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ORIGINAL ARTICLE

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# A phase I trial of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone in combination with gemcitabine for patients with advanced cancer

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**Abstract** *Purpose*: 3-Aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP), a new and potent inhibitor of ribonucleotide reductase (RR), increases the cellular uptake, DNA incorporation, and cytotoxicity of gemcitabine in tumor cell lines. A phase I trial was initiated to determine the safety profile and maximum tolerated doses of 3-AP and gemcitabine when used in combination in patients with advanced cancer. *Study design*: 3-AP and gemcitabine were administered on days 1, 8, and 15 of each 28-day cycle. Initially, 3-AP was infused over 2 h at a fixed dose of 105 mg/m$^2$. Gemcitabine was given over 30 min beginning no less than 1 and no more than 4 h after 3-AP. The first cohort received 3-AP alone in the first cycle. Subsequently, the gemcitabine dose was escalated beginning at 600 mg/m$^2$ in cohorts of three to six patients. Following the gemcitabine 1000 mg/m$^2$ dose level, the study was amended to determine if the 3-AP dose could be escalated above 105 mg/m$^2$. *Results*: 3-AP at 105 mg/m$^2$ administered over 2 h followed in 1–4 h by gemcitabine at 1000 mg/m$^2$ produced a toxicity profile similar to that expected for gemcitabine alone at the same dose. When the dose of 3-AP was escalated to 140 and 185 mg/m$^2$ administered over 2 h and subsequently over 4 h, acute hypotension, hypoxia, and EKG changes including nonspecific ST-T wave changes and mild QT prolongation were observed, and one patient with underlying diffuse coronary artery disease had an asymptomatic myocardial infarction. 3-AP was shown to cause mild, reversible methemoglobinemia. Average end-of infusion serum concentrations for 3-AP at all doses were within the range capable of enhancing gemcitabine cytotoxicity in vitro. Gemcitabine plasma concentrations at end-of-infusion and elimination half-life were consistent with values reported in the literature. Among 22 evaluable patients, one complete response and two partial responses were observed, and an additional patient had prolonged stabilization of a large liver metastasis. *Conclusions*: 3-AP at 105 mg/m$^2$ infused over 2–4 h followed by gemcitabine at 1000 mg/m$^2$ on a days 1, 8, and 15 schedule every 28 days was generally well-tolerated and had a toxicity profile similar to that of gemcitabine alone. 3-AP produced mild to modest methemoglobinemia, which could cause acute symptoms in patients with limited pulmonary or cardiovascular reserve. The combination demonstrated antitumor activity and merits further exploration in phase II trials.

**Keywords** Clinical trial · Phase I · Ribonucleotide reductase · 3-Aminopyridine-2-carboxaldehyde thiosemicarbazone · Gemcitabine · Cancer

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## Introduction

Gemcitabine, 2′,2′-difluoro-2′-deoxycytidine, is a nucleoside analog that has demonstrated modest activity in patients with several types of advanced malignancies, including pancreatic, non-small-cell lung, biliary tract, breast, ovarian, bladder, and germ-cell cancer [5–7, 12, 13, 22, 31, 34, 35, 43, 44]. The most common schedule of administration is a 30-min intravenous (i.v.) infusion weekly for two or three consecutive weeks followed by a week of rest. At the recommended doses of 1000–1250 mg/m$^2$, gemcitabine is well tolerated in most patients. The most common adverse effects include granulocytopenia, thrombocytopenia, anemia, elevations of the transaminases and alkaline phosphatase, nausea, vomiting, fever, and rash. Because of its broad activity and good safety profile, rational approaches to

combine gemcitabine with other agents and further improve its antitumor effects are of substantial interest.

Gemcitabine enters cells through membrane nucleoside transporters and is then phosphorylated to the monophosphate, diphosphate, and triphosphate forms [23, 32, 33]. Gemcitabine's cytotoxicity is dependent on incorporation of the triphosphate into DNA during replication or repair, which results in inhibition of DNA synthesis, and more recently has been reported to poison topoisomerase I activity [26, 38, 41]. The diphosphate is a potent inhibitor of the M1 large subunit of ribonucleotide reductase (RR), the enzyme that catalyzes the chemical reduction of ribonucleotides to deoxyribonucleotides (dNTPs) [17, 24]. Perturbation of cellular dNTP pools inhibits DNA replication and repair, and could further promote incorporation of gemcitabine triphosphate into DNA by reducing intracellular concentrations of the natural substrate dCTP.

In vitro studies and gene expression analysis of patient tumors indicate that overexpression of RR, involving either the M1 or M2 subunit, may be an important mechanism of resistance to gemcitabine [3, 16, 20, 40]. Consistent with these observations, several preclinical studies have shown that prior exposure of tumor cells to RR inhibitors can increase the cytotoxicity of nucleoside analogs, including those nucleoside analogs with their own intrinsic ability to inhibit RR [4, 10, 17, 18, 25, 27, 30, 39, 46, 47, 52]. Recently, Zhou et al. have demonstrated that sequential administration of hydroxyurea, which inhibits the M2 RR subunit, followed by gemcitabine, produces synergistic cytotoxicity against the KB nasopharyngeal carcinoma cell line [54]. Although several trials have explored the activity and safety of sequential administration of RR inhibitors and nucleoside analogs, particularly with ara-C in hematologic malignancies, clinical experience attempting to modulate gemcitabine activity in solid tumors is limited [19, 29, 37, 45, 53].

3-Aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, Triapine; Triapine is a registered trademark of Vion Pharmaceuticals) is a new inhibitor of the M2 RR subunit and a potent iron chelator [15]. In tumor cells, the $IC_{50}$ for 3-AP is approximately 300- to 1000-fold less than that of hydroxyurea, and 3-AP appears to overcome hydroxyurea resistance in selected tumor cell lines [11, 15]. In experiments similar to those conducted with hydroxyurea, sequential exposure of tumor cell lines to 3-AP for 2–16 h followed by gemcitabine for 1 h has been shown to increase gemcitabine cellular uptake and DNA incorporation, and to produce additive or synergistic cytotoxicity [8]. These data provided the basis for a clinical trial of 3-AP as a biochemical modulator of gemcitabine. The pharmacokinetics and toxicity of 3-AP administered by 2-h i.v. infusion once every 4 weeks have been determined in a phase I clinical trial [14]. There were no dose-limiting toxicities (DLTs) at levels as high as 105 mg/m$^2$, which produced peak serum concentrations in the range of 5–8 $\mu M$ with a terminal half-life of approximately 1 h. The 3-AP serum concentrations and duration of exposure were within the range effective in modulating gemcitabine cytotoxicity in vitro. Therefore, the current phase I trial was designed to provide a similar exposure to these agents in vivo and assess the tolerability of this combination.

## Patients and methods

### Patient selection

The study was reviewed and approved by the Human Subject Committees of the two participating institutions. All patients gave their voluntary, written informed consent prior to study participation.

Patients were required to be at least 18 years old and to have histologically documented progressive metastatic or locally advanced cancer that was unlikely to benefit from any currently available standard therapies. Known, active central nervous system (CNS) metastases excluded entry to the study, but patients who had received treatment for CNS metastases, and had been stable for at least 2 months without evidence of new CNS metastases, were eligible. The last dose of chemotherapy, radiotherapy, or any treatment for their malignancy must have been given at least 3 weeks prior to study entry (for nitrosoureas or mitomycin C chemotherapy, at least 6 weeks prior to study entry), and all acute toxicity related to prior drug administration must have returned to baseline. Persistent chronic toxicities from prior chemotherapy could not be greater than grade 1. Patients who had received gemcitabine previously were eligible, but those who had received prior treatment with 3-AP were excluded from study participation. Other eligibility criteria included the following: Eastern Cooperative Oncology Group (ECOG) performance status of 0–1; serum creatinine $\leq$ 1.5 mg/dl; total bilirubin $\leq$ 2.0 mg/dl; alanine and aspartate aminotransferases not more than three times the upper limit of the normal range, or not more than five times the upper limit of normal in the presence of liver metastases; absolute neutrophil count $\geq$1500 $\mu l^{-1}$; platelet count $\geq$100,000 $\mu l^{-1}$; hemoglobin $\geq$10 g/dl and prothrombin time (PT) and activated partial thromboplastin time (aPTT) not more than 1.5 times the upper limit of normal. Males and females of child-bearing potential were required to practice adequate contraception or abstinence, females were required to have a negative serum or urine pregnancy test within 2 weeks prior to beginning treatment on this trial, and nursing women were excluded. Patients were also excluded from participation in the trial if they had a history of myocardial infarction within the previous 3 months or active angina pectoris; uncontrolled congestive heart failure, or arrhythmias other than adequately controlled supraventricular tachycardia; dyspnea at rest; an active infectious process; or prior severe allergic-type reactions to gemcitabine.

## Study design and treatment plan

This was a phase I dose-escalation trial. 3-AP was administered i.v. over 2 or 4 h. Gemcitabine was administered as a 30-min i.v. infusion no less than 1 h and no more than 4 h after completing the 2-h 3-AP infusion, or 0–2 h after completing the 4-h 3-AP infusion. Treatment was administered on days 1, 8, and 15 of each 28-day cycle. Patients who experienced 3-AP-related emesis received prophylactic antiemetics at the discretion of the physician. After an episode of acute dyspnea in the fourth cohort that was believed to be precipitated by gemcitabine (although later attributed to 3-AP), all subsequent patients received dexamethasone 4 mg i.v. prior to each dose of 3-AP. Use of erythropoietin and granulocyte growth factors was permitted at the discretion of the treating physician. Granulocyte growth factors were restricted to a period no earlier than 24 h after administration of 3-AP and gemcitabine and were halted at least 48 h prior to beginning the next treatment.

Dose levels were evaluated by sequential entry of three to six patients per cohort. Only adverse events occurring in the first cycle of treatment were considered for evaluation of DLT. For each new dose level, up to three patients were entered. If none of the first three patients at a dose level developed a DLT, patients were entered to the next higher dose level. If one of the first three patients developed a DLT, up to three more patients were started at that same dose level. If two or more developed a DLT at the same dose, accrual to that dose level and dose escalation was halted, and the previous dose level was expanded as necessary to a total of six patients. The maximum tolerated dose (MTD) was defined as the highest dose level in which fewer than two patients of six developed treatment-related first cycle DLT.

The National Cancer Institute Common Toxicity Criteria version 2.0 was used to grade toxicity. DLT was defined as follows: any grade 3 or greater nonhematologic toxicity, with the exception of noninfectious fever, nausea, vomiting, or constitutional symptoms that could easily be managed with supportive care and resolved within 48 h; elevated alkaline phosphatase more than five times the on-study value; grade 4 neutropenia not resolving grade 3 or less within 96 h or associated with infection; grade 4 thrombocytopenia lasting more than 3 days or associated with clinically significant bleeding; and persisting toxicities of any grade requiring delay of scheduled treatment in a new cycle by more than 1 week. Alopecia or lymphocytopenia of any grade were not considered dose-limiting. Also, grade 3 elevation in transaminases that occurred after the third dose in a cycle and resolved to less than grade 2 within 1 week was considered not dose-limiting.

The doses of each agent in each cohort are shown in Table 1. Initially, the dose of 3-AP was held constant at 105 mg/m$^2$. For the first cohort only, 3-AP was administered without gemcitabine in the first cycle, in order to establish the baseline toxicity profile of weekly 3-AP alone; for the second and subsequent cycles, patients received 3-AP at 105 mg/m$^2$ and gemcitabine at 600 mg/m$^2$, which was the starting dose for the second cohort. The dose of gemcitabine was then escalated in successive cohorts. Once the dose of gemcitabine was escalated to 1000 mg/m$^2$, the study was amended to evaluate 3-AP doses of 140 and 185 mg/m$^2$ in combination with gemcitabine 1000 mg/m$^2$. Acute 3-AP-related toxicity, observed in the second cycle of a patient treated with 140 mg/m$^2$ and the first dose of two patients treated at 185 mg/m$^2$ prompted an amendment to lengthen the 3-AP infusion to 4 h. The longer infusion was applied to patients continuing treatment at the 140 mg/m$^2$ dose and new and continuing patients at the 185 mg/m$^2$ 3-AP dose.

For treatment due on days 8 and 15 of a cycle, full doses were administered if the following criteria were met, otherwise the doses were held: absolute granulocyte $\geq$1000 $\mu l^{-1}$; platelet count $\geq$50,000 $\mu l^{-1}$; liver function tests (AST/ALT) (in patients with on-study values within normal limits) less than grade 3, or in patients with on-study elevated values, less than grade 3 toxicity or not more than twice on-study values; bilirubin $\leq$ 2.5 mg/dl; alkaline phosphatase not more than five times on-study value; creatinine $\leq$ 2.0 mg/dl; constitutional symptoms less than grade 3; and all other non-hematologic major organ toxicity (pulmonary, cardiac, CNS) less than grade 2. Skipped treatments were not made up. If treatment was held on day 8 for drug-related toxicity, the doses were reduced to the next lower dose level for day 15. Dose reductions in an individual patient were permanent for the current and all subsequent cycles. Toxicities, which required holding

**Table 1** Protocol treatment

| Cohort | 3-AP dose (mg/m$^2$) | Gemcitabine dose (mg/m$^2$) | No. of patients | No. of cycles |
|---|---|---|---|---|
| 1 | 105 | 0 (cycle 1), 600 (cycles 2–x) | 3 | 4, 4, 7 |
| 2 | 105 | 600 | 3 | 2, 2, 4 |
| 3 | 105 | 800 | 4 | 2, 2, 4, 6 |
| 4 | 105 | 1000 | 8 | 0[a], 0[a,b], 1[b], 1, 2, 2, 4, 5 |
| 5 | 140 | 1000 | 3 | 1, 2, 4[c] |
| 6 | 185 | 1000 | 5 | 0[a], 1[d], 2[e], 4[f], 12+[f] |

[a]Received one dose only.
[b]3-AP administered over 4 h.
[c]Cycle 2 course 2, 3-AP infusion length increased to 4 h; cycle 2 course 3 dose reduced to 105 mg/m$^2$.
[d]First course of 3-AP over 2 h; subsequently all 3-AP given over 4 h.
[e]All 3-AP over 4 h.
[f]Second and subsequent courses of 3-AP at 105 mg/m$^2$ over 4 h.

treatment and dose reduction in an individual patient, were considered a DLT for the purpose of dose escalation in a new cohort only if they met the criteria described above.

Nadir neutrophil and platelet counts that occurred after the third dose in a cycle and did not meet DLT criteria did not require dose modification in the next cycle. For day 1 of a new cycle, all treatment-related toxicity was required to have resolved to grade 1 or less, with the following exceptions: patients could begin a new cycle with grade 2 fatigue and anorexia, with grade 2 laboratory values that were permitted by the eligibility criteria, and with creatinine $\leq 2.0$ mg/dl. Certain toxicities such as alopecia, lymphocytopenia, and phlebitis that were more than grade 1 at the scheduled beginning of a new cycle did not preclude initiation of a new cycle. The initiation of a new cycle could be delayed for up to 1 week. On day 1 of a cycle, new or additional dose reductions to the next lower dose level were required if, in the preceding cycle, the patient had developed a DLT (for example, after the last treatment on day 15) or required a dose to be held for toxicity. The details of additional adjustments in the doses and schedules of protocol therapy after the unexpected observation of acute cardiopulmonary/hemodynamic reactions are provided in the Results section.

### Study monitoring

Prior to treatment, patients were assessed with a complete history and physical examination; vital signs; scoring of ECOG performance status; complete blood count with platelets and differential; aPTT and PT; serum chemistries including electrolytes and liver function tests; pregnancy test in women of child-bearing potential; electrocardiogram (EKG); chest radiograph; urinalysis; tumor staging studies including computed tomography (CT) scans of chest and abdomen, CT scan or magnetic resonance imaging (MRI) of brain as appropriate to the disease, and tumor markers as appropriate to the disease. Vital signs were obtained 15 min and 2 h after initiation of 3-AP infusion (and at the end of infusion if the 3-AP infusion was given over more than 2 h), then prior to and 30 and 60 min after gemcitabine. For selected patients receiving 3-AP by 4-h i.v. infusion, an EKG was obtained at the end of the first 3-AP infusion, and blood pulse oxygen saturation was monitored at baseline, 1 and 2 h after beginning the first 3-AP infusion, and at the end of the infusion. On days 8, 15, and 22, patients were assessed for interval toxicity and performance status, and blood was drawn for a CBC with platelets and differential and full serum chemistries. On days 8 and 15, the CBC and chemistries were checked prior to treatment. Every other cycle, tumor status was assessed by history, examination, CT scans, and/or other studies as appropriate to the disease. Patients with partial response and those with stable disease were eligible to continue treatment for up to a total of 12 cycles. Patients with complete response were to receive one cycle past the cycle where complete response had been documented. The criteria for response evaluation were based on RECIST [49].

### Drug supply and administration

Gemcitabine was obtained commercially. 3-AP was supplied by Vion Pharmaceuticals. Studies conducted by Vion indicate that 3-AP is stable when diluted to a final concentration of 0.01–2 mg/ml for up to 96 h. 3-AP was diluted in 500 ml normal saline or 5% dextrose in water and administered by i.v. infusion over 2–4 h. Dilutions of 3-AP were performed in glass bottles and not in polyvinyl chloride plastic containers to avoid extraction of the plasticizer, di(ethylhexyl)phthalate (DEHP), by the non-aqueous solvents in the 3-AP formulation. For the same reason, polyethylene-lined administration sets were used for 3-AP infusions.

### Pharmacokinetics

On the first day of the first cycle, blood samples were collected from the arm opposite the drug infusions (or from a peripheral vein if a central i.v. line was in place) immediately before and at the end of the 3-AP infusion, prior to the beginning of gemcitabine administration, and 30 min after the gemcitabine infusion, in order to estimate pharmacokinetic parameters for 3-AP. High-performance liquid chromatography with ultraviolet detection was used to analyze the serum for 3-AP concentration as previously described [14]. For gemcitabine pharmacokinetics, blood samples were collected and analyzed as described in the literature, prior to and at the end of the gemcitabine infusion, and 30, 60, 120, and 240 min after completion of the infusion [1, 51].

## Results

### Patient characteristics

Between July 2001 and March 2003, 26 patients with a median age of 58 years were entered into the study (Table 2). Of these patients, 85% had received prior chemotherapy and 65% had been treated with two or more cytotoxic regimens. Six patients (23%) had previously received gemcitabine alone or in a combination regimen. As expected for a phase I trial, no tumor type was represented by more than three patients.

### Dose escalation and adverse events

The number of patients entered to each dose cohort and the number of cycles administered to each patient are shown in Table 1. Table 3 presents the major hemato-

**Table 2** Patient characteristics

| | |
|---|---|
| Patients entered | 26 |
| Sex | |
| Male | 16 |
| Female | 10 |
| ECOG performance status | |
| 0 | 9 |
| 1 | 17 |
| Age (years) | |
| Median | 58 |
| Range | 26–85 |
| Prior treatment | |
| Radiation | 15 |
| Chemotherapy | 22 |
| Two or more cytotoxic regimens | 17 |
| Gemcitabine | 6 |
| Diagnosis | |
| NSCLC | 3 |
| Pancreas or cholangiocarcinoma | 3 |
| Unknown primary | 3 |
| Head and neck (parotid, adenocystic, mucoepidermoid) | 3 |
| Breast | 2 |
| Colon | 2 |
| Esophageal | 2 |
| Thyroid (anaplastic, Hurthle cell) | 2 |
| Germ cell | 2 |
| Other (sarcoma, ovary, bladder, endometrial) | 4 |

logic and nonhematologic adverse events by initial dose level, and includes events occurring in all cycles received by each of the patients entered at the indicated dose level, including cycles that were administered at lower dose levels. Table 4 lists other nonhematologic adverse events combined for all cycles at all dose levels.

A previous phase I trial had established that a single 2-h i.v. infusion of 3-AP at 105 mg/m$^2$ was associated with minimal toxicity [14]. However, because a weekly schedule had not been evaluated, the first three patients on this trial received 3-AP alone in the first cycle. No clinically significant hematologic toxicity was observed. Following the first 3-AP dose, one patient had a transient sensation of burning in the chest and throat associated with a pulse oxygen saturation decline to 89%. Because this patient had experienced similar reactions with previous chemotherapy, and the reaction did not recur with the second and third weekly doses given without premedication, the hypoxia and symptoms were not attributed to 3-AP administration at that time. Table 5 lists all pulmonary and cardiovascular adverse events observed on this trial for each individual patient.

During the dose escalation of gemcitabine from 600 to 1000 mg/m$^2$ in combination with the 2-h i.v. infusion of 3-AP at 105 mg/m$^2$, adverse events were consistent with the toxicity profile of gemcitabine alone, including anemia, neutropenia, thrombocytopenia, elevations in transaminases, asthenia, and minimal nausea and vomiting. For the latter toxicities, there were no grade 4 events and the incidences of grade 3 thrombocytopenia, neutropenia, or elevated transaminases were all $<20\%$. Two patients, one each at 600 and 1000 mg/m$^2$ of gemcitabine, developed acute reversible reactions following the first gemcitabine dose predominated by dyspnea and hypoxia (pulse oxygen saturation approximately 75%). Both patients had underlying pulmonary disease prior to treatment, and both improved within hours following hospitalization for the acute dyspnea. One of the latter patients was pretreated with dexamethasone and antihistamines prior to subsequent courses of 3-AP and gemcitabine (administered at the same doses), and was able to continue on study for four cycles without complications. For the first course of the fifth cycle, the pretreatment medications were omitted, and the patient developed another episode of dyspnea following 3-AP and gemcitabine; he subsequently elected to discontinue protocol treatment. The second patient to develop dyspnea and hypoxia was noted to have an elevated methemoglobin level (measured by co-oxymetry in an arterial blood gas analyzer) and persistent mild cyanosis even while improving clinically. She was taken off study. For both patients, the dyspnea and hypoxia were initially attributed primarily to gemcitabine, based on the association between gemcitabine and pulmonary events reported in the literature [21, 28, 42, 50]. Subsequently (see below), a relationship between 3-AP administration and methemoglobinemia was established.

**Table 3** Major hematologic and non-hematologic toxicity (all cycles)

| Dose level | No. of patients | Neutrophils | | | | Platelets | | | Hemoglobin | | | AST/ALT | | | Alkaline phosphatase | | | Hypoxia | | | Cardiovascular | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Grade | | | | Grade | | | Grade | | | Grade | | | Grade | | | Grade | | | Grade | | | |
| | | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 4 |
| 105/0[a] | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 105/600[a] | 6 | 1 | 2 | 2 | 0 | 2 | 2 | 0 | 2 | 2 | 1 | 4 | 1 | 0 | 5 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 105/800 | 4 | 0 | 4 | 0 | 0 | 0 | 1 | 1 | 0 | 4 | 0 | 2 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 105/1000 | 8 | 1 | 4 | 0 | 1 | 1 | 0 | 2 | 4 | 1 | 1 | 1 | 3 | 2 | 1 | 1 | 0 | 0 | 0 | 3 | 1 | 0 | 0 | 0 |
| 140/1000 | 3 | 1 | 1 | 0 | 0 | 2 | 1 | 0 | 1 | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 |
| 185/1000 | 5 | 0 | 1 | 1 | 0 | 3 | 0 | 0 | 0 | 3 | 1 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 1 | 1 | 0 | 1 |

[a]Three patients who received 3-AP alone in cycle 1 and 3-AP-gemcitabine in subsequent cycles are included among the six patients in the 105/600 dose level.

**Table 4** Other grade 2 and 3 nonhematologic adverse events ($N = 26$)

| Event | Grade 2 | Grade 3 | Total |
|---|---|---|---|
| Asthenia | 8 | 2 | 10 (38%) |
| Anorexia | 2 | 2 | 4 (15%) |
| Vomiting | 3 | 1 | 4 (15%) |
| Nausea | 2 | 1 | 3 (12%) |
| Diarrhea | 2 | 0 | 2 (7.7%) |
| Mucositis | 2 | 0 | 2 (7.7%) |
| Constipation | 1 | 0 | 1 (3.8%) |
| Fever | 2 | 0 | 2 (7.7%) |
| Rash | 1 | 0 | 1(3.8%) |
| Cough | 2 | 0 | 2 (7.7%) |
| Creatinine | 4[a] | 0 | 4 (15%) |
| Headache | 1 | 0 | 1 (3.8%) |
| Vertigo | 1 | 0 | 1 (3.8%) |

[a]Maximum values 1.8, 1.6, 1.9, and 1.7 mg/dl in patients with baseline values of 1.4, 1.3, 1.5, and 1.3 mg/dl, respectively.

Because a gemcitabine dose of 1000 mg/m$^2$ weekly is within the range employed in accepted single-agent regimens, and the toxicity profile of 3-AP and gemcitabine appeared similar to that expected for gemcitabine alone, an attempt was made to escalate the 3-AP dose to DLT while holding the gemcitabine dose constant. In all three patients treated with 3-AP 140 mg/m$^2$ and gemcitabine 1000 mg/m$^2$, no toxicity higher than grade 2 was observed in the first cycle. However, one patient developed hypoxia (without dyspnea) and hypotension immediately after the 3-AP infusion on day 1 of cycle 2, while rising from a sitting position. An EKG showed a slight prolongation of the QT interval. The event resolved without specific therapy within 5 h. Within days of the latter event, the first patient treated with a 2-h i.v. infusion of 3-AP at 185 mg/m$^2$ developed dyspnea and hypotension, beginning 1 h into the 3-AP infusion. The event also resolved within several hours. The second patient treated with 3-AP 185 mg/m$^2$ over 2 h, an 85 year old man with non-small cell lung cancer, prior thoracic irradiation, and moderate chronic obstructive pulmonary disease, developed transient asymptomatic hypoxia and cyanosis after the 3-AP infusion. Subsequently, the protocol was amended to increase the length of the 3-AP infusion to 4 h for all patients. The patient receiving 3-AP 140 mg/m$^2$ had recurrence of hypotension 3 h in to the 4-h 3-AP infusion; she subsequently tolerated a lower dose of 105 mg/m$^2$ over 4 h. The two patients who had initiated treatment at 185 mg/m$^2$ over 1 h were able to continue treatment with the same dose administered over 4 h.

Two additional patients began treatment with 3-AP 185 mg/m$^2$ administered over 4 h. One patient tolerated the dose without complications, but the second patient, who had a history of diffuse coronary artery disease, developed hypertension and asymptomatic hypoxia during the infusion, and EKG changes following the infusion. Due to the EKG changes, cardiac enzymes were drawn, which showed elevation consistent with myocardial ischemia and infarction. The patient did not receive further treatment on study. After the latter event, all patients on the trial received 3-AP by 4-h i.v. infusion at a dose of 105 mg/m$^2$. Two new patients were entered at this dose in order to gather additional information on acute 3-AP-induced changes in blood oxygen saturation, methemoglobin levels, and EKG. Both patients had transient hypoxia; one patient with limited pulmonary reserve at baseline developed mild transient dyspnea with the first dose of the second cycle. Methemoglobin levels rose to the 10–12% range in both patients after the 3-AP infusion; the treating physician chose to administer methylene blue after gemcitabine in the patient developing mild dyspnea, and following the initial gemcitabine dose in the second patient, with rapid reduction of methemoglobin levels. Neither patient developed EKG changes.

## Response to treatment

Three patients had objective responses to treatment (Table 6). Patient 11 in cohort 2 with metastatic bronchoalveolar carcinoma had reduction of a lung, pleural, and chest wall lesion meeting the criteria for partial response. He was removed from the trial for treatment-related dyspnea, was given gefitinib on a compassionate use protocol as maintenance treatment, and remained free from progression for 18 months. Patient 15 in cohort 3 with esophageal carcinoma metastatic to mediastinal and retroperitoneal lymph nodes achieved a partial response lasting 5 months. Patient 20 in cohort 4 with an unknown primary and disease in the head of the pancreas and liver achieved a complete remission after the fourth cycle lasting 1.5 months. One additional patient, patient 31 on cohort 6 with cholangiocarcinoma metastatic to liver, had reduction of a large 8.5 cm liver mass and reduction of CA19-9 by $>50\%$ without meeting the criteria for partial response. The latter patient remained stable at the time of this report on continuing treatment beyond his 12th cycle.

## Pharmacokinetics

The average end-of-infusion (EOI) 3-AP concentrations by dose and infusion duration are shown in Table 7. Substantial interpatient variability was noted. The elimination half-life is estimated in the range of 1–2 h, consistent with the pharmacokinetic data observed in phase I single-agent trials (data not shown). The small number of patients and limited sampling preclude formal analysis and correlation with adverse events.

The average gemcitabine plasma concentrations at EOI were 31.5, 28.1 and 42.4 $\mu M$ at 600, 800 and 1000 mg/m$^2$ dose levels, respectively. The elimination half-life was rapid and averaged 14.4 min in the first 2 h after completion of the infusion.

**Table 5** Acute 3-AP pulmonary-cardiovascular reactions

| Patient no. | 3-AP dose ($mg/m^2$) and infusion length (h) | Cycle-week | Reaction/symptoms | Pulse $O_2$ saturation (%)[a] | Cardiovascular event[b] | Methemoglobin[c] | Treatment and outcome | Predisposing conditions | Re-exposure and follow-up data |
|---|---|---|---|---|---|---|---|---|---|
| 3 | 105, 2 | 1/1; end of infusion | Burning chest and throat sensation | 89 | None | Not measured | Resolved within 20 min | NSCLC, COPD, prior similar reactions | Received four cycles, no recurrence |
| 11 | 105, 2 | 1/1; 2 h after gemcitabine | Nausea, chills, fever, mild dyspnea, mild confusion; admitted for observation | 78 | None | Not measured | Improved in 3 h, resolved within 12 h | Bronchoalveolar carcinoma, COPD | Pretreated with benadryl and dexamethasone, received four full cycles; recurred cycle 5/ day 1 when pretreatment was omitted associated with pulmonary infiltrate |
| 22 | 105, 2 | 1/1; after completion of gemcitabine | Dyspnea, fever hypertension; admitted to hospital | 75 | None | 12% | Rapid improvement in symptoms but persistent mild cyanosis; resolved completely in 12–16 h | COPD | Taken off study |
| 28 | 185, 2 | 1/1; 1 h into 3-AP infusion | Cough and dyspnea; hypotension to 70/40 mmHg | 89 | Hypotension | Not measured | Infusion halted; symptoms and signs resolved over several hours | None | Received 3-AP 185 $mg/m^2$ over 4 h in subsequent courses uneventfully |
| 27 | 140, 2 | 2/1; just following 3-AP infusion | Dizziness on rising from toilet; hypotension to 88/26 mmHg | 88 | Hypotension; EKG QT interval increase from.37 to.45 s | Not measured | Resolved 5 h post-event | None | Recurred briefly at hour 3 of 4-h 3-AP infusion at 140 $mg/m^2$; tolerated 105 $mg/m^2$ over 4 h |
| 29 | 185, 2 | 1/1; end of 3-AP infusion | Blue lips | 87 | None | Not measured | Resolved rapidly | COPD; desaturates with minimal exercise; age 85 years | Transient, asymptomatic desaturation with subsequent 3-AP 185 $mg/m^2$ over 4 h, not affecting treatment; died 7 days after last treatment with new bilateral pulmonary infiltrates (infection vs progression) |
| 30 | 185, 4 | 1/1; during and following 3-AP infusion | Asymptomatic increase in blood pressure to 193/115 mmHg; EKG ST 1 mm depression; elevation of cardiac enzymes | 92 | Asymptomatic hypertension; EKG ST 1 mm depression; myocardial infarction | Not measured | NA | Known diffuse coronary artery disease; prior history of CHF | Taken off study |
| 31 | 185, 4 | 1/1; after 3-AP infusion | Asymptomatic; EKG showed slightly prolonged QT interval and T-wave inversions laterally | 88 | EKG, slightly prolonged QT interval and T-wave inversions laterally | Not measured | Hypoxia resolved rapidly, EKG changes resolved within 18 h | None | Continued treatment with 3-AP 105 $mg/m^2$ over 4 h + gemcitabine (associated with transient non-specific ST-T wave changes) for ten+ cycles |

**Table 5** (Contd.)

| Patient no. | 3-AP dose ($mg/m^2$) and infusion length (h) | Cycle-week | Reaction/symptoms | Pulse $O_2$ saturation (%)[a] | Cardiovascular event[b] | Methemoglobin[c] | Treatment and outcome | Predisposing conditions | Re-exposure and follow-up data |
|---|---|---|---|---|---|---|---|---|---|
| 33 | 105, 4 | 2/1; 3 h into 3-AP infusion | Mild dyspnea, pallor | 88 (ABG $pO_2$ 63, sat 79%) | EKG normal | 12% | Received gemcitabine uneventfully, then given methylene blue, resolved rapidly | Prior myocardial infarction; dyspnea on exertion, on home oxygen; bilateral carotid stenosis | 4 days later, admitted for ischemic cerebral vascular accident, probably unrelated |
| 34 | 105, 4 | 1/1; 5 min after gemcitabine | Pallor, mild nausea, mild chest pressure | 88–91 (ABG $pO_2$ 83%) | EKG normal | 10.8% | Although minimally symptomatic, given methylene blue; 2 h later, methemoglobin 2.2% | None | Patient elected to discontinue treatment |

[a]Arterial blood gases (*ABG*) were not obtained routinely.
[b]EKGs were not obtained routinely during most of the trial.
[c]Methemoglobin levels were measured by co-oxymetry in an arterial blood gas analyzer; normal levels are 0–2%.

## Discussion

In this phase I trial, we established the safety and feasibility of administering 3-AP prior to each dose of gemcitabine weekly for three consecutive weeks every 28 days. The incidences of grade 3/4 neutropenia, thrombocytopenia, anemia, and transaminase elevations in patients receiving 3-AP in combination with the standard gemcitabine dose of 1000 $mg/m^2$ were similar to the frequencies of these adverse events reported in phase II/III studies of the same dose and schedule of gemcitabine alone. Thus, 3-AP did not substantially alter the toxicity profile of gemcitabine. Surprisingly, 3-AP-related acute toxicity was observed which had not been appreciated in previous phase I trials of 3-AP alone or in combination with other cytotoxic agents. The 3-AP -related adverse events included methemoglobinemia, hypoxia with or without dyspnea secondary to the methemoglobinemia, EKG changes including QT interval increase and ST-T wave changes, and hypotension. Nevertheless, most patients receiving 3-AP at doses of 105 $mg/m^2$ over 2–4 h had minimal to no symptoms, and clinical and laboratory adverse events were rapidly reversible. Thus, for phase II studies, we recommend 3-AP at 105 $mg/m^2$ over 2–4 h followed by gemcitabine 1000 $mg/m^2$ infused over 30 min, starting at least 4 h after the initiation of 3-AP. To minimize risk in phase II trials, the eligibility criteria should exclude patients with clinically significant pulmonary disease such as dependence on supplemental oxygen, severe cardiovascular disease, or glucose-6-phosphate dehydrogenase (G6PD) deficiency [9]. Patients with G6PD deficiency have impaired capacity to generate the NADPH required for NADPH-methemoglobin reductase, which converts methemoglobin back to oxyhemoglobin.

Early in the trial, a causal relationship between 3-AP and methemoglobinemia and hypoxia was not suspected because most patients receiving 3-AP were asymptomatic, some symptomatic patients were rechallenged without complications, and gemcitabine is known to produce pulmonary toxicity in a small percentage of patients [21, 28, 42, 50]. 3-AP-related EKG changes were also not appreciated until late in the trial. As a result, the incidence and dose-relationship of 3-AP-induced methemoglobinemia, hypoxia, and EKG changes were not well-defined. The limited data from this trial suggest that the 105 $mg/m^2$ dose recommended for phase II trials will produce mild, rapidly reversible asymptomatic methemoglobinemia and hypoxia in most patients. Generally, methemoglobin levels in the range that was observed (10–15%) do not produce symptoms unless the patient has compromised cardiopulmonary function. Transient asymptomatic EKG changes may also be a frequent consequence of 3-AP infusions. ST-T wave changes are observed following administration of several cytotoxic drugs and do not preclude safe treatment with the agents [36]. Therefore, with appropriate selection of patients, 3-AP-induced methemoglobinemia and EKG changes should not com-

**Table 6** Summary of patients receiving four or more cycles

| Patient no. | 3-AP/gemcitabine dose (mg/m$^2$) | Age (years) | Sex | ECOG PS | Diagnosis | Metastatic sites | Prior treatment/no. of chemotherapy regimens | No. of treatment cycles | Best response |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 105/600 | 34 | M | 1 | Adenocystic carcinoma, head and neck | Lung, bone | 0 | 4 | SD |
| 3 | 105/600 | 56 | F | 0 | NSCLC | Pleura | 2 (paclitaxel then docetaxel) | 4 | SD |
| 4 | 105/600 | 47 | M | 1 | Low grade mucoepidermoid carcinoma, head and neck | Soft tissue | 3 (gemcitabine) | 7 | SD |
| 11 | 105/600 | 72 | M | 0 | NSCLC, bronchoalveolar | Lung, pleura, chest wall mass | 2 (taxanes) | 4 | PR (maintained on gefitinib) |
| 15 | 105/800 | 59 | M | 1 | Esophagus, adenocarcinoma | Lymph node | 5FU, CDDP + radiotherapy | 6 | PR (5 months) |
| 17 | 105/800 | 49 | M | 0 | Colon, adenocarcinoma | Liver | 2 | 4 | SD |
| 20 | 105/1000 | 70 | F | 1 | Unknown primary, adenocarcinoma | Pancreas, liver | 0 | 5 | CR (1.5 months) |
| 21 | 105/1000 | 36 | M | 1 | Unknown primary, neuro-endocrine | Skin, liver, lung, right groin | 1 | 4 | SD |
| 27 | 140/1000 | 59 | F | 1 | Endometrial adenocarcinoma | Lung, lymph node | 1 | 4 | SD |
| 31 | 185/105/1000 | 57 | M | 0 | Cholangiocarcinoma | Liver, LN | 2 (carboplatin/paclitaxel and carboplatin/docetaxel) | 12+ | SD with regression |
| 33 | 105/1000 | 47 | M | 1 | Anaplastic thyroid carcinoma | Lungs, lymph node, bone, soft tissue | 2 | 4 | SD |

promise patient safety in future studies. Indeed, few acute adverse reactions have been reported in ongoing and completed phase I and II trials of 3-AP administered over 2 h at doses of 96–120 mg/m$^2$ daily for 4–5 days.

Two patients, one receiving 140 mg/m$^2$ over 2 and 4 h, and the other receiving 185 mg/m$^2$ over 2 h, developed transient hypotension. The mechanism responsible for the hypotension is not known. 3-AP is a potent iron chelator, and hypotension has been reported with administration of another iron chelator, desferrioxamine [2]. The longer 4-h infusion at the highest dose of 185 mg/m$^2$ was not associated with hypotension, suggesting that it is related to 3-AP serum concentration levels and not total dose.

In one patient, asymptomatic hypertension and mild hypoxia was observed during the first infusion of 185 mg/m$^2$ over 4 h. A routine postinfusion EKG to document 3-AP-induced effects showed changes suspicious for ischemia. Cardiac enzymes were drawn and indicated a silent myocardial infarction. The patient had known severe but asymptomatic diffuse coronary artery disease, and probably was at risk from any mild cardiovascular stress such as transient 3-AP-induced hypoxia. This latter event, together with transient hypoxia and EKG changes noted in other patients at the highest dose level, prompted a permanent reduction in dose to 105 mg/m$^2$ in all patients. In retrospect, the events observed at 185 mg/m$^2$ of 3-AP administered over 4 h may have been due to patient selection and increased awareness and monitoring for hypoxia and EKG changes. However, because the safety data at 105 mg/m$^2$ administered over 2–4 h was acceptable and the serum concentrations and duration of exposure at this dose were within the range that produced additive or synergistic activity with gemcitabine in vitro, further exploration of higher doses in this trial was not necessary.

The presumed mechanism for 3-AP-induced enhancement of gemcitabine cytotoxicity is increased gemcitabine uptake and incorporation into DNA, which were demonstrated in vitro [8]. The in vitro experiments simulated expected clinical exposure to the drugs; 3-AP was added for periods of 2–16 h, followed by gemcitabine for 1 h, followed by a wash to remove both drugs. Surviving cells were counted 72 h later. Increased cytotoxicity was observed only when cells were exposed to 3-AP first for a period of at least 2 h; in some cell lines maximum effects required pre-exposure to 3-AP for 12–16 h. The mechanisms by which 3-AP enhances gemcitabine uptake and DNA incorporation have not been defined. 3-AP-induced depletion of one or more dNTPs will inhibit DNA synthesis and/or repair, because adequate and balanced supply of all four dNTPs is necessary for chain elongation. The effects on dNTPs and DNA synthesis may alter regulation of nucleoside transporters and activation enzymes such as deoxycytidine kinase. In addition, depletion of intracellular dCTP pools may decrease competition with gemcitabine triphosphate for DNA incorporation. In some cell lines

**Table 7** Pharmacokinetic results for 3-AP (*EOI* end of infusion)

| No. of patients analyzed | No. of courses | Dose (mg/m$^2$) | Infusion duration (h) | Mean EOI concentration (μg/ml) |
|---|---|---|---|---|
| 15 | 18 | 105 | 2 | 1.28 (6.5 μ*M*) |
| 2 | 3 | 105 | 4 | 0.94 (4.79 μ*M*) |
| 3 | 3 | 140 | 2 | 1.85 (9.44 μ*M*) |
| 1 | 1 | 185 | 2 | 2.78 (14.18 μ*M*) |
| 4 | 4 | 185 | 4 | 1.61 (8.2 μ*M*) |

in which dCTP levels do not decline in response to 3-AP, 3-AP enhancement of gemcitabine cytotoxicity can still be demonstrated. In vivo experiments have not yet been conducted, and the relevance of the in vitro observations to animal tumor models or patients remains to be determined. We did not choose to attempt measurement of gemcitabine triphosphate levels in peripheral blood mononuclear cells; however, these studies are contemplated for future trials where tumor tissue is easily accessible (hematologic malignancies), and a trial design can be implemented that permits an intrapatient control with gemcitabine alone.

The design of this phase I trial did not permit a meaningful assessment of 3-AP's contribution to the antitumor activity, a question that will be addressed in phase II and randomized trials. Among the 22 patients who could be evaluated for response, three objective responses were observed, and one other patient with stable disease had evidence of tumor reduction and clinical benefit. Three of the latter were in malignancies known to be sensitive to gemcitabine (non-small-cell lung, cholangiocarcinoma, and an unknown primary, which was probably a pancreatic primary tumor). Currently, phase II studies of 3-AP/gemcitabine are ongoing in patients with metastatic or unresectable pancreatic cancer (no prior chemotherapy) and patients with non-small-cell lung cancer (up to two prior cytotoxic regimens). In these studies, gemcitabine is administered approximately 0–2 h after the completion of a 4-h 3-AP infusion at 105 mg/m$^2$. Although longer exposures to 3-AP prior to gemcitabine produce better antitumor effects in vitro, long single-day i.v. administration schedules on a weekly basis are difficult in a typical outpatient setting. A preliminary single-dose human study of an oral 3-AP formulation has indicated good absorption and thus could be used to provide longer pre-exposure to 3-AP prior to gemcitabine in future trials. Future studies of 3-AP followed by the longer gemcitabine infusions (10 mg/m$^2$/min for 150 min) are also under consideration, based on studies indicating that the longer gemcitabine infusion schedules produce higher intracellular gemcitabine triphosphate concentrations, and possibly greater antitumor activity [48].

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