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CLINICAL TRIAL REPORT

A phase I and pharmacokinetic study of oral 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, NSC #663249) in the treatment of advanced-stage solid cancers: a California Cancer Consortium Study

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Abstract
Background 3-Aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) is a novel small-molecule ribonucleotide reductase inhibitor. This study was designed to estimate the maximum tolerated dose (MTD) and oral bioavailability of 3-AP in patients with advanced-stage solid tumors.
Methods Twenty patients received one dose of intravenous and subsequent cycles of oral 3-AP following a 3 + 3 patient dose escalation. Intravenous 3-AP was administered to every patient at a fixed dose of 100 mg over a 2-h infusion 1 week prior to the first oral cycle. Oral 3-AP was administered every 12 h for 5 consecutive doses on days 1–3, days 8–10, and days 15–17 of every 28-day cycle. 3-AP was started at 50 mg with a planned dose escalation to 100, 150, and 200 mg. Dose-limiting toxicities (DLT) and bioavailability were evaluated.
Results Twenty patients were enrolled. For dose level 1 (50 mg), the second of three treated patients had a DLT of grade 3 hypertension. In the dose level 1 expansion cohort, three patients had no DLTs. No further DLTs were encountered during escalation until the 200-mg dose was reached. At the 200 mg 3-AP dose level, two treated patients had DLTs of grade 3 hypoxia. One additional DLT of grade 4 febrile neutropenia was subsequently observed at the de-escalated 150 mg dose. One DLT in 6 evaluable patients established the MTD as 150 mg per dose on this dosing schedule. Responses in the form of stable disease occurred in 5 (25%) of 20 patients. The oral bioavailability of 3-AP was 67 ± 29% and was consistent with the finding that the MTD by the oral route was 33% higher than by the intravenous route.
Conclusions Oral 3-AP is well tolerated and has an MTD similar to its intravenous form after accounting for the oral

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bioavailability. Oral 3-AP is associated with a modest clinical benefit rate of 25% in our treated patient population with advanced solid tumors.

Keywords 3-AP · Phase I trial · Oral triapine · Ribonucleotide reductase

Introduction

Ribonucleotide reductase (RR) is a highly regulated, omnipresent cellular enzyme in the deoxyribonucleotide de novo synthesis pathway [1]. RR reduces ribonucleotide diphosphates to corresponding deoxyribonucleotide diphosphates, an essential process for DNA synthesis and repair [1, 2]. RR consists of two subunits: M1 (M for human and R for rodent) and M2 (R2). M1 (RRM1) protein is a M_W 170-Kd dimer, containing a binding site for allosteric enzyme regulators [3]. M2 (RRM2) protein is a M_W 88-Kd dimer harboring a tyrosine free radical stabilized by non-heme diferric iron centers crucial for enzyme activity [3]. RR is a rate-limiting enzyme for DNA synthesis, indicating its important role in regulating cellular proliferation [1–5]. A new functional RR protein family member has been cloned [6], designated p53R2 because it contains a p53-binding site. Ultraviolet (UV) light, gamma-irradiation, and doxorubicin treatment induce p53R2 expression by a p53-regulated mechanism [6–8], suggesting its role in the repair of damaged DNA [6]. Cell DNA damage responses also have p53-independent means of increasing RR activity to facilitate timely repair of damaged DNA [7, 8].

Anticancer regimens incorporating RR inhibitors such as hydroxyurea have been successful [9]. However, RR-related leukopenia limits long-term treatment [9–11]. With the intent of lowering RR inhibitor toxicity through increases in drug class potency, investigators have initiated development of the 1,000-fold more potent thiosemicarbazone therapeutic class of RR inhibitors. Novel to this drug class, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, Triapine®, NSC#663249) has seen single-agent activity in phase 1 solid-cancer clinical trials, tolerated at doses of 96–100 mg/m^2 [12–14]. Moreover, 3-AP (25 mg/m^2) given concomitantly with cisplatin (40 mg/m^2) and daily radiation has resulted in significant complete response rates among women with advanced-stage cervical cancer [15]. Pharmacokinetic data for 3-AP indicate that peak serum concentrations of 1–10 μM occur 1–2 h after a 2-h intravenous infusion. Because of the short-lived anticancer therapeutic benefit of RR inhibitors, there has been a clinical desire to develop an oral 3-AP formulation that permits daily dosing.

Here, we report the first phase 1 study evaluating the safety/tolerability of oral 3-AP capsules among patients with advanced-stage solid-cancer patients. We also compare the pharmacologic bioavailability of oral 3-AP.

Methods

Patient selection

Eligibility criteria included histological or cytological confirmation of solid-cancer tumors not amenable to curative surgery, chemotherapy, or radiation. Patients had tumors that were measurable by Response Evaluation Criteria in Solid Tumors (RECIST, v1.0). Patients must have had an Eastern Cooperative Oncology Group (ECOG) performance status score of 0 or 1, be 18 years of age or older, and be able to provide written informed consent. Adequate bone marrow function (neutrophils ≥1,500/μL, platelets ≥100,000/μL, hemoglobin ≥10 gm/dL with transfusions permitted) and kidney function (creatinine ≤1.5 or calculated creatinine clearance ≥50 mL/min) must have been recorded prior to enrollment. Patients had ≤ institutional upper limits of normal bilirubin and ALT, AST, and alkaline phosphatase ≤2.5 × upper limit of normal. Patients were excluded if they were pregnant or breastfeeding women, and also if they had glucose-6-phosphate dehydrogenase deficiency (due to the risk of methemoglobinemia associated with 3-AP [14]), brain metastases, another malignancy (except early-stage squamous cell carcinoma of skin or cervix), or an uncontrolled intercurrent illness (e.g., infection, congestive heart failure, unstable angina, cardiac arrhythmia, congenital or acquired immune deficiency, or psychiatric illness that could potentially impact compliance).

Treatment regimen and strata

All patients received intravenous 3-AP 7 days prior to the first oral cycle at a dose of 100 mg with blood samples drawn over 8 h to determine pharmacokinetics. Oral 3-AP was administered every 12 h for 5 consecutive doses on days 1–3, days 8–10, and days 15–17 of every 28-day cycle. Oral 3-AP started from 50 mg every 12 h and was increased to dose levels of 100, 150, and 200 mg every 12 h in cohorts of 3 patients, expanded to 6 patients if 1 of 3 patients experienced a DLT in the first cycle. There were no intra-patient dose escalations. The initial dosing of 50 mg every 12 h was based on a small, exploratory clinical evaluation of the bioavailability of oral triapine by Vion Pharmaceuticals, and this dose was found to be well below the MTD of 96 mg/m^2/day established by the phase I study of IV daily dosing for 5 days [13]. Patients were asked to fast (except for water) for 2 h prior to dosing and for 1 h after ingesting the 3-AP capsule. All patients were observed clinically for 3–4 h after oral 3-AP administration

during the first week of the first oral treatment cycle. Treatment was continued until progression of disease, unacceptable toxicity, intercurrent illness, declining performance status preventing further treatment, or patient withdrawal. Patients developing emesis with the initial or a subsequent treatment received prophylactic antiemetic treatment prior to every subsequent dose.

3-AP was held if the neutrophil count was <1,000/μL and platelets <50,000/μL or for any ≥grade 2 non-hematologic toxicity except for grade 2 fatigue and anorexia. The dose of 3-AP in the next cycle was permanently reduced one dose level for the following: (a) grade 2 neutropenia and thrombocytopenia, (b) ≥grade 3 neutropenia, or (c) ≥grade 3 thrombocytopenia. CSF use was allowed if cycles were held for neutropenia. Treatment was discontinued permanently for grade 4 non-hematologic adverse events, adverse events clinically necessitating treatment cycle delay for more than 2 weeks or need of more than two dose reductions.

Pharmacokinetics and bioavailability

Intravenous 3-AP pharmacokinetics was determined from the single 100 mg 2-h infusion administered 7 days before oral therapy. Serum samples (5 mL in red top Vacutainer® tubes) were collected from a site contralateral to the site of infusion at the following times: pre-dose, then during the infusion at approximately 0.5, 1, and 2 h (just prior to the end of infusion), and 0.25, 0.5, 1, 2, 4, and 8 h after the end of infusion. Oral 3-AP pharmacokinetics was determined with 5-mL serum samples collected at the following times around the first oral dose: pre-dose, and every 15 min until 2 h, and then at 3, 4, 6, and 8 h following the dose. Serum samples were analyzed for 3-AP levels in the California Cancer Consortium's Analytical Pharmacology Core Facility (APCF) using a validated HPLC/UV assay [12, 13]. Pharmacokinetic analyses were performed with both compartmental and non-compartmental methods using the data from individual patients. Non-compartmental methods were carried out using statistical moment theory and the rule of linear trapezoids. Summary statistics of the pharmacokinetic parameters following either an intravenous or oral triapine dose for the population, including the bioavailability (AUC_{oral}/AUC_{iv}), were derived from the parameters obtained in the individual patients.

Response and toxicity evaluation

Computed tomography or magnetic resonance imaging scans of measurable lesions were obtained at baseline and every 8 weeks. Responses were classified according to RECIST (v1.0, [16]). National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE (v3.0)) was used to grade adverse events. Dose-limiting toxicity (DLT) was defined as ≥grade 3 non-hematologic toxicity (excluding alopecia, controllable nausea and vomiting, and hypertriglyceridemia recovering within 1 week), grade 4 thrombocytopenia, grade 4 febrile neutropenia requiring hospitalization, or treatment delay of >2 weeks as a result of unresolved toxicity. The toxicity must have been definitely, probably, or possibly attributed to the oral 3-AP and have occurred during the first cycle of treatment to be a DLT. Patients removed from study due to symptomatic hypoxia, methemoglobinemia, or hypotension (systolic BP < 85 mmHg) were also considered to have experienced a DLT.

Statistical considerations

The primary objective of this phase I trial was to determine the maximum tolerated dose (MTD) of oral 3-AP. To be evaluable for toxicity, a patient must have received at least 1 complete cycle of treatment and be observed for at least 4 weeks after the start of the first cycle or have experienced a DLT. The maximum tolerated dose (MTD) was defined as the highest dose tested in which no more than 1 of 6 patients evaluable for toxicity experienced a DLT attributable to the oral 3-AP. Dose escalations proceeded according to a standard 3 + 3 design. The phase I trial was closed when 6 patients had been treated at a dose level and evaluated with no more than 1 DLT attributable to 3-AP. For the secondary objective of the study to describe the serum pharmacokinetics and bioavailability of oral 3-AP, the relationship of the AUC with the dose was assessed by least-square regression analysis.

Informed consent and regulatory approval

The study was reviewed and approved by the Cancer Therapy Evaluation Program of the National Cancer Institute, and by the Institutional Review Board at each participating institution. All patients provided written informed consent.

Results

Patient characteristics

Twenty patients were enrolled and treated between February 2007 and May 2009. Baseline patient characteristics are shown in Table 1, including age, gender, performance, and primary sites of solid cancer. On this study, 20 patients had received prior chemotherapy, 13 had received prior radiation, and 12 had received prior cancer-related surgery.

Table 1 Patient characteristics

Patient demographics	
Number of patients	20
Age (years)	
Median (range)	60 (26–81)
Gender	
Male	9
Female	11
Race	
Caucasian	15
Asian	4
African-American	1
ECOG performance	
0	1
Status	
1	14
Prior chemotherapy regimens	
Median (range)	3 (1–9)
Primary site	
Colorectal	4
Pancreas	4
Stomach	2
Breast	1
Cervix	1
Gallbladder	1
Liver	1
Uterus	1
Oropharynx	1
Parotid gland	1
Thyroid	1
Skin	1
Nervous system	1

Treatment administered

Patients were enrolled to four dose levels: 6 patients at dose level 1 (50 mg), one of whom did not complete the first cycle; 4 patients at dose level 2 (100 mg), one of whom did not complete the first cycle; 8 patients at dose level 3 (150 mg), two of whom did not complete the first cycle; and 2 patients at dose level 4 (200 mg). The median number of treatment cycles given was 2 (1 cycle equals 28 days of 3-AP). Therapy was discontinued for disease progression in 10 patients (50%) after a median of 2 cycles (range 2–15) and adverse events in 8 patients (40%) after a median of 1 cycle (range 0–2). There was failure to complete the first cycle in 2 patients (10%), one due to constipation from pain medications and one due to pain and bowel obstruction prior to receiving oral 3-AP. The 8 patients stopping therapy for adverse events included one who received only the initial IV dose.

Table 2 All grade adverse events attributed to treatment

Toxicity	All grades	
	No. of patients	%
Hematologic & infectious		
Hemoglobin (anemia)	7	35
Neutropenia	11	55
Thrombocytopenia	6	30
Infection	3	15
Constitutional		
Fatigue	5	25
Hemorrhage/thrombosis	1/0	5/0
Hypertension/hypotension	1/1	5/5
Hepatic		
Alkaline phosphatase	2	10
(AST/SGOT)	2	10
Gastrointestinal		
Diarrhea	1	5
Anorexia	4	20
Vomiting/nausea	3/5	15/25
Pain		
Headache	4	20
Pulmonary		
Hypoxia	2	10
Renal/metabolic		
Hypokalemia	1	5
Hyperglycemia	3	15

Adverse events

All graded adverse events are shown in Table 2. Grades 3 and 4 adverse events for all patients and by dose level are shown in Table 3. Among the 20 patients enrolled, 27 hematological and infectious adverse events were observed, and the majority (19 of 27 [70%]) were reversible grade 1 or 2. Neutropenia was the most frequent treatment-related hematological toxicity, with initially 2 patients with grade 3–4 adverse events observed at the 150 mg dose level though not dose limiting given no fever requiring hospitalization. One patient had grade 4 neutropenia at the 200 mg dose level though also not dose limiting given no fever requiring hospitalization. Grade 4 thrombocytopenia was also noted at the 200 mg dose level. Dose-limiting grade 4 febrile neutropenia in one patient was not observed until enrollment on the de-escalated 150 mg dose expansion cohort.

Graded non-hematological adverse events exceeding an incidence of 15% included fatigue, nausea and emesis, hypoxia, headache, and hyperglycemia. Significant DLT occurring in the first cycle was observed in 2 patients at the

Table 3 All grade 3–4 adverse events attributed to treatment and by dose

Toxicity	No. of patients (%)				
	All Gr 3–4	50 mg	100 mg	150 mg	200 mg
Hematologic & infectious					
Hemoglobin (anemia)	2 (10)	0	1 (25)	0	1 (50)
Neutropenia	4 (20)	0	0	3 (38)[b]	1 (50)[a]
Thrombocytopenia	1 (5)	0	0	0	1 (50)[a]
I nfection	1 (5)	0	0	0	1 (50)
Constitutional					
Fatigue	1 (5)	0	0	0	1 (50)
Hypertension	1 (5)	1 (17)	0	0	0
Hepatic					
(AST/SGOT)	1 (5)	0	0	0	1 (50)[a]
Gastrointestinal					
Anorexia	1 (5)	0	0	0	1 (50)
Pulmonary					
Hypoxia	2 (10)	0	0	0	2 (100)

[a] Represented grade 4 toxicity

[b] One patient with grade 4 febrile neutropenia occurring after de-escalation from 200 mg dose

Table 4 Treatment summary

Oral 3-AP (mg every 12 h)	No. pts. Treated	No. pts. excluded from cycle one toxicity evaluation	No. pts. excluded from response evaluation[d]	No. completed cycles median (range) (excluding ineligible pts for response)	No. pts. w/DLTs	DLT description	Best responses during therapy (all eligible pts. for response)
50	6	0	1[a]	2 (0–16)	1	2nd Pt: Grd. 3 hypertension	SD–2 PD–3 N\A–1
100	4	1[b]	1[b]	2 (0–3)	0	–	SD–1 PD–2 N\A–1
150	8	2	4	1 (0–9)	1	8th Pt: Grd. 4 febrile neutropenia	SD–2 PD–2 N\A–4
200	2	0	2[c]	0 (0–0)	2	1st Pt: Grd. 3 hypoxia 2nd Pt. Grd. 3 hypoxia 2nd Pt: Grd. 4 AST	N\A–2

[a] Second patient discontinued treatment prior to completing cycle 1 of oral 3-AP (patient experienced a DLT then declined further treatment)

[b] First patient was admitted to hospital prior to completion of first cycle of oral 3-AP

[c] First two patients experienced DLTs and did not complete the first cycle of oral 3-AP

[d] Patients were not eligible for evaluation due to not completing the initial two cycles due to either toxicity or declining further therapy

200 mg dose exhibiting grade 3 hypoxia with decreased O2 saturation at rest requiring continuous supplemental oxygen. Grade 4 AST elevation was also observed in the second patient at the 200 mg dose level also considered to be a DLT.

Dose escalation summary and MTD

Table 4 summarizes the number of patients evaluable for toxicity and DLTs observed on study. The second patient at the starting 50 mg dose level experienced a DLT

resulting in the expansion of the dose level to 3 additional patients. All 3 patients on the expanded level were evaluable for cycle one toxicity and did not experience a DLT. At the 100 mg dose level, 4 patients were accrued, of whom 3 were evaluable for toxicity. No patient experienced a DLT at this dose level, and the decision was made to escalate to 150 mg. All 3 patients accrued at this dose level were evaluable for toxicity, and no patient experienced a DLT. At the 200 mg dose level, the first 2 patients accrued each experienced a DLT. Five additional patients were accrued at the 150 mg dose level, of whom 2 were not evaluable for toxicity. One DLT was observed in the 6 evaluable patients establishing 150 mg as the MTD.

Treatment response

Twelve of the 20 patients with measurable disease were evaluable for response (Table 4). Stable disease as the best response was documented in 5 patients (25%) of the entire enrolled study population. The median duration of stable disease response was 2 months, with the longest progression-free interval of 15 months seen in a patient with pancreatic adenocarcinoma. All twenty (100%) have died in long-term follow-up, with progressive disease confirmed in 10 patients while on study.

Pharmacokinetics of oral 3-AP

Pharmacokinetic data were obtained from 15 patients. A total of 14 of these subjects had data available following both an intravenously and orally administered dose for the determination of oral bioavailability. The pharmacokinetic results are summarized in Table 5 and illustrated in Fig. 1. As shown in the figure, the exposure of orally administered 3-AP increased in a dose-dependent manner. Peak serum concentrations occurred at approximately the same time when the drug was administered orally as when it was given as a 2-h intravenous infusion. The terminal elimination half-lives and mean residence times were also roughly equivalent with the two routes of administration.

The mean oral bioavailability ($F_{oral/iv}$) across all dose levels was 0.69 ± 0.29. At an oral MTD dose of 150 mg, the mean AUC was 10.6 ± 7.6 μM h and was similar to the mean AUC with an intravenous dose of 100 mg of 9.5 ± 3.1 μM h. Mean peak serum concentrations with an oral dose of 150 and an intravenous dose of 100 mg were 5.0 ± 3.8 and 3.5 ± 1.4 μM, respectively.

Discussion

Twice daily oral 3-AP of 150 mg was safely administered to patients with advanced-stage solid cancers. Oral

Table 5 Summary of 3-AP pharmacokinetic results

Patient	IV dose	Oral dose	Intravenous route					Oral route					F (oral/IV)[b]
			C_{max} (μM)	T_{max} (h)	MRT[a] (h)	AUC (μM[c]h)	CLsys (L/h)	C_{max} (μM)	T_{max} (h)	MRT (h)	AUC (μM[c]h)	CL/F (L/h)	
001	100	50	3.5	2.0	3.0	14.5	0.028	1.5	3.0	4.2	5.2	0.020	0.72
002	100	50	2.7	2.0	2.7	8.3	0.016	0.9	0.8	2.6	1.9	0.007	0.46
003	100	50	3.2	2.0	3.0	11.3	0.022	1.5	1.0	2.5	3.5	0.014	0.62
004	100	50	2.9	2.0	2.3	7.2	0.014	0.5	1.5	3.0	1.6	0.006	0.45
005	100	50	2.1	2.0	2.5	5.7	0.011	0.5	1.3	2.6	1.4	0.006	0.50
006	100	50	2.5	2.0	3.1	10.0	0.020	1.1	1.5	3.4	4.1	0.016	0.82
007	100	100	2.8	2.0	2.5	8.8	0.017	2.4	2.0	2.8	6.5	0.025	0.74
008	100	100	5.0	2.0	2.4	4.8	0.009	2.5	1.0	3.0	2.3	0.009	0.48
009	100	150	5.4	2.0	2.5	12.1	0.024	5.9	2.0	3.5	8.0	0.010	0.44
010	100	150	2.4	2.0	2.6	14.1	0.027	2.4	0.8	1.7	21.7	0.028	1.03
011	100	150	4.7	2.0	3.0	7.0	0.014	1.6	6.0	5.4	4.3	0.006	0.41
012	100	150	6.7	2.0	3.5	16.1	0.031	10.1	1.5	3.3	8.5	0.011	0.35
013	100	200	1.9	2.0	2.3	25.6	0.050	0.8	1.5	2.5	56.0	0.055	1.09
014	100	200	3.7	2.0	2.3	10.2	0.020	4.6	3.0	3.9	19.5	0.019	1.28
		Avg.	2.8	2.0	2.8	9.5	0.022	5.0[c]	1.9	3.2	10.6[c]	0.017	0.67
		SD	0.5	0.0	0.3	3.1	0.01	3.8	1.4	0.9	7.6	0.01	0.29

[a] Mean residence time

[b] F normalized to a total oral dose of 100 mg

[c] Average values for patients receiving an oral dose of 150 mg

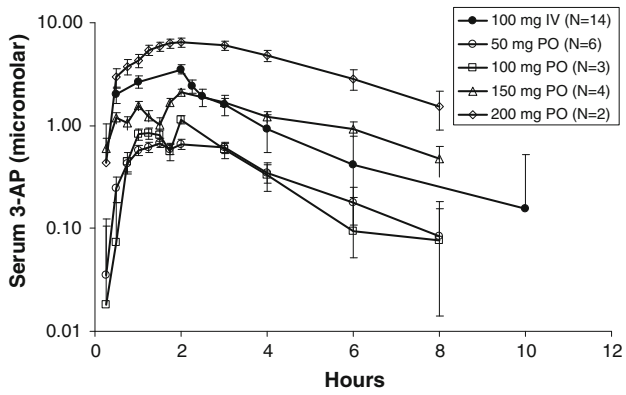

Fig. 1 Mean concentration versus time plots for IV (*closed circles*) and oral (*open symbols*) 3-AP. Peak serum concentrations occurred at the same time when the drug was administered orally or intravenously. The terminal elimination half-life was also roughly equivalent with the two routes of administration

bioavailability was 67% of the administered dose, with 150 mg every 12 h on days 1–3, 8–10, and 15–17 of each 28-day cycle being the MTD. Dose-limiting hypoxia occurred at the 200 mg 3-AP dose level as well as grade 4 neutropenia, thrombocytopenia, and elevated liver enzymes. Stable disease was documented in 5 (25%) of 20 patients, with a median duration of 2 months.

Pharmacologic inhibition of RR has become an attractive "pathbreaking" means of anticancer treatment. Two phase 1 clinical trials of single agent intravenous 3-AP have shown that administration of 3-AP achieves stable disease (30 and 16%) in pretreated advanced-stage patients [12, 13]. An additional phase I trial of intravenous 3-AP in combination with gemcitabine also showed that 42% of patients had at least stable disease as a best response after 4 months of treatment [14]. Likewise, a phase 1 clinical trial of radiation, cisplatin, and three times weekly intravenous 3-AP (25 mg/m^2) showed a durable 18-month complete pelvic tumor response rate in 100% (10 of 10) of women treated with advanced-stage cervical cancer [15]. Given the rapid metabolism of 3-AP (T½ $\approx$ 2.5 h), the most convenient formulation of 3-AP for further phase 3 clinical development would be an oral tablet. In that, our study showed that there was satisfactory bioavailability of oral 3-AP tablets in direct comparison with intravenous delivery further strengthens an argument for oral dosing of 3-AP in future clinical trials. For these reasons, there is interest in investigating daily dosing of oral 3-AP coadministered with radiation in international clinical trials for the treatment of cervical cancer because (a) it permits optimal radiation-drug timed effect [8, 17] and (b) it removes the impediment of refrigeration and intravenous tubing seldom realized in underdeveloped nations where cervical cancer is common.

In this study, intravenous and oral 3-AP pharmacokinetics were compared to determine oral bioavailability. Our results demonstrate that the time course of drug appearance and disappearance from serum is very similar when 3-AP is administered orally or intravenously as a 2-h infusion (Fig. 1). Furthermore, the finding of an average oral bioavailability of 67% is consistent with our finding that the MTD of 3-AP by the oral route is 33% higher than by the intravenous route. 3-AP treatment was scheduled twice daily to provide repeated drug-induced RR inhibition and, thereby, prolonged inhibition of on-demand deoxyribonucleotide synthesis during attempted cell proliferation. From our adverse event and pharmacological data, frequent oral 3-AP dosing at its MTD appears safe and provides precedent for future 3-AP-mediated trials of chemotherapy or radiotherapy sensitization. Perhaps, the most outstanding anticancer benefit of an RR inhibitor like 3-AP is its stalling of DNA damage repair mechanisms, and this will ultimately enhance tumor-directed cytotoxicity [8, 17].

The therapeutic efficacy of single agent 3-AP was low in that only 25% of treated patients attained stable disease as the best response on this study. This finding is consistent with other human anticancer phase 1 and 2 testing of single-agent RR inhibitors (e.g., hydroxyurea, gemcitabine, and 3-AP). Despite our observation of a progression-free interval of 15 months in a single patient with pancreatic cancer, phase II trials of intravenous 3-AP in pancreatic and kidney cancer were not found to exhibit meaningful clinical activity [18, 19]. Combining RR inhibitors, such as a phase II trial of intravenous 3-AP and gemcitabine in relapsed non-small cell lung cancer, also only yielded a 20% stable disease rate [20]. However, substantial therapeutic response gains have been realized when RR inhibitors have been coadministered with cytotoxic chemotherapy and radiation [9, 15, 21]. This discordant finding is most likely attributed to the impeded supply of deoxyribonucleotides demanded by cells for repair of damaged DNA. While deoxyribonucleotide numbers needed to fix damaged DNA vary from a few for double-strand break repair to hundreds for base damage and single-strand gaps, the rate-limiting step in the supply of deoxyribonucleotides is catalyzed by RR. Blockade of RR by 3-AP or other RR inhibitors considerably reduces deoxyribonucleotides furnished de novo on-demand in conditions of DNA damage. Since cells avoid genotoxic stress resulting from large fluctuations in deoxyribonucleotide reserves [22], de novo synthesis of deoxyribonucleotides by RR is a critical early response to DNA damage. Indeed, the two isoforms of the RR small subunit M2 or p53R2 are tuned to cell demands of deoxyribonucleotides. The RR M2 protein is tightly restricted to S-phase replication of DNA by a KEN-box promoting degradation in late mitosis. The RR p53R2 protein is constitutively active throughout the cell

cycle but regulated in its activity by a reversible protein–protein interaction with p53 [23] and in its expression level by a p53-induced transcription mechanism [6]. Preclinical and clinical data collected thus far suggest that the greatest gains in clinical benefit from RR inhibitors occur when cellular DNA is damaged and cell deoxyribonucleotides demands are high. If oral 3-AP proceeds to further clinical testing, it is recommended that 3-AP dosing follows DNA-damaging therapies.

In summary, oral 3-AP dosing at the oral MTD provides drug exposure equivalent to its intravenous form. The tolerable adverse event profile of 3-AP alone at its MTD makes it an attractive drug partner for anticancer trials in combination with chemotherapy and radiation treatments. A phase II trial of intravenous triapine added to days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24, 26, 29, 31, and 33 of cisplatin and radiation therapy in cervical and vaginal malignancies is ongoing [ClinicalTrials.gov identifier NCT00941070]. Based on the data of Kunos et al. [15], cervical cancer would be the most attractive disease to utilize oral 3-AP in combination with cisplatin and radiation.

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