A phase II trial of Triapine® (NSC# 663249) and gemcitabine as second line treatment of advanced non-small cell lung cancer: Eastern Cooperative Oncology Group Study 1503

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Summary

Background—The objective of ECOG 1503 was to determine the response rate of this combination in the second-line treatment of advanced NSCLC.

Methods—Triapine 105 mg/m^2 IV on days 1, 8, and 15, and gemcitabine 1,000 mg/m^2 on days 1, 8, and 15, of a 28 day cycle.

Results—Eighteen patients enrolled. Three patients were not eligible due to protocol violations. No objective antitumor responses were seen. Three patients (20%) experienced stable disease (90% CI 5.7–44%). Median overall survival: 5.4 months (95% CI 4.2–11.6 months); median time

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to progression: 1.8 months (95% CI 1.7–3.5 months). Five patients developed acute infusion reactions to Triapine® related to elevated methemoglobinemia. Patients with *MDR1* variant genotypes of C3435T experienced superior overall survival compared to non-variants (13.3 vs. 4.3 months, respectively, *p*=0.023).

Conclusion—This regimen did not demonstrate activity in relapsed NSCLC. Prolonged survival seen with *MDR1* variant genotypes is hypothesis-generating.

Keywords

Non-small cell lung cancer; Combination chemotherapy; Ribonucleotide reductase; Single nucleotide polymorphism; ATP binding cassette transporter

Introduction

Investigation of more effective agents and combinations for the treatment of non-small cell lung cancer (NSCLC), the leading cause of cancer-related mortality worldwide, remains paramount. Gemcitabine has well-established anti-tumor activity in the first and second line settings, as well as possible utility as maintenance therapy immediately following completion of first line treatment [1–3]. Its low toxicity profile makes it an ideal candidate for combination therapy.

Triapine® (3-aminopyridine-2-carboxaldehyde thiosemicarbazone, 3-AP, NSC# 663249, Vion Pharmaceuticals, Inc., New Haven, CT) is a potent inhibitor of the M2 subunit of ribonucleotide reductase (RRM2), an enzyme that catalyzes the conversion of ribonucleotide diphosphates to their deoxyribonucleotide counterparts and is, hence, critical for DNA synthesis [4,5]. Triapine® is an iron chelator that inhibits the enzymatic activity at the tyrosyl radical of the M2 subunit, and has demonstrated preclinical antitumor activity in models of NSCLC [5,6]. The sequential exposure of NSCLC cell lines to Triapine®, followed by gemcitabine, an inhibitor of the M1 subunit of ribonucleotide reductase (RRM1), has been examined [7,8]. Pretreatment with Triapine® resulted in increased cellular uptake of gemcitabine, synergistic growth inhibition, increased gemcitabine triphosphate pools, and enhanced incorporation of gemcitabine into DNA [7,8]. A combination phase I trial of these agents by Yen et al. identified a recommended phase II dose of 105 mg/m^2 of Triapine® followed 2 to 4 h later by gemcitabine at 1,000 mg/m^2, on days 1, 8, and 15 of a 28 day cycle [9]. One complete response (unknown primary) and two partial responses (bronchioloalveolar carcinoma and esophageal carcinoma) were observed. Pharmacokinetic studies revealed no drug–drug interactions. Treatment-related toxicities included acute reversible hypoxia in three patients felt to be secondary to methemoglobinemia and EKG changes, such as non-specific ST-T wave changes and mild QT$_c$ prolongation. In its function as an iron chelator, Triapine® may be redox active, and thus, may secondarily catalyze the formation of methemoglobin. Therefore, Yen et al. recommended that patients with diminished pulmonary function or a history of G6PD deficiency be excluded from treatment with this combination due to their impaired ability to tolerate Triapine®-related transient hypoxia caused by methemoglobinemia [9].

The ATP-binding cassette transporter superfamily is critical in effecting multidrug resistance by promoting efflux of chemotherapeutics out of tumor cells [10,11]. Two members of this superfamily include P-glycoprotein (P-gp), a product of the multidrug resistance 1 (*MDR*) gene, and a 190 kDa membrane transport protein termed multidrug resistance protein (MRP1). Triapine® appears to be a substrate of P-gp and MRP1 [12]. Single nucleotide polymorphism (SNP) germline variations of *MDR1* and MRP1 may prove

clinically relevant, since certain variations may be associated with reductions in P-gp function [13,14–18].

We sought to describe the clinical efficacy and tolerability of combination therapy with Triapine® and gemcitabine in the second line setting of advanced NSCLC. In addition, we hypothesized that polymorphic variations of *MDR1* and MRP1 may impact the clinical efficacy and toxicity associated with Triapine®.

Patients and methods

Patient selection

Patients at least 18 years of age with histologically confirmed advanced (stage IIIB with pleural or pericardial effusion, stage IV, or recurrent) NSCLC whose disease had progressed during or after treatment with no more than one prior cytotoxic combination chemotherapy regimen and who gave informed consent according to institutional and FDA guidelines were eligible for this study provided that the following criteria were met: Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1; brain metastases, if present, must have been clinically stable after treatment with surgery and/or radiotherapy; adequate bone marrow, liver and renal function; life expectancy of at least 3 months; measureable disease per RECIST criteria; no prior treatment with gemcitabine, no treatment with chemotherapy or radio-therapy within 3 weeks of enrollment and must have recovered from the adverse effects of prior treatment to baseline or grade 1 or less; no other active malignancy; absence of HIV positivity receiving combination antiretroviral therapy; and no uncontrolled intercurrent illness, including dementia, active psychosis, pulmonary disease requiring supplemental oxygen, history of myocardial infarction within the prior 6 months or active cardiac disease. Further, nursing or pregnant women, persons with a history of G6PD deficiency, persons with a history of allergic reactions attributed to compounds of similar chemical or biologic composition to Triapine® or gemcitabine, or persons who used any investigational agent in the month prior to enrollment were excluded. This protocol was approved through institutional ethics review boards of participating ECOG sites.

Treatment plan

Triapine® was supplied by Vion Pharmaceuticals, Inc. (50 mg/vial; 5 mg/ml) and distributed by the Cancer Therapy Evaluation Program, the Division of Cancer Treatment and Diagnosis, National Cancer Institute. Triapine® was further diluted in D5W (final concentration between 0.01–2 mg/ml) in non-polyvinyl chloride (non-PVC) bags and administered as a 2 h infusion through non-PVC tubing.

Treatment consisted of Triapine® 105 mg/m^2 IV over 2 h on days 1, 8, and 15 and gemcitabine 1,000 mg/m^2 over 30 min on days 1, 8, and 15, of a 28 day cycle. Gemcitabine was administered between 4 and 8 h after completion of the Triapine® infusion. Treatment was given only if the ANC≥1,500/μL and platelets ≥100,000/μL on the first day of a new cycle. Dose modifications and delays were specified in the protocol. Treatment was continued for a maximum of six cycles until disease progression, unacceptable toxicity, or withdrawal of consent. Patients who required more than two dose reductions due to toxicity were removed from the study. All toxicities (except alopecia) must have resolved to grade 1 or less prior to the start of the next cycle. All dose reductions were permanent. Patients were to be followed until disease progression or for a maximum of 2 years from the date of registration.

Triapine® iron complexes are believed to be redox active, and thus, may secondarily catalyze hemoglobin oxidation to methemoglobin. Clinical monitoring for methemoglobinemia was performed every 30 min during the Triapine® infusion on day 1 of

cycle 1, and every 60 min for up to 4 h after completion of the infusion. Isolated, mild hypoxemia was treated with supplemental oxygen. The Triapine® infusion was stopped if moderate or severe dyspnea or hypotension (SBP <85 mm Hg) developed. Asymptomatic methemoglobinemia (<5%) did not prompt dose reduction. Patients were removed from protocol treatment with persistent symptoms of hypoxia, dyspnea, or hypotension, or persistent, elevated methemoglobinemia (>15%).

Disease assessment

The objective antitumor response rate (RR) was determined using computed tomography imaging at baseline and after every other cycle of treatment. Bone scans and brain imaging were performed only if clinically indicated. Additional baseline assessment included a history and physical, complete blood count, comprehensive chemistry panel, serum iron studies, ECOG PS, an electrocardiogram, a methemoglobin level, and screening of high risk groups (patients of African, Asian, or Mediterranean origin/ancestry) for G6PD deficiency. In addition, a complete blood count with differential was obtained on days 8 and 15 of each cycle. On day 1 of cycle 1, a methemoglobin level was obtained prior to treatment, at the end of the Triapine® infusion, and then just prior to the gemcitabine administration.

MDR1 genotyping

The methods of assaying peripheral blood at baseline for the common *MDR1* and MRP1 polymorphisms (C1236T, G2677T, and C3435T) have been described [14].

Statistical considerations

The primary endpoint of this study was the best overall confirmed objective RR, defined as the percentage of patients experiencing complete responses (CRs) or partial responses (PRs). Secondary objectives included the rate of stable disease (SD), time to disease progression (TTP), duration of response, overall survival (OS), and safety and tolerability of this combination. All treated patients were evaluable for toxicity assessments. Eligible patients must have received treatment to have been evaluable for response and survival.

In this phase II trial, a true objective RR of at least 25% was considered evidence for further exploration of this regimen. A true RR≤5% was considered evidence of minimal activity not worthy of further study. A two-stage design was employed to terminate the study early if the combination appeared to elicit minimal response. The first stage planned to accrue 20 patients, with 18 patients expected to be eligible. If at least two responses were observed among the initial 18 eligible patients, 28 additional patients were to be entered assuming 25 would be eligible. This regimen would be considered worthy of further study if at least seven total responses were observed among the 43 eligible patients. If the RR of Triapine® and gemcitabine was at least 25%, this design yielded a 92% chance of recommending the regimen for further study. For a response rate of less than 5%, the probability of accepting the regimen was less than 0.005.

Response was evaluated using RECIST criteria. Patients who were unevaluable for response were included in the denominator when computing the rates of RR and SD. Duration of response was defined as the time from the onset of response (CR or PR, whichever status was recorded first) to first documentation of disease progression. Patients with responses but without documented disease progression were censored at the time of last disease evaluation. Patients without responses were excluded from this analysis.

Overall survival was defined as the time from registration to death from any cause. Patients who were alive were censored at the date last known alive. Time to progression was defined as the time from registration to first documentation of disease progression. Patients without

documented progression were censored at the time of last known free of progression. If such a date was not available, patients were censored at the time of registration. Toxicities were graded per the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.

Exact binomial 90% confidence intervals (CIs) were computed for the rates of RR (CR + PR) and SD. The Kaplan and Meier method was used for event–time distributions. Descriptive statistics were used to characterize patient demographics, disease characteristics, and adverse events. Fisher's exact test was used to evaluate associations between genotype and toxicity for *MDR1* and MRP1 polymorphisms. The log-rank test was employed to examine the differences in OS/TTP between genotypes for various *MDR1* and MRP1 polymorphisms. All *p*-values are two-sided. A level of 5% was considered statistically significant.

Results

Patient characteristics and treatment

A total of 18 patients enrolled between July 2004 and January 2005. The study was closed at the first interim analyses in August 2005 due to a lack of efficacy. Table 1 displays the patient demographics. The median age was fairly young, at 61.1 years (range 53.3–79.6). Eighty percent of the patients were male. The median time from completing prior chemotherapy was 4.1 months (range 0.8–14.4 months). Two-thirds of the patients had received prior radiation therapy. All patients received protocol treatment. Nevertheless, among those enrolled, three were ineligible (two patients had baseline scans done prior to the study-defined interval, and the third patient had been treated with more than one prior chemotherapy regimen). Consequently, all efficacy analyses were based on 15 eligible, treated patients. Toxicity analyses included all 18 treated patients. The median number of cycles administered was two. No patients completed the maximum six cycles of treatment. Six patients discontinued treatment due to disease progression, six due to toxicity, two due to patient refusal, and one due to non-compliance.

Toxicity

Table 2 lists toxicities of all grades at least possibly related to study treatment that affected at least two patients. No patients died on study. The most common grade 3/4 toxicities were leucopenia (grade 3–8 patients; grade 4–1 patient), neutropenia (grade 3–8 patients; grade 4–2 patients), acute, reversible hypoxia (grade 3–4 patients), dyspnea (grade 3–2 patients; grade 4–1 patient), fatigue (grade 3–3 patients), and vomiting (grade 3–2 patients). There were no episodes of febrile neutropenia. Five patients experienced acute reactions to Triapine® during the initial infusion. These reactions were characterized by reversible elevations in methemoglobin, transient chest tightness, and hypoxia, reaching grade 3 in three of the five patients. Two of these three patients were removed from protocol treatment. In total, six patients discontinued treatment due to adverse events experienced while on study. These events included leukopenia, neutropenia, and hypoxia stemming from acute reactions to Triapine® infusions.

Treatment efficacy

No patient experienced either a CR or a PR. Eight patients (53%) experienced disease progression, while three patients (20%; 95% CI 5.7–44.0%) had SD. Four patients (27%) were unevaluable for response: one withdrew from study treatment prior to the first disease assessment, two patients had disease assessment scans performed too early, and one patient had inadequate follow-up measurements on repeat imaging and then died.

Fourteen of the 15 eligible patients have died. Twelve patients eventually experienced disease progression. The three patients without disease progression were missing disease assessment data at their last disease assessment, and were censored at the time of registration. Figure 1 displays the median OS of 5.4 months (95% CI 4.2–11.6 months) and Fig. 2 displays the median TTP of 1.8 months (95% CI 1.7–3.5 months).

MDR1 and MRP1 genotyping

Genotyping for *MDR1* polymorphisms C1236T, G2677T, C3435T, and MRP1 G>C was performed on 14 of 15 eligible patients. *MDR1* and MRP1 polymorphism frequencies were consistent with Hardy-Weinberg predictions: C1236T (CC: 0.14; CT: 0.64; TT: 0.22), G2677T (GG: 0.14; GT: 0.64; TT: 0.22), C3435T (CC: 0.14; CT: 0.43; TT: 0.43), MRP > G/C (GG: 0.64; GC: 0.22; CC: 0.14).

For the analyses of survival and toxicity, genotypes were grouped as variant (homozygous) versus non-variant (heterozygous and wild-type). Patients with variant genotypes of C3435T experienced statistically superior OS compared to non-variants (median OS 13.3 vs. 4.3 months, respectively, *p*=0.023, as seen in Fig. 3). Fisher's exact test indicated that non-variant MRP1 genotypes (67%) developed grade ≥3 gastrointestinal toxicity (anorexia and vomiting) more frequently than variants (*p*=0.033). No statistically significant difference in TTP or any other incidence of toxicity, including acute reactions to Triapine® infusions, was observed between genotypes for any polymorphism. Due to the small number of patients who underwent *MDR1* and MRP1 genotyping (*N*=14), these analyses were exploratory in nature. Further, no statistical adjustment for the multiple comparisons was performed.

Discussion

Eastern Cooperative Oncology Group trial 1503 was closed at its first interim analyses due to lack of efficacy. This lack of efficacy was also seen in Ma, et al., a multicenter trial of this regimen in this same population performed in Hong Kong and Singapore [19]. Time to progression in Ma, et al. was slightly longer, at 3.3 months.

Further, our regimen proved moderately toxic, with 6 of 15 patients discontinuing treatment due to toxicity. Neutropenia appeared more commonly in our study, compared to either the phase I study of this combination or Ma, et al., possibly related to prior myelosuppresive radiation therapy that two-thirds of our patients had received [9,19]. Further, five patients (28%) developed acute, symptomatic infusion reactions to Triapine®, consisting of reversible elevated methemoglobinemia, chest tightness, and hypoxia. This syndrome was described in the phase I experience of this regimen, such that those investigators recommended that patients with poor pulmonary, or G6PD deficiency, not receive treatment [9]. Despite the fact that these exclusion criteria were included in ECOG 1503, 28% of our patients still developed this Triapine® infusion reaction. This syndrome was also seen in one-third of patients enrolled in Ma, et al., strongly suggesting that this population is not suited for this regimen [19].

A growing body of evidence suggests that ribonucleotide reductase expression may influence response and survival in a number of malignancies. Over-expression of RRM1 in in vitro and in vivo models of lung cancer was associated with a less malignant phenotype and suppression of tumor growth [20,21]. This was evident clinically, as survival increased correspondingly with RRM1 levels in resected specimens of NSCLC [22]. RRM1 is also the predominant cellular determinant of efficacy of gemcitabine, in that over-expression is associated with in vitro drug resistance [23,24]. Likewise, clinical response to gemcitabine correlates inversely with pretreatment RRM1 expression [25]. However, data examining the

relationship between expression of RRM2 and treatment outcomes are less consistent, with laboratory experiments that both increase or decrease RRM2 expression yielding increased sensitivity of cancer cell lines to gemcitabine [26,27]. This contradiction may relate to the fact that, while expression of RRM1 remains constant through the cell cycle, the expression of the RRM2 gene is tightly controlled during cell cycle progression, with a sharp induction at the end of the G1 phase [24]. Thus, inconsistencies in the timing of sampling may contribute to the seemingly conflicting predictive value of RRM2 seen thus far. Clinically, Souglakos et al. found that low levels of RRM2 mRNA were associated with response to treatment of patients with NSCLC to gemcitabine and docetaxel [28]. The value of RRM2 as a biomarker to predict treatment response should be considered in future studies with Triapine®.

Our exploratory analyses of *MDR1* and MRP1 polymorphisms revealed that the variant alleles of the *MDR1* gene were associated with prolonged survival and that non-variant alleles of MRP1 were associated with increased treatment-related anorexia and vomiting. It is reasonable to speculate that individuals with variant alleles may have decreased efflux of Triapine®, resulting in higher tumor concentrations, and improved response. Likewise, those with the non-variant alleles may have increased efflux, higher plasma concentrations, and increased toxicity. These hypothesis-generating findings require confirmation with a larger sample size and pharmacokinetic data to evaluate possible associations with increased drug exposure.

In summary, our findings mirror those of Ma et al., in that this regimen did not demonstrate activity in a non-selected patient population with relapsed NSCLC. In addition, the syndrome of acute methemoglobinemia related to Triapine® infusion reactions makes this an impractical regimen for this population. However, the prolonged survival of patients with the variant genotypes of *MDR1*, compared to those without, suggest that this combination should be explored in those patients.

Acknowledgments

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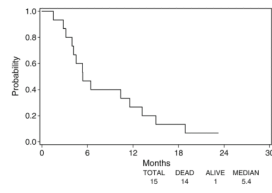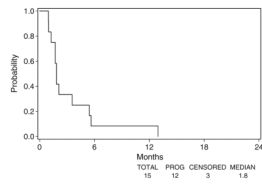
Fig. 1.
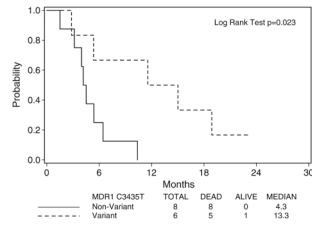Overall survival

Fig. 2.
Time to progression

Fig. 3.
Overall survival by MDR1 C3435T

Table 1

Patient characteristics

	Number	Percent
Sex		
Male	12	80.0
Female	3	20.0
Race		
White	13	86.7
Black	2	13.3
PS		
0	3	20.0
1	12	80.0
Weight loss in previous 6 months		
<5%	1	6.7
5 to <10%	4	26.7
>=20%	1	6.7
Unknown	1	6.7
Disease stage at entry		
IIIB (not recurrent)	1	6.7
IV (not recurrent)	10	66.7
Recurrent	4	26.7
Histology		
Squamous carcinoma	5	33.3
Adenocarcinoma	6	40.0
Non-small cell lung cancer, NOS	4	26.7
Prior chemotherapy	15	100.0
Prior radiation therapy	10	66.7
Prior surgery	4	26.7
Age (median and range)	61.1	53.3–79.6

Table 2

Treatment-related toxicity

Toxicity Type	Treatment Arm A (*n*=18)				
	Grade				
	1, 2	3	4	5	
	(*n*)	(*n*)	(*n*)	(*n*)	
Hemoglobin	16	–	–	–	
Hemolysis	3	–	–	–	
Leukocytes	4	8	1	–	
Neutrophils	3	8	2	–	
Platelets	10	1	1	–	
Hematologic-other	1	1	–	–	
Hypotension	3	–	–	–	
Fatigue	12	3	–	–	
Sweating	2	–	–	–	
Weight loss	2	–	–	–	
Alopecia	5	–	–	–	
Injection site reaction	2	–	–	–	
Rash/desquamation	3	–	–	–	
Anorexia	8	1	–	–	
Constipation	9	–	–	–	
Diarrhea w/o prior colostomy	4	–	–	–	
Muco/stomatitis by exam, oral cavity	2	–	–	–	
Nausea	11	1	–	–	
Vomiting	5	2	–	–	
Hypoalbuminemia	8	–	–	–	
Alkaline phosphatase	5	–	–	–	
ALT, SGPT	3	–	–	–	
AST, SGOT	3	–	–	–	
Hypocalcemia	2	–	–	–	
Creatinine	2	–	–	–	

Toxicity Type	Treatment Arm A (*n*=18)			
	Grade			
	1, 2	**3**	**4**	**5**
	(*n*)	**(*n*)**	**(*n*)**	**(*n*)**
Hyperglycemia	10	–	–	–
Hypokalemia	2	–	–	–
Hyponatremia	3	–	–	–
Neuropathy-sensory	5	–	–	–
Dyspnea	7	2	1	–
Hypoxia	2	4	–	–