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A phase I study of prolonged infusion of triapine in combination with fixed dose rate gemcitabine in patients with advanced solid tumors

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Summary

Purpose—Prolonged exposure of cancer cells to triapine, an inhibitor of ribonucleotide reductase, followed by gemcitabine enhances gemcitabine activity in vitro. Fixed-dose-rate gemcitabine (FDR-G) has improved efficacy compared to standard-dose. We conducted a phase I trial to determine the maximum tolerated dose (MTD), safety, pharmacokinetics (PK), pharmacodynamics (PD), and preliminary efficacy of prolonged triapine infusion followed by FDR-G.

Experimental Design—Triapine was given as a 24-hour infusion, immediately followed by FDR-G (1000 mg/m² over 100-minute). Initially, this combination was administered days 1 and 8 of a 21-day cycle (Arm A, triapine starting dose 120 mg); but because of myelosuppression, it was changed to days 1 and 15 of a 28-day cycle (Arm B, starting dose of triapine 75 mg). Triapine

steady-state concentrations (C_{ss}) and circulating ribonucleotide reductase M2-subunit (RRM2) were measured.

Results—Thirty-six patients were enrolled. The MTD was determined to be triapine 90 mg (24-hour infusion) immediately followed by gemcitabine 1000 mg/m² (100-minute infusion), every 2 weeks of a 4-week cycle. DLTs included grade 4 thrombocytopenia, leukopenia and neutropenia. The treatment was well tolerated with fatigue, nausea/vomiting, fever, transaminitis, and cytopenias being the most common toxicities. Among 30 evaluable patients, 1 had a partial response and 15 had stable disease. Triapine PK was similar, although more variable, compared to previous studies using doses normalized to body-surface-area. Steady decline in circulating levels of RRM2 may correlate with outcome.

Conclusions—This combination was well tolerated and showed evidence of preliminary activity in this heavily pretreated patient population, including prior gemcitabine failure.

Keywords

Triapine; Gemcitabine; Phase I; Clinical Trial

Introduction

Treatment for most advanced solid tumors with the currently available antineoplastic agents, such as the broadly active gemcitabine [1–7], is not satisfactory; and there is a clear need for new agents to improve the efficacy of these drugs. The mechanisms responsible for gemcitabine's anti-tumor effects, and for resistance to gemcitabine, have been investigated extensively [8–11]. Gemcitabine, a deoxycytidine analogue, requires uptake via a nucleoside transporter and is then converted intracellularly to the active metabolites difluorodeoxycytidine di- and tri-phosphate (dFdCDP, dFdCTP), by the enzyme deoxycytidine kinase (dCK). dFdCDP inhibits the M1 subunit of ribonucleotide reductase (RR), an enzyme that converts ribonucleotides to deoxyribonucleotides, thereby decreasing deoxynucleotides (such as deoxycytidine triphosphate, dCTP) available for deoxyribonucleic acid (DNA) synthesis [12]. dFdCTP competes with dCTP for incorporation into DNA, resulting in DNA strand termination and apoptosis.

Based on current understanding of its mechanism of action, the anti-cancer effects of gemcitabine could be enhanced by approaches leading to increased intracellular accumulation and incorporation into DNA. Early preclinical and phase I studies suggest that the accumulation of dFdCTP in peripheral blood mononuclear cells and circulating leukemic blasts was saturated when plasma gemcitabine concentrations exceeded 15–20 µmol/L [13]. Based on that knowledge, improvements of gemcitabine efficacy could be achieved by optimizing its uptake and subsequent activation in tumor cells. Administration of gemcitabine at a fixed-dose-rate (FDR) of 10 mg/m²/min rather than 30-minute bolus infusion typically achieves steady-state plasma levels of 15–20 µmol/L [14]. Tempero et al. conducted a randomized phase II study comparing the activity of FDR gemcitabine to standard 30-minute infusion in patients with advanced pancreatic cancer [15]. The FDR group had a higher response rate, improved survival and time to tumor progression, when compared to the standard group. Hematologic toxicity was the most frequently reported adverse event and occurred more frequently in the FDR group. The median intracellular concentration of dFdCTP in circulating mononuclear cells at the end of each infusion was measured to be 114 µmol/L in standard group vs. 336 µmol/L in the FDR group [15]. Of note, the results of a follow up 3-arm phase III trial suggested only a trend towards a survival benefit for FDR gemcitabine over standard gemcitabine, however the study was possibly underpowered [16].

The activity of the ribonucleotide reductase (RR) enzyme correlates with tumor growth rate [17] and overexpression of RR has been implicated as a mechanism of resistance to gemcitabine [18, 19]. In a number of preclinical experiments, it has been demonstrated that inhibition of cellular RR could enhance the anti-tumor activity of subsequently administered nucleoside analogues [20–24]. Triapine (3-Aminopyridine-2-carboxaldehyde thiosemicarbazone, 3-AP, triapine is a registered trademark of Vion Pharmaceuticals) is a new inhibitor of RR M2-subunit [25], which is significantly more potent than hydroxyurea, the currently available M2-subunit RR inhibitor, in vitro [9, 26] and shows antitumoral effects in hydroxyurea- and gemcitabine-resistant cell lines [9, 26, 27]. In addition, in vitro studies demonstrated that sequential administration of triapine followed by gemcitabine resulted in synergistic or additive antitumoral effects, and this synergism was most evident with prolongation of pre-exposure time to triapine, perhaps resulting in increased cellular and DNA uptake of gemcitabine [28]. Increase in pre-exposure to triapine from 4 to 8 and then 12 h, before administration of gemcitabine, resulted in increasing antiproliferative effects, in vitro. Few phase I clinical trials were conducted in advanced solid tumor malignancies to evaluate the pharmacokinetics and toxicities of triapine, as single-agent or in combination with standard-dose gemcitabine [29–32]. A study that looked at 96-hour triapine infusion defined the MTD to be 120 mg/m²/day every 2 weeks [31]. Triapine was shown to reach its steady state concentration (C_{ss}) within the first few hours of the infusion and this C_{ss} was maintained through the remainder of the infusion. In addition, at 80–160 mg/m² dose, the C_{ss} was within the range of the in vitro IC₅₀ for tumor cell lines (~ 1–2 μM). The phase I study of combined triapine and standard-dose gemcitabine established that triapine at a dose of 105 mg/m² (2–4 hour infusion) does not appear to alter the toxicity of gemcitabine at 1000 mg/m², when administered on days 1, 8 and 15 of a 28-day cycle [32].

Clinical data support improved efficacy of gemcitabine at FDR as compared to standard-dose. Preclinical evidence also demonstrates synergistic effects for triapine followed by gemcitabine administration, which is most evident with prolonged (at least 12 h) pre-exposure time to triapine. Triapine clearance has not been associated with body surface area (BSA) in previous studies using BSA normalized triapine dosing. Therefore, the current phase I study was initiated to study the tolerability of prolonged, non-BSA normalized, infusion of triapine over 24 h, immediately followed by FDR gemcitabine, in patients with advanced solid tumors.

Patients and methods

The study was conducted at the Ohio State University and approved by the Ohio State University institutional review board (IRB).

Eligibility criteria

Patients were required to be ≥ 18 years old with histologically or cytologically confirmed advanced solid tumor with no curable treatment option available. The lapse time between the last dose of prior treatment and enrolment on the study was 3 weeks for radiation therapy and 4 weeks for chemotherapy (6 weeks for nitrosoureas or mitomycin C). Prior treatment with gemcitabine was allowed, if given as a standard (30 min) infusion. Prior exposure to triapine or gemcitabine given at a fixed-dose-rate was not allowed. Patients had Eastern Cooperative Oncology Group performance status (ECOG PS) ≤ 2; life expectancy >12 weeks; leukocytes >3,000/μl; absolute neutrophil count >1,500/μl; platelets >100,000/μl; total bilirubin < institutional upper limit of normal (ULN); AST(SGOT)/ALT(SGPT) <2.5×ULN; serum creatinine within normal institutional limits, or creatinine clearance >60 mL/min, for patients with creatinine levels above institutional normal. Patients with high risk for glucose-6-phosphate dehydrogenase (G6PD) deficiency were screened and those with this diagnosis were excluded, because of the potential risk for severe

methemoglobinemia with triapine. Initially, there were no limitations for number of prior treatments (Arm A), but because of higher than expected myelosuppression, the protocol was amended (Arm B) and no more than 2 lines of systemic chemotherapy (not hormonal or targeted therapies), in the metastatic setting, was allowed. Patients with uncontrolled intercurrent illness, pregnant or breast-feeding females and HIV-positive patients receiving combination anti-retroviral therapy were excluded from the study.

Study design and treatment plan

This was a standard method phase I dose-escalation trial. Treatment was administered on an inpatient basis. Triapine was administered intravenously (i.v.) over 24 h, immediately (0–2 h) followed by gemcitabine i.v. infusion at a fixed rate of 10 mg/m²/min (100 min for 1000 mg/m²). Initially, the 24-hour triapine infusion, which was followed by gemcitabine, was started on days 1 and 8 of a 21-day cycle regimen (Arm A). The starting dose for triapine was 120 mg (dose level 1A), but after dose reductions to dose level -2A (75 mg) because of dose-limiting hematologic toxicities, the protocol was amended and the schedule was changed to a 28-day cycle regimen with treatment started on days 1 and 15 (Arm B). In addition, we limited the number of prior systemic chemotherapies (except for hormonal or targeted therapies) in the metastatic setting to 2, to minimize the risk of myelotoxicity. The starting triapine dose for Arm B was 75 mg (dose level 1B).

We followed a standard 3+3 phase I dose escalation trial. For each new dose level, up to three patients were entered. If none of the first three patients at a dose level developed a dose-limiting toxicity (DLT), patients were enrolled at the next higher dose level. If one of the first three patients developed a DLT, up to three more patients were enrolled at that same dose level. If two or more developed a DLT at the same dose, no more patients were enrolled at that dose level and dose escalation was halted, and the previous dose level was expanded to a total of six patients. The maximum tolerated dose (MTD) was defined as the maximum dose level with fewer than 2 of 6 patients experiencing DLT.

Only adverse events occurring in the first cycle of treatment were considered for evaluation of DLT. The National Cancer Institute Common Toxicity Criteria version 3.0 (CTCAE v3.0) was used to grade toxicities. DLT was defined as Grade 3 non-hematologic or Grade 4 hematologic adverse event with the following exceptions, which was not considered DLT: 1) Grade 3 nausea and Grade 3 vomiting that improved with antiemetic therapy; 2) Grade 3 diarrhea that improved with lomotil; 3) Grade 4 neutropenia that recovered to Grade 3 within 7 days of first identification. Colony-Stimulating-Factors use was allowed according to American Society of Clinical Oncology guidelines, after cycle 1.

Study monitoring and measurement of effect

Before study entry, patients were assessed with a complete history and physical examination (H&P), vital signs, scoring of ECOG PS, complete blood count with platelets and differential, complete serum chemistries including electrolytes, lactate dehydrogenase and liver function tests, serum pregnancy test in women of child-bearing potential, electrocardiogram (ECG), tumor staging studies including computed tomography (CT) scans as appropriate to the disease, G6PD deficiency screening by using standard clinical assays in high risk patients; i.e. patients with African, Asian or Mediterranean origin/ancestry. Considering the risk of acute reactions to triapine, including the risk of methemoglobinemia, patients were closely monitored (vital signs and pulse oximetry) during triapine infusion (every 30 min) and for 2 h (4 h for cycle one) after completion of infusion (every 60 min). A complete H&P and routine laboratory tests were performed on days 1 and 15 of each cycle. Toxicities were monitored and recorded continuously. Patients were evaluated for tumor response every 8 weeks (2 cycles), with full staging studies. The criteria for response

evaluation were based on Response Evaluation Criteria In Solid Tumors (RECIST) version 1.0 [33]. A complete or partial response was confirmed by repeat staging scans in 4 weeks. Patients with partial response and those with stable disease were eligible to continue treatment for 12 cycles, as long as they were tolerating treatment and no evidence of progression. Patients with complete response were to receive one cycle past the cycle where complete response had been documented.

Drug supply and administration

Triapine was supplied by Vion Pharmaceuticals, Inc. and distributed by the Division of Cancer Treatment and Diagnosis (DCTD, CTEP, NCI) to the investigators, in 10 mL amber vials, containing 50 mg of the drug. Prior to administration, triapine was diluted in 500 mL of 0.9 % sodium chloride to a final concentration of 0.01–1 mg/mL. This admixture has been found to be stable for 96 h, when protected from light and stored at room temperature or 2–8°C. Gemcitabine was obtained commercially and reconstituted in 0.9 % sodium chloride. Triapine was administered i.v. over 24 h, immediately (0–2 h) followed by gemcitabine i.v. infusion at a fixed rate of 10 mg/m²/min (100 min for 1000 mg/m²).

Pharmacokinetic studies

Whole blood was collected from each patient within 4 h prior to the end of the 24-hour triapine infusion on day 1 of the first cycle. Plasma was separated and stored at –70°C until analysis. Steady-state triapine levels post-infusion were measured by a validated liquid chromatography-mass spectrometry (LC-MS) assay. The LC-MS system consisted of a Thermo TSQ Quantum Ultra EMR triple quadrupole mass spectrometer and a Shimadzu Prominence HPLC system. Both triapine and internal standard 2-pyridinecarboxaldehyde thiosemicarbazone (2-PAT) were separated on a reverse phase C18 column by isocratic elution with 5 % acetonitrile containing 0.2 % acetic acid. Two parent and product ion pairs 196.2>121.2 and 181.2>106.2 were selected to monitor triapine and 2-PAT in multiple reaction monitoring mode, respectively. Due to strong hydrogen bonding and chelating capabilities of triapine with proteins and metal ions in human plasma [34, 35], EDTA (3 mM) was added to each sample to chelate metal ions prior to sample cleaning by cation exchange solid phase extraction. Plasma concentrations of triapine in patient samples were calculated using a calibration curve of triapine spiked into human plasma prior to extraction. Triapine concentrations were quantifiable between 1 ng/mL to 1000 ng/mL with acceptable precision and accuracy according to FDA guidelines [36]. Clearance (CL) of triapine was calculated as the ratio of infusion rate (dose/infusion time) to steady state concentration.

Quantification of serum human ribonucleotide reductase M2 subunit (hRRM2)

Circulating hRRM2 was evaluated as potential predictive biomarkers for triapine. Patients underwent blood draw immediately before triapine administration on day 1 and immediately after triapine infusion, but prior to gemcitabine infusion, on day 2 of cycles 1 and 2. Peripheral blood mononuclear cell (PBMC) and serum samples were separated and preserved at –70°C. Quantitative assessment of hRRM2 in patients' serum was performed by ELISA [37]. Polystyrene 96-well plates were coated with anti-hRRM2 antibody; at 2 µg/mL concentration, overnight at 4 °C. Unbound sites were blocked during overnight incubation with 2 % bovine serum albumin. Serum or pure RRM2 protein 10 µg/mL (positive control) were added and left to incubate at room temperature for 2 h. After several washes, these plates were incubated with Biotin labeled RRM2 10 ng/mL at room temperature for 1 h. After several washes, these plates were incubated with horseradish peroxidase-conjugated with streptavidin antibody for 0.5 h at room temperature. Plates were then washed and incubated with the substrate solution and spectrophotometric measurements were taken read at 450/630. All samples were measured in duplicate in 2 separate studies. Mean values for the 2 studies were used in the correlation. All washings

were performed 3 times with PBS using a Nunc Immunowasher (Thermo Scientific). All procedures were performed at room temperature.

Statistical considerations

The standard phase I 3+3 design was followed for dose escalation and 3–6 patients were entered per dose level. No intra-patient dose escalation was allowed. Summary statistics (i.e. median and range for continuous variables, and frequency for discrete data) were calculated for patient demographics. The maximum grade for each type of toxicity was recorded for each patient, and frequency tables were provided. Time to progression (TTP) was determined from the date of treatment start to progression. Overall survival (OS) was determined from the date of treatment start to death from any cause or censored at the date of off study. Median TTP and OS was estimated using Kaplan–Meier method. The association between PK parameters (CL or C_{ss}) and categorical variables were studied by using ANOVA. Pearson correlation and linear regression model were used to explore the association between PK parameters and continuous variables.

To evaluate the potential predictive or prognostic value of circulating hRRM2, the baseline hRRM2 levels and its changes during cycle 1, cycle 2, and throughout both cycles were correlated with the clinical outcomes of interest, such as TTP, OS, and disease control rate (DCR = partial response + stable disease). Survival curves were compared using the log-rank test. Fisher's exact test was used to compare DCR according to categorical baseline and changes in hRRM2 levels. Paired *t*-test was used to evaluate differences in hRRM2 levels at specific time-points (cycle 1 day 1 vs. cycle 1 day 2, cycle 2 day 1 vs. cycle 2 day 2).

Results

Patient characteristics

Thirty-six patients with a median age of 59 years were enrolled between July 2006 and April 2010 (Table 1). Two patients (5 %) received no prior chemotherapy and 8 (22 %) received 3 or more prior chemotherapies (up to 8). Fourteen patients (39 %) had received prior gemcitabine. Slightly over half of the patients (19 patients) had lung, pancreas or bladder primary cancers; and 14 patients (39 %) had 3 or more metastatic organ sites.

Adverse events

Table 2 describes dose escalation, DLTs and dose modifications for each dose level. In the initial dose escalation (Arm A), both triapine and gemcitabine were given on days 1 and 8 of a 21-day cycle regimen. No DLTs were observed for the first 3 patients at 120 mg triapine (starting dose level 1A). At 160 mg (dose level 2A), we observed a grade 4 neutropenia and a grade 3 fatigue and dyspnea. Dyspnea was not related to methemoglobinemia and it occurred in a patient with metastatic non-small cell carcinoma of the lung, who had 8 prior systemic chemotherapies, radiation and 2 systemic targeted therapies. Triapine dose was deescalated to 120 mg (level 1A), and one patient experienced grade 4 neutropenia and another patient had grade 4 leukopenia, neutropenia and thrombocytopenia. Therefore, triapine dose was again deescalated to 90 mg (dose level -1A). At this dose level, 2 out of 5 patient experienced DLTs, which were grade 4 leukopenia, neutropenia and thrombocytopenia in one patient and grade 4 neutropenia in the other. Another deescalation to 75 mg triapine was made (level -2A), and the first enrolled patient experienced the same DLTs of grade 4 neutropenia and thrombocytopenia. This patient, who had a diagnosis of metastatic small cell lung cancer with 3 prior systemic chemotherapies, also experienced somnolence, confusion, slurred speech, and tachycardia, after completion of triapine and gemcitabine infusion. Her ECG showed regular sinus tachycardia and her pulse oximetry was at 92 %. With a clinical suspicion of methemoglobinemia, she was started on i.v.

methylene blue. Other causative factors include recent change in her narcotic dosage. Her symptoms eventually resolved. Her head CT scan was non-specific and her methemoglobin measured 1.3 % (normal <1.3 %), which was not elevated at a clinically meaningful level (usually 10–15 %).

At this point, considering the significant hematologic toxicities that we observed with this schedule and knowing that a biweekly gemcitabine schedule is similar in efficacy and less myelosuppressive than weekly [38–40], we amended the trial and the schedule was changed to a 28-day cycle regimen with both drugs given on days 1 and 15 (Arm B). In addition, we limited the number of prior systemic chemotherapies (not hormonal or targeted therapies), in the metastatic setting, to 2 to minimize the risk of myelotoxicity. The starting dose for triapine in Arm B was 75 mg, the same as dose level -2A. At this dose level (1B), 1 out of 6 patients experienced DLT, which was grade 4 neutropenia. We enrolled 4 patients at dose level 2B (triapine 90 mg). One patient was replaced because of rapid progression of disease after day 1 of the first cycle and the other 3 did not experience DLT. At the next dose level (3B, 120 mg triapine), 2 patients were enrolled and both experienced DLTs. One patient had grade 4 leukopenia and neutropenia and the other one had grade 4 neutropenia and grade 3 infection (i.v. catheter-related cellulitis and pneumonia). Deescalation to dose level 2B was made and 3 additional patients were enrolled. One patient experienced grade 4 thrombocytopenia. The other 2 patients mistakenly received treatment at dose level 3B for day 1/cycle 1, and then received appropriate treatment at dose level 2B. Although, none experienced a DLT, both continued treatment on the trial, but were replaced. No further DLTs were noted, defining dose level 2B with 90 mg triapine as the MTD. At this point, we expanded this dose level and enrolled 6 more patients to further define the preliminary activity and safety of this dose level.

Treatment-related toxicities are summarized in Table 3. Nausea, vomiting, fatigue, transaminitis, fever and bone marrow suppression, resulting in anemia, thrombocytopenia, leukopenia, neutropenia and lymphopenia were the most common toxicities. A 74 year-old male with a diagnosis of metastatic gastroesophageal junction carcinoma, who had 3 cycles of chemotherapy on this trial, experienced sudden death, 7 days after the last treatment, thought to be possibly related to the treatment, although no autopsy was performed and this was not confirmed. Twenty-four patients (67 %) required dose delay or dose reduction throughout their treatment on this trial (Table 2).

Response to treatment

Out of 36 enrolled patients, 30 patients were evaluable for response and 6 patients were non-evaluable, mostly due to early removal from the study, secondary to toxicity. Out of 30 evaluable patients, 14 patients (47 %) had progressive disease, 15 (50 %) had stable disease and 1 (3 %) had partial response. The patient with partial response was a 62 year old male with metastatic cholangiocarcinoma of the gallbladder to the liver, who had no prior systemic chemotherapy. He was receiving triapine at the MTD (90 mg) and his partial response lasted for 6 months. The 15 patients with stable disease had a median time to progression of 111 days (95 % CI 84–115 days) and 7 of them had previously progressed on gemcitabine (single agent or combination) chemotherapy. In addition, most (12 patients) had prolonged stable disease more than 3 months. The most common primary sites with stable disease were pancreas (4 patients), lung (3 patients) and bladder (2 patients). One patient with metastatic endometrial adenocarcinoma, previously treated with paclitaxel and carboplatin, showed a 23 % tumor reduction, received 4 cycles and had a time to tumor progression of 202 days. Table 1S (supplement) shows the response to treatment data, number of received cycles, and prior treatment with gemcitabine for each patient. The median number of received cycles was 2, with a range of 1–8 cycles.

Pharmacokinetic analysis

End of infusion steady-state triapine concentrations (C_{ss}) were measured in 31 of the 36 patients enrolled in the study, including 13 and 23 patients treated in Arms A and B, respectively. The administered dose levels are described in Table 2. Plasma steady-state concentrations (C_{ss}) and calculated clearance (CL) of triapine are presented in Table 4. For Arm A, with triapine doses ranging from 75 to 160 mg, mean C_{ss} and CL were 0.43 ± 0.14 μM (rang, 0.24–0.82 μM) and 34.82 ± 10.78 L/hr (range, 13.54–48.23 L/hr), respectively. For Arm B, with triapine doses ranging from 75 to 120 mg, the mean C_{ss} and CL were 0.38 ± 0.14 μM (rang, 0.19–0.72 μM) and 33.64 ± 11.52 L/hr (range, 15.90–53.96 L/hr), respectively. Triapine C_{ss} and CL were comparable between Arms A and B, which was expected since triapine concentrations were determined at 24 h on day 1 prior to the time when treatment differed between the two Arms. With Arm A and B combined, the C_{ss} and CL were 0.40 ± 0.14 (0.21–0.82) μM and 33.72 ± 10.94 (13.54–53.96) L/hr, respectively, for 31 patients receiving 75 to 160 mg triapine over 24-hour i.v. infusion on Day 1.

Performance status (PS) was not significantly associated with either PK parameter, and Sex was found to significantly associate with C_{ss} ($p = 0.03$) but not with CL ($p = 0.14$). Females had higher mean C_{ss} (0.44 ± 0.16 μM , $n = 18$) compared to males (0.33 ± 0.08 μM , $n = 13$). But, when we controlled for the BSA, there was no significant association of C_{ss} and Sex ($p = 0.12$), considering that female patients had a significantly lower mean BSA ($p = 0.03$). We also evaluated Dose level as a categorical variable and found no significant associations with either C_{ss} or CL (p-values 0.69 and 0.21, respectively). Scatter plots for PK parameters vs. Dose are displayed in Fig. 1 (a and b). However, we did observe significant associations with CL versus both Weight and BSA when evaluated with linear regression (p-values 0.05 and 0.04, respectively). C_{ss} was not directly associated with either Weight or BSA (p-values 0.20 and 0.14, respectively), however, it was significantly associated with Dose normalized to both Weight and BSA (p-values 0.04 and 0.05, respectively) (Fig. 1c and d). Normalized doses were not associated with triapine CL (p-values 0.63 and 0.69, respectively). Neither C_{ss} nor CL were significantly associated with Age (p-values 0.16 and 0.06, respectively), although data suggested increasing Age may trend with decreasing CL.

We also evaluated pharmacokinetics against Cycle 1 pretreatment values for serum creatinine, creatinine clearance, total bilirubin, albumin, total protein, and hemoglobin using linear regression. Among all lab variables evaluated, only one significant association was identified, which was a positive correlation between C_{ss} and hemoglobin ($p = 0.02$).

Pharmacodynamic correlates

Circulating hRRM2 data were obtained from 28 patients with complete data from both cycles 1 and 2 obtained for 19 patients. Twenty one patients (75 %) had decline in hRRM2 levels during treatment cycles 1 and 2, with a significant decrease in hRRM2 following treatment on cycle 1 for all patients ($p = 0.0003$), regardless of their RECIST response; but no significant change with cycle 2 ($p = 0.06$). Data regarding the relationship between circulating hRRM2 and clinical outcomes are summarized in Table 5. There was no association between baseline hRRM2 level and DCR, TTP, or OS. Decline in hRRM2 level during cycle 1 was significantly predictive of improved OS ($p = 0.0002$) but not TTP or DCR. Continuous decline in hRRM2 during the first 2 treatment cycles was a significant predictor of DCR ($p = 0.03$), and median TTP ($p = 0.02$), but not median OS ($p = 0.21$). There was no significant correlation between C_{ss} and changes in RRM2 levels for cycle 1 ($p = 0.30$) or maximum changes of hRRM2 levels across cycles 1 and 2 ($p = 0.73$). Table 2S (supplement) shows the details of hRRM2 expression levels and their changes at different time-points throughout the treatment, for each patient.

Discussion

This phase I study was designed to establish the MTD for prolonged (24-hour) infusion of triapine followed by fixed-dose-rate gemcitabine; to determine the safety and tolerability of this combination; to examine the preliminary antitumoral activity of this combination; and to perform limited pharmacokinetic and correlative pharmacodynamic analysis.

This combination was generally well tolerated at the MTD with myelosuppression being the main DLT, as expected in this heavily pretreated patient population. The other common toxicities, such as nausea, vomiting, fatigue, and transaminitis were similar in incidence to the reported toxicities expected with gemcitabine alone. Interestingly, we did not observe any methemoglobinemia or cardiopulmonary toxicity, such as hypotension, hypoxia, and respiratory distress. The MTD on this study was determined to be triapine 90 mg (24-hour infusion) immediately followed by gemcitabine 1000 mg/m² (100-minute infusion), every 2 weeks of a 4-week cycle. Thirty patients were evaluable for response on this study. We observed a disease control rate of 53 % (50 % SD and 3 % PR) and a nearly 4 months median time to tumor progression for patients with SD. About half of these patients had prior disease progression on standard gemcitabine, suggesting preliminary evidence of synergism between fixed-dose-rate gemcitabine and 24-hour infusion triapine that may overcome gemcitabine resistance.

We initially observed significant hematologic toxicities on the Arm A of the protocol, when there was no limitations on prior number of chemotherapy regimens and patients were receiving treatment on days 1 and 8 of a 21-day cycle regimen. After the protocol was amended and the number of prior cytotoxic therapies was limited to 2 or less and the regimen was changed to days 1 and 15 of a 28-day cycle regimen, this combination was much better tolerated with significantly less myelosuppression. Out of 15 patients, who were treated at MTD, 8 required a dose delay or dose reduction at some point throughout their treatment and none of them came off treatment because of toxicity. Single agent triapine, like other ribonucleotide reductase inhibitors, can result in reversible myelosuppression [41], which can explain the higher rate of myelosuppression observed with this combination, as compared to gemcitabine alone. We need to mention that there was a sudden death on this trial, which occurred 7 days after the last treatment on cycle 3 in a 74 year-old male with a diagnosis of metastatic gastroesophageal junction carcinoma. Although no autopsy was performed and given its timing, this was thought to be “possibly” related to both the treatment and the disease.

In contrast, prior to our study other phase I/II trials of this combination with different dose/schedule [32, 42–44], reported more significant toxicities including hypoxia, methemoglobinemia and significant cardiopulmonary toxicity. The initial phase I trial defined the MTD as triapine at 105 mg/m² infused over 2–4 h followed by gemcitabine at 1000 mg/m² over 30 min (standard-dose), on days 1, 8, and 15, for a 28-day cycle [32]. This recommended phase II dose was tested in 4 different phase II solid tumor trials including 2 in non-small cell lung cancer, 1 in advanced pancreas cancer and 1 in biliary cancer [42–45]. Taken together, the only observed responses were in 3 patients with biliary cancers. Of note, in our trial, the only patient with a meaningful PR had metastatic biliary tract carcinoma.

Previous PK studies in humans indicated triapine had linear PK behavior when doses were normalized to BSA [30, 31, 46]. However, significant inter-patient variability and lack of associations between weight or BSA and triapine CL suggested dose normalization may not be required and may contribute to inter-patient variability. One aim of our study was to evaluate the triapine C_{ss} and clearance using a dosing regimen without normalization to body size. As with the previous studies, we observed no significant association of triapine

dose with CL (Fig. 1b). However, we did not observe a clear linear trend for triapine dose and C_{ss} (Fig. 1a). Variability observed for C_{ss} and CL was approximately 30 % coefficient of variation, both within and between dose groups (Table 4). This is not drastically different than the variability reported in the previous studies. However, linearity across dose groups was evident, especially in the report by Murren et al. [30]. Furthermore, in our study, triapine CL was found to be significantly associated with patient weight and BSA, and C_{ss} was significantly associated with dose normalized to weight or BSA. Also, we observed gender was associated with C_{ss} , but this is likely due to female patients having smaller body habitus (BSA) compared to male patients in our study; considering that this correlation was no longer significant when we control for BSA.

The total body CL of 33.72 ± 10.94 L/hr was higher relative to the population estimated CL of 25 L/hr in patients with advanced primary or metastatic tumors, published by Kolesar et al. [47]. The steady-state triapine serum concentrations measured in our study were below mean in vitro tumor growth inhibitory concentrations across multiple cell types (mean GI_{50} 1.6 μ M in a 48-h exposure assay), as previously reported [26]. This may suggest systemic concentrations of triapine were not sufficient to produce the desired hRRM2 inhibitory effects. In fact, cycle 1 hRRM2 levels were inhibited only by an average of 4 % relative to baseline in our study. More investigation will be required to understand why triapine clearance in our study was higher compared to the previous reports in solid tumor patients. This could be related to the differences in the respective assays used for triapine quantification (LC-MS in our study vs. LC-UV in the previous study) [46]. Alternatively, it may reflect differences in triapine volume of distribution or clearance in the respective patient populations. Interestingly, we observed C_{ss} was significantly associated with patients' hemoglobin. As triapine is a metal chelator and has been clearly shown to bind iron, hemoglobin may be an important variable in triapine disposition.

Additionally, we evaluated circulating hRRM2 as a potential predictive biomarker for the clinical efficacy of the combination of triapine and gemcitabine. We show that baseline hRRM2 levels do not appear to predict clinical outcomes. Study treatment resulted in a significant decline in hRRM2 levels with cycle 1. This decline did not predict the response but did correlate with survival. Additionally, our data indicates that continuous decline in circulating hRRM2 during cycles 1 and 2 of treatment correlates with disease control. These results suggest that steady decline in circulating hRRM2 levels may correlate with outcome. These interesting findings need to be explored in future trials to confirm the potential use of circulating hRRM2 on clinical outcomes with the combination of triapine and gemcitabine.

In conclusion, our study defined a safe and tolerable recommended phase II dose (RP2D) of triapine 90 mg (24-hour infusion) immediately followed by gemcitabine 1000 mg/m² (100-minute infusion), every 2 weeks of a 4-week cycle. This RP2D should be tested in future prospective trials with gemcitabine and triapine in various solid tumors, more specifically in patients with biliary cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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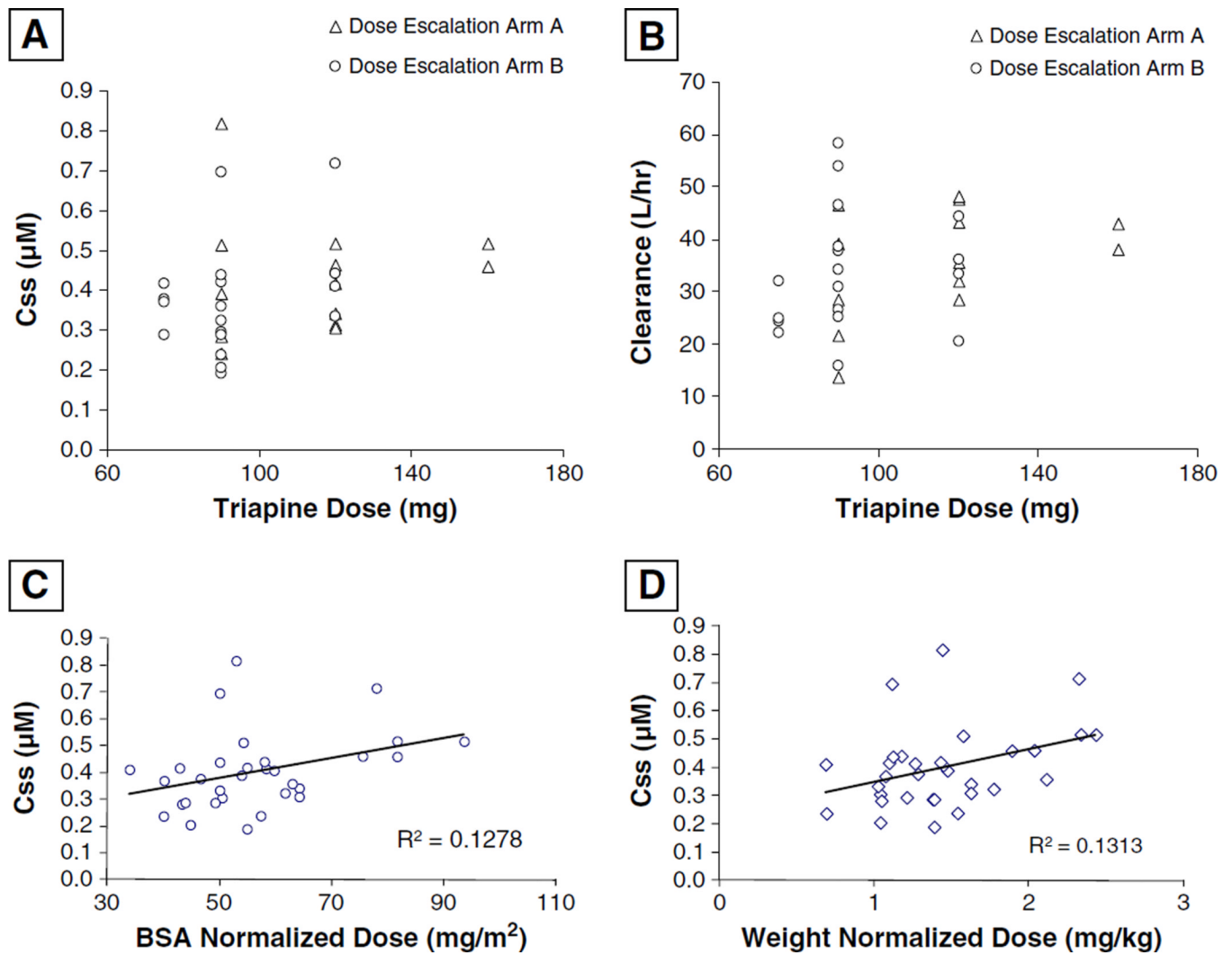


Fig. 1. Plots of triapipe steady-state serum concentrations (C_{ss}) (a) and calculated clearance (b) against dose. Correlative plots of C_{ss} with normalized dose based on body surface area (BSA) (c) and weight (d)

Table 1

Patient characteristics

Characteristics	Number of Patients
Total of Patients Enrolled	36
Median Age (range) in Years	59 (38–81)
Gender	
Female	21
Male	15
ECOG PS ^a	
0	10
1	23
2	3
Primary Tumor Site	
Lung	8
Pancreas	6
Bladder	5
Breast	4
Gallbladder	3
Others ^b	10
Sites of Metastases	
Liver	21
Lung	14
Bone	8
Adrenal Glands	6
Lymph Nodes	16
Soft Tissues	5
Others	7
Number of Metastatic Sites	
1–2	22
3–4	14
Number of Prior Systemic Chemotherapies	
0–2	28
>2 (Up to 8)	8

^aECOG PS: Eastern Cooperative Group performance status

^bOthers: Colon, Rectum, Anus, Stomach, Gastroesophageal Junction, Gastrointestinal Stroma (GIST), Merkel Cell, Cervix, Uterus Endometrium, Unknown Primary

Table 2

Treatment by dose level

Dose Level	Triapine 24-hours i.v. Infusion (mg)	Gencitabine i.v., Fixed-Dose-Rate (mg/m ²)	No. of Patients	No. with DLT ^a	No. with DR ^b or DD ^c	Description of DLTs
-2A	75	1000	1	1	1	Neutropenia, Thrombocytopenia
-1A	90	1000	5	2	4	Neutropenia, Leukopenia, Thrombocytopenia
1A	120	1000	5	2	4	Neutropenia, Leukopenia, Thrombocytopenia
2A	160	1000	2	2	2	Neutropenia, Fatigue & Dyspnea (Grade 3)
1B	75	1000	6	1	4	Neutropenia
2B ^d	90	1000	15	1	8	Thrombocytopenia
3B	120	1000	2	2	1	Neutropenia, Leukopenia, Infection (Grade 3)

^aDLT: Dose limiting toxicity^bDR: Dose reduction^cDD: Dose delay^dIncluding expansion cohort of 6 patients at this dose level

A: Arm A of the study, during which, both drugs were given on days 1 and 8 of a 21-day cycle regimen

B: Arm B of the study, during which, both drugs were given on days 1 and 15 of a 28-day cycle regimen

Table 3

Adverse events

Adverse events	Grade 1/2 N (%)	Grade 3 N (%)	Grade 4 N (%)
Anorexia	5 (14)	2 (6)	0
Fatigue	23 (64)	10 (28)	0
Skin/Rash	9 (25)	1 (3)	0
Nausea/Vomiting	24 (67)	1 (3)	0
Myalgia	7 (19)	2 (6)	0
Infection ^a	2 (6)	3 (8)	0
Dyspnea	2 (6)	3 (8)	1 (3)
Altered Taste	5 (14)	0	0
Pruritus	7 (19)	0	0
Diarrhea	6 (17)	1 (3)	0
Fever	15 (42)	0	0
Febrile Neutropenia	0	2 (6)	0
Chills	8 (22)	0	0
Flu-Like Syndrome	4 (11)	0	0
Leukopenia	15 (42)	13 (36)	5 (14)
Neutropenia	9 (25)	7 (19)	14 (39)
Thrombocytopenia	17 (47)	3 (8)	6 (17)
Anemia	17 (47)	18 (50)	0
Lymphopenia	14 (39)	10 (28)	3 (8)
Transaminitis	15 (42)	1 (3)	0

^aThree cellulitis, one pneumonia, and one urinary tract infection One sudden death (possibly treatment-related), on cycle 3 of treatment at dose level 1B

Table 4

Steady-state triapine levels and calculated triapine clearance

Arm A Scheme			
Dose (mg/m ²)	N	C _{ss} ^a (μM) ^b	CL ^c (L/hr) ^b
75	1	0.41	22.42
90	5	0.45±0.23	29.82±13.19
120	5	0.39±0.09	39.18±8.33
160	2	0.52 and 0.46	38.09 and 42.80
Mean ± SD		0.43±0.14	34.82±10.78
Arm B Scheme			
Dose (mg/m ²)	N	C _{ss} (μM)	CL (L/hr)
75	4	0.36±0.05	25.88±4.28
90	10	0.35±0.15	36.76±13.24
120	4	0.47±0.17	33.62±9.77
Mean ± SD		0.38±0.14	33.64±11.52
Arm A and B Combined Dose (mg/m ²)			
Dose (mg/m ²)	N	C _{ss} (μM)	CL (L/hr)
75	5	0.37±0.05	25.19±4.01
90	15	0.38±0.18	34.45±13.18
120	9	0.44±0.12	35.77±8.53
160	2	0.52 and 0.46	38.09 and 42.80
Mean ± SD (Range)		0.40±0.14 (0.21 →0.82)	33.72±10.94 (13.54→ 53.96)

^aC_{ss}: Serum steady-state concentrations^bValues are mean ± SD^cCL: Calculated triapine clearance

Table 5

Relationship between circulating hRRM2 and clinical outcomes

hRRM2 ^a Levels	DCR ^b % (N=25)	p-value	mTTP ^c (m) (N=28)	95 % CI ^d	p-value	mOS ^e (m) (N=28)	95 % CI	p-value
Baseline level								
>2.41 ^f	50	1.0	4.3	(1.6, 5.0)	0.68	7.9	(6.3, 9.3)	0.94
<2.41	50		3.1	(1.7, 4.6)		8.5	(1.4, 17.2)	
↓with cycle 1								
Yes	60	0.24	3.8	(1.9, 5.0)	0.21	8.5	(6.3, 10.7)	0.0002
No	25		2.4	(1.0, 4.8)		2.1	(1.1, 7.3)	
Continued ^g								
Yes	100	0.031	8.1	(2.9, 8.1)	0.021	9.3	(8.5, 9.3)	0.21
No	37.5		3.0	(1.7, 3.9)		7.3	(3.7, 8.2)	

^ahRRM2.0 Human ribonucleotide reductase M2 subunit^bDCR 0 Disease control rate, which is partial response + stable disease^cmTTP 0 Median time to progression (months)^d95% CI 0 95 % confidence interval^emOS = Median overall survival (months)^f2.41 is the median value for hRRM2 levels^gDenotes continued decline from first measurement on cycle 1 day 1 to last measurement on cycle 2 day 2