Clinical Protocol Number: MDNA55-05

An Open-Label Non-Randomized, Multi-Center Phase-2 Study of Convection-Enhanced Delivery (CED) of MDNA55 in Adults with Recurrent or Progressive Glioblastoma

Product Name:	**MDNA55**
Indication:	**Recurrent or Progressive Glioblastoma**
Version Number:	**Version 6.1; 19 JUL 2019**
IND Number:	**BB IND 007004**
Sponsor:	**Medicenna Therapeutics Inc.** **2 Bloor Street West, Suite 700** **Toronto, ON M4W 3E2** **Canada**

SIGNATURE PAGE

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(Original: Version 6.1; 19 JUL 2019)

An Open-Label Non-Randomized, Multi-Center Phase-2 Study of Convection-Enhanced Delivery (CED) of MDNA55 in Adults with Recurrent or Progressive Glioblastoma

Reviewed and Approved:

[redacted] Advisor, Neurosurgeon and Principal Investigator

_____ **Signature** _____ **Date** (dd/mm/yyyy)

[redacted] Advisor, Neuro-oncologist and Principal Investigator

_____ **Signature** _____ **Date** (dd/mm/yyyy)

[redacted]
Medical Monitor

_____ **Signature** _____ **Date** (dd/mm/yyyy)

Rosemina Merchant, MESc
Chief Development Officer
Medicenna Therapeutics Inc.

_____ **Signature** _____ **Date** (dd/mm/yyyy)

PRINCIPAL INVESTIGATOR PROTOCOL AGREEMENT PAGE

Protocol MDNA55-05

(Original: Version 6.1; 19 JUL 2019)

An Open-Label Non-Randomized, Multi-Center Phase-2 Study of Convection-Enhanced Delivery (CED) of MDNA55 in Adults with Recurrent or Progressive Glioblastoma

I agree to conduct this study as described in this protocol (MDNA55-05), including all future protocol amendments and in accordance with all the Sponsor's guidelines provided to me. The study will be conducted in compliance with 21 CFR Parts 50, 56, and 312 and EU Clinical Trials Directive (EUCTD); the International Council on Harmonization guidelines on Good Clinical Practice [ICH E6 (R2)], and all applicable local regulatory requirements.

I agree to provide all information requested in the Protocol, Work Sheets and the Electronic Document Capture (EDC) system provided to me by the Sponsor in a manner to assure legibility and accuracy, and to this end, I agree to follow all instructions for completing the Work Sheets and EDC within 5 business days of recording the corresponding data.

I also agree that all information provided to me by the Sponsor, including non-clinical data, Investigator's Brochure, Protocol, guidelines, manuals, EDC system, and verbal and written information, will be kept strictly confidential and confined only to personnel involved in conducting this study. It is recognized that this information may be given in confidence to the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) to obtain written and dated approval for the protocol, written informed consent, consent-form updates, subject-recruitment procedures (*e.g.*, advertisements), and any other written information to be provided to the subjects, before initiating this study.

In addition, no reports or information (verbal and written) regarding this study, its progress or results, will be provided to anyone not involved in the study other than to regulatory agencies or other competent authorities as required by law, the Sponsor, or in confidence to the IRB/IEC, without written and authorized permission of the Sponsor.

I agree not to implement any changes to, or deviations from the protocol without prior agreement in writing from the Sponsor and after review and documented approval from the

IRB/IEC, except to eliminate an immediate health hazard to the study subjects or to provide prompt and necessary medical care.

Audits and inspections may be performed at the Investigator site before, during, and after the study by competent authorities and authorized persons of the Sponsor. Informed consent and IRB/IEC approval will assure that competent authorities have access to individual identified patient records as may be required by law.

I acknowledge that the Sponsor of the study, Medicenna Therapeutics Inc., has the right to discontinue the study at any time.

** NOTE: The MDNA55 Investigator's Brochure, Pharmacy Manual, Study Reference Guide and Image Acquisition Guide are cited in this protocol and provide pertinent information regarding the investigational drug and the procedures to be conducted in this study. By signing below, you acknowledge that you have received and read these referenced study documents and will follow all procedures described in this protocol.*

Principal Investigator (Print Name)	**Signature**	**Date** (dd/mm/yyyy)

PERSONNEL RESPONSIBLE FOR CONDUCTING STUDY

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PROTOCOL SYNOPSIS

TITLE:	An Open-Label Non-Randomized, Multi-Center Phase-2 Study of Convection-Enhanced Delivery (CED) of MDNA55 in Adults with Recurrent or Progressive Glioblastoma
RATIONALE:	MDNA55 is a fusion toxin comprising a genetically engineered circularly permuted interleukin-4 (cpIL-4) fused to a modified version of the *Pseudomonas aeruginosa* exotoxin A (PE). MDNA55 binds to the IL-4 receptor (IL4R), over-expressed by cancer cells and non-malignant immunosuppressive cells of the tumor micro-environment (TME), and delivers a potent cell-killing agent, PE. A large percentage of glioblastomas (GBs) and their TME express IL4R in relatively high amounts, making it a relevant target for MDNA55. Intra- and peritumoral infusion minimizes systemic exposure to the fusion toxin, while the image-guided CED technique enhances exposure of active drug throughout the target region. MDNA55 shares many properties with immunotherapies, such as immune checkpoint inhibitors, including the possibility of response following a prolonged (> 3 months) period of pseudo-progression. Given acknowledged difficulties in determination of true progression in patients treated with immunotherapies, the determination of progressive disease (PD) will be informed by use of multimodality MRI and/or biopsy prior to subject withdrawal.
STUDY OBJECTIVES:	**Primary** To assess overall survival (OS) **Secondary** To assess the effect of IL4-R status on overall survival (OS) To assess the safety of MDNA55 following CED To determine the objective response rate (ORR) per Response Assessment in Neuro-Oncology (RANO)-based criteria incorporating advanced imaging modalities. To assess progression-free survival (PFS) **Exploratory** To assess the pharmacokinetics (PK) of MDNA55 in peripheral plasma To assess serum anti-MDNA55 antibody titers and, if elevated, determine neutralizing antibody titers To determine the relationship between clinical outcomes and response assessment status by different sets of imaging-based response criteria To perform additional *ad hoc* efficacy and safety analyses as needed based on the data acquired in this study To assess the performance of the Brainlab catheter during infusion in terms of distribution and convection of the infusate using real time MRI monitoring
KEY ENROLLMENT CRITERIA:	In order to be eligible to participate in this study, male and female subjects ≥ 18 years of age must have primary (*de novo*) GB that has recurred or progressed (per standard RANO criteria), a life expectancy > 12 weeks and a Karnofsky performance status (KPS) ≥ 70. Subjects must have tumor diameter of ≥ 1 cm x ≥ 1 cm (minimum) to 4 cm in any direction by pre-interventional magnetic resonance imaging (MRI) within 14 days of planned treatment and not have features which make the tumor a poor target for CED (*e.g.* significant liquefaction or geometric features not conducive to CED). IL4-R status of tumor will be determined based on archived biopsy from the time of initial diagnosis.

STUDY DESIGN:	This is a single-arm, open-label, multicenter study in approximately 52 adults (at least 46 evaluable) with GB that has recurred or progressed (according to standard RANO criteria). The study will be conducted at up to 12 clinical sites following institutional review board approval and completion of informed consent. The concentration and volume of MDNA55 will be adjusted based on tumor size such that total dose will not exceed the established maximum tolerated dose (MTD) of 240 µg. Doses up to 180 µg have been used in this study to-date, with no evidence of dose dependency for safety. Eligible subjects will undergo surgery associated with catheter placement at which time a tissue biopsy will also be performed. MDNA55 will be infused with the objective of achieving coverage of the tumor and the peritumoral margin to the maximum extent possible as indicated by distribution of a co-infused gadolinium tracer observed by MRI. Pre-treatment catheter trajectory planning will be performed with aim to place up to 4 catheters but a minimum of 2, depending upon the tumor size. Planning for catheter placement will only target the enhancing region of the tumor on MRI. Infusion via each catheter will be initiated at the rate of 3 µL/min/catheter and gradually increased in a stepwise manner. The infusion flow rate can be adjusted at the discretion of the Investigator during real time MRI (with subject maintained under anesthesia) provided that the flow rate per catheter does not exceed 10 µL/min. All functioning catheters should be convecting at similar flow rates. The flow rate should be established such that the duration of infusion is at least 24 hours to a maximum of approximately 48 hours. In the event only one catheter is functioning the flow rate may be increased to complete infusion in 48 hours or less but greater than 24 hours. After the real-time MRI infusion monitoring period is completed, the remainder of the infusion will continue with the subject awake. MRI will be performed upon completion of infusion as a final evaluation of MDNA55 infusate distribution. [redacted]

	[redacted] Post-treatment follow-up assessment of safety will be performed 14 days after CED infusion. Thereafter, efficacy and safety assessments will be performed at 30, 60, 90 and 120 days after CED infusion and every 8 weeks thereafter until 360 days of active follow up have been completed. Subjects who discontinue before the Day 360 visit will undergo all the procedures scheduled for the Day 360 visit at the time of discontinuation. Subjects who complete the Day 360 assessment without disease progression or discontinue early without disease progression will continue to be followed for disease status until progression where possible. After progression (on study or during post-study follow-up), subjects will continue to be followed, where possible, for survival, post-study treatment(s) for GB and imaging for GB until death (or termination of data collection by the Sponsor or withdrawal of consent by the subject).
INVESTIGATIONAL PRODUCT AND ROUTE OF ADMINISTRATION:	MDNA55 drug product is supplied as a sterile frozen solution at a concentration of [redacted] labeled according to country-specific regulatory requirements. For clinical use, MDNA55 drug product will be diluted in Elliotts B® solution to produce an infusate of MDNA55 consisting of [redacted] human serum albumin and [redacted] gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA, Magnevist®). The infusate is prepared at the hospital pharmacy and instructions for its preparation are provided in the Pharmacy Manual. MDNA55 is administered via infusion using CED with precision planning and real-time MRI monitoring of infusate distribution.
DOSAGE AND FREQUENCY:	[redacted] The total dose will not exceed 240 µg (the established MTD). At the discretion of the Investigator a subject may be eligible to receive a second administration of MDNA55.
EFFICACY ENDPOINTS:	**Primary** mOS, defined as the time from treatment until death **Secondary** mOS by tumor IL4-R status OS12 (proportion of subjects surviving 12 months) ORR as determined by an independent central review according to RANO-based criteria incorporating advanced imaging modalities (e.g. diffusion, perfusion and/or TRAMs) PFS, defined as the time from treatment until disease progression or death.

	Other Duration of response (DOR), defined as the time from first response until disease progression or death among those subjects achieving a complete response (CR) or partial response (PR) to treatment. Duration of clinical benefit (DOCB), defined as the time from first response or disease stabilization until disease progression or death among those subjects achieving a complete response (CR), partial response (PR), or stable disease (SD) Additional, exploratory, efficacy analyses may include ORR and PFS based on the Investigator's assessment of response, other time-to-event endpoints (*e.g.*, time to post-study treatment of GB) and exploration of response according to biomarker status.
SAFETY ENDPOINTS:	Serious adverse events (SAEs) and treatment emergent adverse events (AEs) Clinical laboratory results Physical and neurological examinations KPS Electrocardiogram (ECG) Additional, exploratory, safety analyses may include specific AEs of interest and the relationship of safety to plasma MDNA55 pharmacokinetic (PK) parameters and/or evaluation of immune parameters.
OTHER ENDPOINTS:	MDNA55 PK parameters in peripheral plasma Anti-MDNA55 antibody titer in serum Neutralizing antibody titer (if anti-MDNA55 titer observed)
STATISTICAL ANALYSES, POWER AND ANALYSIS POPULATIONS:	**Subject Populations** Following quality review of study images mid-way through recruitment, local tissue reactions, inflammation, immune cell infiltration, edema and/or necrosis, subsequent to MDNA55 administration, was observed in some subjects and has been seen to be indistinguishable from possible tumor growth using the imaging techniques adopted from the outset of this study. Therefore, obtaining reliable imaging data for the primary efficacy analysis has been shown to be confounded. Consequently, under protocol version 6.0, the surrogate endpoint Objective Response Rate (ORR) will be assessed as a secondary outcome measure with the median Overall Survival (mOS) becoming the primary variable. Intent to Treat (mITT) An ITT population will comprise all subjects who receive any amount of study drug will be used for the primary efficacy analyses. A modified ITT population for secondary response analyses (mITT, see below) will comprise all subjects who receive any amount of study drug and have adequate imaging or clinical data for the ORR analysis. *NOTE: Patients who expire or progress clinically prior to the first MRI examination will not be evaluable for any of the response assessments.* Per Protocol (PP) Population The PP population will comprise all subjects in the mITT population who also have no major protocol violations during the study. This population will be finalized prior to the final database lock and primary analysis of the study data. Efficacy analyses will be conducted on this population in support of the primary efficacy results.

	IL4R Analysis Population The IL4R analysis population will be the same as the ITT Population, excluding patients who do not have archived tissue or adequate tissue available for analysis, and will be used for efficacy analyses in subgroups according to IL4R expression status. Safety Population The Safety population will comprise all subjects treated on study. Safety analyses will be presented on this population. **Analyses** The primary efficacy analysis of OS will be assessed according to a single-arm, single-stage design on the ITT population. OS will be determined via Kaplan-Meier estimation, with medians, quartiles, and 95% confidence intervals (CIs) reported. mOS will tested using a single sample one sided log-rank test on a 10% significance level against historical control with null hypothesis of mOS of 8 months and alternative hypothesis of OS > 8 months. With at least 46 evaluable ITT subjects there will be over 80% power for the primary analysis. A secondary analysis of the primary variable will be conducted in the IL4-R population according to IL4-R status. Details of the planned analyses and methods will be published in the SAP (statistical analysis plan) prior to database lock. PFS will be determined via Kaplan-Meier estimation, with medians, quartiles, and 95% confidence intervals (CIs) reported. mPFS will be tested with a single sample one sided log-rank test on a 10% significance level against historical control with null hypothesis of mPFS 4 months and alternative hypothesis of OS > 4 months. Descriptive analysis of the OS and PFS at 6, 9, and 12 months after treatment will be based on the raw proportions of subjects surviving (and progression-free) at those time points as well as Kaplan-Meier estimation. Further analyses will also be conducted by IL4R stratum, including examination of the treatment effect by IL4R level. Efficacy analyses may also explore subject subsets (*e.g*. IL4R level, tumor size, KPS, gender, age, steroid use,) and response by other applicable criteria. Descriptive statistics will be provided for subject demographics and disposition, safety, and exposure data and will include the number of observations, mean, standard deviation, median, and range for continuous variables and number and percent for categorical variables; 95% CIs will be presented where appropriate.

MODIFIED FOR PROTOCOL VERSION 6.1

[redacted]	[redacted]
	[redacted]
[redacted]	[redacted]
	[redacted]
	[redacted]

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ADP	adenosine diphosphate
AE	adverse event
ALT (SGPT)	alanine transaminase (serum glutamate pyruvate transaminase)
AST (SGOT)	aspartate transaminase (serum glutamic-oxaloacetic transaminase)
BUN	blood urea nitrogen
CED	convection-enhanced delivery
CI	confidence interval
CNS	central nervous system
cp	circular permutation/permuted
cpIL-4	circularly permuted version of IL-4 (see also MDNA55)
CR	complete response
CSC	cancer stem cell
CSF	cerebral spinal fluid
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DOCB	duration of clinical benefit
DOR	duration of response
DWI	diffusion weighted imaging
ECG	Electrocardiogram
EDC	electronic data capture
FDA	Food and Drug Administration
FLAIR	fluid-attenuated inversion recovery
Gd-DTPA	gadolinium-diethylenetriamine pentaacetic acid (Magnevist®)
GB	glioblastoma
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HSA	human serum albumin
IHC	Immunohistochemistry
ICH	International Council on Harmonization
IEC	Independent Ethics Committee
IL-4	interleukin-4
IL-4PE	IL-4 fused to a portion of *Pseudomonas* Exotoxin A (code name: MDNA55)
IL4R	IL-4 receptor
INR	International normalized ratio
IRB	Institutional Review Board
IV	intravenous(ly)
KPS	Karnofsky performance status
LDH	lactate dehydrogenase
MDNA55	Study drug; IL-4PE; previously called NBI 3001, PRX321
MDSC	myeloid derived suppressor cell
MedDRA	Medical Dictionary for Regulatory Activities
MGMT	O^6-methylguanine-methyltransferase
MRI	magnetic resonance imaging
MTD	maximum tolerated dose

NCI	National Cancer Institute
NBF	Neutral Buffered Formalin
NIH	National Institutes of Health
OR	objective response
ORR	objective response rate
OS	overall survival
PBS	phosphate buffered saline
PD	progressive disease
PE	*Pseudomonas* exotoxin
PFS	progression-free survival
P-gp	p-glycoprotein
PK	pharmacokinetic
PR	partial response
PsR	pseudo-response
PsP	pseudo-progression
PT	prothrombin time
RANO	Response Assessment in Neuro-Oncology (criteria)
mRANO	modified RANO
RNA	ribonucleic acid
rCBV	relative cerebral blood volume
RCD	real-time convective delivery
SAE	Serious adverse event
SD	stable disease
SOC	System Organ Class
SRC	Safety Review Committee
TAM	tumor associated macrophages
Th2	T-helper cell type-2
TME	tumor microenvironment
TRAM	Tumor Response Assessment Map
US	United States
USP	United States Pharmacopoeia
Vd	volume of distribution
Vd/Vi	volume of distribution: Volume of infusion ratio
VEGF	vascular-endothelial growth factor
Vi	volume of infusion
WBC	white blood cell (count)
WHO	World Health Organization

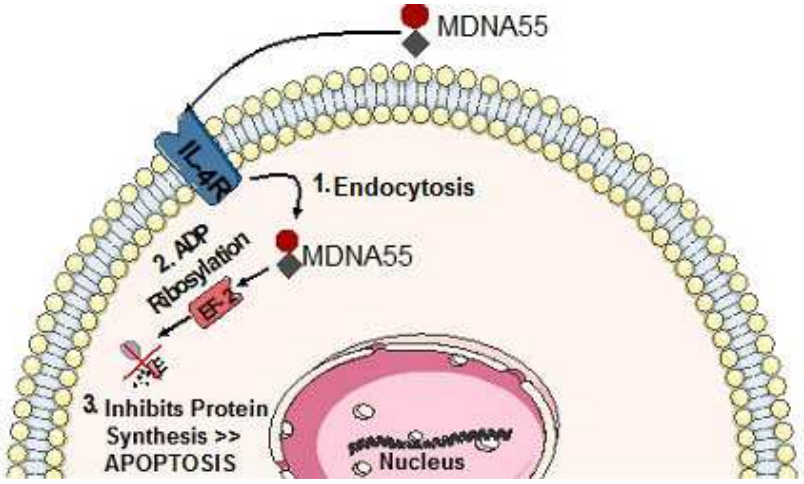
1 Introduction

The study drug, MDNA55, is a fusion protein consisting of a targeting domain linked to a pro-apoptotic cell-killing payload. It was discovered and developed by Drs. Raj Puri (United States [US] Food and Drug Administration [FDA]) and Ira Pastan (National Cancer Institute "NCI") and has been described by various researchers in over 50 publications. It is a therapeutic agent that selectively targets cancer cells that over-express the interleukin-4 receptor (IL4R).

The targeting domain is an engineered circularly permuted version of interleukin-4 (cpIL-4) which is genetically fused to potent payload comprised of a truncated version of the bacterial toxin, *Pseudomonas aeruginosa* exotoxin (PE) A (Kreitman *et al.*, 1994). It was developed for the treatment of glioblastoma (GB) and other adult and pediatric central nervous system (CNS) cancers including immunosuppressive cells of the glioblastoma tumor microenvironment (TME) that frequently over-express the IL-4 receptor (IL4R; Puri *et al.*, 1994; Kohanbash *et al.*, 2013).

The mechanism of action of MDNA55 is well documented (Kreitman *et al.*, 1994; Rand *et al.*, 2000; Puri *et al.*, 2009) and is depicted in Figure 1. MDNA55 binds to IL4R overexpressed on the surface of tumor cells and the entire complex is endocytosed. Following cleavage and activation by furin-like proteases found in high concentrations in the endosome of cancer cells, the catalytic domain of the truncated PE is released into the cytosol where it induces cell death *via* adenosine diphosphate (ADP)-ribosylation of the Elongation Factor-2 and induction of apoptosis through caspase activation (Shapira and Benhar, 2010).

Figure 1: Schematic of MDNA55 Mechanism of Action



Many features of MDNA55 make it a rational choice for the treatment of GB and other primary and metastatic tumors in the CNS:

1. The majority of cancer biopsy and autopsy samples from adult and pediatric CNS tumors, including recurrent GB biopsies, have been shown to over-express the IL4R with little or no IL4R expression in normal adult and pediatric brain tissue (Puri *et al.,* 1996; Kawakami *et al.,* 2002; Joshi, *et al*., 2001; Konanbash *et al*., 2013). Cells that do not express the IL4R target do not bind to MDNA55 and are, therefore, not subject to PE-mediated effects (Kawakami *et al.*, 2002; Puri *et al.,* 2005).

2. Unlike chemotherapeutic agents and radiation, MDNA55's cell-killing ability is not growth-rate dependent (Li and Hall, 2010). Due to its mechanism of action, quiescent cancer stem cells and slower growing non-malignant cells of the TME may be as sensitive to MDNA55 as rapidly dividing tumor cells.

3. O^6-methylguanine-methyltransferase (MGMT) positive cancer cells (harboring unmethylated MGMT promoters and therefore resistant to temozolomide) are sensitive to MDNA55. CNS and non-CNS cancer cell lines such as T98G (glioblastoma), HT-29 (colon cancer), and Mia-Paca-2 (pancreatic cancer) are known to over-express MGMT and are resistant to alkylating agents such as temozolomide (Huang *et al.,* 2012; Kuo *et al.,* 2007; Kokkinakis *et al.,* 2003). However, these IL4R-expressing cell lines show picomolar sensitivity to MDNA55 (Puri *et al.,* 1996; Kreitman *et al.,* 1995; Shimamura *et al.,* 2007), indicating that MDNA55 could provide a treatment option for MGMT positive GB patients.

4. Furin-like proteases are required for cleavage of MDNA55 and activation of the PE toxin (Chironi *et al.,* 1997; Shapira and Benhar, 2010). High expression levels of furin in targeted glioma cells as opposed to normal cells (Mercapide, *et al*., 2002; Wick *et al*., 2004) provides additional tumor specificity and is also a contributory factor to the exceptional picomolar sensitivity of cancer cells to MDNA55.

5. The pro-apoptotic domain of MDNA55 (*i.e.,* PE) is far more potent than chemotherapeutic agents (Li and Hall, 2010). It kills cancer cells by arresting protein synthesis (Shapira and Benhar, 2010), a mechanism not employed by other anti-cancer agents.

6. Internalization of MDNA55 into the target cell occurs *via* a mechanism that is independent of p-glycoprotein (P-gp), a membrane associated protein that is commonly used to transport chemotherapeutic drugs. Mutations in P-gp often lead to cancer cells becoming resistant to traditional chemotherapeutic drugs, a problem not expected with MDNA55

since it does not rely on P-gp for entry into the cell (Strome *et al.,* 2002; de Jong *et al.*, 2003).

7. GB has a robust immunosuppressive TME and may comprise up to 40% of the tumor mass (Kennedy *et al.*, 2013). Recently, it has been shown that malignant gliomas have a T-helper cell type-2 (Th2) bias and are heavily infiltrated by myeloid derived suppressor cells (MDSCs) and tumor associated macrophages (TAMs) and that the IL4/IL4R bias mediates their immunosuppressive functions (Harshyne, *et al.*, 2016). Furthermore, IL4R is up-regulated on glioma-infiltrating myeloid cells but not in the periphery or in normal brain (Kohanbash *et al.*, 2013). Thus, purging Th2 cells, MDSCs, and TAMs using MDNA55 may alleviate the immune block associated with cancer (in a manner similar to immunomodulators such as ipilumimab, pembrolizumab or nivolumab), thereby promoting anti-tumor immunity and aid in long-term disease control.

Safety of MDNA55 has been adequately characterized in non-clinical studies. In addition, safety and efficacy of MDNA55, administered as a single dose by local intra- and peritumoral infusion *via* convection-enhanced delivery (CED) has been evaluated in a total of 72 adults with high grade recurrent gliomas (including 66 adults with recurrent GB) in three prior clinical studies. All nonclinical and clinical studies conducted to date are summarized in the current Investigator's Brochure.

Although not designed for efficacy, the Phase 1/2 clinical studies in adults with recurrent GB treated with MDNA55 generated sufficient data during and post-study to warrant supplemental analysis of tumor response, survival and the effect of tumor response on survival outcomes. Analysis of efficacy is provided in the current Investigator's Brochure.

Results from prior studies show promising anti-tumor effects when MDNA55 is administered *via* CED. Thus, by implementing recent advances in CED, a multi-center, single-arm, open-label study will be carried out in approximately 52 (at least 46 evaluable) patients with GB at recurrence or progression who will receive MDNA55 via intra- and peritumoral infusion using CED. The efficacy of MDNA55 will be investigated with subjects having tumor diameter of ≥ 1 cm x ≥ 1 cm (minimum) to 4 cm in any direction, no more than 2 relapses, and a Karnofsky score of ≥ 70 which are consistent with the inclusion criteria used in recent clinical trials for recurrent GB. Of note, the IL4R tumor expression profile, of subjects treated with MDNA55 in prior clinical studies was not determined. In this study, a retrospective analysis of IL4R expression of the archived tissue obtained at first diagnosis of GB will be conducted to determine the role of the IL4R biomarker on treatment response and patient outcomes. In addition, the IL4R biomarker and other

immunotherapy markers may be retrospectively tested if pre-treatment and/or post-treatment biopsy samples are available.

Planning software will be utilized to optimize catheter placement (Rosenbluth *et al.*, 2012) and small diameter catheters with a stepped design will be used to significantly reduce reflux along the catheter tract (Jahangiri *et al.,* 2016; Krauze *et al*., 2005b) in order to improve distribution of MDNA55. Furthermore, co-infusion of a surrogate tracer (gadolinium-diethylenetriamine pentaacetic acid [Magnevist®] [Gd-DTPA]) during CED will enable effective real-time monitoring of drug distribution thereby ensuring adequate coverage of the tumor and the peritumoral infiltrating margin with MDNA55 (Chittiboina *et al*., 2014; Jahangiri *et al.,* 2016). Use of the latest CED technologies and real-time imaging has the potential to improve MDNA55 distribution, its safety as well as patient outcomes.

1.1 Treatment of Recurrent or Progressive GB

Standard first-line treatment for primary GB includes surgical resection of the bulk tumor to the maximal extent possible consistent with neurological preservation, followed by radiotherapy, often in combination with temozolomide (Stupp protocol; Stupp *et al.*, 2005). When relapse or progression occurs in patients who have undergone the Stupp protocol, therapeutic options are unfortunately limited and generally not effective.

Surgery may be indicated in a minority of relapsed patients with disease that is symptomatic from mass effect, but it results in only limited prolongation of survival (Keles *et al.,* 2004). Survival may be improved by combining surgery with the Gliadel® (carmustine) implant. However, the majority of patients with relapsed disease are not candidates for additional surgery (Weller *et al.*, 2013). The use of Gliadel is thereby limited as surgery is required for Gliadel administration.

Avastin® (bevacizumab) has been seen to improve 6-month progression free survival (PFS) to 42.6% and increase in median overall survival to 9.3 months in patients with recurrent GB. Genentech received accelerated approval from the US FDA for Avastin®, in patients with recurrent GB patients who had failed first-line chemotherapy, based on an overall response rate of 28% (Friedman *et al.,* 2009).

Despite these agents, there is an urgent need for more effective targeted therapies for the treatment of recurrent GB. Intra- and peritumoral infusion *via* CED of targeted fusion toxin, such as MDNA55, is a promising novel tumor-specific therapeutic for the treatment of this disease.

1.2 Targeted Intratumoral Therapy

1.2.1 Fusion Toxins

Fusion toxins fall into the category of targeted therapy and generally consist of highly potent bacterial or plant toxin moieties (payloads) fused to tumor-specific ligands (targeting domains). They represent a novel anti-cancer modality that may offer several advantages over conventional therapies. One such novel fusion toxin in development is MDNA55. Fusion toxins such as MDNA55 take advantage of the selective expression of receptors (*e.g.*, IL4R) on tumor cells, cancer stem cells (CSCs) and tumor microenvironment (TME) (safety and tolerability) with the effectiveness of potent toxins (anti-tumor efficacy). The function of the targeting domain of MDNA55 (*i.e.*, IL-4) is "to guide" the toxin specifically to the tumor cells while sparing normal cells. Fusion toxins such as MDNA55, directed at tumor specific targets therefore, exhibit a relatively wide therapeutic index when compared to conventional chemotherapeutic agents.

MDNA55 has the following unique characteristics:

- Induces tumor shrinkage independent of growth rate. Quiescent CSCs, slow growing cells of the TME and rapidly dividing cancer cells are equally sensitive at the picomolar range (Hall *et al*., 1992)
- Uses a multi-pronged approach to cancer therapy. MDNA55 is able to simultaneously target bulk tumor, deplete CSCs (Merchant *et al.*, 2015) and may also purge TAMs and MDSCs, key components of the TME (Bankaitis and Fingleton, 2015)

New understanding of the role played by the TME in protecting cancer indicates that targeting cancer cells alone will not significantly alter survival outcomes. With a multi-pronged approach, MDNA55 may therefore provide an overall meaningful and durable long-term response in CNS tumors that over-express the IL4R.

1.2.2 Interleukin-4 Receptors as a Drug Target for GB

The study drug, MDNA55, was developed for the treatment of recurrent GB, as various types of CNS tumors have been known to frequently over-express the IL4R and available data demonstrate that a large percentage of GBs express the IL4R at relatively high levels (Puri *et al*., 1994; Puri *et al*., 1996; Joshi *et al*., 2001; Joshi *et al*., 2002). Detailed information on IL4R as a drug target for GB including recent studies evaluating IL4R expression in matched biopsy samples of newly diagnosed and recurrent GB obtained from the same patient are provided in the current

Investigator's Brochure. These data indicate that GB patients continue to express the IL4R at recurrence and in some cases, at much higher levels.

1.2.3 Convection Enhanced Delivery

MDNA55 is a large fusion protein (53 kDa) and as such is not able to cross the blood brain barrier (BBB). In order to by-pass the BBB, localized delivery techniques such as convection enhanced delivery (CED) are being widely developed for CNS diseases. CED improves drug delivery to brain tumors intraparenchymally by utilizing bulk flow, or fluid convection, established as a result of a pressure gradient, rather than a concentration gradient (Yin *et al*., 2011). As such, CED offers markedly improved distribution of infused therapeutics within the CNS compared to direct injection or via drug eluting polymers, both of which depend on diffusion for parenchymal distribution. Additionally, CED obviates the challenges of systemic agents crossing the BBB while minimizing systemic exposure and toxicity. (Fiandaca *et al*., 2008; Yin *et al*., 2011; Vogelbaum and Aghi, 2015). Advantages of CED over diffusion-based delivery include:

- Expanded volume of distribution (Vd); volume of distribution being greater than the volume of infusion (Vi);
- Uniform concentration of the infused therapeutic within the target volume;
- Delivery of the vast majority of the infused therapeutic within the target volume.

CED distribution is enhanced by the arterial pulsations within the brain's perivascular spaces (Hadaczek *et al.,* 2006). Additionally, better understanding of the complexities of the extracellular matrix and its effects on convection has led to improved distribution (Hamilton *et al.,* 2001; Neeves *et al.,* 2007; Nguyen *et al.,* 2001). For example, technical CED infusion parameters, such as cannula size and shape, infusion rate, infusate concentration, and tissue sealing time, have been defined and refined to improve distribution of study agents while limiting potential toxicities and morbidities (Morrison *et al.,* 1999; Chen *et al.,* 1999; Wein *et al*., 2002; Krauze *et al.,* 2005b; Healy and Vogelbaum, 2015; Lewis *et al*., 2016).

1.2.4 Real-time Imaging of Convective Delivery

A major advance in the safe and potentially efficacious use of CED in neurosurgery has been the development of real-time imaging of convective delivery (RCD), which utilizes magnetic resonance imaging (MRI) to visualize the CED process with the aid of co-convected contrast agent (Krauze *et al.,* 2005a; Fiandaca *et al.,* 2009; Nguyen *et al.,* 2003; Krauze *et al.,* 2005b; Murad *et al.,* 2006; Lonser *et al.,* 2007; Chittiboina, *et al*., 2014; Lonser, *et al*., 2015). Use of RCD

allows physicians to directly monitor distribution of therapeutics within the brain. Thus, reflux along the CED catheter or leakage outside the target area, especially at higher flow rates, can be monitored and corrective steps taken, such as retargeting the catheter or altering the rate of infusion (Fiandaca *et al.,* 2008; Varenika *et al.,* 2008).

The RCD technique will be used in this study and represents an important advancement in drug delivery and distribution in the brain. Earlier clinical trials that did not utilize RCD did not achieve adequate distribution of the study drug, which may have caused the studies to not meet their clinical endpoints (Gill *et al.*, 2003; Marks *et al.,* 2010; Sampson *et al.,* 2010). Use of RCD in this trial will enable direct visualization of the study drug distribution, permit uniform tumor coverage and enhanced contact between target cells (GB and TME) and MDNA55.

In many studies using RCD, the surrogate tracer of choice has been gadolinium. Gd-DTPA (Magnevist®) is a contrast agent manufactured by Bayer Healthcare Pharmaceuticals, Inc., and has been used clinically for many years.

Prior *in vitro* and *in vivo* studies (Mardor, *et al.*, 2009; Ding *et al.*, 2010) have shown that Gd-DTPA is biocompatible and safe when co-administered with the MDNA55 (see current Investigator's Brochure). Gd-DTPA, in combination with fusion toxins, has also been safely administered intracerebrally to patients in multiple clinical studies using CED (Lonser *et al.,* 2007; Weber *et al.*, 2003a; Weber *et al.,* 2003b; Sampson, *et al.*, 2011; Chittiboina, *et al.*, 2014). Although gadolinium-based contrast agents are not approved for intracerebral administration, these studies support that Gd-DTPA can be safely administered *via* CED infusion in combination with locally administered therapeutics, such as MDNA55.

In 2017 the EMA (EMA/457616/2017) and FDA (FDA Drug Safety Communication [5-22-2017]) both conducted reviews of gadolinium safety following some evidence of low levels of gadolinium deposition in brain tissues following intravenous administration in animals. No evidence of harm to humans has been described, nevertheless minimizing gadolinium exposure is recommended as a precautionary measure.

1.3 Experience with the Investigational Drug (MDNA55)

1.3.1 Nonclinical Experience with MDNA55

All non-clinical studies of MDNA55 alone or in combination with Gd-DTPA are summarized in the current Investigator's Brochure.

1.3.2 Clinical Experience with MDNA55

To date, a total of 86 adults have received MDNA55, including 72 adults with high grade glioma. All prior clinical studies conducted with MDNA55 are summarized in the current Investigator's Brochure.

Multiple doses of MDNA55 have been intratumorally administered to study subjects in an investigator initiated study; Rand *et al*., 2000) where 9 subjects with histologically confirmed recurrent GB were infused with MDNA55. MDNA55 concentration and infusion volumes ranged from 0.2 to 6.0 µg/mL and 30 to 185 mL, respectively. Two of the 9 subjects in this study received more than one infusion.

MDNA55 has been granted orphan drug status by the US FDA and the European Medicines Agency for the treatment of gliomas and Fast Track designation for the treatment of recurrent GB and AA by the US FDA.

1.4 Study Rationale

This study is designed to test the hypothesis that mOS is improved to a clinically significant extent with MDNA55 administered via CED, as compared to current available treatments for recurrent/progressive GB. The assumptions regarding response to current treatment are based on aggregated mOS data from previous clinical trials in patients with recurrent/progressive glioblastoma weighted according to consistency with the subject inclusion criteria in this trial. The current design is based on a null hypothesis that mOS is 8.0 months versus the alternative hypothesis that mOS is 11.5 months following treatment with MDNA55.

1.4.1 Rationale for Endpoint Methods

Overall Survival is the chosen primary endpoint for this trial as an unequivocal, objective, direct measure of clinical outcome.

In this study ORR will be assessed as a secondary endpoint because MDNA55 shares many properties with immunotherapies, including the possibility of pseudo-progression seen on post-treatment imaging and the possibility of response following prolonged (> 3 months) pseudo-progression (Weber *et al.,* 2003a; 2003b; Okada *et al.,* 2015), both of which can confound the assessment of ORR (Ellingson et al., 2017b). The mode of action of MDNA55 includes initial inflammatory response and necrosis in areas of macroscopic and microscopic infiltrative tumor whereby localized inflammatory responses mimic radiological features of tumor progression with increased enhancement and edema (Weber *et al.,* 2003a; 2003b).

Pseudo-progression is an increase in contrast enhancement on MRI without true underlying tumor progression and may be due to tumor necrosis associated with MDNA55's known mechanism or local tissue reaction due to the CED procedure. Subjects with pseudo-progression may develop brain edema, mass effect and other symptoms that are clinically indistinct from typical disease progression. Failure to recognize this phenomenon may result in premature subject withdrawals from the study, prior to establishing evidence of clinical benefit. To minimize these confounding effects, the application of the RANO criteria in this study will include the use of multi-modality imaging assessments for response analysis and allowing of the designation of non-progression status to patients who have no subsequent signs of disease progression on imaging after initially being considered to have progressive disease (PD). Multi-modality assessments include contrast-enhanced MRI, diffusion MRI, perfusion MRI, spectroscopic MRI, TRAMs, clinical evaluation and tumor tissue biopsy to aid identification of pseudo-progression during early progressive enhancement on T1 images with contrast. Furthermore, in determining PD, there will be less weight on T2-FLAIR images associated with edema. Worsening of clinical symptoms attributed to edema or increased use of steroids to control edema will not automatically be registered as PD.

For the same reason, sites will be strongly recommended that subjects remain in the study for at least 120 days post treatment unless unequivocal evidence of confirmed disease progression is agreed.

1.4.2 Rationale for Dose

[redacted]

[redacted]

[redacted]

The total dose will not exceed 240 µg (the established MTD for the compound).

1.4.3 Rationale for Selection of Catheters to be used for Administration of MDNA55

In earlier clinical studies conducted with MDNA55 and other therapeutic agents, catheters were not designed for effective CED and demonstrated that they were prone to reflux and leakage of the therapeutic agent away from the region of interest. In recent years, smaller diameter MRI-compatible catheters with a stepped design have been introduced which have been shown to dramatically reduce reflux along the catheter tract when compared to the large diameter flexible ventricular catheters used in previous GB studies (Krauze *et al.*, 2005b; Rosenbluth *et al.,* 2011; White *et al.,* 2011; Gill *et al.*, 2013; Jahangiri *et al.,* 2016). This has allowed for improved flow rates (see Section 1.4.5).

In the US, there are essentially 2 step design catheter options and both are being and/or have been used in CED studies; the Brainlab Flexible Catheter [510(k) K123605] and the MRI Interventions SmartFlow® Cannula [510(k) K102101]. The Brainlab catheter is an MRI-compatible flexible catheter that has a rigid stylet design for accurate positioning. This catheter can be left in-place post-surgery and will therefore be employed in this study as it allows continued post-surgical infusion. On the other hand, the SmartFlow® cannula is rigid and can only be used

intra-operatively. Like the Brainlab catheter, it is also MRI compatible, has an identical tip design and is amenable for accurate placement. Both catheters are intended for single use only and not intended for implantation. MDNA55 infusate (prepared in Elliotts B® solution together with 0.02% HSA and 7mM of Gd-DTPA) has been tested and shown to be bio-compatible with the Brainlab catheter and other tubing components comprising the infusion assembly and will therefore be used in this study.

1.4.4 Rationale for Infusion Planning / Catheter Placement Planning

The efficiency of CED in distributing a drug into a tumor depends on correct placement of catheters within the tumor, catheter diameter, flow rate, infusate characteristics, and tissue consistency of the treated area (Wein *et al.,* 2002; Sampson *et al*., 2007; Jahangiri *et al.,* 2016). An incorrectly placed catheter can lead to the infused drug exiting the tumor through sulci or taking a path of least resistance into the cerebrospinal fluid (CSF), resulting in limited drug exposure of the tumor, and lack of efficacy and potential toxicity.

To ensure optimal placement for maximal tumor coverage, Brainlab iPlan® Flow software will be used as the primary tool for generating a pre-treatment plan for placement of catheters (Sampson *et al*., 2007) prior to infusion. Using MRI of the tumor obtained prior to infusion, the software will be used to predict the optimal trajectory for placement of each catheter, making sure to avoid fissures, sulci, and other elements that can contribute to inadequate distribution. iPlan® software helps to predict the placement of the catheter tip according to the catheter placement guidelines (see Section 6.3), to optimize convection of the infusate as well as assist in ensuring safe placement of catheters by avoiding blood vessels, *etc*.

For this study, the iPlan®Flow software and MRI imaging will be employed for pre-treatment catheter trajectory planning. Pre-treatment catheter trajectory planning will be performed with aim to place up to 4 catheters but a minimum of 2, depending upon the tumor size. Planning for catheter placement will only target the enhancing region of the tumor on MRI. MRI perfusion imaging and other advanced imaging modalities may be used to inform on optimal placement of the catheter tips in the active tumor and to support trajectory planning to avoid significant vasculature.

All investigational sites selected for this study have prior CED experience; nevertheless, all sites will be thoroughly trained in the correct use of the catheter, infusion planning, catheter placement and CED infusion with peer-to-peer support of early cases to ensure consistency across study sites.

1.4.5 Rationale for Infusate Flow Rate

Infusion parameters, such as catheter size and shape, infusion flow rate and infusate concentration, have been defined as factors that have an effect on the efficiency of CED. In previous clinical studies with MDNA55, the infusion flow rate of 10 µL/min/catheter was evaluated (using large diameter ventricular catheters that were not designed for CED) to administer large volumes of infusate over several days.

As discussed above (Section 1.4.3) smaller diameter catheters designed specifically for CED of therapeutics into the brain tumor have been developed, which permit higher flow rates with minimal back flow due to their step design Krauze *et al.* (2005b) and Richardson *et al.* (2011) as well as recent studies at UCSF (Butowski *et al.,* 2015), showed that 50 µL/min is a safe flow rate without reflux and affords better manipulation of the dynamics of infusion, allowing for better tumor coverage, especially with larger volumes. Further, Butowski *et al.* (2015) reported that for efficient CED in brain tumors, the maximum volume of drug should be infused in the shortest possible time. In this study, flow rates will be individualized as use of real-time MRI infusion monitoring will enable the Investigator to determine the optimal flow rate of infusate for each subject.

Although the MDNA55 infusate has shown to be bio-compatible with the Brainlab catheters at flow rates of up to 50 µL/min, optimal flow rate for effective intra- and peritumoral distribution of MDNA55 *in vivo* via the Brainlab flexible catheter is not established and may vary from patient to patient due to tumor heterogeneity. Therefore, the rate of infusion will be conservatively assessed with flow rate initiated at 3 µL/min/catheter and gradually increased in a stepwise manner to a maximum of 10 µL/min/catheter (see Section 6.3). The infusion flow rate can be adjusted at the discretion of the Investigator during the real time monitoring period to check leakage, reflux and functioning of all implanted catheters (with subject maintained under anesthesia). The flow rate should be established such that the duration of infusion is at least 24 hours to a maximum of approximately 48 hours. In the event only one catheter is functioning the flow rate may be increased to complete infusion in 48 hours or less but greater than 24 hours. After the real-time MRI infusion monitoring period is completed, the remainder of the infusion will continue with the subject awake. MRI will be performed upon completion of infusion as a final evaluation of MDNA55 infusate distribution.

The rate of infusion may be reduced by 50% or the infusion stopped and restarted at the discretion of the Investigator if the subject shows signs of intolerance. Section 7.5.2.3 provides information relating to interruption or discontinuation of infusion. Reduction to the maximum permitted rate of administration to see drug delivery sustained for up to 48 hours may be considered, guided by

ongoing evaluation of potential toxicities by the SRC and advised to the investigators by the sponsor's medical representative accordingly and recorded in the Study Reference Guide.

1.4.6 Rationale for Real-Time Imaging of Infusate Distribution

NeoPharm conducted a study from 2004 to 2006 using an IL-13-PE fusion protein (cintredekin besudotox, CB), to target the IL-13 decoy receptor, IL-13Ralpha2, known to be over-expressed in GB. This was a randomized Phase 3 trial of GB patients at first recurrence in which CED was used to locally administer CB following surgical resection of the tumor. The study drug did not show survival advantage when compared to Gliadel (Kunwar *et al.*, 2010).

Several reasons have been proposed to explain the lack of efficacy of CB (Chandramohan *et al.*, 2012; Jarboe *et al.,* 2007; Sampson *et al.*, 2010).

- The trial did not include real-time imaging to ascertain if the required amount of drug was delivered to the tumor site (Sampson *et al.,* 2010).
- The estimated coverage of relevant target volumes was low, so that only 20.1% of the penumbra surrounding the resection cavity was covered (Chandramohan *et al.*, 2012).
- Post-trial analysis on catheter positioning revealed only 49.8% of catheters met all positioning criteria (Chandramohan *et al.*, 2012).

Thus, even where IL-13Ralpha2 was present in the tumor (Jarboe *et al.,* 2007), delivery of the drug to the tumor target was suboptimal. These results highlight the need for real-time imaging to assess catheter placement and drug distribution.

The objective in this study will be to achieve maximal coverage of the tumor and peritumoral margin. Therefore, real-time imaging of drug distribution, through co-infusion of a tracer will be employed as a means of assessing infusate distribution.

Preclinical studies using MDNA55 were carried out to evaluate the effect of the imaging agent Gd-DTPA in combination with various concentrations of MDNA55 (Ding *et al.*, 2010) and human serum albumin (HSA) administered by direct infusion into rat brains *via* CED. Results showed that the addition of Gd-DTPA (7 µmol/mL) was well tolerated and that Gd-DTPA did not affect the potency of MDNA55. Feasibility of safely co-infusing Gd-DTPA as a surrogate tracer has been demonstrated in a number of clinical studies (Chittiboina *et al.,* 2014; Lonser *et al.,* 2007; Souweidane 2014; Weber *et al.,* 2003a; Weber *et al.,* 2003b). Therefore, in this study, co-infusion of Gd-DTPA (commercially available Magnevist®) will allow for depiction of overall drug distribution without significant safety concern. Notably, co-infusion of Gd-DTPA will enable

continuous real-time monitoring of MDNA55 distribution and permit real-time adjustment of infusate delivery by either shutting down a non-convecting catheter, repositioning the catheter or adjusting the infusate flow rate).

Under protocol versions 1.0, 2.0 and 3.0 Gd-DTPA was administered at a concentration of 7 mmol for real-time imaging, with the chosen concentration confirmed by the SRC after the treatment of the first 6 study subjects. However, following concerns regarding a possible increase in frequency and intensity of neurotoxicity AEs following adoption of protocol version 3.0, as described in section 1.4.1, and in light of emergent concerns regarding Gd-DTPA, detailed in section 1.2.4, it was decided to lower the concentration of gadolinium as a risk minimization measure. Subsequently, the 2 mmol concentration of Gd-DTPA necessary for adequate imaging will be used for the remainder of the study.

Following a review of infusate-distribution data for subjects enrolled in protocol versions 1.0 to 4.0 inclusive, it was determined that continuous real-time MRI monitoring of MDNA55 for at least the first 3 hours was not necessary. Sites should therefore confirm convection in all catheters as desired and adjust infusion flow rate as required and remove the subject from the MRI scanner once these objectives are met in order to limit subject exposure to anesthesia.

1.4.7 Rationale for No Resection Following CED Infusion

In previous clinical studies carried out with MDNA55 in subjects with recurrent GB, the objective was to target the bulk tumor *in situ* by delivering increasing intra- and peritumoral doses of MDNA55. In the first study (Phase 1) resection was only performed in response to uncontrolled edema. In the second study (Phase 2), the objective was to resect the tumor three weeks post-infusion irrespective of the edema response.

Tumor resection post-treatment neither affected disease outcome nor improved patient survival (see current Investigator's Brochure for more detail). Histological examination of resected tumors showed that by the time they were removed 3 weeks post infusion most tumors consisted mainly of necrotic tissue.

Resection post treatment did not appear to provide a better treatment outcome compared to non-resected subjects while exposing them to the additional risks associated with CNS surgery. Thus, the treatment strategy for this study will consist of intra- and peritumoral administration of MDNA55 without tumor resection.

2 Study Objectives

2.1 Primary Objective

- To assess overall survival (OS)

2.2 Secondary Objectives

Including:

- To assess the effect of IL4-R status on overall survival (OS)
- To assess the safety of MDNA55 following CED
- To determine the objective response rate (ORR) per Response Assessment in Neuro-Oncology (RANO)-based criteria incorporating advanced imaging modalities
- To assess progression-free survival (PFS)

2.3 Exploratory Objectives

Including:

- To assess the pharmacokinetics (PK) of MDNA55 in peripheral plasma
- To assess serum anti-MDNA55 antibody titers and, if elevated determine neutralizing antibody titers
- To perform additional *ad hoc* efficacy and safety analysis as needed based on the data acquired in this study
- To determine the relationship between clinical outcomes and response assessment status by different sets of imaging-based response criteria
- To assess the performance of the Brainlab catheter during infusion in terms of distribution and convection of the infusate using real time MRI monitoring

3 Study Design

This is a single-arm, open-label, multicenter study in approximately 52 (at least 46 evaluable) adults with primary (de novo) GB that has recurred or progressed (according to RANO criteria). The study will be conducted at up to 12 clinical sites following institutional review board approval and completed informed consent.

The concentration and volume of MDNA55 will be adjusted based on tumor size such that total dose will not exceed the established maximum tolerated dose (MTD) of 240 µg. Doses up to 180 µg have been used in this study to-date, with no evidence of dose dependency for safety.

Eligible subjects will undergo surgery associated with catheter placement at which time a tissue biopsy will also be performed. MDNA55 infusate will be infused with the objective of achieving coverage of the tumor and peritumoral margin to the maximum extent possible as indicated by distribution of a co-infused gadolinium tracer observed by MRI. Pre-treatment catheter trajectory planning will be performed with aim to place up to 4 catheters but a minimum of 2, depending upon the tumor size. Planning for catheter placement will only target the enhancing region of the tumor on MRI.

Infusion via each catheter will be initiated at the rate of 3 µL/min/catheter and gradually increased in a stepwise manner. The infusion flow rate can be adjusted at the discretion of the Investigator during real time MRI (with subject maintained under anesthesia) provided that the flow rate per catheter does not exceed 10 µL/min. All functioning catheters should be convecting at similar flow rates. The flow rate should be established such that the duration of infusion is at least 24 hours to a maximum of approximately 48 hours. In the event only one catheter is functioning the flow rate may be increased to complete infusion in 48 hours or less but greater than 24 hours. After the real-time MRI infusion monitoring period is completed, the remainder of the infusion will continue with the subject awake. MRI will be performed upon completion of infusion as a final evaluation of MDNA55 infusate distribution.

[redacted]

Post-treatment follow-up assessment of safety will be performed 14 days after CED infusion. Thereafter, efficacy and safety assessments will be performed at 30, 60, 90 and 120 days after CED infusion and every 8 weeks thereafter until 360 days of active follow up have been completed. Subjects who discontinue before the Day 360 visit will undergo all the procedures scheduled for the Day 360 visit at the time of discontinuation (see section 7.5.4 for specific details concerning early termination visit).

Subjects who complete the Day 360 assessment without disease progression or who discontinue early without disease progression will continue to be followed for disease status until progression. After progression (on study or during post-study follow-up), subjects will continue to be followed for survival and post-study treatment(s) for GB and imaging for GB until death (or termination of data collection by the Sponsor or withdrawal of consent by the subject).

4 Study Population

The population for this study will consist of subjects with histologically proven primary (*de novo*) GB that has recurred or progressed (per RANO criteria) after treatment(s) including surgery and radiotherapy with or without chemotherapy (according to local practice; Stupp protocol, Stupp *et al.*, 2005) and following discontinuation of any previous standard or investigational lines of therapy (up to 2 prior lines of therapy).

4.1 Number of Subjects

Approximately 52 subjects with recurrent or progressive GB will be enrolled in order to achieve 46 evaluable subjects for the primary survival analyses.

4.2 Eligibility Criteria

Prospective subjects must have baseline evaluations performed prior to treatment with study drug and must meet all inclusion and exclusion criteria. In addition, the subject must be thoroughly informed about all aspects of the study, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. Written informed consent must be obtained from the subject before conducting any study-specific procedures.

The following criteria apply to all prospective subjects considered for enrollment into the study unless otherwise specified.

4.2.1 Inclusion Criteria

Prospective subjects will be **eligible** for participation if they meet **all** of the following criteria:

1) Subjects must be ≥ 18 years old and have a life expectancy ≥ 12 weeks
2) Histologically proven, primary (de novo) GB that has recurred or progressed (first or second recurrence, including this recurrence) after treatment(s) including surgery and radiotherapy with or without chemotherapy (according to local practice; Stupp protocol, Stupp *et al.*, 2005) and following discontinuation of any previous standard or investigational lines of therapy
3) Confirmation that archived tissue is available from first diagnosis of GB for biomarker analysis
4) Subjects must have evidence of tumor recurrence/progression as determined by standard RANO criteria following standard therapy:
    a) Includes primary GB
    b) Screening MRI must be performed within 14 days prior to planned infusion, and subjects receiving steroids must be on a stable, or decreasing dose for at least 5 days prior to imaging
c) More than 12 weeks must have elapsed since the completion of radiation therapy at the time of study entry, unless the majority of the new enhancement is outside of the radiation field (for example, beyond the high-dose region or 80% isodose line)
5) Recurrent tumor must be supratentorial, contrast-enhancing GB no smaller than 1 cm x 1 cm (largest perpendicular dimensions) and no larger than 4 cm maximum in a single direction based on MRI taken within 14 days prior to catheter placement

6) Karnofsky Performance Score (KPS) ≥ 70

7) Women of child-bearing potential must have a negative beta-human chorionic gonadotropin pregnancy test documented within 14 days prior to treatment

8) Women and men of child-bearing potential must agree to use adequate contraception: hormonal or barrier method of birth control; abstinence, *etc*. for the duration of study participation and for 6 months post drug administration. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately

9) Requirements for organ and marrow function as follows:

- adequate bone marrow function:
    - leukocytes > 2,000/μL
    - absolute neutrophil count > 1,000/μL
    - platelets > 100,000/μL
- adequate hepatic function:
    - total bilirubin < 1.5 X institutional upper limit of normal (ULN)
    - aspartate transaminase (AST) < 2.5 X institutional upper limit of normal (ULN)
    - alanine transaminase (ALT) < 2.5 X institutional ULN
- adequate renal function:
    - creatinine not to exceed 1.5 X institutional ULN

      OR
    - creatinine clearance: ≥ 60 mL/min/1.73 m^2 for subjects with creatinine levels above institutional ULN
- lymphocytes > 500/μL
- adequate coagulation function
    - international normalized ratio (INR) < 1.4
    - partial thromboplastin time (PTT) ≤ institutional ULN, unless receiving therapeutic low molecular weight heparin (corrected, if necessary, to exclude potential antibody effects)

10) Able to read, understand, and sign the informed consent document before undergoing any study-specific procedures or have a legal representative willing to do so; subjects must be registered prior to treatment with study drug

11) Subjects must be able and willing to undergo multiple brain MRI examinations

12) Subjects must be able and willing to comply with all study procedures

13) Any related toxicities following discontinuation of prior GB therapies must have resolved to CTCAE Grade 1 or lower prior to inclusion in this study

4.2.2 Exclusion Criteria

Subjects will be **ineligible** for participation if they meet **<u>any</u>** of the following criteria:

1 Prior treatment with cytotoxic chemotherapy

- Temozolomide (standard induction and / or maintenance dosing) within the past 4 weeks prior to planned infusion
- "Metronomic" Temozolomide (low-dose, continuous administration) within the past 7 days prior to planned infusion
- Nitrosoureas within the past 6 weeks prior to planned infusion
- Treatment with any other cytotoxic agent within the past 4 weeks prior to planned infusion

2 Prior investigational treatment within the past 4 weeks or prior immunotherapy or antibody therapy within the past 4 weeks prior to planned infusion; Subjects with prior immunotherapy within 6 months of planned infusion must have confirmed evidence of tumor recurrence/progression as determined by iRANO or mRANO criteria.

3 Prior treatment with bevacizumab (Avastin®) or other vascular-endothelial growth factor (VEGF) inhibitors or VEGF-receptor signaling inhibitors within the past 4 weeks prior to planned infusion

4 Prior therapy that included interstitial brachytherapy or Gliadel® Wafers (carmustine implants) within the past 12 weeks prior to planned infusion

5 Prior surgery (including stereotactic radiosurgery and biopsy procedures) within the past 4 weeks prior to planned infusion

6 Ongoing Optune© therapy within 5 days of planned initiation of infusion

7 Secondary GB (*i.e.*, GB that progressed from low-grade diffuse astrocytoma or AA)

8 Known mutation in either the isocitrate dehydrogenase 1 (IDH1) or the IDH2 gene.

9 Tumor in the brainstem (not including fluid-attenuated inversion recovery [FLAIR] changes), an infratentorial tumor, diagnosis of gliomatosis cerebri (highly infiltrative T2 hyperintense tumor with ill-defined margins encompassing at least three lobes of the brain.

10 Multifocal or multicentric satellite tumors with enhancement observed *outside* a 4cm x 4cm area on a single plane (maximum area covered by drug infusion). Multifocal lesions are defined by >1 measurable enhancing lesion (1cm x 1cm perpendicular dimensions) separated by at least 1cm with confluent T2 hyperintensity between the lesions. Multicentric lesions are defined by >1 measurable enhancing lesion (1cm x 1cm perpendicular dimensions) separated by at least 1cm with normal brain between the lesions). Measurable enhancing tumors separated by at least 1cm with any enhancing components >4cm apart are *excluded* from the current study, as these regions will not be covered by the infusion.

11 Tumor with a mass effect (*e.g*. 1-2 cm midline shift)

12 Subjects with tumors for which the preponderance of tissue is not of the type in which convection would be possible (*e.g*. preponderance of cystic component)

13 Tumor with geometric features that make them difficult to adequately cover the tumor volume with infusate using CED catheters according to expert review (*e.g*. on grounds of consistency, location, geometry, relationship to surrounding structures, presence of cyst, etc.); such tumors are likely to include the following:

- tumors that appear to wrap around ventricular structures (such as an “elbow” or “L-shape”) where convection is likely to be compromised
- tumors in which post-surgical enhancement in T1 images in the margins around a resection cavity may be confused with recurring tumor; subjects in whom this enhancement is below 1 cm thickness are excluded
- superficial tumors where direct infiltration of tumor into the cortical surface is apparent on MRI unless the distal margin of the enhancing tumor is ≥ 3cm from the cortical surface (Subjects with superficial tumors where separation of the tumor from the sub-dural space by a continuous layer of intact cortex is apparent on MRI remain eligible)

14 Clinical symptoms that are thought by the Investigator to be caused by uncontrolled increased intracranial pressure, hemorrhage, or edema of the brain

15 Any condition that precludes the administration of anesthesia

16 Known to be human immunodeficiency virus positive

17 On-going treatment with cytotoxic therapy; no additional antineoplastic therapies (including surgical modalities) are planned until there is confirmed evidence of tumor progression (as per modified RANO criteria) after administration of the study drug

18 Concurrent or a history of any significant medical illnesses that in the Investigator's opinion cannot be adequately controlled with appropriate therapy or would compromise the subject's ability to tolerate the study drug therapy and/or put the subject at additional risk or interfere with the interpretation of the results of this trial

19 Known history of allergy to gadolinium contrast agents

20 Presence of another type of malignancy requiring treatment within < 3 years prior to the screening visit, except for adequately treated carcinoma in-situ of the cervix, prostate cancer not actively treated, and basal or squamous cell carcinoma of the skin

21 Unwilling or unable to comply with the requirements of this protocol, including the presence of any condition (physical, mental, or social or geographical) that is likely to affect the subject's returning to the investigational site for follow-up visits including for imaging or other unspecified reasons that, in the opinion of the Investigator or Sponsor, make the subject's enrollment incompatible with study objectives

4.3 Study Duration

Study duration is 12 months for each subject with the day of catheter placement/ start of infusion being designated as Day 0.

Post-treatment follow-up assessment of safety will be performed 14 days after CED infusion. Thereafter, efficacy and safety assessments will be performed at 30, 60, 90 and 120 days after CED infusion and every 8 weeks thereafter until 360 days of active follow up have been completed. Subjects who discontinue before the Day 360 visit will undergo all the procedures scheduled for the Day 360 visit at the time of discontinuation.

Subjects who complete the Day 360 assessment without confirmed disease progression or discontinue early without confirmed disease progression will continue to be followed for disease status until progression where possible. After confirmed progression (on study or during post-study follow-up), subjects will continue to be followed, where possible, for survival, post-study treatment(s) for GB and imaging for GB until death (or termination of data collection by the Sponsor or withdrawal of consent by the subject).

4.4 Subject Withdrawal Criteria

All subjects who receive MDNA55 treatment should be followed for safety and efficacy. Subjects are free to withdraw their consent and discontinue the study at any time without giving a reason and without any disadvantage to future treatment.

The Investigator will withdraw a subject whenever continued participation is no longer in the subject's best interests. Reasons for withdrawing a subject include, but are not limited to the following:

- Confirmed disease progression; before withdrawing a subject for disease progression, Investigators are encouraged to:
    - Ensure confirmatory imaging is obtained following a suitable interval once radiological disease progression is suspected (i.e. PD is not confirmed if a subsequent scan shows disease control)
    - Use all available diagnostic imaging modalities (*e.g*. perfusion MRI, PET scan, spectroscopic MRI) to exclude the possibility of pseudo-progression
    - Within the first 120 days of the study
        - Perform diagnostic biopsy to determine disease status (Vogelbaum *et al.,* 2012; Weller *et al.,* 2016)
        - Discuss all potential subject withdrawals for disease progression with the study's medical monitor
- Occurrence of an AE or a concurrent illness,
- Start of any recurrent GB treatment [excluding low dose bevacizumab (Avastin®) (see section 6.2)]
- Subject's non-compliance or
- Significant uncertainty on the part of the Investigator that continued participation is prudent

Subjects requiring surgical resection for control of physical symptoms should NOT be discontinued from the study if examination of excised tissue finds evidence of tumor necrosis but no residual tumor. Likewise, if low-dose bevacizumab (doses typically used to control radiation induced necrosis) is needed for symptom control and/or steroid sparing (see section 6.2), the

subject need not be withdrawn however subsequent assessments of objective response must be confirmed by repeat imaging at the appropriate interval.

The reason for discontinuation (*e.g.,* withdrawal of consent, lost to follow-up, unable or unwilling to undergo imaging procedures) must be documented fully in the electronic data capture (EDC) form and in the subject's medical records. Subjects who discontinue the study for any reason should undergo the assessments scheduled for the Day 360 visit at the time of discontinuation (see section 7.5.4 for specific details concerning early termination visit).

Subjects who complete the Day 360 assessment without disease progression or who discontinue early without disease progression will continue to be followed for disease status until progression. After progression (on study or during post-study follow-up), subjects will continue to be followed for survival and post-study treatment(s) for GB and imaging for GB until death (or termination of data collection by the Sponsor or withdrawal of consent by the subject).

5 Study Drug

5.1 Investigational Agent: MDNA55

Classification

MDNA55 (IL-4[38-37]-PE38KDEL) is a recombinant fusion toxin, of approximately 53 kDa, consisting of an engineered circularly permuted (cp) version of interleukin-4 (cpIL-4) which is genetically fused to potent payload comprised of a truncated version of the bacterial toxin, *Pseudomonas aeruginosa* exotoxin (PE) A (Kreitman *et al.*, 1994).

Mechanism of Action

The mechanism of action of MDNA55 is well documented (Rand *et al.,* 2000; Kreitman *et al.*, 1994, Puri *et al.,* 2009) and is depicted in Figure 1. MDNA55 binds to IL4R overexpressed on the surface of tumor cells and the entire complex is endocytosed. Following cleavage and activation by furin-like proteases found in high concentrations in the endosome of cancer cells, the catalytic domain of the truncated PE is released into the cytosol where it induces cell death *via* ADP-ribosylation of the Elongation Factor-2 and induction of apoptosis through caspase activation (Shapira and Benhar, 2010).

MDNA55 is produced by a fed batch fermentation process using recombinant *Escherichia coli*. It is expressed intracellularly in the form of inclusion bodies. Following cell lysis and solubilization of the inclusion bodies, crude protein is purified using multiple chromatographic purification steps.

MDNA55 is supplied as a sterile frozen solution at a concentration of [redacted] and labeled according to country-specific regulatory requirements.

Storage and Handling

Vials will be stored at -70°C (± 10°C); MDNA55 is known to be stable for at least 3 years.

Composition of Infusate for CED

MDNA55 drug product will be diluted in Elliotts B® Solution to produce an infusate with a final concentration adjusted to ensure the delivery of a total dose of MDNA55 of up to 240 µg based on tumor size, with [redacted] HSA and [redacted] Gd-DTPA (commercially available Magnevist®; 469.1 mg/mL; [redacted] Details on the preparation of MDNA55 infusate are provided in the Pharmacy Manual.

Side Effects

Complete and updated AE information is available in the current version of the Investigator's Brochure.

6 TREATMENT PLAN

MDNA55 will be administered at a fixed concentration. For patients treated under protocol version 5.0, this will be 6.0 µg/mL, unless advised otherwise by the sponsor's medical representative on behalf of the SRC (see section 5.1) and detailed in an updated pharmacy manual. The study is designed to utilize a volume of infusion (Vi) to achieve maximal coverage of the tumor and peritumoral margin. Use of a surrogate tracer (Gd-DTPA) during infusion of MDNA55 will aim to monitor the pattern of coverage of the tumor and peritumoral margin and estimate the volume of distribution (Vd). [redacted]

6.1 Dose

[redacted] The total dose will not exceed 240 µg (the established MTD).

The study is designed to utilize a volume of infusion (Vi) individualized for each subject based on tumor size in order to achieve maximal coverage of the tumor and peritumoral margin. Use of a surrogate tracer (Gd-DTPA) during infusion of MDNA55 will aim to monitor the pattern of coverage of the tumor and peritumoral margin and estimate the volume of distribution (Vd). The duration of infusion will be at least 24 hours; however, infusion may continue to approximately 48 hours if required.

In the event of any concerns by the SRC regarding the possibility of related adverse events, reductions to the concentration, volume or rate of administration may be advised via the Sponsor's medical representative and detailed in an updated pharmacy manual. Likewise, the SRC may permit usage of concentrations up to 12 µg/mL (provided total dose does not exceed 240 µg (MTD) if ongoing data review suggests a positive benefit-risk.

[redacted]

6.2 Dose Administration

Total volume of infusion (see section 6.1) will be administered via up to 4 catheters surgically placed according to catheter placement guidelines (see Section 6.3).

The infusate will consist of MDNA55, HSA and Gd-DTPA (commercially available Magnevist®) in Elliotts B® Solution.

Customary antibiotic prophylaxis for such a procedure is required and shall be administered in accordance with institutional policy; an example antibiotic protocol is outlined in Table 1.

Table 1: Example Antibiotic and H-2 Antagonist Therapy*

Drug	Dose	Route	Duration
Ceftriaxone Cefazolin Vancomycin	1 to 2 g/day 3 g/day 1 g/day	IV IV IV	Beginning the day of catheter implantation and continuing until the completion of the MDNA55 infusion
Cimetidine Ranitidine Famotidine	300 mg/QID 150 mg/BID 20 mg/BID	Oral Oral Oral	Beginning the day that dexamethasone is started until it is stopped

*To be administered in accordance with institutional policy; Abbreviation: BID = twice daily; IV = intravenous; QID = 4 times daily

Example dexamethasone protocol is outlined in Table 2. Dexamethasone may be used during the study for prophylaxis or treatment of inflammatory tissue responses to MDNA55, with or without evidence of peritumoral edema and / or signs and symptoms suggestive of raised intracranial pressure. For the prevention and / or treatment of Grade ≥3 neurotoxicities mannitol may be used in addition. Example mannitol protocol is outlined in Table 3, if required.

Routine prophylactic use steroids +/- mannitol may be advised by the study SRC if required to manage acute toxicities.

Table 2: Example Dexamethasone Protocol*

Time point	Dexamethasone Dose
Day of catheter placement and postoperative Days 0 and 1:	6 mg every 6 hours
Postoperative Days 2 and 3:	4 mg every 6 hours
Postoperative Days 4 and 5:	3 mg every 6 hours
Postoperative Days 6 and 7:	2 mg every 6 hours
Postoperative Days 8 and 9:	1 mg every 6 hours
Thereafter	taper to off in 8 to 28 days based on neurological exam

*As an example only. To be administered in accordance with institutional policy. If a lower dose regime or more rapid taper is considered effective, these should be used preferentially.

Table 3: Example Mannitol Protocol*

Time point	Mannitol Dose
At any time during the duration of the infusion if subject develops symptoms of increased intracranial pressure Day 0, 1, or 2 depending on duration of planned infusion	25 g every 6 hours for 24 hours; or per institutional practice usually 0.25 to 2 g/kg IV over at least 30 min administered not more frequently than every 6 to 8 hours. + dexamethasone up to maximum of 16 mg daily

*To be administered in accordance with institutional policy

Low-dose bevacizumab (Avastin®), at doses typically used to control radiation induced necrosis (Delishaj *et al.,* 2017) Table 4, may be used for symptom control and/or steroid sparing. Use of low-dose bevacizumab for more than 3 cycles is not recommended due to its potential to confound efficacy results in this study.

Table 4: Example Low-Dose Bevacizumab*

Time point	Bevacizumab Dose**
Not before Day 30 follow up visit	5 mg/kg q2w x 3 cycles (*i.e.* 6 weeks) OR 7.5 mg/kg q3w x 3 cycles (*i.e.* 9 weeks)

* To be administered in accordance with standard of care / institutional policy and only under circumstances described above in this section
** May be repeated as required

6.3 Instructions for MDNA55 Infusate Administration

Pre-treatment catheter trajectory planning using screening/planning MRI and CT scan (registered with iPlan®Flow Infusion planning software) will be performed within 14 days of catheter placement following approval of subject for enrollment. Catheter trajectories will be planned using the following guidelines:

a. The planning processes is designed to ensure all catheter tips are placed within the enhancing tumor; Aim is to place up to 4 catheters but a minimum of 2, depending upon the tumor size.

b. The catheter tip must not be located within necrotic, cystic, or CSF regions including the ventricular system, sulci, or any resection cavity or blood vessel

c. The catheter tip should be placed approximately 1 cm from the ventricular system or sulci in areas where ependyma / pia mater remains intact and ≥ 1 cm away from such areas and previous resection cavities where there is no intact margin

d. Catheter(s) should not cross sulci or blood vessels

e. Suitability of the skull surface (bone condition and prosthetic material from previous surgery) should be considered in planning the locations for the catheter anchor screw points (per reference CT scan)

[redacted]

The approved neuro-navigation system VarioGuide™ by Brainlab will be utilized for the surgical elements of MDNA55 administration (i.e. surgical placement of catheters) on Day 0.

The workflow for study drug administration employing VarioGuide™ is described in Table 4. Table 5 also outlines the required materials for catheter placement and infusion.

[redacted]

Table 5: Procedure using VarioGuide™ Neuro-navigation for Biopsy and Catheter Placement, Followed by CED Infusion

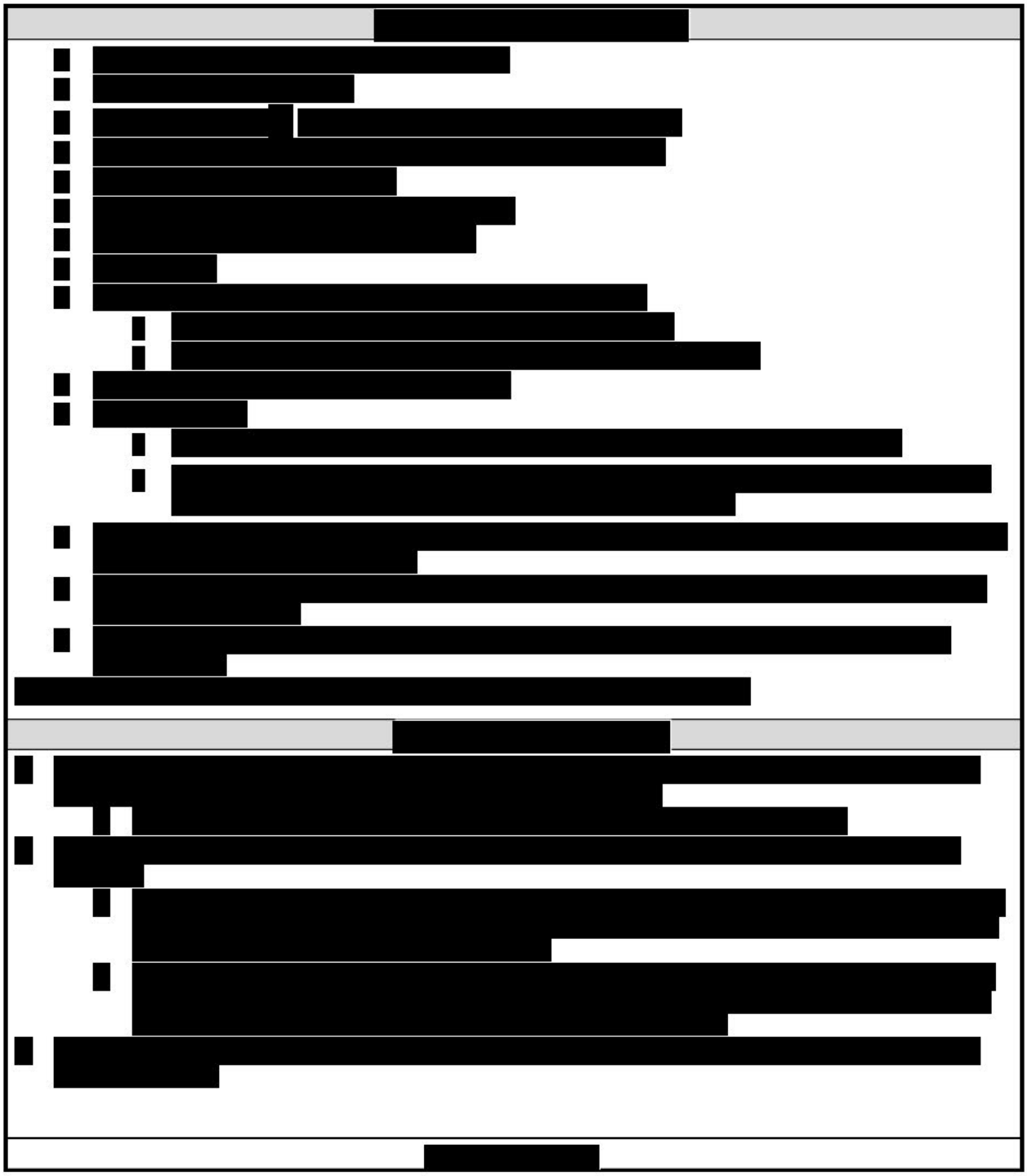


Table 5: Procedure using VarioGuide™ Neuro-navigation for Catheter Placement, Followed by CED Infusion (Cont.)

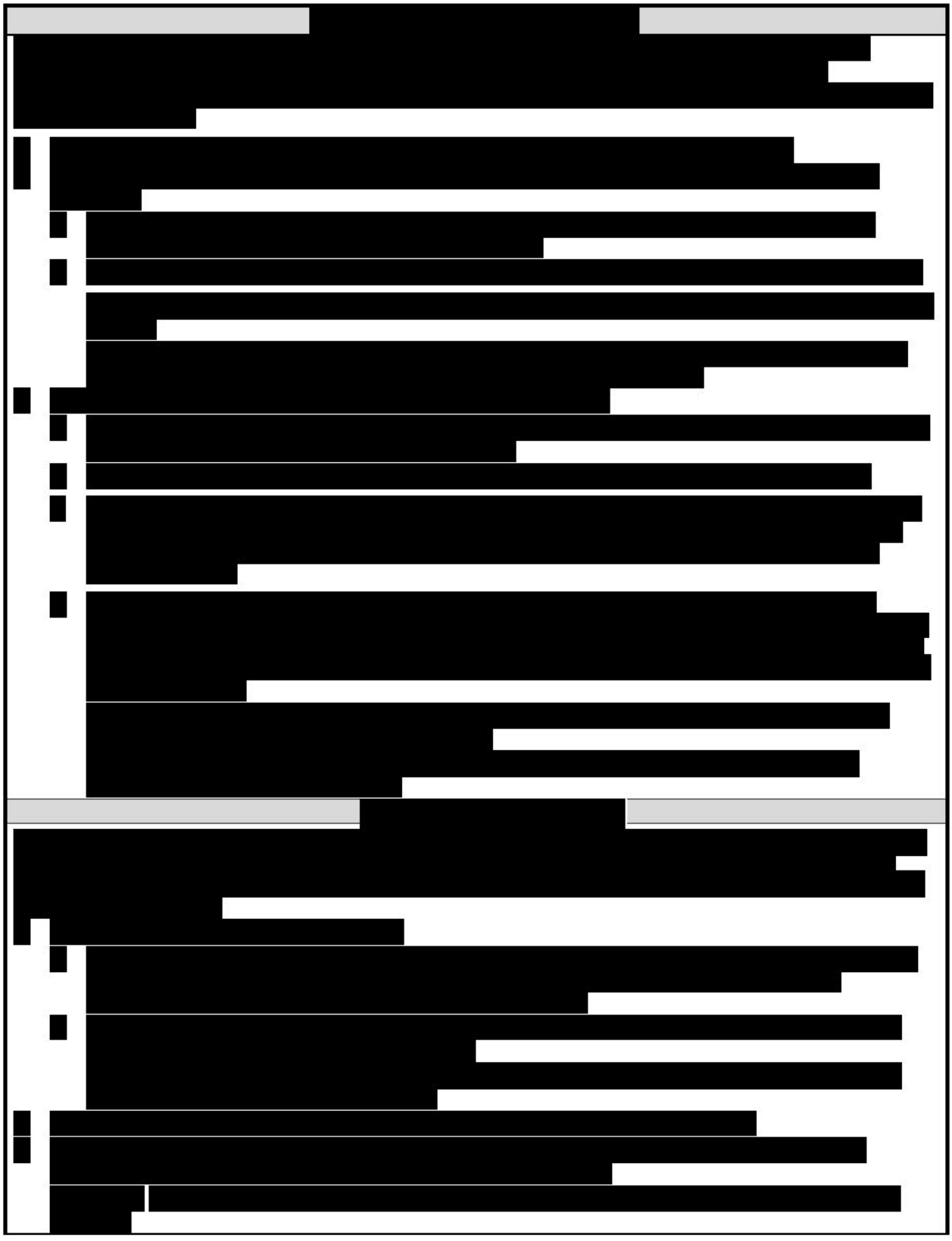
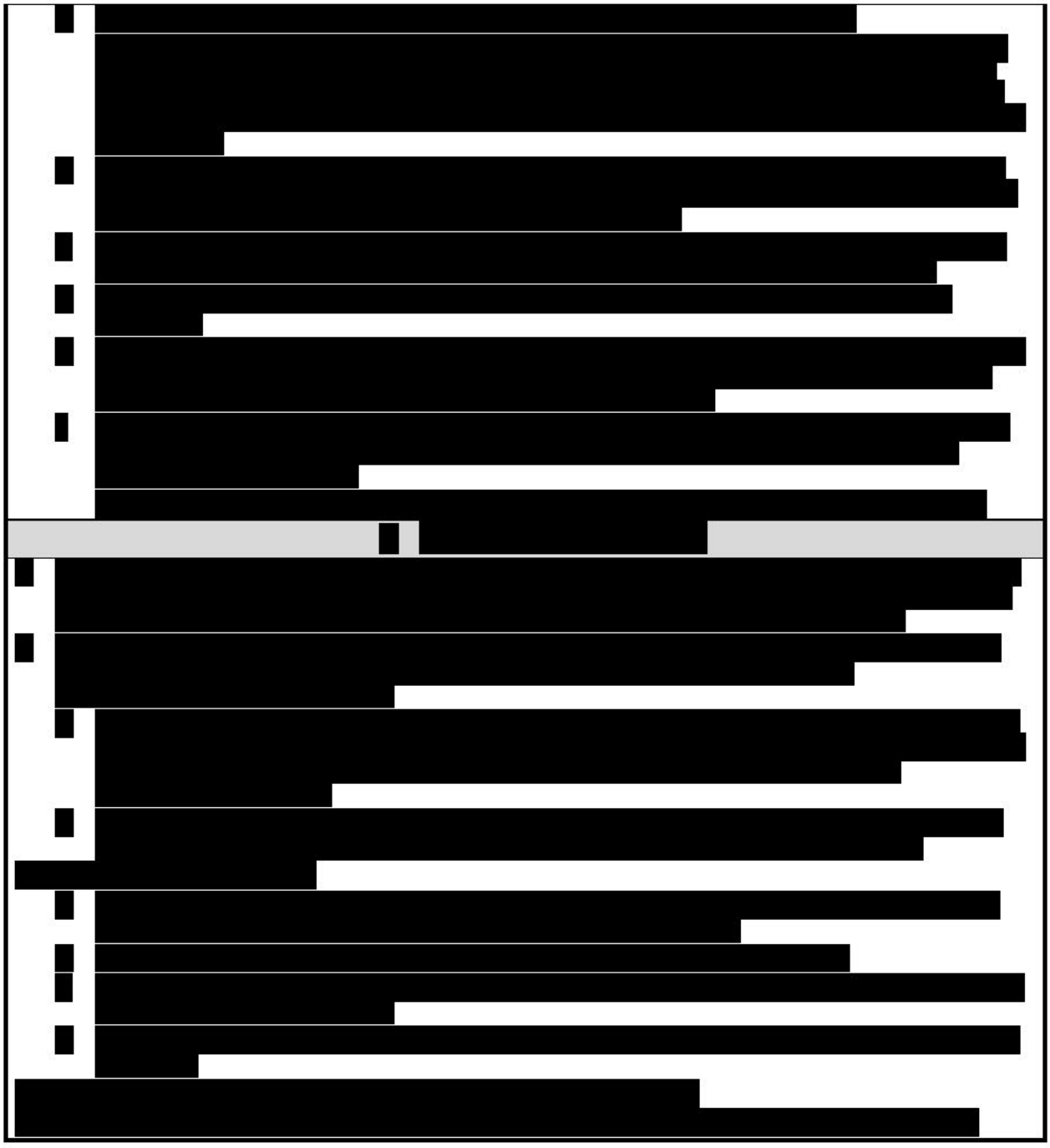


Table 5: Procedure using VarioGuide™ Neuro-navigation for Biopsy and Catheter Placement, Followed by CED Infusion (Cont.)



6.4 Image Acquisition - Schedule and Requirements

MRI will be used in this study to evaluate eligibility, to plan and confirm catheter placement, to assess infusate distribution, and to follow the subject for response, progression or pseudo-progression.

An independent central image review will be performed, for purposes of this protocol, and interpretation of medical images according to a study specific Imaging Charter.

Table 6 summarizes the images which must be acquired for this study. All scans must be obtained using pre-defined imaging protocols to ensure reproducibility at different time points and in a manner consistent with the Consensus Guidelines (Ellingson *et al*., 2015). All image data will be sent to the imaging database via electronic transfer. Training will be provided to the relevant site staff (radiologists, radiology technicians) to ensure that the required MRI scanning parameters are used for the indicated time points.

A study-specific Image Acquisition Guide details the imaging protocol and minimum requirements for the successful acquisition and transfer of image data.

Table 6: Imaging Schedule*

SCAN	PURPOSE
Screening MRI Within 14 days prior to catheter placement	Assess radiologic eligibility criteria Independent central assessment of objective tumor characteristics Catheter trajectory planning using iPlan® Flow Infusion planning software Image acquisition for perfusion and diffusion assessment Electronic image data transfer immediately following acquisition (< 4 hours)
Screening CT Scan [a] Within 14 days prior to catheter placement	Assessed during screening to ensure no prior skull defects or hardware that would preclude catheter placement Catheter trajectory planning to ensure no prior skull defects or hardware in the way of planned entry points for any of the planned catheter trajectories Electronic image data transfer immediately following acquisition (< 4 hours)
Pre-operative Planning MRI [b,c] Within 24 hours prior to catheter placement	Scan for tumor dimensions and volume estimations, using same MRI scanner that will be used for all follow-up MRIs Image acquisition for perfusion and diffusion assessment Finalization of catheter trajectory planning using iPlan® Flow Infusion planning software Pre-operative scan with *in situ* scalp fiducials to assure optimal catheter placement (within 24 hours of catheter placement), where possible, or other validated patient registration tools Electronic image data transfer immediately following acquisition (< 4 hours)
Catheter Placement Confirmation [b] Day 0 - Following surgical placement of catheters	Confirmation of catheters placed by MRI prior to starting infusion Electronic image data transfer following acquisition (< 2 days)
REAL-TIME INFUSION MONITORING [b] Day 0 – initial period of CED Infusion	MRI will be performed for approximately one hour to monitor the distribution of the infusate in real time while subject is maintained under anesthesia (see section 6.3). Repeat MRIs will be collected continuously (at approximately 10-15 minute scanning intervals). Electronic image data transfer following acquisition (< 2 days)
End of Infusion MRI Within 4 hours (ideally within 2 hours) following infusion end time	MRI will be performed after completion of the infusion to allow for a final evaluation of drug distribution and confirmation of final catheter positions Electronic image data transfer following acquisition (< 2 days) *Cont. next page*

SCAN	PURPOSE
Post-Operative MRI Within 18-30 hours following infusion end time	MRI scan for tumor dimensions and volume estimations, using same MRI scanner that will be used for all follow-up MRIs MR image acquisition for perfusion and diffusion assessment Electronic image data transfer immediately following acquisition (< 24 hours)
Follow Up MRI 30, 60, 90 and 120 days post CED infusion and every 8 weeks thereafter until 360 days of active follow up have been completed	Tumor response assessment, including tumor dimensions [d] and volume estimation. Perfusion and diffusion metrics assessment Electronic image data transfer immediately following acquisition (< 24 hours) Past Day 360 or following early withdrawal post study MRIs will be collected and transferred to the imaging database, if possible.
Unscheduled Follow up MRI	May be required to confirm response or progression 4 weeks after initial assessment; or at appropriate interval following discontinuation of bevacizumab (Avastin®) Electronic image data transfer immediately following acquisition (< 24 hours)

* A study-specific Image Acquisition Guide details the imaging protocols and minimum requirements for the successful acquisition and electronic transfer of images to the imaging database

a CT scan acquisition only required when no CT scan is available within 3 months of planned infusion

b [redacted]

c Full quality MRI scanning is required for tumor measurement and catheter trajectory planning. In addition, MRI imaging is needed to register the placement of scalp fiducials which may require only a limited scanning protocol and may be conducted using an intraoperative scanner. Final catheter trajectory plans should be completed sufficiently in advance of surgery to enable timely central peer review;

[redacted]

d RANO-based criteria and site tumor measurement guideline (see Imaging Acquisition Guide)

Screening MRIs will be assessed for determination of disease status, subject eligibility and convectibility. These images will be evaluated at the site and transmitted to the central reviewer to provide independent central assessment of objective tumor characteristics. All image reviews concerning study endpoints will be conducted in a blinded manner by an independent reviewer, without knowledge of the clinical condition or identity of the subject or the local site assessment.

[redacted]

The following criteria will be utilized for independent evaluation of objective tumor characteristics for consideration in determination of eligibility as follows:

- Tumor diameter of ≥ 1 cm x ≥ 1 cm (perpendicular dimensions), minimum, and a maximum size in any single dimension of 4 cm
- Tumor location not infratentorial or involving brainstem
- Exclude regions in the eloquent areas or close to eloquent areas of the brain.
- Diagnosis of gliomatosis cerebri (highly infiltrative T2 hyperintense tumor with ill-defined margins encompassing at least three lobes of the brain)
- Multifocal lesions and multicentric lesions are discouraged, but allowable so long as all boundaries of any multifocal, measurable enhancing lesions are within a 4cm x 4cm area (maximum area covered by drug infusion). Multifocal lesions are defined by >1 measurable enhancing lesion (1cm x 1cm perpendicular dimensions) separated by at least 1cm with confluent T2 hyperintensity between the lesions. Multicentric lesions are defined by >1 measurable enhancing lesion (1cm x 1cm perpendicular dimensions) separated by at least 1cm with normal brain between the lesions.

Independent central review of screening MRIs will be expedited. No subject will be enrolled in the study without this central imaging assessment being performed and reviewed by the Medical Monitor with further advice based on imaging reviews from CED experts, as required, especially in regard to tumors considered not to be good candidates for CED (see Section 4.2.2; exclusion criterion #13). All subjects will be approved for enrollment by the Medical Monitor following comprehensive review of a screening eligibility package comprising the independent central imaging assessment as well as documentation substantiating screening assessments.

Follow up MRIs will be subject to local site review for lesion assessment including tumor measurements in order to ensure consistency in all local tumor assessments (see Image Acquisition Guide).

Follow up MRIs will also be subject to independent central image review for determination of response/progression including tumor volume and perfusion criteria at each follow up time point.

7 Study Procedures and Observations

7.1 Efficacy Evaluation

7.1.1 Survival

After progression (on study or during follow-up), subjects will continue to be followed for survival and post-study treatment(s) for GB and imaging for GB, where possible, until death (or termination of data collection by the Sponsor or withdrawal of consent by the subject).

7.1.2 Tumor Response Assessment

MDNA55 shares many properties with immunotherapies, such as immune checkpoint inhibitors, including the possibility of response following a prolonged (> 3 months) period of pseudo-progression. Given acknowledged difficulties in determination of true progression in patients treated with immunotherapies, the determination of progressive disease (PD) will be informed by use of multimodality MRI and/or biopsy prior to subject withdrawal.

Tumor response will be determined using RANO-based criteria (section 8.1.1) incorporating advanced imaging modalities.

ORR, PFS, duration of response (DOR), and duration of clinical benefit (DOCB) will be assessed based on the independent assessment.

See section 8.1 for information on efficacy endpoints.

7.2 Safety Evaluation

Safety will be evaluated through AE monitoring, clinical evaluations (*i.e.*, vital signs, physical examinations, electrocardiogram [ECG]), laboratory tests (*i.e.*, hematology, serum chemistries, and urinalysis), antibody (serum anti-MDNA55 antibody and neutralizing antibody where applicable) assessments, and plasma drug levels on PK samples from the signing of informed consent until the last study visit (360 days post-CED infusion or termination).

7.2.1 Laboratory Evaluations

All study sites will conduct their own hematology, serum chemistry, and urinalysis tests (within their respective local certified laboratories) at various time points for each subject (see Table 7).

Required clinical laboratory tests for each panel will be as follows:

- Hematology: hemoglobin, hematocrit, platelet count, white blood cell (WBC) count, and WBC differential;
- Coagulation: prothrombin time (PT)/PTT/INR (PTT, corrected, if necessary);
- Serum chemistry: AST, ALT, lactate dehydrogenase (LDH), total bilirubin, indirect bilirubin, alkaline phosphatase, total protein, albumin, sodium, potassium, chloride, carbon dioxide, calcium, phosphorus, blood urea nitrogen (BUN), creatinine, uric acid, and glucose;
- Urinalysis: pH, specific gravity, protein, glucose, ketones, blood, leukocyte esterase, and nitrite. Microscopy required only to follow-up clinically significant abnormal findings; and
- Pregnancy: Serum pregnancy tests will be performed at screening and at 30 and 180 days post CED infusion for all women of childbearing potential.

The Investigator or a designated associate will review all clinical laboratory results, and clinically significant findings will be reported as AEs (see Section 8.2.2) and followed or treated according to institutional guidelines or the treating physician's medical judgment.

7.2.2 Safety Considerations

Physical examinations will be performed at screening, within 24 hours prior to catheter placement and following CED infusion on Days 1 or 2 (according to infusion duration), 14, 30, 60, 90, 120, 180, 240, and 360 (or early termination). The screening and Day 360 (or early termination) examinations will be complete physical examinations; other examinations should be focused, at the discretion of the Investigator, to assess changes from the previous examination. Clinically significant changes in physical examination findings during or after treatment, including transient neurological symptoms, will be reported as AEs.

Other safety assessments will include vital signs. The Investigator or a designated associate will review all vital sign results, and clinically significant findings will be reported as AEs (see Section 8.2.2) and followed or treated according to institutional guidelines or the treating physician's medical judgment. Triplicate twelve-lead ECGs are taken during screening and within 2 hours

following completion of infusion, and clinically significant abnormal findings must be followed by the Investigator.

7.2.3 Pharmacokinetic and Immune Parameter Evaluations

Systemic exposure to MDNA55 is not expected following intra- and peritumoral infusion and circulating MDNA55 has not been detected in previous clinical studies. To continue to evaluate the potential of systemic exposure, blood samples will be collected for analysis at the times indicated in Table 7.

Blood samples will also be collected for testing for the presence of antibodies against MDNA55 (Table 7). If serum anti-MDNA55 antibodies are present, further immunogenicity assessments will be carried out for determination of antibody neutralization potential. Other immune parameters may also be assessed.

All collected blood samples will be processed according to a laboratory manual and samples will be stored at -70°C until instructions are provided to the site to ship the samples to the central testing laboratory (MicroConstants Inc., San Diego, CA).

7.3 Tumor Tissue Analysis

During screening, subject eligibility assessment requires confirmation that archived tumor tissue from initial GB diagnosis is available for the patient. At the time of study treatment, prior to catheter insertion, biopsy samples will be collected, if the neurosurgeon determines it is possible to harvest viable tumor tissue from along the planned trajectory of the CED catheter(s).

Tissue sample archived from initial GB diagnosis and/or tissue sample archived following recurrence will be utilized for retrospective analysis of IL4R expression using immunohistochemistry (IHC). The tissue sample may also be subject to retrospective analysis of other biomarkers, including but not be limited to methylation status of the MGMT gene (Section 7.3.2).

Biomarker analyses will be conducted to determine if there is a correlation between IL4R expression and/or MGMT status and treatment response to MDNA55.

Tissue samples from biopsies taken at the time of MDNA55 infusion will be evaluated for disease status, including assessment of the immunological state of the tumor.

Analyses on other tumor tissue samples obtained during other surgical procedures or local evaluations of disease status (*e.g.* samples from repeat resections or biopsies taken to establish

tissue response status) should be recorded and may be used in sensitivity, sub-group and other exploratory analyses.

7.3.1 Biomarker Analysis including IL4R Expression

Archived tumor tissue specimens from subjects entering the study will be processed by IHC at a CLIA certified laboratory (QualTek Molecular Laboratories, Goleta, CA) for retrospective analysis of IL4R expression to determine if there is a correlation between IL4R expression and tumor response following MDNA55 treatment. Instructions for processing and submitting archived tissue are provided in the Study Reference Guide.

Tissue sections will be graded for IL4R expression by examining staining intensity in a blinded fashion for each specimen using a semi-quantitative scale of 0, 1+, 2+, and 3+ (as well as H-Score). Further quantitative assessment of IL4R staining may include standardized image analysis. Efficacy endpoints (such as PFS, ORR, OS, DOR, DOCB) will be evaluated versus intensity of IL4R expression.

In addition, pre-anesthesia blood sample as well as pre-treatment tumor tissue specimens (from subjects where viable tissue can be harvested at the time of catheter placement) will be processed by Mitra Biotech (Woburn, MA) for exploratory retrospective analysis of the tumor microenvironment by conducting an RNA (ribonucleic acid) based immune profile. Instructions for processing and submitting the blood and biopsy samples are provided in the Study Reference Guide.

7.3.2 O6-Methylguanine-Methyltransferase Analysis

MGMT-expressing cancer cells (harboring unmethylated MGMT promoters and therefore resistant to temozolomide) are sensitive to MDNA55.

Thus, tumor tissue specimens from subjects will be processed at a CLIA certified laboratory for MGMT DNA methylation analysis using a quantitative methylation-specific PCR technique. Primary and secondary endpoints will be analyzed against the MGMT methylation status.

7.4 Evaluation of Progression versus Pseudo-progression

For malignant gliomas, conventional MRI with contrast is currently used to determine radiologic response. While this method has been used to determine overall tumor response, it is known that image interpretation can be confounded by pseudo-progression due to the fact that conventional MRI is unable to differentiate tumor/non-tumor enhancing tissues (Verma *et al.,* 2013; Ellingson *et al.*, 2017b). Therefore, determination of true response may take several months post-treatment

(Floeth *et al.,* 2002; Vogelbaum *et al.,* 2012). Increasingly, evidence supports the designation of non-progression status to patients who have no subsequent signs of disease progression on imaging despite initially being considered to have PD, following treatment with immune-based therapies (Hodi *et al.*, 2018). To address this issue, advanced imaging techniques such as perfusion MRI, PET imaging and spectroscopic MRI have been evaluated for the ability to distinguish between true and pseudo-progression and confirm response in GB patients at earlier time points (Ion-Mărgineanu *et al.,* 2017; Thomas *et al.*, 2015; Boxerman *et al,* 2014).

7.5 Study Visit Schedule

All clinical study evaluations and procedures will be performed according to the schedule of assessments (Table 7) and the instructions listed in the sections following.

All on-study visit procedures are allowed **a window of time** unless otherwise noted. *Treatment or visit delays for weekends, public holidays or weather conditions do not constitute a protocol violation.

Table 7: Schedule of Evaluations and Procedures*

Evaluation	Screen	Hospitalization				Day 14	Day 30, 60, 90 120, then q8w	Long Term F/up
		Before Catheter Placement	Catheter Placement	CED Infusion	End of CED Infusion			
Day	**-14 to 0**	**-24 hrs to 0**	**0**	**0-2**	**1-2 [a]**	**14 [a]**	**[a, b, c]**	**[p]**
Informed Consent [d]	X							
Hospital Registration		X						
Medical/Oncological History	X	X			X			
Operative & Pathology Reports for Index Tumor (resection/biopsy) [e]		X						
Confirmation of archived tissue being available for biomarker analysis	X							
MRI	X[g1]	X[g2]	X[g3]		XX[g4]		X[g5]	
CT	X[g6]							
Physical Exam, KPS	X	X			X[q]	X	X	
Vital Signs (pulse, respiratory rate, weight and blood pressure)	X	X	X[r]	X[r]	X[s]	X	X	
Neurological Exam	X	X			X	X	X	
Standard 12-lead Electrocardiogram (triplicate assessment)	X				X[t]			
Serum Pregnancy Test [h1]	X						X	
Hematology / Serum Chemistry [h2]	X				X[u]	X	X	
Coagulation [h3]	X				X[u]	X	X	
Urinalysis [h4]	X							
Pharmacokinetics [i1]	X			X		X		
Immunogenicity [i2]	X					X	X	
Pre-anesthesia blood sample [i3]			X					
Baseline Conditions [j]	X	X						
Pharmacy Preparation of Infusate [l]			X	X				
Catheter placement with biopsy [k]			X					
CED Infusion: MDNA55/Gadolinium [k]				X				
Catheter(s) Removal [m]					X			
Concomitant Meds/Corticosteroids [n]	X	X	X	X	X	X	X	
Adverse Events [o]			X	X	X	X	X	
Telephone Contact [p]								X

* Treatment or visit delays for weekends, public holidays or weather conditions do not constitute a protocol violation

a Safety assessed after initiation of infusion (during and following CED infusion throughout entire hospitalization period) and safety follow-up to be performed on Days 14 and 30 (± 3 days), and 60, 90, 120, 180, 240, 300 and 360 (± 7 days)

b Scheduled follow-up to be performed with MRI up to 12 months after CED infusion on Day 30 (± 3 days) and Days 60, 90 and 120, then q8w until Day 360 (± 7 days) NOTE: at the Day 300 visit only MRI, physical exam/neurological exam/KPS need be performed

c Subjects who discontinue before the Day 360 visit will undergo all the procedures scheduled for the Day 360 visit if early termination is between follow-up visit time points with the specific provision that MRI will not be required if last MRI performed was within 2 weeks prior to the early termination date. When early termination is in line with a study follow up visit time point that visit will be considered the early termination time point and no other assessments apart from the respective visit date assessments will be required.

d ICF can be signed in advanced of the 14 day screening period

e Operative report to be accessioned

f Archived tissue from the resection of the initial GB diagnosis required for biomarker analysis including IL4R IHC, MGMT DNA methylation, and other translational biomarkers

g All MRIs acquired throughout entire study schedule will follow will be performed according to imaging protocols outlined in the study specific Image Acquisition Guide

g1 Screening MRI to be subject to independent assessment to verify objective radiologic tumor characteristics [*e.g*. tumor size; subjects must have tumor diameter of ≥ 1 cm x ≥ 1 cm (minimum) to 4 cm in any direction, etc.]; additional image reviews of screening MRI pertaining to evaluation of suitability of subjects for convection may be performed by CED experts; if subject approved for enrollment, screening MRI will also be used for catheter placement planning using iPlan® Flow

g2 Pre-operative planning MRI can be performed in one or two MRI exams as required, based on local capabilities; Timing of planning images to support catheter trajectory planning is described in Table 6; Planning MRI images will be registered with iPlan® Flow Infusion planning software to support finalization of catheter trajectory plan.

g3 On Day 0 MRI performed following catheter placement prior to the start of CED infusion as well as during infusion for real-time MRI infusion monitoring (for approximately 1 hour while subject is maintained under anesthesia) (see Section 6.3 for CED infusion procedure)

g4 On Day 1 or Day 2 (depending on duration of infusion) MRI performed within 4 hours (ideally within 2 hours) after completion of infusion relative to infusion end date/time; another MRI performed within 18-30 hours after completion of infusion prior to subject being discharged from the hospital

g5 MRI performed as part of the scheduled follow-up visits on Days 30, 60, 90, 120 and then q8w thereafter until Day 360 / early termination. For patients who are progression free, MRIs will continue as per the institution's standard of care schedule until confirmed disease progression.

g6 CT scan acquisition only required when no CT scan is available within 3 months of planned infusion

h1 Females of child bearing potential only at Screening, Days 30 and Day 180

h2 Hematology: hemoglobin, hematocrit, platelet count, white blood cell (WBC) count, and WBC differential;

Serum chemistry: AST, ALT, lactate dehydrogenase (LDH), total bilirubin, indirect bilirubin, alkaline phosphatase, total protein, albumin, sodium, potassium, chloride, carbon dioxide, calcium, phosphorus, blood urea nitrogen (BUN), creatinine, uric acid, and glucose

h3 Coagulation: prothrombin time (PT)/PTT/INR (PTT, corrected, if necessary)

h4 Urinalysis: pH, specific gravity, protein, glucose, ketones, blood, leukocyte esterase, and nitrite. Microscopy required only to follow-up clinically significant abnormal findings

i1 PK assessed at Screening (baseline), as soon as possible but not more than 1 hour following infusion end time, approximately 3 hours following completion of infusion and then (after the ~3 hour sample collection) every 6 hours ± 2 hours until 24 hours or until subject is discharged from the hospital (whichever occurs first) and at Day 14

i2 Immunogenicity assessed at Screening (baseline) and at Days 14, 30, 120, 240, and 360/early termination

i3 Pre-anesthesia blood draw (within 1 hour of subject be placed under anesthesia), this blood sample collection and processing will use a study specific sample collection kit and require immediate (same day) shipment to central laboratory

j Baseline conditions/symptoms will be collected from Screening until Day 0 before subject is anesthetized for catheter placement surgery; any baseline condition or symptom noted prior to catheter placement will be recorded in the Medical History

k [redacted]

l see Study Pharmacy Manual for infusate preparation and dispensing instructions

m Following completion of infusion (only after the end of infusion MRI and at any time prior to subject's discharge from the hospital), the catheters can be removed and the incisions closed in accordance with institutional practice. Removal of catheters must be performed by a delegated neurosurgeon.

n All concomitant medications will be collected on the Electronic Data capture (EDC) system from the date of informed consent through 30-day safety period. Thereafter, concomitant medications associated with treatment-related SAEs and detailed anti-tumor therapy will be collected. Corticosteroid use will be collected until Day 360 or early withdrawal.

o All AEs will be collected from catheter placement through the end of study visit and all AEs and SAEs will be followed until resolution, stabilization, data cut-off, or death

p Subjects who complete the Day 360 assessment without disease progression or discontinue early without disease progression will continue to be followed for disease status until progression. After progression (documented on study or during post-study follow-up), subjects will continue to be followed for survival, post-study treatment(s) for GB and imaging for GB, where possible, until death (or termination of data collection by the Sponsor or withdrawal of consent by the subject).

q Physical exam and KPS assessments will be performed after completion of infusion when the subject is ambulatory prior to hospital discharge

r Vital signs monitoring (without weight parameter) will be performed according to institutional best practice throughout entire surgical workflow for catheter placement and CED infusion; study specific vital signs check points during the surgical workflow are prior to initiation of anesthesia, following placement of all catheters but prior to patient being loaded into MRI machine for the real-time infusion monitoring segment and approximately 1 hour post initiation of infusion; *NOTE: Site will record any abnormal vitals observed at any time during surgical vital signs monitoring as unscheduled findings.*

s Vital signs taken after completion of infusion when the subject is ambulatory prior to hospital discharge

t ECG (triplicate assessment) will be performed at Screening and immediately following completion of infusion (within 2 hours of infusion end time); triplicate assessment in 3 immediate traces

u Blood samples for hematology, serum chemistry, and coagulation (Section 7.2.1) shall be taken in parallel with either the PK blood collection that is performed within 1 hour following completion of infusion or the PK blood collection that is performed ~3 hours following completion of infusion

7.5.1 Pretreatment Period

7.5.1.1 Informed Consent

Each subject will provide signed informed consent and Health Insurance Portability and Accountability Act (HIPAA) authorization as per institutional and/or Institutional Review Board (IRB)/Independent Ethics Committee (IEC) guidelines and in accordance with the requirements of the International Council on Harmonization guidelines on Good Clinical Practice (ICH E6 GCP) and the principles of the Declaration of Helsinki. Major deviations may not be made from the informed consent provided by the Sponsor unless requested by the IRB/IEC. If translation of informed consent form (ICF) is required, the Sponsor will review a certified back-translation to ensure all elements are present. The IRB/IEC-approval document must be provided to the Sponsor or their representative for regulatory purposes.

Before a subject undergoes any study-related procedures or examinations, the Investigator or approved delegate will explain to each subject (or legal guardian) the nature of the study, its purpose, procedures, expected duration including the necessity to collect information about the subject's treatment and disease after completion of the scheduled last follow-up visit, and the potential benefits and risks of participation.

Subjects will be informed of their right to refuse to consent to the study or to withdraw from the study at any time without any change in the quality of their health care. Subjects will be provided with a copy of the IRB/IEC approved ICF and they will have sufficient time to consider the study information and ask any questions.

If a subject (or legal guardian) agrees to participate in the study, he/she (or legal guardian) will sign and date a statement indicating their informed consent. The statement will also be signed by the Investigator or approved delegate to indicate that he/she fully informed the subject and witnessed the freely provided consent. A copy of the signed ICF will be given to the subject, and a copy will be filed in the medical record. The original signed ICF will be kept on file with the Investigator’s study records.

7.5.1.2 Screening Assessments (within 14 Days of Infusion)

Before a subject can be considered for entry into the study, the Investigator must receive the subject's clinical history, general laboratory results, specific radiological evaluations and diagnoses, and a chronology of all previous therapies for the treatment of GB, including outcomes,

from the referring physician. Subjects should have results from a previous histological diagnosis of initial primary (*de novo*) GB and a pre-study MRI scan providing radiological evidence of recurrence or progression.

A written and signed ICF and HIPAA authorization must be obtained before any study-specific assessments are initiated (See Section 7.5.1.1). The ICF can be signed in advanced of the 14 day screening period.

The following assessments must be carried out within 14 days before catheter placement:

- Screening MRI to assess radiologic eligibility criteria and catheter trajectory planning using iPlan® Flow Infusion planning software
    - Electronic image data transfer immediately following acquisition (< 4 hours)
- CT scan assessed during screening to ensure no prior skull defects or hardware that would preclude catheter placement (CT scan acquisition only required when no CT scan is available within 3 months of planned infusion)
    - Electronic image data transfer (should be concomitant with transfer of screening MRI)
- Verification that archived tumor tissue (from initial GB diagnosis) is available for retrospective biomarker analysis
- **Complete** medical and oncologic history, including history of prior surgical procedures and prior treatments, prior conditions, signs and symptoms and any residual/ongoing toxicity relating to prior treatment(s)
- Complete physical and neurological examinations, including height, weight, mental status, cranial nerves, motor and sensory examinations, and KPS
- Baseline signs and symptoms
- Vital signs (pulse, respiratory rate, weight and blood pressure)
- Standard 12-lead ECG (triplicate assessment)
- Hematology, serum chemistry, and coagulation (Section 7.2.1)
    - If the coagulation values are clinically significant, they must be repeated until within normal limits, unless subject is receiving therapeutic low molecular weight heparin

NOTE: Any test results outside of the reference ranges may be repeated at the discretion of the Investigator

- Collection of blood sample for Immunogenicity analysis (baseline)
- Collection of blood sample for PK analysis (baseline)

Concomitant medications and treatments will be recorded from 14 days before infusion (Day 0) until the Day 360 (or early termination) visit, and AEs will be recorded from the time of signing the informed consent document until the Day 360 (or early termination) visit.

In addition, a serum pregnancy test must be performed within 14 days before infusion (Day 0) for all female subjects of childbearing potential.

While the above assessments are being carried out, screening MRI will be subject to review by CED experts pertaining to evaluation of suitability of tumor for convection. The screening MRI will also be sent to an independent central assessment of objective radiologic tumor characteristics.

Subjects will be approved for enrollment by the Medical Monitor following comprehensive review all screening results.

Following approval of subject for enrollment, Investigator/site will be notified and preliminary pre-treatment catheter trajectory planning using CT scan and screening MRI (registered with iPlan® Flow Infusion planning software) can ensue (see Section 6.3 for catheter placement guidelines).

NOTE: use of iPlan® Flow software is required for catheter trajectory planning.

7.5.2 Treatment Period

7.5.2.1 Pre-operative study Procedures within 24 hours of catheter placement

Within 24 hours prior to catheter placement, subjects will undergo the following procedures and assessments:

- Pre-operative Planning MRI
    - Pre-operative planning MRI can be performed in one or two MRI exams as required, based on local capabilities; Timing of planning images to support catheter trajectory planning is described in Table 6.
    - Planning MRI images will be registered with iPlan® Flow Infusion planning software to support finalization of catheter trajectory plan.

- *NOTE: use of iPlan® Flow software is required for catheter trajectory planning.*
- Brief medical history and physical and neurological examinations including KPS, noting/recording any changes since screening;
- Vital Signs (pulse, respiratory rate, weight and blood pressure)
- Review of Baseline Signs and Symptoms noting/recording any changes or new signs/symptoms since screening in medical history; Any changes to concomitant medications will also be recorded;
- Treatment Plan (study specific source worksheet) will be completed and reported to hospital pharmacy for dispensing infusate at the time required on Day 0.

7.5.2.2 Study Drug Administration (Day 0)

On Day 0 subjects will undergo the following procedures and assessments:

- Pre-anesthesia blood draw
    - Blood sample collection and processing will use a study specific sample collection kit and require immediate shipment to the central laboratory
- Just prior to catheter insertion, core tumor biopsy samples (at least 3 cores) will be collected, if the neurosurgeon determines it is possible to harvest viable tumor tissue from along the planned trajectory of the CED catheter(s)
    - Biopsy sample tissue collection and processing will use a study specific sample collection kit and require immediate shipment to the central laboratory
- Catheter placement and infusion (see Section 6.3 for outline of surgical procedures and instructions for study drug administration via CED with real-time imaging)
    - See Table 1 for example of mandatory antibiotic / H-2 antagonist therapy and Table 2 and Table 3 for optional dexamethasone and mannitol therapy for management of raised intra-cranial pressure (ICP).
    - Throughout entire surgical workflow for catheter placement and infusion vital signs (pulse, respiratory rate and blood pressure) will be performed according to institutional best practice; study specific vital signs check points during the surgical workflow are as follows:

  - Vital signs taken prior to initiation of anesthesia
  - Vitals signs taken following placement of all catheters but prior to patient being loaded into MRI machine for the real-time infusion monitoring segment
  - Vital signs taken approximately 1 hour post initiation of infusion
  - Site will record any abnormal vitals observed at any time during surgical vital signs monitoring as unscheduled findings
- Any AEs experienced or concomitant medications received during the catheter placement procedure or during the infusion will be recorded in the subjects' medical records and EDC system

NOTE: Subjects will likely be admitted to hospital for 2 nights.

7.5.2.3 Interruption or Discontinuation of Infusion

If a subject experiences a clinically significant Grade 3 or 4 AE considered by the Investigator to be related to the study drug or due to infusion procedure, the infusion will be stopped. Infusion may be restarted at the Investigator's discretion if the AE responds to medical management; otherwise, the infusion should be discontinued permanently.

Should an interruption of the infusion be due to observed mass effect / increased intracranial pressure (ICP), treatment with mannitol is suggested (see Table 3) with infusion resuming after recovery (if possible), such that it can be completed within the maximum infusion duration window of approximately 48 hours.

Treatment may be discontinued at the discretion of the Investigator. In the unlikely event that all catheters are placed erroneously (not in line with placement guidelines in section 6.3) or cannot be used (*e.g.* catheters do not function/convect), the procedure will be halted and no infusion given to the subject.

7.5.2.4 End of CED Infusion (Day 1 - 2)

These procedures must be completed at the end of the CED infusion (see Section 6.3):

- A standard 12 Lead ECG (triplicate assessment) will be performed immediately following completion of the infusion (within 2 hours)

- MRI for final evaluation of drug distribution (with catheters maintained in place) will be performed within 4 hours (ideally within 2 hours) following completion of infusion
    - Removal of catheters and closure of incisions in accordance with institutional practice at bedside. *NOTE: catheters are only removed after the end of infusion MRI is performed.*
- Collection of blood samples for PK analysis as soon as possible but not more than 1 hour following infusion end time, approximately 3 hours following completion of infusion and then (after the ~3 hour sample collection) every 6 hours ± 2 hours until 24 hours or until subject is discharged from the hospital (whichever occurs first)
- Blood samples for hematology, serum chemistry, and coagulation (Section 7.2.1) shall be taken in parallel with either the PK blood collection that is performed within 1 hour following completion of infusion or the PK blood collection that is performed ~3 hours following completion of infusion.
- MRI performed within 18-30 hours after infusion end time to assess non-specific enhancement following the infusion
- Brief medical history and physical and neurological examinations including KPS, noting any changes since prior to study drug administration shall be performed when the subject is ambulatory prior to hospital discharge.
- Vital signs (pulse, respiratory rate, blood pressure and weight) taken when the subject is ambulatory prior to hospital discharge.
- Any AEs and new concomitant medications and/or changes to concomitant medications will be recorded throughout entire hospitalization period.

7.5.3 Post-Treatment Follow-up Assessments

Assessment of safety will be performed on Day 14 (± 3 days) after infusion. Both efficacy and safety assessments will be performed on Days 30 (± 3), 60 (± 7), 90 (± 7) and 120 (± 7) and every 8 weeks (± 7) thereafter until at least 360 days of active follow up have been completed after start of infusion. Unscheduled MRI exams may be required for confirmation of overall response status when preliminary CR or PR or PD by repeat assessment 4 weeks (or at appropriate interval for post bevacizumab (Avastin®) therapy) after it is observed (required if preliminary response or preliminary progression is observed at Day 120, 180, 240, 300 or 360). Subjects who undergo

post-treatment surgery to de-bulk the tumor, and if the tumor is shown to be largely necrotic with no viable tumor, will continue to be followed for tumor recurrence by MRI at the scheduled visits in order to establish PFS.

Subjects who complete the Day 360 assessment without disease progression or discontinue early without disease progression will continue to be followed for disease status until progression where possible. After progression (on study or during post-study follow-up), subjects will continue to be followed (by access of available medical records or via periodic telephone contact to the subject/caregiver), where possible, for survival, post-study treatment(s) for GB and imaging for GB until death (or termination of data collection by the Sponsor or withdrawal of consent by the subject).

7.5.3.1 14 Days (± 3 days) After Infusion

Subjects will return 14 (± 3) days after infusion.

Concomitant medications/therapies and AEs will be assessed by asking non-leading questions, and the following procedures will also be performed:

- Vital signs (pulse, respiratory rate, weight and blood pressure)
- Physical and neurological examinations including KPS
- Hematology, serum chemistry, and coagulation (Section 7.2.1)
- Collection of a blood sample for immunogenicity;
- Collection of a blood sample for PK

7.5.3.2 Follow up (for period of 12 months After Infusion)

Following completion of infusion, subjects will return on Days 30 (± 3), 60 (± 7), 90 (± 7) and 120 (± 7), and every 8 weeks (± 7) thereafter until Day 360 to undergo follow-up MRI. For sites that may use TRAMs, delayed contrast extravasation MRI can be performed on Days 30, 60, 90, 120 and 180 for calculation of TRAMs image map to be used for exploratory analyses of tissue response (calculation of TRAMs may be performed at the other follow up visits as or if requested by the Investigator).

At all of the above-named visits concomitant medications/therapies and AEs will be assessed by asking non-leading questions, and the following procedures will also be performed:

- Physical and neurological examinations including KPS

- Vital signs (pulse, respiratory rate, weight and blood pressure)
- Hematology, serum chemistry, and coagulation (Section 7.2.1)
- Serum pregnancy test (female subjects; Day 30 and Day 180 visits only)
- Collection of blood samples for immunogenicity (Day 30, Day 120, Day 240 and Day 360 or early termination)

At any time-point during the follow-up period, should analyses on tumor tissue sample material occur at the discretion of the Investigator (*e.g*. biopsies to establish tissue response status), result should be recorded and material retained for biomarker analysis, where possible.

Other assessments will be performed at the Investigator's discretion and as necessary to follow up any AEs previously recorded.

The Day 360 visit will be equivalent to the early termination visit and will end the subject's safety reporting.

7.5.4 Early Termination Visit

Subjects who discontinue before the Day 360 visit will undergo all the procedures scheduled for the Day 360 visit if early termination is between follow-up visit time points. A new / repeat MRI will not be required if the last MRI performed was within 2 weeks prior to the early termination date, results from this prior MRI will be carried forward for the early termination assessment. When early termination is within 2 weeks following study follow up visit time point (i.e. Day 30, 60, 90, 120, 180 or 240) that visit will be considered the early termination time point and no additional assessments beyond the respective visit date assessments will be required. In the event that a subject is withdrawn from the study prior to the Day 360 visit, post-study follow-up should ensue (see Section 7.5.5 below).

7.5.5 Post-Study Follow-Up

Subjects who complete the Day 360 assessment without disease progression or discontinue early without disease progression will continue to be followed for disease status until progression where possible. After progression (on study or during post-study follow-up), subjects will continue to be followed (by access of available medical records or via periodic telephone contact to the subject/caregiver), where possible, for survival, post-study treatment(s) for GB and imaging for GB until death (or termination of data collection by the Sponsor or withdrawal of consent by the subject).

7.6 Usage of Concomitant Medications

All concurrent medical conditions and complications of the underlying malignancy will be treated at the discretion of the Investigator according to acceptable local standards of medical care. Subjects should receive analgesics, antiemetics, antibiotics, anti-pyretics, and blood products as necessary. Although warfarin-type anticoagulant therapies are permitted, careful monitoring of coagulation parameters is imperative to avoid complications of any possible drug interactions. All concomitant medications, including transfusions of blood products, will be recorded in the EDC system.

Guidelines for treating certain medical conditions are discussed below; however, institutional guidelines for the treatment of these conditions may also be used. The concomitant therapies that warrant special attention are discussed below.

7.6.1 Antiemetic Medications

Dexamethasone and a 5-HT3 blocker (*e.g.,* ondansetron or granisetron) may be administered to subjects as pre-medications unless contraindicated for the individual subject. Antiemetics will also be prescribed as clinically indicated during the study period.

7.6.2 Colony Stimulating Factors

Though unlikely to be needed, the use of granulocyte colony-stimulating factors is permitted to treat subjects with neutropenia or neutropenic fever but not to allow for study eligibility.

7.7 Dietary Restrictions

None.

7.8 Prohibited Medications

The following therapies are not permitted during a subject's participation in the study:

- Other anti-neoplastic therapy, including cytotoxics, targeted agents, endocrine therapy, or other antibodies, including Avastin® (bevacizumab) with any treatment intent. *NOTE: Low-dose bevacizumab (Avastin®) (doses typically used to control radiation induced necrosis, see section 6.2) use is permitted*
- Radiotherapy
- Any other investigational therapy

Should such therapies be administered if a subject is withdrawn from the study due to progressive disease, relevant disease related treatment data will continue to be collected until death or termination of data collection by the Sponsor or withdrawal of consent by the subject.

8 REPORTING AND DOCUMENTATION OF RESULTS

8.1 Evaluation of Efficacy

8.1.1 Overall Survival

The time from start of treatment to the date of death from any cause. For subjects who are not known to have died as of the data-inclusion cut-off date, OS time will be censored at the date of the last contact confirming the subject was alive.

8.1.2 Progression-free survival

The time from start of treatment to the date of confirmed objective progression or death from any cause, whichever occurs first. For subjects who are not known to have died or progressed as of the data-inclusion cut-off date, PFS time will be censored at the date of the last objective progression-free disease assessment prior to the date of any subsequent recurrent GB treatment.

PFS will be determined by RANO-based assessments, adopting the general approaches described for the modified RANO (mRANO) assessment with the response rubric of iRANO (for immunotherapy agents). Additional response assessments will incorporate the use of advanced imaging modalities (e.g. MRI perfusion / diffusion assessments) in the m/iRANO approach, to support differentiation of true vs. pseudo progression / response.

8.1.3 Objective Response Rate

Objective Response Rate (ORR) is the proportion of subjects who achieved a complete response (CR) or partial response (PR) out of all treated subjects.

According to the modified RANO criteria, a responder is defined by radiographic and clinical criteria (Ellingson *et al.*, 2017a). CR and PR together with Stable Disease (SD) and initial and confirmed Progressive Disease (PD) will be first assessed by radiographic changes in tumor size as determined by conventional MRI and further assessed by use of advanced imaging [e.g. diffusion weighted imaging, perfusion using dynamic susceptibility contrast and/or Tumor Response Assessment Maps (TRAMs), etc.] where available. In addition, deterioration of

neurologic function and increase in steroid use will be used to determine PD provided it is confirmed by advanced imaging and/or biopsy since extended duration of pseudo-progression is frequently observed following immunotherapies such as MDNA55 (Weber *et al*., 2003a; 2003b; Okada *et al.,* 2015).

For the purpose of study endpoints, Objective Response (OR) status should be confirmed by repeat examination after at least 4 weeks. Patients who receive low-dose bevacizumab (Avastin®) treatment to control edema, a preliminary OR observed at least 4 weeks post-bevacizumab treatment will require a confirmatory OR after at least 4 additional weeks.

Subjects who remain on study following surgical resection / de-bulking with no histological evidence of recurrent tumor will have a best OR of SD following surgery and will continue to be followed by MRI as scheduled until new recurrence or confirmed PD to determine PFS.

Imaging assessment will be carried out using comprehensive imaging guidelines to help identify pseudo-progression where possible.

A blinded central review will be employed to provide independent assessment of response or progression following treatment with MDNA55 according to RANO-based criteria (Wen *et al.,* 2010; Ellingson *et al.,* 2017a) incorporating advanced imaging modalities. To rule out pseudo-progression, advanced imaging modalities and/or biopsy will be utilized.

8.1.4 Other Time-to-Event Endpoints

The time-to-event endpoints are defined in Table 8.

Table 8: Efficacy Endpoints

Endpoint	Definition
Overall Survival (OS) at fixed times	OS at 6 (OS-6), 9 (OS-9) and 12 (OS-12) months will be estimated.
Progression-Free Survival (PFS) at fixed times	PFS at 6 (PFS-6), 9 (PFS-9) and 12 (PFS-12) months will be estimated.
Duration of response (DOR)	The time from first response until confirmed disease progression or death among those subjects achieving a complete response (CR) or partial response (PR) to treatment
Duration of clinical benefit (DOCB)	The time from first response or disease stabilization until confirmed disease progression or death among those subjects achieving a CR, PR, or stable disease (SD)

The use of MRI is mandatory to determine tumor response and to assess when objective progressive disease has occurred (for use in estimating PFS, DOR, and DOCB).

8.2 Evaluation of Safety

Each subject receiving MDNA55 via CED will be evaluable for safety. Safety parameters include all laboratory tests and hematological abnormalities, physical findings, ECG, imaging parameters and AEs.

Each subject will be assessed periodically for the development of any toxicity as outlined in Section 7.5.

Definitions and reporting procedures for AEs provided in this protocol comply with current ICH E6 and other applicable international and local regulatory requirements. The Medical Monitor will promptly review all information relevant to the safety of MDNA55. The Investigator will carefully monitor each subject throughout the study for AEs and all AEs will be followed until adequately resolved. CTCAE 4.0 will be used to determine severity of AEs and SAEs.

8.2.1 Definitions

8.2.1.1 Adverse Event

An AE (also known as an adverse experience) is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. More specifically, an AE can be any unfavorable and unintended sign (*e.g.,* an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An AE can arise from any use of the drug (*e.g.,* off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

Any condition present before the catheter placement, including pre-existing conditions and pre-study AEs, will be considered medical history and will not be reported as a treatment-emergent AE unless the condition worsens during or after catheter placement. Any worsening (*i.e.,* any clinically significant adverse change in frequency and/or intensity) of a preexisting condition, which is temporally associated with the use of the Sponsor's product, is also an AE.

8.2.1.2 Adverse Reaction

An adverse reaction is defined as 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.

8.2.1.3 Suspected

A suspected adverse reaction is defined as any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of IND safety reporting, "reasonable possibility" indicates that there is evidence to suggest a causal relationship between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

8.2.1.4 Serious

An AE or suspected adverse reaction is considered *serious* if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

Death

Life-threatening AE

- an AE or suspected adverse reaction is considered life-threatening if, in the view of either the Investigator or Sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Inpatient hospitalization or prolongation of existing hospitalization

- applies if the reported AE requires at least a 24-hour in-patient hospitalization or, if in the opinion of the Investigator, prolongs an existing hospitalization. A hospitalization for an elective procedure or a routinely scheduled treatment is not an SAE by this criterion because a “procedure” or a “treatment” is not an untoward medical occurrence. An emergency room visit of less than 24 hours by itself does not constitute a SAE.

A persistent or significant incapacity or substantial disruption of the ability to conduct normal life function

Congenital anomaly/birth defect

- applies if a subject exposed to a medicinal (investigational) product gives birth to a child with congenital anomaly or birth defect.

Medical and scientific judgment should be exercised in determining seriousness in other situations, such as important medical events that may not be immediately life-threatening, result in death, or hospitalization, but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These events should be considered serious and subject to reporting procedures specified below in Section 8.2.2.4.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.

8.2.2 Evaluating and Recording of Adverse Events

At each visit, all AEs that are observed, elicited by the Investigator, or reported by the subject will be recorded in the appropriate section of the EDC System and evaluated by the Investigator and the Medical Monitor.

Minimum information required for each AE includes description of the event, duration (start and end dates), severity, assessment of seriousness, and causal relationship to study drug and delivery/infusion procedure.

If discernible at the time of completing the AE section in the EDC System, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the Investigator and recorded in the appropriate AE section in the EDC System. However, if an observed or reported sign, symptom, or clinically significant laboratory anomaly is not considered by the Investigator to be a component of a specific disease or syndrome, then it should be recorded as a separate AE in the appropriate AE section in the EDC System (clinically significant laboratory abnormalities are those that are identified as such by the Investigator and/or those that require intervention).

8.2.2.1 Relatedness of Adverse Events

The Investigator will assign attribution of the possible association of the event with use of the investigational drug and, separately, for the surgical/infusion procedure (i.e. AEs that occur during catheter placement prior to start of infusion of study treatment versus AEs with onset after start of infusion). Relationship will be determined for each as follows:

- Related: There is evidence to suggest a causal relationship between the drug and the AE, such as:
    - An event that is uncommon and known to be strongly associated with drug exposure (*e.g.*, angioedema, hepatic injury, Stevens-Johnson Syndrome)
    - An event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (*e.g.*, tendon rupture)
- Unrelated: Another cause of the AE is more plausible (*e.g.*, due to underlying disease or occurs commonly in the study population), or a temporal sequence cannot be established with the onset of the AE and administration of the study treatment, or a causal relationship is considered biologically implausible.

In general, AEs that are worsening of baseline or pre-existing conditions are considered unrelated, unless there is reason to believe that the worsening was attributable to the investigational drug. Further, if death occurs due to progressive disease it is not considered attributable to MDNA55.

8.2.2.2 Severity of Adverse Events

The severity of AEs will be graded and recorded by the Investigator using the National Cancer Institute CTCAE version 4.0 guidelines. When specific AEs are not listed in the CTCAE 4.0 they

will be graded by the Investigator as *none*, *mild*, *moderate* or *severe* according to the following grades and definitions:

Grade	Definition
Grade 0	No AE (or within normal limits)
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate; minimal, local, or noninvasive intervention (*e.g.,* packing, cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL)
Grade 3:	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
Grade 4:	Life-threatening consequences; urgent intervention indicated
Grade 5:	Death related to AE

8.2.2.3 Follow-up of Adverse Events

All AEs will be followed with appropriate medical management until resolved. Subjects withdrawn from study for unacceptable AEs will be followed until resolution or stabilization of the AE. For selected AE for which administration of the investigational drug was stopped, a re-challenge of the subject with the investigational drug may be conducted if considered both safe and ethical by the Investigator.

8.2.2.4 Serious Adverse Event Reporting

Within 24 hours of becoming aware of any SAE (regardless of its relationship to investigational product) that occurs during the course of the clinical study up until 12 months from start of infusion, the study personnel at the site must notify the pharmacovigilance vendor, ProPharma, within 24 hours of discovery using a written report (SAE Report Form) via Fax or Email as outlined in table below (the SAE Report Form must be submitted by the site within 24 hours even if it is incomplete). This ensures timely reporting of applicable reports to the applicable Health Authorities. The form must be signed by the Principal Investigator or designee, and if not available, the unsigned report should be faxed/emailed for initial processing and resubmitted after the Investigator's signature has been obtained.

Pharmacovigilance:	Medical Monitor:	Sponsor:
[redacted]	[redacted]	[redacted]

[redacted] will review the SAE documentation received for accuracy and completeness and follow-up with the Investigator to obtain missing information, if required. Upon receiving such notices, the Investigator must review and retain the notice and immediately submit a copy of this information to the IRB/IEC. The Investigator should also comply with IRB/IEC and / or any other specific procedures in their respective institution for reporting any other safety information. Where required, the Investigator must submit safety updates to the local Health Authorities.

All AEs and SAEs will be followed until the event has resolved or until the end of the study whichever occurs first. Follow-up SAE information should be aggressively collected and submitted as soon as new information becomes available by using the follow-up SAE Report Form and faxing/emailing this form to ProPharma. If the follow-up information changes the Investigator's and the Sponsor's assessment of seriousness or causality or relationship to the study drug, this change should be noted on the follow-up SAE Report Form. The initial and any follow-up SAE reports should be placed in the subject's file. For subjects that withdraw from the study, SAEs that occur within 30 days of the date of discontinuation, will be collected.

The Principal Investigator and Sponsor may stop the study at any time if the risk-benefit ratio is no longer favorable for the subject.

SAEs must also be reported on the AE page of the EDC System with corresponding terminology, onset/stop dates and assessment of severity, causality, subject outcome, treatment, and action taken.

If it is determined that the event meets expedited safety reporting criteria (serious, unexpected, and related to study drug expedited reporting to Health Authorities is required.

Serious Unexpected Serious Adverse Reaction (SUSAR) Reporting to Sites:

ProPharma will provide a SUSAR safety packet to all study Investigators at which point site must submit the SUSAR safety packet to their IRB/IEC according to their local requirements.

Site must also observe the statutory reporting time frame for expedited reporting of all SUSARs to FDA:

Fatal or life threatening SUSARs:

Not later than 7 calendar days after the sponsor has information that the case reported fulfils the criteria for a fatal or life-threatening SUSAR, with any follow up information to be reported within a further 8 calendar days.

All other SUSARs:

Not later than 15 calendar days after the sponsor has information that the case fulfilled the criteria for a SUSAR.

8.2.2.5 Pregnancy Reporting

Although pregnancy is not considered an AE, the Investigator (or his or her designee) is responsible for recording in the subject's source document any pregnancy during or within 6 months after completing infusion. All subjects who become pregnant must be followed by the Investigator until the end of the pregnancy to determine the outcome. The outcome of the birth (health of infant) must be reported to the Medical Monitor.

9 Statistical Methods

The primary efficacy analysis of OS will be assessed according to a single-arm, single-stage design on the ITT population. OS will be determined via Kaplan-Meier estimation, with medians, quartiles, and 95% confidence intervals (CIs) reported. mOS will tested using a single sample one sided log-rank test on a 10% significance level against historical control.

A secondary analysis of the primary variable will be conducted in the IL4-R population according to IL4-R status.

PFS will be assessed and summarized as OS.

Descriptive analysis of the OS and PFS at 6, 9, and 12 months after treatment will be based on the raw proportions of subjects surviving (and progression-free) at those time points as well as Kaplan-Meier estimation. Further analyses will also be conducted by IL4R stratum, including examination of the treatment effect by IL4R level.

Efficacy analyses may also explore subject subsets (e.g. IL4R level, tumor size, KPS, gender, age, steroid use,) and response by other applicable criteria.

Descriptive statistics will be provided for subject demographics and disposition, safety, and exposure data and will include the number of observations, mean, standard deviation, median, and range for continuous variables and number and percent for categorical variables; 95% CIs will be presented where appropriate.

9.1 Determination of Sample Size

The sample size is calculated for the primary analysis and based on the following assumptions: uniform accrual over time, no loss to follow-up, exponentially distributed death times, use of the exponential MLE test, follow-up time 12 months and a one-sided test on a 10% significance level. With a null hypothesis of a median OS of 8 months and an alternative hypothesis of 11.5 months the power for the primary test will be over 80% with 46 evaluable subjects.

9.2 Statistical and Analytical Plans

The primary analysis will occur when median survival is reached (> 50% of all subjects have died) or all subjects have completed the Day 360 visit or discontinued prior to completing the Day 360 visit whichever happens last. Collection of follow-up data will continue until all patients have withdrawn from follow-up. Supplementary reports, presenting updated time-to-event data, will be prepared after completion of the 2-year survival follow-up period and beyond, if required.

Descriptive statistics will be presented including the number and percent for categorical variables and the number of observations, mean, standard deviation, median, and range for continuous variables; 95% confidence intervals (CIs) will be presented as appropriate.

9.2.1 Randomization

This study is a single-arm design. All subjects will receive active treatment. No randomization will be performed.

9.2.2 Analysis Populations

Following quality review of study images mid-way through recruitment, local tissue reactions, inflammation, immune cell infiltration, edema and/or necrosis, subsequent to MDNA55 administration, was observed in some subjects and has been seen to be indistinguishable from possible tumor growth using the imaging techniques adopted from the outset of this study. Therefore, obtaining reliable imaging data for the primary efficacy analysis has been shown to be confounded. Consequently, under protocol version 6.0, the surrogate endpoint Objective Response Rate (ORR) will be assessed as a secondary outcome measure with the median Overall Survival (mOS) becoming the primary variable.

9.2.2.1 Intent to Treat (ITT)

An ITT population will comprise all subjects who receive any amount of study drug will be used for the primary efficacy analyses, other purely survival-based endpoints and all safety analyses.

A modified ITT population for secondary imaging-based analyses, including PFS, (mITT, see below) will comprise all subjects who receive any amount of study drug and have adequate imaging or clinical data for the ORR analysis. NOTE: Patients who expire or progress clinically prior to the first MRI examination will not be evaluable for any of the response assessments.

9.2.2.2 Per Protocol (PP) Population

The PP population will comprise all patients in the ITT population (or mITT for imaging-based endpoints) who also have no major protocol violations during the study. This population will be finalized prior to the final lock and primary analysis of the study data. Efficacy analyses will be conducted on this population in support of the primary efficacy results.

9.2.2.3 IL4R Analysis Population

The IL4R Analysis Population will be the same as the ITT Population, excluding patients who do not have archived tissue or adequate tissue available for analysis, and will be used for efficacy analyses in subgroups according to IL4R expression status (Section 9.2.9).

9.2.2.4 Safety Population

The Safety population will comprise all patients treated on study. Safety analyses will be presented on this population.

9.2.3 Missing Data

All available data will be used, and no efficacy data will be imputed. Procedures for handling missing dates will be described in the statistical analysis plan. For the analyses of response, subjects with baseline assessments but no post-treatment assessments will be counted as non-responders. For time to event variables, patients not known to have experienced an event at the time of analysis will be censored as specified in Section 9.2.5.2.

9.2.4 Baseline and Demographic Characteristics

Baseline and demographic characteristics will be summarized and presented descriptively.

9.2.5 Analysis of Efficacy

Details of all planned efficacy analyses and methods will be published in the SAP (statistical analysis plan) prior to database lock.

9.2.5.1 Primary Efficacy Variable

OS will be defined as the time (in weeks) from start of the CED infusion until death from any cause. Subjects not know to have died at the time of the analysis will be censored at the time of last contact.

OS and will be summarized using the Kaplan-Meier method and tested using a single sample one sided log-rank test on a 10% significance level with null hypothesis of OS 8 months and alternative hypothesis of OS > 8 months.

9.2.5.2 Secondary Efficacy Variables

Secondary efficacy variables will include OS variants, ORR and PFS.

ORR will be presented as the percentage of subjects with CRs or PRs by the RANO-based criteria, including those incorporating advanced imaging modalities; only confirmed responses will be presented (i.e., those observed on 2 consecutive MRI scans not less than 4 weeks apart and at appropriate interval following discontinuation of bevacizumab if response was not observed prior to use of bevacizumab).

PFS will be summarized using the Kaplan-Meier method. PFS will be assessed using a single sample log-rank test (against historical control) at 10% significance level with null hypothesis of mPFS 4 months and alternative hypothesis of mPFS > 4 months. PFS will be defined as the time

(in weeks) from start of the CED infusion until radiologic or neurologic disease progression or death from any cause. Subjects not know to have died or experienced disease progression at the time of the analysis will be censored at the time of the last radiologic assessment demonstrating lack of progression or, if during the follow-up period, the time of last contact indicating lack of progression.

Graphical displays and incidence estimates will show PFS and OS at 6, 9, and 12 months.

9.2.5.3 Other Efficacy Variables and Analyses

DOR will be summarized using the Kaplan-Meier method, including graphical displays. DOR will be calculated only for the subset of subjects with a response (CR or PR), and it will be defined as the time (in weeks) from first response until radiologic disease progression or death from any cause. Responders alive and progression-free at the time of the analysis will be censored at the time of the last radiologic assessment demonstrating lack of progression or, if during the follow-up period, the time of last contact indicating lack of progression.

DOCB will be summarized using the Kaplan-Meier method, similar to DOR. DOCB will be calculated only for the subset of subjects with a stable disease or better (CR, PR, or SD), and it will be defined as the time (in weeks) from first response until radiologic disease progression or death from any cause. Censoring will be performed in a fashion similar to DOR.

Additional, exploratory, efficacy variables may include ORR and PFS based on the Investigator's assessment of response and other time-to-event endpoints (*e.g.*, time to post-study treatment of GB). Analyses will also be conducted by IL4R stratum, including examination of the treatment effect by IL4R level.

Efficacy analyses will also explore subject subsets (procedural success, IL4R level, sequence number by site [learning], tumor size, tumor coverage, time of infusion, maximum flow rate, number of catheters, percentage of planned infusate administered, KPS, gender, age, steroid use, immune status) and response by other applicable criteria. Subgroup and sensitivity analyses comparing subjects included before and after protocol version 3.0 may be explored.

9.2.6 Analysis of Safety Variables

Safety variables, including AEs, laboratory results, vital signs, ECGs, antibody assessments, and serum drug levels on PK samples, will be summarized and presented, by study time where appropriate.

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Cancer Therapy Evaluation Program (CTEP) Common Terminology Criteria for Adverse Events NCI CTCAE v4.0 will be used to grade the severity of AEs. Treatment emergent AEs will be summarized by system organ class (SOC) and by preferred terms for all treated subjects and subset of subjects of interest. Certain summaries of the treatment-emergent AEs will also be generated by severity, relationship to study drug, relationship to infusion, catheter placement, volume of infusate, and prior therapies. Proportion of patients experiencing Grade 3 and 4 laboratory test results will be summarized.

9.2.7 Interim Analysis

[redacted]

No formal interim analyses for efficacy have been conducted or are planned.

9.2.8 MRI Analysis

Exploratory analyses will be performed to assess the relationships between planned tissue coverage, actual tissue coverage, tissue toxicity, tissue response (perfusion MRI-derived relative cerebral volume and tracer kinetic (permeability, intravascular and extravascular volumes maps) and clinical results and IL4R expression. These analyses will be descriptive in nature.

9.2.9 Tumor Tissue Analysis

Exploratory analyses will be performed to assess the relationship of IL4R expression levels in tumor tissue with treatment response, tissue response and survival. This analysis will be descriptive in nature.

9.3 Data Quality Assurance

Steps to be taken to assure accuracy and reliability of data include selection of qualified Investigators who have experience with intracranial administration of therapeutic agents and appropriate research centers, and review of protocol procedures with the Investigator and associated personnel before the start of the study. There will be periodic monitoring by a Sponsor representative(s). 100% of data entered into the EDC System and database will be reviewed for accuracy and completeness by a Sponsor representative(s) during on-site monitoring visits, and any discrepancies will be resolved with the Investigator or designees, as appropriate. Data in the clinical trial database will be further checked for accuracy and consistency by a series of computerized and manual procedures.

9.4 Multiplicity control

Multiplicity is controlled by hierarchical testing of the endpoints. First the primary endpoint (OS) will be tested at a 10% one sided significance level. The PFS and the secondary endpoints are then tested in a hierarchical manner (all at a 10% one-sided significance level), first PFS and then secondary endpoints. Testing will only continue if the prior null hypothesis is rejected – thereby controlling the familywise type-I error. Further details will be specified in the SAP.

10 Investigator Requirements and Study Management

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki as stated in 21 CFR §312.120(c)(4); consistent with ICH E6 GCP and all applicable regulatory requirements.

Before initiating this trial, the Investigator will have written and dated approval from the Institutional Review Board for the protocol, written informed consent form, subject recruitment materials, and any other written information to be provided to subjects before any protocol related procedures are performed on any subjects.

The Investigator must comply with the applicable regulations in Title 21 of the Code of Federal Regulations (21 CFR §50, §54, and §312), GCP/ICH guidelines, and all applicable regulatory requirements. The IRB must comply with the regulations in 21 CFR §56 and applicable regulatory requirements.

10.1 Study Initiation

The following documentation must be received by the Sponsor or their representative prior to initiation of the trial:

- The names of Principal Investigators and any Sub-Investigators must appear on the appropriate form complying with local regulations (*e.g.*, US FDA Form 1572) and signed by all Principal Investigators. Investigators must also complete all regulatory documentation as required by ICH E6 GCP and by local or national regulations
- Current (within 2 years) *curricula vitae* of the Principal Investigator and all Sub-Investigators
- IRB/IEC membership list
- Written documentation of IRB/IEC approval of protocol (identified by protocol number and/or title and date of approval) and informed consent document (identified by protocol number and/or title and date of approval)
- A copy of the IRB/IEC-approved informed consent document
- Written documentation of IRB/IEC review and approval of any advertising materials to be used for study recruitment must be received by the Sponsor or their representative. Informed consent forms and any advertising materials must also be reviewed and approved by the Sponsor or their representative prior to seeking approval of IRB/IEC and regulatory agencies
- Current laboratory certification of laboratory performing the hematology, coagulation, and serum chemistry analyses (issuing agency and expiration date), as well as current normal laboratory ranges for all laboratory tests
- A signed Clinical Research (Protocol) Agreement (see page 3 of this protocol)
- Certified translations of IRB/IEC approval letters, pertinent correspondence, and approved informed consent document (when applicable)
- Financial disclosure form for Principal Investigator and all Sub-Investigators, their spouses and dependent children
- Clinical Trial Agreement

10.2 Informed Consent

All participants must be provided a consent form describing the study with sufficient information for each participant to make an informed decision regarding their participation. Participants must sign the IRB/IEC-approved informed consent form prior to participation in any study specific procedure. The participant must receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or Investigator file.

10.3 Protocol Adherence

Once the protocol has been approved, any substantive changes to the protocol made by the Sponsor must be documented in the form of a major amendment. The major amendment must be signed by the Investigator and approved by IRB/IEC and submitted to the FDA prior to implementation. Changes of an administrative nature (no likely impact on patient safety or data integrity) may be instituted as minor amendments.

Each Investigator must adhere to the protocol as detailed in this document, will be responsible for enrolling only those subjects who have met protocol eligibility criteria, and must agree that no changes to the protocol are made, except to eliminate an immediate hazard to the study subjects. If it becomes necessary to alter the protocol to eliminate an immediate hazard to subjects, the Investigator must then notify the Sponsor and IRB in writing within five (5) working days after implementation. The Sponsor will be responsible for updating any participating sites.

10.4 Monitoring Procedures

The Investigator is responsible for the validity of all data collected at his/her site. The purpose of monitoring is to verify that the rights and well-being of human subjects are protected, that trial data are accurate (complete and verifiable to source data), and that the trial is conducted in compliance with the protocol, ICH E6 GCP, and regulatory requirements.

10.4.1 Routine Monitoring

Monitors assigned by the Sponsor or their representative will conduct regular visits to the investigational sites to monitor various aspects of the study. The Investigator must agree to allow Sponsor-authorized personnel direct access to the clinical (or associated) files and clinical trial supplies for all potential and enrolled study subjects to verify EDC System entries. The Investigator should make adequate time and space available for monitoring visits.

The site must complete pages in the EDC System in a timely manner and on an ongoing basis to allow regular review by the study monitor.

Whenever a subject's name is revealed on a document that is to be given to the Sponsor, the name must be blacked out permanently by the site personnel, leaving the initials visible, and annotated with the subject study number as identification.

10.4.2 Inspection and Auditing Procedures

Authorized personnel from external regulatory authorities and quality assurance personnel authorized by the Sponsor may carry out inspections and audits. The purpose of an audit is to ensure that ethical, regulatory, and quality requirements are fulfilled in all studies performed by the Sponsor.

Auditors and inspectors must have direct access to study files, subject's clinical files, and associated study records as well as to clinical supply dispensing and storage areas and any other locations used for the study. In the event that the site is notified directly of a regulatory inspection, the Investigator should notify the Sponsor representative as soon as possible, so that the Sponsor representative can assist with preparations for the inspection.

10.5 Data Recording and Retention of Study Data

The Principal Investigator and/or his/her designee, will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study and will be responsible for recording all data with respect to protocol procedures, drug administration, laboratory data, safety data, and efficacy ratings in the EDC system. The Investigator may formally designate authority to appropriately qualified staff to complete pages in the EDC system, by authorizing and completing the signature log.

The information entered into the EDC system shall be identical to that appearing in original source documents. Source documents will be found in the subject's medical records maintained by institutional personnel. All source documentation must be available for review/monitoring by monitors appointed by the Sponsor or their representative and/or regulatory agencies.

All corrections on source documents must be made in a way that follows the Standard Operating Procedures of the site, does not obscure the original entry. The correct data must be inserted, dated, and initialed by study site personnel. If it is not obvious why a change has been made, a reason must be provided. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID.

In accordance with federal regulations, the Investigator is responsible for the accuracy and authenticity of all clinical and laboratory data entered in the EDC system. The Investigator will approve all completed CRFs to attest that the information contained on the CRFs is true and accurate.

The Investigator must sign the final EDC System “End of study” pages (Principal Investigator signature page) to attest for the accuracy and completeness of all data. These pages must not be signed until after the EDC files have been reviewed 1 final time.

If the Investigator relocates or retires, or otherwise withdraws responsibility for maintenance and retention of study files, the Sponsor must be notified in writing, so that adequate provision can be made with regard to the trial documents.

Trial documents should be retained at least 2 years after the last approval of a marketing application until there are no pending or planned marketing applications, or at least 2 years have elapsed since the formal discontinuation of clinical development of the product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the Sponsor. The Investigator should take measures to prevent accidental or premature destruction of these documents. The archiving arrangements will be addressed by the monitor when closing out the site. The Sponsor will inform the Investigator, in writing, as to when these documents no longer need to be retained.

10.6 Confidentiality of Data

Subjects’ medical information obtained by this study is confidential, and disclosure to third parties other than those noted below is prohibited.

Upon the subject’s permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare.

Data generated by this study must be available for inspection by representatives of the US FDA or other Health Regulatory Authorities, national and local health authorities, the Sponsor or their representative, and the IRB/IEC.

10.7 Study Drug Accountability

All investigational drug required for completion of this study will be provided by the Sponsor. Each site will acknowledge receipt of the drug indicating shipment content and condition. Damaged supplies will be replaced.

The Principal Investigator shall not make the investigational drug available to any individuals other than to qualified study subjects. Furthermore, the Principal Investigator will not allow the investigational drug to be used in any manner other than that specified in this protocol.

Accurate records of all study drug received/inventoried, dispensed from the study site Pharmacy will be maintained.

During the study and at the end of the study, the monitor will verify all inventory and accountability records maintained by the hospital pharmacy. All used drug vials are disposed of at the study site according to the institution's standard operating procedure. Any unused drug vials will be inspected to ensure proper drug dispensing and accuracy of supply. Return unused study drug as directed by the Sponsor or their representative.

All other study materials supplied by the Sponsor, such as catheters and infusion pumps, must be accurately accounted for as well. Unopened, expired, or unused materials as well as infusion pumps will be returned as directed by the Sponsor or their representative.

10.8 Study Completion

The following data and materials are required by the Sponsor or their representative before the study can be considered complete or terminated:

- All test results from screening through the end of the study (*e.g.*, clinical data, all special test results)
- EDC System properly completed by appropriate study personnel
- Study personnel signature and responsibility log
- Completed drug accountability records
- Copies of protocol amendments and IRB/IEC approval/notification, if appropriate
- Documentation of a close letter to the site IRB/IEC by the Principal Investigator
- Completion of all SAE and supplemental follow-up reports in addition to all data queries
- Undertaking by the Investigator to retain/archive study documents as per applicable regulatory requirements

10.9 Study Discontinuation

The Sponsor has the right to terminate this study at any time. Reasons for terminating the study may include (but are not limited to) the following:

- The incidence or severity of AEs in this or other studies indicates a potential health hazard to subjects
- Subject enrollment is unsatisfactory; and/or
- Data recording is inaccurate or incomplete

If appropriate, reimbursement for reasonable expenses will be made.

10.10 Clinical Study Report

The results of this trial will be documented in an integrated statistical and clinical study report that will include a discussion of the study objectives, methodology, clinical observations, and conclusions in relation to the study objectives.

10.11 Ethical Conduct of the Clinical Investigation

The Investigator will ensure that this study is conducted in full conformance with the principles of the "Declaration of Helsinki" and its amendments, or with the laws and regulations of the locality in which the research is conducted, whichever affords the greater protection to the individual.

10.12 Compliance with Financial Disclosure Requirements

By participating in this protocol, the Investigator agrees to provide to the Sponsor accurate financial information to allow the Sponsor to submit complete and accurate certification and disclosure statements as required by local and international guidelines.

10.13 Contractual and Financial Details

The Investigator (and/or, as appropriate, the hospital administrative representative) and/their representative will sign a clinical study agreement prior to the start of the study, outlining overall Sponsor (and/or, as appropriate, the Sponsor representative) and Investigator responsibilities in relation to the study. Financial remuneration will cover the cost per included subject, based on the calculated costs of performing the study assessments in accordance with the protocol, and the specified terms of payment will be described in the contract. The contract should describe

whether costs for pharmacy, laboratory, and other protocol-required services are being paid directly or indirectly.

10.14 Insurance, Indemnity, and Compensation

The Sponsor will provide Product Liability insurance for all subjects included in the clinical study. Where required, a hospital-specific indemnity agreement will be used.

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