University of Illinois Cancer Center

STM-03: PHASE I STUDY OF PROCASPASE ACTIVATING COMPOUND-1 (PAC-1) IN THE TREATMENT OF ADVANCED MALIGNANCIES

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REVISION HISTORY

Revision #	Date	Details of changes	Consent change?
1.1	1/05/2015	Addition of pregnancy tests at the start of each study drug treatment cycle, minor clarifications to Component 1 versus Component 2 eligibility criteria, minor clarifications to SAE reporting, follow-up timeframe revised for consistency, clarification that study drug may not be crushed, clarification to PK sample collection windows.	No
1.2	2/05/2015	Revised protocol Component 2 objective to add "after progression following standard first line therapy", removed reference to "standard of care" in the study procedures calendar. Removal of the MTD +1 Dose Level in Component 2. Eligibility criteria revised to exclude greater than grade 1 (previously grade 3) neuropathies. Revised statement concerning supportive medication use per investigator discretion. Correction to follow-up (6 months from study drug completion) and removed reference to "standard of care" in reference to study procedures.	Yes
2.0	3/20/2015	Revised protocol Component 2 in order to allow PI discretion for dosing of temozolomide. Clarifications made to study schedule of events. Minor revisions made to PK collection and processing [IV catheter use instead of butterfly to obtain PK samples, revisions to collection tube, timeframe for processing (120 minutes), centrifuge time, speed, and temperature]. Removal of the Cycle 1 Day 1 neurocognitive function evaluation (since the same exam is performed at baseline). Clarification that an alternate neuropsychiatrist may conduct the neurocognitive function evaluation under the supervision of the lead neuropsychiatrist, Dr. Julie Janecek. Other minor revisions, including clarifications to the study schedule of events and updates to study footnotes and references.	Yes
3.0	6/02/2015	Added Dr. Holdhoff as Co-Investigator (Principal Investigator at JHU site); deleted Dr. Gallia as JHU Co-Investigator; corrected verbiage in Synopsis to reflect that additional patient cohorts will not be enrolled until all patients at the current dose level complete all planned treatment for Cycle 2; added the testing of zinc at baseline and on Day 1 of each cycle; added 32- and 48-h blood draws for PAC-1 PK analyses; changed verbiage in consent form to inform subjects there would be patients enrolled both at UIC and The Johns Hopkins University School of Medicine	Yes
4.0	9/16/2015	Clarified intention to hold PAC-1 dose during Cycle 1, on Days 2 and 12 during both Component 1 and 2 to ensure more accurate pharmacokinetic analyses at 24, 32, and 48-hours post dosing on Days 1 and 11. Also clarification of PK sample acquisition and processing.	Yes
5.0	9/28/2015	For Component One only, clarified intention to conduct the second set of pharmacokinetic analyses beginning on Day 21 rather than on Day 11 of Cycle 1, and in that way only one PAC-1 dose is skipped (Day 2) instead of two (Days 2 and 12).	Yes
6.0	7/7/2017	In Introduction (Section 5.1) and in Drug Information section (Section 14), added summary of results of an 84-day oral toxicity and toxicokinetic study of PAC-1 conducted in dogs. For Component One added 4 additional dose levels (625, 750, 875, and 1000 mg) for a total of 9 dose levels. For Component 2, first cohort will be started at a PAC-1 dose of 375 mg, one dose level below the highest safely tested dose to date in the single agent PAC-1 component (Component 1). An additional 5 dose levels were added (450, 625, 750, 875, and 1000 mg). Modified Inclusion Criterion #2 to: Diagnosis of advanced solid tumor or hematologic malignancy (limited to lymphoma) that has failed or become intolerant to	Yes

		standard therapy and in which expected median survival is less than 12 months (Component 1 - single agent PAC-1). Modified the term "maximal tolerated dose" to what is more commonly used in Phase 1 trial terminology, "maximum tolerated dose." In Component 2, pharmacokinetic testing will be initiated on days 7 and 12 of cycle 1; the last blood draw will be 24-hour post PAC-1 dosing. Added a third study site at HealthPartners – Regions Cancer Care Center in St. Paul Minnesota (Site PI: Richard Peterson, MD). The consent form was modified to reflect changes described above, including informing subjects of the results of the toxicity/toxicokinetic study in dogs in which microscopic tissue vacuolization changes in different regions of the brain were observed, and that vacuolization was still evident 3 months after dogs were taken off PAC-1. The consent form was also modified to indicate that patients have been dosed up to 450 mg of PAC-1 with no observation of PAC-1 related severe adverse events and no changes in neurologic or neurocognitive function have been associated with PAC-1 dosing in humans to date. The Adverse Event section was updated/edited to provide more clarity and reflect the multisite nature of the protocol. The timing for PK dosing and sample collection for Component 2 was revised and clarified. Where appropriate the Investigational Brochure and consent forms were also updated to reflect the above changes.	
6.1	10/31/2017	Changed PK collection time point, sample aliquoting process and sample volume for Component 2 Day 12 collection. Changed hemoglobin level for study entry from ≥10 g/dL to ≥9.0 g/dL. Specified that PBMCs would be collected only in the MTD expansion cohorts and that exploratory endpoints would be done with these samples. Specified that tumor tissue-based exploratory endpoints would be done only when archived or standard of care tissue samples were available.	Yes
7.0	12/14/2017	Added history of cardiomyopathy to patient exclusion, baseline echocardiogram to be performed for all patients and assessment of liver function tests on D12 of the first 4 cycles for Component 2 patients. Added sponsor review of patient eligibility prior to enrollment and of dose modifications during a treatment cycle. Added clarification that more than 3 patients may be enrolled in a dose cohort to ensure that 3 complete 2 cycles of treatment. Modified PAC-1 doses to be administered for dose reductions to be consistent with current tablet strengths.	Yes
8.0	3/01/2018	Added collection of INR and PTT prior to dosing on Day 1 of each treatment cycle, INR>1.5 x ULN to patient exclusion and a baseline MRI of the brain. Potential patients who are receiving blood thinners must be on a stable dose for at least 2 weeks prior to study registration. The changes were made as additional precautions after a Component 1 patient at the 625mg dose level experienced an intracranial hemorrhage where a possible relationship to PAC-1 could not be ruled out. Modified the second dose level for Component from 450 mg/day to 500 mg/day due to available capsule strengths and ease of use for patients. The DLT section was updated to note that serious adverse events that do not meet the defined criteria for DLT may be classified as a DLT at the discretion of the UICC DSMC.	Yes
9.0	8/07/2018	Updated patient exposure information from the study to be current. Revised SAE information based on new information. Removed the requirement to repeat neurological assessments at Cycle 1 Day 1 unless there is a reported or observed change from screening. Corrected text to be consistent with Recist 1.1. Modified the dose to be administered if a dose reduction is needed to be consistent with the next lower dose level in the escalation scheme.	Yes

10.0	1/23/2019	Updated to remove collection of D12 AST and ALT in Cycles 2 through 4 for Component 2 patients and to include up to 6 additional patients with pancreatic neuroendocrine tumors (PNET) at the MTD in Component 1 (in response to one PNET patient in the dose escalation phase who had a partial tumor response to PAC-1).	Yes (Component 2 only)
11.0	5/10/2019	Updated to reduce the Component 2 DLT observation period to 28 days.	No

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2 SYNOPSIS

2.1 Primary Objective

The primary objectives of this study are to determine the maximum tolerated dose (MTD) of PAC-1 alone in patients with advanced malignancy (Component 1), and the MTD of PAC-1 in combination with temozolomide in patients with high grade glioma (Component 2), by evaluation of toxicity and tolerability.

2.2 Patient Population

- Diagnosis of advanced solid tumor or hematologic malignancy (limited to lymphoma) that has failed or become intolerant to standard therapy and in which median expected survival is less than 12 months (single agent PAC-1, Component 1).
- Diagnosis of high grade glioma: glioblastoma multiforme (GBM) or anaplastic astrocytoma after progression following standard first line therapy (PAC-1 in combination with temozolomide, Component 2) (See ECOG Performance Status).
- ECOG status 0, 1, or 2 (Appendix 4)
- Male or female ≥18 years of age
- Adequate hepatic, renal, and bone marrow function as defined in section 8.1 (inclusion criterion #7)

2.3 Study Design

The study will have two components: dose escalation and safety of PAC-1 alone, and dose escalation and safety in combination with temozolomide. PAC-1 alone (Component 1): Up to 9 dose levels will be tested in Component 1(refer to schema on next page). The maximum tolerated dose (MTD) of PAC-1 will be determined using a modified-Fibonacci dose-escalation 3+3 design. Additional patient cohorts will not be enrolled until all patients at the current dose level complete all planned treatment for cycle 2. The MTD dose level will be expanded to a total of at least 9 patients to ensure safety. Up to 6 additional patients may be included who have pancreatic neuroendocrine tumors to assess for initial signals of efficacy. For all dose cohorts, pharmacokinetics of PAC-1 will be assessed following doses administered on days 1 and 21 of the first cycle.

PAC-1 in combination with temozolomide (Component 2): a modified-Fibonacci dose-escalation 3+3 design starts in Component 2, at a PAC-1 dose of 375 mg, one dose level below the highest safely tested dose to date in the single agent PAC-1 Component 1, and 150 mg/m^2 dose of temozolomide given for the 5 days starting at day 8 of cycle 1 in cohorts of 3-6 patients. PAC-1 dose will be increased according to the Component 1 dose escalation schedule but at no time will the PAC-1 dose exceed the MTD determined in Component 1. The combination cohort that reaches MTD will expanded to at least 9 patients, similar to the PAC-1 alone cohort at MTD. For all dose cohorts,

pharmacokinetics of PAC-1 will be assessed following doses administered on days 7 and 12 of the first cycle. Temozolomide pharmacokinetics will be performed on Day 12 of the first cycle.

2.4 Treatment Plan

PAC-1, alone or in combination with temozolomide, will be given at the assigned dose orally (see schema next page) on days 1-21 of a 28-day cycle. Disease reassessment will be done every 2 cycles (8 weeks). Treatment continues until disease progression, unacceptable toxicity, or patient refusal. In order to ensure accuracy in pharmacokinetic analysis findings, PAC-1 drug administration will be held on Day 2 of Cycle 1 for Component 1 only. Please see Section 12 for more detailed information on PK analyses.

3 SCHEMA

3.1 Dose Finding Component

Table 1. PAC-1 dose finding component (component 1)

	Cycle 1ψ	**Cycle 2**	**Cycle 3 and beyond***
PAC-1[1,2,3]	Days 1-21, morning on empty stomach	Days 1-21, morning on empty stomach	Days 1-21, morning on empty stomach

1 cycle = 28 days
* - Treatment continues until disease progression, unacceptable toxicity, patient refusal or other reason as found in section 10.11
ψ – PAC-1 administration will be held on Day 2 to ensure accuracy of PK analysis.
1 - at assigned dose level (see table below)
2 - disease reassessment done every 2 cycles (8 weeks)
3 - pharmacokinetics performed for each dose cohort, assessed on Days 1 and 21 of the first cycle.

3.2 Dose Levels for PAC-1 alone

Table 2. PAC-1 dose levels (component 1)

Dose Level	PAC-1 Dose (mg)	Number of Patients*
1	75 daily	3-6
2	150 daily	3-6
3	250 daily	3-6
4	375 daily	3-6
5	450 daily	3-6
6	625 daily	3-6
7	750 daily	3-6
8	875 daily	3-6
9	1000 daily	3-6

* MTD cohort will be expanded to 9 patients total to assure safety

3.3 Testing PAC-1 in Combination with Temozolomide (component 2)

Table 3. PAC-1 in combination with Temozolomide (component 2)

	Cycle 1	Cycle 2	Cycle 3 and beyond*
PAC-1[1,2,3,]	Days 1-21, morning on empty stomach,	Days 1-21, morning on empty stomach	Days 1-21, morning on empty stomach
Temozolomide [1,2,4]	Days 8-12, dose timing per PI discretion	Days 8-12, dose timing per PI discretion	Days 8-12, dose timing per PI discretion

* - Treatment continues until disease progression, unacceptable toxicity, patient refusal or other reason as found in section 10.11
1 - at assigned dose level (see table below)
2 - disease reassessment done every 2 cycles (8 weeks)
3 - pharmacokinetics performed for each dose cohort, assessed on Days 7 and 12 of the first cycle,
4 – pharmacokinetics performed for each dose cohort assessed on Day 12 of the first cycle.

3.4 Dose Levels for PAC-1 In Combination With Temozolomide

Table 4. PAC-1 dose levels in combination with temozolomide (component 2)

Dose Level	PAC-1 Dose (mg)[#]	Temozolomide Orally (mg/m^2)	Number of Patients*
1	375 daily	150	3-6
2	500 daily	150	3-6
3	625 daily	150	3-6
4	750 daily	150	3-6
5	875 daily	150	3-6
6	1000 daily	150	3-6

[#] first cohort will be started at a PAC-1 dose of 375 mg, one dose level below the highest safely tested dose to date in the single PAC-1 component (i.e., Component 1). PAC-1 dose will never exceed the current maximum tolerated dose (MTD) determined in Component 1.
* MTD cohort will be expanded to 9 patients total to assure safety.

4 ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
ANC	absolute neutrophil count
ALT	alanine aminotransferase
ASCO	American Society of Clinical Oncology
AST	aspartate aminotransferase
cm	centimeter
CNS	central nervous system
CR	complete response
CTCAE	(NCI) Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
dL	deciliter
DLT	dose-limiting toxicity
DSMC	Data Safety Monitoring Committee (at UICC)
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
h	hour
IND	Investigational New Drug
INR	International normalized ratio
IRB	institutional review board
kg	kilogram
lbs	pounds
mg	milligram
min	minute
mL	milliliter
mm^3	cubic millimeters
NCI	National Cancer Institute
MTD	maximum tolerated dose
PAC-1	first procaspase-activating compound
PET	positron emission tomography
PTT	Partial thromboplastin time
RANO	Response Assessment in Neuro-Oncology
RCI	Reliable Change Index
RECIST	Response Evaluation Criteria in Solid Tumors
SAE	serious adverse event
US	United States
WHO	World Health Organization
wt	weight

5 INTRODUCTION AND RATIONALE

5.1 Introduction

5.1.1 Procaspase-3, Caspase 3, and Their Dysregulation In Many Cancers

Members of the caspase family of cysteine proteases are key players in both the initiation and execution of apoptosis, a programmed form of cell death important in both the development and maintenance of higher organisms.[1] Most critical to apoptosis is the proteolytic conversion of procaspase-3 to caspase-3. As both the intrinsic and extrinsic apoptotic pathways converge to activate procaspase-3, and as caspase-3 has over 100 cellular substrates, the activation of procaspase-3 to caspase-3 is a pivotal and committed event in the apoptotic cascade.[2] Procaspase-3 levels are elevated in a variety of tumors including glioblastoma,[3] breast cancer,[4] colon cancer,[5] lung cancer,[6] lymphoma,[7] neuroblastoma,[8] melanoma,[9] and liver cancer.[10] As a consequence, caspase-3 levels are abnormally low in these tumors, allowing the tumors to grow via rapid cell division.

5.1.2 PAC-1, a Small Molecule, Activates Procaspase-3 in vitro and Induces Apoptosis In Cancer Cells

A compound that would directly activate procaspase-3 to caspase-3 would be predicted to induce cell death. Through the screening of a library of 22,000 small molecules for their ability to induce activation of procaspase-3 *in vitro*, and apoptosis of cancer cells in culture, PAC-1 (first procaspase-activating compound) was discovered to have such characteristics.[11]

5.1.3 There Is a Relationship Between Level of Procaspase-3 and The Activity of PAC-1

Because of the elevation of procaspase-3 in certain tumor types, a procaspase-3 activating compound could have selectivity for killing cancer cells versus normal cells.[12] Indeed, it has been shown that the activity of PAC-1 against cancer cells from primary colon and lung tumors is proportional to their procaspase-3 content - the more procaspase-3 a cancer cell has, the more susceptible it is to PAC-1.[11] Importantly, adjacent margin lung and colon tissue (normal cells) are not susceptible to PAC-1-induced cell death.[11]

5.1.4 PAC-1 Shows Efficacy Across a Wide Range of Cancer Cell Lines

PAC-1 has been shown to kill melanoma (UACC-62, CRL-1792, SK-MEL-5, B16F10), breast cancer (MDA-MB-231, BT-20, Hs578t, BT-549), adrenal cancer (PC-12), lymphoma (U-937, EL4, 17-71, Gl-1, OSW), leukemia (HL-60, Jurkat), cervical cancer (HeLa), osteosarcoma (HOS, 143B, K7M2, Abrams, D17) neuroblastoma (Imr32, SK-N-MC), glioblastoma (D54, 9L, U87), and lung cancer (A549, NCI-H226) cell lines.[2,11,12]

5.1.5 Mechanism of Action by Which PAC-1 Activates Procaspase-3

Structure-activity relationship studies revealed that the activity of PAC-1 *in vitro* and in cell culture is dependent on the presence of the ortho-hydroxy *N*-acyl hydrazone moiety,[12] a functional group known to participate in metal chelation.[13] Indeed, zinc is a powerful inhibitor of procaspase-3 enzymatic activity,[14] and the mechanism by which PAC-1 activates procaspase-3 *in vitro* is through chelation of inhibitory zinc from procaspase-3, which allows procaspase-3 to process itself to the active form.[12,14] This same basic mechanism appears to be operational in cell culture as well: approximately 10% of cellular zinc is not bound tightly but exists as the "labile zinc pool".[15] As zinc from the labile pool has been shown to co-localize with procaspase-3,[15] it appears that PAC-1 chelation of this labile zinc inside the cells enhances procaspase-3 activity, leading to apoptosis.

5.1.6 PAC-1 Has Shown Efficacy in Multiple Animal Models of Cancer, Including Brain Cancers

PAC-1 retarded the growth of tumors (lung and renal) in three different mouse models of cancer, including two models in which PAC-1 was administered orally [11]. It had efficacy in a rat model of glioma (unpublished data) and in a mouse model of extranodal peritoneal and brain lymphoma.[17] It is important to point out that PAC-1 penetrates the blood-brain-barrier,[16] making it a potential anti-cancer treatment for CNS metastases. Treating CNS metastatic cancers is a significant unmet need that PAC-1 has a real possibility of addressing.

5.1.7 PAC-1 Potently Synergizes With Standard-of-Care Therapeutics

The activation of procaspase-3 by PAC-1 has been shown to sensitize cancer cells to many standard-of-care agents. PAC-1 dramatically potentiates the ability of FDA-approved cancer drugs (doxorubicin, etoposide, temozolomide, tamoxifen, bortezomib, carboplatin) and the experimental therapeutics YM155 (a survivin inhibitor) and staurosporine) to kill cancer cells in culture (unpublished data). In addition, several studies have shown that these combinations have efficacy *in vivo*. PAC-1 and doxorubicin synergize to kill cancer cells in culture, and have efficacy in a mouse syngeneic model of osteosarcoma. Additionally, PAC-1 and temozolomide synergize to kill cancer cells in culture, and have efficacy in a mouse syngeneic model of osteosarcoma. Especially pertinent to the second component of this proposed trial, PAC-1 synergized with temozolomide to kill glioblastoma cells in culture, and had efficacy in a rat glioma model (i.e., extended survival times compared to either agent alone) (unpublished data).

5.1.8 Pharmacokinetic Properties of PAC-1

The absorption, metabolism, excretion and pharmacokinetic properties of PAC-1 given orally to SD rat and the Beagle dog have been characterized in a series of Good Laboratory Practices (GLP)-compliant (pivotal) and non-GLP complaint studies. The absorption of PAC-1 was assessed in 2 GLP-compliant toxicology studies in which

toxicokinetics were performed to examine the absorption of PAC-1 given on a daily oral dosing schedule for up to 4 weeks. These studies, in SD rat and Beagle dog examined multiple doses for PK parameters up to 600 mg/kg and 50/35 mg/kg, respectively. These are supported by non-GLP dose range finding studies in SD rats and Beagle dogs. The single dose phases of these studies included doses up to 1000 mg/kg in rats and in dogs. The bioavailability studies were performed in the C57Bl/6 mouse and Hound dog examining single dose oral and IV drug administration. Doses examined in those studies were up to 100 mg/kg in mouse and up to 50 mg/kg in dogs. Intravenous and oral PK study in mature female C57BL/6 mice showed bioavailability at 23% of PAC-1 delivered orally at 100 mg/kg in HPβCD (UIUC-10132011.1). In the research hound dog study PAC-1 when dosed in tablet form without food was shown to have an average bioavailability compared to the IV dose of 24%. In addition, the use of food (meatball) for delivery of PAC-1 tablets or capsules to dogs produced enhanced systemic exposure (approximately 3-fold geomean of $AUC_{0-\infty}$ and more consistent oral availability). PAC-1 was found to be approximately 99.6% protein bound in the SD rat, 99.9% in the Beagle dog, and 99.7% in humans.

Pharmacokinetic analysis of PAC-1 plasma levels in Rats and Dogs was performed in GLP-compliant 28-day toxicity studies. These studies revealed close to exponential elimination rate, half-life in systemic circulation varying between 2.09 and 14.2 hr (females only at the STD) in rats and between 1.82 to 5.16 hr in dogs (depending on dose level and day of administration). The high volume of distribution indicating effective penetration of PAC-1 into tissues. Half-life of PAC-1 increased slightly with dose increase. There was no substantial gender difference in PAC-1 pharmacokinetic parameters. C_{max} and AUC_{0-24} values increased less than dose-proportional in rats but more dose-proportional in dogs (excluding the high dose which was decreased due to toxicity). There was only slight accumulation of PAC-1 after its repeated administration. Respective PAC-1 C_{max}/dose, CL/F and V_d/F values were essentially independent of PAC-1 dose within dose ranges tested in dogs.

PAC-1 is a relatively poor inhibitor of all CYP enzymes tested with only CYP2C19, CYP2D6 and CYP3A4 (Testosterone substrate) being the only CYP tested with IC_{50}'s ~20 μM and all others >20 μM. The relatively weak CYP inhibition by PAC-1 was taken into consideration in determining if any concomitant medications should be excluded in the design of the proposed clinical protocol.

5.1.9 PAC-1 Toxicology Studies

Toxicology of PAC-1 has been characterized and observed in mice, rats, and dogs via different routes of administration. In Sprague-Dawley rats (unpublished data), a 7-day multiple dose toxicity study established that orally-administered PAC-1 at doses ranging from 600-2000 mg/kg when given daily for 7 days demonstrated toxicity in the later stages of the study (i.e., weight loss, anorexia, and in some animals, death). In beagle dogs administered oral PAC-1 at doses of 50 mg/kg and 100 mg/kg (free base), and 100 mg/kg in the salt form, for 7 consecutive days, various signs of toxicity were observed during or after the 7-day regimen when either 100 mg/kg in the free base or salt form was

administered that for the most part was gastro-intestinal related (vomiting, diarrhea, loose stools, and decreased food consumption). A seizure did occur in a female dog on Day 7 of the 100 mg/kg/day dosing regimen (salt form) – the animal recovered.

In a 28-day oral gavage toxicity and toxicokinetic study of oral PAC-1 (doses: 0, 100, 200, and 300 mg/kg/day) with a 2-week recovery period in Sprague-Dawley rats, a Functional Observational Battery was used to assess the functional status of the Central Nervous System in the PAC-1 dose groups. One of the tests, open field activity, showed that in males, there was a statistically significant decrease in mean latency time to move when placed in a novel environment in the 200 and 300 mg/kg/day PAC-1 dose groups when compared with the vehicle control group, in Week 4. This decrease in latency times in the test groups was not considered biologically significant because it was not dose-dependent, and was within the limits observed in a baseline period. In addition, in one male rat at 300 mg/kg PAC-1, moderate hydrocephalus of the lateral ventricles was noted. This may be an incidental finding unrelated to the administration of test article, PAC-1; however, given its observation at the high dose, the fact that PAC-1 penetrates the blood-brain barrier, and the general toxicological findings indicating a potential for CNS toxicity, an association with PAC-1 treatment cannot be ruled out.

In a 28-day oral toxicity and toxicokinetic study of oral PAC-1 (doses: 0, 13, 25, and 50 mg/kg) with a 2-week recovery period in beagle dogs, clinical signs including tremors, uncoordinated movement, decreased activity, and vomiting were observed at the 25 and 50 mg/kg doses during the treatment period. Autopsies conducted after terminal sacrifice revealed that the 25 and 50 mg/kg doses were associated with minimal to mild neurodegeneration at multiple sites in the gray matter of the cerebrum. Pathological findings after a two-week recovery period showed in another set of animals that had been given 50 mg/kg dose of PAC-1 during the treatment period, that neuronal degeneration was still present – however, it was diminished in intensity with less disorganization of the affected parenchyma. The neurodegenerative changes observed in the 28-day oral toxicity study in dogs occurred at the 25 mg/kg and 50 mg/kg doses – the highest non-severely toxic dose in dogs identified in this study was 13 mg/kg (260 mg/m^2). Therefore, the starting dose of PAC-1 in component 1 of the clinical study was 75 mg given once daily on Days 1-21 of a 28-day cycle.

After the initiation of this phase I protocol, an 84-day oral toxicity and toxicokinetic study of PAC-1 with 1 month and 3 months recovery periods in male and female beagle dogs was conducted. Daily oral administration (via capsules) of PAC-1 (VO-100) at 6, 13, or 25 mg/kg for 21 consecutive days with a 7-day wash-out period between cycles and repeated for 3 cycles did not result in any test article-related clinical sign, changes on food intake, blood pressure, body temperature, clinical pathology parameters, gross necropsy, or organ weights. Decreased mean body weight gains were only observed in all test article-treated males in the first week of dosing. Test article-related adverse findings were limited to only the brain. Dose-responsive, minimal to mild test article-related vacuolization occurred in the brains of animals given 13 and 25 mg/kg. The extent and severity of the vacuolization was similar to that seen in the 28-day study (see above paragraph) suggesting that prolonged exposure to PAC-1 does not increase the severity of

this adverse finding. It should also be noted that the observed brain vacuolization did not show evidence of recovery after 3 months of being off study drug.

PAC-1 has been dosed in 31 human patients through 6 dose levels of PAC-1 monotherapy with 24 patients having received PAC-1 for at least 2 dosing cycles (a cycle is 21 days of daily dosing followed by 7 days of no drug). As a result of stable disease responses, three patients received PAC-1 for 4 cycles, one patient received PAC-1 for 6 cycles and two patients received PAC-1 for 10 cycles. PAC-1 has also been dosed in seven patients in the first dose level of PAC-1 in combination with Temozolomide (TMZ) with 5 patients having received the combination for at least 2 dosing cycles. Three patients received PAC-1/TMZ for 4 cycles and one patient received the combination for 6 cycles. One serious adverse event (SAE) of intracranial hemorrhage was initially thought to be possibly associated with PAC-1/TMZ administration but subsequent follow-up indicated that the patient had a metastatic lesion at the site of the bleed which was deemed related to underlying disease. Further, after careful review of all neurological and neurocognitive assessments and data collected through dose level 5 (450 mg) there were no conclusive signs of neurotoxicity associated with PAC-1 dosing. Nevertheless, the brain tissue changes observed in both GLP dog PK/tox studies highlights the need for continued neurologic and neurocognitive testing and observational diligence in detecting any possible neurological changes associated with PAC-1 exposure.

PAC-1 was investigated alone and in combination with TMZ in two rodent (mouse and rat) intracranial models of Glioblastoma Multiforme (GBM). The primary metrics for these studies were increased survival and general tolerance of the drug combination as measured by observation and in the case of the mouse study weight changes. Significant increases in survival times were seen with the PAC-1/TMZ combination over the control and single agent groups. In addition, no observable clinical signs of toxicity or changes in body weights due to the combination of the two test articles were observed. Thus, addition of the second test article did not increase toxicity while significantly increased efficacy.

5.2 Study Rationale

Members of the caspase family of cysteine proteases are key players in both the initiation and execution of apoptosis. Most critical to apoptosis is the proteolytic conversion of procaspase-3 to caspase-3. As both the intrinsic and extrinsic apoptotic pathways converge to activate procaspase-3, and as caspase-3 has over 100 cellular substrates, the activation of procaspase-3 to caspase-3 is a pivotal and committed event in the apoptotic cascade. Procaspase-3 levels are elevated in a variety of tumors including glioblastoma, breast cancer, colon cancer, lung cancer, lymphoma, neuroblastoma, melanoma, and liver cancer. PAC-1 is a small molecule, which enhances procaspase-3 activity *in vitro*, induces apoptotic death of cancer cells in culture, and has efficacy in multiple mouse xenograft models. There is a strong correlation between cellular procaspase-3 levels and the apoptosis-inducing properties of PAC-1. PAC-1 penetrates the blood-brain-barrier, and has been found to have efficacy in brain cancer models. PAC-1 also has a synergistic anti-glioblastoma effect when used in combinations with a front-line chemotherapeutic agent for brain cancers, temozolomide. PAC-1 has shown toxicity in mice, rats and dogs. The toxicity is more typically observed with high doses. However, in one study testing

relatively lower doses of PAC-1 given orally on a daily basis for 28 days in dogs, there was evidence of minimal-mild neurodegeneration. Overall, PAC-1 has a number of attributes which makes this a potentially efficacious anticancer drug in humans. Accordingly, we propose to do a study consisting of two components: to determine the maximum tolerated dose (MTD) of PAC-1 in solid tumor or hematologic malignancy, and to determine the maximum tolerated dose of PAC-1 when combined with the front-line chemotherapeutic agent, temozolomide, in patients with primary brain tumors. Primary endpoints will be evaluation of tolerability and toxicity. Neurological symptoms of CNS toxicity will be assessed throughout the trial.

6 OBJECTIVES

6.1 Primary Objective Component 1

The primary objective of this study component is to determine the maximum tolerated dose (MTD) of PAC-1 as a single agent in patients with advanced, previously treated malignancy, by evaluation of toxicity and tolerability.

6.2 Primary Objective Component 2

The primary objective of this study component is to determine the maximum tolerated dose (MTD) of PAC-1 in combination with temozolomide in patients with high grade glioma: glioblastoma multiforme (GBM) or anaplastic astrocytoma after progression following standard first line therapy, by evaluation of toxicity and tolerability.

6.3 Secondary Objectives Component 1

The secondary objectives of this study component are to determine the clinical response and adverse effects of PAC-1 as a single agent, in patients with advanced, previously treated malignancy.

6.4 Secondary Objectives Component 2

The secondary objectives of this study component are to determine the clinical response and adverse effects of PAC-1 in combination with temozolomide in patients with GBM or anaplastic astrocytoma.

6.5 Correlative Objectives Components 1 and/or 2

- Determine pharmacokinetics of PAC-1 (both components)
- Determine pharmacokinetics of temozolomide (Component 2)
- Evaluate PAC-1 for QT prolongation potential (both components)

- Evaluate neurocognitive functioning as a function of time on treatment in patients administered PAC-1 (Component 1) and PAC-1 in combination with temozolomide (Component 2)

- Correlate procaspase-3 expression in tumor with activity of PAC-1 (both components but only for patients with archived tissue available and/or collected as part of standard of care)

- Correlate activation of procaspase-3 in peripheral blood mononuclear cells with activity of PAC-1 (both components but only for patients in the MTD cohort)

- Correlate status of promoter methylation for methylguanine methyltransferase (MGMT) with PAC-1 and temozolomide activity (Component 2)

7 OVERALL DESIGN AND STUDY PLAN

7.1 PAC-1 Alone (Component 1)

This Phase I dose escalation study will evaluate PAC-1 in patients with advanced malignancy that has failed or become intolerant to standard therapy. PAC-1 is given as a capsule to be taken once daily during the first 21 days of each 28-day cycle. During cycle 1 only, the PAC-1 dose is not administered on day 2 for pharmacokinetic sampling. Treatment continues until disease progression, unacceptable toxicity, patient refusal, or if they pass away either from progression of disease, the therapy itself, or from other causes. Patients, who voluntarily stop the study, have progressive disease, or unacceptable toxicities will be followed for a total of 6 months.

The objective of this component of the study is to determine the MTD of PAC-1. The MTD of PAC-1 will be determined using a modified-Fibonacci dose-escalation 3+3 design. Three patients will initially be enrolled into each dose level cohort but additional patients may be enrolled to ensure that 3 patients are evaluable for DLT assessment and are able to complete 2 full cycles of treatment. Escalation to the next dose will continue unless a patient experiences a DLT, at which time a cohort will be expanded to up to 6 patients who are evaluable for DLT assessment and are able to complete 2 full cycles of treatment. If there are two DLTs experienced during the first cycle of therapy (>33% of patients experiencing DLT), the cohort will be deemed unsafe and one step lower level will be expanded to 6 patients.

If none of the dose levels are acceptable at study completion, a MTD will not be identified, and the drug does not warrant further investigation.

Dose limiting toxicity (DLT) is defined as one of the following events due to study medication occurring during cycle 1(first 28 days) with the exception of neurological toxicity (see below).

- Grade 3 or greater treatment related hematologic toxicity for > 48 hours during the first cycle (28 days) of therapy. If grade 3 or greater cytopenia occurs, daily blood counts will be performed until cytopenia improves ≤ grade 2.

- Cerebrovascular ischemia or hemorrhage of any duration or grade.

- Grade 3 or greater treatment related clinical non-hematological toxicity (excluding ≥ grade 3 nausea, vomiting, or diarrhea without maximal medical intervention and/or prophylaxis) during the first cycle (28 days) of therapy.

- Delay of cycle 2 treatment start by more than 2 weeks due to incomplete hematologic recovery (ANC $\geq$ 1.5 x 10^9/L or platelets $\geq$ 100 x 10^9/L) or unresolved treatment related grade 3 or greater non-hematologic toxicity.

- Grade 2 or greater treatment-related neurological toxicity occurring during the first 2 cycles of therapy and lasting more than 72 hours (for Component 1) and Grade 2 or greater treatment-related neurological toxicity occurring during the first cycle of therapy and lasting more than 72 hours (for Component 2).

Serious adverse events that do not meet the defined criteria for DLT may be classified as a DLT at the discretion of the UICC DSMC. DLT's will be counted based on the number of patients with DLT at a given dose level, not the absolute number of DLTs. No single patient can trigger more than one DLT event.

Additional patient cohorts will not be enrolled until all patients at the current dose level complete all planned treatment for cycle 2 (for Component 1) or for cycle 1 (for Component 2).

Once the MTD of PAC-1 is determined, enrollment will continue until at least 9 patients total are accrued at the MTD to assess for safety. Up to 6 additional patients with pancreatic neuroendocrine tumors may also be included to assess for initial signals of efficacy. Pharmacokinetic analyses will be performed on day 1 and 21 of first cycle for patients in Cohort 1 and on day 7 and 12 of the first cycle for patients in Cohort 2.

7.2 PAC-1 in Combination with Temozolomide (Component 2)

This Phase I dose escalation study will evaluate PAC-1 in combination with temozolomide in patients with diagnosis of primary brain tumor. PAC-1 is given as tablets to be taken once daily during the first 21 days of each 28-day cycle. Pharmacokinetic assay for PAC-1 will be performed during day 7 and 12 of the first cycle. Temozolomide is initiated during cycle 1 on day 8 for 5 days, and each successive cycle. Temozolomide PK will be performed on day 12 of the first cycle. Treatment continues until disease progression, unacceptable toxicity, patient refusal, or if they pass away either from progression of disease, the therapy itself, or from other causes. Patients, who voluntarily stop the study, have progressive disease, or unacceptable toxicities will be followed for a total of 6 months (See Section 10.12 for follow-up requirements).

The objective of this component of the study is to determine the MTD of PAC-1 when combined with temozolomide in patients with a diagnosis of primary brain tumor. A combination of PAC-1 with 150 mg/m^2 dose of temozolomide will be given for 5 days starting on day 8 of each 28 day cycle in cohorts of 3-6 patients who are evaluable for DLT assessment and are able to complete 1 full cycle of treatment. The starting dose of PAC-1 will be 375 mg, one dose level below the highest safely tested dose to date in the single agent PAC-1 component (i.e., Component 1). PAC-1 dose will be increased according to the Component 1 dose escalation schedule but at no time will the PAC-1 dose exceed the current MTD determined in Component 1. The combination cohort that reaches MTD will be expanded to at least 9 patients, similar to the PAC-1 alone cohort at MTD.

8 SELECTION OF PATIENTS

Study entry is open to adults regardless of gender or ethnic background. While there will be every effort to seek out and include women and minorities, the patient population is expected to be no different than that of other advanced hematologic or solid tumor cancer studies (Component 1) at the University of Illinois, the Johns Hopkins University, and HealthPartners – Regions Cancer Care Center or primary brain tumor cancer studies (Component 2) at the University of Illinois, the Johns Hopkins University, and HealthPartners – Regions Cancer Care Center.

8.1 Inclusion Criteria

1. Male or female ≥ 18 years of age

2. Diagnosis of advanced solid tumor or hematologic malignancy (limited to lymphoma) that has failed or become intolerant to standard therapy and in which median expected survival is less than 12 months (**Component 1** - single agent PAC-1). Note: Gliomas are excluded from Component 1 (see exclusion #19)

3. Diagnosis of high grade glioma: glioblastoma multiforme (GBM) or anaplastic astrocytoma after progression following treatment with standard first line therapy (**Component 2** - PAC-1 in combination with temozolomide).

4. Has measurable disease, defined as at least 1 tumor that fulfills the criteria for a target lesion according to RECIST 1.1 (**Component 1**).

5. For patients in study **Component 2** measurable disease RANO criteria will be used.

6. Has an Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2 (see Appendix 4)

7. Has adequate hepatic function defined as total bilirubin ≤ 1.5 mg/dL, serum albumin ≥ 3.0 gm/dL, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 1.5 × upper limit of normal (ULN) or ≤ 3 x ULN for subjects with known hepatic metastases

8. Has adequate renal function defined as serum creatinine $\leq 1.5 \times$ ULN

9. Has adequate bone marrow function defined as a hemoglobin ≥ 9.0 g/dL, absolute neutrophil count (ANC) $\geq 1.5 \times 10^9$/L, and platelet count $\geq 100 \times 10^9$/L

10. Patients taking antiepileptic drugs (AED) must be on stable doses of AED for at least two weeks prior to registration and have no episode of seizures for at least 14 days prior to registration. Because some AEDs enhance or inhibit enzymes that may affect PAC-1 metabolism, those AEDs will not be permitted in this study. The AEDs that are and are not permissible are in Appendix 6.

11. Patients taking any blood thinner(s), must be on a stable dose for at least two weeks prior to registration.

12. Patient must be able to take oral medication and to maintain a fast as required for 2 hours before and 1 hour after capsule(s) administration

13. Must be willing and able to comply with study visits and procedures

14. Has read, understood and signed the informed consent form (ICF) approved by the Institutional Review Board/Independent Ethics Committee (IRB/IEC)

15. Women of childbearing potential (WOCP) must not be pregnant (confirmed by a negative pregnancy test, with a serum *B*-HCG with a sensitivity of 50 mL U/L within 7 days of study treatment) or breast-feeding. In addition, a medically acceptable method of birth control must be used such as an oral, implantable, injectable, or transdermal hormonal contraceptive, an intrauterine device (IUD), use of a double barrier method (condoms, sponge, diaphragm, or vaginal ring with spermicidal jellies or cream), or total abstinence during the study participation and for one month after last dose of study drug(s). Women who are postmenopausal for at least 1 year or surgically sterile (bilateral tubal ligation, bilateral oophorectomy, or hysterectomy) are not considered to be WOCP.

16. Men who are not surgically or medically sterile must agree to use an acceptable method of contraception. Male patients with female sexual partners who are pregnant, possibly pregnant, or who could become pregnant during the study must agree to use condoms at least one month after the last dose of study drug. Total abstinence for the same study period is an acceptable alternative.

17. Prior systemic treatments for metastatic disease are permitted but may not be ongoing, including targeted therapies, biologic response modifiers, chemotherapy, hormonal therapy, or investigational therapy (see Exclusion #20).

18. Willingness to donate blood for biomarker studies related to the type of therapies used in this trial and the tumor types being treated

8.2 Exclusion Criteria

19. Had surgery within 4 weeks prior to study treatment except for minor procedures (hepatic biliary stent placement is allowed)

20. For Component 1 (PAC-1 alone), gliomas are excluded, as well as any history of brain metastases, seizures or underlying brain injury (e.g., traumatic brain injury, or hemorrhagic or ischemic stroke)

21. Patients may not have received cytotoxic chemotherapy, targeted therapies, biologic response modifiers, chemotherapy, and hormonal therapy within the last 3 weeks, or nitrosureas within the last 6 weeks prior to study treatment.

22. Has a known hypersensitivity to temozolomide (this criterion applies only in Component 2).

23. Has INR >1.5 x ULN

24. Has a history of blood clots, pulmonary embolism, or deep vein thrombosis unless controlled by anticoagulant treatment (patient must be on stable dose for 2 weeks)

25. Has any history of cardiomyopathy or a history of an arterial thromboembolic event within the prior six months including cerebrovascular accident, transient ischemic attack, myocardial infarction, or unstable angina.

26. Has uncontrolled human immunodeficiency virus (HIV) (defined as HIV RNA >500 copies/ml and CD4+ count<200/mm^3 on antiretroviral therapy) infection or hepatitis B (defined as ALT > 1 x ULN, and HBV DNA >2000 IU/ml) or hepatitis C (defined as ALT > 1 x ULN, persistent viremia on antiviral therapy) infections.

27. Has any clinically significant infection, i.e., any acute viral, bacterial, or fungal infection that requires specific treatment (anti-infective treatment has to be completed ≥ 7 days prior to study entry)

28. Has any other severe, uncontrolled medical condition, including uncontrolled diabetes mellitus (defined as a Hemoglobin A1C≥ 9% in patients with a prior history of diabetes, 28 days prior to study) or clinical signs of unstable congestive heart failure (Stage III-IV of the New York Heart Association Functional Classification) (Appendix 5).

29. Radiation therapy to more than 25% of the bone marrow. Whole pelvic radiation is considered to be over 25%.

30. Prior allogeneic bone marrow or organ transplantation.

31. > Grade 1 peripheral neuropathy within 14 days before enrollment.

32. Pregnant or breastfeeding – temozolomide is Pregnancy Category D – can cause fetal harm. Confirmation that the subject is not pregnant must be established by a negative serum beta-human chorionic gonadotropin (beta-hCG) pregnancy test result obtained during screening. Pregnancy testing is not required for post-menopausal or surgically sterilized women.

33. Patient has received other investigational drugs within 14 days prior to study treatment.

34. Other severe acute or chronic medical or psychiatric conditions or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the patient inappropriate for enrollment in this study.

35. Abnormalities on 12-lead electrocardiogram (ECG) considered by the investigator to be clinically significant (such as acute ischemia, left bundle branch block, ventricular arrhythmias) or baseline prolongation of the rate-corrected QT interval (e.g., repeated demonstration of QTc interval > 480 milliseconds).

36. Presence of any non-healing wound, fracture, or ulcer within 28 days prior to the first dose of study drug.

37. Has any condition that, in the opinion of the investigator, might jeopardize the safety of the patient or interfere with protocol compliance

38. Has any mental or medical condition that prevents the patient from giving informed consent or participating in the trial

9 REGISTRATION

Registration will occur after the patient has signed the patient consent and eligibility is confirmed, but before any treatment has been administered. To be eligible for registration to this study, the patient must meet each criterium listed on the eligibility checklist based on the eligibility assessment documented in the patient's medical record. Site personnel will submit a completed Patient Enrollment Form which will be reviewed and approved by the Sponsor's Medical Monitor prior to initiating patient dosing. A copy of the eligibility checklist and/or the Patient Enrollment Form is maintained by each local site and should be filed for each individual subject within the subject shadow chart.

9.1 Registration in OnCore

Upon completion of the screening evaluation, eligibility checklist, including the Patient Enrollment Form signed by the Sponsor's Medical Monitor, and obtaining consent, the site study coordinator or designee will enroll the patient into the OnCore® data management system.

The PAC-1 (and temozolomide, Component 2) dose levels will be assigned at registration.

9.2 Patients Who Do Not Begin Study Treatment

If a patient signs consent and is registered to the study, and is later found not able to begin the planned study treatment, for whatever reason, the patient will be removed from study and treated at the physician's discretion. The patient will be considered a screen/baseline failure and be replaced. The reason for removal from study will be clearly indicated in the OnCore® data management system.

If a patient begins treatment, and then is discontinued for whatever reason, the patient must be followed per section 10.12.

10 TREATMENT PLAN

10.1 Administration of Study Drugs

A new treatment cycle will only be initiated when all of the following conditions are met:

- ANC $\geq$ 1,500/mm^3
- Platelets $\geq$ 100,000/mm^3
- INR$\leq$1.5 xU:N
- Non-hematologic treatment related toxicities have improved to grade 1 or resolved (CTCAE v. 4)

Refer to section 10.8 for guidelines for dose modifications based on treatment-related delays and worse grade toxicities of the previous cycle.

10.2 PAC-1 (Components 1 and 2)

Dosing will occur in 28-day cycles. In Component 1, PAC-1 (PO) will be dosed at 75-1000 mg daily in the morning on days 1-21 in each cycle. In Component 2, the first PAC-1 dose will be 375 mg, one dose level below the highest safely tested dose to date in the single agent PAC-1 component (Component 1), and the maximum dose will not exceed the MTD determined in Component 1. PAC-1 will be taken in the morning on days 1-21 in each cycle. During cycle 1 for each dose cohort, subjects will be instructed to administer their daily dose at the same approximate time (+/- 1 hour) each morning. Subsequent cycles must meet the criteria found in section 10.8.1 and may begin 1 day earlier or up to 2 days later to accommodate scheduling issues. The study drug will be administered in the morning and on an empty stomach with the patient remaining NPO (nothing by mouth), except for water and prescribed medications, for 2 hours before and 1 hour after each dose. Patients will be instructed to take each oral dose of PAC-1 with 8 ounces (1 cup, 240 mL). Missed doses will not be made up. All tablets are to be ingested

as dispensed by research team (e.g. tablets may not be broken or crushed by subject); patients who have difficulty swallowing tablets will be excluded from the study. If vomiting occurs during the course of treatment, no re-dosing of the patient is allowed before the next scheduled dose. Missed doses will not be made up. Patients in Components 1 will not receive their PAC-1 dose on day 2 of Cycle 1. The dose on this day is being held in order to accurately define the absorption and metabolism of PAC-1 in patients at 24, 32, and 48 hours post drug administration.

10.3 Temozolomide (Component 2)

Temozolomide (PO) will be dosed at 150 mg (adjusted for body size area [m^2]) daily for 5 days starting on day 8 at cycle 1, and then for each successive cycle. Cycles must meet the criteria found in section 10.8.1 and may begin 1 day earlier or up to 2 days later to accommodate scheduling issues. Temozolomide will be administered (dose timing per investigator discretion) on an empty stomach with the patient remaining NPO (nothing by mouth), except for water and prescribed medications, for 2 hours before and 1 hour after each dose. Patients will be instructed to swallow the capsules whole, in rapid succession, without chewing them. Patients will be instructed to take the temozolomide capsule(s) with 8 ounces (1 cup, 240 mL) of water and to administer their daily dose at the same approximate time (+/- 1 hour) each day. If vomiting occurs during the course of treatment, no re-dosing of the patient is allowed before the next scheduled dose. Missed doses will not be made up. Patient will be given a prophylactic dose of the anti-emetic, ondansetron (4 mg tablet, 1 or 2) to be taken 30-60 minutes before ingesting temozolomide.

10.4 Monitoring

Patients will be required to come every 4 weeks to the clinic to meet with their oncologist, have their weight and vital signs (blood pressure, pulse, temperature, respirations, and pulse oximetry) measured, have blood drawn for CBC and coagulation testing, and pick up their drug(s) for the next four weeks. Patients at baseline and at the start of each monthly cycle will also see a study neurologist (e.g. Dr. Nicholas at UIC) or a trained medical professional skilled in doing neurological assessments under the supervision of the neurologist for a complete neurological examination (Appendix 7), that includes use of the Mini Mental Status Examination (Appendix 8). During these clinic visits (except C1D1), the patient will also see a neuropsychologist, or a trained medical professional skilled in doing such assessments under the supervision of the neuropsychologist, who will administer 3 cognitive tests (Appendix 9). The Reliable Change Index (RCI), described in a summary paper on neurocognitive testing in clinical trials developed by the Health Services and Research Outcomes (HSRO) Subcommittee of the Radiation Therapy Oncology Group (RTOG), will be used to monitor neurocognitive changes and as a guide for dose reductions (see sections 12.4 and Appendix 10).

Patients will keep a drug diary to record each dose of PAC-1 (and temozolomide in Component 2) administered and time of day when administered, and any side effects they

are experiencing. The oncologist will record information from the diaries, discuss any concerns the patient has regarding the treatment, and will also collect any unused medication during these visits. Patients will be closely monitored for toxicities. We will assess toxicity using CTCAE version 4.0 (evs.nci.nih.gov).

10.5 Supportive Measures

PAC-1 alone (Component 1): Patients will be told if they experience any symptoms that they associate with the tablet being taken that they should contact the Principal Investigator or a member of her research study team immediately.

PAC-1 in combination with temozolomide (Component 2): Temozolomide is associated with adverse effects. Prophylactic antiemetic therapy with ondansetron 4 mg tablet (1 or 2) will be prescribed to be taken 30-60 minutes before temozolomide and every 8 hours as needed.

For both Component 1 and Component 2, supportive medications, such as acetaminophen for fever, meperidine for chills, anti-emetics for nausea and vomiting, and loperamide for diarrhea, may be given per Principal Investigator discretion to patients depending on the side effects they report.

10.6 Precautions

Patients should not drive, operate dangerous tools or machinery, or engage in any other potentially hazardous activity that requires full alertness and coordination if they experience sedation while enrolled in this study.

Patients are to be instructed to refrain from using alcohol while enrolled in this study.

One Component 1 patient who was in the 625mg/day cohort experienced an intracranial hemorrhage where a possible relationship to PAC-1 could not be ruled out. Although it did not meet the stated definition of a DLT because it occurred after cycle 2 of therapy, the independent DSMC recommended that the 625mg/day cohort be expanded to include 3 additional patients. In order to monitor and ensure patient safety, patients were subsequently required to have a baseline brain MRI for future comparison if needed. Coagulation tests were also required to be done prior to dosing on Day 1 of each treatment cycle. Subsequent follow-up for this patient revealed a metastatic lesion at the site of the bleed so causality was reassigned to underlying disease. Although the DSMC no longer requires the baseline brain MRI or coagulation tests, patients will continue to have these done as a safety precaution.

Temozolomide can cause fetal harm when administered to a pregnant woman. Based on its mechanism of action, temozolomide is expected to result in adverse reproductive effects. Temozolomide was teratogenic, embryotoxic, and fetotoxic in mice and rabbits. If a woman becomes pregnant or suspects that she is pregnant while participating in this study, she must inform the investigator immediately as detailed in section 15.17 and must permanently discontinue study drug. Effective barrier contraception must be practiced

during the entire study treatment period and through one month after the last dose of study drug by females of childbearing potential and male patients (even if surgically sterilized (i.e., status post vasectomy) or the patient must completely abstain from heterosexual intercourse.

Refer to section 15.17 for reporting pregnancy in a female patient or the partner of a male patient.

10.7 Drug Dose Level Assignment

10.7.1 PAC-1 alone (Component 1)

Dose level assignment will occur at the time of study registration.

Table 5. Dose Levels for PAC-1 alone (component 1)

Dose Level	PAC-1 Dose (mg)	Number of Patients*	Dose Reduction Level^
1	75 daily	3-6	Not applicable
2	150 daily	3-6	Not applicable
3	250daily	3-6	Not applicable
4	375 daily	3-6	250 daily
5	450 daily	3-6	250 daily
6	625 daily	3-6	450 daily
7	750 daily	3-6	625 daily
8	875 daily	3-6	750 daily
9	1000 daily	3-6	875 daily

^Required dose reductions are described in Table 7.

* MTD cohort will be expanded to 9 patients total to assure safety, pharmacokinetics analysis will be performed in all cohorts (assessed on Days 1 and 11 or 21 of the first cycle depending on the protocol version).

Toxicity and adverse events will be classified according to NCI's Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE v 4).

Dose limiting toxicity (DLT) is defined as one of the following events due to study medication occurring during cycle 1 (first 28 days) with the exception of neurological toxicity (see below):

- Grade 3 or greater treatment related hematologic toxicity for > 48 hours during the first cycle (28 days) of therapy.
- Cerebrovascular ischemia or hemorrhage of any duration or grade.

- Grade 3 or greater treatment related clinical non-hematological toxicity (excluding ≥ grade 3 nausea, vomiting, or diarrhea without maximal medical intervention and/or prophylaxis) during the first cycle (28 days) of therapy.

- Delay of cycle 2 treatment start by more than 2 weeks due to incomplete hematologic recovery (ANC ≥ 1.5 x 10^9/L or platelets ≥ 100 x 10^9/L) or unresolved treatment related grade 3 or greater non-hematologic toxicity.

- Grade 2 or greater treatment related neurological toxicity occurring during the first 2 cycles of therapy and lasting more than 72 hours (for Component 1) and Grade 2 or greater treatment-related neurological toxicity occurring during the first cycle of therapy and lasting more than 72 hours (for Component 2).

DLT's will be counted based on the number of patients with DLT at a given dose level, not the absolute number of DLTs. No single patient can trigger more than one DLT event.

Additional patient cohorts will not be enrolled until all patients at the current dose level complete all planned treatment for cycle 1.

10.7.2 PAC-1 In Combination With Temozolomide (Component 2)

Table 6. Dose Levels for PAC-1 + Temozolomide combination (component 2)

Dose Level	PAC-1 Dose (mg)#	Temozolomide Orally (mg/m2)	Number of Patients*	Dose Reduction Level^
1	375 daily	150	3-6	250 daily
2	500 daily	150	3-6	250 daily
3	625 daily	150	3-6	500 daily
4	750 daily	150	3-6	625 daily
5	875 daily	150	3-6	750 daily
6	1000 daily	150	3-6	875 daily

first cohort will be started at a PAC-1 dose of 375 mg, one dose level below the highest safely tested dose to date in the single PAC-1 component (Component 1). PAC-1 dose will never exceed the current maximum tolerated dose (MTD) determined in Component 1.
^Required dose reductions are described in Table 7.
* MTD cohort will be expanded to 9 patients total to assure safety.

Enrollment will follow a 3+3 design. Dose limiting toxicity (DLT) is defined in section 7.1.

10.8 Dose Delays/Modifications

10.8.1 Start of a New Cycle

Toxicity and adverse will be classified according to NCI's Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE v 4). A copy of the CTCAE can be downloaded from the CTEP home page (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

A new treatment cycle will only be initiated when all of the following conditions are met:

- ANC $\geq$ 1,500/mm^3
- platelets $\geq$ 100,000/mm^3
- INR$\leq$1.5 xULN
- non-hematologic treatment related toxicities have improved to $\leq$ Grade 1 or to the patient's baseline values (except alopecia)

If blood counts are below this threshold, blood work is to be repeated weekly until counts are at an acceptable level. Treatment will be restarted with appropriate dose modifications. If treatment is unable to restart within 3 weeks of the planned treatment date, the patient will be permanently discontinued from study therapy. Note: For cycle 1 only, the inability to restart therapy within 2 weeks of the planned date is a DLT (per section 10.7.1); however the patient is allowed an additional week of recovery and is allowed to remain on study if treatment restarts within 3 weeks.

For subjects who present to the clinic with toxicity during a current treatment cycle, the study drug may be modified or stopped per the discretion of the Principal Investigator, in consultation with the Sponsor's Medical Monitor, if the toxicity is determined to be clinically significant.

Table 7. PAC-1 adverse event dose modifications

CTCAE v 4 Adverse Effects Terms & Descriptions)	PAC-1 Dose Modifications
Any other grade 3 non-hematologic toxicity	Hold dose until improvement to grade ≤1 then decrease dose as noted in Tables 5 and 6
Grade ≥ 2 neurologic toxicity detected at any time while on study treatment	Hold dose until improvement to grade ≤1 then decrease dose as noted in Tables 5 and 6
Neurocognitive functioning reduction that exceeds the threshold of the Reliable Change Index*	Decrease dose as noted in Tables 5 and 6 per section 12.4
Any grade cerebrovascular ischemia or hemorrhage	Discontinue treatment
Any other grade 4 non-hematologic toxicity	Discontinue treatment
Neutrophils <500 cells/mm^3	Hold dose until improvement to grade ≤1 then decrease dose as noted in Tables 5 and 6
Febrile Neutropenia	Hold dose until improvement to grade ≤1 then decrease dose as noted in Tables 5 and 6
Platelets <25,000/mm^3 or platelets <50,000/mm^3 with bleeding	Hold dose until improvement to grade ≤1 then decrease dose as noted in Tables 5 and 6
Any other grade 3 hematologic toxicity	Hold dose until improvement to grade ≤1 then decrease dose as noted in Tables 5 and 6
Any other grade 4 hematologic toxicity	Discontinue treatment

* See Section 12.4 (Evaluation of Neurocognitive Functioning) and Appendix 10.

Up to 3 dose reductions are allowed. If subsequent cycles require additional dose reductions, treatment will be discontinued.

Table 8. Temozolomide (Component 2) adverse event dose modifications

CTCAE v 4 Adverse Effects Terms & Descriptions	Temozolomide Modifications
Any grade 3 non-hematologic and non-neurologic toxicity (with exception of alopecia, nausea, vomiting)	Hold dose until improvement to grade ≤1 then decrease dose to 100 mg/m^2
Any grade 4 non-hematologic toxicity (with exception of alopecia, nausea, vomiting)	Discontinue treatment
Neutrophils <1000 cells/mm^3	Hold dose until improvement to grade ≤1 then reduce dose to 100 mg/m^2
Platelets <50,000/mm^3	Hold dose until improvement to grade ≤1 then reduce dose to 100 mg/m^2

Up to 1 dose reduction is allowed. If subsequent cycles require additional dose reductions, treatment will be discontinued.

10.9 Permitted Concomitant Medications and Procedures

Myeloid growth factors to treat patients with neutropenia according to the American Society of Clinical Oncology (ASCO) Guidelines are permitted. Myeloid growth factors should be avoided (if medically appropriate) in Cycle 1 until patients have developed a DLT or dose-limiting Grade 4 neutropenia.

In Component 1, prophylactic antiemetic agents may be administered after cycle 2 at the discretion of the investigator. If an antiemetic is needed, the first line antiemetic agent will be ondansetron 4 mg, tablet. In component 2 ondansetron 4 mg tablet (1 or 2) will be taken 30 to 60 minutes before the dose of temozolomide is ingested. All other manifestations of the patient's malignancy should be treated at the discretion of the investigator.

Medications with potential CNS effects are not prohibited in this study, but it is recommended that their use be minimized to avoid confusion in the interpretation of CNS effects should they occur during the course of treatment with PAC-1 alone or in combination with temozolomide. Those medications would include CNS depressants, including benzodiazepines and other sedative-hypnotics (zolpidem), tricyclic antidepressants, and muscle relaxants.

In appropriate settings, such as combinations with agents known to produce frequent thrombocytopenia, restricted uses of anticoagulants should be considered.

All other medical conditions should be treated at the discretion of the investigator in accordance with local community standards of medical care.

10.9.1 The Effects of PAC-1 on Other Drugs

There are no known preclinical or human studies that have examined the effects of PAC-1 on other drugs.

10.9.2 The Effects of Other Drugs on PAC-1

There are no known preclinical or human studies that have examined the effects of other drugs on PAC-1.

10.9.3 The Effects of Temozolomide on Other Drugs.

The package insert of temozolomide (TEMODAR®) did not have a section on the effects of TEMODAR® on other drugs.

10.9.4 The Effects of Other Drugs on Temozolomide

In a multiple-dose study, administration of TEMODAR® Capsules with ranitidine did not change the C_{max} or AUC values for temozolomide or MTIC (a metabolite of temozolomide). A population analysis indicated that administration of valproic acid decreases the clearance of temozolomide by about 5%. A population analysis did not demonstrate any influence of co-administered dexamethasone, prochlorperazine, phenytoin, carbamazepine, ondansetron, H_2-receptor antagonists, or phenobarbital on the clearance of orally administered temozolomide.

10.9.5 Prohibited Concomitant Medications

Patients should not receive other anti-cancer therapy (cytotoxic, biologic, or radiation) while on treatment in this study.

10.10 Management of Clinical Events and Supportive Care Guidelines

Maximum patient care is to be given to all patients.

Patients should receive full supportive care during the study, including transfusion of blood and blood products, treatment with antibiotics, analgesics, erythropoietin, or bisphosphonates, when appropriate.

Although acetaminophen at doses of ≤2 grams/day is permitted, it should be used with caution in patients with impaired liver function.

10.10.1 Nausea and Vomiting

Prophylactic antiemetic therapy with ondansetron (4 mg tablet) will be used if necessary with PAC-1 only after cycle 2 in Component 1 and anytime in Component 2. Prophylactic antiemetic therapy with ondansetron (4 mg tablets, 1 or 2) will be used before temozolomide administration because nausea and emesis are common side effects of temozolomide. Because of the potential of benzodiazepines to cause sedation, the use of benzodiazepines for antiemetic prophylaxis should be reserved for patients who cannot be satisfactorily managed otherwise.

10.10.2 Diarrhea

Antidiarrheal medications will not be used prophylactically; however, patients will be instructed to take loperamide, 4 mg, at the occurrence of the first loose stool and then 2 mg every 2 hours until they are diarrhea-free for at least 12 hours. During the night, patients may take 4 mg of loperamide every 4 hours. Fluid intake should be maintained to avoid dehydration.

10.10.3 Pneumocystis carinii pneumonia prophylaxis

For patients with diagnosis of glioblastoma multiforme and are undergoing prophylactic treatment for pneumocystis carinii pneumonia (PCP), prophylaxis against PCP should be continued. There may be a higher occurrence of PCP when temozolomide is administered during a longer dosing regimen. All patients receiving temozolomide (component 2), particularly patients receiving steroids, will be observed closely for the development of PCP. All patients on temozolomide who develop lymphocytopenia (lymphocyte count less than 500/µL) will initiate PCP prophylaxis.

10.11 Duration of Therapy

Patients will receive protocol therapy unless:

- Extraordinary Medical Circumstances: If at any time the constraints of this protocol are detrimental to the patient's health, protocol treatment should be discontinued.
- Patient withdraws consent.
- There is evidence of progressive disease or unacceptable toxicity.
- The treating physician thinks a change of therapy would be in the best interest of the patient.
- More than a 3-week delay between cycles.

10.12 Follow-Up

For both Component 1 (PAC-1 alone) and Component 2 (PAC-1 in combination with temozolomide), a final study visit will occur 30 days (+/-1 week) after the last dose of study drug with follow-up for survival every 2 months until 6 months from completion of study drug. This visit will end study participation unless there is ongoing toxicity at least possibly related to study treatment. In this case, the patient will be followed as medically appropriate until resolution or stabilization of the adverse event.

11 STUDY PARAMETERS

11.1 Study Procedures

Table 9. Summary of procedures before, during and after dosing

	Baseline, within 14 days of enrollment	Cycle 1[1, ψ], Day 1	Cycle 2 Day 1	Cycle 3 Day 1 and each cycle thereafter	Every 8 weeks (± 1 week)	30 days (+ 1 week) after final dose of drug(s)[2, 12]
Signed consent	x					
Medical history	x	x	x	x		x
Review of prior therapy	x					
Review of concurrent therapy	x					
Physical exam	x	x	x	x		x
Vital signs	x	x	x	x		x
Neurologic and Mini Mental State Examination assessment	x	X[3]	x	x		x
Weight	x	x	x	x		x
Height	x					
Concomitant meds review	x	x	x	x		x
Performance status	x	x	x	x		x
Symptom and toxicity	x	x	x	x		X[4]
CBC w/ diff and coagulation (INR and PTT)[5]	x	x	x	x		x
Comprehensive metabolic panel (CMP)[6]	x	x	x	x		x
Zinc	x	x	x	x		

	Baseline, within 14 days of enrollment	**Cycle 1[1, Ψ], Day 1**	**Cycle 2 Day 1**	**Cycle 3 Day 1 and each cycle thereafter**	**Every 8 weeks (± 1 week)**	**30 days (+ 1 week) after final dose of drug(s)[2, 12]**
Urinalysis	**x**	**x**	**x**	**x**		**x**
Serum pregnancy test for females of child-bearing potential	**x**	**x**	**x**	**x**		
ECG / Echocardiogram[7]	**x**					
MRI of the brain (Component 1 patients)[8]	**X**					
Appropriate X-rays or scans to assess disease status	**X[9]**				**x**	
PAC-1 administration[10]		**x**	**x**	**x**		
Temozolomide administration[11]		**x**	**x**	**x**		
Promoter methylation of MGMT (PAC-1 and Temozolomide Combination study)						**x**

1 - For cycle 1 only, tests and procedures do not need to be repeated if done within 3 days of day 1 of the 28-day cycle
2 - For patients who leave treatment with a response, repeat appropriate disease assessment every 6-12 weeks until progression or start of a new treatment
3 – Neurological assessment will only be repeated if there is a reported or observed change from the screening assessment.
4 - For patients with unresolved treatment-related toxicity, follow as medically appropriate or stabilization
5 - Taken prior to dosing on Day 1 of each cycle
6 - CMP consists of: glucose, calcium, BUN, creatinine, sodium, potassium, chloride, CO2, serum calcium, serum total protein, serum albumin, bilirubin, ALP, AST, and ALT. For Component 2 patients, AST and ALT should be assessed on Day 12 of Cycle 1.
7 – All patients should have a baseline ECG and echocardiogram performed at baseline. Postdose assessment may be done as clinically indicated.
8. – All Component 1 patients should have a MRI of the brain performed at baseline. Postdose assessment may be done as clinically indicated.
9. - Within 4 weeks of study enrollment. Refer to Appendix 1 for solid tumors. Refer to Appendix 3 for lymphoma.
10 - PAC-1 in both Components 1 and 2 taken one time daily in the morning, days 1-21 of the 28-day cycle (subjects should fast 2 hours before and 1 hour after each dose). On Cycle 1 of Day 2, PAC-1 should not be taken (see footnote Ψ).
11 - Temozolomide started in cycle 1, and taken once a day for days 8-12 of the 28-day cycle (subjects should fast 2 hours before and 1 hour after each dose)
12 - For both Component 1 and Component 2 subjects who begin study drug, a final study visit will occur 30 days (+/-1 week) after the last dose of study drug with follow-up for survival every 2 months until 6 months from completion of study drug. See Section 10.12 for details.
Ψ – During Cycle 1 (Component 1 only), PAC-1 administration should be held on Day 2 to ensure accurate pharmacokinetic analysis.

11.2 Research Related Procedures:

Table 10. Summary of research related procedures before during and after dosing

	Baseline within 14 days of enrollment	**Cycle 1**			**All cycles thereafter (Day 1)**	**Once during or after duration of therapy**	**30 days (± 1 week) after final dose of PAC-1**
		Day 1 (Comp. 1) Day 7 (Comp. 2)	**Day 12 (Comp. 2 Only)**	**Day 21 (Comp. 1 Only)**			
PAC-1 PK[1]		x	x	x			
Temozolomide PK[2]			x				
ECG (see 12.3)		x	x	x			
Neurocognitive Function Evaluation[3]	x				x		x
Correlate procaspase-3 expression to PAC-1 activity[4]	x					x	
Correlate procaspase-3 activity in PBMCs to PAC-1 activity[5]		x	x	x			

1: PK analysis done on PAC-1 for all dose cohorts. Refer to Section 12 for further details on samples collection and processing.
2: PK analysis done on temozolomide for all dose cohorts in Component 2. Refer to Section 12 for further details on samples collection and processing.
3: A neuropsychologist, or a trained medical professional skilled in doing such assessments under the supervision of the neuropsychologist, will administer the following tests that evaluate neurocognitive functioning to the subject: the Hopkins Verbal Learning Test-Revised, the Trail Making Test (Forms A and B), and the Controlled Oral Word Association test.
4: Correlative study done on archived or standard of care biopsy collected at any time before, during or after duration of therapy in both Components 1 and 2.
5. PBMCs only collected in dose expansion cohort and PBMC correlative studies only preformed on these samples.

12 CORRELATIVE STUDIES

12.1 Pharmacokinetics

The pharmacokinetics (PK) and pharmacodynamics (PD) of PAC-1 will be assessed following doses administered on days 1 and 21 of the first cycle in Component 1 and on days 7 and 12 of the first cycle in Component 2. The PK and PD of temozolomide will be assessed following the dose administered on day 12 of the first cycle in Component 2. The PK and PD analyses will occur with each dose cohort during components 1 and 2.

For Component 2, administration of PAC-1 and temozolomide on Day 12 of Cycle 1 will occur in the morning. Temozolomide administration will occur at least 2 hours before PAC-1. The dose timing on this day is being specified to accommodate a sampling schedule that minimizes the number of blood draws needed from the patient. Information on the time and number of PAC-1 and temozolomide doses received for the 96 hours prior to the dose on day 12 will be obtained from subjects. Patients in Component 1 will not receive their PAC-1 dose on day 2 of Cycle 1. The dose on this day is being held in order to accurately define the absorption and metabolism of PAC-1 in patients at 24, 32, and 48 hours post drug administration. Actual and nominal sample collection times will be recorded on the case report forms and sample tubes.

12.1.1 Sample Collection

Component 1: Beginning on the mornings of days 1 and 21 of the first cycle in Component 1, blood samples (10 mL) will be collected immediately prior to oral ingestion of PAC-1 and at the following time points after ingestion: 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 32, and 48 hours. These time points are based on PAC-1 pharmacokinetics in dogs.

Component 2: Pharmacokinetic sample collections during Component 2 will occur under steady-state conditions in the mornings on days 7 (PAC-1 only) and 12 (PAC-1 and temozolomide) of the first cycle.

- Day 7, Cycle 1, Component 2: Subjects receive only PAC-1.
    - Blood samples (10 mL) are collected immediately prior to oral ingestion of PAC-1 and at the following time points after ingestion: 0.5, 1, 2, 3, 4, 6, 8, 10, and 24 hours.
- Day 12, Cycle 1, Component 2: Subjects receive both PAC-1 and temozolomide.
    - Temozolomide administered 2 hours before PAC-1.
    - Blood samples (10 mL) are collected immediately prior to oral ingestion of temozolomide, 1 hour before ingestion of PAC-1, and at the following time points after ingestion of PAC-1: 0.5, 1, 2, 3, 4, 6, 8, 10, and 24 hours. The sample volume is 10-mL at each time point.

The manner in which blood will be collected is as follows: Blood will be drawn from an upper extremity peripheral vein using an intravenous catheter with the exception of the

32 and 48 h blood draws in Component 1 – those blood draws will be done via needle sticks. Patients will not be given their dose of PAC-1 on Day 2 during Component 1 so as to not interfere with the PK/PD analyses for 24, 32, and 48 hours post administration. The next dose of PAC-1 should be given immediately after the 48-hour PK is drawn on Day 3 of Component 1.

When the catheter is being used, it will be kept patent between individual blood draws with normal saline. A waste sample, approximately 2 mL, will be collected through the catheter and discarded prior to each sample. For the 32 and 48 h post-dose blood draws, separate needle sticks will be used. If it becomes necessary, PK/PD samples can be drawn from the patients' port access. However, in these cases, a waste sample of approximately 10 mL should be collected and discarded prior to each sample.

For all samples record actual time drawn in addition to the nominal sample time. Prior to 24 hours, the samples will be drawn +/- 15 minutes from the designated time point. A +/- 2-hour collection window is permitted for the 24, 32, and 48 hour samples.

Pharmacokinetic Sample Processing

Refer to the separate PK Laboratory Operations Manual for detailed sample collection and processing information.

Blood samples (10 mL) will be collected in purple- top (potassium EDTA) tubes. The tube should be gently inverted a few times for complete mixing with the anticoagulant. The tube should be kept on wet ice to minimize hemolysis. Within 120 minutes (Cohort 1) or 30 minutes (Cohort 2) of draw, centrifuge each blood sample at approximately 3,000 X g for 5-10 minutes at 25 ^{o}C (room temperature). Plasma should be aliquoted (~1 mL) into 2 plastic tubes and immediately frozen at -80^{o}C. When samples will also be analyzed for temozolomide, i.e. samples collected during Component 2, Cycle 1, Day 12, plasma should be aliquoted into four rather than 2 tubes, with 2 tubes containing 0.1 ml of 1N HCl to chemically stabilize temozolomide, and immediately frozen at -80 ^{o}C. The plasma aliquots going into the tubes with 1N HCl should be exactly 1 mL. Plasma concentrations of PAC-1 and its metabolites and temozolomide will be using validated high performance liquid chromatographic-tandem mass spectroscopy (LC-MS/MS) assays under GLP conditions at the Toxicology Research Laboratory, University of Illinois at Chicago.

12.1.2 Pharmacokinetic Analysis

Non-compartment analysis: Non-compartmental analysis of the PAC-1 plasma concentration-time data following doses on days 1 and 21 for cycle 1 for each dose cohort during Component 1 and on days 7 and 12 for cycle 1 for each dose cohort during Component 2. Also during Component 2, temozolomide plasma concentration data on day 12 of cycle 1 will be performed using WinNonlin 6.3 (Pharsight, St Louis, MO) for subjects in whom complete PK profiles are obtained. Pharmacokinetic parameters to be estimated following each dose include: 1). area under the PAC-1 plasma concentration-

time curve from time 0 to 48 hours (AUC_{0-48}) for days 1 and 21 of Component 1 and area under the PAC-1 plasma concentration-time curve from time 0 to 24 hours (AUC_{0-24}) for days 7 and 12 of Component 2, 2) area under the temozolomide plasma concentration-time curve from 0 to 24 hours (AUC_{0-24}) 3). area under the PAC-1 plasma concentration-time curves from time 0 to infinity ($AUC_{0-\infty}$) on day 1 (Component 1) and day 7 (Component 2) only, 4). maximum PAC-1 and temozolomide plasma concentrations (C_{max}), 5). terminal elimination rate constant for both drugs (λ_z), and 6). oral clearance (CL/F) for both drugs.

Statistical Analysis: The influence of multiple dosing (e.g., Component 1: Day 1 $AUC_{0-\infty}$ vs. Day 21 AUC_{0-48}) and concurrent administration of temozolomide and dose on the pharmacokinetics of PAC-1 will be evaluated using a generalized linear models approach. PAC-1 AUC_{0-48}, $AUC_{0-\infty}$, C_{max} and CL/F will be log transformed before statistical analysis. Geometric means will be calculated for the parameters on each study day. The 90% confidence intervals for the geometric mean ratios will be constructed for every comparison.

PK Modeling: PAC-1 PK data will be fit for each subject and the population to an appropriate model using nonlinear mixed effects modeling as implemented in NONMEM (version7.2). One and two-compartment models with zero, first or serial input functions will be evaluated. For the population analysis, first order conditional estimation, Monte Carlo expectation maximization and Monte Carlo Bayesian methods will be explored for estimating the maximum likelihood. Subjects contributing at least one blood sample with documented dosing history will be included in the PK analysis. Estimates of the pharmacokinetic parameters for each subject will be derived from both the individual and population pharmacokinetic analyses and used to estimate PAC-1 exposures (i.e. $AUC_{0-24 \text{ or } 48}$) during the study.

12.2 Pharmacodynamic Assessment

The pharmacodynamic (PD) effects of PAC-1 (Component 1: days 1 and 21 of Cycle # 1; Component 2: days 7 and 12), and temozolomide (Component 2: day 12 of Cycle #1), will be used to assess the systemic responses of individuals following PAC-1 or temozolomide doses administered orally. Systemic variables are defined as plasma concentrations of PAC-1 or temozolomide, which will be measured and used to assess whether PAC-1 or temozolomide has affected the production of these biological markers. Measures of toxicity (e.g., changes in WBC counts, differential cell populations, platelets, etc.) are also considered systemic PD variables.

Pharmacodynamic data will be fit to an appropriate model, using maximum likelihood estimation. To determine whether any relationship exists between systemic activity of drug and biomarkers, the individual baseline corrected maximum biomarker concentrations will be compared to individual estimates of PAC-1 or temozolomide exposure.

12.3 Evaluation of PAC-1 for its QT prolongation potential

QT evaluation will be performed only in MTD expansion during PK collection times by ECG immediately prior to the PAC-1 oral dose ingestion, and at the following time points after ingestion: 1, 2, 3, 4, 6, and 24 hours (+/-10 minutes). This will be done on Days 1 and 21 of the first treatment cycle in Component 1), and on Days 7 and 12 of the first treatment cycle in Component 2.

12.4 Evaluation of Neurological/ Neurocognitive Functioning

As described in the Introduction, a study with dogs indicated oral PAC-1 administered in a repeated dosing study was associated with minimal to mild neurodegeneration. We will assess neurological and cognitive functioning throughout the trial in both components. The neurological assessment which includes the use of the Mini-Mental State Examination[18], will be done by a neurologist (Dr. Nicholas at UIC) or by a trained medical professional skilled in doing such assessments under the supervision of the neurologist.

The Neurocognitive Function Evaluation, which will consist of the Hopkins Verbal Learning Test-Revised[19], the Trail Making Test[20], and the Controlled Oral Word Association test[21], will be performed at baseline, on Day 1 of each cycle (except C1D1), and 30 days after the final dose of PAC-1 by a neuropsychologist or by a trained medical professional skilled in doing such assessments under the supervision of the neuropsychologist. Refer to the separate Neurocognitive Test Administration and Training Manual.

The Hopkins Verbal Learning Test-Revised (HVLT-R) is used to assess memory (immediate recall [i.e., learning], delayed free recall, and recognition). There are 6 alternate forms of the HVLT-R all of which will be used in alternating fashion to minimize the impact of practice effects. The Trail Making Test Part A (TMT-Part A) is used to assess visual and spatial scanning, attention, sequencing, and speed, and Trail Making Test Part B (TMT – Part B) is used to assess executive/frontal lobe skills. The Controlled Oral Word Association test (COWA) is used to assess language/verbal fluency. There are 2 alternate forms of the COWA and we will use both forms in alternating fashion to minimize the impact of practice effects. The HVLT-R, TMT and COWA have been used in numerous Radiation Therapy Oncology Group clinical trials in which neuropsychological functioning has served as an outcome measure (http://www.rtog.org/LinkClick.aspx?fileticket=6YFnX8hIh2M%3d&tabid=139, last accessed November 15, 2014). Published normative data is available for each test to standardize patient performance after adjusting for relevant demographic characteristics.[22, 23, 24] These three tests were recommended to assess cognitive functioning in patients with cancer by an International Cognition and Cancer Task Force.[25]

In order to determine whether PAC-1 dose needs to be reduced in a subject, we will use an approach that was described in a summary paper developed by the Health Services and

Research Outcomes Subcommittee of the Radiation Therapy Oncology Group (RTOG).[26]. In it they describe a method to determine clinically significant changes in performance in the HVLT-R, TMT-Part A and B, and COWA. It is done by use of a distribution-based statistical method, the Reliable Change Index (RCI).[26, 27] The RCI is derived from the standard error of measurement of each test and represents the 90% confidence interval for the difference in raw score from baseline to the next assessment that would be expected if no real change occurred. Changes that exceed the RCI represent a decline or improvement in performance. The equation for calculating the RCI is as follows:

$RCI = 1.64(SEdiff)$, where $SEdiff = [2(SEM^2)]^{1/2}$ and $SEM = SD_1[(1-r_{xy})^{½}.]$

SEdiff is the standard error of difference, SEM is the standard error of measurement, SD is the standard deviation, and r_{xy} is the test-retest reliability statistic. The RCI belongs to a family of similar statistical methodologies to determine a clinically meaningful effect at the within subject level.

Changes that exceed the RCI on any of the tests[28] (HVLT-R, TMT-Part A and B, and COWA, Appendix 10) indicating a decline in performance relative to baseline at the start of any cycle will trigger a decrease in the dose of PAC-1 according to the dose reduction schedule enumerated in Table 7.

12.5 Correlate Procaspase-3 Expression in Tumor with Activity of PAC-1

Correlation between procaspase-3 expression on diagnostic tumor biopsy samples, when available, and PAC-1 activity will be performed. To the extent available through archived or collected as standard of care tissue biopsy, tumor samples prior to, during or following treatment in either Component 1 or Component 2 will be evaluated for procaspase-3 expression and apoptosis by immunohistochemical staining, and then correlated with clinical outcome measured in tumor responses.

12.6 Correlate Procaspase-3 Activity in Peripheral Blood Mononuclear Cells with Activity of PAC-1

Correlation between change in procaspase-3 activation and PAC-1 activity in peripheral blood mononuclear cells from baseline to Day 21 (Component 1) or Day 12 (Component 2) will be performed.

12.7 Correlate promoter methylation status of MGMT with Activity of PAC-1 and Temozolomide

Correlation between methylation status of MGMT (obtained as a standard test for prognosis of high grade gliomas) and PAC-1 and temozolomide activity will be performed at the end of component 2.

13 STUDY ENDPOINTS

13.1 Primary Study Endpoint Component 1

MTD, defined as the highest tolerated dose of PAC-1 tested alone in patients with advanced, previously treated malignancies.

13.2 Primary Study Endpoint Component 2

MTD, defined as the highest tolerated dose of PAC-1 tested in combination with temozolomide in patients with high grade glioma.

13.3 Secondary Study Endpoints Components 1 and 2

- Preliminary information will be collected for treatment related toxicity and tolerance. Toxicity will be graded using the NCI's Common Terminology Criteria for Adverse Events version 4 (CTCAE v4). Treatment tolerance will be based on the number of treatment delays and dose reductions. Information will be presented in a tabular and descriptive manner.

- Disease response in patients with solid tumors will be assessed every 8 weeks while on study treatment using either the Response Evaluation Criteria In Solid Tumors version 1.1 (RECIST 1.1- Appendix 1). Disease response in patients with lymphoma will be assessed every 8 weeks while on study treatment using the Deauville PET Criteria (see Appendix 3)

- For Component 2 disease response will be assessed by RANO criteria (Appendix 2).

13.4 Correlative Study Endpoints

- Pharmacokinetics of PAC-1 assessed on Days 1 and 21 (Component 1) and Days 7 and 12 (Component 2) of the first cycle

- Pharmacokinetics of temozolomide assessed on Day 12 of the first cycle (Component 2 only)

- Evaluation of PAC-1 for its QT prolongation potential (Components 1 and 2)

- Neurocognitive functioning (Components 1 and 2)

- Procaspase-3 expression in tumor (Components 1 and 2)

- Activation of procaspase-3 in peripheral blood mononuclear cells in relation to activity of PAC-1 (Components 1 and 2)

- Promoter methylation status of MGMT and Activity of PAC-1 and Temozolomide combination (Component 2 only)

14 DRUG FORMULATION AND PROCUREMENT

14.1 Temozolomide

14.1.1 Other names

Temodar®

14.1.2 Classification

Alkylating anti-neoplastic agent that is a prodrug

14.1.3 How Supplied

Capsules are supplied in amber glass bottles with child-resistant polypropylene caps or child-resistant sachets.

14.1.4 Availability

Temozolomide is a FDA-approved medication for patients with primary brain tumors and will be prescribed by treating physician. Temozolomide is available in strengths of 5, 20, 100 and 250 mg capsules. Each strength is a different capsule size. Each capsule contains temozolomide in combination with anhydrous lactose NF, colloidal silicon dioxide NF, sodium starch glycolate NF, tartaric acid NF and stearic acid NF. Temozolomide capsules are supplied in amber glass bottles containing 14 capsules per bottle for the 5, 20 and 100 mg strengths. The 250 mg strength is available with 5 capsules per bottle.

14.1.5 Description

Temozolomide is not directly active but undergoes rapid nonenzymatic conversion at physiologic pH to the reactive compound MTIC, which is an active metabolite of dacarbazine. Unlike dacarbazine, formation of MTIC from temozolomide does not require metabolic activation (liver). The extent of MTIC formation and its overall contribution to cytotoxicity are unclear. The cytotoxicity of MTIC is thought to be primarily due to alkylation of DNA. Alkylation (methylation) occurs mainly at the O6 and N7 positions of guanine.

14.1.6 Storage, Handling, and Accountability

Store TEMODAR Capsules at 25°C (77°F); excursions permitted to 15-30°C (59-86°F) [see USP Controlled Room Temperature]. Care should be exercised in the handling and preparation of temozolomide. Capsules should not be opened. If capsules are accidentally opened or damaged, rigorous precautions should be taken with the contents to avoid inhalation or contact with the skin or mucous membranes. If it gets on the skin, wash it immediately with soap and water. The medication should be kept away from children and pets.

14.1.7 Administration

Oral (capsules are swallowed with water)

14.1.8 Risks

Hematological: thrombocytopenia, leukopenia

GI: nausea, vomiting, anorexia, constipation, diarrhea, mouth sores, stomatitis

Hepatic: increased liver enzymes (reversible)

Skin: rash, alopecia

Other: Fatigue, headache, shortness of breath, internal bleeding, pain when swallowing, pneumocystis carinii, fever, increased blood sugar, chills, cough, body ache and seizure.

14.1.9 Warnings and Precautions

Myelosuppression

Patients treated with temozolomide may experience myelosuppression, including prolonged pancytopenia, which may result in aplastic anemia, which in some cases has resulted in a fatal outcome. In some cases, exposure to concomitant medications associated with aplastic anemia, including carbamazepine, phenytoin, and sulfamethoxazole/trimethoprim, complicates assessment. Prior to dosing, patients must have an absolute neutrophil count (ANC) greater than or equal to 1.5 x 10^9/L and a platelet count greater than or equal to 100 x 10^9/L. A complete blood count should be obtained on Day 22 (21 days after the first dose) or within 48 hours of that day, and weekly until the ANC is $\geq$ 1.5 x 10^9/L and platelet count $\geq$ 100 x 10^9/L. Geriatric patients and women have been shown in clinical trials to have a higher risk of developing myelosuppression.

Myelodysplastic Syndrome

Cases of myelodysplastic syndrome and secondary malignancies, including myeloid leukemia, have been observed.

Pneumocystis carinii Pneumonia

For treatment of newly diagnosed glioblastoma multiforme: For patients with diagnosis of glioblastoma multiforme and are undergoing prophylactic treatment for pneumocystis carinii pneumonia (PCP), prophylaxis against PCP should be continued. There may be a higher occurrence of PCP when temozolomide is administered during a longer dosing regimen. However, all patients receiving temozolomide, particularly patients receiving steroids, should be observed closely for the development of PCP regardless of the

regimen. Patient with leucopenia (lymphocyte count lower than 500/μL) will receive PCP prophylaxis.

Laboratory Tests

For the 28-day treatment cycles, a complete blood count should be obtained prior to treatment on Day 1 of each cycle. Blood counts should be performed weekly until recovery if the ANC falls below 1.5 x 10^9/L and the platelet count falls below 100 x 10^9/L.

Use in Pregnancy

Temozolomide can cause fetal harm when administered to a pregnant woman. Administration of temozolomide to rats and rabbits during organogenesis at 0.38 and 0.75 times the maximum recommended human dose (75 and 150 mg/m^2), respectively, caused numerous fetal malformations of the external organs, soft tissues, and skeleton in both species.

14.2 PAC-1

14.2.1 Other names

First procaspase-activating compound, VO-100

14.2.2 Classification

Ortho-hydroxyl *N*-acyl hydrazone that enhances the enzymatic activity of procaspase-3 and induces apoptosis in cancer cells.

14.2.3 How Supplied

PAC-1 will be supplied as 250 mg dose with a 125 mg dose score mark tablets. Approximately 1000 tablets are bulk-packaged into 950 cc HDPE Bottles with five 1-Gram desiccant packets per bottle, capped with 53 mm child-resistant closures with FS M1/.035 Pulp Liner, induction sealed, and labeled. Tablets will be distributed to patients by licensed pharmacist according to the clinical protocol and the separate Pharmacy Manual.

14.2.4 Availability

PAC-1 will be provided by Vanquish Oncology, Inc. with approval of the FDA for investigational purposes (IND# 120544)

14.2.5 Description

PAC-1 will be provided in strengths of 250 mg tablets. Each tablet contains PAC-1 in combination with Avicel PH 101 & 200 (microcrystalline cellulose filler, NF), Pearlitol

100 SD (mannitol filler, USP), Explotab (sodium starch glycolate disintegrant, NF), Cabosil (fumed silica glidant, NF), hydroxypropyl cellulose (binder, NF) and Sodium Stearyl Fumarate (lubricant, NF). PAC-1 tablets will be supplied to the experimental pharmacy where the correct dose for one cycle will be packaged for each patient.

14.2.6 Storage, Handling, and Accountability

Bottles containing PAC-1 tablets will be provided to the investigative site. Bottles will be stored at room temperature, 15ºC to 30ºC upon arrival at the site. A packing list will be included with the shipment of clinical study material. Upon receipt of study drug, the site will inspect the shipment for any damage, and compare contents against the packing list. The site will acknowledge receipt of the shipment by contacting Vanquish Oncology, Inc., noting any discrepancies or damages.

14.2.7 Administration

Oral (tablets are swallowed with water).

14.2.8 Risks

The risks of oral PAC-1 in humans are unknown. However, PAC-1 has shown toxicity in mice, rats, and dogs. In a rat study, deaths occurred in 2 out of 12 rats administered 600 mg/kg/day over a 7-day interval – it should be noted this is a dose over 10 times higher than the planned maximum daily dose to be tested in this trial, 3000 mg (i.e., 50 mg/kg for a 60 kg individual). In one dog study involving repeated oral dosing of PAC-1, gastro-intestinal symptoms were observed (diarrhea, vomiting) accompanied by weight loss. As well, one of the dogs in that study had a seizure that was resolved by administration of diazepam. In another dog study, daily administration of oral PAC-1 at doses of 25 and 50 mg/kg for 28 days was associated with minimal-mild neurodegeneration at multiple sites in the gray matter of the cerebrum. An 84-day oral toxicity and toxicokinetic study of PAC-1 with 1 month and 3 months recovery periods in male and female beagle dogs was recently completed. Daily oral administration (via capsules) of PAC-1 at 6, 13, or 25 mg/kg for 21 consecutive days with a 7-day wash-out period between cycles and repeated for 3 cycles dose-responsive, minimal to mild test article-related vacuolation in different sections of the brains of animals given 13 and 25 mg/kg. The extent and severity of the vacuolization was similar to that seen in the 28-day study. The observed brain vacuolization did not show evidence of recovery after 3 months of being off study drug. No PAC-1 associated neurological or neurocognitive changes have been observed through dose level 5 (450 mg) of Component 1 of this study.

14.2.9 Warnings and Precautions

Observation and assessment of hematological and non-hematological markers of toxicity in research dogs raised a possibility that PAC-1 may cause neurodegenerative changes in the brain, and therefore neurological (e.g. Dr. Nicholas at UIC) and neurocognitive examinations will be performed at baseline, every cycle of therapy (except C1D1), and 30 days after completion of therapy. Personnel with similar expertise will be included in

the study during Components 1 and 2 at Johns Hopkins and at HealthPartners – Regions Cancer Care Centers.

One Component 1 patient who was in the 625mg/day cohort experienced an intracranial hemorrhage where a possible relationship to PAC-1 could not be ruled out. In order to monitor and ensure patient safety, patients should have a baseline brain MRI for future comparison if needed. Coagulation tests will be done prior to dosing on Day 1 of each treatment cycle.

15 ADVERSE EVENT DOCUMENTATION AND REPORTING

The NCI -CTCAE; Version 4.0 will be used for grading toxicities unless otherwise specified. Patients will be monitored throughout the treatment and follow-up period for occurrence of AEs (acute, delayed, and/or cumulative), as well as for changes in clinical status, vital sign measurements, and laboratory data. Safety parameters to be measured/assessed include vital sign measurements, physical examinations, concomitant medications, hematology, serum chemistries, urinalysis, pregnancy testing, and ECOG performance status.

When an investigational agent(s) is used in combination with a commercial agent(s), the combination is considered investigational. Reporting of adverse events follows the guidelines for investigational agents.

15.1 Definition

The following definitions are based on the Code of Federal Regulations Title 21 Part 312.32 (21CFR312.32(a)).

Adverse Event: Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. AEs include:

- Suspected adverse drug reactions. These may be serious or not serious.
- Reactions from drug overdose, abuse, withdrawal, sensitivity, or toxicity.
- Significant changes or abnormalities, when compared to baseline, in structure (sign), function (symptom), clinical laboratory results, ECG results, or physiological testing.
- This includes any worsening of a pre-existing condition temporally associated with the use of study drug.
- Other medical events, regardless of their relationship to the study drug, such as injury, surgery, accidents, extensions of symptoms, or apparently unrelated illnesses.

15.2 Adverse Event/Serious Adverse Event Reporting Period

Findings existing prior to signing informed consent will be recorded as medical history. For the purpose of data collection, all untoward events that occur after informed consent through 30 days after the last dose of study drug are to be recorded on CRFs by the investigational site. This requirement includes AEs from unscheduled as well as scheduled visits.

Investigators will seek information on adverse events at each patient contact. All adverse events, whether reported by the patient or noted by study personnel, will be recorded in the patient's medical record. Adverse events will then be reported in OnCore®:

- After informed consent has been obtained but prior to initiation of study drug, only serious adverse events caused by a protocol-mandated intervention should be reported.

- After initiation of study drug, all adverse events/serious adverse events, regardless of relationship to study drug, will be reported until 30 days after the last dose of PAC-1/temozolomide.

- After a period of 30 days from the last dose of PAC-1/temozolomide, Investigators should report any deaths, serious adverse events, or other adverse events of concern that are believed to be related to prior treatment with study drug.

- Subjects will be monitored for survival status for up to 6 months after the last dose of PAC-1/temozolomide.

15.3 Evaluating Adverse Events

The investigator will determine the seriousness, intensity, and causality of an AE associated with the use of the study drug (i.e., events where there is a reasonable possibility that the event may have been caused by the study drug) based on the definitions that follow.

The adverse event severity grading scale for the NCI CTCAE (v4.0) will be used for assessing adverse event severity.

15.4 Serious Adverse Events

(Notify sponsor or designee within 24 hours of first awareness)

The SAE definition and reporting requirements are in accordance with the ICH Guideline for Clinical Safety Data Management, Definitions, and Standards for Expedited Reporting, Topic E2A, with Title 21 Part CFR 312.32, and the Guidance for Industry and Investigators Safety Reporting Requirements for INDs and BA/BE Studies.

SAE: An adverse event is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- **Death:** This includes any death that occurs while the patient is “on study” as well as any death that occurs within 30 days after the last dose of study drug.
    - ***Note***: Death is an outcome of an AE, and not an AE in itself. The event(s) that caused death (e.g., illness, accident) is the SAE. Death due to any other cause(s) must also be reported as an outcome of the reportable SAE.
- **Life-threatening adverse event:** An AE or suspected adverse reaction is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the patient or patient at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death).
- **Inpatient hospitalization or prolongation of existing hospitalization:** In the absence of an AE, the investigator should not report hospitalization or prolongation of hospitalization. This is the case in the following situations:
    - Hospitalization or prolongation of hospitalization is needed for a procedure required by the protocol
    - Hospitalization or prolongation of hospitalization is part of routine procedure followed by study center
    - Hospitalization for survey visits or annual physicals
    - A hospitalization planned before the start of the study for a pre-existing condition that has not worsened does not count as an SAE.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital anomaly/birth defect
- Important medical event: An event that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, it may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
- Some serious events will not be reported as SAEs, including:
    - Disease progression
    - Death due to disease progression occurring more than 30 days after the last dose of study drug

- Medical or surgical procedures when the condition that leads to the procedure is an AE
- Pre-existing diseases, or conditions or laboratory abnormalities present or detected prior to the screening visit, that do not worsen
- Situations for which an untoward medical occurrence has not occurred (e.g., hospitalization for elective surgery, social and/or convenience admissions)

15.5 Suspected Unexpected Serious Adverse Reactions (SUSAR)

(Notify sponsor or designee within 24 hours of first awareness)

A suspected unexpected serious adverse reaction is any adverse drug event, the specificity or severity of which is not consistent with those noted in the current protocol and/or Investigator's Brochure (IB). This refers to any AE that has not been previously observed (e.g., included in the IB), rather than from the perspective of such an event not being anticipated from the pharmacological properties of the product.

15.6 Unexpected Adverse Events

Unexpected Adverse Events as defined by HHS regulations 45 CRF part 46 include any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- Related or possibly related to participation in the research (*possibly related* means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Note: The major discord between the FDA and OHRP definitions is whether or not the underlying disease is included when considering expectedness.

15.7 Non-Serious Adverse Events

All other AEs, not fulfilling the previous definitions, are classified as non-serious.

15.8 Protocol-Related Adverse Events

AEs that are not test drug related may nevertheless be considered by the investigator or the Medical Monitor to be related to the conduct of the clinical study. That is, the event may be related to the fact that a patient is participating in the study. For example, a protocol-related AE may be an event that occurs during a washout period or that is related to a procedure required by the protocol.

15.9 Relationships/Causality to Study Drug

The investigator will attempt to assess the relationship of the event to study drug using a 5- point scale.

15.9.1 Assessment of Attribution

When assessing whether an adverse event is related to a medical treatment or procedure, the following attribution categories are utilized:

Definitely: A causal relationship that can only be the result of the investigational medicinal product and there is no other plausible cause of the AE.

Probable: A causal relationship is clinically / biologically highly plausible and there is a plausible time sequence between onset of the AE and administration of the investigational medicinal product and there is a reasonable response on withdrawal.

Possible: A causal relationship is clinically / biologically plausible and there is a plausible time sequence between onset of the AE and administration of the investigational medicinal product.

Unlikely: A causal relation is improbable and another documented cause of the AE is most plausible.

Unrelated: A causal relationship can be definitely excluded and another documented cause of the AE is most plausible.

15.10 Recording Adverse Events

All AEs (including SAEs) are to be accurately recorded in OnCore®. The date of onset as well as the duration of the event also should be recorded. In addition, the method used to treat the AE and the outcome of the AE also will be noted. The investigator will assess the relationship of the event to study drug (not related, unlikely-related, possibly related, probably related, or definitely related).

15.11 Adverse Event Monitoring and Follow-up

The investigator will follow all patients who experience adverse events until there is a return to the patient's baseline condition, Grade 1 severity or until a clinically satisfactory resolution has been achieved. The appropriate follow-up visits must be scheduled and the

specific tests repeated or performed as necessary. Where a diagnosis is possible, it is preferable to report this diagnosis rather than a series of terms (signs/symptoms) relating to the diagnosis.

15.12 Laboratory Abnormalities

15.12.1 Non-Clinically Significant (NCS) Laboratory Abnormalities

All laboratory results must be filed in the patient's medical record and be monitored. The investigator must review laboratory results in a timely manner demonstrated by signature/date and assignment of clinical significance assessment. Non-clinically-significant laboratory abnormalities, i.e., minor deviations from the normal range, are expected and it is likely that no medical intervention will be required. Such results will not be considered to be AEs.

15.12.2 Clinically Significant (CS) Laboratory Abnormalities

Any laboratory abnormality that is considered to be clinically significant by the investigator will be recorded in OnCore®. A clinically significant abnormal test result will be considered an AE if:

- It is not associated with an already reported AE, diagnosis or pre-existing condition
- There is a change in concomitant medication or intervention as needed, in direct response to the laboratory result
- The investigator exercises his/her discretion to make significance determinations for any patient laboratory result or result that requires intervention

All such lab abnormalities will be repeated and assessed by the investigator, or licensed (MD), as soon as possible for "seriousness" and if they meet the regulatory definition of "serious", they will be reported as SAEs following regulatory and protocol requirements. Repeat laboratory tests may be run in order to monitor the result.

15.12.3 Serious Laboratory Abnormalities

Any lab abnormality meeting the regulatory definition of "serious" must be recorded on both in OnCore® and the SAE Form. If a patient experiences a serious toxicity or dies, the FDA will be notified within 24 hours, as required.

15.13 Preexisting Medical Conditions

A preexisting medical condition is one that is present at the screening visit for this study. Such conditions should be recorded as part of the patient's medical history in OnCore®. A preexisting medical condition should be recorded as an adverse event only if the frequency, severity, or character of the condition worsens during the study. When

recording such events in OnCore®, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

15.14 Other Unanticipated (Unexpected) Problems/Events

Federal regulations [45CFR46.103 (b) (5) and 21CFR56.108 (b) (1)] require IRBs to ensure that researchers promptly report "any unanticipated problems involving risk to subjects or others" (UPIRSOs). UPIRSOs are defined as any problem or event which in the opinion of the local researcher was *unanticipated, reflects new or increased risk to the subjects and at least possibly related* to the research procedures.

The following problems/events as reportable to the site's local IRBs per each IRB's policies and procedures:

- Any accidental or unintentional change to the IRB-approved protocol that increases risk or has the potential to recur
- Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject
- Any publication in the literature, safety monitoring report (including Data and Safety Monitoring Reports), interim result or other finding that indicates an unexpected change to the risk/benefit ratio of the research
- Any breach in confidentiality that may involve risk to the subject or others
- Any complaint of a subject that cannot be resolved by the research staff
- Any other possibly related event which in the opinion of the investigator constitutes an unanticipated risk

Expedited (Rapid) Reporting: Certain events may require rapid notification to entities providing patient safety oversight (e.g. IRB, FDA) as detailed in section 15.15. For the IRB this is 5 working days. For studies under an IND, it is 7 or 15 calendar days.

15.15 Required Reporting: Local Site IRB, FDA, Vanquish Oncology, Inc., OnCore

Table 11. Summary of required reporting

Agency	Criteria for reporting	Timeframe	Form to Use	Submission address/ fax numbers	Copy AE to:
Local Site IRB	UPIRSO: Events which are Unanticipated Problems, Involving Risk to Subjects or Others (e.g. serious and unexpected adverse events)	5 Working Days or per Local Site IRB's requirements	Local Site IRB's Form	Local Site IRB	UICC's CTO Regulatory Coordinator
	Other Problems or Events meeting the definition of UPIRSO in section 15.1	5-15 Working Days per IRB requirements	Local Site IRB Forms		
FDA	Unexpected and fatal or life threatening suspected adverse reaction	As soon as possible but no later than 7 Calendar-Day	Medwatch Form FDA 3500A for Mandatory Reporting/ OnCore (Paper report can be generated from system)	Fax SAE to IND/IDE Regulatory Health Project Manager, Techiya (Thea) Toaff, R.N., B.S.N., Division of Oncology Products 1 at 1-301-796-9845. E-mail: techiya.toaff@fda.hhs.gov – follow-up with written report submitted as an amendment to: MedWatch, 5600 Fishers Lane, Rockville, MD 20852-9787	
	1) Serious and unexpected suspected adverse reaction or 2) increased occurrence of serious suspected adverse reactions over that listed in the protocol or investigator brochure or 3) findings from other sources (other studies, animal or in vitro testing)	As soon as possible but no later than 15 Calendar-Day			
	All other events per CRF 312.33	At time of IND annual report	Summary format	Submit as part of the IND annual report	
Note: Events due to the disease under treatment or an underlying medical condition will not require expedited reporting to the FDA for the purposes of this study					

Table 11. Summary of required reporting, continued

Vanquish Oncology, Inc.	All serious adverse events , regardless of expectedness or relationship with any study drug per section 15.16	Within 24 hours of knowledge	MedWatch Form FDA 3500A for Mandatory Reporting/ OnCore (Paper report can be generated from system)	Vanquish Oncology, Inc. SAE and Pregnancy Reporting Contact Information: Vanquish Oncology, Inc. Pharmacovigilance 2001 S. First St., Suite 201 Champaign, IL 61820 Phone: (510) 219-6200 Fax: (855) 250-2953	UICC's CTO Regulatory Coordinator
	Pregnancy in a female patient or the partner of a male patient per section 15.17		Pregnancy Reporting Form OnCore (Paper report can be generated from system)		
Local Site Clinical Trials Office	All serious events regardless of expectedness or attribution through 30 days (+1 week) after the last dose of study drug(s)	Within 24 hours of knowledge	OnCore (Paper report can be generated from system)	Local Site's Regulatory Coordinator/Specialist	Local Site Regulatory File Binder/eBinder

15.16 Procedures for Reporting SAEs to Vanquish Oncology, Inc.

Adverse events (AEs) may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures must be reported to Vanquish Oncology, Inc. Pharmacovigilance (or designee). AEs which are serious must be reported to Vanquish Oncology, Inc. Pharmacovigilance (or designee) from first dose of PAC-1 alone (Component 1) or PAC-1 in combination with temozolomide (Component 2) up to and including 30 days after administration of the last dose(s) of the drug(s). When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event. Any SAE that occurs at any time after completion of PAC-1 alone treatment (Component 1) or PAC-1 in combination with temozolomide treatment (Component 2) or after the designated follow-up period that the investigator and/or sub-investigator considers to be related to any study drug must be reported to the Vanquish Oncology, Inc. Pharmacovigilance (or designee).

Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the trial are not to be considered AEs unless the condition deteriorated in an unexpected manner during the trial (e.g., surgery was performed earlier or later than planned). All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

This is a Vanquish Oncology, Inc.-initiated study. The principal investigators conducting the study at the local sites are Oana Danciu, MD (UIC), Matthias Holdhoff, MD, PhD (JHU), and Richard Peterson, MD (HealthPartners Regions).

The Principal Investigator at each site must report all SAEs that occur at their respective sites, regardless of expectedness or relationship with any study drug, to Vanquish Oncology, Inc. Pharmacovigilance (or designee) as soon as possible, but no later than 5 calendar days of the investigator's observation or awareness of the event. In the event that this is a multisite study, the investigator is responsible to ensure that the SAE reports are sent to Vanquish Oncology, Inc. Pharmacovigilance (or designee) from all sites participating in the study. Sub-investigators at each site must report all SAEs to their site's Principal Investigator so that the principal investigator can meet his/her foregoing reporting obligations to Vanquish Oncology, Inc. Pharmacovigilance, unless otherwise agreed between the principal investigator and sub-investigator(s). Vanquish Oncology, Inc. Pharmacovigilance (or designee) may request follow-up information to a reported SAE, which the local site Principal Investigator will be responsible for providing to Vanquish Oncology, Inc. Pharmacovigilance (or designee).

The SAE report must include event term(s), serious criteria, and the investigator's or sub-investigator's determination of both the grade of the event(s), the expectedness of the event (s) and the relationship of the event(s) to study drug administration.

The grading for each SAE, including any clinically significant lab abnormality, will be determined by using the NCI CTCAE version 4.

Relationship to all study drugs for each SAE will be determined by the investigator or Sub-investigator at the occurring site by responding yes or no to the question: Is there a reasonable possibility that the AE is associated with the study drug.

The Principal Investigator at the occurring site must also provide Vanquish Oncology, Inc. Pharmacovigilance with a copy of all communications with applicable regulatory authorities related to the study or study drug(s), including, but not limited to, telephone conversation logs, as soon as possible but no later than 5 calendar days of such communication.

The Cancer Center SAE form (paper form generated from OnCore) and the Medwatch Form FDA 3500A for Mandatory Reporting will be used.

15.17 Procedures for Reporting During Pregnancy and Birth Events

If a woman becomes pregnant or suspects that she is pregnant while participating in this study, she must inform the investigator immediately and must permanently discontinue study drug(s). All pregnancies and suspected pregnancies must be reported to Vanquish Oncology, Inc. Pharmacovigilance (or designee) immediately using the appropriate Pregnancy Report Form (see Section 15.15 for contact information). The pregnancy must be followed for the final pregnancy outcome (i.e., delivery, still birth, miscarriage) and Vanquish Oncology, Inc. Pharmacovigilance will request this information from the investigator.

16 STUDY DATA COLLECTION AND MONITORING

16.1 Data Management

This study will report clinical data using the OnCore® data management system utilizing study specific case report forms. Access to OnCore® will be granted to each local site by UICC upon the local site's initiation of the study. Key study personnel are trained on the use of case report forms and will comply with protocol specific instructions for data collection.

Patient demographics, patient specific study treatment calendars, adverse events and other information required for IND annual reporting will be maintained with the OnCore data management system.

16.2 Case Report Forms

This study will utilize electronic CRFs (eCRFs) via the OnCore® data management system. Participant data will be collected using the protocol specific electronic case report forms (eCRFs). eCRF data will be approved by UICC's Principal Investigator and

the study biostatistician prior to release for use. The Study Coordinator or designee at each local site will be responsible for registering the patient into the OnCore® data management system at time of study entry, completing eCRFs based on the patient specific calendar, and updating the patient record until patient death or end of required study participation.

16.3 Data Safety Monitoring Plan (DSMP)

The study's Data and Safety Monitoring Plan will be in compliance with the University of Illinois Cancer Center's Data & Safety Monitoring Plan (DSMP).

For the purposes of data and safety monitoring, this study is classified as high risk (under a locally held IND). Therefore, the following requirements will be fulfilled:

The UICC PI will complete and submit a quarterly Trial Progress Report to the Cancer Center Data and Safety Monitoring Committee (DSMC) with the understanding the Cancer Center Protocol Review Committee (CCPRC) may require more frequent reporting. The Data Safety Monitoring Committee will include a neuro-oncologist who will evaluate the results of neurocognitive testing conducted during a given quarter. In addition, there will be an annual audit performed of all neurologic and neurocognitive evaluations.

The UICC PI will comply with at least twice yearly monitoring of the project by the Cancer Center monitoring services.

The Local Sites' PIs will oversee the submission of all reportable adverse events by each site's Clinical Trials Office (CTO), the local site IRB, Vanquish Oncology and the FDA. He/she will also ensure that all information if promptly recorded in OnCore® to ensure that the UICC DSMC has access to the most current information at all times.

In addition, at the time of the continuing review with Local Site IRB, a copy of the DSMC report with any attachments will be submitted to any appropriate committees per local site policies and procedures

The sponsor, Vanquish Oncology, will maintain its own monitoring of all study sites with special focus paid to the accurate administration and data collection of the neurologic and neurocognitive tests. An annual review will be performed by an independent expert of all neurologic and neurocognitive data.

16.4 IND Annual Reports

In accordance with regulation 21 CFR § 312.33, the sponsor will submit a progress report annually. The report will be submitted within 60 days of the anniversary date that the IND went into effect. Included as a part of the annual report for this IND will be initial reporting of all deaths due to disease at any time and all deaths (regardless of cause)

occurring more than 2 years after the PAC-1 alone treatment (Component 1) or treatment of PAC-1 in combination with temozolomide (Component 2).

16.5 Monitoring

Sites will permit study-related monitoring, audits, and inspections by the study's Sponsor and/or any designees, the local IRB, government regulatory bodies, and University of Illinois compliance groups. The investigator will make available all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

16.6 Record Retention

The investigator will retain study records including source data, copies of case report form, consent forms, HIPAA authorizations, and all study correspondence in a secured facility for at least 6 years after the study file is closed with the IRB and FDA.

In addition, the Clinical Trials Office (CTO) will keep a master list of all patients participating in the study through the OnCore® database with sufficient information to allow retrieval of the medical records for that patient from the study sites.

Please contact the CTO before destroying any study related records.

17 STATISTICAL CONSIDERATIONS

The primary endpoint is safety and MTD of PAC-1, alone (Component 1) and in combination with temozolomide (Component 2). We will establish that the PAC-1 dose is safe if there is a less than 33% chance for DLT at a given dose level during the first cycle of therapy.

18 CONDUCT OF THE STUDY

18.1 Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

18.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in

order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, consent, written information given to the patients, safety updates, progress reports, and any revisions to these documents will be provided to the IRB by the Investigator.

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*These references are in manuals or other books. A copy can be made available upon request.

Appendix 1. Response Evaluation Criteria in Solid Tumors (RECIST)

Tumor response will be defined by the Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1

The evaluation criteria for tumor response are based on the Response Evaluation Criteria in Solid Tumors (RECIST) Quick Reference. The RECIST guidelines resulted from meetings between European Organization for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of the United States (NCI) (Lit 11). They can be found on the Internet at the following location http://imaging.cancer.gov/clinicaltrials/imaging/, last accessed December 2, 2012.

Evaluation of measurable and non-measurable lesions

Objective response

Measurable disease - the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Measurable lesions - lesions that can be accurately measured in at least one dimension with longest diameter >20 mm using conventional techniques or >10 mm with spiral CT scan.

Non-measurable lesions - all other lesions, including small lesions (longest diameter <20 mm with conventional techniques or <10 mm with spiral CT scan), i.e., bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques; and:

- 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.

- 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.

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

Methods of Measurement

- CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI 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.

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

- When the primary endpoint of the study is objective response evaluation, ultrasound (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.

- The utilization of endoscopy and laparoscopy 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 specialized centers. However, such techniques can be useful in confirming complete pathological response when biopsies are obtained.

- 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 when all lesions have disappeared.

- Cytology and histology can be used to differentiate between PR and CR in rare cases (e.g., after treatment to differentiate between residual benign lesions and residual malignant lesions in tumor types such as germ cell tumors).

Baseline documentation of "Target" and "Non-Target" lesions

- All measurable lesions up to a maximum of two lesions per organ and 5 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.

- All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Response Criteria – target lesions	**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 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
Response Criteria – non-target lesions	**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 (1)

1 - 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 later on by the review panel (or study chair).

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 PD the smallest measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

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

- 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) to confirm the complete response status.

Confirmation

- The main goal of confirmation of objective response is to avoid overestimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed.

- To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.

- In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in this study protocol as every 6 weeks (2 cycles).

Duration of overall response

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

Duration of stable disease

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

The clinical relevance of the duration of SD varies for different tumor types and grades. Therefore, it is highly recommended that the protocol specify the minimal time interval required between two measurements for determination of SD. This time interval should take into account the expected clinical benefit that such a status may bring to the population under study.

Appendix 2. RANO Response Criteria

Criterion	CR	PR	SD	PD
T1 gadolinium enhancing disease	None	≥ 50% ↓	< 50% ↓ but < 25% ↑	≥ 25% ↑*
T2/FLAIR	Stable or ↓	Stable or ↓	Stable or ↓	↑*
New lesion	None	None	None	Present*
Corticosteroids	None	Stable or ↓	Stable or ↓	NA†
Clinical status	Stable or ↑	Stable or ↑	Stable or ↑	↓*
Requirement for response	All	All	All	Any*

- Abbreviations: RANO, Response Assessment in Neuro-Oncology; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; FLAIR, fluid-attenuated inversion recovery; NA, not applicable.
- ↲* Progression occurs when this criterion is present.
- ↲† Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration.

J Clin Oncol 28:1963-1972.

Appendix 3. Response Evaluation Criteria for Hodgkin Lymphoma

Tumor response will be defined by the Deauville PET Criteria (NCCN Guidelines Version 2.2013

DEAUVILLE PET CRITERIA

Score	PET/CT scan result
1	No uptake above background
2	Uptake ≤ mediastinum
3	Uptake > Mediastinum but ≤ liver
4	Uptake moderately increased compared to the liver at any site
5	Uptake markedly increased compared to liver at any site
X	New areas of uptake unlikely to be related to lymphoma

Source: Barrington SF, Qian W, Somer EJ, et al. Concordance between four European centres of PET reporting criteria designed for use in multicentre trials in Hodgkin lymphoma. *European Journal of Nuclear Medicine and Molecular Imaging* 2010;37:1824-33.

Appendix 4. ECOG Performance Status

ECOG Score	Performance Status
0	Asymptomatic
1	Symptomatic, fully ambulatory
2	Symptomatic, in bed < 50% of the day
3	Symptomatic, in bed > 50% of the day but not bedridden
4	Bedridden
5	Dead

Appendix 5. NYHA Functional Classification

Class I: patients with no limitation of activities; they suffer no symptoms from ordinary activities.

Class II: patients with slight, mild limitation of activity; they are comfortable with rest or with mild exertion.

Class III: patients with marked limitation of activity; they are comfortable only at rest.

Class IV: patients who should be at complete rest, confined to bed or chair; any physical activity brings on discomfort and symptoms occur at rest.

Reference: Hurst JW, Morris DC, Alexander RW. The use of the New York Heart Association's classification of cardiovascular disease as part of the patient's complete Problem List. *Clin Cardiol*. 1999 Jun;22(6):385-90.

Appendix 6. List of Anti-Epileptic Drugs (AEDs) Permitted and Not Permitted in Component 2

Permitted: Non-Inducing AEDs
Keppra (levetiracetam)
Vimpat (lacosamide)
Lamictal (amotrigine)
Zonegran (zonisamide)
Felbatol (felbamate)

Not Permitted: Enzyme-Inducing AEDs
Tegretol (carbamazapine)
Dilantin (phenytoin)
Phenobarbital and primidone
Trileptal (oxacarbazepine)
Topamax (topiramate): only at high doses
Sabril (vigabatrin): speeds metabolism, enzyme-induction assumed

Not Permitted: AEDs With Weak Inhibitory Function
Depakote (divalproex sodium or valproate semisodium)
Depakene (valproic acid)
Stavzor (valproic acid delayed release)
Depacon (valproate sodium or sodium valproate)

Appendix 7. Neurological Examination Template

Subject ID Number: _____ Subject Initials: _____

DOB:

Mental status			
	Normal	**Deficit**	**Comments on Abnormalities**
A. Alertness/Attention			
B. Orientation			
C. Memory			
D. Language			
E. Knowledge			

Cranial nerves					
	R Normal	**L** Normal	**R** Deficit	**L** Deficit	**Comments on Abnormalities**
F. II, see visual system					
G. III, IV, VI					
H. V					
I. VII					
J. VIII					
K. IX, X					
L. XI					
M. XII					

N. Muscle strength (5/5 unless specified). Comment on deltoid, triceps, biceps, and grip in the UEs, and in the LEs on extension and flexion of hip, knee, and ankle.					
	R Normal	**L** Normal	**R** Deficit	**L** Deficit	**Comments on Abnormalities**
Upper extremity					
Lower extremity					

NEUROLOGICAL EXAMINATION (page 2)

O. Muscle Tone/Bulk/ Abnormal movements					
	R Normal	**L** Normal	**R** Deficit	**L** Deficit	**Comments on Abnormalities**
Upper extremity					
Lower extremity					

P. Coordination					
	R Normal	**L** Normal	**R** Deficit	**L** Deficit	**Comments on Abnormalities**
Upper extremity (FNF/RAM)					
Lower extremity (HKS)					

Gait , including tandem			
	Normal	**Deficit**	**Comments on Abnormalities**

Q. Sensory – Upper Extremity					
	R Normal	**L** Normal	**R** Deficit	**L** Deficit	**Comments on Abnormalities**
Vibration					
Light touch					
Pin					
Proprioception					

R. Sensory – Lower Extremity					
	R Normal	**L** Normal	**R** Deficit	**L** Deficit	**Comments on Abnormalities**
Vibration					
Light touch					
Pin					
Proprioception					

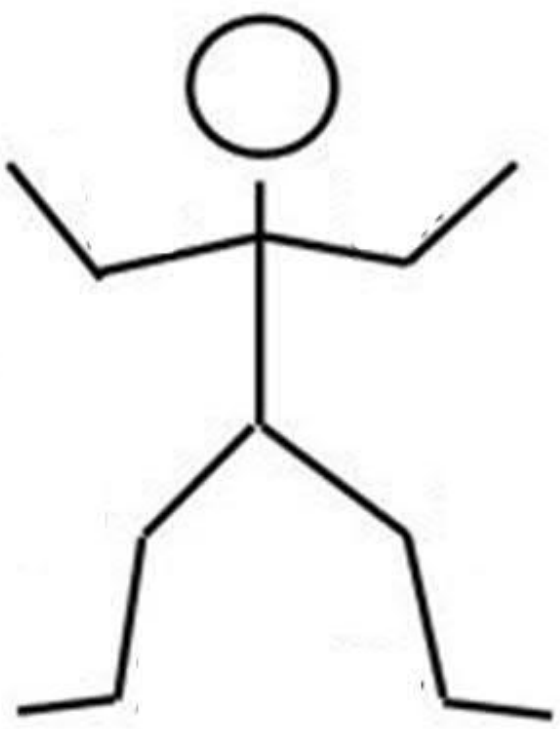
NEUROLOGICAL EXAMINATION (page 3)

S. Romberg test

	Positive	**Negative**	

T. MMSE score

U. Deep Tendon Reflexes (note plantar response with arrows)



Examiner's name (print):

Examiner's name (signature):

Appendix 8. Mini-Mental State Examination

Abilities

Orientation: What is the: (year) (season) (date) (day) (month)?

Orientation: Where are we: (state) (county) (town) (building) (floor)?

Registration: Learn: "apple, table, penny."_____# of trials

Attention and Calculation: Subtract serial 7's: (100, 93, 86, 79, 72); or spell "WORLD" backwards

Recall: Recall: "apple, table, penny."

Naming: Name: "pencil" and "watch."

Repetition: Repeat: "no ifs, ands or buts."

Three-Stage Command: "Take this paper in your right hand, fold it in half, and put it on the floor."

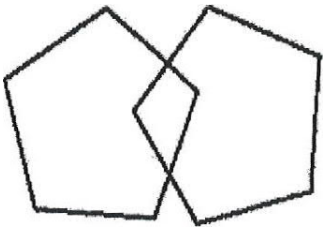
Reading: Read and obey: "Close your eyes."

Writing: write a sentence on the back of this card.

Copying: Copy the design on the back of this card

Total out of 30: abnormal if < 24; if < 8th grade, then <21 is considered abnormal.)

Close your eyes.



Spencer, M.P. and Folstein, M.F. The Mini-Mental State Examination. In: P.A. Keller and L.G. Ritt (Eds), Innovations in Clinical Practice: A Source Book. Vol. 4. Sarasota, FL: Professional Resource Exchange, Inc., 1985, 307-308.

Appendix 9. Cognition Tests

Hopkins Verbal Learning Test-Revised[19]

UIC Medical Center
Neuropsychology Service
Tel (312) 996-6217
912 South Wood Street, MC913
Chicago, IL 60612-7327

HVLT-R

FORM 1 - four legged animals, precious stones, human dwellings

Name: _____ **Date:** _____ **Age:** ____ **Examiner:** _____

Trial 1:
"This is a test of memory. I am going to read a list of words. Pay attention and try to remember as many of them as you can. You can say the words back to me in any order you wish. It will be difficult to remember all of the words, just do the best you can. Any questions?" Read the list at a 2-second inter-stimulus interval, and then say, **" Now tell me as many of the words as you can."**

Trial 2 & 3:
"That was fine. Now I would like to see whether you can remember more of the words if you have another chance. I will present the list again, just like before. Try to remember as many words as you can, including the words you just gave me."

Delayed Recalled (wait for 20-25 minutes):
"Remember those words I read to you before? I want to see how many you can remember now. I know it sounds difficult, but try to remember as many of the words as you can. Go ahead."

	Trial 1	Trial 2	Trial 3	Delay
LION				
EMERALD				
HORSE				
TENT				
SAPPHIRE				
HOTEL				
CAVE				
OPAL				
TIGER				
PEARL				
COW				
HUT				

Trial 3 Completed
_____

Delayed Recall
(20-25 minutes)
_____

Test continued on back...

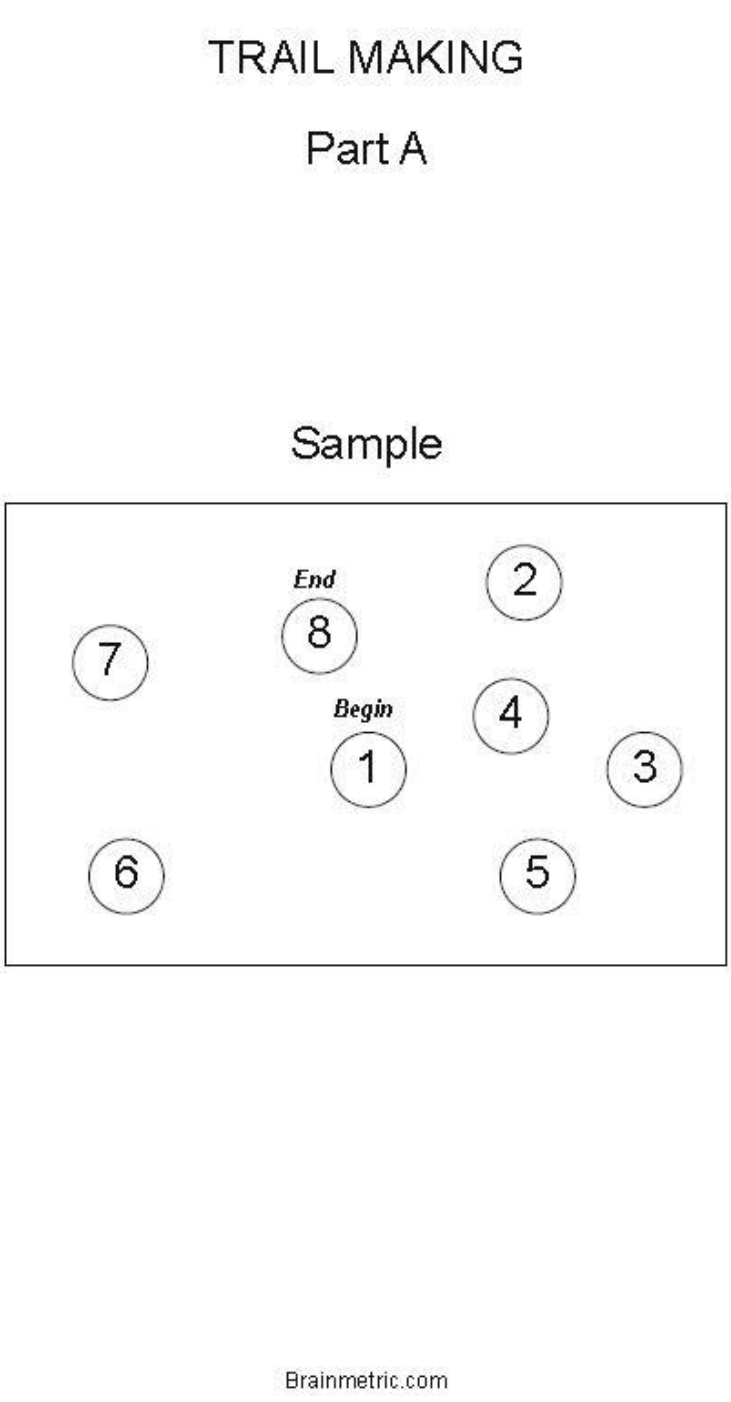
Recognition Memory:
"Now I am going to make things a little easier. I have some more words to read to you. Some of them were on the list I read before, and others are new words I didn't read before. Your job is to tell the difference. Say yes for those words that were on the list, and say no for those words that were not previously presented. Yes or no, was _____ on the list I read before?" Present each word, one at a time, reading across the list from left to right. If the patient says he is not sure, insist that he guess yes or no.

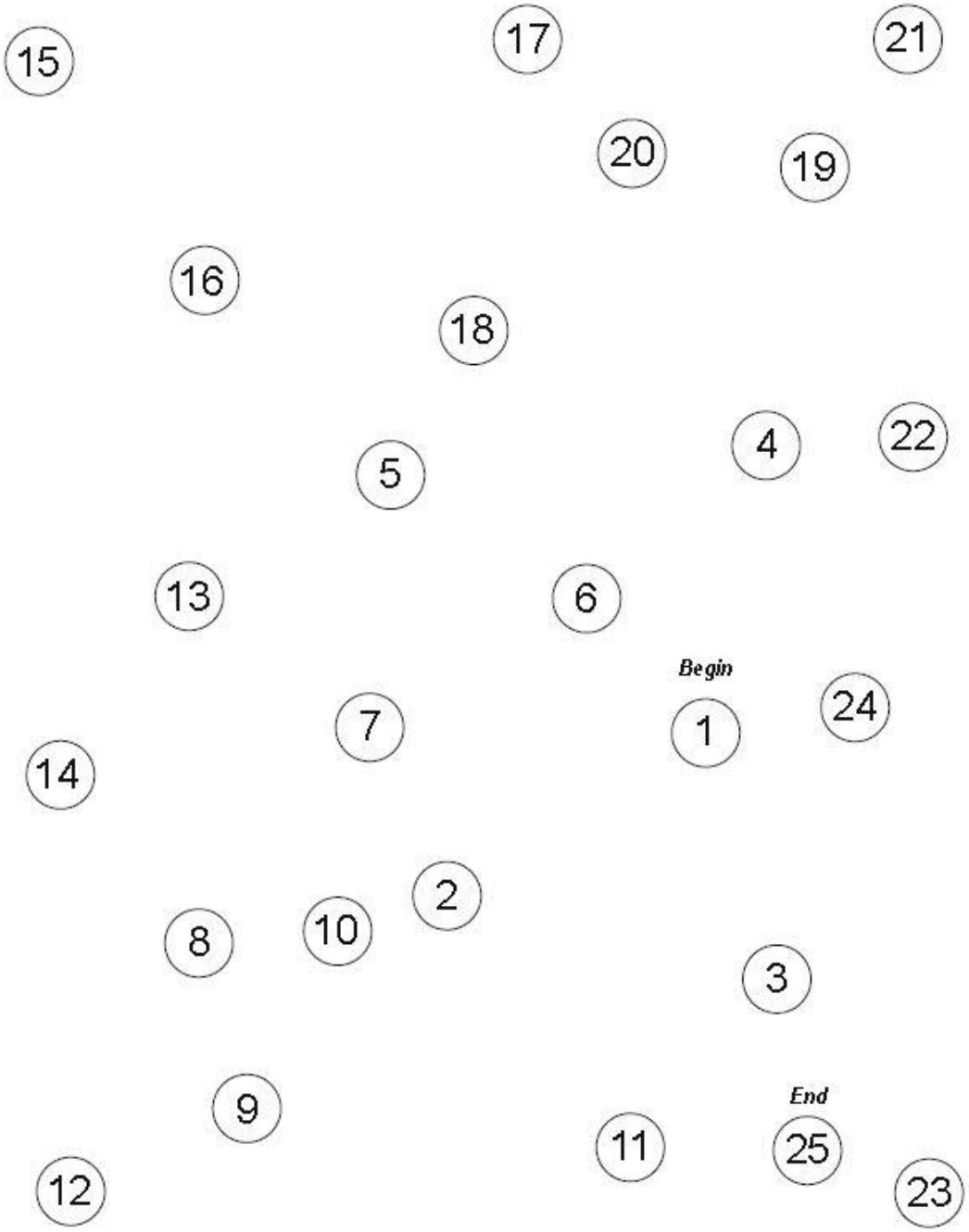
(Circle all "Yes" responses)

HORSE	ruby*	**CAVE**	balloon	coffee	**LION**	house*
OPAL	**TIGER**	boat	scarf	**PEARL**	**HUT**	**EMERALD**
SAPPHIRE	dog*	apartment*	penny	**TENT**	mountain	cat*
HOTEL	**COW**	diamond*				

	Raw	Z Score
Trial 1	_____	_____
Trial 2	_____	_____
Trial 3	_____	_____
Total Trials 1-3	_____	_____
Delayed Recall	_____	_____
Recognition Hits	_____	_____
False Positives	_____	_____
Discrimination (Hits – FP)	_____	_____

Trail Making Test[20]




15
17
21
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19
16
18
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13
6
Begin
24
7
1
14
2
10
8
3
9
End
11
25
12
23

Controlled Oral Word Association test[21]

UIC Medical Center
Neuropsychology Service
Tel (312) 996-6217

Verbal Fluency - CFL
912 South Wood Street, MC913
Chicago, IL 60612-7327

Name: _____ **Age:** ____ **Date:** _____ **Examiner:** _____

I'm going to say to you a letter of the alphabet. After I do, I want you to tell me as many of the words as you can think of that begin with that letter. There are only two rules. One, you are not allowed to say any words that are proper nouns or begin with a capital letter. So no names of people like "Ralph" or places like "Rome". Second, you cannot use a form of a word you've already said. For example, if the letter were "R" you could say "Run", but then you could not say "Running". If there are no questions, we can begin. Tell me as many words as you can think of that begin with the letter _____.

Begin timing for one (1) minute, marking every 15 second interval.

	C	F	L
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
Total	_____	_____	_____

Total CFL _____ **Z Score** _____

Appendix 10. Report From the Health Services and Research Outcomes Subcommittee of the Radiation Therapy Oncology Group on the Reliable Change Index, and Table 1 From Wefel et al. (reference below) Describing the Tests and Defining the RCI's For Each of the Tests

NEUROCOGNITIVE TESTS:

Hopkins Verbal Learning Test-Revised (HVLT-R)
Trail Making Test (TMT)
Controlled Oral Word Association (COWA)

This summary was last revised 5 January 2011.

DISCLAIMER:
This summary has been placed online by the Health Services and Research Outcomes (HSRO) Subcommittee of the Radiation Therapy Oncology Group (RTOG). It is meant to be an aid to others in the designing of clinical trials. This summary may not be redistributed without appropriate attribution. Furthermore, the posting of this catalog of assessment tools on the RTOG web site does not imply that the evaluation instruments themselves are in the public domain and are not subject to copyright protection. You must determine the proprietary status of each tool prior to incorporating it in a clinical protocol.

Brief overview:
A Neurocognitive Chair is required in order to have a neurocognitive outcome on a protocol. The neuropsychologist should be consulted to help determine relevant issues of study design such as test selection, testing time points, outcome hypotheses, analytical approaches, etc; and, the Neurocognitive Chair is required in order to obtain permission to use the tests from the test publishing companies.

Neurocognitive tests are objective, standardized tests of neurologic/cognitive function. The HVLT-R, TMT and COWA have been used in numerous RTOG studies to assess cognitive function. However, there are numerous other tests of cognitive function that vary in many characteristics including psychometric properties (reliability, validity etc) as well as the domains of cognition they assess. The study neuropsychologist should determine the optimal test(s) for each study. The HVLT-R is a test of verbal learning and memory. The TMT measures information processing speed and executive function. The COWA is a measure of verbal fluency that requires expressive language and executive functions. A number of outcome variables can be obtained and evaluated from each of the tests. Published normative data is available for each test to standardize patient performance after adjusting for relevant demographic characteristics.

Psychometric properties/Validation:
Numerous published articles and books have described the psychometric properties and validity related evidence in support of these measures and their respective measurement of domains of cognitive function (for example [1, 2, 3, 4, 5]).

Normative data:
Numerous normative studies have been published for each test (for example[1, 2, 3, 4, 5]). The following normative studies have been used to evaluate test performances in several RTOG trials[2, 4, 5]. The study neuropsychologist should determine the optimal published normative data to use for each study.

Clinically significant changes:
One way to determine clinically significant changes is to use a distribution-based statistical method such as the Reliable Change Index (RCI)[6]. The RCI is derived from the standard error of measurement of each test and represents the 90% confidence interval for the difference in raw score from baseline to the next assessment that would be expected if no real change occurred. Changes that exceed the RCI represent a decline or improvement in performance. The equation for calculating the RCI is as follows:

$$RCI = 1.64(SEdiff), \text{ where } SEdiff = [2(SEM^2)]^{1/2} \text{ and}$$

$$SEM = SD_1[(1-r_{xy})^{1/2}]$$

where SEdiff is the standard error of difference, SEM is the standard error of measurement, SD is the standard deviation, and r_{xy} is the test-retest reliability statistic. The following published studies have been used to derive RCI for the HVLT-R, TMT and COWA (insert references). Note, the RCI belongs to a family of similar statistical methodologies to determine a

clinically meaningful effect at the within subject level. The study neuropsychologist should be consulted to determine the optimal methodology to use for the purpose of each study.

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References:

1. Mitrushina, M, Boone, KB, et al. *Handbook of Normative Data for Neuropsychological Assessment, Second Edition*. New York, Oxford University Press, 2005.
2. Benedict RHB, Schretlen D, Groninger L, Brandt J. Hopkins Verbal Learning Test--Revised: Normative data and analysis of inter-form and test-retest reliability. *The Clinical Neuropsychologist*. 1998;12:43-55
3. Benton AL, Hamsher KDS. Multilingual aphasia examination. Iowa City, AJA Associates, 1989

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References:
1. Mitrushina, M, Boone, KB, et al. *Handbook of Normative Data for Neuropsychological Assessment, Second Edition*. New York, Oxford University Press, 2005.
2. Benedict RHB, Schretlen D, Groninger L, Brandt J. Hopkins Verbal Learning Test--Revised: Normative data and analysis of inter-form and test-retest reliability. *The Clinical Neuropsychologist*. 1998;12:43-55
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Table 1. Overview of neurocognitive tests

Test	Description	Possible range of score	Reliable change index threshold (from baseline)
Hopkins Verbal Learning est-Revised (HVLT-R)	The Hopkins Verbal Learning Test-Revised (HVLT-R)[15] is a learning and memory test, in which the patient is asked to learn and recall a list of 12 words over three trials.		
Total Recall (HVLT-R TR)		0–36	±5 words[15]
Delayed Recall (HVLT-R DR)	Spontaneous recall is assessed before and after a delay. Recognition discriminability is also assessed after a delay. Four alternate versions of the test were used to minimize practice effects over time.	0–12	±3 words
Delayed Recognition (HVLT-R RECOG)		12–+12	±2 words
Trail Making Test (TMT)	The Trail Making Test (TMT)[28] Part A (TMTA) assesses visual scanning and motor tracking requiring focused attention. Patients are required to sequentially connect numbered dots in ascending order that are randomly scattered across the test page. Part B (TMTB) includes a divided attention component requiring mental flexibility (i.e., executive function). On this subtest, dots with numbers and letters are randomly scattered on the test page. Patients are required to alternate between connecting numbers and letters in an ascending sequential order. Both tests require the patient to complete the sequence as fast as possible. TMTA was discontinued after 3 min and TMTB was discontinued after 5 min for patients that had difficulty in order to reduce patient burden.		
Part A (TMTA)		1–2750	±12 s[29]
Part B (TMTB)		1–3750	±26 s
Controlled Oral Word Association (COWA)	The Controlled Oral Word Association [COWA][30] test assesses lexical fluency. Given a specific letter of the alphabet, patients are required to produce as many words as possible that begin with that letter. There are two alternate forms of the COWA, each with three unique letter exemplars.	0 – unlimited	±12 words[17]

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