Phase I clinical trial of temozolomide and methoxyamine (TRC-102), an inhibitor of base excision repair, in patients with advanced solid tumors

Jennifer R. Eads, Smitha S. Krishnamurthi, Joel Saltzman, Joseph A. Bokar, Panos Savvides, Neal J. Meropol, Joseph Gibbons, et al

Investigational New Drugs
Novel Anti-Cancer Therapeutics and Therapies

ISSN 0167-6997

Invest New Drugs
DOI 10.1007/s10637-020-00962-x

Springer

Investigational New Drugs
https://doi.org/10.1007/s10637-020-00962-x

PHASE I STUDIES

Phase I clinical trial of temozolomide and methoxyamine (TRC-102), an inhibitor of base excision repair, in patients with advanced solid tumors

Jennifer R. Eads[1] · Smitha S. Krishnamurthi[1] · Joel Saltzman[1] · Joseph A. Bokar[1] · Panos Savvides[2] · Neal J. Meropol[3,4] · Joseph Gibbons[1] · Henry Koon[1] · Neelesh Sharma[5] · Lisa Rogers[1] · John J. Pink[3] · Yan Xu[6] · Jan H. Beumer[7] · John Riendeau[1] · Pingfu Fu[3] · Stanton L. Gerson[1] · Afshin Dowlati[1]

Received: 17 May 2020 / Accepted: 11 June 2020

Summary
Temozolomide (TMZ) generates DNA adducts that are repaired by direct DNA and base excision repair mechanisms. Methoxyamine (MX, TRC-102) potentiates TMZ activity by binding to apurinic and apyrimidinic (AP) sites after removal of N^3-methyladenine and N^7-methylguanine, inhibiting site recognition of AP endonuclease. We conducted a phase I trial to determine the maximum tolerated dose and dose-limiting toxicities (DLTs) of intravenous MX when given with oral TMZ. Patients with advanced solid tumors and progression on standard treatment were enrolled to a standard 3 + 3 dose escalation trial assessing escalating doses of TMZ and MX. Tumor response was assessed per RECIST and adverse events (AEs) by CTCAEv3. Pharmacokinetics (PK) of MX and COMET assays on peripheral blood mononuclear cells were performed. 38 patients were enrolled—median age 59.5 years (38–76), mean number of cycles 2.9 [1–13]. No DLTs were observed. Cycle 1 grade 3 AEs included fatigue, lymphopenia, anemia, INR, leukopenia, neutropenia, allergic reaction, constipation, psychosis and paranoia. Cycle 2–13 grade 4 AEs included thrombocytopenia and confusion. A partial response was seen in 1 patient with a pancreatic neuroendocrine tumor (PNET) and six additional patients, each with different tumor types, demonstrated prolonged stable disease. MX PK was linear with dose and was not affected by concomitant TMZ. TMZ 200 mg/m^2 daily × 5 may be safely administered with MX 150 mg/m^2 intravenously once on day 1 with minimal toxicity. Further studies assessing this drug combination in select tumor types where temozolomide has activity may be warranted.

Keywords Temozolomide · Methoxyamine · TRC-102 · Phase I · DNA repair

This research was presented at the annual ASCO meeting in 2015: Eads JR, Krishnamurthi SS, Saltzman J, Bokar JA, Meropol NJ, Gibbons J, Koon H, Sharma N, Pink JJ, Xu Y, Beumer JH, Beatty K, Kane D, Rodal MB, Czalkiewicz J, Riendeau J, Fu P, Gerson SL, Dowlati A. Phase I clinical trial of temozolomide and methoxyamine (TRC-102) in patients with advanced solid tumors. J Clin Oncol, 2015 ASCO Annual Meeting Proceedings.

✉ Afshin Dowlati
Afshin.dowlati@case.edu

[1] Case Comprehensive Cancer Center, Case Western Reserve University, University Hospitals Seidman Cancer Center, 11100 Euclid Avenue, Lakeside 1200, Cleveland, OH 44106, USA

[2] St. Joseph's Hospital and Medical Center, University of Arizona Comprehensive Cancer Center, University of Arizona, Phoenix, AZ, USA

[3] Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH, USA

[4] Flatiron Health, New York, NY, USA

[5] Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA

[6] Case Comprehensive Cancer Center, Cleveland State University, Cleveland, OH, USA

[7] UPMC Hillman Cancer Center, University of Pittsburgh, Pittsburgh, PA, USA

Published online: 17 June 2020

Introduction

Temozolomide (TMZ) is an oral alkylating agent that is commonly used in the management of glioblastoma multiforme, melanoma and PNETs [1–3]. It acts as a DNA damaging agent where it generates three main DNA adducts—O^6-methylguanine (O^6-mG), N^7-methylguanine (N^7-mG) and N^3-methyladenine (N^3-mA). O^6-mG is repaired by O^6-methylguanine-DNA-methyltransferase (MGMT) while N^7-mG and N^3-mA are repaired via the base excision repair (BER) mechanism [4]. Under normal conditions, the base excision repair process involves methylpurine DNA glycosylases recognizing methyl groups in N^7-mG and N^3-mA and cleaving the glycosidic bond, thereby generating apurinic/apyrimidinic (AP) sites. These AP sites are then acted upon by AP endonuclease where the damaged DNA site is repaired by a DNA beta-polymerase and sealed by DNA ligase [5]. Disruption of any step in this process results in a dysfunctional BER mechanism leaving these DNA adducts unrepaired and the cell at increased risk of cell death from double strand breaks. As such, inhibition of any component of the BER mechanism with a novel agent has the potential to potentiate the cytotoxicity generated by the administration of temozolomide and may result in an improved antitumor response.

Methoxyamine is a small molecule that acts by binding the sugar aldehyde at AP sites generated during chemotherapy-induced damage, specifically damage generated by temozolomide, pemetrexed, fludarabine and 5-fluorouracil [6–8]. This prevents AP endonuclease from recognizing the damaged DNA site, thereby leaving the DNA unrepaired. Preclinical models in human tumor xenografts demonstrate that methoxyamine potentiates the activity of all of these agents by showing significant slowing in tumor growth [9–11]. To date, phase I trials assessing the combinations of pemetrexed and methoxyamine (NCT00692159) [12] and fludarabine and methoxyamine (NCT01658319) [13] have been conducted and both combinations have been deemed safe and tolerable. Given our hypothesis that administration of methoxyamine along with temozolomide may result in greater clinical outcomes for patients with temozolomide sensitive malignancies, we conducted a phase I clinical trial in patients with advanced solid tumor malignancies to determine the maximum tolerated dose (MTD) and DLTs of intravenous methoxyamine when given with oral temozolomide.

Patients and methods

Patient eligibility

Patients with an advanced solid tumor who had progressed on all standard lines of therapy and for whom no remaining chemotherapeutic, radiation or surgical options were feasible were included in the study. All cancer diagnoses must have been histologically confirmed. Patients must have been free of chemotherapy or radiation for at least three weeks prior to starting study treatment with the exception of prior mitomycin-C or nitrosourea use in which case 6 weeks must have elapsed. Prior temozolomide therapy was permitted. All prior adverse events must have resolved to at least a grade 1 per the Common Terminology Criteria for Adverse Events (CTCAE) version 3 criteria prior to starting study treatment. Patients must have been at least 18 years of age and had an ECOG performance status of 0–2. All patients must have had a life expectancy of at least 12 weeks and patients with central nervous system involvement were not permitted. Adequate bone marrow, renal and hepatic function was required.

Patients were treated according to an Institutional Review Board approved protocol and informed consent was obtained for all patients prior to conducting any study procedures. The study was conducted according to good clinical practice and the declaration of Helsinki. It is registered at ClinicalTrials.gov (NCT00892385). Patients were recruited from University Hospitals Cleveland Medical Center and several affiliated sites in the Cleveland area.

Study design and treatment approach

Seven dose levels were assessed in this standard 3 + 3 dose escalation trial (Appendix A, Table 4), and two different administration schedules were employed (Fig. 1). Temozolomide was escalated from 150 to 200 mg/m^2 by mouth once daily on days 1–5 and methoxyamine was escalated from 15 to 150 mg/m^2 intravenously once on day 1. Patients assigned to dose levels 1–3 were treated according to a 42 day treatment cycle where temozolomide was administered orally on day 1 followed by administration of MX intravenously over one hour on day 4 (delayed treatment schedule). Patients then received temozolomide orally once daily for 5 days on days 15–19 along with MX over a one-hour intravenous infusion on day 15 (concomitant treatment schedule). This treatment schedule was employed so as to allow for delayed and concomitant pharmacokinetic evaluation to ensure that no interaction was observed between the two agents. Temozolomide was administered within 5 min of the MX infusion where appropriate. Subsequent treatment cycles for dose levels 1–3 as well as all treatment cycles for dose levels 4–7 (when it was determined that no interaction existed between temozolomide and methoxyamine) were 28 days with administration according to the concomitant treatment schedule. The study was designed to assess a maximum methoxyamine dose of 150 mg/m^2

Fig. 1 Study schema

Dose Levels 1-3 (DLT period of 42 days during cycle 1)

Cycle 1: 42 day cycle

	D1	D2	D3	D4	D5		D15	D16	D17	D18	D19
MX				X			X				
TMZ	X						X	X	X	X	X

Subsequent cycles: 28 day cycle

	Day 1	Day 2	Day 3	Day 4	Day 5
MX	X				
TMZ	X	X	X	X	X

Dose Levels 4-7 (DLT period of 28 days, cycle length 28 days)

	Day 1	Day 2	Day 3	Day 4	Day 5
MX	X				
TMZ	X	X	X	X	X

daily × 1 based on pharmacokinetics and dose limiting toxicity data observed in a prior phase I study of methoxyamine and pemetrexed [12].

Safety evaluation

The National Cancer Institute CTCAE version 3.0 criteria were used to determine treatment related adverse events. Dose limiting toxicities (DLTs) were defined as any of the following occurring in a patient during the first treatment cycle: any grade 3 non-hematologic or grade 4 non-hematologic toxicity (thought to be MX related); any grade 4 anemia, neutropenia or thrombocytopenia lasting greater than 7 days and thought to be due to either MX alone or combination TMZ and MX.

The MTD was defined as the highest dose tested in which zero or 1 patient experienced a DLT attributable to the study drug combination when at least 6 patients were treated at that dose level and evaluable for toxicity.

Due to preclinical animal toxicity data suggesting that there may be neurologic toxicity observed in patients receiving MX, all patients were evaluated by a neurologist prior to initiation of treatment. Additionally, all patients were hospitalized for 24 h on day 1, with assessment by an oncologist for the presence of neurologic symptoms and neurologic checks were conducted every 4–8 h by the treatment nurse. Patients exhibiting a ≥ grade 2 neurologic toxicity were seen by a neurologist.

Pharmacokinetics

Pharmacokinetic analysis quantifying MX levels in human plasma were measured using an LC-MS method [14]. During the delayed treatment component of dose levels 1–3 (TMZ given day 1, MX given day 4 of cycle 1), blood samples were obtained at baseline and at the following time points to assess the pharmacokinetics of MX following a delayed exposure to TMZ: 15, 30, 45, 59, and 70 min, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192 and 216 h following MX dosing. To evaluate the pharmacokinetic interaction between TMZ and MX, blood samples were obtained during the concomitant treatment phase (TMZ daily × 5 days on days 15–19, MX once on day 15) at the following time points: pre-MX and TMZ dose, 15, 30, 45, 59 and 70 min, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192 and 216 h following MX dosing. During dose levels 4–7, blood samples were obtained during the concomitant treatment schedule at the following time points: pre-MX dose, 0.5, 1, 2, 4, 8, 24, 48, 72, 96 and 120 h following MX dosing.

Pharmacodynamics

To assess the DNA damage induced by exposure to MX and TMZ in vivo, an alkaline COMET (single cell gel electrophoresis) assay was conducted on patient peripheral blood mononuclear cells [15–17]. The COMET assay distinguishes

undamaged DNA from DNA harboring either single strand or double strand breaks by the detection of differential migration as the DNA passes through an electric field. We hypothesized that exposure of DNA to temozolomide and methoxyamine would result in differential migration of DNA prior to and following treatment due to the accumulation of double strand breaks. For patients assigned to dose levels 1–3, during the delayed treatment period, blood samples were obtained on day 1 prior to TMZ, then again 2 h and 24 h following TMZ. Samples were then obtained on day 4 prior to MX treatment as well as 2 h, 4 h and 24 h following treatment. During the concomitant treatment phase, samples were obtained prior to TMZ and MX treatment as well as 2 h, 4 h, 24 h and 122 h following treatment. For patients assigned to dose levels 4–7, samples were obtained prior to TMZ and MX treatment as well as 2 h, 4 h and 24 h following treatment.

Efficacy evaluation

Patients with measurable disease were evaluated for response with a CT of the chest, abdomen and pelvis on an every 8 week basis and response to treatment was based on the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0 [18].

Statistical analysis

Tradition 3 + 3 phase I design was used. Toxicity data and efficacy data were tabulated. Interval data were summarized by mean and range / standard deviation (SD). Paired interval data were examined using Wilcoxon rank sum test. The association between dose level and laboratory correlates (COMET) was estimated using Pearson correlation coefficient. All tests were two-sided and p value ≤ 0.05 were considered statistically significant.

Results

Patient characteristics

A total of 38 patients were enrolled in this study. Twenty-one patients were assigned to receive treatment per the delayed followed by concomitant treatment schedule. Once it was determined that there was no pharmacokinetic interaction between TMZ and MX, the subsequent 17 patients were assigned to receive treatment only with concomitant scheduling. Median age was 59.5 years (range 38–76) and the median number of treatment cycles received was 2.9 (range 1–13). Patient baseline characteristics are shown in Table 1.

Table 1 Patient baseline characteristics

	Number of patients (%) ($n = 38$)
Gender	
Male	17 (45)
Female	21 (55)
Race	
White	31 (82)
African American	6 (16)
Asian	1 (2)
Primary site of disease	
Colorectal	11 (29)
Lung	6 (16)
Pancreas	5 (13)
Head and neck	4 (11)
Soft tissue	3 (8)
Neuroendocrine	2 (5)
Melanoma	1 (2)
Breast	1 (2)
Ovarian	1 (2)
Cholangiocarcinoma	1 (2)
Gastroesophageal	1 (2)
Endometrial	1 (2)
Unknown primary	1 (2)

Adverse events and dose limiting toxicities

During dose level 1, one patient experienced a grade 3 psychosis and grade 4 confusion with subsequent expansion of the cohort. A second patient at dose level 1 experienced an allergic reaction that was thought not to be related to study treatment and the dose level 1 cohort was expanded to 10 evaluable patients. There were no further DLTs observed throughout the study and 6 evaluable patients were able to be treated at the final dose level. As such, dose level 7 (TMZ 200 mg/m^2 daily × 5 days plus MX 150 mg IV daily × 1 on day 1) was deemed the MTD and the recommended phase II dose (RP2D). Other grade 3 AEs not meeting the definition of a DLT or occurring outside of the DLT window included anemia (3%), elevated INR (3%), leukocytes (3%), lymphopenia (5%), neutrophils (3%), constipation (3%), fatigue (5%) and paranoia (3%). There was one grade 4 thrombocytopenia that also occurred outside of the DLT window. Full adverse event data of grade 3 and 4 AEs as well as grade 1 and 2 AEs experienced in at least 5% of patients are shown in Table 2.

Pharmacokinetics

Methoxyamine pharmacokinetic profiles suggests single compartment behavior with straight log-concentration vs

Table 2 Adverse events (all grade 3/4 AEs and grade 1/2 AEs seen in ≥5% of patients)

Adverse event	Toxicity grade: number (%), $n = 38$		
	1–2	3	4
Hematologic			
ALT, SGPT	3 (8)		
AST, SGOT	3 (8)		
Creatinine	2 (5)		
Hemoglobin	18 (47)	1 (3)	
INR		1 (3)	
Leukocytes	2 (5)	1 (3)	
Lymphopenia	7 (18)	2 (5)	
Neutrophils	2 (5)	1 (3)	
Platelets	7 (18)		2 (5)
Non-hematologic			
Allergic reaction	1 (3)	1 (3)	
Anorexia	6 (16)		
Confusion			1 (3)
Constipation	4 (11)	1 (3)	
Diarrhea	2 (5)		
Dyspnea	2 (5)		
Fatigue	9 (24)	2 (5)	
Hot flashes	2 (5)		
Mucositis (oral)	2 (5)		
Nausea	9 (24)		
Neurology-paranoia		1 (3)	
Pain-headache	2 (5)		
Psychosis	1 (3)?	1 (3)	
Weight loss	2 (5)		

time profiles up to almost ten days after dosing with parallel profiles across dose levels (Fig. 2). The pharmacokinetics of methoxyamine were linear across the dose range studied, with maximum plasma concentration (C_{max}) and area under the plasma concentration vs time curve (AUC) going up with dose, and dose normalized C_{max} and clearance trending horizontally across dose levels (Fig. 3). The average clearance of methoxyamine is 34.5 L/h/m^2 (SD 15.6) and the half-life ($t_{1/2}$) was 46.9 h (SD 11.5) (Table 3). Pharmacokinetic assessment during the delayed treatment period as well as the concomitant treatment period from dose levels 1–3 demonstrated that the pharmacokinetics of methoxyamine are not affected by the co-administration of temozolomide (geometric mean ratio of combination/monotherapy $C_{max} = 0.76$ (SD 0.85), $P = 0.16$; geometric mean ratio of AUC = 0.88 (SD 0.36), $P = 0.39$; geometric mean ratio of t½ = 1.08 (SD 0.19), $P = 0.40$) as assessed by Wilcoxon paired testing (Fig. 4, and Table 3).

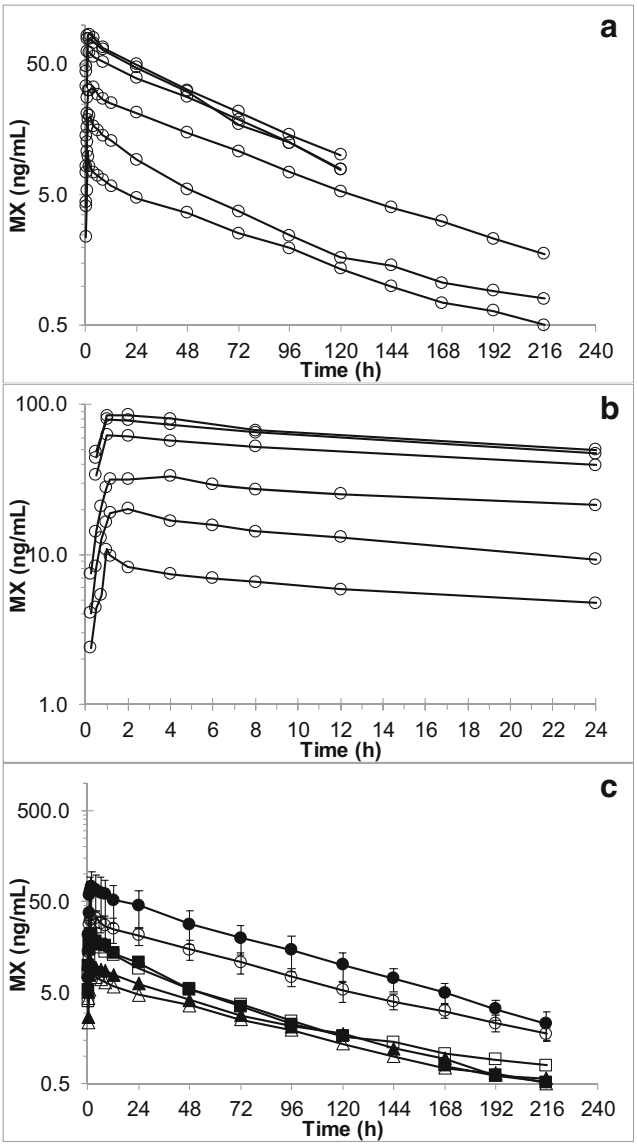

Fig. 2 Mean concentration versus time profiles of methoxyamine across dose levels of 15 ($N = 10$), 30 ($N = 3$), 60 ($N = 3$), 90 ($N = 3$), 120 ($N = 3$), and 150 ($N = 3$) mg/m^2, from bottom to top. **a** all dose levels of the combination of methoxyamine with temozolomide. **b** close-up of the first 24 h. **c** comparison of single agent (solid symbols) and in combination with temozolomide (open circles) of 15 (triangles), 30 (squares), and 60 (circles, with standard deviation error bars) mg/m^2

Pharmacodynamics

COMET assays were performed for all patients starting in dose level 4. No correlation between dose level and either COMET outcome or response to therapy was observed.

Efficacy

A partial response of 8 months duration was observed in one patient with a PNET. Disease control was observed in 6 patients showing prolonged stable disease: ovarian cancer

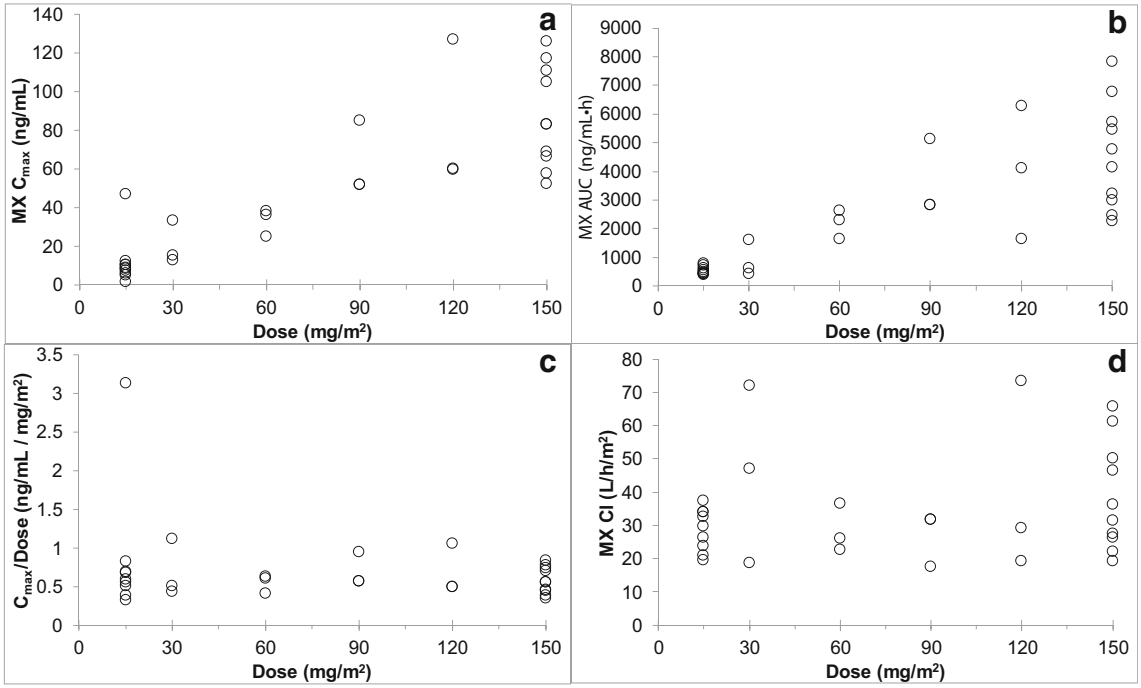

Fig. 3 Pharmacokinetics of methoxyamine in combination with temozolomide. Maximum plasma concentration (C_{max}) (**a**) and area under the plasma concentration vs time profile (AUC) (**b**) plotted against dose, and dose-normalized C_{max} (**c**) and clearance (**d**) plotted against dose

(12.5 months), PNET (9 months), small bowel NET (5.5 months), non-small cell lung cancer (5.5 months) and one patient each with a pancreatic adenocarcinoma and a squamous cell carcinoma of the head and neck (4 months). Figure 5 depicts the duration of treatment patients received while on this study.

Table 3 Methoxyamine plasma pharmacokinetic parameters

Dose	MA single agent				MA in combination with TMZ				Ratio[2] combination/single agent		
TMZ / MA (mg/m^2)	C_{max} (ng/mL)	$AUC_{0\text{-}inf}$ (ng/mL•h)	t½ (h)	Cl (L/h/m^2)	C_{max} (ng/mL)	$AUC_{0\text{-}inf}$ (ng/mL•h)	t½ (h)	Cl (L/h/m^2)	C_{max}	$AUC_{0\text{-}inf}$	t½
150 / 15 ($N = 11$)	11.4 (5.1)	656 (310)	51.6 (14.2)	27.7 (12.1)	11.7 (12.8)	549 (133)	53.0 (13.6)	28.7 (6.4)	0.83 (1.03)	1.05 (0.33)	1.00 (0.15)
150 / 30 ($N = 4$)	23.2 (4.4)	897 (332)	37.9 (11.4)	36.2 (9.9)	20.5 (11.2)	892 (639)	43.8 (10.5)	45.8 (26.8)	0.87 (0.37)	0.84 (0.31)	1.10 (0.12)
150 / 60 ($N = 3$)	72.9 (34.5)	4161 (1646)	45.1 (4.5)	16.2 (7.1)	33.1 (7.1)	2200 (513)	60.4 (3.1)	28.4 (7.3)	0.49 (0.16)	0.55 (0.10)	1.34 (0.09)
150 / 90 ($N = 3$)					62.8 (19.2)	3611 (1323)	40.6 (6.8)	26.9 (8.1)			
150 / 120 ($N = 3$)					82.2 (38.8)	4012 (2325)	37.7 (6.6)	40.5 (28.8)			
150 / 150 ($N = 3$)					80.0 (26.6)	4111 (1632)	41.0 (2.7)	41.1 (18.0)			
200 / 150 ($N = 7$)					90.0 (27.5)	4769 (2067)	43.7 (10.2)	37.4 (17.0)			
Total ($N = 18$–15)	–	–	47.5 (13.3)	27.7 (12.3)			46.9 (11.5)	34.5 (15.6)	0.76 (0.85)	0.88 (0.36)	1.08 (0.19)
								P-value[1]	0.159	0.389	0.169

mean (standard deviation) unless otherwise indicated

[1] 2-tailed Wilcoxon exact signed rank test; C_{max}, $N = 16$; $AUC_{0\text{-}inf}$, $N = 15$; t½, $N = 15$

[2] geometric mean, normal standard deviation

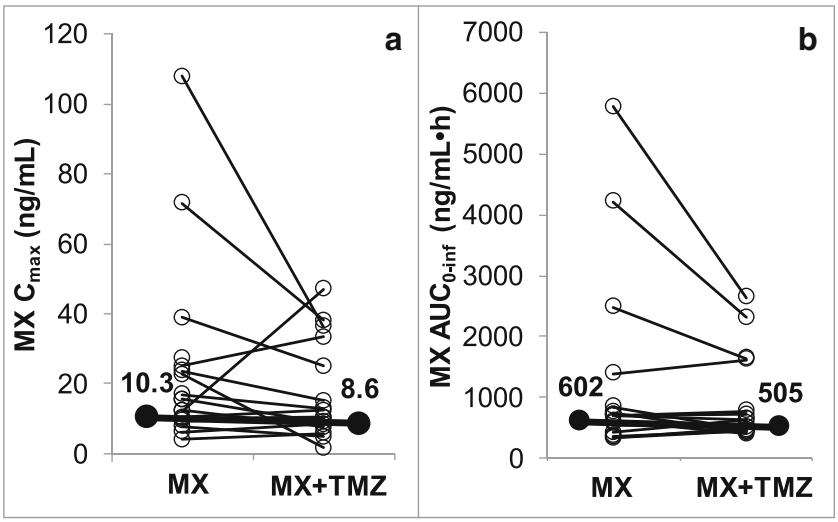

Fig. 4 Within patient comparison of C_{max} (**a**, N = 16, $P = 0.159$) and AUC (**b**, N = 15, $P = 0.389$) of methoxyamine (MX) as single agent and in combination with temozolomide (MX + TMZ) from patients treated at 15, 30, and 60 mg/m^2 methoxyamine; open circles indicate individual patients; solid circles indicate medians

Discussion

This study is the first to report the safety and tolerability of oral temozolomide when administered in combination with intravenous methoxyamine. Seven dose levels, escalating methoxyamine and then temozolomide were planned and patients were treated at all levels without the development of DLTs. In a prior phase I study assessing combination methoxyamine and pemetrexed therapy [12], hemolytic anemia was determined to be a DLT but we did not observe this in this trial. Anemia was a commonly observed adverse event occurring in 51% of all patients but was primarily grade 1–2 (48%) and with no evidence of hemolysis. Other common grade 1 and 2 hematologic toxicities included thrombocytopenia (18%) and lymphopenia (18%) and grade 4 thrombocytopenia occurred in 2 patients, however these events were outside of the DLT period. Most commonly observed grade 1 and 2 non-hematologic toxicities included anorexia (16%), fatigue (24%) and nausea (24%). Overall grade 3 and 4 toxicities were very limited. Given the minimal toxicity observed with this treatment regimen, we concluded that methoxyamine 150 mg/m^2 intravenously on day 1 may be safely administered in combination with temozolomide 200 mg/m^2 orally daily × 5 days in a 28 day treatment cycle and is the RP2D.

Preclinical studies conducted in animals suggested that methoxyamine-induced neurotoxicity may be an issue. We therefore conducted neurotoxicity monitoring throughout the course of the trial with no detection of methoxyamine-induced neurotoxicity.

Two dosing schedules were evaluated in this trial in an effort to determine the pharmacokinetic relationship between methoxyamine and temozolomide. In the first three dose levels, patients received treatment via a delayed dosing schedule in the first 14 days of treatment, followed by a concomitant

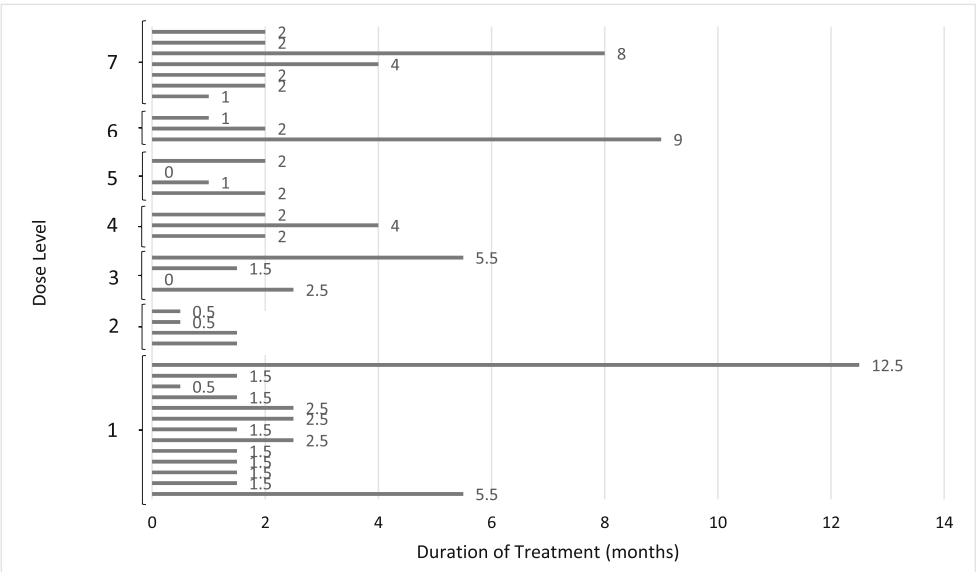

Fig. 5 Swimmer plot denoting length of treatment of patients receiving temozolomide and methoxyamine, Partial response seen in patient with a pancreatic neuroendocrine tumor at dose level (DL) 7 of 8 months duration. Prolonged stable disease seen in a patient with ovarian cancer (DL 1, 12.5 months), pancreatic neuroendocrine tumor (DL 7, 9 months), small bowel neuroendocrine tumor (DL 3, 5.5 months), non-small cell lung cancer (DL 1, 5.5 months), pancreatic adenocarcinoma (DL 4, 4 months) and squamous cell carcinoma of the head and neck (DL 7, 4 months)

dosing schedule in the subsequent 28 days of treatment. Pharmacokinetic analysis could not detect an impact of temozolomide on the pharmacokinetics of methoxyamine. At the 60 mg/m^2 dose, it may seem that there is a temozolomide-methoxyamine interaction, because especially the C_{max} and AUC-ratios (combi/single agent) appear to be distinct from unity (0.49 (SD 0.16), and 0.55 (SD 0.10), respectively). Unfortunately, no more data was collected at the higher dose levels and ultimate MTD. However, if we put the data at 60 mg/m^2 in the context of the other dose levels, we notice that the single agent methoxyamine clearance value of 16.2 L/h/m^2 at 60 mg/m^2 is a low value outlier relative to clearance values at lower doses for the single agent, and any of the other doses in the combination. Thus, the observed ratio values are likely attributable to outlier single agent clearance values in a small cohort of 3 patients, as opposed to some temozolomide-associated drug-drug interaction on methoxyamine. Methoxyamine demonstrated linear pharmacokinetics with dose over a 10-fold dose range.

Methoxyamine administered in an oral form is also being investigated in combination with temozolomide (NCT01851369) [19]. In this trial, each agent is given by mouth once daily for 5 days during a 28-day treatment cycle. Pharmacokinetic studies from this study similarly demonstrate that there is no interaction between temozolomide and methoxyamine and that the C_{max} rises with dose in a linear fashion. The MTD achieved in the oral methoxyamine study was methoxyamine 150 mg/m^2/day × 5 days and temozolomide 150 mg/m^2/day × 5 days with the observed DLT being anemia, including hemolytic anemia. The finding of hemolytic anemia is not unexpected as this was the observed DLT in the methoxyamine and pemetrexed phase I study [12]. In our study utilizing a single dose of intravenous methoxyamine, we were able to achieve higher (and more standardly used) doses of temozolomide, perhaps due to the lower overall methoxyamine exposure. Based on the outcomes of these two phase I studies and given the overlap in toxicity profile of temozolomide and methoxyamine (particularly anemia), it seems unlikely that further escalation of methoxyamine would be reasonable if standard temozolomide doses were to be implemented. This further justifies our RP2D level of methoxyamine 150 mg/m^2 intravenously × 1 and temozolomide 200 mg/m^2 daily × 5 over a 28 day treatment cycle.

We attempted to correlate the pharmacodynamic outcome of DNA damage induced by methoxyamine and temozolomide with response to therapy using a COMET assay conducted on peripheral blood mononuclear cells. COMET data was analyzed for patients enrolled to dose levels 4–7. We did not observe any relationship between the extent of DNA damage detected in peripheral blood mononuclear cells via the COMET assay and response to treatment. One major limitation to this pharmacodynamic assessment and perhaps an explanation for lack of association between degree of DNA damage and response to therapy is that the COMET assay was conducted on circulating mononuclear cells as opposed to tumor specimens. In a separate phase I trial of methoxyamine administered in combination with fludarabine, some association was observed between degree of DNA damage detected via COMET and response to therapy [13]. This is likely due to the fact that patients in this trial had hematologic malignancies, which were more likely to be affected by the study treatment than a solid tumor cohort. Future studies assessing the methoxyamine and temozolomide combination should incorporate pharmacodynamic studies that directly assess DNA damage occurring in the tumor itself. One such assay would be to quantitatively assess AP sites in tissue specimens before and after treatment. Development of such an assay is underway [20].

While the primary objective of this phase I study was safety and tolerability, several patients did derive clinical treatment response benefit. One patient with a PNET had a partial response with an associated 8 month progression free survival (PFS). A second PNET patient had stable disease, but had a PFS of 9 months. Temozolomide-based therapy known to be effective for the management of PNETs with median PFS ranging from 11 months to 18 months [21–23]. These survival data however were based on populations of PNET patients receiving treatment in various stages of their standard treatment course, not part of a phase I trial for patients with no remaining treatment options. As such, it is unclear what the median PFS may be in patients whom have received temozolomide and methoxyamine in a comparable PNET population and our data suggest that further evaluation is warranted. Prolonged periods of stable disease were also observed in patients with small bowel NET (5.5 months), non-small cell lung cancer (5.5 months), squamous cell carcinoma of the head and neck (4 months) and pancreatic adenocarcinoma (4 months).

This phase I study demonstrates that intravenous methoxyamine and oral temozolomide may be safely administered together with minimal toxicity at standard full doses of temozolomide. As methoxyamine appears to be minimally toxic, multiple phase II trials utilizing methoxyamine are ongoing. Pemetrexed-based studies include methoxyamine, pemetrexed, cisplatin and radiation in lung cancer (NCT02535325) and methoxyamine, pemetrexed and cisplatin in mesothelioma (NCT02535312). A phase II study assessing temozolomide and methoxyamine in patients with recurrent glioblastoma is also ongoing (NCT02395692). Given our findings of two favorable treatment responses in patients with PNETs, further trials investigating the combination of temozolomide and methoxyamine in this patient population with improved pharmacodynamic markers are warranted.

Funding information This project was supported NCI grants R21 CA126149 and 5R01 CA86357 and by the Case Comprehensive

Cancer Center (CCCC) (P30CA043703), the Translational Research and Pharmacology Core Facility of the CCCC, the UPMC Hillman Cancer Center Cancer Pharmacokinetics and Pharmacodynamics Facility (CPPF) and the following awards—P30CA47904, 5K12CA076917–17.

Compliance with ethical standards

Conflict of interest Jennifer Eads reports no conflict of interest.

Smitha S. Krishnamurthi reports no conflict of interest.

Joel Saltzman reports no conflict of interest.

Joseph A. Bokar reports no conflict of interest.

Panos Savvides reports no conflict of interest.

Neal J. Meropol is an employee of Flatiron Health, Inc., an independent subsidiary of the Roche Group. NJM holds equity interest in Flatiron Health and Roche.

Joseph Gibbons reports no conflict of interest.

Henry Koon is an employee of Bristol Myers Squibb.

Neelesh Sharma is an employee of Novartis.

Lisa Rogers reports no conflict of interest.

John J. Pink reports no conflict of interest.

Yan Xu reports no conflict of interest.

Jan H. Beumer reports no conflict of interest.

John Riendeau reports no conflict of interest.

Pingfu Fu reports no conflict of interest.

Stanton L. Gerson is inventor of a patent on methoxyamine. The intellectual property on methoxyamine has been licensed by Case Western Reserve University to Tracon Pharmaceuticals, Inc., and Dr. Gerson owns stock in, and is a paid consultant for Tracon Pharmaceuticals, Inc.

Afshin Dowlati reports research support (to institution) by Ipsen, Tesaro, Takeda, Astra Zeneca, Bayer, Eli-Lilly, Abbvie, Seattle Genetics, Bristol Myers-Squibb, Regeneron, Amgen, Symphogen. Dr. Dowlati reports advisory role for Seattle Genetics, Takeda, Abbvie, Bristol Myers-Squibb and Astra Zeneca.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology (NCCN guidelines). Central Nervous System Cancers, version 1.2016
2. National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology (NCCN guidelines). Melanoma, version 3.2016
3. National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology (NCCN guidelines). Neuroendocrine Tumors, version 2.2016
4. Wang Y, Liu L, Wu C, Bulgar A, Somoza E, Zhu W, Gerson SL (2009) Direct detection and quantification of abasic sites for in vivo studies of DNA damage and repair. Nucl Med Biol 36:975–983
5. Curtin NJ (2012) DNA repair dysregulation from cancer driver to therapeutic target. Nat Rev Cancer 12:801–817
6. Yan L, Bulgar A, Miao Y, Mahajan V, Donze JR, Gerson SL, Liu L (2007) Combined treatment with temozolomide and methoxyamine: blocking apurinic/pyrimidinic site repair coupled with targeting topoisomerase IIα. Clin Cancer Res 13:1532–1539
7. Bulgar AD, Weeks LD, Miao Y, Yang S, Xu Y, Guo C, Markowitz S, Oleinick N, Gerson SL, Liu L (2012) Removal of uracil by uracil DNA glycosylase limits pemetrexed cytotoxicity: overriding the limit with methoxyamine to inhibit base excision repair. Cell Death Dis 12:e252. https://doi.org/10.1038/cddis.2011.135
8. Bulgar AD, Snell M, Donze JR, Kirkland EB, Li L, Yang S, Xu Y, Gerson SL, Liu L (2010) Targeting base excision repair suggests a new therapeutic strategy of fludarabine for the treatment of chronic lymphocytic leukemia. Leukemia 24:1795–1799
9. Liu L, Bulgar A, Donze J, Adams BJ, Theuer CP, Gerson SL (2007) Prevention of base excision repair by TRC102 (methoxyamine) potentiates the anti-tumor activity of pemetrexed in vitro and in vivo. J Clin Oncol 25(suppl: abst 12005)
10. Taverna P, Liu L, Hwang HS, Hanson AJ, Kinsella TJ, Gerson SL (2001) Methoxyamine potentiates DNA single strand breaks and double strand breaks induced by temozolomide in colon cancer cells. Mutat Res 485:269–281
11. Liu L, Nakatsuru Y, Gerson SL (2002) Base excision repair as a therapeutic target in colon cancer. Clin Cancer Res 8:2985–2991
12. Gordon MS, Rosen LS, Mendelson D, Ramanathan RK, Goldman J, Liu L, Xu Y, Gerson SL, Anthony SP, Figg WD, Spencer S, Adams BJ, Theuer CP, Leigh BR, Weiss GJ (2013) A phase 1 study of TRC-102, an inhibitor of base excision repair, and pemetrexed in

Appendix

Table 4 Dose escalation strategy

Dose level	Temozolomide (mg/m^2/day) × 5 days	Methoxyamine (mg/m^2) × 1
Level − 1	100	15
Level 1	150	15
Level 2	150	30
Level 3	150	60
Level 4	150	90
Level 5	150	120
Level 6	150	150
Level 7	200	150

patients with advanced solid tumors. Investig New Drugs 31:714–723
13. Caimi P, Cooper B, William BM, Campagnaro EL, Creger RJ, Afable M, Xu Y, Pink J, Dowlati A, Lazarus HM, de Lima M, Gerson SL (2014) Phase I trial of the base-excision repair blocker methoxyamine (TRC-102) combined with fludarabine in relapsed/refractory chronic lymphocytic leukemia (CLL) and lymphoid malignancies. Blood 124:4688
14. Yang S, Savvides P, Liu L, Gerson SL, Xu Y (2012) Development and validation of an LC-MS/MS method for pharmacokinetic study of methoxyamine in phase I clinical trial. J Chromoatogr B Analyt Technol Biomed Life Sci 901:25–33
15. Singh NP, McCoy MT, Tice RR, Schneider EL (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175:184–191
16. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF (2000) The single cell/gel comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 35:206–221
17. Komet User Guide, Kinetic Imaging. June 2005, version 6.0.1
18. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG (2000) New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 92:205–216
19. Meehan RS, Chen AP, O'Sullivan Coyne GH, Collins JM, Kummar S, Anderson L et al (2016) A phase 1 trial of TRC102 (methoxyamine HCl) with temozolomide (TMZ) in patients with solid tumors and lymphomas. J Clin Oncol 34(suppl; abstr 2556)
20. Condie AG, Yan Y, Gerson SL, Wang Y (2015) A fluorescent probe to measure DNA damage and repair. PLoS One 10: e0131330. https://doi.org/10.1371/journal.pone.0131330
21. Chan JA, Stuart K, Earle CC, Clark JW, Bhargava P, Miksad R, Blaszkowsky L, Enzinger PC, Meyerhardt JA, Zheng H, Fuchs CS, Kulke MH (2012) Prospective study of bevacizumab plus temozolomide in patients with advanced neuroendocrine tumor. J Clin Oncol 30:2963–2968
22. Kulke MH, Stuart K, Enzinger PC, Ryan DP, Clark JW, Muzikansky A, Vincitore M, Michelini A, Fuchs CS (2006) Phase II study of temozolomide and thalidomide in patients with metastatic neuroendocrine tumors. J Clin Oncol 24:401–406
23. Strosberg JR, Fine RL, Choi J, Nasir A, Coppola D, Chen DT, Helm J, Kvols L (2011) First-line chemotherapy with capecitabine and temozolomide in patients with metastatic pancreatic endocrine carcinomas. Cancer 117:268–275