	Total	Grade 3	Grade 3†	Grade 4†
					n = 11	
Cardiovascular						
Transient flushing	0	1	1	1	0	0
Electrocardiogram QTc ≥ 0.48 seconds	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 x upper limit of normal)	0	2	2	0	0	1
Neurological						
Confusion	0	2	2	1	0	0
Sensory neuropathy	0	1	1	1	0	0
Dermatological						
Skin decubitus ulcer	0	1	1	0	0	0

*
Adverse events were summarized by the dose to which the patient was initially assigned. Grade 3 adverse events were evaluated using the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (NCI CTCAE).

†
Grade 3 adverse event not resolving to grade 0-2 over 2 days, considered dose-limiting toxicity. Grade 4 toxicity considered dose-limiting toxicity.