Published in final edited form as:

Cancer Chemother Pharmacol. 2010 October ; 66(5): 973–980. doi:10.1007/s00280-010-1250-z.

The maximum tolerated dose and biologic effects of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) in combination with irinotecan for patients with refractory solid tumors

Brian S. Choi¹, Dona B. Alberti¹, William R. Schelman¹, Jill M. Kolesar¹, James P. Thomas², Rebecca Marnocha¹, Jens C. Eickhoff¹, S. Percy Ivy³, George Wilding¹, and Kyle D. Holen¹

¹University of Wisconsin Paul P. Carbone Comprehensive Cancer Center

²Ohio State University

³Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment and Centers, National Cancer Institute

Abstract

Purpose—3-AP is a ribonucleotide reductase inhibitor and has been postulated to act synergistically with other chemotherapeutic agents. This study was conducted to determine the toxicity and antitumor activity of 3-AP with irinotecan. Correlative studies included pharmacokinetics and the effects of ABCB1 and UGT1A1 polymorphisms.

Methods—The treatment plan consisted of irinotecan on day 1 with 3-AP on days 1-3 of a 21-day cycle. Starting dose was irinotecan 150 mg/m² and 3-AP 85 mg/m²/d. Polymorphisms of ABCB1 were evaluated by pyrosequencing. Drug concentrations were determined by HPLC.

Results—Twenty-three patients were enrolled, 10 men and 13 women. Tumor types included 7 patients with pancreatic cancer, 4 with lung cancer, 2 with cholangiocarcinoma, 2 with mesothelioma, 2 with ovarian cancer, and 6 with other malignancies. Two patients experienced dose-limiting toxicity (DLT) at dose level 1, requiring amendment of the dose escalation scheme. Maximal tolerated dose (MTD) was determined to be 3-AP 60 mg/m²/d and irinotecan 200 mg/m². DLTs consisted of hypoxia, leukopenia, fatigue, infection, thrombocytopenia, dehydration and ALT elevation. One partial response in a patient with refractory non-small cell lung cancer was seen. Genotyping suggests that patients with wild-type ABCB1 have a higher rate of grade 3 or 4 toxicity than those with ABCB1 mutations.

Conclusions—The MTD for this combination was 3-AP 60 mg/m²/d on days 1-3 and irinotecan 200 mg/m² on day 1 every 21 days. Antitumor activity in a patient with refractory non-small cell lung cancer was noted at level 1.

Keywords

Triapine®; 3-AP; irinotecan; ABCB1; UGT1A1

INTRODUCTION

Ribonucleotide reductase (RR) is an enzyme that is responsible for the reduction of ribonucleotides to deoxyribonucleotides. This reaction is the rate-limiting step for DNA synthesis, and hence RR plays a key role in the regulation of cell growth and proliferation. Based on studies performed on *E. coli* and mouse enzymes, RR is thought to be a tetrameric holoenzyme composed of an α_2 -homodimer called R1 and a β_2 -homodimer called R2. The subunit R1 is the active site of the reductase component, while R2 contains an iron center and a tyrosyl radical that are required for enzymatic activity (1). Inactivation of RR results in depletion of deoxyribonucleotides, inhibition of DNA synthesis, and subsequently blockage of cell division (2). As tumor cells are highly proliferative and require increased quantity of dNTPs, inhibition of RR is an ideal target for anticancer therapy. The clinical importance of RR is clearly evident in non-small cell lung cancer, where increased expression of RR mRNA led to poor response to gemcitabine and decreased survival (3). Many of the existing chemotherapeutic drugs are nucleoside analogs that inhibit RR by binding R1, either at the active site (gemcitabine and cytarabine) or the allosteric effector site (fludarabine and cladribine). Among the drugs that are currently in clinical use, the only one that inhibits RR by inactivating R2 is hydroxyurea (HU), which acts by destroying the tyrosyl radical. Its clinical efficacy is limited by a relatively low affinity for RR, short half-life, and development of rapid resistance (4).

3-Aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) is a novel small-molecule inhibitor of ribonucleotide reductase (RR). In addition to direct inhibition of tumor growth, it is postulated that 3-AP enhances the cytotoxic effect of other chemotherapeutic drugs by disrupting DNA repair. Preclinical studies demonstrated potentiation of several agents including cisplatin, doxorubicin, and etoposide (5). Irinotecan is a topoisomerase I modulator effective against a wide range of solid tumors, particularly colorectal, esophageal, small-cell lung, and ovarian cancers (6-9). Topoisomerase inhibitors not only damage the DNA, but also inhibit DNA synthesis; therefore, the combination of 3-AP and irinotecan has the potential of inhibiting DNA repair by two different mechanisms. We conducted this study to evaluate the safety, toxicity, maximal tolerated dose, pharmacokinetics, and possible antitumor activity of 3-AP administered with irinotecan. In addition, the effects of ABCB1 polymorphisms on pharmacokinetics (PK) and toxicity were explored.

PATIENTS AND METHODS

Patient Selection

This Phase I clinical trial was approved by the Human Subjects Committee at the University of Wisconsin and meets the ethical standards of the 1964 Declaration of Helsinki. All patients gave informed consent prior to being enrolled in the study.

Patients were required to have a histologically confirmed, measurable or evaluable solid tumor malignancy that was metastatic or unresectable and for which standard chemotherapy measures do not exist or were no longer effective. All patients were at least 18 years of age, had Eastern Cooperative Oncology Group performance status 2 or less, and had life expectancy of greater than 12 weeks. Prior irinotecan was not allowed. Patients were required to have normal organ and bone marrow function defined as follows: leukocytes $\geq 3,000/\mu\text{L}$; absolute neutrophil count $\geq 1,500/\mu\text{L}$; platelets $\geq 100,000/\mu\text{L}$; total bilirubin within normal limits; AST/ALT $\leq 2.5 \times$ upper limit of normal; and creatinine ≤ 1.5 mg/dl or creatinine clearance ≥ 50 mL/min/1.73 m². Patients could not have had chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosureas or mitomycin C) prior to entering the study. Toxicity from prior agents had to be resolved to grade 1 or less. Women of child-bearing potential and all men agreed to practice adequate contraception, and pregnant or

breastfeeding women were excluded. All patients were screened for glucose-6-phosphate dehydrogenase (G6PD) deficiency and excluded if G6PD was below the lower limit of normal, as 3-AP can induce hemolysis and methemoglobinemia in patients with G6PD deficiency (10). The protocol was later amended so that patients were also required to have a baseline screening test for UGT1A1 and excluded if homozygous for UGT1A1*28, which is known to increase irinotecan toxicity (11). Patients with HIV receiving combination antiretroviral therapy, prior myocardial infarction, or severe pulmonary disease requiring oxygen were excluded. Patients could not have uncontrolled intercurrent illness including, but not limited to, active infection, congestive heart failure, unstable angina, cardiac arrhythmia, or psychiatric illness. Patients were not allowed to be on enzyme-inducing anticonvulsant agents. Patients with central nervous system metastasis were excluded unless the subject was more than 6 months from definitive therapy, radiographically and clinically stable, and not receiving acute steroid therapy or taper.

Treatment Plan

Each patient on this study was scheduled to receive therapy on a 21-day cycle: irinotecan infused over 1 hour on day 1 and 3-AP infused over 2 hours on days 1, 2, and 3. The 3-AP infusion was started immediately following irinotecan on day 1. Treatment continued until disease progression; intercurrent illness preventing further therapy; unacceptable toxicity; treatment delay >2 weeks; patient decision to withdraw from the study; or any general or specific change in the patient's condition that renders further treatment unacceptable in the judgment of the investigator. A patient was considered evaluable for the determination of the MTD if he or she received one complete cycle of therapy. However, any patient who could not complete a full course of therapy due to toxicity was still considered evaluable for toxicity purposes.

This clinical trial was a phase I dose-escalation study of 3-AP and irinotecan. Patients were enrolled in cohorts of three, beginning at dose level 1 (3-AP 85 mg/m² and irinotecan 150 mg/m²) with continued escalations to occur based on toxicities noted. Unexpectedly, two patients developed dose-limiting toxicities (DLTs) at dose level 1 and therefore the dose-escalation schema was amended as shown on Table 1. Accrual then resumed at dose level -1 (3-AP 60 mg/m² and irinotecan 100 mg/m²) with a standard 3 + 3 design: if none of the three patients at a given dose level develops a DLT, another cohort of three would be treated at the next higher dose level. The escalation continues until a DLT is noted, at which point the cohort is expanded to six and the MTD was defined as the highest dose level at which 0/6 or 1/6 patients experience a DLT.

The National Cancer Institute Common Toxicity Criteria, version 2.0, were used for the grading of toxicities. Dose-limiting toxicity was defined as grade 3 or 4 leukopenia lasting ≥7 days; grade 3 or 4 thrombocytopenia lasting ≥7 days; grade 3 or 4 diarrhea not responsive to maximal anti-diarrheal medications; febrile neutropenia (ANC ≤1000 and temperature ≥38.5°C); and other grade 3 or 4 non-hematologic toxicity thought to be related to the study drugs.

Study Monitoring and Measurement of Effect

Prior to treatment, all patients had a complete history and physical exam, complete blood count with differential, basic chemistries including liver function tests, baseline electrocardiogram, pulse oximetry, and tumor staging with computed tomography (CT) or other appropriate imaging. Patients were screened for G6PD deficiency. All patients were tested for UGT1A1 genotype, but homozygosity for UGT1A1*28 was not added as an exclusion criterion until 16 patients were already enrolled.

3-AP is associated with an acute reaction (hypoxia and/or hypotension) during or shortly following the infusion. All patients were monitored clinically for 3-4 hours after each dose of 3-AP during the first cycle of therapy. 3-AP infusion was stopped if systolic blood pressure was <85 mmHg or the patient developed dyspnea. Asymptomatic hypoxia was treated with supplemental oxygen, but if the oxygen saturation did not return to >92% the treatment was stopped. A methemoglobin level was monitored in all patients pre-treatment on day 1 of the first cycle, at the end of 3-AP infusion, and at 2, 4.5, and 22 hours after the infusion. Patients with persistent methemoglobinemia >15% were removed from study.

Patients underwent weekly complete blood count and ANC. The dose was reduced by one level for the subsequent cycle for ANC <1500 for ≥ 7 days, febrile neutropenia, or platelet count <50,000 for ≥ 7 days. Recurrent toxicity (as noted above) required an additional decrease of one dose level. Patients developing recurrent toxicity after two dose reductions were removed from study. Routine use of colony stimulating factor (G-CSF or GM-CSF) was not allowed.

Non-hematologic grade 3 or 4 toxicity (except nausea and vomiting controlled with anti-emetics) required dose reduction by one level. A second occurrence required an additional dose reduction. Patients who developed grade 3 or higher toxicity for the third time were removed from study.

In this study, patients were evaluated every 6 weeks to look for response to therapy or disease progression. Any partial or complete response was confirmed 4 weeks later. Tumor assessment was performed in strict adherence to the Response Evaluation Criteria in Solid Tumors guidelines (12).

Pharmaceutical Information

3-AP was supplied by Vion Pharmaceuticals, Inc., and distributed by the Cancer Therapy Evaluation Program of the National Cancer Institute. 3-AP injection was supplied in 10-mL amber vials containing 50 mg of 3-AP. Prior to administration, the drug was diluted in saline or 5% dextrose in water to a concentration of 0.01-2 mg/mL. Intact vials or diluted solutions were stored in a refrigerator (2-8 °C).

Pharmacokinetics

Sample Preparation—Blood samples for evaluation of 3-AP were collected pre infusion, 1 hour into the infusion, 1-2 minutes just before the end of the infusion, and at 10, 20, 30, 45 minutes and 1, 2, 4.5, 6, 8, 10, and 22 hours after the end of the infusion. At each time point specified, two 5mL green top (heparinized) vacutainers were drawn from the arm opposite from the infusion arm for pharmacokinetic analysis of 3-AP. Plasma was separated by centrifugation at approximately $1200 g \times 15$ minutes. Duplicate plasma aliquots of 2mL each were frozen in labeled polypropylene cryotubes at -70°C until assay. The erythrocyte phase was aliquoted and stored at -70°C until analysis.

3-AP—HPLC with UV detection was used to analyze the serum and erythrocyte samples for 3-AP concentration by the method of Murren (13). A Spectra Physics P2000 HPLC system was used. Chromatographic separation was achieved using a Supelco Discovery C18 column (5 μM , 250 mm $\times$ 4.6 mm; Supelco, St. Louis, MO) with detection at 400 nm. Plasma or erythrocyte samples (0.5 ml) were extracted with 1.0 ml of methanol (containing 4 mM EDTA). After centrifugation, the extract was concentrated to dryness and was reconstituted with 0.25 ml of a solvent consisting of 10% acetonitrile and 90% mobile Phase A [20 mM potassium phosphate buffer, 15 mM 1-heptanesulfonic acid, and 1 mM EDTA (pH 3.0)].

The reconstituted solution sample (30 μ l) was then injected into the HPLC system. External calibration standards were prepared in pooled control human plasma and were processed identically to test samples. The validated assay is linear over 0.02–10 μ g/ml for plasma ($r^2 = 0.99$), with an intraday variability ranging from a coefficient of variation (CV) of 0.41%–3.4% and an interday variability ranging from a CV of 2.60%–5.5%. The lower limit of quantitation was 0.078 μ g/ml and an absolute recovery from plasma of 92%.

Data analysis—Pharmacokinetic modeling and parameter calculations were conducted using WinNonlin software program (Pharsight Corporation, Mountain View, CA) with non-compartmental methods. The following PK parameters were computed: area under the serum concentration-time curve (*AUC*) from time zero to the last data point with extrapolation to infinity, peak serum concentration ($C_{\max}$), elimination half-life ($T_{1/2}$), volume of distribution at steady state (V_{ss}), and total body clearance (*Cl*). Descriptive statistics (mean and SD) were calculated and were used to characterize the pharmacokinetic parameters. The *AUC* and $C_{\max}$ were dose adjusted. Analysis of Variance (ANOVA) was used to evaluate differences of PK parameters between genotypes. The association between genotypes and toxicities (DLT vs. no DLT) was assessed using Fisher's exact test. All statistical tests were two-sided, and $p < 0.05$ was used to indicate statistical significance.

Genotyping

Pyrosequencing assays for the common ABCB1 polymorphisms C1236T, G2677T/A, and C3435T (16). Samples were collected at baseline in DNA PAXgene tubes (Qiagen, Valencia, CA), and DNA was extracted as recommended as by the manufacturer.

PCR Amplification—Specific oligonucleotide primers for amplification by PCR of ABCB1 gene fragments from genomic DNA were derived from known sequences (GenBank accession no: AC005068) using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). PCR for pyrosequencing was performed in 40 μ l reactions containing 20 μ l PCR Master Mix (Promega, Madison, WI), 10 pmol forward and reverse primer (IDT Technology, Coraville, IA), 14 μ l of nuclease free water and 10–100 ng of genomic DNA. PCR amplification was performed under the following conditions: initial denaturation at 95°C for 5 minutes, 50 cycles of denaturation at 95°C for 30 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 30 seconds, followed by a final extension step at 72°C for 5 minutes.

Pyrosequencing—The pyrosequencing primers were designed using SNP Primer Design Software Version 1.01 (<http://www.pyrosequencing.com>). Briefly, 35 μ l of biotinylated PCR product was immobilized on streptavidin-coated Sepharose beads (Amersham Biosciences, Piscataway, NJ) with binding buffer (10 mM Tris-HCl, 2 M NaCl, 1 mM EDTA, and 0.1% Tween 20, pH 7.6). After room temperature incubation with constant agitation for 10 minutes, the strands were separated and treated with 70% ethanol, denaturation solution (0.2 M NaOH) and washing buffer (10 mM Tris-Acetate, pH 7.6). The beads, containing the biotinylated template, were released into wells with a 40 μ l mixture of annealing buffer (20 mM Tris acetate, 2 mM magnesium acetate tetrahydrate, pH 7.6) and 21 pmol of sequencing primer (IDT Technology, Coraville, IA). Incubation was carried out at 80°C for 2 minutes. Genotyping was subsequently performed using a PSQ 96 SNP Reagent Kit and PSQ 96MA system (Biotage AB, Uppsala, Sweden). Genotypes were resolved on the basis of peak height measurements using PSQ96 SNP Software, version 1.2 AQ. Subjects were categorized as wild-type, heterozygote or variant.

RESULTS

Patient Characteristics

Twenty-three patients were enrolled in the study between October 2004 and July 2007 (Table 2). The majority of patients had gastrointestinal (particularly pancreatic) or lung primary tumors. All patients had prior chemotherapy, and 17 (74%) had received two or more chemotherapy regimens. The median age was 59 (range 29-72) years, and 10 (43%) were men. Although the study allowed patients with ECOG performance status of 2, all of the patients who entered the study had performance status of 0 (17%) or 1 (83%).

Treatment Summary

All 23 patients were evaluable to determine the MTD of 3-AP plus irinotecan. Table 1 summarizes the number of treatment courses that each patient received, any dose modifications, and reason for discontinuing therapy. The study initially accrued at dose level 1 (3-AP 85 mg/m² and irinotecan 150 mg/m²), but the first patient developed a DLT (grade 3 hypoxia). The protocol was then amended to evaluate dose level -1, with 3-AP reduced to 60 mg/m² and irinotecan to 100 mg/m². The first three patients on dose level -1 tolerated the treatment well without a DLT. Therefore, three additional patients were evaluated at the original dose level 1, but another DLT occurred (grade 4 leukopenia lasting 7 days). Three more patients were then evaluated at dose level -1 (total of 6 at this dose) to ensure safety, with subsequent dose escalations modified as shown on Table 1. Additional DLTs occurred in one patient each at dose level -1 and 3 (grade 4 fatigue and both grade 4 infection and grade 3 fatigue, respectively). Two of the initial cohort of three at dose level 4 developed DLT, one patient with grade 3 platelets >7 days and the other patient with grade 3 dehydration. At this point the study was amended to expand the cohort despite a possible second DLT, because the investigators felt that the grade 3 dehydration was more likely to be from the underlying disease than the treatment. The next patient enrolled after the amendment also had DLT (grade 3 ALT), and the study was terminated. Therefore, the MTD was determined to be 3-AP 60 mg/m² and irinotecan 200 mg/m².

Adverse Events

Adverse events that were definitely, probably, or possibly related to the study treatment and affecting 3 or more patients are listed in Table 3. The most common non-hematologic toxicities irrespective of severity were nausea (70%), diarrhea (61%), vomiting (57%), abdominal pain or cramping (57%), fatigue (57%), and dizziness or lightheadedness (30%). Grade 3 or 4 non-hematologic events included nausea, vomiting, diarrhea, fatigue, hypoxia, abdominal pain/cramp, thrombosis, infection, hyperbilirubinemia, and elevated ALT. Grade 3 or 4 hematologic toxicities included neutropenia in 8 patients, thrombocytopenia in 2 patients, and anemia in one patient. Only one patient experienced methemoglobinemia exceeding 15%; that patient was treated at dose level 1, the only level at which the dose of 3-AP was 85 mg/m²/d.

Antitumor Activity

Of the 23 patients enrolled in the study, 20 patients were evaluable for measurement of treatment effect. The best response for each patient is shown in Table 1. One patient with non-small cell lung cancer had a confirmed PR, which was maintained until he developed disease progression at approximately 6 months from study entry. Fifteen patients had SD but only one, with pancreatic cancer, received more than 4 cycles of therapy.

Pharmacokinetics of 3-AP and ABCB1 Genotype

The 3-AP PK data were performed in 20 patients, 16 of them receiving the dose of 60 mg/m² and 4 receiving 85 mg/m². The mean PK parameters representing all dose levels are shown in Table 4. There was no statistically significant difference between plasma and RBC after adjusting for dose.

Three common polymorphisms of the ABCB1 gene were studied: C1236T, G2677T, and C3435T. Data were available for 19 patients (4 patients had DNA that did not amplify). The distribution of patients among the 7 possible haplotypes is shown in Table 5. For the analysis of PK and toxicity, all-wild-type haplotype (CC GG CC) was compared to the all-variant haplotype (TT TT TT).. As shown in Table 5, ABCB1 haplotype had a significant effect on both PK and the occurrence of DLT. Patients with the wild-type haplotype had higher dose-adjusted C_{max} and AUC of 3-AP, and 3 of 4 of the DLTs in the 19 patients analyzed occurred in the wild-type group.

DISCUSSION

3-AP is a novel small-molecule inhibitor of RR that acts by destroying the tyrosyl radical of the R2 subunit. It belongs to a class of iron chelators known as heterocyclic carboxaldehyde thiosemicarbazones, and its activity depends on the formation of a ferrous-3-AP complex (17). In preclinical studies 3-AP was shown to have an increased affinity for RR, up to 1000-fold potent than HU, with a broad spectrum of antitumor activity in leukemia and solid tumors (5). In addition to direct inhibition of tumor growth, it is postulated that 3-AP enhances the cytotoxic effect of other chemotherapeutic drugs by disrupting DNA repair. Indeed, in preclinical experiments 3-AP potentiated several agents, including cisplatin, etoposide, and doxorubicin (5). Here, we report a phase I trial of combining 3-AP with irinotecan, including pharmacokinetic and pharmacogenomic data with regards to ABCB1 and UGT1A1.

In our study, the MTD of 3-AP was significantly lower than previously reported. Other phase I studies with single-agent 3-AP demonstrated safety with single 2-hour IV infusions of up to 105 mg/m² every 4 weeks (18); daily 2-hour infusions up to 96 mg/m² for 5 days every two weeks (13); and continuous 96-hour infusions up to 120 mg/m²/day every two weeks (19). Similarly, the MTD of irinotecan was lower than the standard dose, which in most studies is between 300-350 mg/m² every 3 weeks. The lower MTDs suggest that 3-AP and irinotecan are at least additive in terms of toxicity, if not synergistic. The most frequent grade 3 or 4 side effects of this regimen were nausea, vomiting, diarrhea, and myelosuppression, all of which are typical of irinotecan. 3-AP infusions were generally well-tolerated, with only 2 patients developing grade 3 hypoxia.

Partial response to treatment was seen in only 1 patient with non-small cell lung cancer, who remained on therapy for about 6 months. The duration of stable disease was generally short, with only 1 patient receiving more than 4 cycles of therapy. The paucity of response is not surprising given this group was heavily pre-treated, and most of whom had tumor types that are not known to be sensitive to irinotecan. Considering the modest antitumor activity and substantial dose-reduction of irinotecan, further study of this particular regimen is not being pursued.

The gene ABCB1, also known as MDR1, encodes for the ubiquitous efflux pump P-glycoprotein (P-gp). Single-nucleotide polymorphisms of this gene can affect not only drug resistance but also drug exposure and disease susceptibility, although the precise role of ABCB1 remains controversial (20). Analysis of the ABCB1 haplotype in our study indicates that polymorphisms of this gene do in fact influence drug exposure and toxicity from 3-AP,

with wild-type patients experiencing higher plasma concentrations and being more likely to experience a DLT when compared to the variants. Emerging evidence suggests that the ABCB1 haplotype (C1236T, G2677T, C3435T) may contribute to subtle changes in protein folding and function that lead to altered substrate binding, specificity and drug effect (21). In general wild-type individuals have more P-gp and may be able to pump more drug out of the cell, resulting in higher plasma concentrations, increased toxicity and less activity. This is consistent with our previous work, where variants had a longer progression free survival compared to wild-type individuals (22). In addition to substrate specificity, dose may contribute to pharmacokinetic variability with variant genotypes, as our previous work using a lower dose of 3-AP did not show a correlation between genotype and plasma concentrations (23).

Acknowledgments

This study was supported by the following grants: U01CA062491 “Early Clinical Trials of Anti-Cancer Agents with Phase I Emphasis”, NCI; 1ULRR025011 “Clinical and Translational Science Award of the National Center for Research Resources”, NIH; CTEP Translational Research Initiative Funding 24XS090.

The authors would like to thank Marcy Pomplun and Zhisheng Jiang of the University of Wisconsin Paul P. Carbone Comprehensive Cancer Center (UWCCC) Analytical Instrumentation Laboratory for Pharmacokinetics, Pharmacodynamics & Pharmacogenetics (3P Lab) for support in the acquisition of 3-AP pharmacokinetics for this research.

The authors would also like to thank the nurses and research specialists of the UWCCC Phase I Program for their efforts in conducting and managing this trial.

REFERENCES

1. Kolberg M, Strand KR, Graff P, Andersson KK. Structure, function, and mechanism of ribonucleotide reductases. *Biochimica et Biophysica Acta (BBA) - Proteins & Proteomics* 2004;1699:1–34.
2. Cory J, Carter G. Drug action on ribonucleotide reductase. *Advances in Enzyme Regulation* 1985;24:385–401. [PubMed: 3915189]
3. Rosell R, Danenberg KD, Alberola V, Beppler G, Sanchez JJ, Camps C, et al. Ribonucleotide reductase messenger RNA expression and survival in gemcitabine/cisplatin-treated advanced non-small cell lung cancer patients. *Clin Cancer Res* 2004;10:1318–1325. [PubMed: 14977831]
4. Shao J, Zhou B, Chu B, Yen Y. Ribonucleotide reductase inhibitors and future drug design. *Current Cancer Drug Targets* 2006;6:409–431. [PubMed: 16918309]
5. Finch RA, Liu M, Grill SP, Rose WC, Loomis R, Vasquez KM, et al. Triapine (3-aminopyridine-2-carboxaldehyde-thiosemicarbazone). A potent inhibitor of ribonucleotide reductase activity with broad spectrum antitumor activity. *Biochem Pharmacol* 2000;59:983–991. [PubMed: 10692563]
6. Cunningham D, Pyrhönen S, James RD, Punt CJ, Hickish TF, Heikkilä R, et al. Randomised trial of irinotecan plus supportive care versus supportive care alone after fluorouracil failure for patients with metastatic colorectal cancer. *Lancet* 1998;352:1413–1418. [PubMed: 9807987]
7. Ilson DH, Bains M, Kelsen DP, O'Reilly E, Karpeh M, Colt D, et al. Phase I trial of escalating-dose irinotecan given weekly with cisplatin and concurrent radiotherapy in locally advanced esophageal cancer. *J Clin Oncol* 2003;21:2926–2932. [PubMed: 12885811]
8. Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, et al. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. *N Engl J Med* 2002;346:85–91. [PubMed: 11784874]
9. Bodurka DC, Levenback C, Wolf JK, Gano J, Wharton JT, Kavanagh JJ, et al. Phase II trial of irinotecan in patients with metastatic epithelial ovarian cancer or peritoneal cancer. *J Clin Oncol* 2003;21:291–297. [PubMed: 12525521]
10. Foltz LM, Dalal BI, Wadsworth LD, Broady R, Chi K, Eisenhauer E, et al. Recognition and management of methemoglobinemia and hemolysis in a G6PD-deficient patient on experimental anticancer drug Triapine. *Am J Hematol* 2006;81:210–211. [PubMed: 16493607]

11. Ando Y, Saka H, Ando M, Sawa T, Muro K, Ueoka H, et al. Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. *Cancer Res* 2000;60:6921–6926. [PubMed: 11156391]
12. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;92:205–216. [PubMed: 10655437]
13. Murren J, Modiano M, Clairmont C, Lambert P, Savaraj N, Doyle T, et al. Phase I and pharmacokinetic study of triapine, a potent ribonucleotide reductase inhibitor, administered daily for five days in patients with advanced solid tumors. *Clin Cancer Res* 2003;9:4092–4100. [PubMed: 14519631]
14. Poujol S, Pinguet F, Malosse F, Astre C, Ychou M, Culine S, et al. Sensitive HPLC-fluorescence method for irinotecan and four major metabolites in human plasma and saliva: Application to pharmacokinetic studies. *Clin Chem* 2003;49:1900–1908. [PubMed: 14578322]
15. Hu ZP, Yang XX, Chen X, Chan E, Duan W, Zhou SF. Simultaneous determination of irinotecan (CPT-11) and SN-38 in tissue culture media and cancer cells by high performance liquid chromatography: Application to cellular metabolism and accumulation studies. *J Chromatogr B Analyt Technol Biomed Life Sci* 2007;850:575–580.
16. Green H, Soderkvist P, Rosenberg P, Horvath G, Peterson C. Mdr-1 single nucleotide polymorphisms in ovarian cancer tissue: G2677T/A correlates with response to paclitaxel chemotherapy. *Clin Cancer Res* 2006;12:854–859. [PubMed: 16467099]
17. Shao J, Zhou B, Di Bilio AJ, Zhu L, Wang T, Qi C, et al. A ferrous-triapine complex mediates formation of reactive oxygen species that inactivate human ribonucleotide reductase. *Mol Cancer Ther* 2006;5:586–592. [PubMed: 16546972]
18. Feun L, Modiano M, Lee K, Mao J, Marini A, Savaraj N, et al. Phase I and pharmacokinetic study of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) using a single intravenous dose schedule. *Cancer Chemother Pharmacol* 2002;50:223–229. [PubMed: 12203104]
19. Wadler S, Makower D, Clairmont C, Lambert P, Fehn K, Sznol M. Phase I and pharmacokinetic study of the ribonucleotide reductase inhibitor, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone, administered by 96-hour intravenous continuous infusion. *J Clin Oncol* 2004;22:1553–1563. [PubMed: 15117978]
20. Chinn LW, Kroetz DL. ABCB1 pharmacogenetics: Progress, pitfalls, and promise. *Clin Pharmacol Ther* 2007;81:265–269. [PubMed: 17259950]
21. Fung KL, Gottesman MM. A synonymous polymorphism in a common MDR1 (ABCB1) haplotype shapes protein function. *Biochim Biophys Acta* 2009;1794:860–871. [PubMed: 19285158]
22. Traynor AM, Lee JW, Bayer GK, Tate JM, Thomas SP, Mazurczak M, et al. A phase II trial of Triapine(R) (NSC# 663249) and gemcitabine as second line treatment of advanced non-small cell lung cancer: Eastern Cooperative Oncology Group Study 1503. *Invest New Drugs* 2010;28:91–97. [Epub ahead of print]. [PubMed: 19238328]
23. Schelman WR, Morgan-Meadows S, Marnocha R, Lee F, Eickhoff J, Huang W, et al. A phase I study of Triapine in combination with doxorubicin in patients with advanced solid tumors. *Cancer Chemother Pharmacol* 2009;63:1147–1156. [PubMed: 19082825]

Table 1

Dose Escalation and Treatment Summary

Patient	Dose Level	Irinotecan mg/m ²	3-AP mg/m ²	Dose Reductions	DLT	Number of Treatments	Best Response	Reason for Study Exit
1	-1	100	60	None		2	SD	PD ^a
2				None		4	SD	Pt withdrew ^b
3				None		2	SD	PD ^a
4				None		2	SD	PD ^a
5				Yes	Yes	2	SD	PD ^a
6				None		1	PD	PD
7	1	150	85	Yes	Yes	1	N/A ^d	PD ^a
8				None		8	PR	PD
9				Yes	Yes	2	PD	PD
10				None		2	SD	PD ^a
11	2	150	60	None		2	PD	PD
12				None		3	SD	Other ^e
13				None		2	SD	PD
14	3	200	60	None		3	SD	Pt withdrew ^b
15				None		4	SD	PD
16				None	Yes	1	N/A ^d	Toxicity
17				None		2	SD	PD
18				None		2	SD	Other ^e
19				None		4	SD	Toxicity
20	4	250	60	None		2	SD	PD ^a
21				Yes	Yes	2	PD	PD
22				None	Yes ^c	1	N/A ^d	Pt withdrew ^b

Patient	Dose Level	Irinotecan mg/m ²	3-AP mg/m ²	Dose Reductions	DLT	Number of Treatments	Best Response	Reason for Study Exit
23				Yes	Yes	6	SD	Other ^e

^aProgression based on clinical judgment of treating physician but not as defined by RECIST

^bPatients' reason for withdrawing from study – rising tumor marker (2), need for hernia surgery (14), and side effects from therapy (22)

^cInitially designated as DLT, but later attributed to the disease

^dTreatment discontinued before the first disease evaluation

^eOther – no further clinical benefit (11), need for radiotherapy for pain control (18), and decision to treat primary tumor with radiotherapy (23)

Table 2

Patient Characteristics

Characteristic	No. of patients (n=23)	%
Sex		
Male	10	43%
Female	13	57%
ECOG Performance Status		
0	4	17%
1	19	83%
Age, years		
Median	59	
Range	29-72	
Prior therapy		
Chemotherapy	23	100%
Radiotherapy	9	39%
Tumor type		
Pancreatic	7	30%
Cholangiocarcinoma	2	9%
Ovarian	2	9%
Esophageal	1	4%
Lung	4	17%
Sarcoma	1	4%
Unknown primary	1	4%
Lymphoma	1	4%
Gastric	1	4%
Mesothelioma	2	9%
Cervical	1	4%

Table 3

Incidence toxicity by dose level. Events that are unlikely to be related to treatment were excluded.

Number of patients experiencing toxicity (all grades [grade 3 or 4])												
	Dose Level -1(n=6)		Dose Level 1(n=4)		Dose Level 2(n=3)		Dose Level 3(n=6)		Dose Level 4(n=4)		Total	
Nausea	4	1	3	1	2	0	4	2	3	1	16	5
Diarrhea	4	0	3	0	1	0	4	2	2	0	14	2
Vomiting	4	1	1	1	1	0	4	1	3	1	13	4
Abdominal pain or cramp	4	0	4	1	0	0	2	0	3	0	13	1
Fatigue	5	1*	2	0	1	0	3	1*	2	0	13	2
Lightheaded/ Dizziness	2	0	1	0	1	0	3	0	0	0	7	0
Flushing	2	0	0	0	1	0	1	0	0	0	4	0
Hiccups	0	0	1	0	2	0	1	0	0	0	4	0
Dehydration	2	0	0	0	0	0	1	0	1	0	4	0
Dyspnea	1	0	0	0	0	0	2	0	1	0	4	0
Hypoxia	0	0	1	1*	0	0	2	1	0	0	3	2
Cough	0	0	1	0	1	0	0	0	1	0	3	0
Anorexia	1	0	1	0	0	0	0	0	1	0	3	0
Chills	0	0	0	0	0	0	3	0	0	0	3	0
Diaphoresis	1	0	0	0	0	0	2	0	0	0	3	0
Neuropathy sensory,	1	0	1	0	1	0	0	0	0	0	3	0
Headache	1	0	0	0	1	0	1	0	0	0	3	0
Flatulence	0	0	1	0	0	0	0	0	2	0	3	0
Leukocytes	1	0	3	2	0	0	2	2	3	3	9	7
Neutrophils	2	1	3	2*	1	1	2	1	3	3	11	8
Hemoglobin	0	0	2	0	0	0	0	0	3	1	5	1
Platelets	0	0	1	1	0	0	1	0	1	1*	3	2

* DLT

Table 4

PK Parameters for All Dose Levels of 3-AP (60 mg/m², n=16, and 85 mg/m², n=4)

	Plasma		Erythrocyte	
	Mean	SD	Mean	SD
T _{1/2} (hours)	6.16	2.36	6.90	3.15
T _{max} (hours)	0.05	0.11	0.06	0.11
C _{max} (mg/mL/mg/m ²)	1.67	0.63	1.84	0.69
AUC _{0-∞} (mg•hr/mL/mg/m ²)	4.42	3.15	4.53	3.9
Cl (mL/min/m ²)	0.20	0.11	0.15	0.10
V _{ss} (L/m ²)	103	47	96.2	50

There is no significant difference between plasma and erythrocyte concentrations

Table 5

3-AP PK and DLT according to ABCB1 genotype

ABCB1 Genotype		Plasma PK Parameters		
C1236T	G2677T	C3435T	n	DLT
CC	GG	CC	3	3
CC	GG	CT	2	1
CT	GT	CC	1	0
CT	GT	CT	3	0
CT	GT	TT	1	0
TT	TT	CT	1	0
TT	TT	TT	8	0
			C _{max} (µg/mL/mg/m ²)	AUC (µg·hr/mL/mg/m ²)
			1.90 ± 0.37	7.81 ± 1.53
			2.19 ± 0.06	6.46 ± 2.77
			1.88	1.62
			1.33 ± 0.33	3.10 ± 1.00
			1.76	1.51
			1.00	1.55
			1.22 ± 0.44	2.65 ± 1.53

The dose adjusted C_{max} and AUC are significantly higher in the CC GG CC haplotype compared to the TT TT TT haplotype, p=0.04 and 0.009, respectively. The association between genotype and DLT is statistically significant when analyzed individually (p=0.029) or as a group (p<0.0001).