STUDY PROTOCOL

Protocol Title:	A Phase I Study of Ad-RTS-hIL-12, an Inducible Adenoviral Vector Engineered to Express hIL-12 in the Presence of the Activator Ligand Veledimex in Subjects with Recurrent or Progressive Glioblastoma or Grade III Malignant Glioma
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Phase:	I
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Sponsor:	ZIOPHARM Oncology, Inc. [redacted]
Medical Monitor:	[redacted]
	[redacted]
Safety Reporting:	[redacted]

PROTOCOL SYNOPSIS

Clinical Protocol ATI001-102

Title

A Phase I Study of Ad-RTS-hIL-12, an Inducible Adenoviral Vector Engineered to Express hIL-12 in the Presence of the Activator Ligand Veledimex in Subjects with Recurrent or Progressive Glioblastoma or Grade III Malignant Glioma

Protocol Number

ATI001-102

Clinical Phase

Phase I

Investigational Product(s)

Adenovirus-[redacted]-human interleukin-12 (Ad-RTS-hIL-12) and veledimex (RTS activator ligand)

Ad-RTS-hIL-12 is a replication-incompetent adenoviral vector containing the hIL-12 gene under the control of the RTS inducible promoter turned on in the presence of the activator ligand, veledimex. Veledimex is a small molecule RTS specific transcription factor that stabilizes the RTS promoter components and allows transcription of the hIL-12 target gene. Since target gene expression is dependent on the dose and frequency of veledimex administration, the expression of hIL-12 can be modulated (turned on and off) by the veledimex dose and schedule.

Research Hypothesis

Among subjects with recurrent or progressive glioblastoma or Grade III glioma, Ad-RTS-hIL-12 and veledimex can be safely administered and can induce signals of immune activity.

Primary Objective

- To determine the safety and tolerability of varying dose levels of intratumoral Ad-RTS-hIL-12 and oral veledimex doses in subjects with recurrent or progressive glioblastoma or Grade III malignant glioma

Secondary Objectives

- To determine the veledimex maximum tolerated dose (MTD) when given with varying doses of intratumoral Ad-RTS-hIL-12
- To determine the veledimex pharmacokinetic (PK) profile
- To determine the veledimex concentration ratio between the brain tumor and blood
- To evaluate cellular [redacted] immune responses elicited by Ad-RTS-hIL-12 and veledimex
- To determine investigator assessment of response, including tumor objective response rate (ORR) and progression-free survival (PFS)

- To determine overall survival (OS)

Study Design

This is a Phase I study of varying dose levels of Ad-RTS-hIL-12 administered by intratumoral injection and varying veledimex doses administered orally in subjects with recurrent or progressive glioblastoma or Grade III malignant glioma. This study will investigate two intratumoral Ad-RTS-hIL-12 doses [2 x 10^{11} viral particles (vp) and 1 × 10^{12} vp] and escalating veledimex doses to determine the safe and tolerable dose based on the safety profiles observed in the presence of variable corticosteroid exposure.

This study is divided into three periods: the Screening Period, the Treatment Period and DLT evaluation period (Days 0-28), and the Follow-up Period. After the informed consent form (ICF) is signed, subjects will enter the Screening Period to assess eligibility. Eligible subjects will be stratified into one of two groups, according to clinical indication for tumor resection. Subjects who are scheduled for a standard of care craniotomy and tumor resection (Group 1) will receive one veledimex dose before the resection procedure. Samples (tumor, cerebrospinal fluid (CSF) (if available), and blood) will be collected during the resection procedure to determine the veledimex concentration ratio between the tumor, the CSF (when available), and the blood. Ad-RTS-hIL-12 by dose cohort (either 2 x 10^{11} vp or 1 x 10^{12} vp) will be administered by [redacted] injection. After Ad-RTS-hIL-12 injection, subjects will continue on oral veledimex for 14 days. Subjects not scheduled for tumor resection (Group 2) will receive Ad-RTS-hIL-12 (2 × 10^{11} vp or 1 × 10^{12} vp) by stereotactic injection and then will continue on oral veledimex for 14 days

Note: Subjects in Group 1 will receive up to a total of 15 veledimex doses: one veledimex dose 3 (± 2) hours before the craniotomy procedure (first dose prior to craniotomy will be a dose specific to the assigned cohort) and tumor resection (prior to Ad-RTS-hIL-12 administration), and up to 14 veledimex doses after Ad-RTS-hIL-12 administration. Subjects in Group 2 will receive up to a total of 14 veledimex doses after Ad-RTS-hIL-12 administration.

Group 1: Subjects scheduled for craniotomy and tumor resection:

Subjects with a clinical indication for tumor resection will receive veledimex dose specific to the assigned cohort 3 (± 2) hours before the craniotomy procedure, on an empty stomach (excluding other medications). At the time of tumor resection, brain tumor, CSF (if available), and blood samples will be collected to determine the veledimex concentration ratio between brain tumor, CSF (if available), and blood.

For Cohorts 1 and 2, immediately after tumor resection, Ad-RTS-hIL-12 2 x 10^{11} vp will be administered by [redacted] injection into approximately two sites within the residual tumor for a total volume of 0.1 mL. The total amount delivered to each site will be recorded in the CRF. In the event that less than the planned total injected volume is administered, the reason will be provided. The day of Ad-RTS-hIL-12 administration is designated as Day 0.

For Cohorts 3, 4, 5 and 6, immediately after tumor resection, Ad-RTS-hIL-12 1 x 10^{12} vp will be administered by [redacted] injection into multiple sites in a divided dose within the residual tumor (day of Ad-RTS-hIL-12 administration is designated as Day 0). The planned total injected volume for these cohorts is 0.5 mL given in approximate aliquots of 0.1 mL at each injection site. The total amount delivered to each site will be recorded in the CRF. In the event that less than the planned total injected volume is administered, the reason will be provided. When available, an intra-operative magnetic resonance imaging (MRI) can be performed to guide the Ad-RTS-hIL-12 injection to areas of contrast-enhancing tumor tissue.

After the Ad-RTS-hIL-12 injection, veledimex will be administered orally for 14 days. The first postresection veledimex dose is to be given on Day 1, preferably with food. Subsequent veledimex doses are to be taken once daily, in the morning and within approximately 30 minutes of a regular meal.

Group 2: Subjects who will NOT undergo tumor resection:

Subjects who will not undergo tumor resection will receive Ad-RTS-hIL-12 by standard stereotactic surgery on Day 0. Ad-RTS-hIL-12 (2×10^{11} vp or 1×10^{12} vp) will be administered by stereotactic injection. The day of Ad-RTS-hIL-12 administration is designated as Day 0. In Cohorts 1 and 2, it will be delivered into approximately two intratumoral sites for a total volume of 0.1 mL. In Cohorts 3, 4, 5 and 6 it will be delivered into multiple intratumoral sites in a divided dose to deliver 0.5 mL volume in approximate aliquots of 0.1 mL at each site. The total amount delivered to each site will be recorded in the CRF. In the event that less than the planned total injected volume is administered, the reason will be provided. Care should be taken to avoid intraventricular or basal cisternal injection or other critical locations.

After the Ad-RTS-hIL-12 injection, veledimex will be administered orally for 14 days. The first veledimex dose is to be given on as Day 1, preferably with food. Subsequent veledimex doses are to be taken once daily, in the morning and within approximately 30 minutes of a regular meal.

Subject enrollment into Group 2 will start no earlier than after two subjects have completed 28 days in Group 1, Cohort 1. Enrollment into Group 2 cohorts is otherwise independent of enrollment into Group 1 cohorts.

This study will explore four veledimex dose cohorts; 20 mg, 40 mg, 80 mg, and 120 mg. Subject enrollment and veledimex dose escalation will proceed according to a standard 3+3 design modified to independently evaluate two stratified subject groups that may exhibit different safety and tolerability profiles. In each cohort, the first subject will be monitored for the 14 days of treatment and will be observed an additional 7 days post the last veledimex dose before the second and third subjects are enrolled in that same cohort. The dose-limiting toxicity (DLT) evaluation period is defined as 28 days post Ad-RTS-hIL-12 injection (Day 0 – Day 28). Determination of safety and recommendation to dose escalate will occur after all dosed subjects in a cohort have been evaluated for at least 28 days post Ad-RTS-hIL-12 injection. The details on the dose escalation schedule are provided in Section 6.2 Table 5 and the dose escalation decision rules provided in Section 6.3 and summarized in Table 6.

Study Oversight for Safety Evaluation:

This study will implement an independent data and safety monitoring board (DSMB) to provide unbiased and objective input on the safety evaluation of the high grade glioma population. In the context of an open label study, the responsibility of the DSMB is to provide an objective determination of safety at the end of each dose cohort and make a recommendation to proceed to the next higher veledimex dose level or to discontinue the study. A separate charter will describe the activities of this committee.

The first level of safety oversight will occur through the site investigator and medical monitor. A formal Safety Review Committee (SRC) comprised of the study investigators, the medical monitor and other appropriate sponsor representatives will provide the overall safety oversight. Additional external medical and scientific experts may also be invited to participate in the reviews as needed. A separate charter will outline the SRC activities. Briefly, the SRC will evaluate patient safety within each cohort, and within each stratification group of the same cohort. If no significant safety events occur with the first patient of each cohort, the second and third subjects will be enrolled and treated. If a significant safety event occurs with the first patient in each cohort, the SRC will convene to evaluate the safety event(s) and make a recommendation and decision on the enrollment of the second and third subjects in that same cohort. At the end of each cohort (Group 1 and 2 separately), the SRC will convene to review the safety data after the final patient in a cohort, within a group, has completed veledimex dosing and has been monitored for 28 days after the Ad-RTS-hIL-12 injection.

At the end of each cohort, the safety summary will be communicated to the DSMB. The DSMB, will be guided by a DSMB charter and, within three days of receipt, will evaluate the safety data and make a

recommendation to the SRC regarding continuation to the next dose cohort, amendment of the dose escalation schedule, or discontinuation of the study. The DSMB recommendation will be communicated to the SRC and appropriate action will be taken. A written record of the DSMB recommendation will be provided to the SRC and maintained on file with the sponsor. See Section 3.3.

Dose-limiting toxicity (DLT) is defined as:

An event, occurring within the first 28 days (Day 0 - Day 28) that meets one of the following conditions:

- Any local reaction that requires operative intervention and felt to be attributable to Ad-RTS-hIL-12 and/or veledimex
- Any local reaction that has life-threatening consequences requiring urgent intervention or results in death and felt to be attributable to Ad-RTS-hIL-12 and/or veledimex
- Any Grade 3 or greater non-hematological adverse event that is at least possibly related to the study drug
- Any Grade 4 hematologic toxicity that is at least possibly related to the study drug and lasts at least 5 days
- Grade 3 or higher thrombocytopenia at least possibly related to the study drug

Note: Diagnostic brain tumor biopsy is not considered a DLT. Seizures, headache and cerebral edema are commonly observed in this population and will be recorded according to grade of toxicity, but will not be considered a DLT unless they are deemed as most likely to be drug-related as the main contributory factor.

Expansion cohorts are not prospectively planned in this study. A decision to enroll additional subjects, as part of an expansion cohort, at the determined veledimex MTD and the Ad-RTS-hIL-12 dose will be made by the SRC only after the MTD has been identified for one or both groups and safety evaluated. If an expansion cohort is implemented, the veledimex dose may be delayed or reduced for individual subjects in the event of toxicity. If ≥ 33% of subjects in the expansion cohort experience DLTs, according to the definition used in the dose-escalation phase, additional subjects may be enrolled at the next lower dose tested in the dose escalation phase, or at an intermediate dose, as recommended by the SRC. All DLTs should be reported to the sponsor within 24 hours of investigator/site awareness.

Number of Centers

Approximately 8 to 12 centers

Number of Subjects

Up to 72 subjects may be enrolled.

Study Population

The eligible study population includes adult subjects with recurrent or progressive glioblastoma or Grade III malignant glioma (anaplastic astrocytoma, anaplastic oligodendroglioma, and anaplastic oligoastrocytoma,) for which there is no alternative curative therapy.

Inclusion Criteria:

1. Male or female subject ≥ 18 and ≤ 75 years of age
2. Provision of written informed consent for tumor resection, stereotactic surgery, tumor biopsy, samples collection, and treatment with investigational products prior to undergoing any study-specific procedures
3. Histologically confirmed supratentorial glioblastoma or other World Health Organization (WHO) Grade III or IV malignant glioma from archival tissue
4. Evidence of tumor recurrence/progression by MRI according to response assessment in neuro-oncology (RANO) criteria after standard initial therapy
5. Previous standard of care antitumor treatment including surgery and/or biopsy and chemoradiation. At the time of registration, subjects must have recovered from the toxic effects of previous treatments as determined by the treating physician. The washout periods from prior therapies are intended as follows: (windows other than what is listed below should be allowed only after consultation with the Medical Monitor)
    a. Nitrosoureas: 6 weeks
    b. Other cytotoxic agents: 4 weeks
    c. Antiangiogenic agents, including bevacizumab: 4 weeks
    d. Targeted agents, including small-molecule tyrosine kinase inhibitors: 2 weeks
    e. Experimental immunotherapies (e.g., PD-1, CTLA-4): 3 months
    f. Vaccine-based therapy: 3 months
6. Able to undergo standard MRI scans with contrast agent before enrollment and after treatment
7. Karnofsky Performance Status ≥ 70
8. Adequate bone marrow reserves and liver and kidney function, as assessed by the following laboratory requirements:
    a. Hemoglobin ≥ 9 g/L
    b. Lymphocytes > 500/mm^3
    c. Absolute neutrophil count ≥ 1500/mm^3
    d. Platelets ≥ 100,000/mm^3
    e. Serum creatinine ≤ 1.5 x upper limit of normal (ULN)
    f. Aspartate transaminase (AST) and alanine transaminase (ALT) ≤ 2.5 x ULN. For subjects with documented liver metastases, ALT and AST ≤ 5 x ULN
    g. Total bilirubin < 1.5 x ULN
    h. International normalized ratio (INR) and activated partial thromboplastin time within normal institutional limits

9. Male and female subjects must agree to use a highly reliable method of birth control (expected failure rate less than 5% per year) from the Screening Visit through 28 days after the last dose of study drug. Women of childbearing potential (perimenopausal women must be amenorrheic for at least 12 months to be considered of non-childbearing potential) must have a negative pregnancy test at screening.

Exclusion Criteria:

1. Radiotherapy treatment within 4 weeks or less prior to starting first veledimex dose
2. Subjects with clinically significant increased intracranial pressure (e.g., impending herniation or requirement for immediate palliative treatment) or uncontrolled seizures
3. Known immunosuppressive disease, autoimmune conditions, and/or chronic viral infections [e.g., human immunodeficiency virus (HIV), hepatitis]
4. Use of systemic antibacterial, antifungal, or antiviral medications for the treatment of acute clinically significant infection within 2 weeks of first veledimex dose. Concomitant therapy for chronic infections is not allowed. Subjects must be afebrile prior to Ad-RTS-hIL-12 injection; only prophylactic antibiotic use is allowed perioperatively.
5. Use of enzyme-inducing antiepileptic drugs (EIAED) within 7 days prior to the first dose of study drug. Note: Levetiracetam (Keppra®) is not an EIAED and is allowed
6. Other concurrent clinically active malignant disease, requiring treatment, with the exception of non-melanoma cancers of the skin or carcinoma *in situ* of the cervix or nonmetastatic prostate cancer
7. Nursing or pregnant females
8. Prior exposure to veledimex
9. Use of medications that induce, inhibit, or are substrates of CYP450 3A4 within 7 days prior to veledimex dosing without consultation with the Medical Monitor
10. Presence of any contraindication for a neurosurgical procedure
11. Unstable or clinically significant concurrent medical condition that would, in the opinion of the investigator or medical monitor, jeopardize the safety of a subject and/or their compliance with the protocol. Examples include, but are not limited to, unstable angina, congestive heart failure, myocardial infarction within 2 months of screening, ongoing maintenance therapy for life-threatening ventricular arrhythmia or uncontrolled asthma.

Investigational Product(s), Dose and Mode of Administration

Adenovirus-[redacted]human interleukin-12 (Ad-RTS-hIL-12) and veledimex (RTS activator ligand)

Ad-RTS-hIL-12 is a replication-incompetent adenoviral vector containing the hIL-12 gene under the control of the RTS inducible promoter activated in the presence of the activator ligand, veledimex. Veledimex is a small molecule RTS specific transcription factor that stabilizes the RTS promoter components and allows transcription of the hIL-12 target gene. Since target gene expression is dependent on the dose and frequency of veledimex administration, the expression of hIL-12 can be modulated (turned on and off) by the optimal veledimex dose and schedule.

Ad-RTS-hIL-12 Dose:

This study will explore varying dose levels of Ad-RTS-hIL-12. The Ad-RTS-hIL-12 will be administered by either [redacted] injection into residual tumor sites immediately after tumor resection (Group 1) or by stereotactic injection at intratumoral sites (Group 2).

Veledimex Dose Cohorts post Ad-RTS-hIL-12 injection

Veledimex will be administered orally. Group 1 will receive veledimex 3 (± 2) hours before the planned craniotomy (first dose prior to craniotomy will be a dose specific to cohort), and will resume veledimex dosing as appropriate for the cohort after Ad-RTS-hIL-12 administration. Group 2 will receive veledimex only after Ad-RTS-hIL-12 administration at dose levels specific to their cohort. Group 2 will start recruitment only after the first two subjects have completed 28 days in Group 1, Cohort 1.

This study will explore the following dose cohorts given after Ad-RTS-hIL-12 administration:

Cohort	Ad-RTS-hIL-12[a] (Day 0)	Veledimex[b] Group 1 (Days 0 through 14) Group 2 (Days 1 through 14)
	Dose (vp)	Total Daily Dose (mg)
1	2×10^{11}	20
2	2×10^{11}	40
3	1×10^{12}	20
4	1×10^{12}	40
5	1×10^{12}	80
6	1×10^{12}	120

Ad-RTS-hIL-12 = Adenovirus-[redacted]-human interleukin-12, PK = pharmacokinetics, vp = viral particles
[a]: Intratumoral injection
[b]: Dose levels may be modified based on additional clinical and nonclinical PK data (Section 6.4)

Study Assessments and Criteria for Evaluation

Safety Assessments:

Safety will be evaluated in the Overall Safety Population (OSP) and the Evaluable Safety Population (ESP) using National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4.03. Safety assessments will be based on medical review of adverse event (AE) reports and the results of vital signs, physical and neurologic examinations, electrocardiograms (ECGs), clinical laboratory tests, and monitoring the frequency and severity of AEs. The incidence of AEs will be tabulated and reviewed for potential significance and clinical importance. The reporting period of safety data will be from the date of ICF signature through the initial Follow-up Period.

Tumor Response Assessments:

The ESP will be evaluated for investigator assessment of objective response rate (ORR), progression-free survival (PFS) and overall survival (OS). Response will be assessed using the RANO/iRANO criteria (Appendix 16.4 and Appendix 16.5).

Immune Response Assessments:

Immunological and biological markers, such as levels of IL-12, interferon-gamma (IFN-γ), [redacted] will be assessed in pre- and posttreatment serum samples.

[redacted] Schedule of Study Procedures.

Pharmacokinetic Evaluations:

Veledimex PK parameters will be evaluated at each dose level in the dose escalation and any proposed expansion cohorts for Group 1 and Group 2 subjects. PK sampling times are outlined in Section 11.4 and Table 8.

[redacted]

Statistical Methods

Analysis Populations:

- The Overall Safety Profile includes all subjects who have received at least one dose of veledimex (pretumor resection and/or post-stereotactic procedure) and/ or all subjects who received Ad-RTS-hIL-12.
- The Evaluable Safety Population includes subjects who have received Ad-RTS-hIL-12 and at least one dose of veledimex after Ad-RTS-hIL-12 administration.
- The Pharmacokinetics Population (PKP) includes all subjects who receive veledimex.

Safety Analysis:

The OSP will be used to perform safety evaluations for all safety variables of all subjects who received at least one dose of veledimex and/or all subjects who received Ad-RTS-hIL-12.

The ESP will be used to make determinations for dose escalation to subsequent higher veledimex doses for each group (Group 1 or Group 2) separately based on a standard 3 + 3 design, as previously described.

For the first veledimex dose cohort, a minimum of three ESP subjects must be eligible and must have received veledimex dosing. In addition, DLT evaluation must also be performed on these subjects according to the rules of the protocol in both Group 1 and Group 2, respectively.

Safety variables will be tabulated and presented by dose cohort and by group. Exposure to study drug and reasons for discontinuation of study treatment will be tabulated. All treatment-emergent AEs will be coded according to the system organ class and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA), and will be tabulated by number and percent of subjects, and according to relationship to the study drugs, severity, and seriousness. Laboratory parameters will be summarized by visit. Vital signs and physical examination data will be presented by visit.

Tumor Response, Overall Survival, Pharmacodynamic and PK Analyses:

Tumor response analysis will be performed on the ESP. Investigator assessment of ORR and PFS will be determined for each dose cohort according to RANO/iRANO criteria (Appendix 16.4 and Appendix 16.5). Overall survival (OS) is defined as the duration of time from the first dose of study drug to the date of death, or, for subjects who are still alive two years post first dose of study drug, they will be censored at the last follow-up contact date. A two-sided confidence interval will be computed for the ORR. PFS and OS will be analyzed using the Kaplan-Meier method.

Pharmacodynamic and PK analysis will be performed on the PKP. [redacted] Veledimex PK parameters will be determined based on blood levels of veledimex using standard methods.

Sample Size Determination

The choice of the number of subjects was based on the standard 3+3 modified to independently evaluate two stratified subject groups that may exhibit different safety and tolerability profiles.

Up to 72 subjects may be enrolled into this study, including three to six subjects per group enrolled in each of the planned dose level cohorts. Subjects who withdraw from the study during the DLT evaluation period (Days 0 - 28) for reasons other than toxicity or disease progression may be replaced.

Study Duration

The duration of this study from the time of initiating subject screening until the completion of survival follow up is anticipated to be approximately 48 months, including 24 months for enrollment and 24 months of follow-up.

The start of study is defined as the date when the first subject is consented into the study and the study stop date is the date of database lock.

SCHEDULE OF STUDY PROCEDURES

	Screening Period	Treatment Period								Initial Follow-up Period			Long Term Follow-up Period
Activity	**Day -28 to -1**	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4-6**	**Day 7**	**Day 8-13**	**Day 14**	**Day 15**	**Day 28 ± 7**	**Day 56 ±7**	**Every 8 weeks**
Clinical Assessments													
Informed Consent	X												
Medical/Cancer History[a,b]	X												
Physical Exam[c], including targeted neurological exam	X	X	X	X	X		X		X		X		
Karnofsky PS[d]	X	X							X		X		
Height (only at Screen) and Weight	X						X		X		X		
Vital Signs[e]	X	X	X	X	X		X		X		X		
Adverse Events[f]	X →	→	→	→	→	→	→	→	→	→	→	X	
Concomitant Medications[b,f]	X →	→	→	→	→	→	→	→	→	→	→	X	X[g]
Survival Status[g]	X →	→	→	→	→	→	→	→	→	→	→	→	→
Clinical Laboratory[u]													
Pregnancy Test[h]	X	X											
Hematology Panel[i]	X	X		X	X		X		X		X		
Coagulation Panel[j]	X	X			X		X		X				
Serum Chemistry Panel[k]	X	X		X	X		X		X		X		
Urinalysis Panel[l]	X	X							X				
ECG[m]	X	X			X				X				
Registration[n]	X												
Study Drug Administration													
Ad-RTS-hIL-12		X[o,p]											
Veledimex Dose Group 1		X	X[p,q]	X	X	X	X	X	X[r]				
Veledimex Dose Group 2			X[p,q]	X	X	X	X	X	X[r]				
Veledimex Dose Compliance/Subject Diary[r]			X	X	X	X	X	X	X				
PK/PD/Immune Assessments													
[redacted]	[redacted]				[redacted]								
Veledimex PK blood sample[s, u]		X[t]	X	X	X		X		X	X			
Serum Cytokine profile[u]		X[t]			X		X		X		X		
[redacted]		[redacted]					[redacted]		[redacted]		[redacted]		
MRI Scans[v,w]	X[v,w]			X[v,w,x]					X[v]		X[w]	X[w]	X[w]
Tumor Sample[u] (Group 1 only)		X											
CSF Sample[y] (Group 1 only)		X											

[a]: Medical history includes demographic information, medical and surgical history. Cancer history includes current cancer diagnosis, prior treatment [regimen(s), doses, start and stop dates and any associated residual toxicity], and best response for each regimen.
[b]: Medications received in the period preceding consent (~28 days) in addition to those ongoing at screening will be captured in the CRF.
[c]: A complete physical examination including a neurological exam and mental status is required at baseline. Targeted neurological exams thereafter.
[d]: See Appendix 16.1
[e]: Blood pressure, pulse, temperature, and respiration will be recorded. Blood pressure should be monitored closely, with hydration as needed to prevent hypotension after veledimex administration. Subjects must be instructed to maintain adequate oral hydration on and between veledimex dosing days; sites must closely monitor subjects' hydration status.
[f]: Monitoring and recording of concomitant medications and adverse events (AEs) and serious adverse events (SAEs) will be conducted throughout the study. Concomitant medications given and AEs/SAEs that occur following signed informed consent form (ICF) through the initial Follow-up Period (e.g. Day 56 visit) must be recorded in the CRF. AEs that are ongoing at the end of the Initial Follow-up Period and are considered drug related should be followed until resolution or no resolution is expected.
[g]: Patients will be followed to document start of a new anticancer therapies and survival status for 2 years following administration of Ad-RTS-hIL-12.
[h]: Females of childbearing potential will have a serum pregnancy test at the Screening Visit and a urine or serum pregnancy test on Day 0, with a negative pregnancy outcome required prior to first dose of study drug (either veledimex (Group 1) or injection of Ad-RTS-hIL-12 (Group 2)).
[i]: Hematology Panel: complete blood count, white blood count with differential, red blood cell count, hematocrit, hemoglobin, red blood cell indices, mean corpuscular volume, and platelet count.
[j]: Coagulation Panel: activated partial thromboplastin time, international normalized ratio, erythrocyte sedimentation rate and C-reactive protein.
[k]: Serum Chemistry Panel: aspartate transaminase, alanine transaminase, lactate dehydrogenase, alkaline phosphatase, creatinine, total bilirubin, total protein, albumin, amylase, blood urea nitrogen, glucose, sodium, potassium, chloride, calcium, phosphorus and bicarbonate.
[l]: Urinalysis Panel (dipstick): appearance, pH, specific gravity, glucose, protein/albumin, blood, ketones, bilirubin, nitrates, and leukocyte esterase. In addition, a microscopic exam for casts, crystals and cells may be done if clinically indicated.
[m]: Standard 12-lead ECG; single measurement at each time point
[n]: Centralized registration of eligible subjects will be completed prior to first dose of study drug (either veledimex (Group 1) or injection of Ad-RTS-hIL-12 (Group 2)), according to a process defined by the sponsor.
[o]: Ad-RTS-hIL-12 intratumoral injection should be administered by [redacted] injection for Group 1 subjects and intracranial stereotactic injection for Group 2 subjects. Subjects must be instructed to maintain adequate oral hydration during the Treatment Period; sites must closely monitor subjects' hydration status. Because of the potential for toxicity (e.g., fevers, chills, fatigue and dehydration), administration of prophylactic antipyretics is recommended after injection of Ad-RTS-hIL-12.
[p]: Each subject will be carefully monitored for any local reactions and/or hypersensitivity reactions following the Ad-RTS-hIL-12 injection and veledimex administration. Subject should be instructed to call the clinical site if headache, hemiparesis, seizure or other local reactions develop anytime and especially between study visits.
[q]: The first postresection veledimex dose is to be given on the next day, designated as Day 1, preferably with food. Subsequent veledimex doses are to be taken once daily, in the morning and within 30 minutes of a regular meal.
[r]: Study sites must determine compliance of veledimex dosing. Subjects should be instructed to document veledimex dosing compliance in a subject diary, including the time subject took the once daily dose, the time subject ate breakfast, the number of capsules taken, whether subject missed any veledimex doses and the study day and reason for any missed doses. Study drug container(s) with any remaining capsules should be returned to the study staff, so that staff can properly assess dose compliance.
[s]: The PK sampling schedule is provided in Table 8, Section 11.4.
[t]: Day 0, PK, and cytokine blood samples should be obtained prior to Ad-RTS-hIL-12 injection.
[redacted]
For details, see Section 11.1 through Section 11.4 and the Laboratory Manual.
[v]: Appropriate cancer staging procedures should be performed during screening. All imaging should be of diagnostic quality. The brain is to be imaged using the same method(s) used throughout the study. Measurable target lesions should be selected and measured per RANO/iRANO guidelines. (Appendix 16.4 and 16.5). A repeat scan to confirm progression should be completed at 4 weeks (per RANO) and preferably again at 12 weeks (per iRANO) after first documentation of progression. Additional tumor response assessments as well as a posttreatment diagnostic brain biopsy may be performed at the discretion of the investigator as part of providing standard of care treatment in accordance with current iRANO guidelines.
[w]: The Day 28 (± 7 days) and Day 56 (± 7 days) MRI scans are required for all subjects, including those with unconfirmed disease progression, to ensure that more slowly declining tumor burden in response to therapy is noted. For 2 years, subjects without confirmed disease progression should continue to have tumor assessments performed every 8 weeks as per standard practice until disease progression has been identified (first documentation) and confirmed (12 weeks after first documentation). MRI scans should be available for collection upon sponsor request.
[x]: The MRI scan designated on Day 2 should be taken within 72 hours of Ad-RTS-hIL-12 administration and will be considered the baseline scan for tumor response assessments.
[y]: Additional tumor, CSF (if available) and blood samples to be collected, if available, as part of standard of care procedures.

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ABBREVIATIONS

Abbreviation	Definition
Ad	Adenovirus
AE	adverse event
ALT	alanine transaminase
ALP	alkaline phosphatase
ALVAC	canarypox virus
aPTT	activated partial thromboplastin time
AST	aspartate transaminase
AUC	area under the curve
AUC_{last}	area under the curve up to the last measureable concentration
BBB	blood brain barrier
BID	*bis in die* (twice daily)
BUN	blood urea nitrogen
CBC	complete blood count
C_{max}	maximum plasma concentration observed
CD	cluster of differentiation
CL	Clearance
CR	complete response
CRF	case report form
CRP	C-reactive protein
CSF	cerebrospinal fluid
CTCAE	Common Terminology Criteria For Adverse Events
DLT	dose-limiting toxicity
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
EcR	ecdysone receptor
eCRF	electronic case report form
EDC	electronic data capture
EIAED	enzyme-inducing antiepileptic drugs
EMCV	encephalomyocarditis virus
ESR	erythrocyte sedimentation rate
ESP	Evaluable Safety Population
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HED	human equivalent dose

Abbreviation	Definition
HGG	high-grade gliomas
HIPAA	Health Insurance Portability and Accountability Act
hIL-12	human interleukin-12
HIV	human immunodeficiency virus
ICF	informed consent form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
INF-β	interferon-beta
IFN-γ	interferon-gamma
IHC	Immunohistochemistry
IL	Interleukin
IV	Intravenous
INF	Interferon
INR	international normalized ratio
IP-10	interferon gamma-induced protein 10
IRB	Institutional Review Board
IRES	internal ribosome entry site
LDH	lactate dehydrogenase
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
NCI	National Cancer Institute
NK	natural killer
ORR	objective response rate
OS	overall survival
OSP	Overall Safety Population
PD	progressive disease
PFS	progression-free survival
PI	Principal Investigator
PK	Pharmacokinetic
PKP	Pharmacokinetic Population
PO	per oral
PR	partial response
QD	*quaque die* (every day)
QT interval	measure of the time between the start of the Q wave and the end of the T wave in the electrical cycle of the heart

Abbreviation	Definition
QTc interval	corrected measure of the time between the start of the Q wave and the end of the T wave in the electrical cycle of the heart
RANO	response assessment in neuro-oncology (immune response assessment in neuro-oncology (iRANO))
rAD	recombinant adenovirus
RBC	red blood cell
[redacted]	[redacted]
RXR	retinoid X receptor
SAE	serious adverse event
SD	stable disease
SPD	sum of products of diameters
SRC	safety Review Committee
$t_{1/2}$	terminal phase half-life
TEAE	treatment-emergent adverse event
TGFβ	transforming growth factor β
Th1	T-helper cell type 1
TNFα	tumor necrosis alpha
T_{max}	time at which the maximum concentration is observed
ULN	upper limit of normal
UA	unable to assess
V_d	volume of distribution
Vp	viral particles
WBC	white blood cell
WHO	World Health Organization

1. INTRODUCTION

1.1 Disease Background

High-grade gliomas (HGG) are the most common primary brain tumors and account for approximately 85% of all newly diagnosed malignant brain tumors annually. The HGG category typically describes World Health Organization (WHO) classified Grade 3 and 4 tumors of which glioblastomas make up 60% to 70%, anaplastic astrocytomas 10% to 15%, and several other anaplastic subtypes the rest (Central Brain Tumor Registry). WHO Grade 4 gliomas include glioblastoma (also known as glioblastoma multiforme), giant cell glioblastoma, and gliosarcoma, while Grade 3 gliomas include anaplastic forms of astrocytoma, oligodendroglioma, and oligoastrocytoma (Louis 2007). Glioblastoma is by far the most frequent malignant glioma and is associated with a particularly aggressive course and dismal prognosis. The current standard of care treatment is based on surgical resection with the intent to remove as much of the tumor as is feasible. Resection is then followed by radiotherapy and concomitant adjuvant temozolomide. However, such aggressive treatment is associated with only modest improvements in survival. Newly diagnosed glioblastoma subjects have a median overall survival (OS) of 11 to 17 months and 2-year OS rate between 15% and 27%. To date, no salvage treatment has been validated by Phase III data for recurrent or progressive glioblastoma. For such subjects, the median OS is 6 to 7 months, and median progression-free survival (PFS) is 2 to 3 months.

The lack of standard and validated salvage therapies has prompted the use of nitrosureas, temozolomide rechallenge, bevacizumab, and other targeted agents that are unsatisfactory treatment options. This likely reflects the complexities of disease heterogeneity, and the treatment limitations of brain tumors given the low activity of antineoplastic agents, de novo or acquired drug resistance, the sensitivity of the brain to irreversible damage in response to treatment and the presence of the blood brain barrier (BBB), which maintains the brain as a privileged compartment. Surgical resection may be offered for subjects with recurrent disease, with the goal of alleviating mass effect, improving symptoms, and achieving cytoreduction. Surgical resection, however, is limited by the infiltrative nature of the disease and the lack of clear margins delimitating the tumor. Given the poor overall prognosis and lack of effective treatments, new therapeutic approaches for malignant gliomas are needed.

A role for immunotherapy has been explored in treating brain tumors. Localized activation of immune modulators is particularly compelling since the specificity and efficiency of the immune system against tumors may spare normal cells and minimize toxicity from systemic agents. Early clinical trials for multiple vaccine platforms have demonstrated feasibility and safety, and suggested that future studies should be explored in the setting of minimal disease, combining immunotherapeutic approaches with surgery, radiation and chemotherapy (Mitchell et al. 2008).

1.2 Interleukin-12 and Cancer Immunotherapy

Interleukin-12 (IL-12) is a pro-inflammatory cytokine and has been recognized as a master regulator of cell mediated immunity in response to intracellular pathogens and neoplastic transformation. Structurally, IL-12 is a heterodimeric protein composed of p35 and p40 subunits covalently linked to form the biologically active IL-12p70 molecule (Carra et al. 2000). The expression of the p40 subunit is tightly regulated and requires specific priming and amplification signals through complex combinatorial matching of Toll receptor agonists and specific cytokines, thus limiting the cell types that can produce native biologically active IL-12 to activated antigen-presenting cells, neutrophils, and

macrophages (Trinchieri 2003). On a secondary level, IL-12 production can also be negatively regulated through various mechanisms including production of IL-10 and transforming growth factor β (TGFβ).

Initial studies identified that IL-12 was produced by innate immune cells in response to pathogens and that it led to the production of interferon gamma (INF-γ) and tumor necrosis alpha (TNFα) by T and natural killer (NK) cells (Micallef et al.1996, Trinchieri 2003). When it was discovered that IL-12 could drive naïve T-helper cell differentiation to the inflammatory T-helper cell type 1 (Th1) phenotype (Hsieh et al. 1993), a role for IL-12 was established as a bridge between innate immune cells and the adaptive immune response through polarization of naïve CD4+ cells. More recent data demonstrate additional functional roles of IL-12 directly influencing CD8+ T-cell differentiation (Curtsinger et al. 2003, Kalinski et al. 1999) and the reactivation and survival of memory CD4+ T-cells (Yoo et al. 2002). This is particularly important in the context of the tumor microenvironment where high levels of IL-12 have been shown to repolarize antigen-experienced CD4+ T-cells back to the functional antitumor Th1 phenotype (Wesa et al. 2007).

Evidence that IL-12 is able to trigger innate and adaptive immunity and modulate the tumor microenvironment supports the relevance of IL-12 as an important immunotherapeutic agent. Its ability to activate and recruit dendritic cells that facilitate the cross-priming of tumor antigen-specific T-cells, along with its influence on NK and $CD8^+$ T-cell cytotoxic activities and antigen-specific antitumor responses (Mosmann et al. 1989, Trinchieri 1995, Tsung et al. 1997, Mailliard et al. 2002) warrant further study in cancer therapy. Additionally, IL-12 has also been shown to stimulate the production of superoxides and nitric oxide and possess potent antiangiogenic activity through INF-γ (Voest et al. 1995, Wigginton et al. 1996, Coughlin et al. 1998). The potent antitumor activity of IL-12 has been well documented in various cancer mouse models including melanoma, mammary carcinoma, sarcoma, and colon and renal carcinoma (Colombo et al 2002). The potent nature of its biological activity and signaling complexity has also prompted the study of different delivery mechanism with a focus on intratumoral delivery and tumor microenvironment modulation.

Based on such data, human studies of IL-12 as an anticancer agent were initiated. The first of these studies was a Phase I dose escalation of intravenous (IV) administered recombinant human IL-12 in subjects with either melanoma or renal cell carcinoma. The study reported a transient complete response in melanoma and a partial response in renal cell carcinoma with significant toxicities. The Phase II trial observed similar toxicities, and two IL-12 related deaths prompted the Food and Drug Administration (FDA) to suspend the trial (Atkins et al 1997, Leonard et al 1997). Additional studies confirmed that systemic administration of recombinant IL-12 resulted in significant toxicity, limiting its potential for clinical development (Salem et al. 2006). These results prompted the investigation of alternative delivery routes focusing on locoregional administration either by subcutaneous injection or intratumoral delivery implementing IL-12 as a direct anticancer therapeutic or as an adjuvant to vaccination.

1.3 Local IL-12 Delivery and Development of an Inducible IL-12 Immunotherapy

The potent effects of cytokines, particularly IL-12, as mediators of an anticancer immune response remain compelling. This is especially true since the advent of immunotherapies such as anti-CTLA-4 and anti-PD1 antibodies provide proof of concept that inhibiting immune checkpoints translates into clinical benefit. IL-12 biology including the level of activation, location of initial expression, immune

effector function, and biologically active combination with other cytokines remain incompletely understood.

Several human studies that have implemented the local delivery of cytokines or chemotherapeutic agents have already shown that such an approach reduces systemic toxicity and produces signals of clinical benefit. One particularly relevant trial implemented a gene transfer strategy to express interferon beta (INF-β) locally in glioma tumors, and thus achieved high intratumoral INF-β expression without systemic toxicity. The INF-β was constitutively expressed through a replication-defective serotype 5 adenoviral vector under the control of a cytomegalovirus promoter achieving transduction of both dividing and non-dividing cells. The investigators reported the approach proved feasible and well tolerated; however, although the INF-β transduction was variable among subjects, it was associated with apoptosis (Chiocca et al. 2008).

In the current Phase I study, we are exploring a local treatment strategy for high-grade gliomas with the goal of extending the IL-12 therapeutic window and reducing its systemic toxicity. This strategy employs an inducible serotype 5 adenoviral vector (Ad-RTS-hIL-12) expressing the human interleukin 12 (hIL-12) gene under an inducible promoter named [redacted] (RTS®). The replication-incompetent Ad-RTS-hIL-12 vector is injected locally in the tumor lesion and initiates hIL-12 transcription only in the presence of the promoter specific oral activator ligand, veledimex. With this system, the IL-12 expression level can be modulated by the dose and frequency of veledimex administration, making it feasible to lower or terminate IL-12 expression in the event of severe or unexpected toxicities.

1.4 Adenoviral Vectors for Gene Therapy

1.4.1 Adenoviral Safety

Adenoviral vectors have been used extensively to deliver a variety of gene products to human subjects, including cancer subjects. Although adenoviral vectors are immunogenic, virtually all recipients have pre-existing humoral immunity to adenoviruses and they are generally considered a safe and well tolerated vehicle for gene delivery. To date, numerous studies utilized adenoviral vectors to achieve intratumoral expression of a variety of genes. In a Phase I/II clinical trial of subjects with prostate cancer, direct intraprostatic injection of a replication-defective adenoviral vector encoding bacterial nitroreductase (dose levels 5×10^{10}-1×10^{12} viral particles [vp]) was well tolerated, with minimal adverse events (AEs) (Patel et al. 2009). A Phase I study of subjects with oral leukoplakia implemented multiple intraepithelial injections of recombinant adenovirus (rAd)-p53 (1×10^{8} vp/cm^2) and demonstrated good tolerance of the vector, with no evidence of dose-limiting toxicity (DLTs) (Zhang et al. 2009). Another Phase I/II study of subjects with chemoradiation-resistant advanced esophageal carcinoma, intratumoral injections of adenovirus vector containing p53 (Ad.5CMV-p53) were well tolerated at doses ranging from 10×10^{11} to 25×10^{11} vp, with no DLTs, and generally mild to moderate adverse events (AEs) (Shimada et al. 2006). The most common AEs were fever (all 10 subjects), pain (30% of subjects), and hyperglycemia, which was attributed to the use of total parental nutrition (30% of subjects). Hypocalcemia was reported in two subjects (20%) and one subject each (10%) experienced activated partial thromboplastin time (aPTT) elongation, abnormally high serum amylase, and abnormally high serum creatinine.

In a Phase I study of subjects with advanced pancreatic, colorectal, or primary liver tumors, intratumoral injection of an adenoviral vector encoding hIL-12 (Ad.IL-12) was well tolerated at doses of up to 3×10^{12} vp. Common AEs were similar to symptoms observed with gene delivery by other

adenoviral vectors, including transient, mild to moderate fever, malaise, sweating, and lymphopenia (Sangro et al. 2004).

A recent randomized, open-label, Phase III study compared a regimen of surgical resection, adenovirus-mediated gene therapy (intraoperative perilesional sitimagene ceradenovec), intravenous ganciclovir, and standard of care interventions versus surgical resection plus standard of care interventions in 250 subjects with newly diagnosed high-grade glioma amenable to resection. Results showed that although the time to death or re-intervention was prolonged, OS was not improved for the investigational regimen relative to the standard of care regimen (Westphal et al. 2013). However, the authors concluded that the treatment had a positive risk benefit ratio, with similar AEs (hemiparesis [often transient], hyponatremia, and seizures) compared with the standard of care regimen. Neither cerebral hemorrhages nor hematomas were observed.

1.4.2 Safety of Intratumoral Injection of IL-12 Gene Vectors

In contrast with the systemic toxicity resulting from administration of recombinant IL-12 protein, local administration of IL-12 via injection of plasmid or adenoviral vectors containing the hIL-12 gene has proven to be well tolerated in subjects with various cancers, and therefore appears to provide an effective delivery method for this potent immunomodulatory cytokine. Several studies have investigated the safety of intratumoral expression of IL-12 in subjects with metastatic melanoma. A Phase I study investigated intratumoral expression of IL-12 together with the co-stimulatory molecule B7.1 via two separate canarypox virus viral vectors (ALVAC) in subjects with metastatic melanoma and reported mild to moderate injection site reactions, fever, chills, myalgia, and fatigue as AEs (Triozzi et al. 2005). However, all subjects also developed antibodies to ALVAC. Notably, serum IL-12 and INF-γ levels were not increased after treatment. Another Phase I trial showed that delivery by electroporation of a plasmid containing IL-12 to tumors in subjects with metastatic melanoma resulted in minimal systemic toxicity, with transient pain after electroporation being the most common AE (Daud et al. 2008). Results from another Phase I study showed that intratumoral injection of DNA encoding hIL-12 in subjects with metastatic melanoma was well tolerated overall (Heinzerling et al. 2005). Eight of nine subjects experienced a transient response at the intratumoral injection site, and some subjects who had tumor responses also showed some increases in systemic in IL-12, interferon gamma-induced protein 10 (IP-10), and IFN-γ.

Localized production of IL-12 also has been reported as well tolerated in subjects with other malignancies. For example, a Phase I study in 17 subjects with metastatic pancreatic, colorectal, or primary liver cancer examined intratumoral injection of dendritic cells engineered to secrete IL-12 via a rAD vector (Mazzolini et al. 2005). In that study, the most common AEs were lymphopenia, fever, and malaise. Subjects also developed antibodies to the adenoviral capsid proteins. Intraperitoneal injection of a plasmid containing the hIL-12 gene in women with chemotherapy-resistant, recurrent, ovarian cancer also was found to be generally safe and well tolerated (Anwer et al. 2010). Low-grade fever and abdominal pain were the most common AEs. Plasmid DNA was not detected in the subjects' serum samples, and treatment-related increases in IFN-γ levels were observed in pleural fluid, but not in serum. Similar data were reported in a study of subjects with advanced pancreatic, colorectal, or primary liver malignancies who received intratumoral injections of adenoviral vectors encoding hIL-12 at doses ranging from 2.5×10^{10} to 3×10^{12} vp (Sangro et al. 2004). In that study, treatment was well tolerated and a maximum tolerated dose (MTD) was not reached. Transient lymphopenia was observed in 86% of subjects, and the severity was increased at higher vector doses. Transient, mild to moderate fever, sometimes accompanied by malaise and sweating, was observed in ~ 60% of subjects during the first 2 days after the injection. Five of the 21 subjects (24%) experienced nausea and/or

vomiting on the day of the injection. No cumulative toxicity was observed. These events were deemed related to injection of the virus and not to transgene expression.

[redacted]

[redacted]

[redacted]

1.5.3 Veledimex

Veledimex is a diacylhydrazine that is fully active at the RTS receptor. Drug product is formulated as a semi-solid containing veledimex as a dry powder and excipients. This formulation has been encapsulated in gelatin capsules for oral administration in clinical trials.

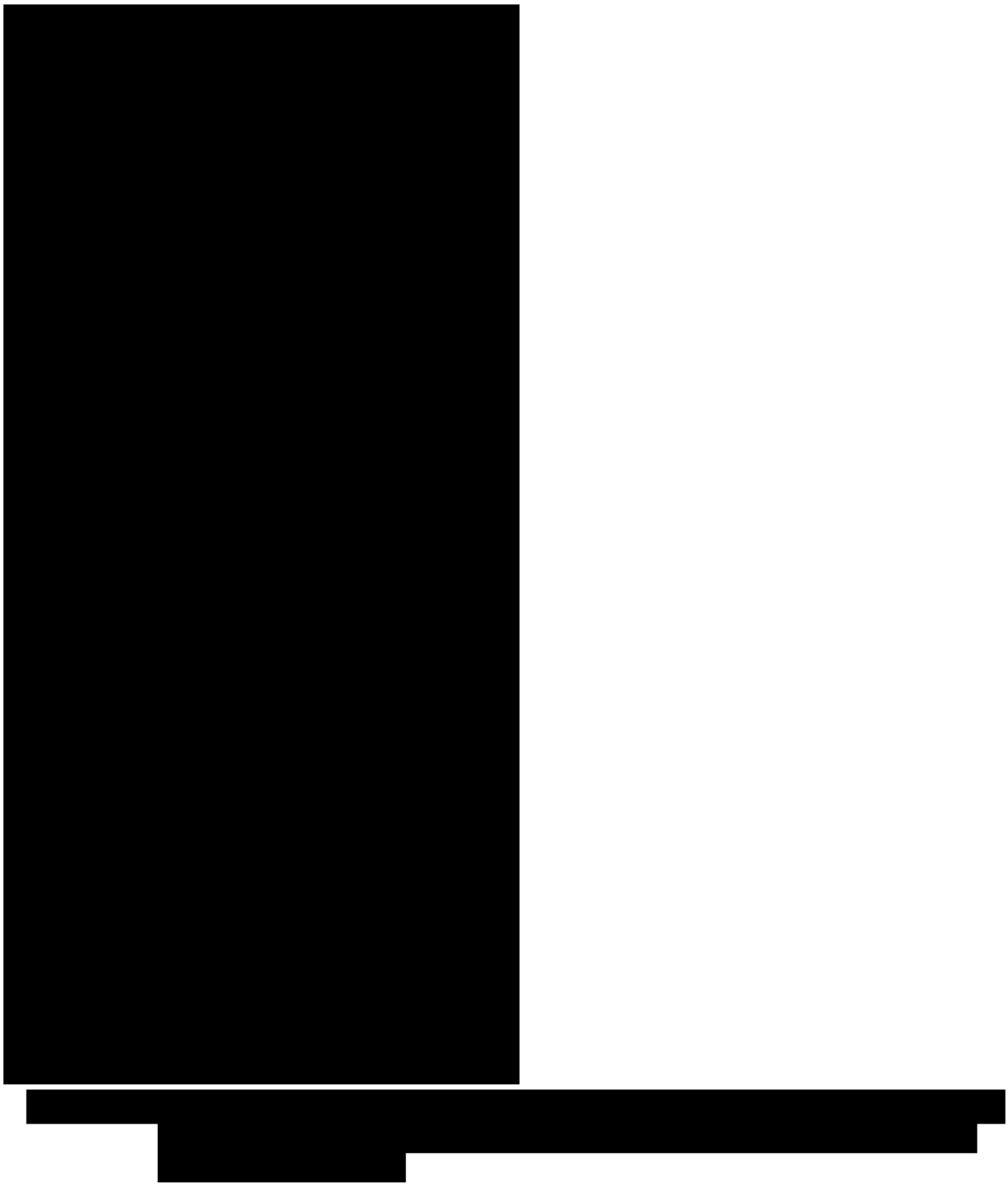
Nonclinical studies *in vitro* and *in vivo* demonstrate that veledimex interacts with the receptor component EcR of RTS to induce the activation of therapeutic gene transcription, leading to the production of transgene messenger RNA and, ultimately, protein (Anderson et al. 2000, Palli et al. 2003, Karzenowski et al. 2005).

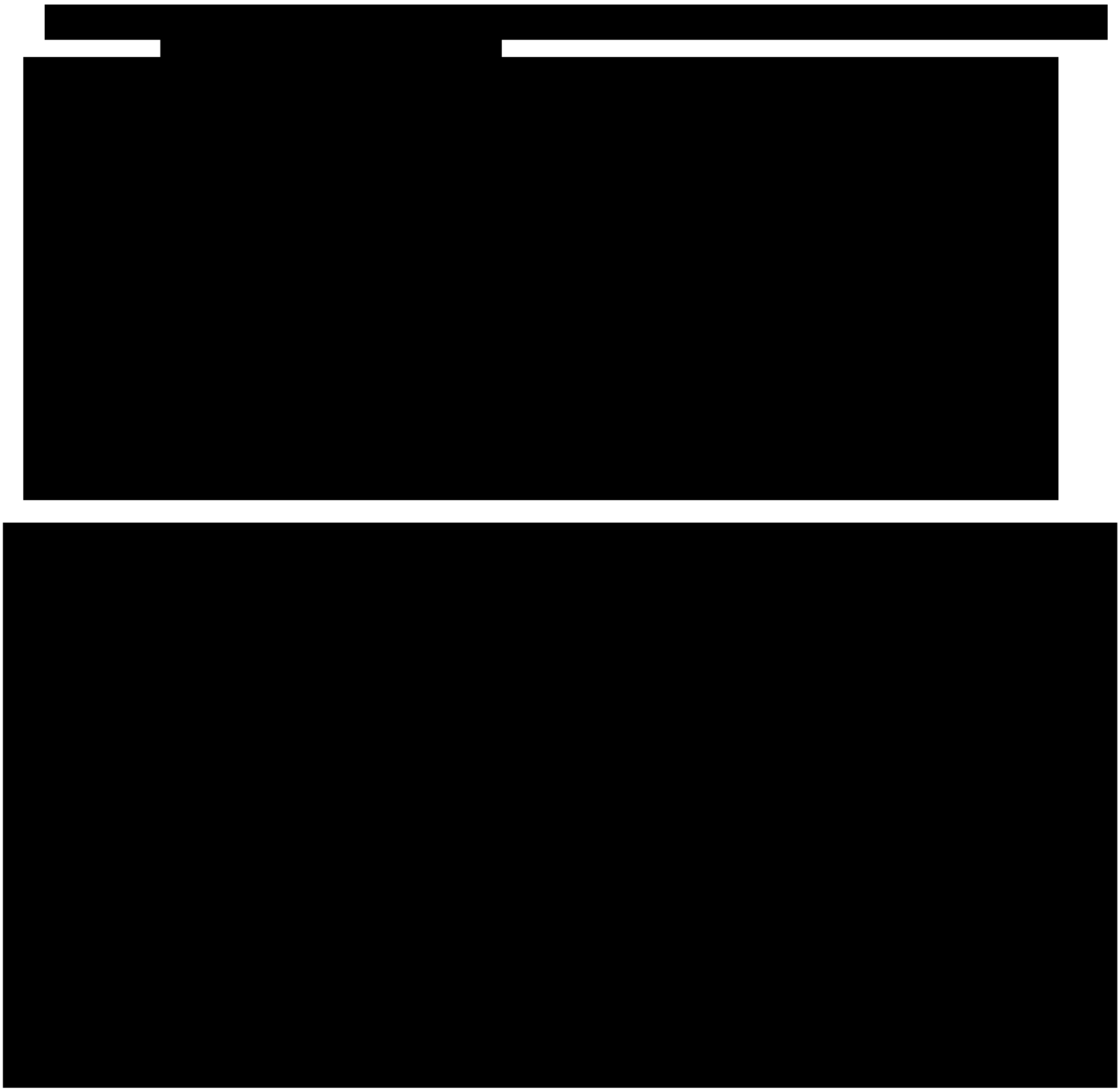
[redacted]

[redacted]

[redacted]

[redacted]





[redacted]

1.7 Rationale for Starting Dose and Dose Escalation Schedule-Glioblastoma Patient Starting Dose Rationale

[redacted]

[redacted]

Based on our animal modeling and our clinical experience with Ad-RTS-hIL-12 and veledimex, we expect a significant safety margin and tolerability profile. However, we will also implement rigorous safety monitoring and will review incoming data on an ongoing basis.

In summary, these results support the administration of Ad-RTS-hIL-12 at 2 x 10^{11} vp intracranial intratumorally with a starting dose of veledimex administered PO at 20 mg QD x 14 days.

[redacted]

[redacted]

[redacted]

[redacted]

Summary of Safety

Please see the Investigator's Brochure for the most current safety information.

[redacted]

[redacted]

2. STUDY OBJECTIVES

2.1 Primary Objective

- To determine the safety and tolerability of varying doses of antitumoral Ad-RTS-hIL-12 plus oral veledimex doses in subjects with recurrent or progressive glioblastoma or Grade III malignant glioma.

2.2 Secondary Objectives

- To determine the MTD of veledimex when administered with varying doses of intratumoral Ad-RTS-hIL-12 dose
- To determine the veledimex PK profile
- To determine the veledimex concentration ratio between brain tumor and blood
- To evaluate cellular [redacted] immune responses elicited by Ad-RTS-hIL-12 and veledimex
- To determine investigator assessment of response including tumor Objective Response Rate (ORR) and PFS
- To determine OS

3. STUDY DESIGN

3.1 Overall Study Design

This is a Phase I study of varying doses of Ad-RTS-hIL-12 administered by intratumoral injection and varying veledimex doses administered orally in subjects with recurrent or progressive glioblastoma or Grade III malignant glioma. This study will investigate an Ad-RTS-hIL-12 dose of 2 x 10^{11} vp and 1 × 10^{12} vp and escalating veledimex doses to determine the veledimex MTD based on the observed safety profile and in the presence of variable corticosteroid exposure.

Ad-RTS-hIL-12 is a replication-incompetent adenoviral vector containing the hIL-12 gene under the control of the RTS inducible promoter turned on in the presence of the activator ligand veledimex. Veledimex is a small molecule RTS specific transcription factor that stabilizes the RTS promoter components and allows transcription of the hIL-12 target gene. Since target gene expression is dependent on the dose and frequency of veledimex administration, the expression of hIL-12 can be modulated (turned on and off) by the veledimex dose and schedule.

This study is divided into three periods: the Screening Period, the Treatment Period, the DLT evaluation period (Days 0-28), and the Follow-up Period. After the Informed Consent Form (ICF) is signed, subjects will enter the Screening Period to assess eligibility. Eligible subjects will be stratified according to clinical indication for tumor resection. Subjects will then be enrolled into one of six cohorts within each group. The start of study procedures will occur when the first subject is consented into the study and the study will stop as of the database lock date.

Subjects who are scheduled for a craniotomy for tumor resection (Group 1) will receive one veledimex dose before the resection procedure. This initial dose will be representative of the respective cohort dose level. Samples (tumor, CSF if available, and blood) will be collected during the resection procedure to determine the veledimex PK just prior to Ad-RTS-hIL-12 administration. After Ad-RTS-hIL-12 injection, subjects will continue on oral veledimex for 14 days. Subjects in Group 1 will receive up to a total of 15 veledimex doses: one veledimex dose on Day 0 3 (± 2) hours before the craniotomy procedure (and tumor resection) and up to 14 veledimex doses after Ad-RTS-hIL-12 administration.

Group 2 will include subjects who will NOT undergo tumor resection. Subject enrollment into Group 2 will start no earlier than after two subjects have completed 28 days in Group 1, Cohort 1. Enrollment into Group 2 cohorts is otherwise independent of enrollment into Group 1 cohorts. Subjects in Group 2 will receive Ad-RTS-hIL-12 (2 × 10^{11} vp or 1 × 10^{12} vp) by stereotactic injection first, and then will start oral veledimex on Day 1 and continue for up to 14 days.

Group 1: Subjects scheduled for craniotomy and tumor resection:

Subjects with a clinical indication for tumor resection will receive veledimex dose specific to the assigned cohort 3 (± 2) hours before the craniotomy procedure, on an empty stomach (excluding other medications). At the time of tumor resection, brain tumor, CSF (if available), and blood samples will be collected to determine the veledimex concentration ratio between brain tumor, CSF, and blood.

Immediately after tumor resection, subjects in Cohorts 1 and 2 will receive Ad-RTS-hIL-12 2 x 10^{11} vp. This will be administered by [redacted] injection into approximately two sites within the residual tumor for a total volume of 0.1 mL. The total amount delivered to each site will be recorded

in the CRF. In the event that less than the planned total injected volume is administered, the reason will be provided. The day of Ad-RTS-hIL-12 administration is designated as Day 0.

For Cohorts 3, 4, 5 and 6, immediately after tumor resection, Ad-RTS-hIL-12 1 x 10^{12} vp will be administered by [redacted] injection into multiple sites in a divided dose within the residual tumor (day of Ad-RTS-hIL-12 administration is designated as Day 0). The planned total injected volume for these cohorts is 0.5 mL given in approximate aliquots of 0.1 mL at each injection site. The total amount delivered to each site will be recorded in the CRF. In the event that less than the planned total injected volume is administered, the reason will be provided. When available, an intra-operative magnetic resonance imaging (MRI) can be performed to guide the Ad-RTS-hIL-12 injection to areas of contrast enhancing tumor tissue.

After the Ad-RTS-hIL-12 injection, veledimex will be administered orally for 14 days. The first postresection veledimex dose is to be given on Day 1, preferably with food. Subsequent veledimex doses are to be taken once daily, in the morning and within approximately 30 minutes of a regular meal.

Group 2: Subjects who will NOT undergo tumor resection:

Subject enrollment into Group 2 will start no earlier than after two subjects have completed 28 days in Group 1, Cohort 1. Enrollment into Group 2 cohorts is otherwise independent of enrollment into Group 1 cohorts.

Subjects who will not undergo tumor resection will receive Ad-RTS-hIL-12 by standard stereotactic surgery on Day 0. Tumor biopsy is recommended to reconfirm malignant glioma. Ad-RTS-hIL-12 (2×10^{11} vp or 1×10^{12} vp) will be administered by stereotactic injection. The day of Ad-RTS-hIL-12 administration is designated as Day 0. In Cohorts 1 and 2, it will be delivered into approximately two intratumoral sites for a total volume of 0.1 mL. In Cohorts 3, 4 and 5 it will be delivered into multiple sites in a divided dose to deliver up to 0.5 mL volume in approximate aliquots of 0.1 mL at each site. Care should be taken to avoid intraventricular or basal cisternal injection or other critical locations.

After the Ad-RTS-hIL-12 injection, veledimex will be administered orally for 14 days. The first veledimex dose is to be given on Day 1, preferably with food. Subsequent veledimex doses are to be taken once daily, in the morning and within approximately 30 minutes of a regular meal.

This study will explore four veledimex dose cohorts of 20 mg, 40 mg, 80 mg and 120 mg once daily (Table 5).

3.2 Dose Escalation Cohorts

This study will implement a standard 3 + 3 design modified to independently evaluate two stratified subject groups that may exhibit different safety and tolerability profiles. The dose escalation rules apply to subjects who have received Ad-RTS-hIL-12 and at least one veledimex dose post Ad-RTS-hIL-12 administration. In each cohort, the first subject will be monitored for the 14 days of treatment and will be observed an additional 7 days post last veledimex dose before the second and third subjects are enrolled in that same cohort. The DLT evaluation period is defined as 28 days post Ad-RTS-hIL-12 injection (Day 0 – Day 28). Determination of safety and recommendation to dose escalate will occur after all dosed subjects in a cohort have been evaluated for at least 28 days post

Ad-RTS-hIL-12 injection. The details on the dose escalation schedule are provided in Section 6.2 and the dose escalation decision rules provided in Section 6.3.

3.3 Study Oversight for Safety Evaluation

This study will implement an independent DSMB to provide additional unbiased and objective input on the safety evaluation of study population. In the context of an open label study, the responsibility of the DSMB is to provide an objective determination of safety at the end of each dose cohort and make a recommendation to proceed to the next higher veledimex dose level, amend the dose escalation schedule or discontinue the study. A separate DSMB charter will describe the activities of this committee.

The first level of safety oversight will occur through the site investigator and medical monitor. A formal SRC, guided by the SRC charter, will include the study investigators, the medical monitor and other appropriate sponsor representatives and will provide overall safety oversight. Additional external medical and scientific experts may also be invited to participate in the reviews as needed and appropriate and as decided by the SRC. The SRC will evaluate patient safety within each cohort, and within each stratification group of the same cohort. If no significant safety events occur with the first patient of each cohort, the second and third subjects will be enrolled and treated. If a significant safety event occurs with the first patient in each cohort, the SRC will convene to evaluate the safety event(s) and make a recommendation and decision on the enrollment of the second and third subjects in that same cohort

At the end of each cohort (Group 1 and Group 2 separately), the SRC will convene to review all the safety data from each patient treated in that cohort, separately for each group. At the end of each cohort, the SRC will provide the safety summary and a recommendation to the DSMB. The DSMB, within 3 days of receipt, will evaluate the safety data and make a recommendation to the SRC regarding continuation to the next dose cohort, amendment of the dose escalation schedule, or discontinuation of the study.

The DSMB recommendation will be communicated to the SRC and appropriate action will be taken. A written record of the DSMB recommendation will be provided to the SRC and maintained on file with the sponsor.

4 SUBJECT SELECTION

The eligible study population includes adult subjects with recurrent or progressive glioblastoma or Grade III malignant glioma (anaplastic astrocytoma, anaplastic oligodendroglioma, and anaplastic oligoastrocytoma) for which there is no alternative curative therapy.

4.1 Inclusion Criteria

1. Male or female subject ≥ 18 and ≤ 75 years of age
2. Provision of written informed consent for tumor resection, stereotactic surgery, tumor biopsy, samples collection, and treatment with investigational products prior to undergoing any study-specific procedures
3. Histologically confirmed supratentorial glioblastoma or other WHO Grade III or IV malignant glioma from archival tissue
4. Evidence of tumor recurrence/progression by MRI according to response assessment in neuro-oncology (RANO) criteria after standard initial therapy
5. Previous standard of care antitumor treatment including surgery and/or biopsy and chemoradiation. At the time of registration, subjects must have recovered from the toxic effects of previous treatments as determined by the treating physician. The washout periods from prior therapies are intended as follows: (windows other than what is listed below should be allowed only after consultation with the Medical Monitor)
    a. Nitrosoureas: 6 weeks
    b. Other cytotoxic agents: 4 weeks
    c. Antiangiogenic agents including bevacizumab: 4 weeks
    d. Targeted agents including small-molecule tyrosine kinase inhibitors: 2 weeks
    e. Experimental immunotherapies (e.g., PD-1, CTLA-4): 3 months
    f. Vaccine-based therapy: 3 months
6. Able to undergo standard MRI scans with contrast agent before enrollment and after treatment
7. Karnofsky performance status ≥ 70
8. Adequate bone marrow, liver, and kidney function, as assessed by the following laboratory requirements:
    a. Hemoglobin ≥ 9 g/L
    b. Lymphocytes > 500/mm^3
    c. Absolute Neutrophil Count ≥ 1,500/mm^3
    d. Platelets ≥ 100,000/mm^3
    e. Serum creatinine ≤ 1.5 x upper limit of normal (ULN)
    f. Aspartate transaminase (AST) and alanine transaminase (ALT) ≤ 2.5 x ULN. For subjects with documented liver metastases, ALT and AST ≤ 5 × ULN
    g. Total bilirubin < 1.5 x ULN

h. International normalized ratio (INR) and aPTT normal institutional limits

9. Male and female subjects must agree to use a highly reliable method of birth control (expected failure rate less than 5% per year) from the Screening Visit through 28 days after the last dose of study drug. Women of childbearing potential (perimenopausal women must be amenorrheic for at least 12 months to be considered of non-childbearing potential) must have a negative pregnancy test at screening.

4.2 Exclusion Criteria

1. Radiotherapy treatment within 4 weeks or less prior to veledimex dosing
2. Subjects with clinically significant increased intracranial pressure (e.g., impending herniation or requirement for immediate palliative treatment) or uncontrolled seizures
3. Known immunosuppressive disease, autoimmune conditions, and/or chronic viral infections [e.g., human immunodeficiency virus (HIV), hepatitis]
4. Use of systemic antibacterial, antifungal, or antiviral medication for the treatment of acute clinically significant infection within 2 weeks of first veledimex dose. Concomitant therapy for chronic infections is not allowed. Subject must be afebrile prior to Ad-RTS-hIL-12 injection; only prophylactic antibiotic use is allowed perioperatively.
5. Use of enzyme-inducing antiepileptic drugs (EIAED) within 7 days prior to the first dose of study drug. Note: Levetiracetam (Keppra®) is not an EIAED and is allowed
6. Other concurrent clinically active malignant disease, requiring treatment, with the exception of non-melanoma cancers of the skin or carcinoma *in situ* of the cervix or nonmetastatic prostate cancer
7. Nursing or pregnant females
8. Prior exposure to veledimex
9. Use of medications that induce, inhibit, or are substrates of cytochrome p450 (CYP450) 3A4 within 7 days prior to veledimex dosing without consultation with the Medical Monitor
10. Presence of any contraindication for a neurosurgical procedure
11. Unstable or clinically significant concurrent medical condition that would, in the opinion of the investigator or medical monitor, jeopardize the safety of a subject and/or their compliance with the protocol. Examples include, but are not limited to: unstable angina, congestive heart failure, myocardial infarction within 2 months of screening, ongoing maintenance therapy for life-threatening ventricular arrhythmia, or uncontrolled asthma.

4.3 Subject Enrollment

Up to 72 subjects may be enrolled.

4.4 Withdrawal of Subjects from Study Treatment and/or Study

The sponsor may terminate this study at any time. The investigator and/or the subject have the right to terminate the subject's participation in the study at any time. Efforts should be made to ask subjects who discontinue study drug to be available to complete the Follow-up assessments.

A subject may withdraw or be withdrawn from the study treatment prematurely for any of the following reasons:

- Principal Investigator (PI) determines further participation is not in subject's best interest (e.g., subject experiences rapid clinical deterioration in the absence of confirmed disease progression)
- Subject has confirmed disease progression

A subject must be withdrawn in the event of any of the following:

- Subject withdraws informed consent.
- Any treatment-related AEs that meet withdrawal criteria
- Substantial noncompliance with study requirements
- Subjects with a confirmed positive pregnancy test
- Any intercurrent illness that would, in the judgement of the investigator or sponsor's medical monitor, affect assessments of clinical status to a significant degree and require discontinuation of protocol therapy
- Subjects who did not receive the Ad-RTS-hIL-12 injection

- *Note: Any subject who wishes to withdraw from the study treatment may do so at any time, but will be asked to be available for the safety, tumor response, and survival Follow-up assessments.*

Every effort should be made to follow subjects who withdraw from study treatment for ongoing treatment-related AEs. Subjects who withdraw during the treatment period should continue to have study assessments as clinically indicated.

4.5 Replacement of Subjects

Subjects who withdraw from the study during the DLT evaluation period (Days 0 to 28) for reasons other than toxicity or disease progression may be replaced. All dosed subjects will be included in the overall safety assessment.

4.6 Premature Termination of Study or Study Site

The sponsor has the right to close the study at any time, although this should occur only after mutual consultation between the sponsor and the investigators. The Institutional Review Board (IRB) or Independent Ethics Committee (IEC) must be informed of such action. Should the study or center be closed prematurely, all study materials (completed, partially completed, and blank case report forms (CRF), study medication, etc.) must be stored or disposed of according to the sponsor's instructions. Events that may trigger premature termination of the study or closure of a center include, but are not limited to the following: new toxicity findings; decision to re-challenge patient who has experienced a Grade 4 event; interim analysis results; noncompliance with the protocol; changes in the development plans for the study drug; slow recruitment; and poor-quality data.

5 INVESTIGATIONAL PRODUCT

The investigational product has two components: the Adenovirus-[redacted] human interleukin-12 (Ad-RTS-hIL-12) and veledimex (RTS activator ligand). Ad-RTS-hIL-12 is a replication-incompetent adenoviral vector containing the hIL-12 gene under the control of the RTS inducible promoter activated in the presence of the activator ligand, veledimex. Veledimex is a small molecule RTS specific transcription factor that stabilizes the RTS promoter components and allows transcription of the hIL-12 target gene. Since target gene expression is dependent on the dose and frequency of veledimex administration, the expression of hIL-12 can be modulated (turned on and off) by the optimal veledimex dose and schedule.

Please refer to the Pharmacy Manual for additional information.

5.1 Preparation of Ad-RTS-hIL-12

Ad-RTS-hIL-12 will be supplied in single-dose vials. Information regarding the preparation of the Ad-RTS-hIL-12 dose is provided in the Pharmacy Manual.

5.2 Preparation of Veledimex

Sponsor will provide veledimex capsules to be dispensed by the study site pharmacy to subjects for oral administration.

5.3 Handling and Storage

Study drugs must be stored in a restricted access area under the storage conditions indicated in the Investigator's Brochure or Pharmacy Manual. All necessary precautions while handling potentially toxic compounds must be strictly followed.

5.4 Monitoring of Subject Adherence and Managing Missed Veledimex Doses

The first veledimex dose following Ad-RTS-hIL-12 injection is expected to be administered when the subject is at the clinical site, under careful medical supervision by the clinic staff to ensure that the subject does not have difficulty swallowing the capsules. Thereafter, subjects may be allowed to self-administer the remaining once daily doses as described. Subjects are to be instructed to take the appropriate number of capsules in the same way for each of the remaining treatment period days, and may be reminded to do so by phone on non-visit days.

Subjects should NOT make up any missed doses.

Subjects should be instructed to document veledimex dosing compliance in a subject diary, including the time subject took the once daily dose, the time subject ate breakfast, the number of capsules taken, whether subject missed any veledimex doses, and the study day and reason for any missed doses. Study drug container(s) with any remaining capsules should be returned to the study staff on Day 15, so that staff can properly assess dose compliance.

5.5 Disposition of Unused Drug

All unused study drug should be destroyed at the study site in accordance with standard institutional practice and in accordance with United States Occupational Safety and Health Administration

procedures, after full accountability has been documented. Any study drug destruction at study site must be documented and the records maintained in the investigator's study file.

5.6 Accountability and Dispensation

The investigator must maintain accurate records accounting for the receipt and dispensation of study drugs. The investigational materials are to be prescribed only by the investigator or the sub-investigators named on FDA Form 1572, and may only be dispensed by authorized personnel at the institution(s) listed therein. Under no circumstances will the PI allow the investigational drug(s) to be used for purposes or in subjects other than as directed by the protocol.

6 TREATMENT PLAN

6.1 Ad-RTS-hIL-12 Dosage and Administration Procedures

Subjects will be stratified according to clinical indication for standard of care tumor resection. Subjects who are scheduled for standard of care craniotomy and tumor resection (Group 1) will receive one veledimex dose before the resection procedure. Samples [tumor, CSF (if available), and blood] will be collected during the resection procedure just prior to Ad-RTS-hIL-12 injection to determine the veledimex concentration ratio between brain tumor, CSF, and blood. After Ad-RTS-hIL-12 injection, subjects will start oral veledimex for 14 days. Subjects not scheduled for tumor resection (Group 2) will receive Ad-RTS-hIL-12 by stereotactic injection first, and then will receive oral veledimex for 14 days.

In the event that Ad-RTS-hIL-12 injection is not performed, subject will not continue with postresection veledimex dosing.

Subjects Undergoing Tumor Resection (Group 1):

- Subjects will be given a cohort-specific dose of veledimex by mouth, on an empty stomach (excluding other medications) 3 (± 2) hours before craniotomy. The actual time of veledimex administration should be noted and recorded.
- Surgical planning will be performed on a diagnostic MRI acquired prior to the surgery as per standard of care.
- At the time of tumor resection, tumor, CSF (if available), and blood samples will be collected.
- Immediately after tumor resection, when available, an intraoperative MRI can be performed to identify contrast enhancing or T2/FLAIR hyper intense residual tumor. If intraoperative MRI is not available, the neurosurgeon will select sites for injection.
- Subjects in Cohorts 1 and 2 will receive Ad-RTS-hIL-12 2 x 10^{11} vp. This will be administered by [redacted] injection into approximately two sites within the residual tumor for a total volume of 0.1 mL selected by the neurosurgeon. When available an intra-operative MRI can be performed to guide the Ad-RTS-hIL-12 injection to areas of contrast-enhancing tumor tissue.
- For Cohorts 3, 4, 5 and 6, immediately after tumor resection, Ad-RTS-hIL-12 1 x 10^{12} vp will be administered by [redacted] injection into multiple sites in a divided dose within the residual tumor at sites selected by the neurosurgeon. The planned total injected volume for these cohorts is 0.5 mL given in approximate aliquots of 0.1 mL at each injection site. When available, an intra-operative MRI can be performed to guide the Ad-RTS-hIL-12 injection to areas of contrast-enhancing tumor tissue.
- The day of Ad-RTS-hIL-12 administration is designated as Day 0.
- After tumor resection and Ad-RTS-hIL-12 injection, veledimex will be administered orally for 14 days. The first postresection veledimex dose is to be given on Day 1, preferably with food. Subsequent veledimex doses are to be taken once daily, in the morning and within approximately 30 minutes of a regular meal.

Subjects NOT Undergoing Tumor Resection (Group 2):

- Surgical planning will be performed on a diagnostic MRI acquired prior to the stereotactic procedure.
- On the day of Ad-RTS-hIL-12 administration (Day 0), subjects will be anesthetized and prepared for standard stereotactic surgery.
- Subjects in Cohorts 1 and 2 will receive Ad-RTS-hIL-12 2 x 10^{11} vp. It will be administered by stereotactic injection into approximately two intratumoral sites to deliver up to 0.1 mL volume. In Cohorts 3,4,5 and 6, Ad-RTS-hIL-12 1×10^{12} vp will be administered by stereotactic injection into multiple sites in a divided dose to deliver up to 0.5 mL volume in approximate aliquots of 0.1 mL at each site. Care should be taken to avoid intraventricular or basal cisternal injection or other critical location. In case of multifocal tumors, one leading lesion should be selected for injection.
- After the Ad-RTS-hIL-12 injection, veledimex will be administered orally for 14 days. The first veledimex dose is to be given on Day 1, preferably with food. Subsequent veledimex doses are to be taken once daily, in the morning and within approximately 30 minutes of a regular meal.

Subjects should be carefully monitored for possible local reactions and/or hypersensitivity reactions, according to standard practice. Intracranial bleeding or other procedure-related events should be evaluated before the first veledimex dose is given post Ad-RTS-hIL-12 administration. Any changes in neurological status should be reported to the investigator immediately, either during hospitalization or once subject is discharged. Subjects should be instructed to call the study physician or study nurse if they develop any symptoms after they are released from the hospital.

Proper hydration is critical while subjects are on veledimex. It is important that subjects are instructed repeatedly to maintain adequate oral hydration on and between veledimex doses. Study sites must closely monitor subjects for proper hydration. Blood pressure should be monitored regularly.

Administration of prophylactic antipyretics is strongly recommended during the first week after Ad-RTS-hIL-12 injection.

6.2 Veledimex Dosage and Administration

Subjects Undergoing Tumor Resection (Group 1):

- Before Ad-RTS-hIL-12 administration: a cohort-specific oral dose of veledimex 3 (± 2) hours, on an empty stomach (excluding other medications), before craniotomy and tumor resection.
- After Ad-RTS-hIL-12 administration: Dose veledimex as assigned by dose cohort. The first postresection veledimex dose is to be given on Day 1, preferably with food. Subsequent veledimex doses are to be taken once daily, in the morning and within approximately 30 minutes of a regular meal.

Subjects NOT Undergoing Tumor Resection (Group 2)

- After Ad-RTS-hIL-12 administration: Group 2 will start dosing subjects only after two subjects have completed 28 days in Group 1, Cohort 1. Veledimex dose will be administered as assigned by dose cohort. The first veledimex dose is to be given on Day 1, preferably with food. Subsequent veledimex doses are to be taken once daily, in the morning and within approximately 30 minutes of a regular meal.

Subjects in Group 1 will receive up to a total of 15 veledimex doses: The initial veledimex dose (cohort-specific) will be administered 3 (± 2) hours before the craniotomy procedure and before Ad-RTS-hIL-12 administration, and up to 14 veledimex doses after Ad-RTS-hIL-12 administration. Subjects in Group 2 will receive up to a total of 14 veledimex doses after Ad-RTS-hIL-12 administration. All veledimex doses will be recorded and safety monitored.

Dose Escalation Schedule

Four veledimex dose levels will be explored in this study. Each dose cohort is expected to enroll at least three subjects, per group, in each dose level. The prospectively defined veledimex dose levels are 20 mg, 40 mg, 80 mg, and 120 mg. The veledimex dose is to be taken by mouth once daily in the form of veledimex capsules . The dose escalation cohorts are outlined in Table 5.

TABLE 5 VELEDIMEX DOSE-ESCALATION COHORTS POST AD-RTS-HIL-12 INJECTION

Cohort	Ad-RTS-hIL-12[a] (Day 0)	Veledimex[b] Group 1 (Days 0 through 14) Group 2 (Days 1 through 14)
	Dose(vp)	Total Daily Dose (mg)
1	2×10^{11}	20
2	2×10^{11}	40
3	1×10^{12}	20
4	1×10^{12}	40
5	1×10^{12}	80
6	1×10^{12}	120

vp = viral particle
[a]: Intratumoral Injection
[b]: Dose levels may be modified based on additional clinical and nonclinical data (Section 6.4)

The DSMB will review the safety data at the end of each dose cohort and provide a recommendation before any new subjects can be enrolled in the next higher dose cohort (Sections 3.2 and 3.3).

6.3 Dose Escalation Decision Rules

This study has been designed to assess the safety and tolerability of varying doses of Ad-RTS-hIL-12 and veledimex. The dose escalation rules apply to subjects who have received Ad-RTS-hIL-12 and at least one veledimex dose post Ad-RTS-hIL-12 administration. The veledimex dose escalation

schedule will follow a 3 + 3 design modified to independently evaluate two stratified subject groups that may exhibit different safety and tolerability profiles. In each cohort, the first subject will be monitored for the 14 days of treatment and will be observed an additional 7 days post the last veledimex dose before the second and third subjects are enrolled in that same cohort. At the end of each cohort, the SRC will convene to review the safety data after the final patient in a cohort, within a group, has completed veledimex dosing and has been monitored for 28 days after the Ad-RTS-hIL-12 injection.

Each dose cohort will enroll three subjects. If no DLTs occur among the first three subjects in a cohort, within the same group, dosing may proceed to the next higher dose cohort after the SRC and DSMB have provided their recommendation to proceed. In any cohort, if one DLT occurs among the first three subjects within the same group, three additional subjects of the same group will be enrolled. If one or more of the three additional subjects experience a DLT (i.e., two DLTs in Group 1 or two DLTs in Group 2), the dose escalation in the group with the two DLTs will stop. The SRC will then convene to evaluate safety, determine if the MTD has been reached per Section 6.6, and decide whether to stop treatment or de-escalate veledimex dosing in that group. The SRC may also seek an ad hoc safety evaluation and recommendation by external experts and/or the DSMB as needed. Enrollment in the group that did not experience DLTs (zero or one DLT per corhort), will continue to a total of three or six evaluable subjects, respectively, to assess the dose escalation separately.

The MTD is defined as the dose level below the dose in which more than 33% of subjects of the same cohort and group experience DLTs, with an independent assessment of Group 1 and Group 2 subjects. If two DLTs occur in the same cohort in a group the dose escalation will stop in the group experiencing the DLTs. The MTD will then be explored in six subjects of the same group at the next lower dose level and will be declared to be the dose in which less than 33% of subjects experience DLTs. Intra-subject dose escalation is not permitted. An intermediate dose cohort may be explored during the dose escalation phase, as deemed necessary and as recommended by the SRC.

During the dose escalation phase, the DSMB will recommend proceeding with the next dose cohort, de-escalating to a lower dose or stopping the study. If an expansion cohort is implemented, the SRC may recommend a dose reduction for any individual subject who experiences a DLT. If DLTs are observed in ≥ 33% of the subjects treated in the expansion phase, the SRC and/or DSMB may recommend enrolling additional subjects at a dose below the MTD defined in the escalation phase, thereby establishing a new MTD, or may stop the study. A decision to enroll additional subjects, as part of an expansion cohort, at the determined veledimex MTD and the Ad-RTS-hIL-12 dose will be made by the SRC after the MTD has been identified for one or both groups and safety evaluated. If an expansion cohort is implemented, the veledimex dose may be delayed or reduced for individual subjects in the event of toxicity. If ≥ 33% of subjects in the expansion cohort experience DLTs (according to the definition used in the dose escalation phase), additional subjects may be enrolled at the next lower dose tested in the dose escalation phase, or at an intermediate dose, as recommended by the SRC.

TABLE 6 DOSE ESCALATION DECISION RULES

Subjects with DLTs (Any Dose Level)	Escalation Decision Rule
No subject experience a DLT	Enroll the next higher dose level cohort (after DSMB recommendation).
1 subject experiences a DLT	Enroll at least three more subjects in the same group at this dose level cohort. If zero or one of the three additional subjects experience a DLT, proceed to the next planned dose level cohort (after DSMB recommendation).
2 or more subjects experience a DLT	If two subjects experience a DLT (two DLTs in Group 1 or two DLTs in Group 2), then **STOP** dose escalation in the cohort experiencing the DLTs and do the following: • The SRC will conduct a safety review to evaluate safety and determine whether the cohort experiencing the two DLTs has met the definition of the MTD (Section 6.6) Based upon this review, the SRC will make a recommendation that the cohort experiencing the ≥ two DLTs either continue at the existing dose, at a lower dose level or other measures to be undertaken including discontinuation of treatment. After completion of a cohort, if it has been determined that the MTD has not been reached, escalation to the next cohort will proceed once recommended by the SRC and authorized by the DSMB. If it has been determined that escalation should not proceed, dose de-escalation will be undertaken. .

6.4 Modification of Dose Escalation Schedule

This trial implements a design that is aimed to identify the veledimex pharmacokinetic profile in human subjects so that a more accurate determination can be made of the veledimex distribution ratio between the tumor, CSF (if available), and blood. A single cohort-specific veledimex dose will be administered to subjects undergoing standard of care tumor resection 3 (± 2) hours prior to the craniotomy and Ad-RTS-hIL-12 injection. At the time of the tumor resection, samples of the tumor, CSF (if available), and blood will be collected to determine the level of veledimex in these compartments.

A period of at least 1 week will be required to analyze the veledimex concentration ratio in brain tumor, CSF, and blood from subjects in Group 1. Interpretable data from at least three subjects should be sufficient to provide an initial estimate of the veledimex concentration in human brain tissue and the ratio of veledimex in the tumor, CSF (if available), and blood. Based on this initial estimate and data from subsequent cohorts, dosage level may be modified after review by the SRC and DSMB.

Three interpretable and properly analyzed samples are required before the planned dose escalation schedule can be modified. An assessment and evaluation of the veledimex ratio and the safety profile will be conducted after the first three evaluable patient tumor samples are available. If there is a significant change in the veledimex ratio (greater or less than 50%), the question to modify the dose escalation schedule will be proposed to the SRC and the DSMB. The dose escalation schedule will be modified accordingly after consultation with the SRC and DSMB. If a determination can be made on three interpretable and properly analyzed samples, then the veledimex dose before the Ad-RTS-hIL-12 injection in subjects who are scheduled for standard of care resection may be discontinued.

6.5 Dose Limiting Toxicity

- Dose Limiting Toxicities are defined as events that occur during the first 28 days (Day 0 – Day 28) that meets one of the following criteria:
    - Any local reaction that requires operative intervention and felt to be attributable to Ad-RTS-hIL-12 and/or veledimex
    - Any local reaction that has life-threatening consequences requiring urgent intervention or results in death and felt to be attributable to Ad-RTS-hIL-12 and/or veledimex.
- Any Grade 3 or greater non-hematological adverse event that is at least possibly related to the study drug
- Any Grade 4 hematologic toxicity that is at least possibly related to the study drug and lasts at least 5 days
- Grade 3 or higher thrombocytopenia at least possibly related to the study drug

  Note: Diagnostic brain or tumor biopsy is not considered a DLT. Seizures, headaches, and cerebral edema are commonly observed in this patient population and will be recorded according to grade of toxicity, but will not be considered a DLT unless they are deemed as most likely to be drug related as the main contributing factor.
- Safety and tolerability will be assessed by the incidence and severity of AEs, with severity assigned according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4.03. Details provided in Section 10.
- If multiple toxicities are seen, the DLT should be defined as the most severe toxicity experienced.
- All DLTs should be reported to the sponsor within 24 hours of investigator or site awareness.

6.6 Maximum Tolerated Dose

The veledimex MTD given in combination with either Ad-RTS-hIL-12 2 x 10^{11} vp or 1 x 10^{12} vp dose will be that dose at which fewer than 33 % of subjects experience a DLT.

The MTD in this study will be determined in the context of variable corticosteroid exposure and will be reported as such. Investigational treatment of the high-grade glioma patient population requires that steroid use be allowed to manage brain tumor-related symptoms or edema, especially after tumor resection (Section 7). It is expected that the use and dose of corticosteroids will vary among trial sites and among subjects, and therefore, the extent and dose of steroids is not specified in this protocol. The treating physician will make this determination as it is safe and appropriate for the individual subject and should consider the minimum steroid dose or the lowest amount that controls the subject's symptoms.

6.7 Dose Modifications and Dose Delays

Treatment dose delays and dose reductions for individual subjects will be allowed in the event of a DLT, according to the criteria shown in Table 7.

If ≥ 33% of subjects experience DLTs in a cohort within a group, the SRC will convene to determine the course of action. The medical monitor or investigators may request a meeting of the SRC at other times, as appropriate.

TABLE 7 CRITERIA FOR DOSE DELAY AND DOSE REDUCTION

Event	Course of Action
Any local injection reaction that requires operative intervention	Hold veledimex and contact the medical monitor for instruction on resuming dosing
Any local injection reaction that has life-threatening consequences requiring urgent intervention or results in death	Hold veledimex and contact the medical monitor for instruction on resuming dosing
Any drug-related Grade 3 or higher AE[a]	Hold veledimex and contact medical monitor for instruction on resuming dosing

AE = adverse event
[a:] Excluding nausea and/or vomiting in subjects who did not receive optimal treatment with anti-emetics

6.8 Safety Monitoring and Adverse Effect Management

Each subject receiving Ad-RTS-hIL-12 and/or at least one dose of veledimex will be included in the Overall Safety Population (OSP). The MTD will be determined from the Evaluable Safety Population (ESP). Parameters used in the safety analysis of all populations will include all laboratory tests, physical examination, imaging scans, and spontaneous reports of AEs reported by subjects. Each patient will be assessed according to the scheduled study procedures and any additional visits as a result of AEs. Adverse events will be assessed according to the NCI CTCAE v4.03 criteria.

6.9 Severity Grading and Management of Local Reactions

Injection of agents into tissue carries a potential risk of local reactions that may be characterized as intense immunologic reaction at or near the injection site. Local reactions will be graded according to the NCI CTCAE v4.03 criteria.

As with all signs and symptoms, events should be recorded and graded as AEs according to NCI CTCAE v.4.03 criteria. Study stopping rules will not apply to a specific event if it is clearly unrelated to the Ad-RTS-hIL-12 injection.

6.10 Prophylactic Antipyretic and/or Analgesic Administration

The use of antipyretics and/or analgesics is allowed as a prophylactic measure perioperatively. Antipyretics and/or analgesics can be used anytime during study treatment, as indicated and required for patient safety and must be recorded as concomitant medications. Please refer to exclusion criteria for acute clinically significant and/or chronic infections.

NOTE: Since fever and flu-like symptoms (e.g., fever, headache, chills, dehydration, etc.) are commonly experienced following adenoviral vector administration, it is highly recommended that subjects be treated with prophylactic antipyretic and/or analgesic medication prior to Ad-RTS-hIL-12 injection and during the first week after injection.

Please refer to Appendix 16.2 for the recommended regimen for the prophylactic administration of antipyretics and/or analgesics.

7 CONCOMITANT THERAPY

Information on concomitant medications, including all medications, blood products, vitamins, and other supplements, will be collected through the Screening, Treatment, and the Initial Follow-up Period of this study.

Subjects experiencing brain tumor-related symptoms or edema should be treated with corticosteroids as per standard practice. The treating physician should consider the minimum starting steroid dose for study subjects, if determined that it is safe and appropriate for that individual patient. For study subjects who require a higher starting steroid dose, efforts should be made to taper steroids to the lowest amount that controls the subjects' symptoms, as determined to be safe and appropriate by the treating physician

7.1 Permitted Medications

Subjects may receive standard treatments, including palliative and supportive care for any illness or symptom management during study treatment, including:

- Corticosteroids are permitted for brain tumor-related symptoms. The treating physician should consider the minimum steroid dose for study subjects, if determined that it is safe and appropriate for that individual patient. For study subjects who require a higher steroid dose, efforts should be made to taper steroids to the lowest amount that controls the subjects symptoms, as determined to be safe and appropriate by the treating physician.
- Antidiarrheal therapy is permitted for study drug-induced diarrhea
- Anti-emetics are permitted for study drug-induced nausea and vomiting

NOTE: Care should be given when prescribing medications that are classified as CYP450 3A4 inducers, inhibitors, or substrates due to potential interactions with the study drug. In the event that one is prescribed, consultation with the Medical Monitor is advised. All medications should be recorded in the case report form as indicated in the completion guidelines.

7.2 Prohibited Medications

The following medications are prohibited during the study:

- Any other investigational agent or anticancer therapy (chemotherapy, radiotherapy, etc.) while receiving study treatment
- Palliative radiotherapy is not permitted while on study
- Enzyme inducing anti-epileptic drugs (EIAED) are listed in Appendix 16.2 and are NOT permitted.

  NOTE: Care should be given when prescribing medications that are classified as CYP450 3A4 inducers, inhibitors, or substrates due to potential interactions with the study drug. In the event that one is prescribed, consultation with the Medical Monitor is advised. All medications should be recorded in the case report form as indicated in the completion guidelines.

8 STUDY PROCEDURES

8.1 Written Informed Consent

The provided written ICF must be signed before any protocol specific procedures and assessments can be performed. A copy of the signed ICF will be given to the subject and a copy should be filed in the medical record. The original ICF should be kept on file with the study reports. Standard of care evaluations performed as part of the subject's routine treatment prior to signing the ICF can be used if they were conducted within the timeframe of the screening period. Refer to Section 13.8 for further information.

8.2 Subject Registration

Centralized registration of subjects will be completed according to a process defined by the sponsor. Eligible subjects are to be enrolled and assigned a unique study identification number before the planned intratumoral Ad-RTS-hIL-12 injection. Once assigned, a subject's identification number will not be reused.

8.3 Schedule of Procedures and Observations

Screening assessments must be performed within 28 days prior to the Ad-RTS-hIL-12 injection. Any screening tests, exams, or procedures outside of this range may be repeated at the investigator's discretion. All study visits must be completed as described in the protocol while subjects are taking veledimex capsules. Follow-up assessments are allowed a window of ± 7 days.

8.3.1 Study Tests, Exams, and Procedures

Demographics, Medical and Cancer History, and Concomitant Medications

Each subject's complete medical history will be documented during screening, including demographic information, relevant medical history, current primary cancer diagnosis, and prior cancer treatments (chemo- and immunotherapies, radiation therapy, surgeries, and any associated residual toxicities). In addition, concomitant medications, including blood products, vitamins, and other supplements received during the screening period (28 days) prior to initiating study treatment will be recorded. Concomitant medications will continue to be collected through the Initial Follow-Up period (e.g. Day 56).

Physical Examinations

A complete physical examination will also include a neurological examination.

Vital Signs, Height, and Weight

Vital signs will include blood pressure, pulse rate, temperature, and respiration rate. Subject's blood pressure is to be monitored closely, with hydration as needed to prevent hypotension for 72 hours after administration of Ad-RTS-hIL-12. Assessment of vital signs is required prior to injection of Ad-RTS-hIL-12, and prior to veledimex dosing. Height and weight will be measured and recorded according to Schedule of Study Procedures.

Karnofsky Performance Status

The Karnofsky Performance Status measures the ability of cancer subjects to perform ordinary tasks. Scores range from 0 to 100 with a higher score meaning that the patient is better able to carry out daily activities. The Karnofsky Performance Status is used to determine a patient's prognosis, to measure changes in a patient's ability to function, or to decide if a patient could be included in a clinical trial. Subjects must have a Karnofsky Performance Status score of ≥ 70 at the Screening Visit to be included in the study (Appendix 16.1).

Pregnancy Testing

Females of childbearing potential will have a serum pregnancy test at the Screening Visit and a urine or serum pregnancy test on Day 0, with a negative pregnancy outcome prior to study drug initiation.

Monitoring of Adverse Events

Monitoring and recording of AEs and serious adverse events (SAEs) will be conducted throughout the study. Adverse events and SAEs that occur following the signing of the ICF through the Initial Follow-up Period (e.g. Day 56) must be recorded on the AE CRF.

Definitions, documentation, and reporting of AEs and SAEs are described in Section 10.

Proper hydration is critical. It is important that subjects are instructed repeatedly to maintain adequate oral hydration on and between veledimex doses; study sites must closely monitor subjects' hydration status. Blood pressure should be monitored regularly.

Administration of prophylactic antipyretics is strongly recommended during the first week after Ad-RTS-hIL-12 injection.

Clinical Laboratory Assessments

The hematology panel comprises a complete blood count (CBC), including white blood cell (WBC) count with differential, red blood cell (RBC) count, hematocrit, hemoglobin, red blood cell indices, mean corpuscular volume (MCV), and platelet count.

The serum chemistry panel comprises the following parameters: AST, ALT, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatinine, total bilirubin, total protein, albumin, amylase, blood urea nitrogen (BUN), glucose, sodium, potassium, chloride, calcium, phosphorus, and bicarbonate.

The coagulation panel includes activated partial thromboplastin time (aPTT) and INR.

The urinalysis panel (dipstick) includes appearance, pH, specific gravity, glucose, protein/albumin, presence of blood, ketones, bilirubin, nitrates, and leukocyte esterase. In addition, a microscopic exam for casts, crystals, and cells may be done if clinically indicated.

MRI

Subjects should be able to undergo MRI scans with contrast agent at screening and during study participation. MRI scans should be available for collection upon sponsor request.

[redacted]

[redacted]

Electrocardiogram

A standard, single, 12-lead electrocardiogram (ECG) for evaluation of the QT/QTc interval will be performed.

8.3.2 Screening Period: Assessments

The screening exams, tests, and procedures that must be done within 28 days of Ad-RTS-hIL-12 injection are:

- Signed informed consent form
- Medical/cancer history
- Physical examination (including neurological examination)
- Height and weight
- Vital signs
- ECG
- Karnofsky Performance Status
- History of prior treatments and any associated residual toxicity
- Medications taken within 28 days of Ad-RTS-hIL-12 injection
- Adverse Events
- Concomitant medications
- Serum pregnancy test
- **Hematology Panel including**: complete blood count (CBC), white blood cell (WBC) count with differential, red blood cell (RBC) count, hematocrit, hemoglobin, red blood cell indices, mean corpuscular volume (MCV), and platelet count
- **Serum Chemistry Panel including**: aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatinine, total bilirubin, total protein, albumin, blood urea nitrogen (BUN), glucose, sodium, potassium, chloride, calcium, phosphorus, and bicarbonate.
- **Coagulation Panel including**: partial thromboplastin time (PTT), international normalized ratio (INR) ESR – erythrocyte sedimentation rate and CRP - C - reactive protein.
- **Urinalysis Panel (dipstick) including**: appearance, pH, specific gravity, glucose, protein/albumin, presence of blood, ketones, bilirubin, nitrates, and leukocyte esterase. In addition, a microscopic exam for casts, crystals, and cells may be done if clinically indicated
- [redacted]
- Subject Registration

- MRI scan for surgical planning and/or stereotactic procedures

8.3.3 Treatment Period: Day 0 (Ad-RTS-hIL-12 injection)

- Physical Examination (including neurological examination)
- Karnofsky Performance Status
- Vital Signs
- Adverse Events Evaluation
- Concomitant medications
- Urine or Serum Pregnancy Test
- Hematology Panel
- Coagulation Panel
- Serum Chemistry Panel
- Urinalysis Panel
- ECG
- Group 1 subjects undergoing a tumor resection will take a cohort specific dose of veledimex 3 (± 2) hours prior to resection, on an empty stomach (excluding other medications). Tumor, CSF (if available), and blood samples will be collected at time of resection. Intratumoral Ad-RTS-hIL-12 will be administered by [redacted] injection.
- Group 2 subjects who will not undergo tumor resection will receive Ad-RTS-hIL-12 by stereotactic injection
- Group 1 only: Blood Sample for Veledimex PK
- Blood Sample for Evaluation of Serum Cytokine Profile
- [redacted]
- Tumor and CSF (if available) for Group 1 subjects only

8.3.4 Treatment Period: Day 1

- Once Daily Veledimex (in the morning with food) AND compliance diary
- Physical Examination (including targeted neurological evaluation)
- Vital Signs
- Adverse Events Evaluation
- Concomitant Medications
- Blood Sample for Veledimex PK

8.3.5 Treatment Period: Day 2

- Once Daily Veledimex Dose (in the morning with food) AND compliance diary

- Physical Examination (including targeted neurological evaluation)
- Vital Signs
- Adverse Events Evaluation
- Concomitant Medications
- Hematology Panel
- Serum Chemistry Panel
- Blood Sample for Veledimex PK
- MRI scan (to be done within 72 hours of Ad-RTS-hIL-12 administration and to be used as the baseline MRI for tumor response assessment)

8.3.6 Treatment Period: Day 3

- Once Daily Veledimex Dose (in the morning with food) AND compliance diary
- Physical Examination (including targeted neurological evaluation)
- Vital Signs
- Adverse Events Evaluation
- Concomitant Medications
- Hematology Panel
- Serum Chemistry Panel
- Coagulation Panel
- [redacted]
- Blood Sample for Veledimex PK
- Blood Sample for evaluation of Serum Cytokine Profile
- ECG

8.3.7 Treatment Period: Days 4 through 6

- Once Daily Veledimex Dose (in the morning with food) AND compliance diary
- Adverse Events evaluation
- Concomitant Medications

8.3.8 Treatment Period: Day 7

- Once Daily Veledimex Dose (in the morning with food) AND compliance diary
- Physical Examination (including targeted neurological evaluation)
- Vital Signs
- Weight

- Adverse Events Evaluation
- Concomitant Medications
- Hematology Panel
- Serum Chemistry Panel
- Coagulation Panel
- Blood Sample for Veledimex PK
- Blood Sample for evaluation of Serum Cytokine Profile
- [redacted]

8.3.9 Treatment Period: Days 8 through 13

- Once Daily Veledimex Dose (in the morning with food) AND compliance diary
- Adverse Events Evaluation
- Concomitant Medications

8.3.10 Treatment Period: Day 14

- Once Daily Veledimex Dose (in the morning with food) AND compliance diary
- Physical Examination (including targeted neurological evaluation)
- Karnofsky Performance Status
- Weight
- Vital Signs
- ECG
- Adverse Events Evaluation
- Concomitant Medications
- Hematology Panel
- Serum Chemistry Panel
- Coagulation Panel
- Urinalysis Panel
- Veledimex Dose Compliance
- Blood Sample for Veledimex PK
- [redacted]
- Blood Sample for evaluation of Serum Cytokine Profile
- MRI

8.3.11 Follow-up Period: Day 15

- AE evaluation
- Concomitant medications
- Blood sample for veledimex PK

8.3.12 Follow-up Period: Day 28 (± 7 days)

- Physical Examination (including targeted neurological examination)
- Karnofsky Performance Status
- Weight
- Vital Signs
- Adverse Events Evaluation
- Concomitant Medications (including any new anti-cancer therapies)
- Hematology Panel
- Serum Chemistry Panel
- Blood Sample for evaluation of Serum Cytokine Profile
- [redacted]
- MRI scan

8.3.13 Follow-up Period: Day 56 (± 7 days) and every 8 weeks (± 7 days) thereafter

- AE evaluation (Day 56 only)
- Documentation of any anti-cancer therapies
- MRI Scan
    - Subjects should continue to be followed until confirmed disease progression has been documented
        - Documentation of total daily dose of steroids taken for the two weeks prior to the MRI scan
- Survival Follow-up
    - Subjects should continue to be followed for 2 years after enrollment.

8.3.14 Unscheduled Visits Collections

In the event of subject termination or an unscheduled visit for a drug-related AE, an unscheduled visit kit should be obtained for cytokines [redacted] for CSF evaluation, if applicable.

9.0 TUMOR RESPONSE ASSESSMENTS

9.1 Tumor Response

The secondary time-to event endpoints of this study include Investigator assessment of ORR, PFS and OS.

Tumor response will be evaluated radiographically using MRI scans to determine tumor response and to assess the time of objective disease progression (estimate of PFS). A baseline MRI will be performed within 72 hours of Ad-RTS-hIL-12 administration (Day 2). The Ad-RTS-hIL-12 injected lesion and/or other measureable brain lesions will be measured according to the RANO/iRANO criteria guidelines attached in Appendix 16.3 and Appendix 16.5. MRI scans will be collected and stored at the study site and each subject will be evaluated for response by the study investigator. Subjects should be imaged throughout the study using the same method(s) as were used for the screening and baseline MRIs. Independent tumor response assessments, as well as posttreatment tumor biopsies, may occur as available and at the discretion of the investigator. A repeat scan to confirm progression should be completed at 4 weeks (per RANO) and preferably again at 12 weeks (per iRANO) after first documentation of progression. Consideration should be given to performing a diagnostic brain biopsy, which should be performed in accordance with the current iRANO guidelines.

Response is defined by radiographic and clinical criteria. Complete response (CR) or partial response (PR) will be first assessed by radiographic changes that indicate a reduction of bidimensional tumor size as per RANO/iRANO criteria. In addition, changes in neurologic function and steroid use will be considered to determine stable disease (SD).

Tumor response assessments will occur at 4 weeks (Day 28 ± 7 days), 8 weeks (Day 56 ± 7 days), and every 8 weeks thereafter for all subjects, including those who may have experienced a dose delay or missed a dose, until the occurrence of confirmed tumor progression, initiation of alternative therapy, or one year, whichever occurs first.

9.2 Tumor Response Evaluation and Pseudo-Progression

The interpretation of MRI findings in subjects with treated brain tumors has an inherent uncertainty that stems from the pseudo-progression phenomena. Pseudo-progression is a term used to describe the appearance of radiographic disease progression due to increase contrast enhancement on MRI without true tumor progression. The increase in contrast enhancement can be influenced by several parameters including differences in radiologic technique, the amount of contrast agent used, the timing of the contrast agent administration relative to the imaging, postsurgical changes, infarction, treated related inflammation, seizure activity, sub-acute radiation effects, radiation necrosis, and corticosteroid use. Consideration of these factors by experts and clinical experience is likely to identify these subjects. In this study, pseudo-progression is unlikely to impact the duration of therapy since the veledimex treatment period lasts 14 days and the first tumor assessment MRI will be done on Day 28 (± 7 days). Imaging assessments will be performed using RANO/iRANO criteria.

10 SAFETY ASSESSMENTS

Safety evaluation will include all subjects enrolled in the study using NCI CTCAE v.4.03 criteria for reporting AEs.

The OSP will include all subjects who have received at least one dose of veledimex (pretumor resection and/or post-stereotactic procedure) and/or all subjects who have received Ad-RTS-hIL-12.

The ESP will include subjects who have received both Ad-RTS-hIL-12 and at least one dose of veledimex after Ad-RTS-hIL-12 administration.

10.1 Adverse Events and Definitions

Adverse Event

Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related. An AE can be any unfavorable and unintended sign including an abnormal laboratory finding, symptom, disease temporarily associated with the use of a medical treatment or procedure, and any worsening of a pre-existing condition regardless of causality to study drug. An AE is also known as an adverse experience.

Suspected Adverse Reaction

Any AE for which there is evidence to suggest a causal relationship (reasonable possibility) between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

Adverse Reaction

Any AE caused by the use of a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

Unexpected Adverse Reaction

Any AE that is (a) not listed in the Investigator's Brochure, (b) not listed with the specificity and severity that is being observed, (c) not consistent with the risk information described in the general investigational plan or elsewhere in the current application (in the absence of an investigator brochure), and (d) listed as occurring with a class of drugs, but not specifically mentioned as occurring with the particular drug under investigation.

10.2 Evaluation of Adverse Events

Adverse events include:

- Suspected adverse drug reactions
- Reactions from study drug overdose, abuse, withdrawal, sensitivity, or toxicity
- Significant changes or abnormalities when compared to baseline, in signs, symptoms, clinical laboratory results, or physiological testing. This includes any worsening of a pre-existing condition temporally associated with the use of study drug.

- Other untoward medical events, regardless of their relationship to the study drug, such as injury, events that require surgery, accidents, extensions of symptoms, or apparently unrelated illnesses

The following considerations apply when identifying an AE:

- Anticipated day-to-day fluctuations of pre-existing conditions, including the disease under study, that do not represent a clinically significant exacerbation or worsening need not be considered AEs.
- In the event that a constellation of symptoms results in a confirmed diagnosis, the diagnosis (not the symptoms) should be recorded as the AE term.
- If a diagnosis cannot be established, the symptoms should be recorded as the AEs.
- If an ongoing symptom has been included in the medical history, an associated severity grade and frequency should also be documented so that a worsening in severity or frequency of a symptom can be readily identified as an AE.
- Progression of disease is not itself an AE; however, the presenting sign or symptom of the disease progression should be documented as an AE (e.g., increase in pain). However, if a subject experiences progression of disease that results in death, "progression of disease" may be reported as an SAE if, by medical opinion, the term best describes the cause of death.

Adverse events will be followed through the Day 56 Visit. AEs that are drug-related should be followed until resolved or no resolution is expected.

10.3 Determination of Seriousness

10.3.1 Serious Adverse Event

An AE is considered an SAE if at least one of the following conditions applies:

- Death: An AE that results in death during the active study period or within 30 days following study drug administration. In addition, a reported death at any time post-study that is thought to be related to study drug administration.
- Life-threatening AE: An AE that places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (i.e., this does not include a reaction that, had it occurred in a more severe form, might have caused death).
- Permanent, persistent, or significant disability: A disability is defined as any substantial disruption of a person's ability to conduct normal life functions.
- Inpatient hospitalization or prolongation of existing hospitalization: In general, hospitalization refers to admission of a subject into a hospital for at least a 24-hour stay. Hospitalizations for routine blood transfusions, hospitalization for an elective or diagnostic procedure, or surgery for a pre-existing condition that has not worsened, are not considered SAEs. Emergency room visits that do not result with admission are not considered as SAEs.
- A congenital anomaly/birth defect: A fixed, permanent impairment established at or before birth.

- Important medical event: Events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they jeopardize the subject and require medical or surgical intervention to prevent a life-threatening situation, hospitalization or death.
- New cancer: Occurrence or diagnosis of a new cancer during the trial is considered an SAE. (This does not pertain to metastasis of current disease);
- Any AE associated with an overdose of the study drug: An overdose of study drug is defined as an occurrence of administered dose exceeding that which is prescribed by the investigator per protocol.

10.3.2 Non-Serious Adverse Event

An AE that does not fulfill the criteria for a SAE is classified as a non-serious AE.

10.4 Determination of Severity

The severity of AEs will be assessed according to the NCI CTCAE, v. 4.03. If the AE is not defined in the NCI CTCAE, v. 4.03, the investigator will determine the severity of an AE based on the following definitions:

- Mild (Grade 1): The AE is noticeable to the subject, but does not interfere with routine activity. The AE does not require discontinuing administration or reducing the dose of the study drug.
- Moderate (Grade 2): The AE interferes with routine activity, but responds to symptomatic therapy or rest. The AE may require reducing the dose, but not discontinuation of administration of the study drug.
- Severe (Grade 3): The AE significantly limits the subject's ability to perform routine activities despite symptomatic therapy. In addition, the AE leads to discontinuation of administration or reducing the dose of the study drug.
- Life-threatening (Grade 4): The AE requires discontinuing administration of the study drug. The subject is at immediate risk of death.
- Death (Grade 5): The subject dies as a direct result of the complication or condition.

10.5 Determination of Causality

The investigator will use medical consideration to determine the potential relationship of the AE to the study drugs based on his/her clinical judgment. Assessment of causality will be based upon the following:

- Alternative possible causes of the AE, including the subject's underlying disease or comorbid conditions, other drugs, other host, and environmental factors
- The temporal sequence between the exposure to study drug and the AE
- Whether the clinical or laboratory manifestations of the AE are consistent with known actions or previously reported toxicity of the study drug or similar drugs

- Whether the AE resolved or improved with decreasing the dose or stopping the study drug (i.e., dechallenge); or recurred or worsened with re-exposure to the drug (i.e., rechallenge).

Relationship assessments that indicate "Not Related" to investigational product:

- None: The event is related to an etiology other than the investigational product (the alternative etiology must be documented in the study subject's medical record and/or SAE form).
- Unlikely or Remote: The event is unlikely to be related to the investigational product and likely to be related to factors other than investigational product.

Relationship assessments that indicate "Related" to investigational product:

- Possible: There is an association between the event and the administration of the investigational product and there is a plausible mechanism for the event to be related to investigational product; but there may also be alternative etiology, such as characteristics of the subject's clinical status or underlying disease.
- Probable: There is an association between the event and the administration of investigational product, a plausible mechanism for the event to be related to the investigational product and the event could not be reasonably explained by known characteristics of the subject's clinical status or an alternative etiology is not apparent.
- Definite: There is an association between the event and the administration of investigational product, a plausible mechanism for the event to be related to the investigational product and causes other than the investigational product have been ruled out and/or the event re-appeared on re-exposure to the investigational product.

For AEs that occur prior to the administration of investigational product, an assessment of protocol relatedness must be made. AEs may occur as a result of procedures required during the screening process (e.g., blood collection, washout of an existing medication) prior to the initial administration of investigational product. For AEs that occur before administration of investigational product, only those that are assessed by the investigator as protocol-related should be reported to the sponsor. The following guidelines should be used by investigators to assess the relationship of an AE to a protocol-required procedure:

- Protocol-related: The event occurred due to a procedure or intervention that was described in the protocol for which there is no alternative etiology present in the subject's medical record.
- Not protocol-related: The event is related to an etiology other than the study procedure (the alternative etiology must be documented in the study subject's medical record).

10.6 Documenting Adverse Events

All AEs, including SAEs, are to be accurately recorded on the Adverse Event page of the subject's electronic CRF from the time the subject signs the informed consent through the Follow-up Period. Each event will be assessed for serious criteria, severity, and causality (Section 10.5). The date of onset, as well as the duration of the event will be recorded. In addition, treatments provided to the subject, actions taken with the study drugs, and the outcome of the AE will also be noted.

10.7 Reporting Dose-Limiting Toxicities and Serious Adverse Events

Time Frame for Reporting

All DLTs and SAEs must be reported to the sponsor or sponsor designee within 24 hours of awareness, regardless of initiation of new anticancer therapy including the following:

- Any death or SAE experienced by the subject from the signing of informed consent to 30 days after the last dose of study drug, regardless of relationship to study drug.
- Any death or SAE that the investigator becomes aware of, and believes to be study drug-related, that occurs more than 30 days after the subject last received study drug.

All DLTs and SAEs must be reported to the following fax line within 24 hours of awareness:

[redacted]

[redacted]

Questions or assistance with completing forms can be directed to ZIOPHARM Oncology, [redacted]

Additional data concerning the SAE (e.g., diagnostic test reports, hospital summaries, etc.) must be promptly reported (within 24 hours of receipt) to the sponsor or sponsor's designee, until resolution of the SAE. Should the FDA or National Regulatory Authorities require that the sponsor submit additional data on the event, the investigator will be asked to provide those data to the sponsor in a timely fashion.

Information to be Provided by the Investigator

Within 24 hours of becoming aware of the SAE or subject death, the investigator must notify the sponsor or designee and transmit information to the sponsor or designee. Information (initial and follow-up) should be provided on an electronic and/or paper SAE Report form signed and dated by the investigator. The SAE Report form and copies of source documents with subject identifiers redacted will be transmitted by fax. A hospital discharge summary should be provided if the subject was hospitalized. An SAE report will be considered final once all relevant information has been received and reviewed by the sponsor.

The SAE report form is provided in the investigator study files. A DLT specific form will be used to report DLTs and is also provided in the investigator files. Please refer to the investigator study files for instructions on how to complete these forms. The investigator will provide all of the following information related to the event:

- Investigator identification
- Subject identification (e.g., subject number, initials, sex, age or date of birth)
- Information regarding study drug administration (e.g. start/stop date, dose, and frequency)
- Cycle and Day of SAE or DLT occurrence documentation on SAE or DLT report forms
- Description of event

- Action taken with the study drugs in relation to the SAE or DLT
- Outcome of the SAE or DLT

In addition to the above information, the investigator must provide, for each event term, an assessment of:

- Severity/intensity
- Relationship to the study drug (causality assessment)

10.8 Sponsor and Investigator Responsibility for Reporting Adverse Events

All AEs and SAEs will be reported to regulatory authorities, IRBs/IECs, and investigators in accordance with all applicable global laws and regulations. The investigator must submit all Safety Letters received from the sponsor to his/her IRB/IEC per agreements and local requirements. The investigator must keep copies of all safety reports/letters, including correspondence with ZIOPHARM and the IRB/IEC, in the study file.

10.9 Follow-up Information for Adverse Events

Appropriate diagnostic tests should be performed and therapeutic measures, as medically indicated, should be instituted. Appropriate consultation and follow-up evaluations should be carried out until the event has resolved, stabilized, returned to baseline, or is otherwise explained by the investigator.

Required Follow-up for Adverse Events

All treatment-related AEs-and SAEs will be collected through the Follow-up Period (i.e., 42 days after the end of veledimex treatment) or until:

- The event resolves
- The event returns to baseline, if a baseline value is available
- The event stabilizes (following consultation and agreement by the ZIOPHARM medical monitor)
- The event can be attributed to factors other than the study drug or other than study procedure

10.10 Pregnancies

Subjects who become pregnant during the study should immediately discontinue participation in the study. The sponsor should be immediately notified.

An initial Pregnancy Report form and a Pregnancy Outcome Form are to be completed by the investigator or designee. The Pregnancy Report form and the completion guidelines will be provided in the investigator study files. Please refer to the investigator study files for details on how to complete these forms.

10.11 Overdose

Investigational product overdose of study subject, with or without associated AEs/SAEs, should be reported within 24 hours of awareness to sponsor (ZIOPHARM Oncology, [redacted]

[redacted] All AEs or SAEs as a result of overdose should be reported as described previously in Sections 10.6 and 10.7.

11 IMMUNE RESPONSE AND PHARMACOKINETIC ASSESSMENTS

11.1 Serum Cytokine [redacted] Assessments from Blood

Blood will be drawn and evaluated from all subjects according to the Schedule of Study Procedures and the laboratory manual for the following:

- Serum IL-12, IFN-γ, [redacted]
- [redacted]

[redacted]

11.4 Veledimex Pharmacokinetic Assessment

Veledimex PK assessment will occur for all subjects during study treatment. Whole blood samples will be collected at the time points specified in the Schedule of Veledimex Pharmacokinetic Sampling Times (Table 8). Veledimex plasma concentrations will be determined using a fully validated liquid chromatography-mass spectrometry assay.

TABLE 8 SCHEDULE OF VELEDIMEX PHARMACOKINETIC SAMPLING TIMES

Sample	Day 0	Day 1	Day 2	Day 3	Day 7	Day 14	Day 15
1	During Resection	Predose[a]	Predose[a]	Predose[a]	Predose[a]	Predose[a]	Scheduled Visit[b]
2	-	3 to 5 hours after dose	-	-	-	3 to 5 hours after dose	-

[a] ≤ 30 minutes prior to veledimex dose
[b] 24 hours post last dose (Day 14)

11.5 Veledimex

Pharmacodynamic and PK analysis will be performed on the pharmacokinetic population (PKP). Available immunologic and biologic response marker data will be summarized by dose cohort and by visit. Veledimex PK parameters will be determined based on blood levels of veledimex using standard methods and will include, but are not limited to, the maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), half-life ($t_{1/2}$), area under the curve (AUC), volume of distribution (V_d), and clearance (CL). Where possible, descriptive statistics of the PK parameters will be provided; individual subject veledimex concentrations, actual sampling times, and PK parameters will be listed.

[redacted]

12 STATISTICAL METHODS

This study is designed as a Phase I dose escalation according to a standard 3 + 3 design to determine if the MTD will be reached in up to four veledimex dose cohorts; 20 mg, 40 mg, 80 mg and 120 mg. It is modified to independently evaluate the safety of two groups of recurrent HGG subjects previously described:

- Group 1: Subjects scheduled for craniotomy and tumor resection
- Group 2: Subjects who will not undergo tumor resection

The statistical difference between the groups is that Group 1 subjects will receive a cohort-specific dose of veledimex 3 (± 2) hours before the craniotomy procedure on Day 0 on an empty stomach (excluding other medications). At the time of tumor resection, tumor CSF (if available), and blood samples will be collected to determine the veledimex concentration in these samples. This will not occur for Group 2 subjects because they will not undergo a standard of care tumor resection.

In each cohort, starting on Day 1 the first subject will be monitored for the 14 days of treatment and will be observed an additional 7 days post the last veledimex dose before the second and third subjects are enrolled in that same cohort. Evaluation for the determination to dose escalate will occur after all dosed subjects in a cohort have participated on-study for at least 28 days postdose from Day 1, unless the subject discontinued due to a DLT.

12.1 Populations for Analysis

The following subject populations will be evaluated and used for presentation and analysis of the data:

- The OSP includes all subjects who have received at least one dose of veledimex (pretumor resection and/or post-stereotactic procedure) and/or all subjects who received Ad-RTS-hIL-12.
- The ESP includes subjects who have received Ad-RTS-hIL-12 and at least one dose of veledimex after Ad-RTS-hIL-12 administration.
- The PKP includes all subjects who receive veledimex.

12.2 Sample Size and Power Calculations

The sample sizes chosen were based on clinical consideration for a standard 3 + 3 dose escalation design to determine the MTD for two separate groups of subjects independently evaluable for safety and dose escalation. Power calculations for comparison of AE rates are simply too inexact to be clinically meaningful because the AE rates are unknown and cannot even be approximately projected. If an AE occurs at a rate of 1% or 10%, then the chance for observing such an AE among six subjects receiving that dose will be 6% and 47% respectively. If no AE is observed in any of the six subjects, then the true incidence is at most 24% with 80% confidence and 32% with 90% confidence.

12.3 Endpoints

12.3.1 Primary Endpoint

The primary endpoint is the assessment of safety of Ad-RTS-hIL-12, administered by intratumoral injection plus veledimex administered orally, as determined by the AE rate and the occurrence of DLTs analyzed specifically for each group.

12.3.2 Secondary Endpoints

Secondary endpoints are as follows:

- Investigator assessment of ORR and PFS
- OS
- Veledimex concentration ratio between brain tumor, CSF, and blood (Group 1, Day 0)
- Veledimex PK estimates starting Day 1, as previously described
- Correlative measures of immune response including serum cytokine levels [redacted]

12.4 Analyses

12.4.1 Baseline Characteristics

Demographic and baseline disease characteristic data will be summarized. Data to be tabulated will include at least demographic features such as sex, age, and race, as well as disease-specific status and medical history.

Categorical data will be summarized using counts and percentages based on non-missing values. For continuous variables, the mean, median, standard deviation, minimum, and maximum values will be presented. Data will be summarized by dose cohort and by group (Group 1 or Group 2) and according to the defined populations for analysis.

12.4.2 Safety Analyses

The ESP will be used to make determinations for dose escalation to subsequent higher veledimex doses for each group (Group 1 or Group 2) separately based on a standard 3 + 3 design, as previously described.

For the first veledimex dose cohort, a minimum of three ESP subjects must be eligible and have received veledimex dosing. In addition, DLT evaluation must also be performed on these subjects according to the rules of the protocol in both Group 1 and Group 2, respectively. As previously described, all subjects (Group 1 and Group 2) must have a minimum of 28 days of postdose observation experience or discontinued the trial due to a DLT before the data will be summarized based on a safety template to be created for an SRC review. The SRC during or after its review may request additional data before it makes a recommendation to the DSMB. The sponsor will coordinate the logistics of all SRC and DSMB interactions in order to expedite obtaining a dose escalation recommendation from the DSMB according to the rules of the SRC and DSMB charter.

The OSP will be used to perform safety evaluations for all safety variables of all subjects who received at least one dose of veledimex and/or all subjects who received Ad-RTS-hIL-12.

Safety evaluations will be based on the incidence, intensity, and type of AEs and SAEs. Clinically significant changes in the subjects' physical examinations, vital signs, and ECG evaluations, and abnormal laboratory values will be captured as AEs. Safety will also be assessed based on medical history and prior/concomitant medications.

The safety evaluation period extends from the date the patient signs the ICF until approximately 1 year after receiving study drug from Day 1 dosing, unless the patient discontinues the trial due to one of the following reasons:

- Documented progression
- Symptomatic deterioration also denoted symptomatic progression
- AEs that the investigator feels will subsequently make the subject noncompliant with the protocol planned Schedule of Study Procedures
- Loss to follow-up
- Noncompliance with the protocol
- Other reason not listed above

All treatment-emergent AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and will be tabulated by number and percent of subjects, and according to relationship to the study drugs, severity, and seriousness. Treatment-emergent is defined as any AE that occurs during or after administration of the first dose of study drug through the evaluation period for safety defined above, regardless of relationship to study drug; or any event that is present at baseline that worsens in intensity or is subsequently considered to be drug related by the investigator. Deaths, SAEs, and AEs resulting in study discontinuation will be listed.

Subjects who discontinue the trial as defined above will be followed for safety up to 30 days after discontinuation and until all safety events that have started during the safety evaluation period are classified as resolved or the end of the study is reached. After the conclusion of the safety evaluation period is triggered by a discontinuation event, the subject continues to be followed only for OS.

Listings of vital signs and physical examination data will be presented by visit.

12.4.3 Tumor Response Analyses

Tumor response analysis will be performed on the ESP. Investigator assessment of ORR and PFS will be determined for each dose cohort according to RANO/iRANO criteria. OS is defined as either the duration of time from the first dose of study drug to the date of death or to the last follow-up contact date if the subject has not died, in which case the subject is censored if still alive up to 2 year from the first dose of study drug received. A two-sided confidence interval will be computed for the ORR. PFS and OS will be estimated using the Kaplan-Meier method for appropriately-sized subject groups.

Following completion of the study, best response will be determined for each subject in accordance with RANO/iRANO guidelines and the ORR will be presented for all ESP subjects and for each group of subjects. Where applicable, summary data of PFS, OS, and durability of response will be

determined using Kaplan-Meier methodology; otherwise, a listing by-subject of each dosing cohort for Group 1 and Group 2 will display the data obtained. Two-sided confidence intervals will be computed for the ORR. Descriptive statistics will be performed on all of the cohorts combined and by Group 1 and Group 2.

12.4.4 Multi-Center Study

Tumor response and safety data will be presented for each dose cohort by specific group pooled over all study centers.

12.4.5 Adjustments for Covariates

No adjustments for covariates will be made.

12.4.6 Procedures for Handling Missing, Unused, and Spurious Data

No imputation of values for missing data will be performed. Standard clinical monitoring and data management practices will be used to ensure the integrity of data.

12.5 Procedures for Reporting Deviations to Original Statistical Analysis Plan

A formal statistical plan for the analysis and presentation of data from this study will be prepared prior to database lock. Deviations from the statistical analyses outlined in this protocol will be indicated in this plan; any further modifications will be noted in the final clinical study report.

13 STUDY MANAGEMENT

13.1 Electronic Case Report Forms and Source Documentation

For each subject, electronic case report forms (eCRFs) and corresponding source records will be maintained at each clinical site. The sponsor or designee will provide the study sites with secure access to and sufficient training on the electronic data capture (EDC) application, to permit site personnel to enter or correct information in the eCRFs for the subjects for whom they are responsible.

The eCRFs should be completed in a timely manner, and every effort should be made to have forms completed and up-to-date in anticipation of a visit by the sponsor's monitor. Specific instructions will be provided to the site. All requested information must be entered on the eCRF in the spaces provided. If an item is not available or is not applicable, it should be documented as such; do not leave a space blank.

It is the investigator's responsibility to ensure the accuracy, completeness, clarity, and timeliness of the data reported in the subject's eCRF. Through the EDC application, the investigator must provide formal approval of all subject information in the eCRFs and changes to the eCRFs to endorse the final submitted data for the subjects for whom he/she is responsible. The audit trail entry will show the user's identification information and the date and time of any corrections.

eCRF completion may be delegated to other study personnel; however, such delegation must be documented in writing. If, for any reason, certain data are lacking to complete an individual report form, the investigator will provide a written statement explaining the reasons for the lack of data.

Sponsor or designee will retain the eCRF data and corresponding audit trails. A copy of the final archival eCRF in the form of a compact disk or other electronic media will be placed in the investigator's study file.

13.2 Good Clinical Practice

The study will be conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines, applicable regulatory requirements, and ZIOPHARM policies.

13.3 Sponsor Monitoring

After satisfactory receipt of all necessary regulatory paperwork, the sponsor's monitor will arrange that all study material be delivered to the study site at a mutually convenient time. An initiation visit by ZIOPHARM and its monitoring personnel will be made. At this meeting, all personnel expected to be involved in the conduct of the study will undergo an orientation to include review of study protocol, instruction for CRF completion and overall responsibilities, including those for drug accountability and study file maintenance.

Throughout the course of the study, the sponsor's monitor will make frequent contact with the investigator, and this will include telephone and/or onsite visits. During these visits, CRFs will be reviewed for completeness and adherence to protocol. As part of the data audit, it is expected that source documents (e.g., hospital records, office records) will be made available for review by the monitor. The monitor also will perform drug accountability checks, and may periodically request

review of the investigator's study file to assure completeness of documentation in all respects of study conduct.

Upon study completion, the monitor will arrange for a final review of the study files, after which the file should be secured by storage for the appropriate period as specified in Section 13.5. The investigator or appointed delegate will receive the sponsor's representative during these onsite visits and will cooperate in providing the documents for inspection and responding to inquiries that may arise as part of this review. The investigator will also permit inspection of the study files by authorized representatives of the FDA.

13.4 Duration of the Study

The duration of this study from the time of initiating subject enrollment until the completion of survival follow-up is anticipated to be approximately 48 months, including 24 months for enrollment and 2 years of further follow-up.

The overall duration is expected to be up to 2 years for an individual subject, including the following:

- Screening period of up to 28 days
- Study treatment period of 2 weeks (Days 0 through 14)
- Assessment of safety through the Follow-up Period
- Assessment of tumor response at Day 28 (± 7 days), Day 56 (± 7 days), and every 2 months thereafter until the occurrence of confirmed tumor progression
- Survival status through 2 years

In addition, subjects who discontinue or complete study treatment without objective evidence of disease progression should continue to be followed until confirmed disease progression has been documented. Subjects will be followed for survival status for 2 years after enrollment, whichever occurs first. The active study period refers to the study period from informed consent through the Initial Follow-up Period.

13.5 Records Retention

Records of drug disposition, CRFs, and reports of the clinical trial must be maintained by the investigator for a period of at least 2 years following the date on which the test article is approved by FDA for marketing for the purposes that were investigated in the study. If no application is to be filed or if the application is not approved for such indication, the records must be stored for two additional years and then returned to ZIOPHARM. No records will be destroyed, but will be indefinitely stored.

13.6 Institutional Review Board/ Independent Ethics Committee

This protocol and the study ICF must be reviewed and approved by the Institutional Biosafety Committee, where applicable, and IRB/IEC prior to the start of the study, and a copy of the approval letter supplied to ZIOPHARM. During the course of the study, the investigator shall make timely and accurate reports to the IRB/IEC on study progress at intervals not exceeding 1 year, as well as satisfying any other local IRB/IEC reporting regulations. Copies of all reports to, and correspondence with, the IRB/IEC must be provided to ZIOPHARM. Further, within 3 months of the completion or

early termination of the study, a final report should be made to the IRB/IEC and ZIOPHARM by the investigator.

All protocol revisions must originate with and be documented by ZIOPHARM. If the requested revision is an amendment, the investigator must sign it. The FDA will be notified of all revisions by ZIOPHARM. The investigator must submit the amendment to his/her IRB/IEC for review and approval prior to implementation. Documentation of approval signed by the chairperson or designee of the IRB/IEC must be sent to ZIOPHARM.

It is the investigator's obligation to maintain an IRB/IEC correspondence file and to make this available for review to ZIOPHARM representatives as part of the routine study monitoring process.

13.7 Confidentiality and HIPAA

The written ICF will explain that study data will be stored in a database, maintaining confidentiality in accordance with national data legislation. All data processed by ZIOPHARM, or its representatives, will be identified by subject number and study code.

The written ICF will also explain that, for data verification purposes, authorized representatives of ZIOPHARM, a regulatory authority (FDA), and/or the IRB/IEC may require direct access to parts of the hospital or clinic records relevant to the study that include the subject's medical history.

The written ICF will be accompanied by or include a separate document incorporating United States Health Insurance Portability and Accountability Act (HIPAA)-compliant wording by which the subjects authorize the use and disclosure of their Protected Health Information.

13.8 Informed Consent

13.8.1 FDA Informed Consent Requirements

The investigator or his/her staff will explain the nature of the study, its purpose and associated procedures, the expected duration, and the potential risks involved to the prospective subject prior to enrollment. The ICF should also indicate that, by signature, the prospective subject or, where appropriate, a legal guardian, permits access to relevant medical records by the sponsor and by representatives of the FDA. If a prospective subject does not understand English, an appropriate translation into his or her primary language must be made available. The investigator or designee will obtain written, informed, and witnessed consent. The prospective subject will have ample time and opportunity to ask questions. The prospective subject will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide a reason for this decision. Following the discussion regarding the study, the prospective subject will be asked if he/she is willing to sign and personally date a statement of informed consent. Only if the prospective subject voluntarily agrees to sign the informed consent statement and has done so, may he/she enroll into the study. A copy of his/her signed and dated informed consent will be provided to each prospective subject. The signed ICF is to remain in the investigator's file.

The ICF and any other written information provided to the subjects will be revised whenever important new information becomes available that may be relevant to the subject's consent, or if there is an amendment to the protocol that necessitates a change to the content of the subject's informed consent. The investigator will inform the subject of changes in a timely manner and will ask the subject to

confirm continuation of his/her participation in the study by his/her signature on the revised ICF, if applicable. Any written ICF and written information must receive IRB/IEC approval/favorable opinion in advance of use.

13.8.2 Subject Informed Consent Form

ZIOPHARM will provide a sample subject ICF for modification, as appropriate, by the investigator.

14 PROTOCOL APPROVAL PAGE

A Phase I Study of Ad-RTS-hIL-12, an Inducible Adenoviral Vector Engineered to Express hIL-12 in the Presence of the Activator Ligand Veledimex in Subjects with Recurrent or Progressive Glioblastoma or Grade III Malignant Glioma

With the exception of a change intended to eliminate an immediate hazard to subjects, the study shall be conducted as described in the approved protocol. All deviations from the protocol will be documented in the CRF. Any significant deviation or deviation related to dosing or safety evaluation will be reported to ZIOPHARM and documented in the CRF.

I agree to the terms of this study protocol. I will conduct the study according to the procedures specified herein, and according to principles of Good Clinical Practice and local regulations and requirements.

Study Site Center Name: _____

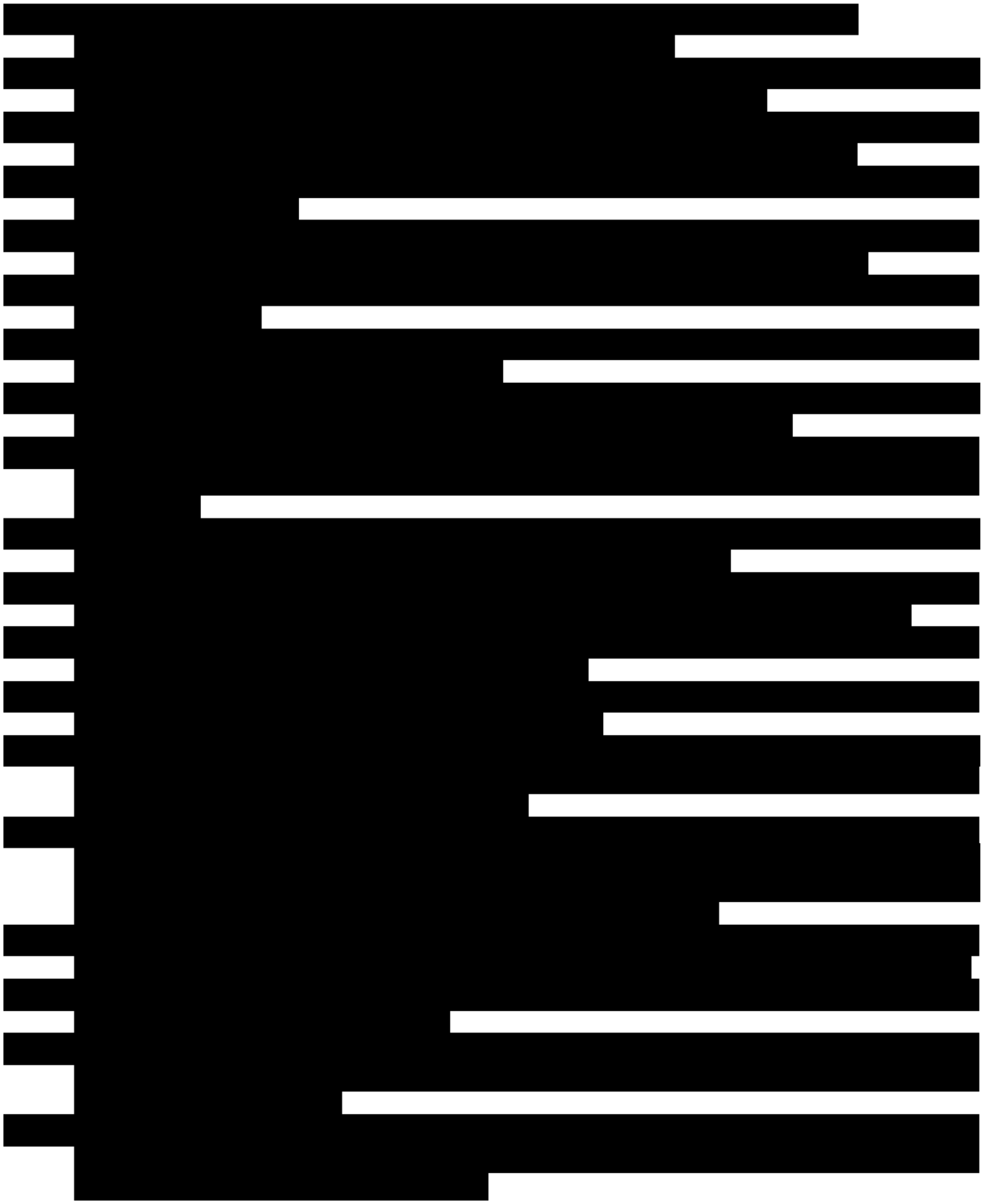
Principal Investigator

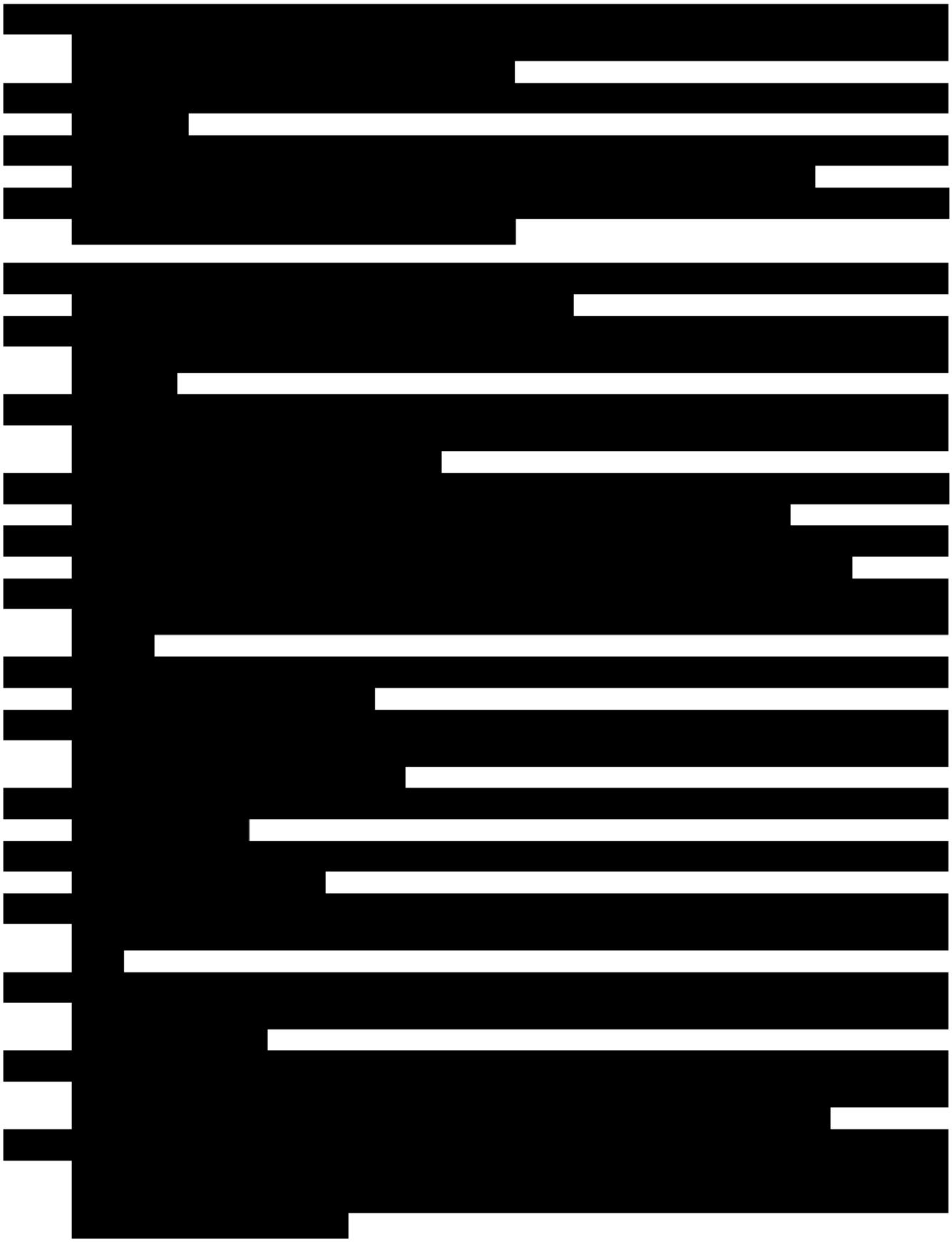
Print Name: _____

Signature: _____

Date: _____

15 REFERENCES





16 APPENDICES

16.1 Karnofsky Performance Status

Karnofsky Performance Status Scale Definitions Rating (%) Criteria		
Able to carry on normal activity and work; no special care needed.	100	Normal no complaints; no evidence of disease
	90	Able to carry on normal activity; minor signs of symptoms of disease
	80	Normal activity with efforts; some signs of symptoms of disease
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work
	60	Requires occasional assistance, but is able to care for most of his personal needs
	50	Requires considerable assistance and frequent medical care
Unable to care for self; requires equivalent of institutional or hospital care; diseases may be progressing rapidly.	40	Disabled; requires special care and assistance
	30	Severely disabled; hospital admission is indicated although death not imminent
	20	Very sick; hospital admission necessary; active support treatment necessary
	10	Moribund; fatal processes progressing rapidly
	0	Dead

Oxford Textbook of Palliative Medicine, Oxford University Press. 1993, 109.

16.2 Prohibited Enzyme-Inducing Antiepileptic Drugs and Enzyme-Inhibiting Antiepileptic Drugs

Enzyme-inducing Antiepileptic Drugs	Enzyme-inhibiting Antiepileptic Drugs
Dilantin® (phenytoin)	Depakene®, Depacon®, Depakote®, Stavzor®, Valproic® (divalproex sodium or valproate)
Lamictal® (lamotrigine)[a]	
Mysoline® (primidone)	
Sabril® (vigabatrin)	
Solfoton®, Luminal® (phenobarbital)	
Tegretol® (carbamazepine)	
Topamax® (topiramate)[b]	
Trileptal® (oxacarbazepine)	

[a]: Barely induces uridine diphosphate-glucuronosyltransferase enzymes, rarely has clinical impact
[b]: Effect is only significant at higher doses

16.3 Recommended Regimen for Antipyretic and/or Analgesic Prophylaxis

Recombinant adenoviral vectors have the potential to elicit potent cellular and humoral immune responses. While the mechanism responsible for these effects is poorly understood, transient low-grade fevers are common after systemic rAD vector administration and temperatures up to 104° F with chills and generalized malaise have been observed with treatment. Because low-grade fever is very likely to occur, prophylaxis with acetaminophen is strongly recommended.

While meta-analyses suggest that ibuprofen is a better anti-pyretic medication than acetaminophen, acetaminophen also prevents and/or reduces a fever. Acetaminophen is available without a prescription in 325 mg or 500 mg tablets. Each site should follow the institutional protocol for the administration of acetaminophen.

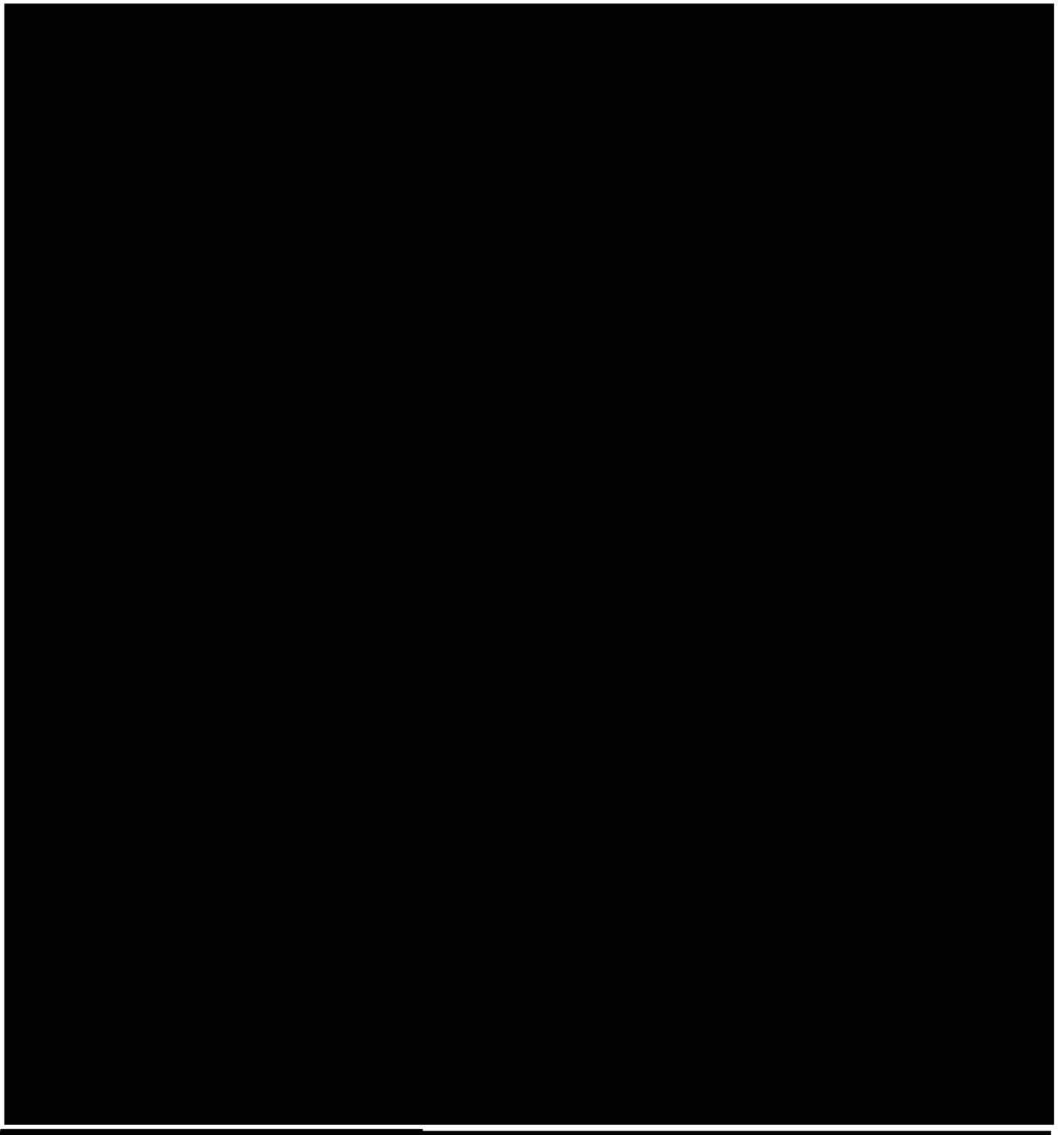
Adverse events are rare, but some people are allergic to the medication. Overdoses may cause liver failure. Therefore, people with liver disease and chronic alcohol users should avoid this medication.

Common brand names of acetaminophen include Aspirin Free Anacin®, Feverall®, Genapap®, Panadol®, Tempra®, and Tylenol®.

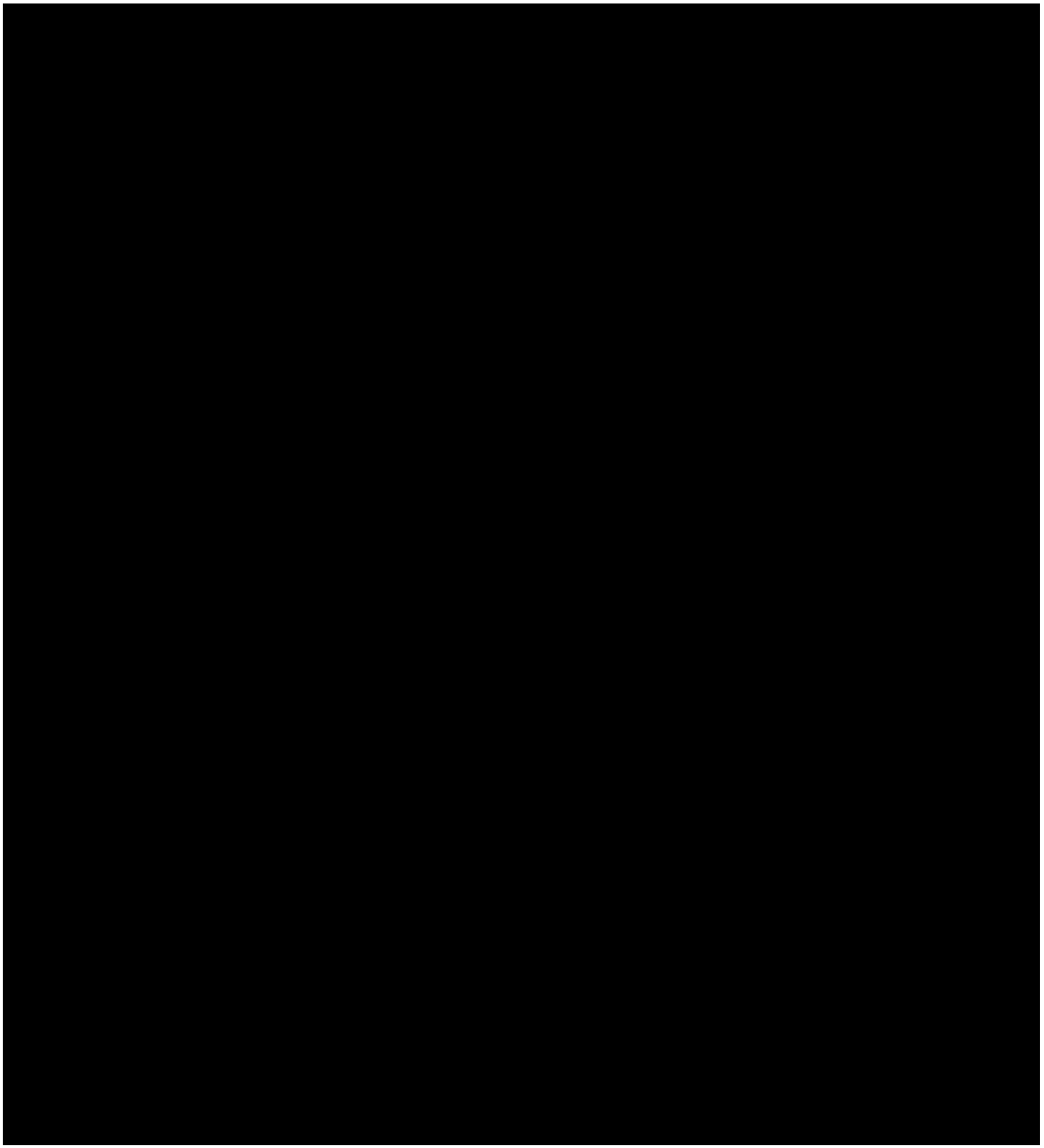
In general, a fever can be treated with acetaminophen. Alternating doses of ibuprofen with acetaminophen will also effectively control fever and prevent accidental overdose. If a fever occurs in spite of prophylactic medication or does not respond to usual doses of acetaminophen, then a combination of both acetaminophen and ibuprofen may be needed to stop the fever.

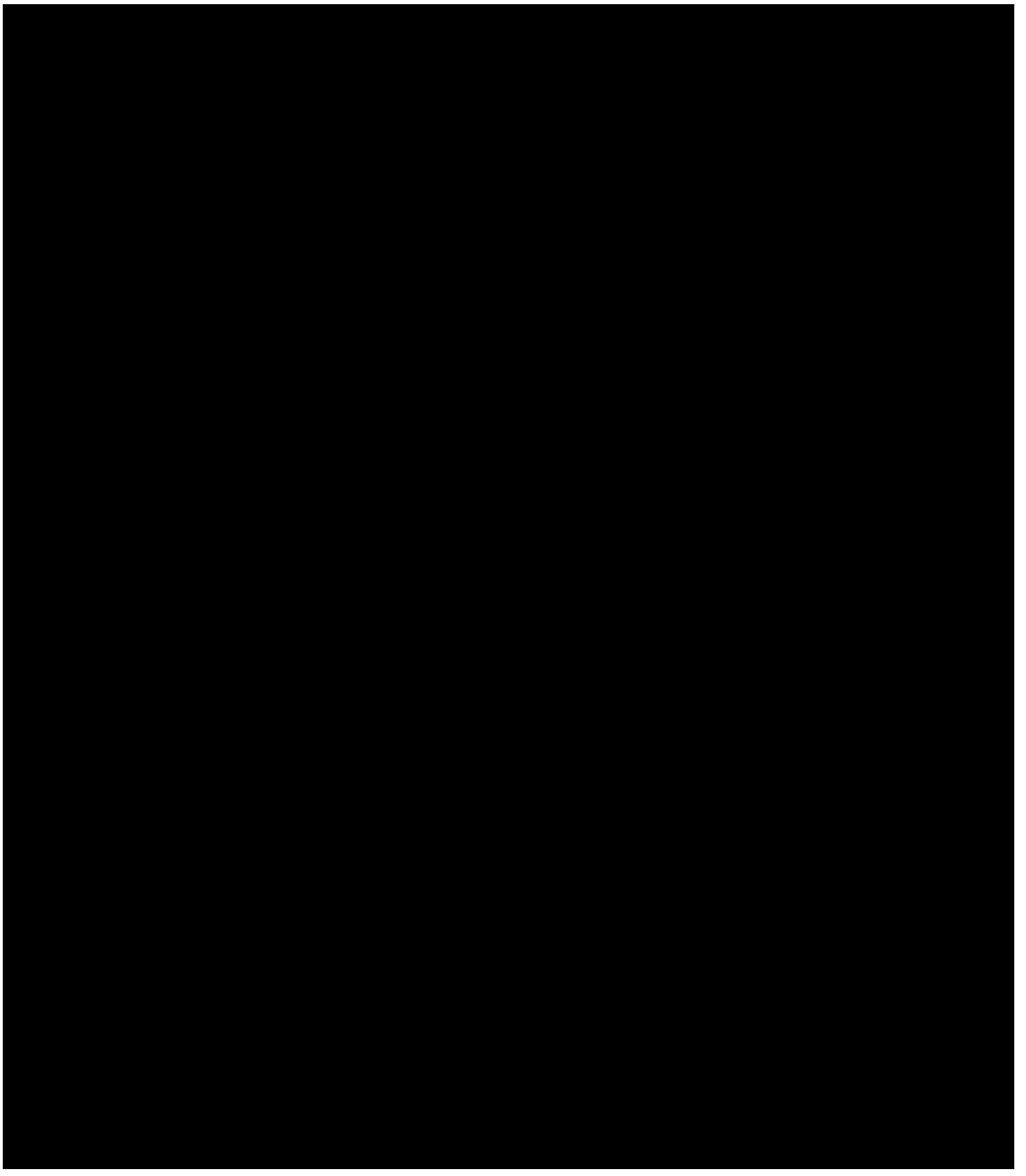
16.4 Updated Response Criteria for High-grade Glioma: Response Assessment in Neuro-oncology Working Group (Attached)

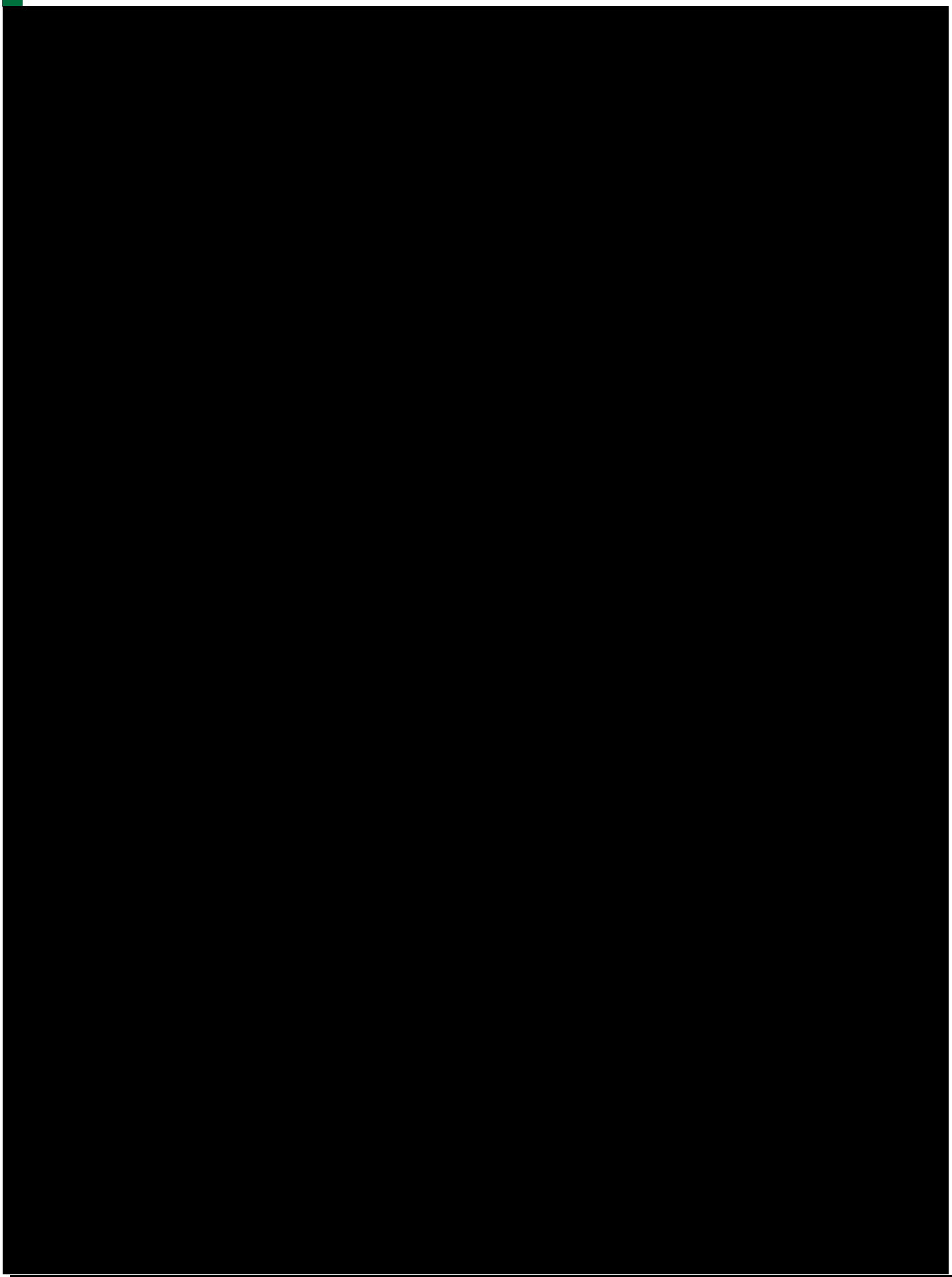
16.5 Immunotherapy Response Assessment in Neuro-Oncology: a Report of the RANO Working Group (Attached)



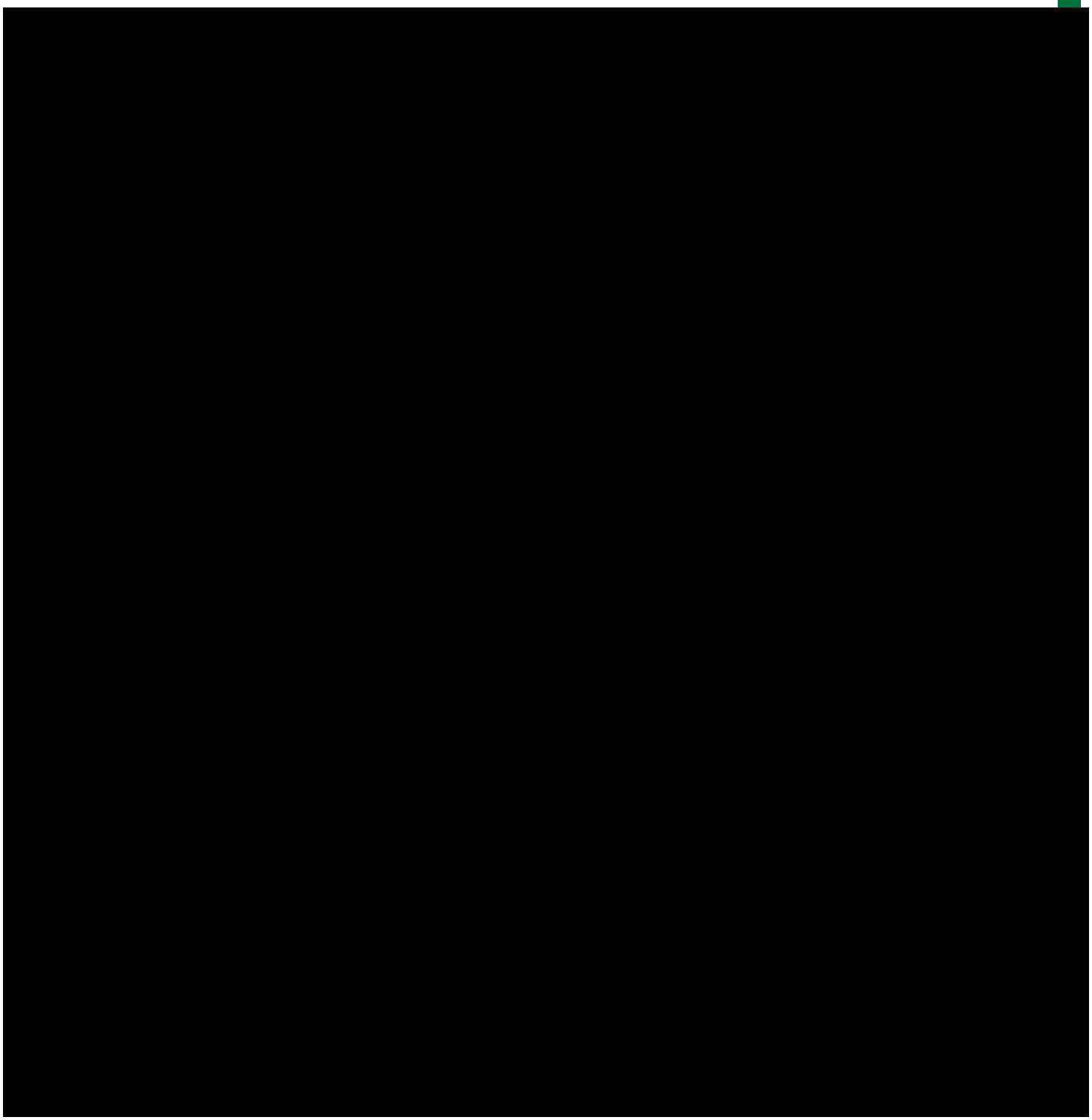
[redacted]







e



Protocol Amendment for Ad-RTS-IL-12 + Veledimex (Amendment 1)

Effective Date: 14 April 2015

Summary of Changes

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
Title Page, Page Headings and Footers	• Title change for Medical Monitor to Executive Vice President and Chief Medical Monitor • Date change made to the protocol date and to the header on each page.	Updated Protocol Amendment
Global	• Schedule of Assessment and Section 8 in the protocol were corrected for consistency between the two. • Wherever the collection of CSF is mentioned, *if available* has been added. • After all edits were made, numbering of tables and sections were updated in the document.	Updated Protocol Amendment
Primary Objective	To determine the safety and tolerability of varying dose levels of Ad-RTS-hIL-12~~a single intra tumor Ad-RTS-hIL-12 dose (1.0×10^{12} vp) plus escalating~~ and oral veledimex doses in subjects with recurrent or progressive glioblastoma or Grade III malignant glioma	Addition of lower IL-12 dose due to new nonclinical information
Secondary Objective	To determine the veledimex maximum tolerated dose (MTD) when given with varying doses of ~~a single intra tumor~~ Ad-RTS-hIL-12. ~~dose (1.0×10^{12} vp)~~	Addition of lower IL-12 dose due to new nonclinical information
Protocol Synopsis Study Design	This is a Phase I study of varying dose levels of Ad-RTS-hIL-12, administered by intra tumor injection, and varying ~~escalating~~ veledimex doses, administered orally, in subjects with recurrent or progressive glioblastoma or Grade III malignant glioma. This study will investigate ~~a single~~ two intra tumor Ad-RTS-hIL-12 doses (2×10^{11} and 1.0×10^{12} vp) and escalating veledimex doses to determine the safe and tolerable ~~veledimex~~ dose based on the safety profiles observed. This study is divided into three periods: the Screening Period, the Treatment Period and the Follow-up Period. After the Informed Consent is signed, subjects will enter the Screening Period to assess eligibility. Eligible patients will be stratified to one of two groups, according to clinical indication for tumor resection. Patients who are scheduled for a standard of care craniotomy and tumor resection (Group 1) will receive one veledimex dose before the resection procedure. Samples (tumor, cerebrospinal fluid (CSF) (if available) and blood) will be collected during the resection procedure to determine the veledimex concentration ratio between the tumor, the CSF and the blood. ~~Approximately 1.0×10^{12}~~ Ad-RTS-hIL-12 by dose cohort (either 2×10^{11} or 1×10^{12} vp) ~~viral particles (vp)~~ will be administered by [redacted] injection. After Ad-RTS-hIL-12 injection, patients will continue on oral veledimex for 14 days. Patients not scheduled for tumor resection (Group 2) will receive ~~approximately 1.0~~1 $\times 10^{12}$ Ad-RTS-hIL-12 viral particles by stereotactic injection and then will continue on oral veledimex for 14 days. Note: Subjects in Group 1 will receive up to a total of 15 veledimex doses: one veledimex dose 3 ± 2 hours before the craniotomy procedure (first dose prior to craniotomy will be a dose specific to the assigned cohort) and tumor resection (prior to Ad-RTS-hIL-12 administration), and up to 14 veledimex doses after Ad-RTS-hIL-12 administration. Subjects in Group 2 will receive up to a total of 14 veledimex doses after Ad-RTS-hIL-12 administration.	Addition of lower IL-12 dose due to new nonclinical information

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	Group 1: Patients scheduled for craniotomy and tumor resection. Patients with a clinical indication for tumor resection will receive veledimex dose specific to the assigned cohort 3 ± 2 hours before the craniotomy procedure, on an empty stomach (excluding other medications). At the time of tumor resection, brain tumor, CSF (if available) and blood samples will be collected to determine the veledimex concentration ratio between brain tumor, CSF and blood. Immediately after tumor resection, subjects in Cohorts 1 & 2 will receive ~~approximately~~ Ad-RTS-hIL-12 2 x 10^{11} vp. This will be administered by free-hand injection into approximately two sites within the residual tumor for a total volume of 0.1 mL. The day of Ad-RTS-hIL-12 administration is designated as Day 0. ~~Immediately after tumor resection, approximately 1.0 × 10^{12} Ad-RTS-hIL-12 viral particles will be administered by [redacted] injection into multiple sites (approximately five selected sites) within the residual tumor (day of Ad-RTS-hIL-12 administration is designated as Day 0). The planned total injection volume is up to 0.5 mL given in approximate aliquots of 0.1 mL at each injection site.~~ For Cohorts 3, 4, 5 and 6, immediately after tumor resection, Ad-RTS-hIL-12 1 x 10^{12} vp will be administered by [redacted] injection into multiple sites (approximately five selected sites) within the residual tumor (day of Ad-RTS-hIL-12 administration is designated as Day 0). The planned total injected volume for these cohorts is 0.5 mL given in approximate aliquots of 0.1 mL at each injection site. When available an intra-operative MRI can be performed to guide the Ad-RTS-hIL-12 injection to areas of contrast enhancing tumor tissue. After the Ad-RTS-hIL-12 injection, veledimex will be administered orally for 14 days. The first post resection veledimex dose is to be given on the day immediately following Ad-RTS-hIL-12 administration designated as Day 1, preferably with food. Subsequent veledimex doses are to be taken once daily, in the morning and within 30 minutes of a regular meal. *Group 2: Patients who will not undergo tumor resection* Patients who will not undergo tumor resection will receive Ad-RTS-hIL-12 by standard stereotactic surgery on Day 0. ~~Approximately~~ Ad-RTS-hIL-12 ~~1.0~~1 × 10^{12} ~~Ad-RTS-hIL-12~~ vp ~~viral particles~~ will be administered by stereotactic injection into approximately five intra tumor sites to deliver up to 0.5 mL volume in approximate aliquots of 0.1 mL at each site. Care should be taken to avoid intraventricular or basal cisternal injection. After the Ad-RTS-hIL-12 injection, veledimex will be administered orally for 14 days. The first veledimex dose is to be given on the day immediately following Ad-RTS-hIL-12 administration designated as Day 1, preferably with food. Subsequent veledimex doses are to be taken once daily, in the morning and within 30 minutes of a regular meal. This Group 2 will start dosing patients only after two patients have completed 28 days in Group 1, Cohort 3.	

<table>
<tr><th>Section in Amended Protocol</th><th>Revised or Deleted Text/Section</th><th>Rationale for Change</th></tr>
<tr><td></td><td>Dose-limiting toxicity (DLT) is defined as: one was added
• Diagnostic brain tumor biopsy is not considered a DLT

Expansion cohorts are not prospectively planned in this study. A decision to enroll additional subjects, as part of an expansion cohort, at the determined veledimex MTD and the ~~fixed~~ Ad-RTS-hIL-12 dose ~~(1.0 × 10¹² vp)~~ will be made by the SRC only after the MTD has been identified for one or both groups and safety evaluated.</td><td></td></tr>
<tr><td>Study Synopsis Number of subjects:</td><td>Up to ~~48~~ 72 subjects may be enrolled.</td><td>Addition of 2 cohorts</td></tr>
<tr><td>Protocol Synopsis Investigational Products, Dose and Mode of Administration</td><td>Ad-RTS-hIL-12 Dose
This study will explore ~~one~~ varying dose levels of Ad-RTS-hIL-12 ~~dose. Approximately 1.0 × 10¹² Ad-RTS-hIL-12 viral particles (0.5 mL)~~ . The Ad-RTS-hIL-12 will be administered by either [redacted] injection into residual tumor sites immediately after tumor resection (Group 1) or by stereotactic injection at intra tumor sites (Group 2).

<table>
<tr><th rowspan="2">Cohort</th><th>Ad-RTS-hIL-12[a] (Day 0)</th><th>Veledimex[b] (Days 1-14)</th></tr>
<tr><th>Dose(vp)</th><th>Total Daily Dose (mg)</th></tr>
<tr><td>1</td><td>2 x 10¹¹</td><td>20</td></tr>
<tr><td>2</td><td>2 x 10¹¹</td><td>40</td></tr>
<tr><td>3</td><td>1 × 10¹²</td><td>20</td></tr>
<tr><td>4</td><td>1 × 10¹²</td><td>40</td></tr>
<tr><td>5</td><td>1 × 10¹²</td><td>80</td></tr>
<tr><td>6</td><td>1 × 10¹²</td><td>120</td></tr>
<tr><td colspan="3">[a] Intra Tumor Injection
[b] Oral Administration Post Ad-RTS-hIL-12 on Days 1-14. Dose levels may be modified based on additional clinical and nonclinical PK data (Section 6.4)</td></tr>
</table></td><td>Addition of lower IL-12 dose due to new nonclinical information</td></tr>
</table>

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
Introduction Section 1.7	**Glioblastoma Patient Starting Dose Rationale** The current plan for the Phase I study in glioblastoma is to begin dosing with veledimex PO at 20 mg/day + Ad-RTS-hIL-12 at 2 x 10^{11} vp administered IT. [redacted] for our Phase I study in patients with recurrent or progressive glioblastoma or Grade 3 malignant glioma. Based on our animal modeling and our clinical experience with Ad-RTS-hIL-12 and veledimex, we expect a significant safety margin and tolerability profile. However, we will also implement rigorous safety monitoring and will review incoming data on an ongoing basis. ~~The Ad-RTS-hIL-12 dose of approximately 1.0 × 10^{12} viral particles (vp) was~~	Addition of lower IL-12 dose due to new nonclinical information as provided in revised IB, ver. 4.1

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	~~selected based on nonclinical and clinical evidence indicating that this would be within an effective range, as well as data from other trials in which doses of adenoviral based vectors at doses up to 3×10^{12} viral particles were well tolerated, with no DLTs, following intra tumor administration (Sangro et al. 2004; Patel et al. 2009).~~ In summary, these results support the administration of Ad-RTS-hIL-12 at 2 x 10^{11} vp IC intratumorally with a starting dose of veledimex administered PO at 20 mg QD x 14 days.	
1.8 Introduction: Clinical Studies of Ad-RTS-hIL-12 and Veledimex in Malignancies	**Melanoma Patient Population** Protocol ATI001-101 is a Phase 1/2 study evaluating the safety and tolerability of Ad-RTS-hIL-12 administered by intra tumor injection at a constant dose (~~1.0~~1 × 10^{12} vp) plus escalating veledimex doses (5mg, 20mg,, 80, 100mg, 120 and 160 mg) in subjects with unresectable Stage III or IV melanoma. The primary objective is the evaluation of safety and tolerability while secondary objectives include assessment of anti-tumor activity, PK profile, immunological effect and schedule determination. Please see Investigator Brochure for most current information on this study. [redacted]	Updated safety information from updated investigator brochure, ver. 4.1

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
[redacted]	[redacted]	[redacted]

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	[redacted] Please see the Investigator Brochure for the most current information on this study. [redacted] **Summary of Safety** Please see the Investigator Brochure for the most current safety information. [redacted] [redacted] ~~2.~~ [redacted]	

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	~~EVENTS IN ONE SUBJECT), ANEMIA AND~~ [redacted]	
Section 3 Study Design	This is a Phase I study of varying doses of Ad-RTS-hIL-12, administered by intra tumor injection, and varying ~~escalating~~ veledimex doses administered orally, in subjects with recurrent or progressive glioblastoma or Grade III malignant glioma. This study will investigate an ~~single~~ Ad-RTS-hIL-12 dose of 2×10^{11} and ~~1.0~~1 $\times 10^{12}$ viral particles and escalating veledimex doses to determine the veledimex MTD based on the observed safety profile and in the presence of variable corticosteroid exposure. *Group 1: Patients scheduled for craniotomy and tumor resection* Patients with a clinical indication for tumor resection will receive veledimex dose specific to the assigned cohort 3 ± 2 hours before the craniotomy procedure, on an empty stomach (excluding other medications). At the time of tumor resection, brain tumor, CSF (if available) and blood samples will be collected to determine the veledimex concentration ratio between brain tumor, CSF and blood. Immediately after tumor resection, subjects in Cohorts 1 & 2 will receive Ad-RTS-hIL-12 2×10^{11} viral particles. This will be administered by [redacted] injection into approximately two sites within the residual tumor for a total volume of 0.1 mL. The day of Ad-RTS-hIL-12 administration is designated as Day 0. For Cohorts 3, 4, 5 and 6, immediately after tumor resection, Ad-RTS-hIL-12 1×10^{12} viral particles will be administered by [redacted] injection into multiple sites (approximately five selected sites) within the residual tumor (day of Ad-RTS-hIL-12 administration is designated as Day 0). The planned total injected volume for these cohorts is 0.5 mL given in approximate aliquots of 0.1 mL at each injection site. When available an intra-operative MRI can be performed to guide the Ad-RTS-hIL-12 injection to areas of contrast enhancing tumor tissue.	Addition of lower IL-12 dose due to new nonclinical information and clarification of language for cohorts

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	After the Ad-RTS-hIL-12 injection, veledimex will be administered orally for 14 days. The first post resection veledimex dose is to be given on the day immediately following Ad-RTS-hIL-12administration designated as Day 1, preferably with food. Subsequent veledimex doses are to be taken once daily, in the morning and within 30 minutes of a regular meal. ~~Patients with a clinical indication for tumor resection will receive veledimex dose specific to the assigned cohort 3 ± 2 hours before the craniotomy procedure, on an empty stomach (excluding other medications). At the time of tumor resection, brain tumor, CSF and blood samples will be collected to determine the veledimex concentration ratio between brain tumor, CSF and blood. Immediately after tumor resection, subjects in cohorts 1 & 2 will receive approximately 2 x 10^{11} Ad-RTS-hIL-12 viral particles administered by [redacted] injection into multiple sites (approximately two selected sites) within the residual tumor (day of Ad-RTS-hIL-12 administration is designated as Day 0). The planned total injection volume For cohorts 1 & 2 is up to 0.1 mL given in divided dose at each injection site. For cohorts 3, 4, 5 and 6, immediately after tumor resection, approximately 1 x 10^{12} Ad-RTS-hIL-12 viral particles will be administered by [redacted] injection into multiple sites (approximately five selected sites) within the residual tumor (day of Ad-RTS-hIL-12 administration is designated as Day 0). The planned total injected volume for these cohorts is 0.5 mL given in approximate aliquots of 0.1 mL at each injection site. When available an intra-operative MRI can be performed to guide the Ad-RTS-hIL-12 injection to areas of contrast enhancing tumor tissue.~~ ~~Patients with a clinical indication for tumor resection will receive a cohort specific oral dose of veledimex 3 ± 2 hours before the craniotomy procedure, on an empty stomach (excluding other medications). At the time of tumor resection, tumor, CSF and blood samples will be collected to determine the veledimex PK. Neuropathology can reconfirm malignant glioma diagnosis from frozen tissue; surgeon will proceed with Ad-RTS-hIL-12 injection in case sample is insufficient for diagnosis. Immediately after tumor resection, Cohort 1 and 2 subjects will receive approximately 2 x 10^{11} Ad-RTS-hIL-12 and Cohorts 3-6 will receive approximately 1.0 × 10^{12} Ad-RTS-hIL-12 viral particles. These doses will be administered by [redacted] injection into multiple sites (approximately five selected sites) within the residual tumor. The planned total injection volume will be up to 0.5~~	

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	~~mL given in approximate aliquots of 0.1 mL at each injection site. When available an intra-operative MRI can be performed to guide the Ad-RTS-hIL-12 injection to areas of contrast enhancing tumor tissue.~~ ~~After the Ad-RTS-hIL-12 injection, veledimex will be administered orally for 14 days. The first post resection veledimex dose is to be given on the day immediately following Ad-RTS-hIL-12 injection designated as Day 1, preferably with food. Subsequent veledimex doses are to be taken once daily, in the morning and within 30 minutes of a regular meal.~~ *Group 2: Patients who will NOT~~not~~ undergo tumor resection* Patients who will not undergo tumor resection will receive Ad-RTS-hIL-12 by standard stereotactic surgery. ~~Neuropathology~~ Tumor biopsy is recommended to ~~can~~ reconfirm malignant glioma~~ from frozen tissue.~~ ~~Approximately~~ Ad-RTS-hIL-12 ~~1.0~~1 × 10^{12} ~~Ad-RTS-hIL-12~~ viral particles will be administered by stereotactic injection into approximately five intra tumor sites to deliver up to 0.5 mL volume in approximate aliquots of 0.1 mL at each site. Extreme care should be taken to avoid intraventricular or basal cisternal injection or other critical locations.	
Section 6 Treatment Plan and Table 5	Patients Undergoing Tumor Resection (Group 1) 1. 3 ± 2 hours before craniotomy, patients will be given a cohort specific dose of veledimex by mouth, on an empty stomach (excluding other medications). The actual time of veledimex administration should be noted and recorded. 2. Surgical planning will be performed on a diagnostic MRI acquired prior to the surgery as per standard of care. 3. At the time of tumor resection tumor, CSF (if available) and blood samples will be collected. 4. Immediately after tumor resection, when available, an intraoperative MRI can be performed to identify contrast enhancing or T2/FLAIR hyper intense residual tumor. If intraoperative MRI is not available, the neurosurgeon will select sites for injection. 5. Patients in Cohorts 1 & 2 will receive Ad-RTS-hIL-12 2 x 10^{11} vp. This will be administered by free-hand injection into approximately two sites within the residual tumor for a total volume of 0.1 mL selected by the neurosurgeon. When available an intra-operative MRI can be	Addition of lower IL-12 dose due to new nonclinical information

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
	performed to guide the Ad-RTS-hIL-12 injection to areas of contrast enhancing tumor tissue. 6. For Cohorts 3, 4, 5 and 6, immediately after tumor resection, Ad-RTS-hIL-12 1 x 10^{12} vp will be administered by [redacted] injection into multiple sites (approximately five selected sites) within the residual tumor at sites selected by the neurosurgeon. The planned total injected volume for these cohorts is 0.5 mL given in approximate aliquots of 0.1 mL at each injection site. When available an intra-operative MRI can be performed to guide the Ad-RTS-hIL-12injection to areas of contrast enhancing tumor tissue. 4.7. ~~Approximately 1.0 × 10^{12} Ad-RTS-hIL-12 viral particles will be administered by [redacted] injection into multiple sites (approximately five intra tumor sites) selected by the neurosurgeon. When available, an intraoperative MRI can be used to guide the injections to contrast enhancing tumor sites. The planned total injection volume will be up to 0.5 mL given in approximate aliquots of 0.1 mL at each injection site.~~ The day of Ad-RTS-hIL-12 administration is designated as Day 0. Two additional cohorts have been added at a lower starting dose for Ad-RTS-hIL-12: **Cohort** | **Ad-RTS-hIL-12[a] (Day 0) Dose(vp)** | **Veledimex[b] (Days 1-14) Total Daily Dose (mg)** 1 | 2 x 10^{11} | 20 2 | 2 x 10^{11} | 40 3 | 1 × 10^{12} | 20 4 | 1 × 10^{12} | 40 5 | 1 × 10^{12} | 80 6 | 1 × 10^{12} | 120 [a] Intra Tumor Injection [b] Oral Administration Post Ad-RTS-hIL-12 on Days 1-14. Dose levels may be modified based on additional clinical and nonclinical PK data (Section 6.4)	

Section in Amended Protocol	Revised or Deleted Text/Section	Rationale for Change
Section 6.4 Modification of dose escalation schedule	A period of at least one week will be required to analyze the veledimex concentration ratio in brain tumor, CSF and blood from patients in Group 1. ~~This study adopts a starting veledimex dose for Cohort 1 of 20 mg total daily veledimex and a planned dose escalation schedule for Cohort 2 at 40 mg, Cohort 3 at 80 mg, and Cohort 4 at 120 mg, given once daily.~~ Interpretable data from at least three subjects should be sufficient to provide an initial estimate of the veledimex concentration in human brain tissue and the ratio of veledimex in the tumor, CSF (if available) and blood. Based on this initial estimate and data from subsequent cohorts, dosage level may be modified after review by the SRC and DSMB.	Addition of lower IL-12 dose due to new nonclinical information
Section 6.6 Maximum Tolerated Dose	The veledimex MTD given in combination with either Ad-RTS-hIL-12 2 x 10^{11} or 1 x 10^{12} vp ~~a constant Ad-RTS-hIL-12~~ dose will be that dose at which fewer than 33 % of subjects experience a DLT.	Addition of lower IL-12 dose due to new nonclinical information
Section 10.4 Determination of Severity	The following was deleted: ~~Adverse events should be reported using the maximum intensity of the event (e.g. if a subject reported nausea lasting 3 days, 1 start date and stop date should be recorded along with the maximum intensity experienced for that event over that 3-day timeframe).~~	Information regarding the capture of adverse events is included in the eCRF Completion Guidelines.

PROTOCOL AMENDMENT SUMMARY

Protocol Title:	A Phase I Study of Ad-RTS-hIL-12, an Inducible Adenoviral Vector Engineered to Express hIL-12 in the Presence of the Activator Ligand Veledimex in Subjects with Recurrent or Progressive Glioblastoma or Grade III Malignant Glioma
Protocol Number:	ATI001-102
Study Drugs:	Adenovirus-[redacted]-human interleukin-12 (Ad-RTS-hIL-12) and veledimex (RTS activator ligand)
Date of Protocol:	Amendment 2: 29 January2016 Amendment 1: 14 April 2015 Original: 12 February 2014

NOTE TO INVESTIGATORS

Amendment 2 dated 29 January 2016 will be used to conduct the study in place of any preceding version of this protocol. ZIOPHARM Oncology will implement this version as of 01 February 2016. This protocol should be submitted to your IRB promptly.

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AMENDMENT 2

ATI001-102

1. Summary and Rationale for Changes

ZIOPHARM is amending the clinical protocol for the ATI001-102 study, "A Phase I Study of Ad-RTS-hIL-12, an Inducible Adenoviral Vector Engineered to Express hIL-12 in the Presence of the Activator Ligand Veledimex in Subjects with Recurrent or Progressive Glioblastoma or Grade III Malignant Glioma" to provide updated guidance to the investigator based on review of DLTs with the SRC and DSMB; to incorporate currently approved iRANO guidelines, correct inconsistencies between the synopsis and the body of the protocol and to clarify the use of CYP450 3A4 medications. Additionally updates including correction of typos, administrative edits and formatting errors were made.

These updates included:

- addition of the word "intratumoral" preceding Ad-RTS-hIL-12 throughout as well as the change from "intra tumor" to "intratumoral"
- the clear designation of the day of Ad RTS hIL 12 administration as "Day 0" (as indicated in the Schedule of Study Procedures) throughout the protocol
- the follow-up period was divided into two portions – Initial Follow up Period and Long Term Follow up as certain procedures only took place up to Day 56
- the addition of abbreviations after the first instance of a phrase and replacement of the words with the abbreviations in later sections of the protocol
- grammatical corrections
- formatting of tables and figures, including alphabetizing the legends and addition of abbreviations under the majority of tables.

2. Tabular Summary of Key Revisions Implemented in the Amended Protocol

Section in Amended Protocol	Revision	Rationale for Change
Title Page and Footers	**FROM:** Amendment 1: 14 April 2015 **TO:** Amendment 2: 29 January 2016	Update to current protocol version and date of amendment.
Synopsis Study Design	**Addition of text:** "in the presence of variable corticosteroid exposure." **FROM:** This study will investigate two intra tumor Ad RTS hIL 12 doses [2 x 10^{11} viral particles (vp) and 1 × 10^{12} vp] and	Updated to align with Section 3.1 of the protocol.

Section in Amended Protocol	Revision	Rationale for Change
	escalating veledimex doses to determine the safe and tolerable dose based on the safety profiles observed. **TO:** This study will investigate two intratumoral Ad RTS hIL 12 doses [2 x 10^{11} viral particles (vp) and 1×10^{12} vp] and escalating veledimex doses to determine the safe and tolerable dose based on the safety profiles observed **in the presence of variable corticosteroid exposure.**	
Synopsis Study Design	**FROM:** "This Group 2 will start dosing patients only after two patients have completed 28 days in Group 1, Cohort 3." **TO**: "Subject enrollment into Group 2 will start no earlier than after two subjects have completed 28 days in Group 1, Cohort 1. Enrollment into Group 2 cohorts is otherwise independent of enrollment into Group 1 cohorts."	Text regarding Cohort 3 was an administrative error when the new dosing cohorts were added in Amendment 1. The change clarifies the enrollment timing. This new language was add in Section 3.1 as well.
Synopsis and Section 13.4	**ADDITION OF:** Study Duration The duration of this study from the time of initiating subject screening until the completion of survival follow up is anticipated to be approximately 48 months, including 24 months for enrollment and 24 months of follow up. The start of study is defined as the date when the first subject is consented into the study and the study stop date is the date of database lock.	Updated to align with the body of the protocol.
Synoposis Study Objectives and Section 2.2	**REMOVAL OF:** CSF from secondary objectives.	CSF is not available for all subjects.
Synopsis, Study Design and Section 3.1	**ADDITION OF:** The total amount delivered to each site will be recorded in the CRF. In the event that less than the planned total injected volume is administered, the reason will be provided.	As the protocol indicated that up to a specific amount of drug would be delivered, language was added to ensure that the data being collected would reflect actual volume delivered.
Synopsis, Section 3.1, 3.2, 4.5	**FROM:** 28 days (Day 0-14, plus 14 days) of DLT evaluation period **TO:** 14 days (Day 0-14) of DLT evaluation period	The initial period that is being reviewed to make the determination from dosing subject 1 to allowing enrollment of 2 and 3 is an additional 7 over the 14 day evaluation period, therefore,

Section in Amended Protocol	Revision	Rationale for Change
		the DLT evaluation period was updated to reflect the intention of the 14 day review for subsequent subjects as opposed to a longer time of review for subsequent subjects.
Synopsis, Section 3.3	**FROM:** If a significant safety event occurs with the first patient in each cohort, the SRC will convene to evaluate the safety event(s) and make a recommendation and decision on the enrollment of the second and third subjects in that same cohort. If any cohort requires the enrolment of 6 subjects, the SRC will evaluate safety and make a recommendation and decision between the stratification groups. The SRC may also seek an ad hoc safety evaluation and recommendation by external experts and/or the DSMB as needed. At the end of each cohort, the SRC will convene to review the safety data after the third patient in a cohort, within a group, has completed veledimex dosing and has been monitored for 28 days after the Ad-RTS-hIL-12 injection. **TO**: If a significant safety event occurs with the first patient in each cohort, the SRC will convene to evaluate the safety event(s) and make a recommendation and decision on the enrollment of the second and third subjects in that same cohort. At the end of each cohort (Group 1 and 2 separately), the SRC will convene to review the safety data after the final patient in a cohort, within a group, has completed veledimex dosing and has been monitored for 28 days after the Ad RTS hIL 12 injection.	Removal of additional sentences that represented potential conflict about timing of meetings. Clarified to represent that the SRC will meet at the end of a cohort, regardless of how many subjects are enrolled. Sentence that the SRC can get expert advice not necessary for the protocol.
Synopsis, Section 4.1	**The following text was added regarding the washout period:** windows other than what is listed below should be allowed only after consultation with the Medical Monitor	The washout periods in the inclusion criteria are a guideline and the specific drug and its relative half-life should be reviewed to make eligibility determination – the new language allows for updated guidance in relation to washout period to be considered as new therapies become available.
Synopsis, Section 4.2	**FROM:** Use of medications that induce, inhibit or are substrates of CYP450 3A4 within seven days prior to veledimex dosing.	The new language allows the Medical Monitor and Investigator to discuss appropriate washout periods

Section in Amended Protocol	Revision	Rationale for Change
	TO: Use of medications that induce, inhibit, or are substrates of cytochrome p450 (CYP450) 3A4 within 7 days prior to veledimex dosing without consultation with the Medical Monitor	for CYP450 3A4 medications or alternate therapies/schedules to be discussed.
Section 7.2	**Added Text:** NOTE: Care should be given when prescribing medications that are classified as CYP450 3A4 inducers, inhibitors, or substrates due to potential interactions with the study drug. In the event that one is prescribed, consultation with the Medical Monitor is advised. All medications should be recorded in the case report form as indicated in the completion guidelines.	Update necessary to allow for Institutions to treat subjects according to their standard practice, with consultation with the Medical Monitor.
Synopsis, Section 6.1	**The following text was added to the veledimex column of the dose cohort table:** Group 1 (Days 0 through 14) Group 2 (Days 1 through 14)	Clarification.
Synopsis Study Design and Section 6.5	**FROM:** Dose-limiting toxicity (DLT) is defined as: • Any local reaction that requires operative intervention and felt to be attributable to Ad-RTS-hIL-12 and/or veledimex • Any local reaction that has life-threatening consequences requiring urgent intervention or results in death and felt to be attributable to Ad RTS hIL 12 and/or veledimex • Any study drug related Grade 3 adverse event (AE) • Diagnostic brain tumor biopsy is not considered a DLT Note: Seizures, intracranial venous thrombosis, and cerebral edema are commonly observed in this patient population and will be recorded according to grade of toxicity, but will not be considered a DLT unless they are deemed drug related **TO:** Dose-limiting toxicity (DLT) is defined as: An event, occurring within the treatment period (Day 0 - Day 14) that meets one of the following conditions: • Any local reaction that requires operative intervention and felt to be attributable to Ad RTS hIL 12 and/or veledimex • Any local reaction that has life threatening consequences requiring urgent intervention or results in death and felt to be attributable to Ad RTS hIL 12 and/or veledimex • Any study drug-related Grade 3 or higher adverse event (AE) persisting for more than 5 days (i.e. not resolving to Grade 2 or lower) despite optimal medical support	Language was updated to clarify that a one-time lab value or otherwise transient grade 3 or higher event would not be a DLT. Additionally, headache was added as a known observation in this population and intracranial venous thrombosis was removed at the request of the DSMB. Language about the SRC being involved in the evaluation of DLTs in order to make a determination of the MTD was added in alignment with Section 6.6 of the protocol.

Section in Amended Protocol	Revision	Rationale for Change
	• Optimal medical support will include the timely temporary or permanent discontinuation of veledimex in conjunction with changes in administration of steroids (or other supportive care) as applicable. In the event that the medical support provided does not meet these conditions, the SRC will review the event to make a DLT determination. Note: Diagnostic brain tumor biopsy is not considered a DLT. Seizures, ~~intracranial venous thrombosis,~~ **headache** and cerebral edema are commonly observed in this population and will be recorded according to grade of toxicity, but will not be considered a DLT unless they are deemed as most likely to be drug related as the main contributory factor.	
Section 9.1	**FROM:** Tumor Response, Pharmacodynamic and PK Analyses: Tumor response analysis will be performed on the ESP. Investigator assessment of ORR and PFS will be determined for each dose cohort according to RANO criteria (Appendix 16.5) from the date of ICF signature through the Follow-up Period. A two sided confidence interval will be computed for the ORR. PFS and OS will be analyzed using the Kaplan Meier method. **TO:** Tumor Response, Overall Survival, Pharmacodynamic and PK Analyses: Tumor response analysis will be performed on the ESP. Investigator assessment of ORR and PFS will be determined for each dose cohort according to RANO/iRANO criteria (Appendix 16.5 and Appendix 16.6). Overall survival (OS) is defined as the duration of time from the first dose of study drug to the date of death, or, for subjects who are still alive two years post first dose of study drug, they will be censored at the last follow-up contact date. A two sided confidence interval will be computed for the ORR. PFS and OS will be analyzed using the Kaplan Meier method.	Updated to include current guidelines (iRANO). Please note: Use of iRANO was added to the RANO guidelines throughout the protocol (represented as RANO/iRANO) with the exception of the inclusion criteria which specifies RANO alone. Similar language was updated in the Synopsis.
Section 9.1 and Schedule of Study Assessments	**The following text was added:** A repeat scan to confirm progression should be completed at 4 weeks (per RANO) and preferably again at 12 weeks (per iRANO) after first documentation of progression. Consideration should be given to performing a diagnostic brain biopsy, which should be performed in accordance with the current iRANO guidelines.	Updated to align with current guidelines (iRANO).
Section 13.4, Schedule of	**FROM:** In addition, subjects who discontinue or complete study treatment without objective evidence of disease progression	Clarification of the overall survival data collection. The protocol inadvertently linked

Section in Amended Protocol	Revision	Rationale for Change
Study Procedures	should continue to be followed until confirmed disease progression has been documented, or an alternate anticancer therapy has been initiated, or one year after enrollment, whichever occurs first. The active study period refers to the study period from informed consent through the Follow-up Period. **TO:** In addition, subjects who discontinue or complete study treatment without objective evidence of disease progression should continue to be followed until confirmed disease progression has been documented. Subjects will be followed for survival status for 2 years after enrollment, whichever occurs first. The active study period refers to the study period from informed consent through the Initial Follow up Period.	survival to progression or new anti-cancer therapy. The language was updated to clarify that data will be requested during alternate anticancer therapy and for 2 years. Similar language was updated in the Synopsis.
Schedule of Study Procedures	The footnotes of Schedule of Study Procedures were updated to align with the body of the protocol (see track changes attached)	Clarification to align with protocol changes.
Synopsis, Section 3.1, Section 6.1	**FROM:** For Cohorts 3, 4, 5 and 6, immediately after tumor resection, Ad-RTS-hIL-12 1 x 10^{12} viral particles will be administered by [redacted] injection into multiple sites (approximately five selected sites) within the residual tumor…The planned total injected volume for these cohorts is 0.5 mL given in approximate aliquots of 0.1 mL at each injection site. **TO:** For Cohorts 3, 4, 5 and 6, immediately after tumor resection, Ad RTS hIL 12 1 x 10^{12} vp will be administered by [redacted] injection into multiple sites in a divided dose within the residual tumor …The planned total injected volume for these cohorts is 0.5 mL given in approximate aliquots of 0.1 mL at each injection site.	Clarification.
Throughout	**FROM:** Within 30 minutes of a regular meal. TO: Within approximately 30 minutes of a regular meal.	Update to allow for expected normal variation.
Section 10.2	**ADDED Text:** Adverse events will be followed through the Day 56 Visit. AEs that are drug-related should be followed until resolved or no resolution is expected.	To align with Section 10.7 which requires reporting of SAEs through 30 days post last dose of study drug (i.e. Day 44). Following AEs through Day 56 fulfills the SAE reporting requirements conservatively.

Section in Amended Protocol	Revision	Rationale for Change
Appendix 16.2	**Appendix was removed**	Updated to be in line with revised guidance with relation to CYP450 3A4 medications.
Appendix 16.5 and 16.6	**Appendix 16.5 was replaced with the RANO guidelines and Appendix 16.6 (iRANO guidelines) was added**	Included to align with protocol revisions.

PROTOCOL AMENDMENT SUMMARY

Protocol Title:	A Phase I Study of Ad-RTS-hIL-12, an Inducible Adenoviral Vector Engineered to Express hIL-12 in the Presence of the Activator Ligand Veledimex in Subjects with Recurrent or Progressive Glioblastoma or Grade III Malignant Glioma
Protocol Number:	ATI001-102
Study Drugs:	Adenovirus-[redacted]-human interleukin-12 (Ad-RTS-hIL-12) and veledimex (RTS activator ligand)
Date of Protocol:	Amendment 3: 14 March 2016 Amendment 2: 29 January 2016 Amendment 1: 14 April 2015 Original: 12 February 2014

NOTE TO INVESTIGATORS

Amendment 3 dated 14 March 2016 will be used to conduct the study in place of any preceding version of this protocol. ZIOPHARM Oncology will implement this version as of 15 March 2016. This protocol should be submitted to your IRB promptly.

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AMENDMENT 2

ATI001-102

1. Summary and Rationale for Changes

ZIOPHARM is amending the clinical protocol for the ATI001-102 study, "A Phase I Study of Ad-RTS-hIL-12, an Inducible Adenoviral Vector Engineered to Express hIL-12 in the Presence of the Activator Ligand Veledimex in Subjects with Recurrent or Progressive Glioblastoma or Grade III Malignant Glioma" to provide updated guidance to the investigator on the DLT evaluation period and the definition of DLT. Additionally updates including correction of typos, administrative edits and formatting errors were made.

These updates included:

- grammatical corrections
- renumbering of Appendices to correct administrative error (Appendices inadvertently skipped from 16.1 to 16.3 in Protocol Amendment 2)

2. Tabular Summary of Key Revisions Implemented in the Amended Protocol

Section in Amended Protocol	Revision	Rationale for Change
Title Page and Footers	**FROM:** Amendment 2: 29 January 2016 **TO:** Amendment 3: 14 March 2016	Updated to reflect current protocol version and date of amendment.
Synopsis, Section 3.1, 3.2, 4.5	**FROM:** 14 days (Day 0-14) of DLT evaluation period **TO:** 28 days (Day 0-14, plus 14 days) of DLT evaluation period	The DLT evaluation period was updated to allow for a longer time of review for subjects.
Synopsis Study Design and Section 6.5	**FROM:** Dose-limiting toxicity (DLT) is defined as: An event, occurring within the treatment period (Day 0 - Day 14) that meets one of the following conditions: • Any local reaction that requires operative intervention and felt to be attributable to Ad RTS hIL 12 and/or veledimex • Any local reaction that has life threatening consequences requiring urgent intervention or results in death and felt to be attributable to Ad RTS hIL 12 and/or veledimex • Any study drug-related Grade 3 or higher adverse event (AE) persisting for more than 5 days (i.e. not resolving to Grade 2 or lower) despite optimal medical support	Language was updated [redacted]

Section in Amended Protocol	Revision	Rationale for Change
	• Optimal medical support will include the timely temporary or permanent discontinuation of veledimex in conjunction with changes in administration of steroids (or other supportive care) as applicable. In the event that the medical support provided does not meet these conditions, the SRC will review the event to make a DLT determination. **TO:** An event, occurring within the first 28 days (Day 0 - Day 28) that meets one of the following conditions: • Any local reaction that requires operative intervention and felt to be attributable to Ad RTS hIL-12 and/or veledimex • Any local reaction that has life threatening consequences requiring urgent intervention or results in death and felt to be attributable to Ad RTS hIL-12 and/or veledimex • Any Grade 3 or greater non-hematological adverse event that is at least possibly related to the study drug • Any Grade 4 hematologic toxicity that is at least possibly related to the study drug and lasts at least 5 days • Grade 3 or higher thrombocytopenia at least possibly related to the study drug	
Section 4.4	**The following text was added:** Subjects who withdraw during the treatment period should continue to have study assessments as clinically indicated.	Updated to clarify how a subject should be followed if he/she discontinues treatment but remains on study.
Section 6.3	**FROM:** An intermediate dose cohort may be explored during the dose escalation phase, as deemed necessary and as recommended by the DSMB and the SRC. **TO:** An intermediate dose cohort may be explored during the dose escalation phase, as deemed necessary and as recommended by the SRC.	Correcting administrative error; updated to align with the rest of the protocol.
Schedule of Study Procedures	**The following clarification was added:** MRI Scans are collected during long term follow-up.	Administrative error corrected, footnote text was correct, however, the X was missing in the table.
Schedule of Study Procedures	**FROM:** Tumor Sample[u] **TO:** Tumor Sample[u] (Group 1 only)	Correction; “Group 1 only” specification was inadvertently removed in Protocol Amendment 2.