Local Protocol #: CASE 1Y05

TITLE: A Phase I Study of Methoxyamine and Temozolomide in Patients with Advanced Solid Tumors

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Supplied Agent(s):	Methoxyamine (NSC 3801) IND #: 73,575
Other Agent:	Temozolomide, commercially available

SCHEMA

Cycle 1 and subsequent cycles.

Methoxyamine (MX) will be given as a single one hour infusion every 28 days.

Temozolomide (TMZ) will be given orally for 5 days every 28 days on days 1-5. It will be administered within five minutes of initiation of methoxyamine

Cycles are concluded every 28 days.

Week1	**Day1**	**2**	**3**	**4**	**5**
MX	↔				
TMZ	↓	↓	↓	↓	↓

Note:
Treatment toxicities for cycle 1 will be considered for dose limiting toxicity (DLT) attribution. Dose modifications are allowed for cycle 2 and beyond.

Cohort A (eligible patients with no CNS disease)

DOSE-ESCALATION SCHEDULE		
Dose Level	Dose	
	TMZ (mg/m^2/day) x 5days	MX (mg/m^2)
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

Cohort B (eligible patients with CNS disease)

DOSE-ESCALATION SCHEDULE		
Dose Level	Dose	
	TMZ (mg/m^2/day) x5days	MX (mg/m^2)
Level -1	75	15
Level 1	100	15
Level 2	150	15

Level 3	150	30
Level 4	150	60
Level 5	150	120
Level 6	150	150
Level 7	200	150

For each cohort, 3 patients will be entered sequentially to each dose level.

- If none of the 3 patients at a dose level experience dose limiting toxicity (DLT) during the first cycle, new patients may be entered at the next dose level.

- If 1/3 patients experiences DLT during the first cycle, up to 3 more patients will be treated at the same dose level. If the 1st patient in the expanded cohort (4th at the given DL), experiences no DLT, then the remaining 2 patients can start treatment without a necessary delay.
    - As of protocol v22, if 1/3 patients experience any DLT that is not of a CNS nature in Cohort B dose level 6, we will continue dose escalation to dose level 7 since we have already treated three subjects in Cohort A dose level 6 and seven subjects in Cohort A dose level 7, none of whom experienced non-CNS toxicities

- If 2 or more experience DLT during the first cycle, no further patients are started at that dose and the MTD is the highest dose level in which <2 (of 6) patients develop DLT.

- If the final dose level is deemed the MTD, 6 patients will be treated at this dose level, even if a DLT has not been observed.

Select non-hematologic grade 3 or 4 or hematologic grade 4 drug-related toxicities (CTC version 3.0) occurring in the first 28 days of study will be considered a DLT.

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1. OBJECTIVES

1.1 To determine the maximum tolerated dose of methoxyamine given in conjunction with temozolomide in patients with and without CNS disease.

1.2 To determine the dose limiting toxicities of the combination of methoxyamine and temozolomide in patients with and without CNS disease.

1.3 To determine the pharmacokinetics of these two agents when given alone or in combination, as well as the pharmacokinetic profile of methoxyamine after a single one-hour IV administration.

1.4 To determine relative DNA damage, as single or double strand breaks by comet assay in blood mononuclear cells which will serve as a surrogate for tumor response to the drug combination.

2. BACKGROUND

Base excision repair (BER) is a fundamental cellular process that reduces the cytotoxicity of alkylating agent chemotherapy. Heretofore, no therapeutic agents have targeted this DNA repair pathway.

Methoxyamine (MX, NSC-3801) is a small organic amine that inhibits base excision repair (BER) by covalently binding to apurinic/apyrimidinic (AP) sites in DNA (1). It has been demonstrated that MX alters the AP site structure by forming a covalent linkage with the aldehyde moiety to form an MX-bound AP site that is refractory to the catalytic activity of the apurinic/apyrimidinic endonuclease (APE), leading to the interruption of BER. Therefore, MX has been studied as a structural modulator of AP sites to enhance therapeutic effect of alkylating agents such as temozolomide (TMZ, NSC-362856).

TMZ is a therapeutic alkylating agent that is efficacious in the treatment of gliomas, melanomas, carcinoid tumors and Hodgkin's lymphoma. However, drug resistance remains a critical problem, often resulting in treatment failure in clinical use. A major obstacle to effective treatment with TMZ in cancer is the presence of elaborate mechanisms of DNA repair. For instance, TMZ forms O^6-methylguanine (O^6mG), 7-methylguanine (N7mG), and 3-methyladenine (N3mA) DNA adducts that are repaired by at least two mechanisms. The O^6mG DNA adduct, a cytotoxic and genotoxic lesion, is repaired by O^6-methylguanine DNA-methyltransferase (MGMT). MGMT activity is a major mechanism of resistance to alkylating agents. In contrast, cell death caused by O^6mG adducts is promoted by the mismatch repair (MMR) system, such that, deficiency in MMR is associated with pronounced resistance to alkylating agents. N7mG and N3mA DNA adducts are removed by the base excision repair (BER) pathway. BER, is an important drug resistant factor because it recognizes a variety of substrates and has the ability to rapidly and efficiently repair these DNA lesions. BER is the major pathway that protects cells against the potentially harmful effects of spontaneously and chemically occurring DNA damage, including (*i*) inappropriate bases; (*ii*) abasic sites that are

formed by enzyme-catalyzed, spontaneous or chemical-induced base release; and (*iii*) DNA single strand breaks produced during the processing of damaged DNA. Efficient BER minimizes the impact of these lesions in normal and tumor cells. Thus, when BER is disrupted, these abundant N-methylated DNA adducts and intermediates generated in the repair process become highly cytotoxic. Most importantly, BER disruption is able to bypass other resistance factors such as MMR defects and high MGMT activity.

In several preclinical studies, we have demonstrated improved therapeutic efficacy of alkylating agents by blocking BER with MX (2,3). We have demonstrated in *in vivo* studies that MX potentiates the antitumor effect of TMZ in human tumor xenografts in nude mice. Mice carrying SW480 colon cancer tumors (AGT expressing, MMR wt and p53 mutant) treated with MX (2 mg/kg) plus TMZ (120 mg/kg) had cessation of tumor growth lasting 50±13 days and very slow regrowth, yielding tumor growth delays of 70±14 days ($p < 0.002$) compared with untreated tumors. In contrast, TMZ (120 mg/kg) combined with O6benzylguanine (BG, a new therapeutic agent being tested in clinical trials), gave rise to a tumor growth delay (T2x-C2x) of 25±2.4 days but was accompanied by significant weight loss (maximum body weight loss from 26 to 20 g, 23%) and very low leukocyte counts (90% decreased) 5 days after the last treatment. Thus, compared to BG+TMZ, treatment with MX+TMZ was associated with less body weight loss and less myelosuppression (3). Therefore, MX inhibition of DNA BER is a promising strategy to sensitize tumor cells to therapeutic alkylating agents.

In this first in human phase I clinical trial, methoxyamine was administered as a 5-day continuous intravenous infusion based on animal data. Dosing started at a MX dose of 15 mg/m^2/day for 5 days and plasma samples were collected and analyzed for MX using validated methods. Pharmacokinetic parameters were derived using a single compartment iv infusion model and plasma methoxyamine concentrations from 5 patients across 15 dosing cycles using WINNONLIN PK model 2 software.

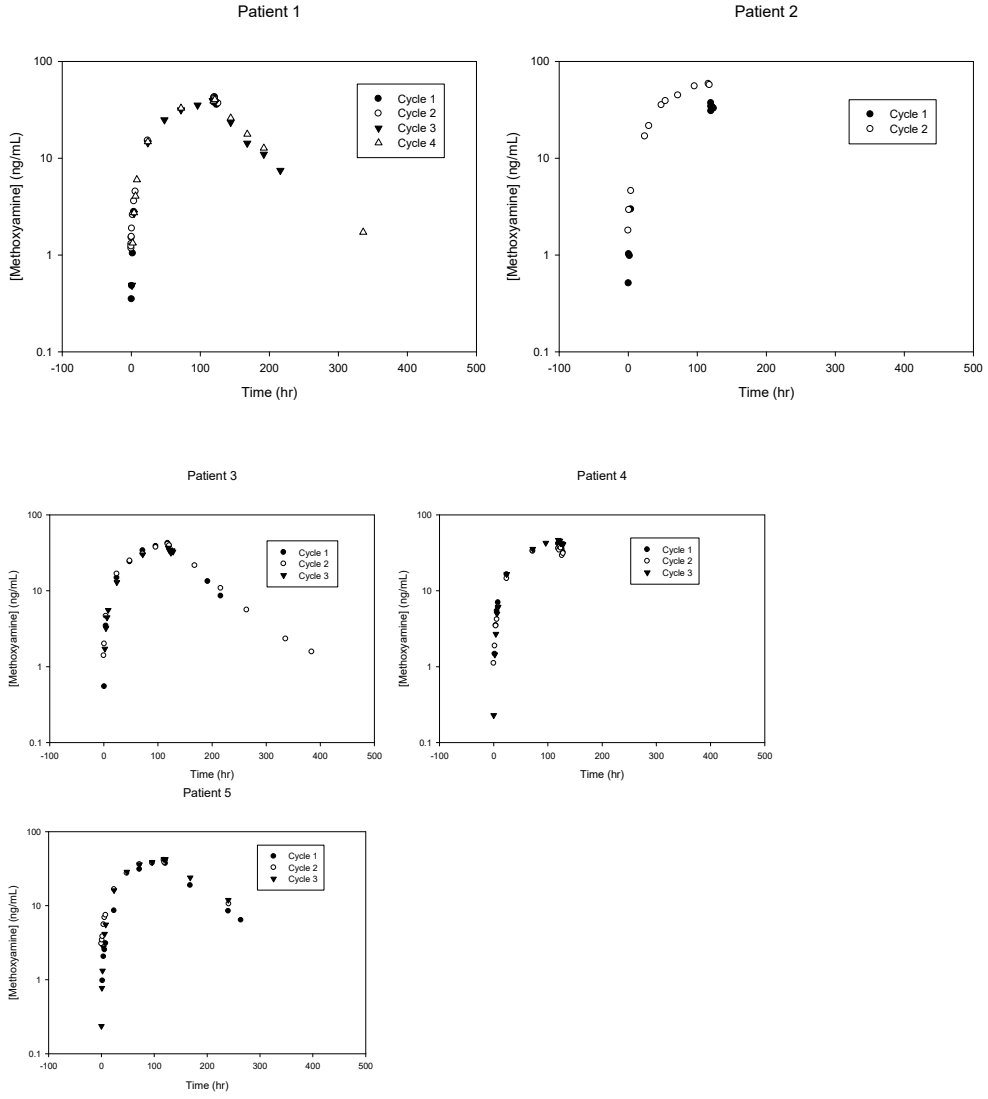
Pharmacokinetic studies obtained from the 5 patients who enrolled in the study demonstrated an unexpected prolonged half-life of methoxyamine (4). In humans, the half life (t½) of MX was estimated to be 45.3 hours (range: 32.1 - 68.8 hours) which represents a 10-fold increase when compared to the MX half life observed in dogs (t½ = 4.5 hours) and a 143-fold increase when compared to the MX half life observed in rats (t½ = 19 min). The volume of distribution (Vss) was estimated to be 1,437 L and the clearance (CL) was estimated to be 22.47 L/h. The Cmax (across all patients) was 41.4 ng/mL. Individual pharmacokinetic exposures and derived PK parameters are presented for each cycle for each patient in table 1, demonstrating very little intra- and interpatient variability.

Table 1. Individual pharmacokinetic exposures and derived PK parameters

Patient	Cycle	Cmax (ng/mL)	AUC (hr*ng/mL)	Vss (L)	$t_{1/2}$ (hr)
1	1	40.57	5,296	1,125	33.05
	2	40.68	5,572	1,290	39.85
	3	38.06	5,132	1,324	37.69
	4	39.93	5,544	1,355	41.66

2	1	34.33	4,878	1,851	48.71
	2	60.11	10,015	1,313	68.76
3	1	41.24	5,826	1,251	43.93
	2	40.95	6,049	1,422	49.70
	3	35.98	4,996	1,443	41.64
4	1	41.58	5,499	1,147	34.97
	2	36.90	4,787	1,211	32.13
	3	45.93	6,646	1,277	47.06
5	1	38.33	5,876	2,006	54.47
	2	42.16	6,122	1,679	47.49
	3	44.19	7,169	1,865	61.79

Figure 1. Methoxyamine concentration profiles, presented separately for each patient.



[Note] Time points may vary from patient to patient and cycle to cycle

The long half-life of methoxyamine in human plasma indicates that iv administration over a shorter time period than 5 days should achieve plasma concentrations needed for activity during the five day period of concurrent temozolomide dosing. A 60 minute infusion is desirable to facilitate patient convenience and accrual to the trial. The estimated dose of methoxyamine given over 60 minutes required to produce a Cmax equal to the observed Cmax of 41.40 ng/mL obtained with 5 day continuous IV administration was therefore calculated as follows, and simulation data are included in appendix C:

Amount of Methoxyamine (A) needed to produce an observed C_{max} of 41.40 ug/L by bolus injection:

$A = C_{max} \times V_{ss} = 41.40\ \mu g/L \times 1{,}437.27\ L = 59{,}502.98\ \mu g = 59.50\ mg$

Additional methoxyamine (A_{inf}) required to compensate for methoxyamine eliminated during a 60 minute infusion period:

$A_{inf} = A(1-e^{-kt}) = 59.50\ mg\ (1-e^{-(0.0153/h)(1\ h)}) = 0.90\ mg$

$(k = 0.693/T_{1/2} = 0.693/45.26\ h = 0.0153/h)$

Therefore, the total methoxyamine dose (A_{total}) given over 60 minutes by intravenous infusion needed to maintain a C_{max} of 41.4 ng/mL over 60 minutes was estimated as:

$A_{total} = A + A_{inf} = 59.50\ mg + 0.90\ mg = 60.40\ mg$

The estimated total dose of methoxyamine given over a 60 minute i.v. infusion needed to produce a Cmax of 41.40 ng/mL (60.40 mg) corresponds to 30 mg/m^2 (assuming an average BSA of 2 m^2). Decreasing this calculated dose by 50% to 15mg/m^2 provides a rational and safe starting methoxyamine dose to administer intravenously over 60 minutes.

In sum, a single dose of MX administered by 60 minute iv infusion in patients enrolled at dose level 1 (15 mg/m^2), represents a conservative initial dose level based on human PK parameters obtained from five patients who received methoxyamine at doses of 15 mg/m^2/day IV over five days for a total of fifteen cycles.[4] This dose was calculated so that expected peak concentration following a single 60 minute IV infusion at dose level 1 will be approximately half the observed maximum concentration observed following 5 day intravenous MX infusion.

Temozolomide is now standard treatment for patients with high-grade gliomas[5] and is often used in patients with metastatic brain tumors as a radiosensitizer or as a single agent therapy. Temozolomide has excellent brain penetration. However, more than half of all GBM patients have MGMT-induced resistance to this agent.[6] The FDA noted that there was neurotoxicity as DLT from combining temozolomide with methoxyamine in animal toxicology studies and they advised us to exclude patients with brain metastasis or glioma from the initial Phase I study. We now propose to expand the scope of this study while maintaining the original aims by adding a "dose behind" cohort of patients with brain metastasis or glioma. This cohort will be treated with a 1 hr infusion MX dose that is one dose level below that of patients currently enrolling with advanced malignancies but

without brain disease. Patients will receive the dose-level appropriate temozolomide dose. However, since many of these patients will have received prior temozolomide, the initial dose level -1 will be 100 mg/m^2 of TMZ orally on days 1-5 of a 28-day cycle and 15 mg/m^2 of MX i.v. over one hour on day 1. Once patients without brain disease have accrued to dose level 2 of MX, we will begin to enroll patients with brain metastasis or gliomas at dose level 1.

3. PATIENT SELECTION

Given the animal toxicity data and CNS involvement of a proportion of eligible patients, two cohorts of eligible patients will be enrolled in the trial:

- Cohort A will include patients **with no** CNS disease
- Cohort B will include patients **with** CNS disease (including recurrent or progressive malignant gliomas and/or brain metastatic disease). Brain metastases do not have to be new to participate in this study.

Inclusion of two separate cohorts was chosen, because this design will allow us to address toxicity concerns generated by the animal studies in a cohort of patients with no CNS disease (cohort A) where development of potential treatment-related CNS toxicity will not be confounded by pre-existing or disease-progression-related symptoms. Inclusion of patients with CNS disease in cohort B will allow us to evaluate the combination in the group of patients where TMZ is currently extensively evaluated and used.

3.1 Eligibility Criteria

3.1.1 Patients must have a histologically confirmed solid tumor that is considered incurable and is not amenable to conventional surgical, radiation therapy or chemotherapy treatment programs.

3.1.2 Prior chemotherapy and/or radiation are allowed. At least 3 weeks must have elapsed since prior large-field radiation therapy; patients must have been off previous anti-cancer therapy for at least 3 weeks (6 weeks for mitomycin-C and nitrosoureas); and recovered from all treatment related toxicity to $\leq$ grade 1 according to NCI CTCAE version 3.0 (with the exception of alopecia and radiation-induced taste changes). Prior temozolomide treatment is not restricted.

3.1.3 Age $\geq$18 years.

3.1.4 ECOG performance status $\leq$2 (Karnofsky $\geq$50%, see Appendix A).

3.1.5 Life expectancy $\geq$ 12 weeks.

3.1.6 Patients must have normal organ and marrow function as defined below:

- absolute neutrophil count $\geq$ 1,500/μl
- platelets $\geq$ 100,000/μl
- hemoglobin $\geq$ 10.0 g/dl
- total bilirubin $\leq$ 1.5 mg/dl
- AST(SGOT) $\leq$ 2.5 X institutional upper limit of normal
- creatinine $\leq$ 1.5 mg/dl

and/or

- creatinine clearance $\geq$ 60 mL/min/1.73 m^2

3.1.7 Patients with known primary or metastatic CNS disease, are eligible for participation in cohort B, but not in cohort A.

3.1.8 Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

3.1.9 Ability to understand and the willingness to sign a written informed consent document.

3.2 **Exclusion Criteria**

3.2.1 Patients may not be receiving any other investigational agents or have received other investigational agents for at least 3 weeks.

3.2.2 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.3 Pregnant and lactating women are excluded from this study because the methoxyamine and temozolomide combination is likely to be teratogenic.

3.2.5 NYHA classification III or IV heart disease

3.2.6 Patients with known primary or metastatic CNS disease (cohort B) are not eligible if they have a mini mental status exam score < 15 (per Appendix D) or evidence of leptomeningeal disease.

3.2.7 Patients with pre-existing neurologic toxicity > grade 1 (as per

CTCAE, version 3.0) are not eligible for participation in cohort A. Patients screened for participation in cohort B with pre-existing neurologic toxicity > grade 2 (as per CTCAE, version 3.0) are not eligible, unless pre-existing neuroloxic toxicity is documented in detail and patient's participation in the trial has been approved by the neuro-oncology team at participating institutions.

3.3 Inclusion of Women and Minorities

Both men and women of all ethnic groups are eligible for this trial.

4. TREATMENT PLAN

To enter eligible patients on study, investigators will register patients by contacting the study coordinator for this trial. The following information will be requested: a registration card, copy of the informed consent, and copy of the signed eligibility criteria from the study protocol. These must be submitted prior to a patient starting treatment and placed in the chart.

4.1 Agent Administration

For all cycles, TMZ will be given orally for 5 days every 28 days. MX will be given as a single one-hour IV infusion every 28 days. Temozolomide will be administered within 5 minutes following the initation of methoxyamine.

Patients will be treated and monitored in the inpatient setting (Clinical Research Unit) during MX administration (on cycle 1 day 1). A physician will be onsite within the hospital in case an adverse event occurs.

Cohort A (eligible patients with no CNS disease)

DOSE-ESCALATION SCHEDULE		
Dose Level	Dose	
	TMZ (mg/m2/day) x5days	MX (mg/m^2)
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

Cohort B (eligible patients with CNS disease)

DOSE-ESCALATION SCHEDULE		
Dose Level	Dose	
	TMZ (mg/m^2/day) x5days	MX (mg/m^2)
Level -1	75	15
Level 1	100	15
Level 2	150	15
Level 3	150	30
Level 4	150	60
Level 5	150	120
Level 6	150	150
Level 7	200	150

For each cohort, 3 patients will be entered sequentially to each dose level.

- If none of the 3 patients at a dose level experience dose limiting toxicity (DLT) during the first cycle, new patients may be entered at the next higher dose level.
- If 1/3 patients experience a DLT during the first cycle, up to 3 more patients will be treated at the same dose level. If the 1st patient in the expanded cohort (4th at the given DL), experiences no DLT, then the remaining 2 patients can start treatment without a necessary delay.
    - As of protocol v22, if 1/3 patients experience any DLT that is not of a CNS nature in Cohort B dose level 6, we will continue dose escalation to dose level 7 since we have already treated three subjects in Cohort A dose level 6 and seven subjects in Cohort A dose level 7, none of whom experienced non-CNS toxicities
- If 2 or more experience DLT during the first cycle, no further patients are started at that dose and the MTD is the highest dose level in which <2 (of 6) patients develop DLT.
- If the final dose level is deemed the MTD, 6 patients will be treated at this dose level even if a DLT has not been observed.

Select non-hematologic grade 3 or 4 or hematologic grade 4 drug-related toxicities (CTC version 3.0) occurring within the first 28 days of the study will be considered a DLT.

Cohort A, Dose level 1: Two DLTs were observed in Dose Level 1 of Cohort A: grade 3 psychosis and grade 3 allergic reaction. Upon further review of the hypersensitivity reaction, it is believed that this was an idiosyncratic event, and further expansion of this

dose level is warranted. Dose level 1 of Cohort A will be expanded to 10 evaluable patients. Further dose escalation will occur only if less than 4 out of the 10 patients experience a DLT. Enrollment to subsequent dose levels will follow the rules outlined above.

4.2 Definition of Dose-Limiting Toxicity (DLT) and Criteria for Dose Escalation

DLT in a given patient is defined as any grade III non-hematologic (thought to be MX related) or IV non-hematological toxicity or any grade IV anemia, neutropenia or thrombocytopenia lasting greater than 7 days, that is felt to be related to MX or the MX + TMZ drug combination occurring during the first cycle of treatment.

MTD (maximum tolerated dose) will be defined based on the toxicities observed during the first cycle of treatment

Definition of MTD: The MTD is defined as the highest dose tested in which none or only one patient experienced DLT attributable to the study drug combination, when at least six patients were treated at that dose and are evaluable for toxicity. The MTD is one dose level below the highest dose tested in which 2 or more patients experienced DLT attributable to the study drug. At least 6 patients will be treated at the MTD.

Management and dose modifications associated with the above adverse events are outlined in Section 5.

Special consideration is given to the evaluation of neurologic toxicity given the animal toxicity data and CNS involvement of a proportion of eligible patients.

Specifically:

- all patients will be evaluated by a staff Neurologist prior to initiation of treatment
- patients will undergo frequent (every 4-8 hours) neurologic checks by a CRU RN during the 1-day inpatient CRU stay on the first day of treatment.
- Patients will be evaluated by the covering Oncology clinician during the 1-day inpatient CRU stay on cycle 1.

A neurology consult will be obtained for any observed treatment-related neurologic toxicity ≥ grade 2. For patients enrolled in cohort B (patients with CNS disease) with pre-existing neurologic toxicity cleared for participation by the neuro-oncology team, a neuro-oncology or neurology consult will be obtained for any observed treatment-related worsening in neurologic toxicities.

Dose escalation will proceed within each cohort according to the following scheme. DLT is defined above.

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level.
≥2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter at least 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose. o As of protocol v22, if 1/3 patients experience any DLT that is not of a CNS nature in Cohort B dose level 6, we will continue dose escalation to dose level 7 since we have already treated three subjects in Cohort A dose level 6 and seven subjects in Cohort A dose level 7, none of whom experienced non-CNS toxicities
≤1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

Cohort A, Dose level 1: Dose level 1 of Cohort A will be expanded to 10 evaluable patients. Further dose escalation will occur only if less than 4 out of the 10 patients experience a DLT. Enrollment to subsequent dose levels will follow the rules outlined in Sec. 4.1.

4.3 Supportive Care Guidelines

Compazine or ondansetron in the usual doses may be used to manage constitutional symptoms of nausea and vomiting. Steroids may be added based on the treating physicians discretion. Blood products may be administered at the treating physician's discretion. Growth factors will not be administered during cycle 1, but can be administered in subsequent cycles based on treating physician's judgment.

4.4 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may

continue until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator.

5. DOSING DELAYS/DOSE MODIFICATIONS

On the day of starting each cycle, neutrophil and platelet hematologic parameters must have returned to eligibility values. Furthermore, grade 3 non-hematologic toxicity that is felt to be drug related must return to a grade 2 or better. If these criteria are not met, therapy will be held by 1 week increments for a maximum of 2 weeks. If treatment cannot be given during that time frame the patient will be removed from study.

Any grade 4 hematologic toxicity will result in a dose reduction to the lower dose level for methoxyamine/temozolomide. If this occurs at dose level 1, the dose of temozolomide will be decreased to dose level -1 (75 mg/m2/d for 5 days if in the CNS cohort; 100mg/m2 for 5 days if in the non-CNS cohort).

6. AGENT FORMULATION AND PROCUREMENT

6.1 Temodar (Temozolomide)

6.1.1 Availability: Temodar is commercially available.

6.1.2 Chemical Name - The chemical name of temozolomide is 3,4-dihydro-3-methyl-4-oxoimidazo[5,1-d]-*as*-tetrazine-8-carboxamide.

6.1.3 Molecular Formula - $C_6H_6N_6O_2$

6.1.4 Mechanism of Action - Temozolomide is not directly active but undergoes rapid non-enzymatic conversion at physiologic pH to the reactive compound MTIC. The cytotoxicity of MTIC is thought to be primarily due to alkylation of DNA. Alkylation (methylation) occurs mainly at the O^6 and N^7 positions of guanine.

6.1.5 Pharmacokinetics - Temozolomide is rapidly and completely absorbed after oral administration; peak plasma concentrations occur in 1 hour. Food reduces the rate and extent of temozolomide absorption. Mean peak plasma concentration and AUC decreased by 32 % and 9 %, respectively, and Tmax increased 2- fold (from 1.1 to 2.25 hours) when

temozolomide was administered after a modified high fat breakfast. Temozolomide is rapidly eliminated with a mean elimination half- life of 1.8 hours and exhibits linear kinetics over the therapeutic dosing range. Temozolomide has a mean apparent volume of distribution of 0.4 L/kg (% CV= 13%). It is weakly bound to human plasma proteins; the mean percent bound of drug- related total radioactivity is 15%.

6.1.6 How Supplied - TEMODAR (temozolomide) Capsules are supplied in amber glass bottles with child-resistant polypropylene caps containing the following capsule strengths:

TEMODAR (temozolomide) Capsules **5 mg:** opaque white bodies with green caps. The capsule body is imprinted with two stripes, the dosage strength, and the Schering-Plough logo. The cap is imprinted with "TEMODAR".

5 and 14 capsule bottles.
5 count - NDC# 0085-3004-02
14 count - NDC# 0085-3004-01

TEMODAR (temozolomide) Capsules **20 mg:** opaque white bodies with yellow caps. The capsule body is imprinted with two stripes, the dosage strength, and the Schering-Plough logo. The cap is imprinted with "TEMODAR".

5 and 14 capsule bottles.
5 count - NDC# 0085-1519-02
14 count - NDC# 0085-1519-01

TEMODAR (temozolomide) Capsules **100 mg:** opaque white bodies with pink caps. The capsule body is imprinted with two stripes, the dosage strength, and the Schering-Plough logo. The cap is imprinted with "TEMODAR".

5 and 14 capsule bottles.
5 count - NDC# 0085-1366-02
14 count - NDC# 0085-1366-01

TEMODAR (temozolomide) Capsules **140 mg:** opaque white bodies with blue caps. The capsule body is imprinted with two stripes, the dosage strength, and the Schering-Plough logo. The cap is imprinted with "TEMODAR".

5 and 14 capsule bottles.
5 count - NDC# 0085-1425-01
14 count - NDC# 0085-1425-02

TEMODAR (temozolomide) Capsules **180 mg:** opaque white bodies with orange caps. The capsule body is imprinted with two stripes, the dosage strength, and the Schering-Plough logo. The cap is imprinted with "TEMODAR".

5 and 14 capsule bottles.
5 count - NDC# 0085-1430-01

14 count - NDC# 0085-1430-02

TEMODAR (temozolomide) Capsules **250 mg:** opaque white bodies with white caps. The capsule body is imprinted with two stripes, the dosage strength, and the Schering-Plough logo. The cap is imprinted with "TEMODAR".

5 capsule bottles.
5 count - NDC# 0085-1417-01

Store at 25° (77° F); excursions to 15°-30° C (59°-86° F).
[See USP Controlled Room Temperature]

6.1.7 Route of Administration – Oral

6.1.8 Toxicity - The incidence of adverse events in the 158 patients in the Anaplastic Astrocytoma study for whom data are available. In the absence of a control group, it is not clear in many cases whether these events should be attributed to temozolomide or the patients' underlying conditions, but nausea, vomiting, fatigue and hematologic effects appear to be clearly drug-related. The most frequently occurring side effects were nausea, vomiting, headache and fatigue. The adverse events were usually NCI Common Toxicity Criteria (CTC) Grade 1 or 2 (mild to moderate in severity) and were self-limiting, with nausea and vomiting readily controlled with antiemetics. The incidence of severe nausea and vomiting (CTC grade 3 or 4) was 10% and 6%, respectively. Myelosuppression (thrombocytopenia and neutropenia) was the dose-limiting adverse event. It usually occurred within the first few cycles of therapy and was not cumulative.

Myelosuppression occurred late in the treatment cycle and returned to normal, on average, within 14 days of nadir counts. The median nadirs occurred at 26 days for platelets [range 21-40 days] and 28 days for neutrophils [range 1-44 days]. Only 14% (22/158) of patients had a neutrophil nadir and 20% (32/158) of patients had a platelet nadir which may have delayed the start of the next cycle. (See **WARNINGS**). Less than 10% of patients required hospitalization, blood transfusion or discontinuation of therapy due to myelosuppression.

In clinical trial experience with 110-111 women and 169-174 men (depending on measurements), there were higher rates of Grade 4 neutropenia (ANC < 500 cells/μL) thrombocytopenia (< 20,000 cells/μL) in women than men in the first cycle of therapy: (12% versus 5% and 9% versus 3%, respectively).

In the entire Safety database for which hematologic data exist (N= 932), 7% (4/61) and 9.5% (6/63) of patients over age 70 experienced Grade 4 neutropenia or thrombocytopenia in the first cycle, respectively. For patients less than or equal to age 70, 7% (62/871) and 5.5% (48/879) experienced Grade 4 neutropenia or thrombocytopenia in the first cycle, respectively.

Data released by the European Organization for Research and Treatment of Cancer (EORTC) in January 2014 shows that amongst 44 patients receiving temozolomide

worldwide, hepatic injury, including fatal hepatic failure was identified in patients receiving temozolomide. These events are rare but for patients with liver function abnormalities, the risk/benefit of receiving temozolomide should be considered prior to initiation of this agent.

6.2. **METHOXYAMINE HYDROCHLORIDE**:

6.2.2.1.Chemistry and Manufacturing Introduction:

Methoxyamine hydrochloride: a small organic amine is soluble in water.
Synonymous: O-methylhydroxyamine hydrochloride
Specifications:

Molecular Formula	$CH_5NO.HCl$
Molecular Weight	83.5
Physical description	White crystal
Melting point	149-153 °C
Moisture	1.0% Max
Purity (GC)	98.2%

There are not any chemical or manufacturing differences between the drug product proposed for clinical use and the drug product used in the animal toxicology trials.

6.2.2.2. **Drug Substance:**

NSC 3801 is Methoxyamine hydrochloride. Following is a description of the drug substance

Non-Proprietary Names:	n.a.
Chemical Names:	Methoxyamine hydrochloride, O-methylhydroxyamine hydrochloride
Other Names:	TRC102
CAS Number:	593-56-4
NCI Code:	NSC 3801
Chemical Structure:	CH_3-O-NH_2•HCl

6.2.2.3. **Manufacturer of the Drug Substance:**

Manufactured for: Tracon Pharamceuticals
4510 Executive Drive, Suite 330
San Diego, CA 92121

Phone: 858/550-0780
Fax: 858/550-0786

6.2.2.4. **Drug Product:**

1. Components and Composition

Methoxyamine HCl Injection (also referred to as TRC102 Injection)

The drug product is a sterile solution of methoxyamine hydrochloride in Water For Injection filled into 10 mL single-use vials. Each vial contains 10 mL of a 15 mg/mL solution for a total of 150 mg of methoxyamine hydrochloride per vial. Vials of Methoxyamine HCl Injection are labeled with the following.

Methoxyamine HCl Injection (TRC102 Injection)
10 mL per vial at 15 mg/mL

_____	_____	xxx-xx-xxxx
Protocol Number	Patient Number	Lot No.

For IV/Single use only
Instructions for Use: Give as directed per protocol
Caution: New Drug-Limited by Federal (or United States) law to investigational use
Store at 25ºC; excursions permitted to 15-30 ºC
TRACON Pharmaceuticals, Inc., San Diego, CA 92121 USA

2. Methoxyamine HCl Injection Storage and Shipping

Methoxyamine HCl Injection should be stored upright at controlled room temperature (25ºC; excursions permitted to 15-30 ºC).

3. TRC102 Injection Preparation

Methoxyamine HCl Injection will be prepared in the pharmacy and diluted with normal saline in a 250 mL bag. Following dilution in normal saline, Methoxyamine HCl Injection will be administered at room temperature within 24 hours of preparation if refrigerated or 8 hours of preparation if maintained at room temperature. Depending on the dose and patient weight, multiple vials may be required for a single dose.

The following formulae should be used to calculate the volume of Methoxyamine HCl Injection to be added to normal saline:

- Patient weight (kg) × dose level (mg/kg) divided by Methoxyamine HCl Injection (mg/mL) = volume of Methoxyamine HCl Injection (mL) to be administered.

The volume of Methoxyamine HCl Injection to be administered can be rounded up or down to the nearest 0.1 mL. If an increment of 0.05 mL is calculated, the volume should be rounded up. The calculated volume of Methoxyamine HCl Injection is to be diluted in normal saline. A 250 mL normal saline bag is recommended for this purpose. Appropriate judgment

should be exercised in withdrawing an adequate amount of saline (if any) necessary to permit injection of the appropriate volume of diluted antibody into the appropriate sized normal saline bag.

4.The absorption, distribution, metabolism, and excretions of methyoxyamine.

Methoxyamine is a small organic amine; it is very soluble in aqueous solution and plasma. A [^{14}C]-MX protein-binding study in mouse plasma at 37°C indicated insignificant protein binding. 100% of radiolabelled MX was recovered from the samples after Centrifree processing, indicating a lack of reactivity with plasma proteins.

6.2.3. **Toxicology information:**

Methoxyamine (MX) is a novel biochemical inhibitor of the base excision repair (BER) pathway. It has potential as a chemotherapeutic agent through its ability to synergize with compounds whose DNA damage is normally repaired by BER, such as the alkylating agent, temozolomide (TMZ). *In vitro* studies and xenograft models have confirmed synergistic cytotoxicity in tumor cells treated with both MX and TMZ.

(a) **The toxicity of MX was studied in Fischer 344 rats.** MX was administrated as a 5-day continuous IV infusion in sterile water. Twelve dose groups, including vehicle control, 0.25, 0.5, 1.25, 2.5, 5, 10, 15, 20, 50, 200 and 500 mg/kg/day were studied using 4-5 males/dose group. Body weight was measured baseline, and on days 1-5, 8, and 12. Blood was drawn for clinical pathology baseline and on day 8 and 12. Animals were sacrificed on day 12. Histopathology was performed on animals that received vehicle control, 2.5, 20 and 50 mg/kg/day.

Mortality: all rats in the 500 mg/kg/day group were found dead or sacrificed moribund on day 1. All animals in the 200 mg/kg/day group were found dead on day 2.

Clinical Observations, Body Weights: animals given 500 mg/kg/day had bradycardia dyspnea, convulsions, prostration and/or were comatose within the first few hours of the beginning of dosing. There were no statically significant changes in body weight in any group compared to the vehicle control group.

Hematology and Clinical chemistry: these parameters are not available for animals given 200 mg/kg/day. Animals that received 500 mg/kg/day that were sacrificed moribund had elevated AST/ALT (4-6X). A decrease in RBC (25-30%) was noted on day 12 in rats given 50 mg/kg/day.

Histopathology: no drug-related lesions were seen in animals given 20 or 50 mg/kg/day.
Conclusions: the maximum tolerated dose of MX given as a 5-day continuous iv infusion in rats is greater than 50 mg/kg/day (> 300 mg/m2/day) but less than 200 mg/kg/day (< 1200 mg/m2/day).

(b) **Multiple dose toxicity study of methoxyamine and temozolomide in rats and dogs**. The effect of escalating doses of MX on the pharmacology and toxicity of a constant dose of TMZ was assessed in rats and dogs on a 5-day schedule. TMZ was given orally, and MX was administered as a continuous iv infusion.

In rats: vehicle control, MX alone (100 mg/kg/day), TMZ alone (30 mg/kg/day), or 25, 50, 75, or 100 mg/kg/day MX in combination with 30 mg/kg/day TMZ were studied using 10 rats/sex/dose group. Body weights were determined baseline, and on days 1-5, 8, 15, 22, and 33. Blood was drawn for clinical pathology on days 8, 15, 22 and 33. Animals were sacrificed on day 8 or 33, and histopathology was performed.

In dogs: vehicle control, MX alone (15 mg/kg/day), TMZ alone (2.5 mg/kg/day), 5 or 15 mg/kg/day MX in combination with 2.5 mg/kg/day TMZ were studied using 2 males and 2 females/dose group. Due to adverse clinical signs seen in males given 15 mg/kg/day ±TMZ, females in these dose groups received 10 mg/kg/day MX±TMZ. Body weights were determined at baseline, and on days 1-5, 8, 12, 19, 26, and 33. Body temperatures were measured baseline, immediately prior to and at 2 and 4 hours after each TMZ dose, and on day 6-8, 12, 15, 19, 26 and 33. Food consumption was measured daily on days 1-5 and weekly thereafter. Blood was drawn for clinical pathology on days 8, 12, 15, 19, 26 and 33. One animal/sex/group was sacrificed on day 8 or 33, and histopathology was performed.

In summary, in both rats and dogs, MX alone (1200-3000 mg/m2/day in rats and 300 mg/m2/day in dogs) caused dose-limiting neurotoxicity (convulsions, tremors, ataxia, and/or head tilt). TMZ alone caused minimal anemia in dogs (50 mg/m2/day) and minimal to moderate neutropenia in rats (180 mg/m2/day). In rats, TMZ-induced neutropenia increased with increasing doses of MX. Lethality also increased in rats with increasing doses of MX in combination with TMZ. The maximum tolerated dose (MTD) in rats is 25 mg/kg/day MX + 30 mg/kg/day TMZ. In dogs, the combination of MX + TMZ did not produce lethality. The MTD is ~10-15 mg/kg/day MX + 2.5 mg/kg/day TMZ. The recommended clinical starting dose of MX is 15 mg/m2/day when given as a 5-day continuous iv infusion in combination with orally administered TMZ. This dose is one-tenth the MTD in the most sensitive species (rat).

Pharmacokinetic interaction studies were performed with TMZ and MX in F344 rats and dogs (M.D. Anderson Cancer Center). TMZ concentrations in rat and dog plasma were analyzed after treatment with TMZ and MX. Results showed that MX did not appear to alter the pharmacokinetics of TMZ in either rats or dogs. (Plasma samples were obtained for TMZ analysis 30 min post-TMZ on day 5 and 10 min before the end of the MX infusion on day 6 for MX analysis.)

(c) In vitro **studies.** *In vitro* bone marrow assays were conducted to determine the effect of MX on the myelotoxicity of TMZ. Granulocyte-macrophage colony-forming units (CFU-GM) were obtained from two human and two murine donors. The study provided evidence that TMZ is likely more potent to murine than human CFU-GM. Single agent

MX was well tolerated in both murine and human CFU-GM. Combination of TMZ with MX shows that MX mildly enhances TMZ toxicity to CFU-GM in both species.

7. CORRELATIVE/SPECIAL STUDIES

This protocol will utilize the Translational Research Core (TRC) of the Case Comprehensive Cancer Center for the collection, processing, storage, and distribution of patient samples before and after therapy.

Translational Research Core
ATTN Erin Hohler
University Hospitals of Cleveland
11100 Euclid Avenue
Seidman Cancer Center, Room 3608
Cleveland, OH 44106
Telephone: 216-286-3889/216-386-3890
Fax: 216-286-5772
Pager: 33471
E-mail: emh14@case.edu

The TRC will schedule patient sample collection in direct communication with the clinical trials research nurse. For UH patients a representative from the TRC will be present during patient sampling, wherein every sample will be given a patient identifier, and collected in the appropriate tubes or vials.

1. Pharmacokinetic studies

PK blood sampling schedule for Temozolomide (TMZ) and Methoxyamine (MX) during cycle 1 are shown in the table that follows. Depending upon the PK data from initial patients, the timing of blood draws for correlative studies may be revised for future patients. PK data during and following single one-hour MX infusion may be obtained in all subsequent cycles (cycles 2 and beyond) as necessary.

Preclinical data have not provided any evidence of significant drug interactions between TMZ and MX. However, this needs to be confirmed. TMZ is not stable in plasma, so plasma sample isolation must be carried out without delay. Plasma from one tube of whole blood will be used for both TMZ and MX determinations.

Collection Instructions for MX and TMX determinations:

1. Draw 5 ml of blood into a lithium heparinized, chilled syringe.
2. Immerse the tube in an icewater bath for 2 min.
3. Centrifuge at 1500 x g and 4 deg. C. for 5 min.
4. Transfer 1.0 ml of plasma into each of two Nunc brand cryostorage tubes.
5. To one tube, to be used for TMZ determination, add 0.1 ml of cold 1 M HCl.
6. Cap, mix, label, and store frozen at -60˚C.

a) Methoxyamine determination

An LC-MS/MS method for quantitative determination of MX in human plasma was developed. In this method, MX and its stable isotope methoxyl-d_3-amine (MX-d3 as internal standard) were directly derivatized in human plasma with 4-(*N,N*-diethylamino)benzaldehyde. The derivatized MX and IS were extracted by methyl-*tert*-butyl ether, and separated isocratically on a Xterra C18 column (2.1 x 100 mm) using an aqueous mobile phase containing 45% acetonitrile and 0.4% formic acid at a flow rate of 0.200 ml/min. Quantitation of MX was carried out by multiple-reaction-monitoring (MRM) mode of positive turbo-ion-spray tandem mass spectrometry. This method has been validated according to FDA guidelines for bioanalytical method. The linear calibration range for MX was 1.25-500 ng/ml in human plasma with a correlation coefficient $\geq$ 0.9993. The intra- and inter-assay precision (%CV) at three concentration levels (3.50, 45.0 and 450 ng/ml) ranged 0.9-1% and 0.8-3%, respectively. The stability studies showed that MX met the acceptable criteria under all tested conditions.

b) Temozolomide determination

Analyses will be performed in the Core Pharmacology laboratory, directed by Dr. Hoppel.

2. DNA strand break determination by comet assay.

The comet assay is based on the ability of denatured, cleaved DNA fragments to migrate out of fixed cells under the influence of an electric field. Undamaged DNA migrates slower and remains within the confines of the nuclei when a current is applied. This assay is a simple assay but sensitively detects both single and double stranded DNA breaks, resulting from AP sites as well as other alkali labile DNA adducts in individual cells. To perform this assay, the cell suspension (1x 10^5 /ml cold PBS) is mixed with 1% low melting point agarose at 42 °C at a ratio of 1:10 (v/v) and immediately pipetted (75 µl) onto a CometSlide. When the agarose has set, slides are electrophoresed in both alkali and neutral solution. DNA is stained using SYBR Green and the images of comets are visualized and captured using a microscope coupled with a digital camera. DNA damage is assessed based on evaluation of the DNA "comet" tail shape and migration distance that are analyzed using Komet version 6.0 software. In previous *in vitro* studies with human lymphocytes, we have shown that DNA strand breaks are correlated with the levels of AP sites in cells treated with TMZ. A 2 hr treatment with TMZ plus MX generated higher levels of DNA strand breaks (up to 2-fold greater), compared to TMZ alone. This demonstrates that the comet assay can be used to monitor drug effects on DNA damage in clinical studies. Analyses for DNA strand breaks by comet assay will be performed in the Translational Research Core Facility lab in the Wolstein Research Building.

Collection Instructions:

1. Immediately deliver tubes to the **Translational Research Core Laboratory, Seidman Cancer Center, Room 3608, (phone number 216-286-3889)** for processing and analysis by comet assay.

Cycle 1	Week 1	Day1	2	3	4	5	6
	MX	↔					
	TMZ	↓	↓	↓	↓	↓	
Correlative Studies	Pharmacokinetic Studies	0 30min 1h 2h 4h 8h	24h	48h	72h	96h	120h
	Comet assay	0 2h 4h	24h				

8. STUDY CALENDAR

Baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Scans and x-rays must be done ≤ 4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

	Pre-study	C1 Wk 1	C1 Wk 2	C1 Wk 3	C1 Wk 4	Cx Wk 1 [f]	Cx Wk 2	Cw Wk 3	Cx Wk 4	Off study
Physical exam	X	X				X				X
Neurologic exam	X [d]									
MMSE	X [i]									
Vital signs	X	X								X
Heights	X									
Weight	X	X								X
Performance Status	X	X								X
CBC w/diff, plts	X	X	X	X	X	X [j]			X [j]	X
Serum chemistry [a]	X	X	X	X	X	X				X
Serum tumor specific tumor markers [g]	X									X
EKG (as indicated)	X	X								
Adverse event evaluation		X	X	X	X	X				
Tumor measurements [h]	X	Tumor measurements are repeated at week 8, then every 8 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease.								X
B-HCG	X [b]									
Pharmacokinetics [e]**, Lab Correlates**		X [c]								

a: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
b: Serum pregnancy test (women of childbearing potential).
c: See section 7.0. Pharmacokinetics will be done during week 1.
d: All patients will be evaluated by a staff Neurologist prior to initiation of treatment and then will undergo frequent (every 4-8 hours) neurologic checks by a CRU RN during the 1-day inpatient CRU stay on cycle 1, day1.
e: Pharmacokinetics will be obtained for the first cycle only.
f: Subsequent cycles and associated evaluations may be conducted within +/- 2 days of the previous cycle.
g: Tumor specific tumor markers to be obtained per physician discretion (CEA, CA 19-9, CA 125, etc) if appropriate.
h. For cohort A, CT of the chest, abdomen and pelvis will be obtained. For cohort B, CT of the chest, abdomen and pelvis as well as MRI of the brain will be obtained if malignancy is a non-CNS primary. For primary brain tumors, CT of the chest, abdomen and pelvis not required.
i. MMSE to be done at screening for subjects in Cohort B only. These subjects must have a score of < 15. See appendix D.
j. CBCs to be done Days 1 and 22 for Cycles 2 and beyond, for both Cohorts.

9. MEASUREMENT OF EFFECT

Although response is not the primary endpoint of this trial, patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients should be reevaluated every 8 weeks. For measurement of effect purposes, baseline imaging studies are the ones obtained pretreatment. If a patient has a complete response, partial response or stable disease, a confirmatory scans will be obtained at least 4 weeks following initial documentation of an objective response. Imaging evaluations will then return to an every 8 week schedule. All responses will be reviewed by the Cancer Center Data Safety and Toxicity Committee.

9.1 Definitions

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [*JNCI* 92(3):205-216, 2000]. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria. Note: Lesions are either measurable or non-measurable using the criteria provided below. The term "evaluable" in reference to measurability will not be used because it does not provide additional meaning or accuracy.

9.1.1 Measurable Disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as $\geq$20 mm with conventional techniques (CT, MRI, x-ray) or as $\geq$10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

9.1.2 Non-measurable Disease

All other lesions (or sites of disease), including small lesions (longest

diameter <20 mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

9.1.3 Target Lesions

All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

9.1.4 Non-target Lesions

All other lesions (or sites of disease) should be identified as **non-target lesions** and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as non-measurable lesions. Measurements of these lesions are not required but the presence or absence of each should be noted throughout follow-up.

9.2 Guidelines for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). In the case of skin lesions, documentation by color photography, including a ruler to

estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI. These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

Ultrasound (US). When the primary endpoint of the study is objective response evaluation, US should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in reference centers. However, such techniques can be useful to confirm complete pathological response when biopsies are obtained.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific additional criteria for standardized usage of prostate-specific antigen (PSA) and CA-125 response in support of clinical trials are being developed.

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

9.3 Response Criteria

9.3.1 **Evaluation of Target Lesions**

Complete Response (CR):Disappearance of all target lesions

Partial Response (PR):	At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD
Progressive Disease (PD):	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

9.3.2 **Evaluation of Non-target Lesions**

Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level
Incomplete Response/ Stable Disease (SD):	Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits
Progressive Disease (PD):	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

Although a clear progression of "non-target" lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail, and the progression status should be confirmed at a later time by the review panel (or study chair).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

9.3.3 **Evaluation of Best Overall Response**

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as

reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria (see section 9.3.1 and 9.4.1).

Target Lesions	Non-target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Note:

- Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration." Every effort should be made to document the objective progression, even after discontinuation of treatment.

- In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

9.4 Confirmatory Measurement/Duration of Response

9.4.1 Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed at a minimum of 4 weeks after the criteria for response are first met. In the case of SD, confirmatory scans must have met the SD criteria at least once at a minimum interval of 4 weeks (see section 9.3.3).

9.4.2 Duration of Overall Response

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

9.4.3 **Duration of Stable Disease**

Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

9.5 Evaluation of response in patients with CNS disease (cohort B)

In addition to evaluation of response using RECIST criteria, additional analyses will be performed on patients with CNS disease enrolled in cohort B. More specifically, contrast-enhanced MRI or CT scans and neurological examinations will be used to determine the response to therapy.

The following modified definitions of response will be used in the additional analyses:

- Complete Response: Complete disappearrance of all tumor on MRI/CT scan, off all glucocorticoids with a stable or improving neurological examination for a minimum of 4 weeks.

- Partial Response: Greater than or equal to 50% reduction in tumor size on MRI/CT scan, on a stable or decreasing dose of glucocorticoids, with a stable or improving neurological examination for a minimum of 4 weeks.

- Progressive Disease: Progressive neurological abnormalities not explained by causes unrelated to tumor progression (e.g. anticonvulsant or corticosteroid toxicity, electrolyte abnormalities, hyperglycemia, etc.) or a greater than 25% increase in the size of the tumor by MRI/CT scan. If neurological status deteriorates, on a stable or

- increasing dose of steroids, or if new lesions appear on serial MRI/CT, further study treatment will be discontinued.

- Stable Disease: A patient whose clinical status and MRI/CT scan do not meet the criteria for *Complete Response*, *Partial Response or Progressive Disease.*

10. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

This protocol will adhere to the policies of the Case Comprehensive Cancer Center Data and Safety Monitoring Plan in accordance with NCI regulations.

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (*Section 10.1*) and the characteristics of an observed AE (*Section 10.2*) will determine whether the event requires expedited reporting **in addition** to routine reporting.

10.1 Comprehensive Adverse Events and Potential Risks Lists

10.1.1 CAEPR

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single, complete list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system.

In addition to the comprehensive list, a subset, the Agent Specific Adverse Event List (ASAEL), appears in a separate column and is identified with ***bold*** and ***italicized*** text. This subset of AEs (the ASAEL) contains events that are considered 'expected' for expedited reporting purposes only.

10.1.1.1 Comprehensive Adverse Events and Potential Risks List (CAEPR) for Methoxyamine

The Comprehensive Adverse Event and Potential Risks list (CAEPR) was developed to provide a single, complete list of reported and/or potential adverse events associated with an agent using a uniform presentation of events by body system. The table below identifies reported adverse events (AEs) that may be expected to occur with Methoxyamine and/or Temozolamide. The subset of AEs that is used to guide expedited reporting requirements [i.e., the Agent Specific Adverse Event List (ASAEL)], as presented in the right hand column, is identified with ***bold*** and ***italic*** text. This subset contains events that are considered expected and may not need to be reported via expedited AE reporting.

Category (Body System)	Adverse Events with Possible Relationship to Methoxyamine (CTCAE v3.0 Term)			"Expected" Adverse Events (see above)
	Likely	Less Likely	Rare but Serious	

	(> 20%)	(≤ 20%)	(< 3%)	
ALLERGY/IMMUNOLOGY				
			Allergic reaction	
BLOOD AND LYMPHATIC SYSTEM DISORDERS				
			Febrile neutropenia	
CONSTITUTIONAL SYMPTOMS				
DERMATOLOGY/SKIN				
ENDOCRINE				
GASTROINTESTINAL				
HEMORRHAGE/BLEEDING				
HEPATOBILIARY/PANCREAS				
INFECTION				
METABOLIC/LABORATORY				
NEUROLOGY				
			hallucinations	
PAIN				
PULMONARY/UPPER RESPIRATORY				
VASCULAR				

10.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 3.0. A copy of the CTCAE version 3.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

- **'Expectedness'**: AEs can be 'Unexpected' or 'Expected' *(see Section X.1 above)* for expedited reporting purposes only. 'Expected' AEs (the ASAEL) are ***bold and italicized*** in the CAEPR *(Section X.1.1)*.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

10.3 SERIOUS ADVERSE EVENT (SAE) REPORTING

All adverse clinical experiences, whether observed by the investigator or reported by the patient, must be recorded, with details about the duration and intensity of each episode, the action taken with respect to the study agent(s), and the patient's outcome. The investigator must evaluate each adverse experience for its relationship to the study agents and for its seriousness.

The investigator must evaluate all abnormal laboratory results for their clinical significance. If any abnormal laboratory result is considered clinically significant, the investigator must provide details about the action taken with respect to the combination of drugs and about the patient's outcome.

Adverse events will use the descriptions and grading scales found in the revised NCI Common Toxicity Criteria (CTC), version 3.0.

Severity Ratings

The investigator will evaluate the severity of each adverse experience using the following definitions:

- Mild-noticeable to the patient, does not interfere with the patient's daily activities, usually does not require additional therapy, dose reduction, or discontinuation of the study drug.
- Moderate-interferes with the patient's daily activities, possibly requires additional therapy, but does not require discontinuation of study drug.
- Severe-severely limits the patient's daily activities and may require discontinuation of the study drug.

Serious, Life-threatening, or Unexpected Adverse Experience (21 CFR 314.80)

- A "serious" adverse event (experience) or reaction is any untoward medical occurrence that at any dose: results in death, is life threatening, results in persistent, significant and/or permanent disability/incapacity, requires inpatient hospitalization or prolongation of existing hospitalization, or is a congenital anomaly/birth defect. A "life-threatening" adverse experience places the patient at immediate risk of death in the judgment of the investigator.
- The definition of serious adverse event (experience) also includes *important medical event*. Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic

bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

- An "unexpected" adverse experience is one not identified in nature, severity, or frequency in the Investigator's Brochure or the product package insert for the study drug.

Relationship to Study Drug

- Probably Related-the experience occurs within a reasonable time period following drug administration or follows a known response for the drug and cannot be reasonably explained by known patient characteristics (including use of concomitant medications). The definition of "related" being that there is a reasonable possibility that the drug caused the adverse experience.
- Unknown Relationship-the etiology of the experience is not known and the experience does not occur within a reasonable time period following drug administration and does not follow a known response pattern for the drug.
- Definitely Not Related-the experience is not known to be caused by the study drug.

Investigator Reporting Responsibilities

The conduct of the study will comply with all FDA safety reporting requirements.

All adverse experience reports must include the patient number, age, sex, weight, severity of reaction (mild, moderate, severe), relationship to study drug (probably related, unknown relationship, definitely not related), date and time of administration of test medications and all concomitant medications, and medical treatment provided. The investigator will record this information on FORM 3500 A (or Medwatch form) and will provide reports of adverse experiences on a regular basis during the study conduct. The investigator is responsible for evaluating all adverse events to determine whether criteria for "serious" and "unexpected" as defined above are present.

Adverse Drug Reaction Reporting

Toxicity will be scored using CTCAE Version 3.0 for toxicity and adverse event reporting. A copy of the CTCAE Version 3.0 can be downloaded from the CTEP homepage (http://ctep.info.nih.gov). All appropriate treatment areas have access to a copy of the CTCAE Version 3.0. All adverse clinical experiences, whether observed by the investigator or reported by the patient, must be recorded, with details about the duration and intensity of each episode, the action taken with respect to the test drug, and the patient's outcome. The investigator must evaluate each adverse experience for its relationship to the test drug and for its seriousness.

The investigator must appraise all abnormal laboratory results for their clinical significance. If any abnormal laboratory result is considered clinically significant,

the investigator must provide details about the action taken with respect to the test drug and about the patient's outcome.

Investigator Reporting to the FDA:

Adverse drug reactions should be reported promptly to the Food and Drug Administration (FDA) in writing by each investigator/physician engaged in clinical research if the type of effect is **Serious, Unlisted/unexpected event, and at least possibly associated to the drug** and has not previously been reported in the Investigators brochure, or reference safety information document, or literature. A clear description of the suspected reaction should be provided along with an assessment as to whether the event is drug or disease related.

The investigator/physician must also call the FDA as soon as an adverse reaction occurs. The phone number is (301) 594-5778. A recorder is available after hours. Report these reactions to the FDA within ten (10) working days both verbal and written.

The address of the Food and Drug Administration is:

FDA
Division of Oncology
HFD-150
1451 Rockville Pike
Rockville, MD 20852-1448

The phone number of the FDA is (301) 594-5778. Please ask to speak with Maureen Pelosi in the Division of Oncology

10.4 DATA SAFETY AND MONITORING PLAN

This protocol will adhere to the policies of the Case Comprehensive Cancer Center Data and Safety Monitoring Plan in accordance with NCI regulations. The Data and Safety Toxicity Committee will review all serious adverse events and toxicity reports as well as annual reviews.

11.0 REGULATORY AND DATA REPORTING REQUIREMENTS

Data will also be collected using the Oncore database at the Case Comprehensive Cancer Center.

Initial Enrollment Forms – at time of enrollment

Patient History Form– within 2 weeks of enrollment

Lab Form – Weekly during treatment

Course Assessment Form – For each treatment cycle

Physical Assessment, Vital Signs and Toxicity Form – For each treatment cycle

Response Form – At the time response is assessed/confirmed

Off Treatment Form – When patient comes off treatment

Survival Form –Patients to be followed annually for survival

12. STATISTICAL CONSIDERATIONS

For cohort A, a maximum of 36 patients can theoretically participate on this study, based on 6 dose levels with a maximum of 6 patients at each dose level. However, given the fact that a 10-fold increase in MX is being tested in 6 consecutive dose escalation levels it is highly unlike that the maximum number of patients will need to be enrolled at each level.

For cohort B, a maximum of 42 patients can theoretically participate on this study, based on 7 dose levels with a maximum of 6 patients at each dose level. However, given the fact that a 1.5- fold increase in TMZ and a 10-fold increase in MX is being tested in 7 dose escalation levels it is highly unlike that the maximum number of patients will need to be enrolled at each level.

The following table gives the probabilities of escalating under given true but unknown underlying rates of DLT:

	True Underlying DLT Rate				
	0.1	0.2	0.3	0.4	0.5
Escalate to next full dose	0.91	0.71	0.49	0.32	0.17
Do not escalate; prior dose level is MTD	0.09	0.29	0.51	0.68	0.83

Thus, if the true underlying rate of DLT is 20%, there will be a 71% chance of escalating to next full dose and a 29% chance that the lower dose will be chosen as the MTD.

The baseline levels (at 0h) of AP sites and DNA strand breaks will be compared to the levels at various time points (i.e. 2h, 4h and 122h) after treatment by paired T-test or Wilcoxon rank sum test. The predictive value of PK parameters (e.g. AUC, C_{max}), levels of AP sites and levels of DNA strand breaks on clinical response and grade 3/4 toxicity will be examined by logistic regression after adjusting the dose level; their predictive value on time to event outcomes, such as disease progression free survival, will be tested using (extended) Cox model after controlling other factors including dose level.

REFERENCES

1. L Liu, P Taverna, CM Whitacre, S Chatterjee, and SL Gerson. Pharmacologic disruption of base excision repair sensitizes mismatch repair-deficient and –proficient colon cancer cells to methylating agents. Clin Cancer Res 5:2908-2917, 1999.
2. L Liu, Y Nakatsuru, and SL Gerson. Base excision repair as a therapeutic target in colon cancer. Clin Cancer Res 8:2985-2991, 2002.
3. L Liu, JR Donze, and SL Gerson. Methoxyamine enhances antitumor efficacy of BCNU in colon tumor xenografts. Proc Amer Assoc Cancer Res 43:794 (#3938), 2002.
4. P Savvides, Yan Xu, Lili Liu, Stanton Gerson. Unexpected prolonged half life of the base-exsicion repair inhibitor Methoxyamine (MX) given with Temozolomide(TMZ) in the first in human phase 1 clinical trial. Proc Amer Assoc Cancer Res (submitted, full abstract included in appendix B)
5. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma.N Engl J Med. 352: 987-96, 2005.
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APPENDIX A

Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B

Submitted Abstract:

Unexpected prolonged half life of the base-excision repair inhibitor Methoxyamine (MX) given with Temozolomide (TMZ) in the first in human phase 1 clinical trial

Author Block: *Panos S. Savvides*, *Yan Xu*, *Lili Liu*, *Stanton Gerson*. Ireland Cancer Ctr., Cleveland, OH, Ireland Cancer Center, Cleveland, OH

Abstract:
Background: MX is the first base excision repair (BER) inhibitor evaluated in humans. MX blocks the BER pathway by covalently binding to apurinic/pymidinic (AP) sites in DNA. In several preclinical studies, improved therapeutic efficacy has been demonstrated with various chemotherapeutic agents including alkylating agents, such as TMZ. Initial results and correlative studies of the first in humans administration of MX are presented.

Methods: This ongoing phase I dose-escalation trial investigates the safety, pharmacokinetic (PK) and pharmacodynamic (PD) profile of MX given as an intravenous continuous infusion (CI) for 5 days in combination with TMZ given orally for 5 days on a 28-day cycle. PD markers, including analysis of AP sites measured on DNA extracted from patients' mononuclear cells (PBMCs) as well as DNA strand break determined by comet assay at multiple time points during the 5-day treatment with MX and TMZ, are included.

Results: 5 patients with metastatic solid tumors have enrolled, 4 at dose-level (DL) 1 (TMZ 100 mg/m2/day, x5days and MX 15 mg/m2/day, x5days CI) and 1 at DL 2 (TMZ 150 mg/m2/day, x5days, MX at same dose as in DL 1). Primary tumors include colon cancer (n=2), head and neck cancer (n=1), radiation-associated sarcoma (n=1), thymic carcinoma (n=1). Treatment has been well tolerated. No treatment-related AEs leading to dose reduction, interruption or discontinuation were reported. No grade 3 or higher treatment related toxicities or AEs have occurred. Grades 1-2 toxicities observed include: nausea, vomiting, fatigue, fever, dyspnea on exertion, anemia, leukopenia, lymphopenia, hypophosphatemia, dyspnea on exertion, nystagmus.

PK analyses on samples from the 5 patients included in the study revealed a distinct PK profile different from the one observed in rats and dogs, where half-life of MX was estimated to be 19 minutes and 4.5 hours respectively. In humans, MX half life is estimated to be 45.3 hours (range: 32 - 68.8 hours).

An increase in formation of AP sites over time in cells was detected after patients received a single dose of TMZ (100 mg/m2), reaching the highest levels 2 or 4 h after TMZ in different patients.

Administration of the combination of TMZ and MX resulted in at least 60% reduction in detectable AP sites, due to the binding of MX to AP sites to form MX-bound AP sites.

Comet assay in PBMCs following treatment with the combination of MX and TMZ detected a 1.5 to 3-fold increase of DNA strand breaks compared to single dose of TMZ.

Conclusions: Initial data from the first in humans MX phase I clinical trial demonstrate that MX in combination with TMZ is well tolerated. MX has a distinct PK profile in humans, corresponding to a 10-fold increase in estimated half-life when compared to the half-life observed in dogs. PD demonstration of MX's biologic activity on patients' mononuclear cells has been demonstrated even at the first dose level.

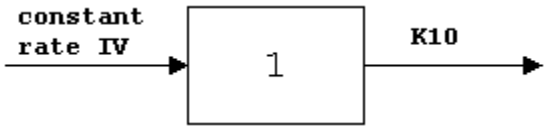
APPENDIX C

Simulation of One-Hour Infusion for a Cmax of 41.40 µg/L

User_Defined_Settings

PK Model 2 - [Untitled6]
Simulating data

1 compartment IV-Infusion, no lag time, 1st order elimination



C(T)=(D/TI)/V/K10*(EXP(-K10*TSTAR)-EXP(-K10*T))

Parameter	Units	Estimate	VIF	Sqrt[VIF]_P_%
V	L	1437.27	0.13	0.81
K10	1/hr	0.015315	0.000000	2.75

Worksheet: Non-Transposed Final Parameters
(20-Jan-2009)

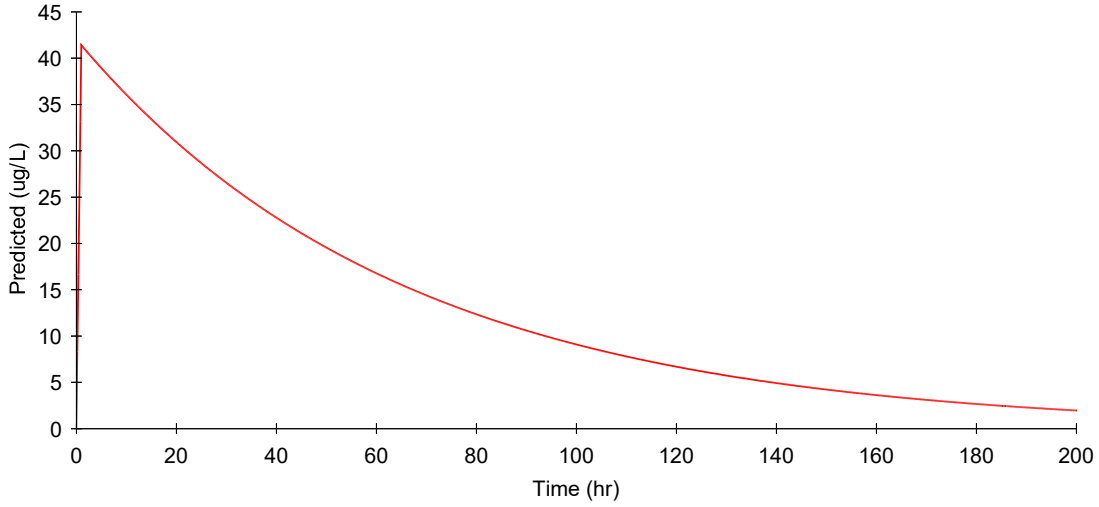
Constant	Value (mg)
Number of Doses	1
Dose #1	59.961
Start Time of Dose #1	0
End Time of Dose #1	1

Worksheet: Dosing
(20-Jan-2009)

Time_obs (hr)	Time (hr)	Predicted (ug/L)	VIF
0	0.0000	0.0000	0.0000
0.1	0.1000	4.1687	0.0011
0.2	0.2000	8.3310	0.0045
0.4	0.4000	16.6365	0.0179
0.6	0.6000	24.9166	0.0399
0.8	0.8000	33.1713	0.0703
1	1.0000	41.4008	0.1091
1.2	1.2000	41.2742	0.1074
1.4	1.4000	41.1480	0.1057
1.6	1.6000	41.0222	0.1041
1.8	1.8000	40.8967	0.1026
2	2.0000	40.7716	0.1011
5	5.0000	38.9408	0.0826
10	10.0000	36.0702	0.0663
15	15.0000	33.4113	0.0630
20	20.0000	30.9484	0.0677
24	24.0000	29.1094	0.0750
36	36.0000	24.2225	0.1032
45.26	45.2600	21.0199	0.1229

60	60.0000	16.7723	0.1417
72	72.0000	13.9566	0.1449
96	96.0000	9.6639	0.1287
120	120.0000	6.6915	0.0992
144	144.0000	4.6334	0.0699
168	168.0000	3.2083	0.0463
200	200.0000	1.9653	0.0250

Parameter	Units	Estimate	VIF	Sqrt[VIF]_P_%
AUC	hr*ug/L	2724.07573	4547.78338	2.48
K10_HL	hr	45.259956	1.546513	2.75
Cmax	ug/L	41.400843	0.109058	0.80
CL	L/hr	22.011503	0.000298	2.48
AUMC	hr*hr*ug/L	179234.144	85014379.981	5.14
MRT	hr	65.296315	3.218864	2.75
Vss	L	1437.270000	0.134888	0.81



X vs. Predicted Y
(20-Jan-2009)

APPENDIX D

University Hospitals
Case Medical Center

APPENDIX III – FUNCTIONAL INDICES
Mini-Mental State Examination (MMSE)

This exam should be administered and filled out by the doctor or nurse. You will need two blank sheets of paper in addition to the two worksheets attached to this questionnaire.

Name: _____ Date: _____

ORIENTATION:

Instructions: Ask the date. Then ask specifically the parts omitted, e.g., "Can you also tell me what season it is?"

1. What is the (year) (season) (date) (day) (month) _____ (5)

Instructions: Ask in turn "Can you tell me the name of this hospital?" (town, county, etc.). One point for each correct response.

2. Where are we? (state) (county) (city) (hospital) (floor) _____ (5)

REGISTRATION:

Instructions: Ask the patient if you may test his/her memory. Then say the names of three unrelated objects, clearly and slowly, about one second for each. After you have said all three, ask the patient to repeat them. This first repetition determines his/her score (0-3) but keep saying them until he/she can repeat all three, up to six trials. If he/she does not eventually learn all three, recall cannot be meaningfully tested.

3. Repeat after me: ball – flag – tree

Record the number recited initially. _____ (3)

Did the patient learn the three words? **YES** **NO**

How many trials were required to learn the words? _____

ATTENTION AND CALCULATION:

Instructions: Ask the patient to begin with 100 and count backwards by 7. Stop after five subtractions. Score the total number of correct answers (93 – 86 – 79 – 72 – 65).

4. Serial 7's. One point for each correct answer. Stop after five answers.

If the patient cannot or will not perform this task, ask him/her to spell the word "world" backwards. The score is the number of letters in correct order, e.g., dlrow = 5, dlorw = 3.

Alternatively, spell WORLD backwards: D – L – R – O – W _____ (5)

Continued on the reverse side

Name: _____ Date: _____

RECALL:

5. Ask for the three objects repeated above.
   Give one point for each correct.

_____
(3)

LANGUAGE:

Instructions: *Naming:* Show the patient a wrist watch and ask him/her what it is. Repeat for pen. Score 0-2.

6. Name these items: (watch and pen)

_____
(2)

Instructions: *Repetition:* Ask the patient to repeat the phrase after you. Allow only one trial. Score 0-1.

Repeat after me: ("no ifs, ands, or buts")

_____
(1)

Instructions: *3-Stage command:* Give the patient a piece of plain blank paper and repeat the command. Score 1 point for each part correctly executed.

Take the piece of paper in your right hand, fold it in half and put it on the floor.

_____
(3)

Instructions: Give the patient item "A" and ask him/her to read and obey. 1 point.

Read and obey item "A". (Close your eyes.)

_____
(1)

Instructions: Give the patient a piece of paper and ask him/her to write a sentence.

Write a sentence:

_____
(1)

Copy the design attached:

_____
(1)

Total score _____
(30)

(J Psychiatr Res 12:189-198, 1975.)

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Close your eyes

Name: _____ Date: _____

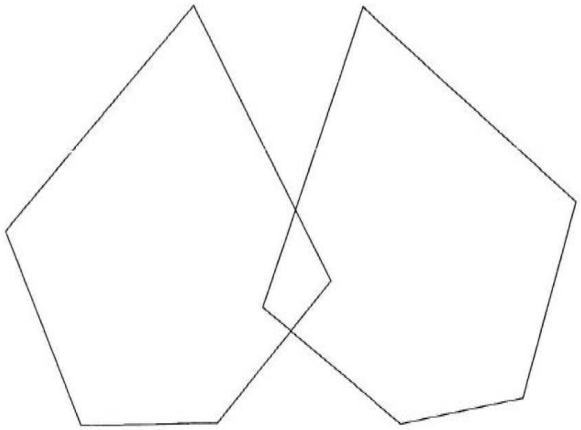

Figure drawn by patient